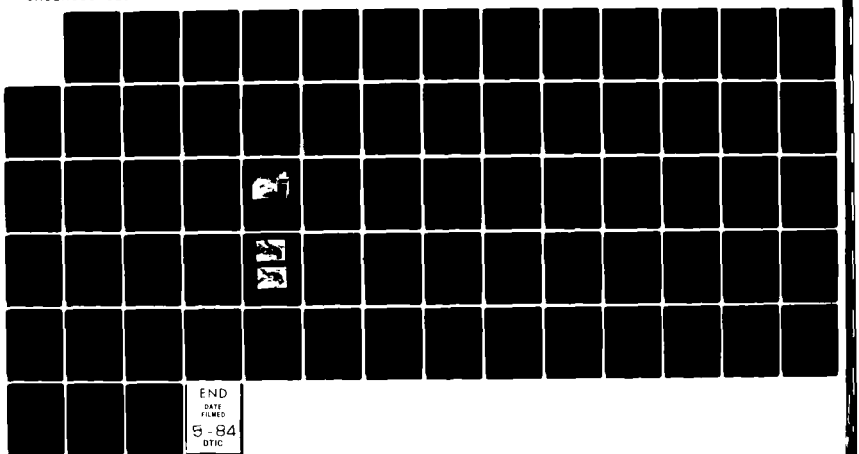
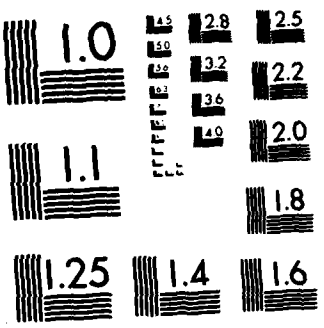
AD-A140 102 IN-VIVO TECHNIQUES FOR MEASURING ELECTRICAL PROPERTIES 1/1
OF TISSUES(U) GEORGIA INST OF TECH ATLANTA ENGINEERING
EXPERIMENT STATION E C BURDETTE ET AL. SEP 80
UNCLASSIFIED DAMD17-78-C-8044 F/G 6/16 NL



END
DATE FILMED
5-84
DTIC



(1)

AD _____

IN-VIVO TECHNIQUES FOR MEASURING ELECTRICAL PROPERTIES OF TISSUES

ANNUAL REPORT

E. C. Burdette, P. G. Friederich, F. L. Cain

September 1980

Supported by

US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-78-C-8044

Georgia Insitute of Technology
Engineering Experiment Station
Atlanta, Georgia 30332

Approved for public release; distribution unlimited

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A140 702	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) In-Vivo Techniques for Measuring Electrical Properties of Tissues	5. TYPE OF REPORT & PERIOD COVERED Annual 1 July 79--30 June 80
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) E.C. Burdette, P.G. Friederich, and F.L. Cain	8. CONTRACT OR GRANT NUMBER(s) DAMD17-78-C-8044
9. PERFORMING ORGANIZATION NAME AND ADDRESS Engineering Experiment Station Georgia Institute of Technology Atlanta, Georgia 30332	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62777A.3E162777A878.BB.013
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, Maryland 21701	12. REPORT DATE September 1980
	13. NUMBER OF PAGES 71
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report) Unclassified
	15a. DECLASSIFICATION DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for Public Release; Distribution Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Approved for Public Release; Distribution Unlimited

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

In-Vivo probe
Dielectric properties
In-situ tissues
Antemortem/Postmortem studies
Renal blood flow
Electromagnetic energy
Monopole antenna

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The overall objectives of this research investigation are to further develop and extend the capabilities of the recently-developed in-vivo probe measurement technique and to use this technique to study the possible effects of induced physiological changes on tissue dielectric properties. During the second year of the program, the research investigations performed included (1) development of techniques to further reduce systemic measurement errors, (2) development of a method for maintaining uniform probe contact pressure during in-situ dielectric measurements of tissue, (3) examination of the potential necessity of time-

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. ABSTRACT

locking data sampling to the animal's respiratory cycle, (4) studies of antemortem/postmortem dielectric characteristics of the pial surface of dog brain, (5) investigations of the effects of changing renal blood flow on kidney dielectric properties, and (6) initial studies of the effects of auditory stimulation on dielectric properties of the auditory cortex in dogs.

Significant changes in dielectric properties of dog cortex were observed as a function of time postmortem; sacrifice using KCl and $CaCl_2$ produced different effects on dielectric properties immediately post injection which were consistent with their different mechanisms of physiological action. Mean changes in dielectric constant and electrical conductivity postmortem were 9.5% and 16.5%, respectively. In studies of the effects of renal blood flow on dielectric properties of kidney cortex and medulla, without exception a decrease in renal flow rate resulted in a measurable increase in dielectric constant and permittivity. Both in-vitro and in-situ renal studies were conducted, with emphasis on characterizing dielectric changes in the isolated perfused kidney. Generally, greater effects were observed in medullar rather than cortical tissue; the least effect of flow rate/perfusion pressure on dielectric properties was observed on the cortical surface. No correlation between acoustic stimuli and dielectric property changes in dog auditory cortex was observed. However, the lack of positive results do not necessarily mean that there was no effect. No changes in cortical EEG response was observed during auditory stimulation. This lack of cortical response was attributed to the existence of significant CNS depressant effects (of the anesthetic agent used) especially on higher centers. Use of halothane anesthesia (or a decerebrate preparation) and selection of an optimal stimulus paradigm should correct the problem, thus permitting evaluation of sensory stimulation.

FOREWORD

The research on the second year of this four-year program was carried out by personnel of the Biomedical Research Branch of the Electronics Technology Laboratory of the Engineering Experiment Station at the Georgia Institute of Technology, Atlanta, Georgia 30332. Mr. F. L. Cain served as the Principal Investigator and Mr. E. C. Burdette served as the Associate Principal Investigator. The program, which is sponsored by the U.S. Army Research and Development Command, Fort Detrick, Frederick, Maryland 21701, under Contract No. DAMD17-78-C-8044, is designated by Georgia Tech as Project A-2171.

This Annual Technical Report covers the work which was performed from 1 July 1979 through 30 June 1980 . This work was made possible through the combined efforts of many people at the Walter Reed Army Institute of Research (WRAIR), at the Emory University School of Medicine, and at the Georgia Institute of Technology. The authors would especially like to thank Dr. L. E. Larsen and Mr. J. H. Jacobi at WRAIR and Dr. V. P. Popovic at the Emory University School of Medicine, all of whom contributed significantly to the second year's success of this research program.

Respectfully submitted,

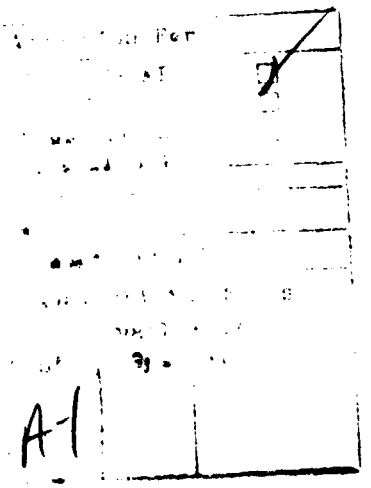
Everette C. Burdette

E. C. Burdette
Associate Principal Investigator

Approved:

Fred L. Cain

Fred L. Cain
Principal Investigator
Associate Director,
Electronics Technology Laboratory



SUMMARY

The overall objectives of this research investigation are to further develop and extend the capabilities of the recently-developed in-vivo probe measurement technique and to use this technique to study the possible effects of induced physiological changes on tissue dielectric properties.

During the second year of the program, the research investigations performed included (1) development of techniques to further reduce systemic measurement errors, (2) development of a method for maintaining uniform probe contact pressure during in-situ dielectric measurements of tissue, (3) examination of the potential necessity of time-locking data sampling to the animal's respiratory cycle, (4) evaluation of data recording methods, (5) studies of antemortem/postmortem dielectric characteristics of the pial surface of dog brain, (6) investigations of the effects of changing renal blood flow on kidney dielectric properties, and (7) initial studies of the effects of auditory stimulation on dielectric properties of the auditory cortex in dogs.

Significant changes in dielectric properties of dog cortex were observed as a function of time postmortem; sacrifice using KCl and $CaCl_2$ produced different effects on dielectric properties immediately post injection which were consistent with their different mechanisms of physiological action. Mean changes in dielectric constant and electrical conductivity postmortem were 9.5% and 16.5%, respectively.

In studies of the effects of renal blood flow on dielectric properties of kidney cortex and medulla, without exception, a decrease in renal flow rate resulted in a measurable increase in dielectric constant and conductivity. Both in-vitro and in-situ renal studies were conducted, with emphasis on characterizing dielectric changes in the isolated perfused kidney. Generally, greater effects were observed in medullar rather than cortical tissue; the least effect of flow rate/perfusion pressure on dielectric properties was observed on the cortical surface.

No correlation between acoustic stimuli and dielectric property changes in dog auditory cortex was observed. However, the lack of positive results do not necessarily mean that there was no effect, because no changes in cortical EEG response were observed during auditory stimulation (which

would have been indicative of locally-increased blood flow). This lack of cortical EEG response to sensory stimulation confirms the existence of significant CNS depressant effects (especially on higher centers) due to the anesthesia used. It is also believed that the stimulus paradigm may not have been optimal for illiciting a measurable cortical response. Use of halothane anesthesia (or a decerebrate preparation) and an optimal stimulus paradigm should correct the problem, thus permitting evaluation of sensory stimulation effects on cortical dielectric properties.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

SECTION I
INTRODUCTION

The research described in this Annual Report is the result of efforts performed during the second year of this four-year research program. The overall objectives of this research investigation are to further develop and extend the capabilities of the recently-developed in-vivo probe dielectric property measurement technique [1-4] and to use this technique to study the resultant effect of induced physiological changes in organs or organ systems on local tissue dielectric properties within that system.

It is well known that the interaction of an electromagnetic (EM) field with a biological system is largely determined by the dielectric properties of that system. Accurate in-situ dielectric property information would be of significant benefit in many ways. Real-time in-situ measurement of living tissues could be used for the detection of pathophysiological conditions in tissues, for measuring changes in certain normal physiological processes, for differentiating between normal and diseased tissues, or for elucidating pharmaco-physiological effects due to drugs. Also, it is important to note that tissue dielectric properties play a key role in electromagnetic imaging, both dosimetric and diagnostic in nature. Specific differences or changes in in-situ dielectric properties within a single tissue type or among tissues are reflective of the ability of EM imaging methods to discern the existence of pathophysiological conditions in intact tissues, organs, or organisms. Differences between in-situ living tissue properties and in-vitro properties are of key importance in dosimetry determinations (in both magnitude and distribution) via EM imagery. Finally, in dosimetry determinations with respect to potential EM radiation hazards and in treatment planning for cancer patients using hyperthermia induced by RF or microwave fields,an accurate knowledge of the respective in-situ tissue dielectric properties is essential to an accurate determination of absorbed power.

A. Background

The determination of the dielectric properties of biological tissues has in the past been limited to measurements over restricted frequency ranges on separate excised tissue samples. These previous results served to validate electrical impedance models of tissue structure, but were not capable of yielding information on the actual living in-situ tissue dielectric properties. In addition, the influence of surrounding tissue organization, blood perfusion, and the existence of pathological conditions could not be ascertained from these in-vitro measurements.

Questions addressing the importance of in-situ tissue dielectric property data as opposed to data obtained from measurements of excised tissues have long been of concern to investigators. As early as 1922, Osterbout described significant changes in the dielectric characteristics of biological materials following death due to the loss of membrane function and the breakdown of cellular structure [5]. Rajewsky's low frequency measurements showed a deterioration of the dielectric properties concomitant with a significant decrease in metabolic rate [6]. However, the reported change did not begin until approximately one day following death of the organism. Recent in-situ low frequency measurements by Burdette, et al. [7] revealed changes in electrical conductivity of the liver within an hour following death, while one day was required for the observation of changes in the dielectric permittivity. The observed changes in low frequency dielectric properties are caused by a breakdown of cellular membranes, which are primarily responsible for the low frequency characteristic properties (i.e., α and β dispersions). In other measurements, Larsen, Jacobi, and Krey [8] have reported changes in permittivity dispersion over the 3-30 MHz frequency range associated with physiological and drug-induced pathophysiological changes in cellular memberanes in living cell suspensions. Schwan and Foster [9] state that the high frequency data are relatively unaffected by death of the tissue because at microwave frequencies and that the dielectric characteristics of tissue are predominately due to the water and protein contents of the tissue. However, some of their data [9,10] do show magnitude changes in dielectric properties

following death. They also indicate that changes in the tissue do occur upon excision which may affect its dielectric properties. Factors which may play a role in dielectric changes due to excision include blood loss and moisture loss.

It is true that at high frequencies the tissue dielectric characteristics largely reflect those of water. However, in measurements performed in-situ (under the present contract), changes in the dielectric characteristics of brain tissue at microwave frequencies were seen immediately upon termination of the experimenal animal [11,12]. These initial changes were followed by a slower, gradual reduction in permittivity and conductivity over a longer period of time (two hours). The initial change was attributed to blood loss, while the slower changes were attributed to a combination of tissue water loss and autolysis. From those in-situ studies of permittivity, there appear to be significant influences on tissue dielectric characteristics due to the fact that the tissue is living and in-situ, even at microwave frequencies.

Extensive work at Georgia Tech has been devoted to studying and developing a small (2mm diameter) probe that operates over a wide frequency range (1 MHz to 10,000 MHz) [1-4]. The development of this measurement probe provides the opportunity for obtaining in-situ dielectric data over a range of physiological conditions and offers the potential of using dielectric measurements for the detection of local tissue pathology. With this probe technique, it also should be possible to correlate the time rate of change of the in-vivo tissue electrical properties with tissue blood flow, in a manner similar to that of impedance plethysmography.

During the present program, under Army Medical Research and Development Command support, the measurement capabilities of the in-situ dielectric probe have been significantly improved. The versatility of the probe was increased through the use of a high-quality flexible probe cable, and systemic and cable/connector measurement errors were accounted for through the development and application of a data correction and processing program which corrects the impedance data measured by the probe for those errors. Methods were developed to virtually eliminate fluid accumulation at the tip of the measurement probe, thereby improving

measurement accuracy and repeatability. An investigation to determine the minimum sample volume necessary for accurate dielectric measurement results was conducted for probes having diameters over the 1.5 - 6.0 mm range. Techniques for rapid data acquistion and processing were studied and developed, and a spring-loaded probe holder which maintains the probe contact pressure on the tissue being measured at a nearly constant value was developed and tested. In-situ measurements of different locations within dog brain were performed which yielded information about the existence of significantly different dielectric properties for the various types of brain matter, which in turn impacts EM-hazards dosimetry determination.

Several investigations of physiological significance were performed during the current research program. The primary study conducted involved the effect of physiological changes at death on in-situ dielectric properties. The results of *antemortem/postmortem* experiments using two methods of sacrifice (KCl and $CaCl_2$) were reported in Quarterly Progress Reports 6 and 7. Significant dielectric changes occurred postmortem for both KCl and $CaCl_2$ sacrifice, with differences between the two sacrifical methods being observed. Measurements of in-situ dielectric changes during death were also performed in canine gray and white matter. Changes in in-situ dielectric impedance due to the presence of vasculature at the probe measurement site were also examined during in-situ measurements on the pia mater of canine brains. Other investigations, which are presently ongoing, include studies of changes in renal dielectric properties concomitant with changes in renal blood flow and experiments involving measurement of in-situ dielectric properties of the auditory cortex during acoustical stimulation.

B. Research Objectives

The objectives of this four-year research program are to further develop and extend the in-vivo probe measurement technique for determining tissue dielectric characteristics, to use this technique to study possible changes in dielectric properties resulting from physiological changes, and to investigate differences between tissue dielectric properties determined in-vivo versus those determined in-vitro. Specific areas of

investigation include (1) extending the capabilities of the recently developed in-vivo probe measurement techniques [1-3], (2) elucidating differences between the in-situ dielectric proerties of living tissue and dielectric properties of tissue determined in-vitro, and (3) correlating changes in the in-vivo dielectric properties of brain with physiological changes (regional cerebral blood flow) due to hypercapnic and anoxic conditions and to non-noxious sensory stimuli. A longer-term objective is the extension of the probe technique as a method for quantitative determination of local blood flow changes in kidney (and possibly brain) without the need for radioactive tracers and tissue excision.

C. Summary of First Year Efforts

The tasks performed during the first-year investigations include (1) the development of techniques for increased flexibility in probe positioning, (2) implementation of methods to account for measurement errors associated with the microwave measurement instrumentation, (3) the development of techniques for rapid data collection and processing, (4) evaluation of the effects of surface fluid accumulation around the probe, (5) fabrication of different diameter probes for measurements of tissue samples of varied volumes, (6) probe dielectric measurements of living and non-living brain tissue, and (7) preliminary measurements of canine brain dielectric properties as a function of induced physiological changes. These efforts are briefly summarized below.

Under previous research efforts [2,3], it was determined that probe positioning was a critical factor in the performance of accurate in-vivo dielectric measurements. Further, it was determined that positioning of the probe needed to be made more compatible with measurement conditions associated with animals larger than mice or rats, which were positioned beneath a fixed measurement probe. Several alternative methods involving the use of semi-rigid or flexible coaxial cable attached to the in-vivo measurement probe were evaluated. The technical requirements of a suitable cable for use with the probe were the following: a length adequate to allow the probe to be positioned on a large experimental animal (dog) and still permit convenient location of the network analyzer, adequate

cable flexibility for ease in repositioning, introduction of minimal phase variations due to cable movement, low attenuation and VSWR, and the ability to withstand sterilization. Following an examination of cables from nearly a dozen manufacturers, it was determined that the best-suited cable was the Gore-Tex ®flexible cable. A three-foot length cable, including connectors, was tested and found to perform satisfactorily over the frequency range examined (2-4 GHz).

An investigation of the accuracy of the results obtained from measurements of standard dielectric materials indicated a need to evaluate the residual systemic errors associated with the network analyzer measurement system [4]. An existing model [3] for reduction of microwave measurement errors (directivity, source match, frequency tracking) associated with the network analyzer, reflectometer, and interconnecting cables was further developed to include multiple-load data in the determination of the directivity error. Also, the systemic error correction model was incorporated in a microprocessor-based data acquisition/data processing system. The error correction is performed through measurements of terminations (short circuits, open circuit, and a sliding matched load) for which the reflection coefficients are known. From these measurements, the error terms are computed using the error correction model and the measured tissue sample data are corrected to account for the systemic measurement errors.

The semi-automated microprocessor-based data acquisition system was upgraded during the first year of the program. Specifically, a digital frequency locking capability was added to the system, an external keyboard/printer was interfaced, and the computer algorithm was rewritten to increase system flexibility and to accommodate additional system hardware. Reflection coefficient data from test samples measured by the probe are automatically collected and processed, and corrected dielectric property information outputted. Measurements may be made either over swept frequency bands between 0.1 GHz and 10 GHz or as a function of time at a single frequency.

Effort was also directed toward the evaluation of the effects of fluid accumulation in the vicinity of the probe. Excessive fluid accumulation affects both the accuracy of the dielectric measurements and the repeatability of the measurements. It was determined that fluid accumulation around the probe did not appreciably effect the results of dielectric measurements of high-loss tissues (muscle, kidney). However, significant effects of fluid accumulation were observed in the results of measurements on relatively low-loss tissues (fat). After investigating several methods of preventing the fluid accumulation, a simple approach was developed which yielded accurate and consistent results.

Attention was also directed to probe fabrication techniques and factors influencing measured sample volume and useful frequency range. In addition to sources of measurement error previously mentioned, errors in measurement accuracy have resulted from factors such as improper attachment of probe connectors and variations in the manufactured quality of materials from which the probes were fabricated. During the first year of this program, additional measures for ensuring good probe fabrication techniques were implemented.

Finally, preliminary in-situ measurements of living and non-living brain and of in-vivo dielectric properties of brain under conditions of induced physiological change were performed as a part of the first-year efforts. All measurements were performed at a frequency of 2450 MHz using a probe having a diameter comparable to that of a 16-guage hypodermic needle. In-situ dielectric measurements of both living and non-living canine brain were performed on the dura, on the pia, and in gray and white matter. In the case of dural measurements, one case of normal and anoxic conditions was examined. Measurements of dielectric properties as a function of time after death were performed both in brain and on the pial surface in separate experiments.

D. Summary of Second Year Efforts

During the second year of the program, the research investigations performed included (1) development of techniques to further reduce systemic measurement errors, (2) development of a method for maintaining constant probe contact pressure on tissue during in-situ dielectric measurements,

(3) examination of the potential necessity of time-locking data sampling to the animal's respiratory cycle, (4) evaluation of analog and digital data sampling and recording methods, (5) studies of antemortem/postmortem dielectric characteristics of the pial surface of dog brain, (6) comparison of pial surface measurements with measurements of homogenized samples of whole-brain tissues, (7) investigations of the effects of changing renal blood flow on kidney dielectric properties, and (8) initial studies of the effects of auditory stimulation on dielectric properties of the auditory cortex in dogs.

During the evaluation of the effects of surface fluid accumulation on probe dielectric property measurement accuracy in the first year of the program, it was determined that probe contact pressure on the tissue was also a factor in the accuracy and repeatability of measured results. This was further apparent during experiments in which antemortem/postmortem dielectric changes in dog brain were studied. Following cessation of blood flow, the brain receeded slightly into the skull, resulting in a change in probe contact pressure and a concomitant change in measured dielectric properties. A six percent maximum change was measured between the conditions of initial probe contact with the pia and an applied pressure producing a 0.15-inch depression of the pia using the 0.085-inch diameter probe. An approximate change of four to five percent in conductivity was also observed under the above stated probe contact conditions. To minimize these effects of probe contact pressure on measured tissue dielectric characteristics, a mechanical "spring-loaded in-situ probe holder" was designed to maintain the probe contact on the tissue at a constant or nearly constant pressure. The contact force (and therefore, contact pressure) of the probe with the tissue is determined by the spring constants. Appropriate springs were selected to achieve complete probe/tissue contact at a pressure which could be consistently maintained, but produce only a slight depression of the pia. Using the spring-loaded probe holder, the probe contact induced variations in measured in-situ dielectric properties were on the order of one percent.

Methods for further reducing systemic measurement errors associated with the microwave network analyzer were investigated during the second

year. Following implementation of the vector measurement error correction model during the first year of the program, the only remaining systemic measurement error source of significance was noise present in the measurement of the phase angle of small-magnitude reflection coefficients. Two approaches to the solution of this problem were examined. The first approach involved the use of an active filter to remove high frequency noise from the phase signal. However, filtering of noise in this manner both added complexity to the measurement system and reduced the maximum data sampling rate. The second approach utilized a software filtering technique in which the reflection coefficient phase data were multiply sampled at each frequency of interest and the statistical mean of these samples was computed and stored. In this manner, the actual data sample rate was not affected and no additional hardware was required. The software filter greatly reduced the noise present in the phase measurement of small complex reflection coefficients.

The Commodore PET microcomputer initially used with the probe dielectric measurement system for control and data acquisition purposes lacked sufficient memory to permit storage of computed dielectric property data for the duration of an experiment. Several possible solutions to the need for an increased data storage capability were examined, and the necessity for time-locking data sampling to the respiratory cycle of the animal was evaluated. The primary question concerned selection of a digital sampling frequency high enough to record the rapid dielectric and physiological changes which occured immediately upon terminating the animal. However, it was also noted that throughout most of the period during which experimental data were recorded, those changes took place very slowly. Although the optimal solution would be the use of a digital recording system which automatically increased sampling frequency at the onset of a physiological change, it was determined that by recording the dielectric property data in both analog and digital form, it was possible to record the more rapid dielectric property changes in analog form, permitting the use of a slower digital sampling rate. Two modifications to the recording methodology were incorporated. The Commodore PET microcomputer (8K Bytes memory) was replaced by a Hewlett-Packard 9835A Desktop Computer (48K Bytes memory). The additional memory in the HP-9835A provided

adequate data storage for sampling dielectric property data at a rate of one point per minute during a ninety-minute experiment. Simultaneous with digital recording of dielectric property data, the amplitude and phase of the complex reflection coefficient and various physiological parameters were also recorded in analog form. Recording data in this manner permitted both on-line digital dielectric property computations and continuous monitoring of dielectric parameters during periods of rapid physiological change. Further, it was not necessary to time-lock the digital sampling of dielectric data to the respiratory cycle of the animal because of the correlated continuous analog recording of dielectric property data.

Studies of antemortem/postmortem dielectric property changes in dog brain were conducted during the second year, and a limited investigation of vascular effects on measured in-situ tissue impedance was performed. Each of the antemortem/postmortem experiments involved measurement of the in-situ dielectric properties of dog brain with the dura mater and arachnoid removed and the probe placed directly on the pia mater over the ectosylvian gyrus. All dielectric measurements were performed as a function of time at a frequency of 2450 MHz using a 0.085-inch diameter probe.

In-situ dielectric measurements were performed over a two-hour period. Both dielectric property data and physiological data were recorded for 30 minutes prior to sacrificing the animal. This ensured physiological stability of the animal and permitted recording in-situ dielectric data under conditions of homeostasis. Sacrifice was performed by injecting a 20 cc bolus of saturated KCl or $CaCl_2$ into the femoral vein. All dielectric and physiological parameters were monitored for 90 minutes postmortem, with death being defined to be the time at which all systemic pressures were zero. A comparison of results obtained with KCl sacrifice to those obtained with $CaCl_2$ sacrifice can be found in Tables II and III in Section III of this report. The overall trend in both groups is similar. Both conductivity and dielectric constant gradually decrease as a function of time postmortem. However, in cases of $CaCl_2$ sacrifice, the values peak immediately upon intravenous injection and change more than in the KCl cases. While nominal values for the dielectric constant are about 57 in both cases, the $CaCl_2$ sacrifice results in an increase to about 62.3, or a change of approximately

10%. This peak occurs from 6-10 seconds before systemic pressures reach zero. This indicates that the $CaCl_2$ injection causes a stronger and more abrupt change in blood flow to the brain, as would be expected from the different mechanisms by which the K^+ and Ca^{2+} ions cause death.

Studies of the effects of changing renal blood flow on the dielectric properties of kidney cortex and medulla were also performed. A decrease in total renal flow consistently produced an increase in both relative dielectric constant and conductivity, and an increased renal flow produced the opposite results. Measurements on renal tissue were performed both in-situ in living dogs and in-vitro using isolated, perfused kidneys. Measured renal dielectric changes were typically greater in the medulla than in the cortex.

In a limited study of the effects of acoustical stimulation on dielectric properties of the auditory cortex in dogs, no correlation between acoustic stimuli and dielectric property changes was observed. However, this lack of correlation does not necessarily mean that no correlation exists because the cortical EEG recordings taken during auditory stimulation showed no change in the electrical activity of the auditory cortex. It is believed that the problem was due to the CNS depressant effects of anesthesia used (pentobarbital sodium or α-chloralose) on higher cortical centers and to the use of a sub-optimal stimulus paradigm, thus preventing the normal cortical response to sensory stimuli. The use of a different anesthetic agent (or of a decerebrate preparation) and a different stimulus paradigm should correct the problem, thus permitting evaluation of sensory stimulation on the dielectric properties of canine cortex.

In the following sections of this report, the Second-year investigations are detailed. Section II discusses further refinement of the probe dielectric measurement system. In Section III, the experimental investigations are described and results therefrom presented. Conclusions from results and recommendations for future studies are presented in Section IV.

SECTION II

PROBE DIELECTRIC MEASUREMENT SYSTEM

In this section, the dielectric measurement probe and system are discussed with respect to design modifications and refinements relevant to investigations made during the second year of this research program. Specifically, probe design refinements and factors influencing measurement accuracy and data processing are addressed. Additional efforts directed at measurement accuracy improvement and at increasing the applicability of the probe dielectric measurement technique to physiological studies included (1) development of methods for further systemic measurement error reduction, (2) additional refinement of probe design, (3) development of a method for maintaining constant, uniform probe contact pressure during in-situ measurements, (4) examination of possible need to "time-lock" data sampling to respiratory cycle, (5) determination of data sampling rate and storage requirements, and (6) evaluation of analog versus digital data recording.

Prior to discussing refinements made to the probe dielectric measurement system and technique, it is appropriate to briefly mention the theoretical basis underlying the probe dielectric measurement method. The detailed development of the probe is described in a series of reports on previous work performed for the Army [1-3],and a complete analysis of the theoretical aspects of the probe design is presented in Reference 4. The probe dielectric measurement technique is based on the application of an antenna modeling theorem to the characterization of unknown dielectric media [13]. Simply stated, the antenna modeling theroem equates the terminal impedance of an antenna operating at frequency f in a lossy dielectric material to its terminal impedance in free space at frequency nf, where $n = \sqrt{\varepsilon^*/\varepsilon_o}$ is the complex index of refraction of the material (ε^* is the complex permittivity of the material and ε_o is the permittivity of free space). In this application of the antenna modeling theorem, the dielectric medium is assumed to be non-magnetic since the magnetic permeability of most biological material is equal to that of free space and magnetic losses are essentially non-existent. For probes fabricated as an open-circuited transmission line, transmission line analysis can be used to obtain the same result as the

antenna modeling theorem for the special case of an infinitesimally short antenna [4].

A. Probe Design Refinements and Measurement Error Reduction

Early in the second year of this research program, emphasis was placed on further refining the probe dielectric measurement method for studying in-situ dielectric property changes under varying physiological conditions. These refinements consisted of fabrication of probes using hardened stainless steel tubing, development of a "spring-loaded probe holder" and stand, and development of methods for further reduction of systemic measurement errors.

In Annual Technical Report No. 1 [11], probe fabrication techniques were discussed in terms of useful frequency range and minimum required measurement-sample volume considerations. Numerous factors affect the performance of any particular probe with respect to useful frequency range, impedance, and resulting measurement accuracy. Further, following probe calibration, it is important to prevent the occurrence of any physical change in the probe which may affect its inherent electrical characteristics. Probes fabricated from 0.085-inch semi-rigid coaxial cable having a copper outer conductor performed well in all respects but one. The inherent electrical characteristics of these probes changed over time with handling. Thus, it was necessary to continually recalibrate these probes in order to ensure their proper performance and measurement accuracy. The use of a stainless steel hypodermic needle as the outer conductor of the probe provided added rigidity to the probe and considerably reduced the frequency of required recalibration. Rigid 0.085-inch diameter coaxial cable having a stainless steel outer conductor and copper inner conductor was purchased from Uniform Tubes, Inc., of Collegeville, PA. Probes were fabricated in the same manner as described on page 17 of Annual Technical Report No. 1 [11]. The center conductor and teflon dielectric are first removed from the cable, and an SMA connector is attached (soldered) to the outer conductor. Next, the center pin of the SMA connector is soldered to one end of the copper center conductor. While disassembled, the center and outer conductors of the probe are gold-plated. Finally, the probe is assembled and the tip of the probe trimmed and finished. The performance of probes fabricated from the 0.085-inch diameter stainless steel cable was equivalent to that of probes fabricated from copper semi-rigid cable. The new gold-plated

stainless steel probes maintained calibration longer and are more rigid than their copper counterparts.

During the evaluation of the effects of surface fluid accumulation on probe dielectric property measurement accuracy in the first year of the program, it was determined that probe contact pressure was also a factor in the accuracy and repeatability of measured results. This was further apparent during experiments in which antemortem/postmortem dielectric changes in dog brain were studied. Following cessation of blood flow, the brain receeded somewhat into the skull resulting in a large change in probe contact pressure and a concomitant significant change in measured dielectric properties. During this reporting period, effects of probe contact pressure against the pia were investigated. A six percent maximum change (from a nominal in-situ dielectric constant of 57) was measured between the conditions of initial probe contact with the pia and an applied pressure producing a 0.15-inch depression of the pia using the 0.085-inch diameter probe. An approximate change of four to five percent in conductivity (from a nominal value of 21 mmho/cm) was also observed under the above stated probe contact conditions (Table I). To minimize these effects of probe contact pressure on measured tissue dielectric characteristics, a mechanical device was designed to maintain the probe contact on the tissue at a constant or nearly constant pressure. This mechanical device, termed a "spring-loaded in-situ probe holder", is diagrammed in Figure 1. The contact force (and therefore, contact pressure) of the probe with the tissue is determined by the spring constants. Appropriate springs were selected to achieve complete probe/tissue contact at a pressure which could be consistently maintained, but produce only a slight depression of the pia. A dual spring system (one on either side of a central piston) was employed in order to permit following tissue movements either pushing against or moving away from the probe. Using the spring-loaded probe holder (Figure 1), the probe contact induced variations in measured in-situ dielectric properties were on the order of one percent. These results are presented in Table I.

Methods for further reducing systemic measurement errors associated with the microwave network analyzer were investigated during the second year of the program. Following implementation of the vector measurement

TABLE I

REDUCTION IN VARIABILITY OF MEASUREMENTS MADE USING SPRING-LOADED PROBE HOLDER

TEST CONDITION	NOMINAL VALUE		PERCENT VARIABILITY	
	K	σ(mmho/cm)	K	σ
Probe rigidly attached to electrode carrier; pressure variations causing 0.00 to 0.38 cm deflection of the pia	57.1	21.6	6%	4-5%
Probe mounted in spring-loaded probe holder; pia surface deflection 0.2 ± 0.08 cm over entire travel of device	57.1	21.6	1-2%	1%

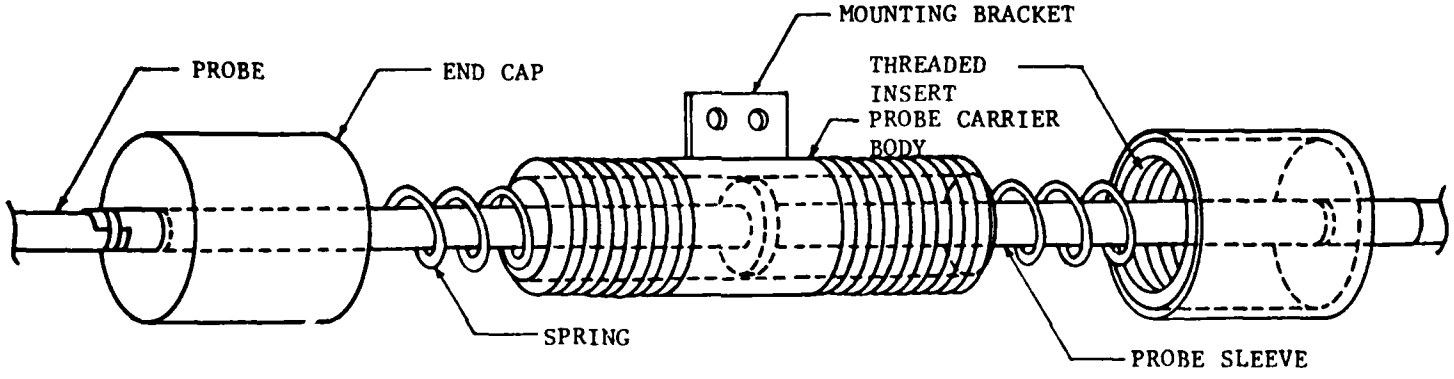


Figure 1. Schematic diagram of spring-loaded in-situ probe holder.

error correction model during the first year of the program, the only remaining source of systemic measurement error of significance was noise present in the measurement of the phase angle of small-magnitude reflection coefficients. Two approaches to the solution of this problem were exa' ed. First, a circuit incorporating an active filter was designed which rem d high frequency noise from the phase signal. However, filtering of noise in this manner both adds complexity to the measurement system and reduces the maximum data sampling rate. The second approach utilized a software filtering technique in which the reflection coefficient phase data were multiply sampled at each frequency of interest and the statistical mean of these samples was computed and stored. In this manner, the actual data sample rate was not affected and only a slight increase in total data acquisition time was incur d over a given frequency range. For measurements performed as a function of time, the phase and amplitude data comprising a "single" time point were sampled at the maximum sample rate, with time gaps of predetermined arbitrary length being set between each "single" time point. The software filter significantly reduced the noise present in the phase measurement of small complex reflection coefficients when only 3-4 points were averaged. Increasing the number of averaged data samples to 10-15 effectively eliminated the noise in the phase signal.

B. Analog and Digital Data Acquisition and Processing Methods

During the second year of this research program considerable effort was directed toward evaluation of various methods for collecting and processing both the dielectric property and physiological data. In particular, the advantages and disadvantages of analog versus digital data recording were examined. Other aspects of data collection and analysis addressed included the need for a variable digital sampling rate, additional mass memory, and possible methods for better correlating in time changes in measured in-situ dielectric properties with physiological changes.

The stored quantity of dielectric property data was dependent upon the available memory in the microcomputer system for mass storage. Several possible solutions to the data mass storage problem were examined, and the necessity for time-locking data sampling to the respiratory cycle

of the animal was evaluated. The primary question concerning digital sampling of the measured complex reflection coefficient data (from which the dielectric property data were subsequently computed) was the required frequency of sampling to ensure that no information relating to physiological changes in the tissue was lost. During periods of homeostasis, effectively no change in dielection properties was observed, and during most of the period following termination of the animal, changes in dielectric properties occurred slowly. However, throughout and immediately following the period during which the animal was terminated, many significant physiological and biochemical changes occurred. Of particular significance were the rapid changes in cardiovascular parameters which were reflected in in-situ dielectric property measurements. During that period, data should be rapidly sampled (on the order of tens of milliseconds). A desirable solution to the problem would be the implementation of a digital recording system which automatically increased data sampling rate at the onset of physiological change. As an interim solution, two approaches were simultaneously implemented. First, the Commodore PET Microcomputer was replaced by a Hewlett-Packard 9835A Desktop Computer. The HP-9835A has 48K of available memory for program and data storage, whereas the PET has available less than 8K of memory. This additional memory provided adequate data storage for a sampling rate of one point per minute during a ninety-minute recording of dielectric properties under homeostatic conditions, rapid physiological changes (termination), and slower physiological and biochemical changes (post-termination). Second, during each experiment, a simultaneous analog recording of measured reflection coefficient phase and amplitude was made and time-correlated to the recorded physiological parameters. Recording data in this manner permitted both real-time dielectric property computations (digital sampling) and continuous monitoring of dielectric parameters during periods of rapid physiological change. Further, it was not necessary to time-lock the digital sampling of dielectric data to the respiratory cycle of the animal because of the correlated continuous analog recording of dielectric property data.

As a part of the antemortem/postmortem dielectric studies conducted

during this reporting period, measured in-situ dielectric property data were recorded in both analog and digital form. The rapid cardiovascular changes which take place when an experimental animal is sacrificed are reflected in the measured in-situ dielectric properties. These changes were readily observed on the analog recordings, but were less evident on the digitally recorded data because of the low sampling frequency (one point per minute) being used.

A single-frequency data acquisition program was written which can be operated either in an automatic mode or a user-prompted mode. In the user-prompted mode, data are sampled whenever the user taps a key on the keyboard. In the automatic mode, the user selects a data sampling rate prior to executing the program. Upon execution, the program automatically samples data at the predetermined rate. For the antemortem/postmortem investigations, the automatic mode was used, but with one modification- - two sampling rates were employed. Before termination of the animal, a sampling rate of one data point (consisting of ampltide, phase, temperature) per minute was used. At the initiation of sacrifice, data were sampled at the rate of 12 points per minute (one each five seconds) for a ten-minute period. At ten minutes postmortem, the sampling rate was changed to one point per minute once again. The digitally-recorded data were compared to analog recordings (from a stripchart recorder having a 3-4 Hz frequency response) to determine if the 12 points-per-minute digital sampling rate was adequate. The most rapid in-situ dielectric property changes occurred immediately following injection of a bolus of $CaCl_2$(which resulted in rapid termination of the experimental animal) and lasted for less than three minutes. The maximum frequency of these changes was 0.3-0.6 Hz,and very good correlation between digitally-recorded and analog data was achieved using the new data acquisition software. (Efforts are now being directed toward increasing the digital sampling rate to 30 points per minute.)

Based on the analyses of these data (analog physiological, analog dielectric, digital dielectric) from a limited number of experiments, an attractive solution to the problem of needed continuous dielectric data recording was formulated. This solution would involve recording dielectric property information directly and continuously in analog form using an

analog computer. However, this solution would require the development of additional hardware. The merits of this approach with respect to digital data acquisition and processing methods will be evaluated during the third year of this research program. An alternative approach would be to incorporate a 16-channel multiplexed data acquisition system (for both dielectric and physiological data) with either a programmable sampling rate or a fixed rate which is gated on and off. This second approach will also be evaluated during the third year.

SECTION III
EXPERIMENTAL INVESTIGATIONS

This section summarizes the experimental investigations conducted during the second year of this research effort. These investigations consisted of (a) antemortem/postmortem studies of canine brain dielectric properties, in which the effects of two different modes of sacrifice (KCl and $CaCl_2$) were contrasted and compared; (b) brain blood flow studies, in which the effects of changes in blood flow on the measured dielectric properties of the pial surface were investigated; (c) auditory stimulation studies, during which attempts were made to determine effects of single-frequency auditory tones sounded in a dog's ear on the dielectric properties of the auditory cortex via changes in local blood flow; and (d) renal flow studies, wherein canine kidneys were placed on an external perfusion circuit and dielectric properties were measured under various controlled flow conditions. For each investigation, laboratory procedures are outlined and experimental results are presented.

A. Antemortem/Postmortem Studies

Each dog used in the studies of brain electrical property changes postmortem was prepared in much the same manner as described in Annual Technical Report No. 1 [11]. A block diagram of the dielectric property measurement system is shown in Figure 2. Experimental subjects were obtained from Emory University, Department of Physiology,and surgical and measurement procedures were performed at Georgia Tech in the Engineering Experiment Station Animal Surgical Laboratory. Each dog was initially anesthetized with 30 mg/kg body weight of pentobarbital sodium (Nembutal), and supplemental dosages of 30-60 mg were administered as necessary to maintain a constant level of anesthesia at Geudel's Stage III, Level 3 [14]. The femoral vessels of one side were cannulated to allow monitoring of arterial and venous blood pressures. Electrocardiogram (ECG) needle electrodes were placed in two forelimbs and one hind limb in a Lead II configuration to record heart rate and ECG. A thermistor temperature probe was inserted rectally to the level of the colon to measure systemic temperature.

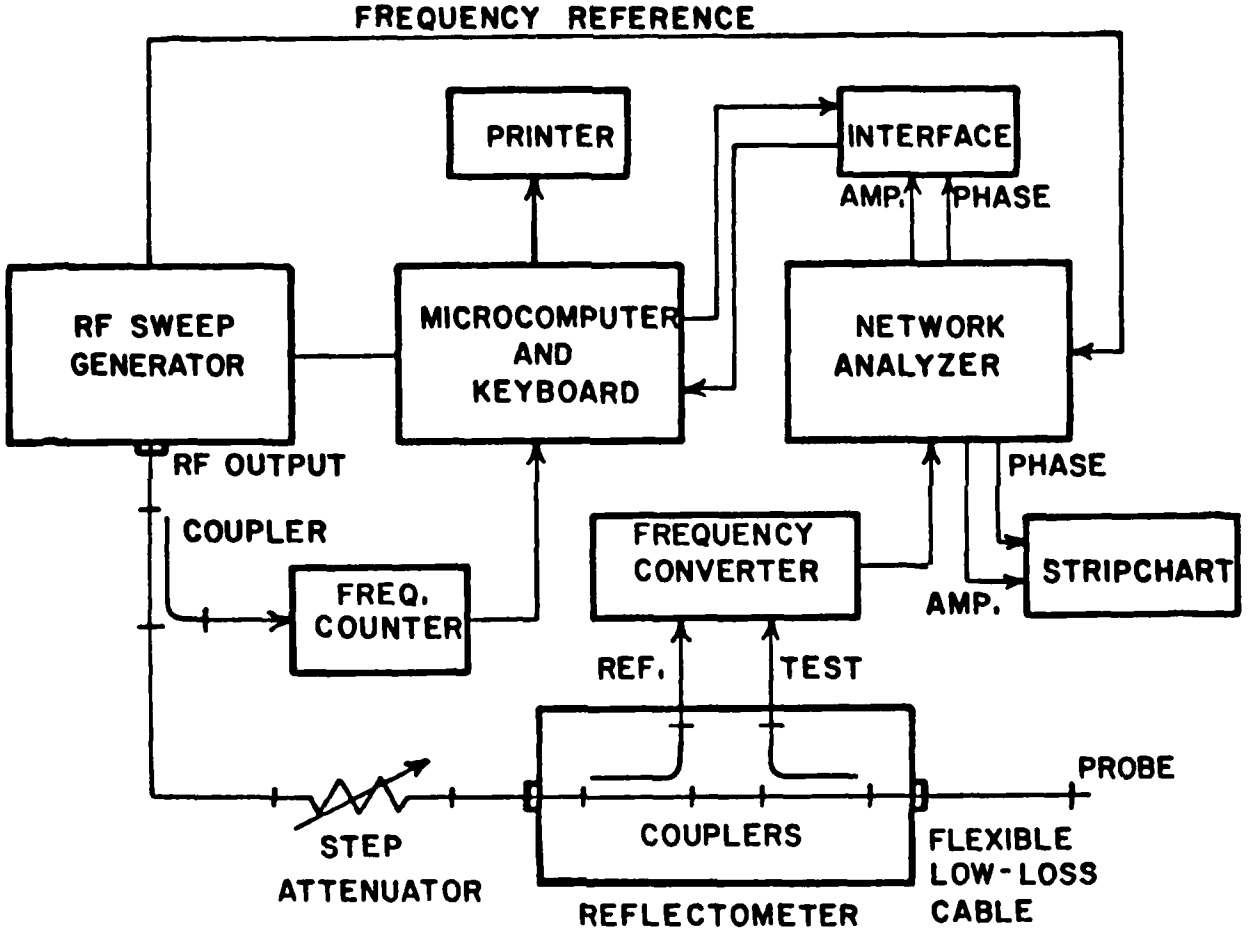


Figure 2. Block diagram of microcomputer-controlled impedance measurement instrumentation used with the in-vivo dielectric measurement probe.

With the dog in a prone position, the head was placed in a David Koph Model 1504 stereotaxic apparatus acquired during the first year of the research program as shown in Figure 3. The head's position was fixed in space by eye bars, ear bars, and a mouthpiece. Following positioning of the head, a midline incision was made through skin from the eyes back to 2-3 cm behind the lamboidal ridge. The skin and muscle layers were resected by scraping with a bone curette until most of the top left side of the skull was exposed. Using a 3/4-inch trephine, a hole was then drilled through the skull at a location midway between the eyes and the lamboidal ridge and about 2 cm laterally left of midline. Finally, the pial membrane on the surface of the brain was exposed by carefully puncturing and cutting away the tough outer dural membrane and allowing the intervening cerebro-spinal, fluid (CSF) to drain away. A dura hook, miniature tweezers, and fine scissors were used for this procedure. At this point, the pial surface was covered with saline-moistened 4-inch by 4-inch gauze pads and the animal's condition allowed to stabilize for 15-30 minutes while the in-vivo probe measurement system was calibrated.

The system was calibrated using standard terminations to correct for errors as described in Annual Technical Report No. 1 [11]. Following system calibration and stabilization of the dog's physiological condition, the pial membrane was exposed,and its dielectric properties were measured to determine the effects of local blood flow on tissue electrical characteristics. This procedure will be described in Sub-section B of this section. When the in-vivo measurements of blood flow effects were completed, the dielectric measurement probe was placed over a vessel (0.5-mm diameter) on the epipial layer of the pia,and the complex reflection coefficient phase and amplitude were measured for approximately ten minutes to establish a "baseline". All dielectric property measurements were performed at a frequency of 2.45 GHz. After the in-vivo baseline dielectric property values were established, the dog was sacrificed by femoral injection of a bolus of saturated solution consisting of 30-50 cc of either KCl or $CaCl_2$. The dielectric properties of the pial surface

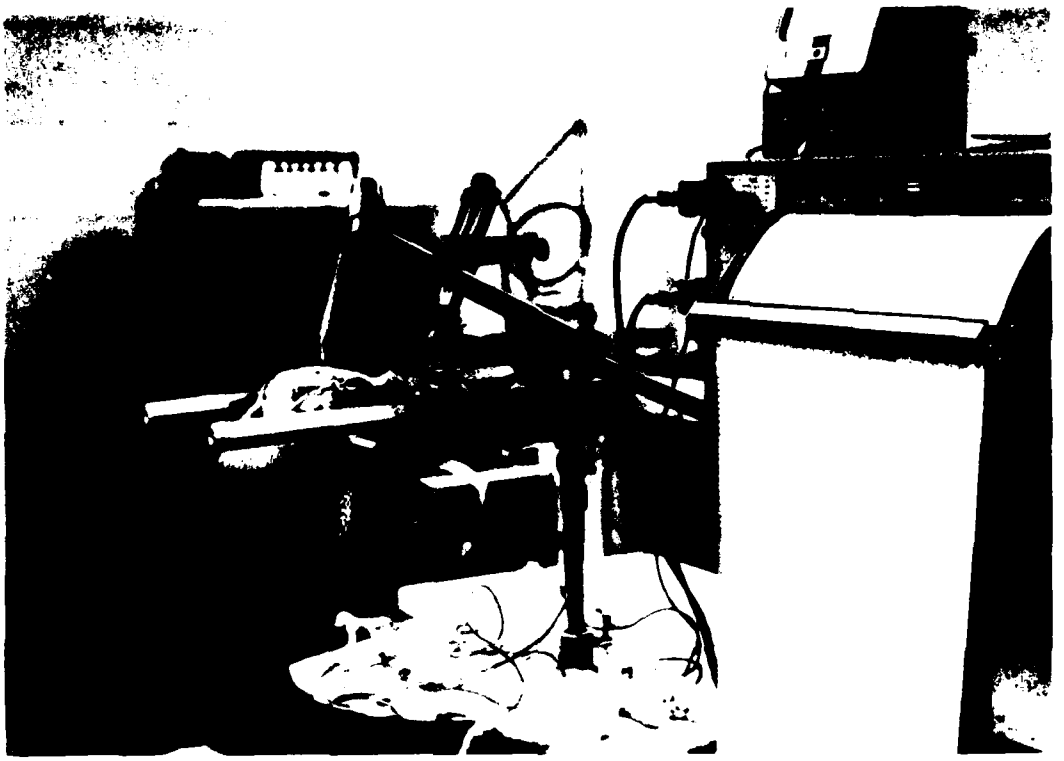


Figure 3. Dog's head positioned in stereotaxic unit (David Koph 1504) during in-situ dielectric measurements of brain tissue performed using dielectric measurement probe.

were monitored with the probe at the same location as during baseline measurements for a period of approximately 90 minutes after arterial pressure dropped to zero.

The spring-loaded probe holder described in Section II (Figure 1) of this report proved extremely useful in monitoring antemortem and postmortem dielectric changes which occurred in the brain as a result both of the method of sacrifice and of blood draining from the brain following cessation of arterial pressure. Both factors had pronounced effects on the physical appearance of the brain's surface and on its spatial position. The initial expansion and later recession of the brain upon injection of $CaCl_2$ caused significant variation in the probe's contact pressure at the measurement site. As noted in Annual Technical Report No. 1 [11], probe contact pressure variations can have a significant, measureable effect on recorded electrical properties. The spring-loaded probe holder largely eliminated the problem associated with probe/sample contact variations. This was particularly important when measuring the results following sacrifice by $CaCl_2$ injection intravenously because of the mechanism by which the Ca^{2+} ions cause death. The presence of an excessive concentration of Ca^{2+} ions causes the cardiac myofibrils to undergo a forceful, sustained contraction. The result of this contraction is a rapid expansion of the brain with the surge of blood, followed by a less rapid recession as the blood slowly leaves the brain (due to gravity). Thus, the measurement probe must track the brain's surface through a displacement of several millimeters. Without the mobile capability provided by the spring-loaded probe holder, such tracking would be impossible.

A comparison of the results obtained from animals sacrificed with KCl and $CaCl_2$ may be found in Tables II and III. The relative dielectric constant, K, and conductivity, σ, (both measured at 2.45 GHz), in each case are given as functions of time elapsed after the fatal injection. Both the relative dielectric constant and the conductivity are seen to decrease gradually after death occurs. This change appears more rapid in cases of $CaCl_2$ sacrifice, however.

TABLE II

SUMMARY OF POSTMORTEM IN-SITU DIELECTRIC STUDIES WITH KCl SACRIFICE†

Time After Injection (min)	Dielectric* Constant	Conductivity* (mmho/cm)	Brain Temperature (°C) ± 5.0	Colonic Temperature (°C) ± 5.0
0.1	58.15	20.41	36.4 ± 1.2	36.1 ± 1.0
0.2	57.72	20.38	-	-
0.4	57.53	20.43	-	-
0.6	57.16	20.13	-	-
1.0	57.64	20.36	36.2 ± 1.1	36.1 ± 1.2
3.0	58.51	20.66	36.3 ± 1.2	36.0 ± 1.3
10	58.63	19.56	36.5 ± 1.5	36.0 ± 2.1
20	56.66	18.34	36.0 ± 1.5	35.9 ± 2.1
40	54.63	17.12	35.6 ± 1.7	35.6 ± 2.3
60	52.08	16.60	35.7 ± 1.6	34.9 ± 2.6
90‡	52.32	17.59	35.4 ± 1.7	33.6 ± 2.2

† Average values from 8 dogs.

‡ Average values from 5 dogs.

* Standard deviation is shown in Figures 6 and 7.

TABLE III

SUMMARY OF POSTMORTEM IN-SITU DIELECTRIC STUDIES WITH $CaCl_2$ SACRIFICE†

Time After Injection (min)	Dielectric * Constant	Conductivity * (mmho/cm)	Brain Temperature (°C) ± 5.0	Colonic Temperature (°C) ± 5.0
0.1	59.91	25.74	40.0 ± 1.3	37.9 ± 0.8
0.2	62.22	26.46	-	-
0.4	58.80	24.74	-	-
0.6	58.35	24.49	-	-
1.0	57.50	24.13	38.7 ± 2.5	37.7 ± 0.0
3.0	55.09	23.05	37.5 ± 2.1	37.7 ± 0.7
10	54.06	22.04	35.5 ± 3.5	37.4 ± 0.2
20	53.36	21.56	36.3 ± 2.4	37.3 ± 0.3
40	53.19	20.97	37.9 ± 0.4	37.1 ± 0.1
60	53.05	21.22	37.1 ± 1.3	36.8 ± 0.1
90	52.57	20.09	36.4 ± 1.7	36.3 ± 0.1

† Average values from 5 dogs.

* Standard deviation is shown in Figures 4 and 5.

The average pre-sacrifice values from all dogs in both groups taken together were a relative permittivity of 57.10 $\pm$ a standard error of 1.35, and a conductivity of 21.65 mmho/cm $\pm$ a standard error of 1.04 mmho/cm. These errors indicate the variability among baseline values (pre-sacrifice). Note that standard errors in Tables II and III would be influenced by both the variation among baseline values and the variation among the dogs' responses to the lethal injections. Since it was desired to look at postmortem effects separately, a normalization procedure was adopted. For each individual dog, the individual baseline value was subtracted from all postmortem values. The resulting data represented plus or minus change from baseline. These normalized data were averaged and plotted in Figures 4 - 7. Thus, the graphs represent the average changes caused by each mode of sacrifice, and the error bars are a measure of the variability of the responses to each type of injection. Variations caused by individual baseline differences have been eliminated from these plots.

Figures 4 and 5 show the effects of $CaCl_2$ injection on observed dielectric properties. As discussed above, Ca^{2+} induced death is associated with a surge of blood to the brain. This corresponds to a sudden, rapid rise in the values of these properties. Figure 4 shows that, following injection, K increases by an average of 5.1 relative dielectric units in just 12 seconds after the fatal injection is initiated. Over the course of the following 90 minutes, the dielectric constant falls to a value 3.9 relative dielectric units below baseline, or a total drop of 9.0 units from its peak value. Similarly, as shown in Figure 5, the conductivity experiences a rapid initial rise of 2.8 mmho/cm, and then gradually drops to a value 3.6 mmho/cm below baseline and 6.4 mmho/cm below its peak value.

In contrast to the results obtained following $CaCl_2$ sacrifice, response measured in dogs sacrificed by injection of KCl did not exhibit the sudden sharp rise characteristic of $CaCl_2$ sacrifice. Starting at a normalized baseline of zero (0), the relative dielectric constant underwent an apparent rise of as much as 1.1 units as displayed in Figure 6. However, as the standard error bars on the graph indicate, this small

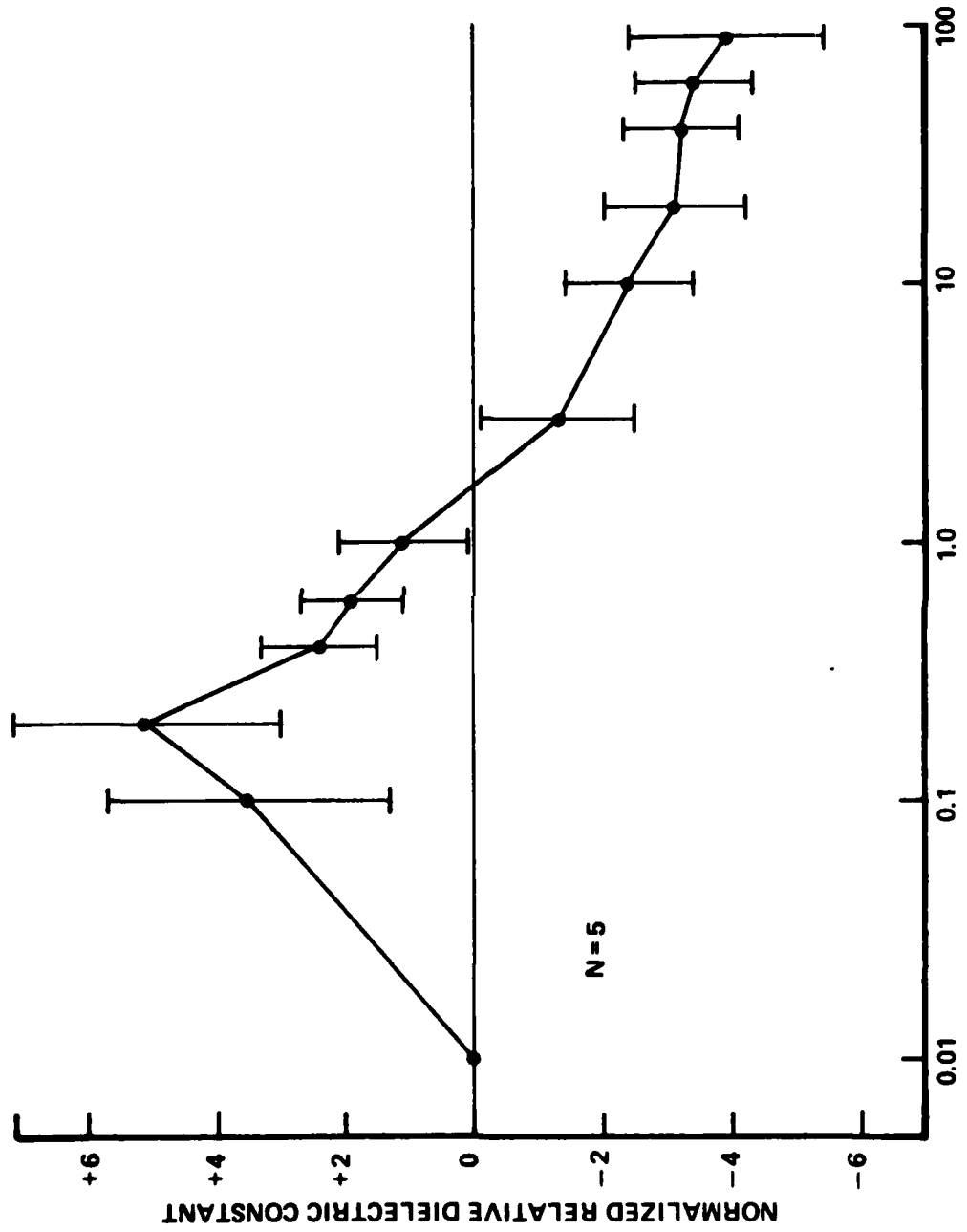


Figure 4. Mean normalized relative dielectric constant of pial surface of dog brain measured as a function of time after lethal injection of $CaCl_2$. Bars indicate plus or minus one standard deviation. Refer to text for details.

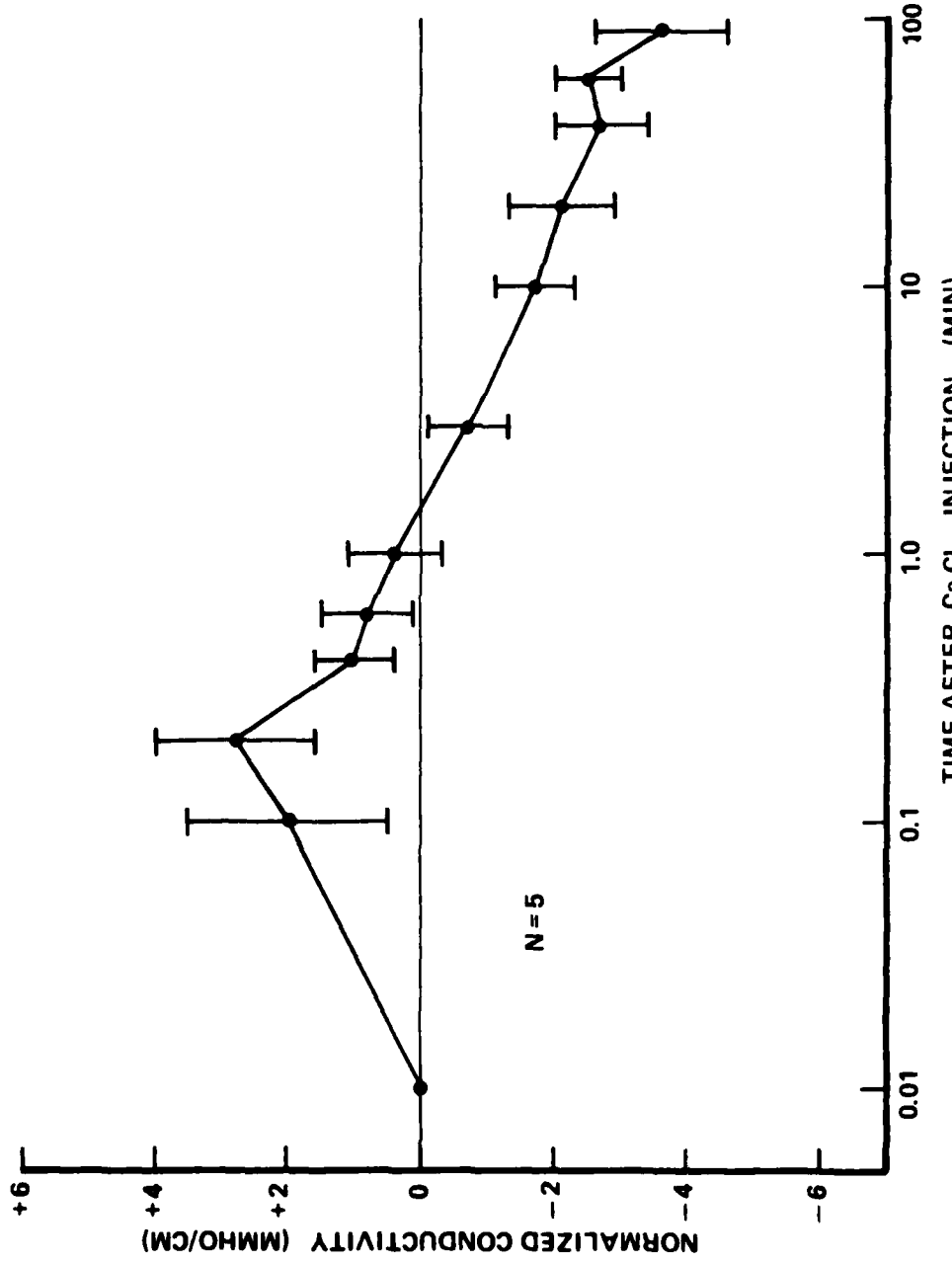


Figure 5. Mean normalized conductivity of pial surface of dog brain measured as a function of time after lethal injection of $CaCl_2$. Bars indicate plus or minus one standard deviation. Refer to text for details.

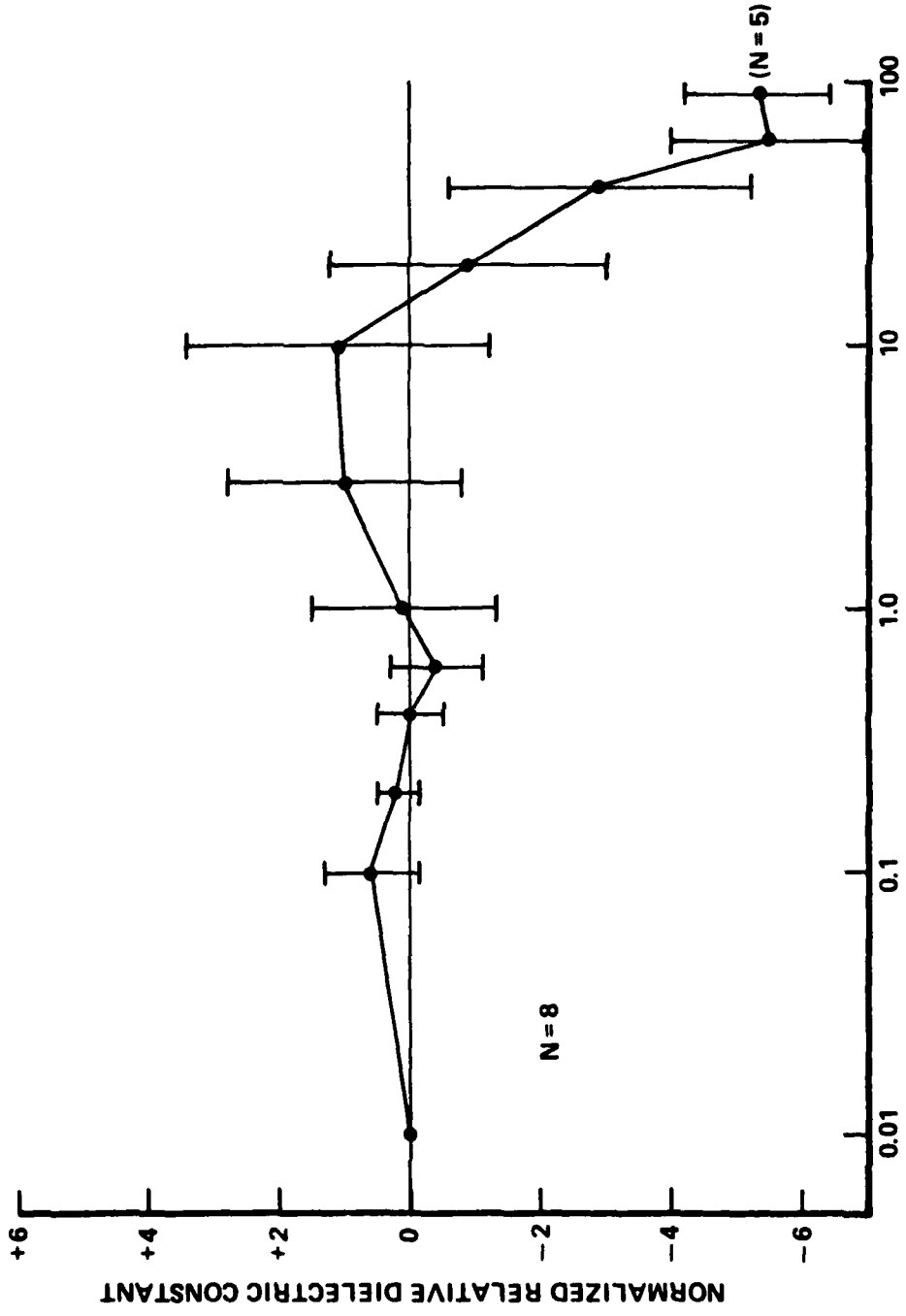


Figure 6. Mean normalized relative dielectric constant of pial surface of dog brain measured as a function of time after lethal injection of KCl. Bars indicate plus or minus one standard deviation. Refer to text for details.

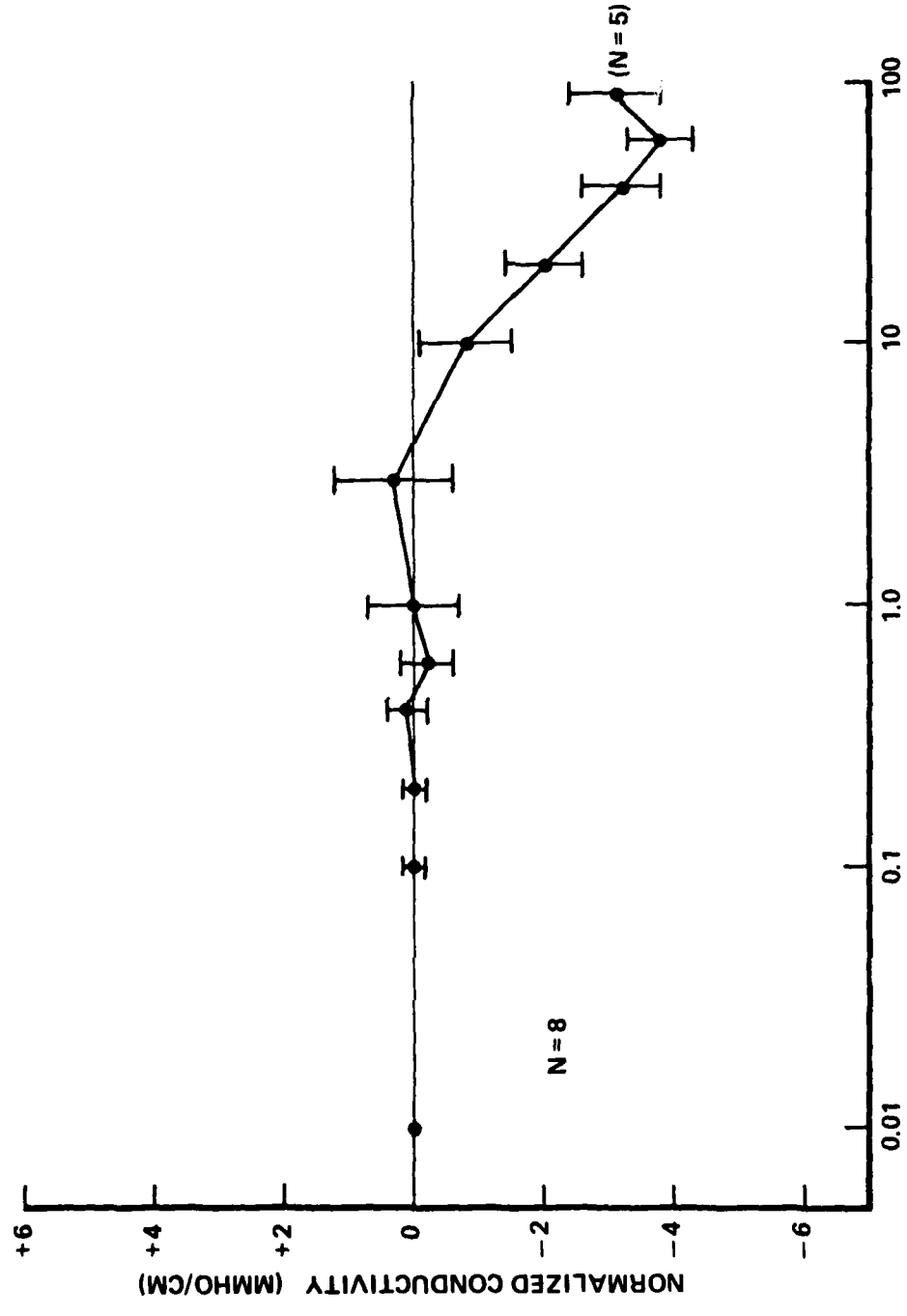


Figure 7. Mean normalized conductivity of pial surface of dog brain measured as a function of time after lethal injection of KCl. Bars indicate plus or minus one standard deviation. Refer to text for details.

measured rise in dielectric constant is probably not statistically significant. Approximately 10 minutes following death, however, an easily identifiable declining trend in measured dielectric constant begins. The value of the relative dielectric constant falls to 5.5 units below the mean baseline value. Figure 7 shows that the conductivity of canine pial tissue as a function of time elapsed after KCl sacrifice exhibits the same type behavior as the dielectric constant. After a statistically insignificant rise of 0.3 mmho/cm, it drops to 3.8 mmho/cm below its mean baseline in-vivo value.

Parallels can be drawn among the physiological action of each ion species used for sacrifice, its effect on blood flow to the brain, and resulting changes in dielectric properties. The mechanism by which Ca^{2+} ions act has been explained previously. The subsequent surge of blood also accounts for the sudden sharp increase in dielectric properties immediately after injection of $CaCl_2$. An excessive presence of K^+ ions, on the other hand, has the physiological effect of interfering with the propagation of the action potential in the myocardium. In cases where KCl is injected to sacrifice an animal, sufficient concentration is used to cause both SA block and AV block. The heart goes into fibrillation and death soon follows. Thus, when K^+ ions are used as the agent of sacrifice, one sees neither the sharp increase of blood volume in the brain nor the associated rise in dielectric property values.

Regardless of the method of sacrifice, however, both groups of dogs used in this experiment exhibited the same long-term trend from about 3 minutes postmortem until the end of the observation period at about 90 minutes postmortem. The trend was for the dielectric property values to gradually decrease. The fact that this trend appeared in both sets of dogs, regardless of whether death was caused by K^+ ions or Ca^{2+} ion, indicates that postmortem decreases are due to (1) blood flow changes and (2) concomitant metabolic changes associated with death itself rather than the mode of action of the ion species used for sacrifice.

In several cases, after postmortem data had been collected for a short period, the left hemisphere of the brain was removed and homogenized for in-vitro measurement of dielectric properties. This was accomplished by enlarging the hole in the skull with rongeurs, excising the brain tissue, homogenizing it first with a syringe and then with a tissue homogenizer, and placing the homogenized brain tissue in a beaker. The beaker was partially immersed in a temperature-controlled water bath, and the probe tip was immersed in the homogenized tissue. Measurements were performed at a frequency of 2.45 GHz as a function of temperature from $25^{\circ}C$ to $40^{\circ}C$. The average dielectric profile of the values obtained from four dog brains exhibited little change with temperature, having a mean relative dielectric constant of 43.3 (s.d. = 0.57) and a mean conductivity of 15.2 mmho/cm (s.d. = 0.23) over the entire temperature range. The mean dielectric property data are presented as a function of temperature in Table IV.

Dielectric property measurements taken with the probe inserted in the intact brain to various depths indicate decreasing values for K and σ at deeper insertions. In-situ values of K = 32.3 and σ = 12.1 corresponding to deep brain (white matter), K = 43.0 and σ = 17.4 corresponding to shallow brain (gray matter), and K = 57.2 and σ = 19.3 corresponding to pia mater were found. These results are displayed in Table V. As expected, the values measured for homogenized brain tissue lie near the middle of this range, since all tissue types were present in the homogenized samples. The lower values found for the tissue at deeper locations in the brain can be associated with the fact that blood flow is somewhat reduced in deep brain with respect to cortex. Since the homogenized tissue was measured almost immediately after excision, it is unlikely that significant water loss occurred due to exposure of the tissue (water loss would be reflected as a decrease in measured in-vitro dielectric constant and conductivity).

B. Brain Blood Flow Studies

The surgical procedure for exposing the brain for measurement of blood-flow effects was identical to that used in the antemortem/post-mortem studies. This fact, and the desire to minimize the number of animals sacrificed allowed us to obtain both types of measurements from

TABLE IV

DIELECTRIC PROPERTIES OF HOMOGENIZED CANINE BRAIN

N	TEMPERATURE (°C)	K ±	S.E.	σ ± (mmho/cm)	S.E.
1	25	46.88	-	16.75	-
3	26	42.64	2.39	14.73	1.01
3	27	43.32	2.07	15.13	0.90
4	28	42.94	1.81	14.98	0.75
3	29	43.88	1.96	15.18	0.89
4	30	43.39	1.69	15.16	0.75
4	31	43.37	1.69	15.16	0.75
4	32	43.40	1.65	15.16	0.75
4	33	43.49	1.65	15.17	0.75
4	34	43.49	1.62	15.17	0.74
4	35	43.59	1.58	15.32	0.68
4	36	43.60	1.55	15.32	0.67
4	37	43.63	1.52	15.44	0.67
4	38	43.59	1.43	15.58	0.67
4	39	43.62	1.38	15.58	0.66
2	40	41.53	0.54	14.95	0.54
Mean (all temperatures)	26-40	43.30	0.29	15.20	0.12

TABLE V

IN-SITU DIELECTRIC MEASUREMENTS

LIVING CANINE BRAIN

MEAN VALUES ± SEM

DURA MATER		
	$K' = 49.9 \pm 1.9$	$\sigma = 20.7 \pm 1.3$
PIA MATER		
	$K' = 52.7 \pm 0.9$	$\sigma = 19.3 \pm 0.7$
*SHALLOW BRAIN (GREY MATTER)		
	$K' = 43.0$	$\sigma = 17.4$
DEEP BRAIN (WHITE MATTER)		
	$K' = 32.3 \pm 1.7$	$\sigma = 12.1 \pm 0.8$

* N=1 DOG

the same group of subjects. Of the total 14 dogs studied during this reporting period, 8 dogs were measured in the blood flow studies over a combined frequency range of 0.1 - 4.0 GHz. In addition, a large base of single frequency data at 2.45 GHz was collected (note that this is the frequency at which the postomortem data were collected).

Two conditions were measured in these studies: (1) blood flow through macroscopic vasculature in epipial layer of pia, and (2) epipial layer of pia absent of macroscopic vasculature. To measure the "blood flow present" condition (first condition), the "open-circuit" probe was placed directly over a small vessel (< 0.5 mm diameter) on the pial surface of the brain. Comparison measurements were made with the probe on the pial surface beside the vessel, but with no vessel under the probe tip, to represent the second condition - absence of flow through macroscopic vasculature. These two conditions are pictured in Figure 8.

Results of these measurements are displayed in Figures 9 and 10, along with some comparison points for canine gray matter measured in-situ. The data for these comparison points were collected during the first year effort, as described in Annual Technical Report No. 1. Figure 9, is a plot of the relative dielectric constant as a function of frequency in the range 0.1 - 4.0 GHz. A clear, statistically significant difference exists between the means of measurements taken over a blood vessel on the pial surface and those taken on the pia without the vessel present. The presence of a small vessel in the measurement volume of the dielectric probe causes a rise of about 3 relative dielectric units. This difference is fairly consistent over the frequency range measured. Similarly, as shown in Figure 10, the presence of a small vessel in the measurement field is associated with a conductivity about 2 mmho/cm higher than the conductivity with no vessel present. This difference appears to diminish with increasing frequency, owing to the increasing dominance of "free" water over bound water. Free water is that which is unassociated with larger molecules in a solution, and bound water is that which is associated with proteins, enzymes, or other macromolecules. The conductivity, whether measured with the probe located over or not over a vessel, increases with frequency from near 10 mmho/cm at 150 MHz to nearly 30 mmho/cm at

(a) PROBE DIRECTLY ON EPIPIAL LAYER OF PIA

(b) PROBE PLACED OVER 0.5-mm DIAMETER VESSEL ON PIAL SURFACE

Figure 3. In-situ dielectric measurement probe in position on pial layer of dog brain.

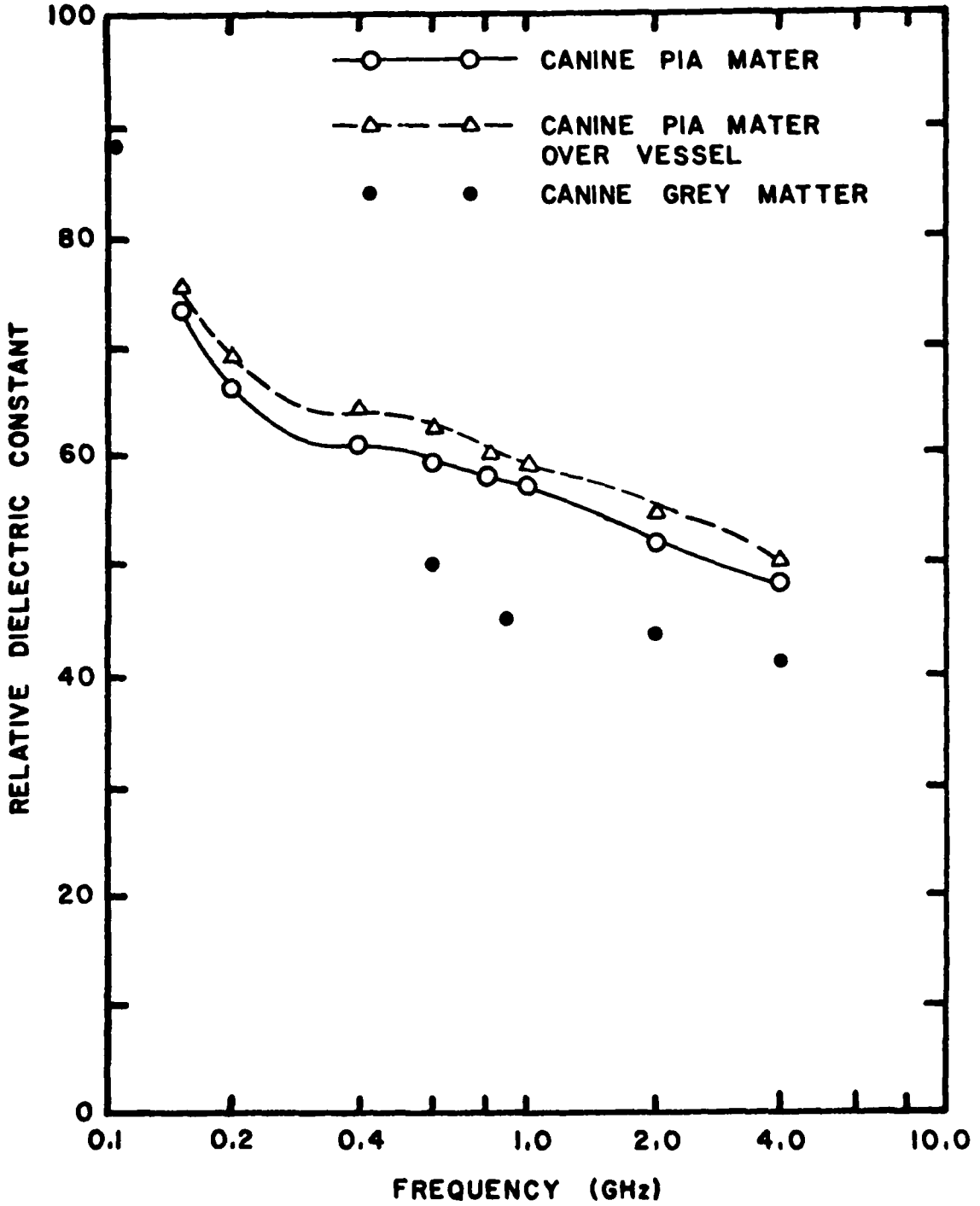


Figure 9. Relative dielectric constant of in-situ canine brain (pia mater) at 36°C compared to 37°C in-vitro canine brain (gray matter) data from [9].

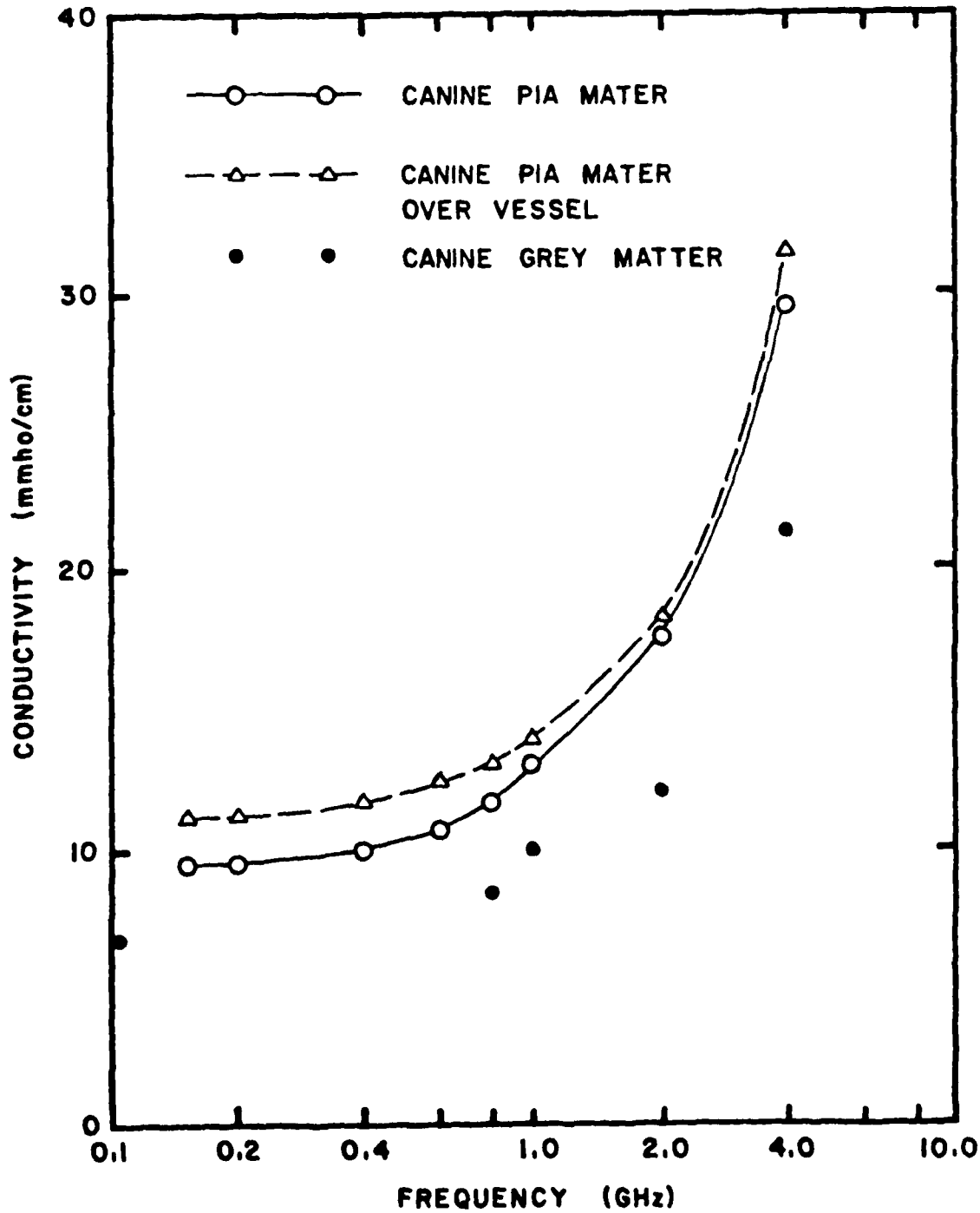


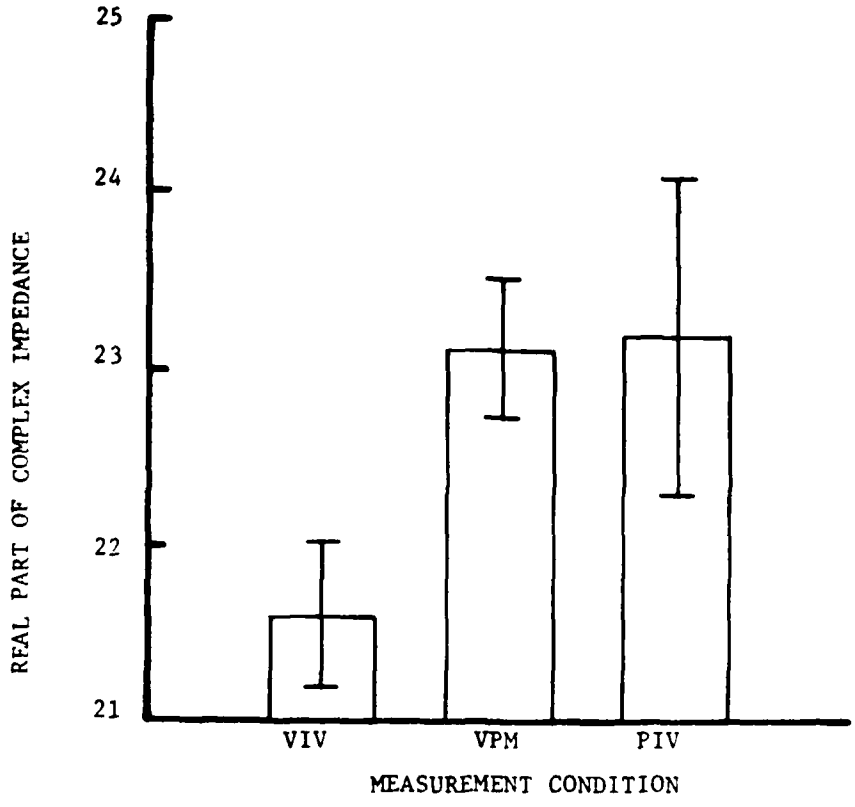
Figure 10. Conductivity of in-situ canine brain (pia mater) at 36°C compared to 37°C in-vitro canine brain (gray matter) data from [9].

4.0 GHz. In the same frequency range, the relative dielectric constant for both conditions decreases from approximately 75 to near 50 relative dielectric units.

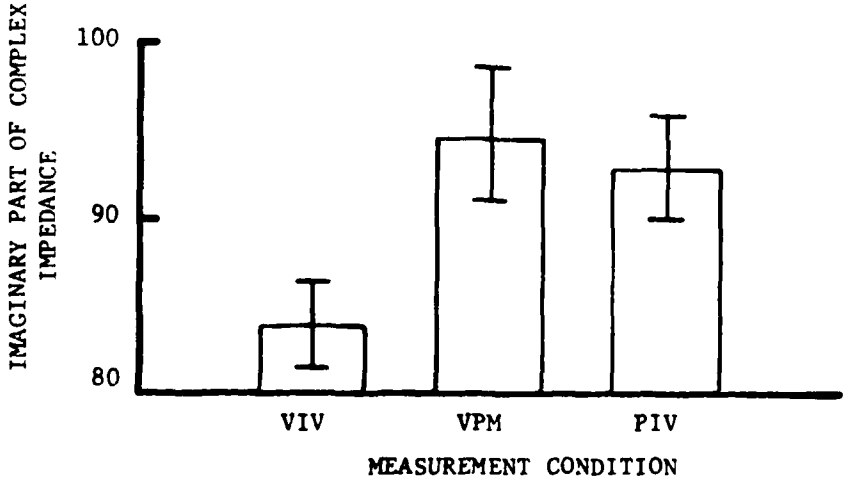
Because the postmortem studies were done at 2.45 GHz, a comparison of the effect of vessel present/no vessel present conditions on in-vivo electrical properties was also done at this single frequency. Taken together with postmortem data, an interesting pattern emerges, as illustrated in Figure 11. This figure compares complex impedances, from which dielectric properties are computed. As was expected, a significant difference was found between complex impedances measured at sites over a blood vessel and sites not over a blood vessel. In addition, the postmortem condition immediately after sacrifice, which was measured at a site over a small vessel, was found to be remarkably similar to the living tissue when no vessel was present. Presumably this is because blood had drained out of the vessel in the postmortem case. Thus, the electrical differences measured in the pia can probably be attributed to presence or lack off of significant blood flow in the measurement region included within the probe's fringing field.

C. Auditory Stimulation

From the results presented in the previous subsection, it is apparent that blood flow can have a significant effect on the dielectric properties of tissue. It is also known that auditory stimulation increases blood flow to the auditory cortex. Based on results from the measurement of blood-flow conditions in the brain, it was decided to attempt to measure differences in dielectric properties of the auditory cortex produced by increased blood flow due to auditory stimulation. In this way, we hoped to determine just how sensitive dielectric properties were to changes other than gross blood volume decrease due to death. The set up used for the acoustical stimulation experiments is shown in Figure 12. Sound was produced for these experiments by a signal generator and a small floating-coil transducer. The transducer was a type originally used as the receiver element in a telephone, reconfigured for use as a speaker element in the onboard stereo found on some commercial airline flights. Two of these transducers were provided to us by Delta Airlines at no cost.



(a) Real part of measured complex impedance



(b) Imaginary part of measured complex impedance

Figure 11. Canine brain complex impedance measured in-vivo and in-situ postmortem on the pia at a frequency of 2450 MHz. Measurement conditions consisted of probe on small (0.5-mm diameter) vessel in-vivo (VIV), probe on vessel postmortem (VPM), and probe on pia in-vivo (PIV) with no vessel beneath probe.

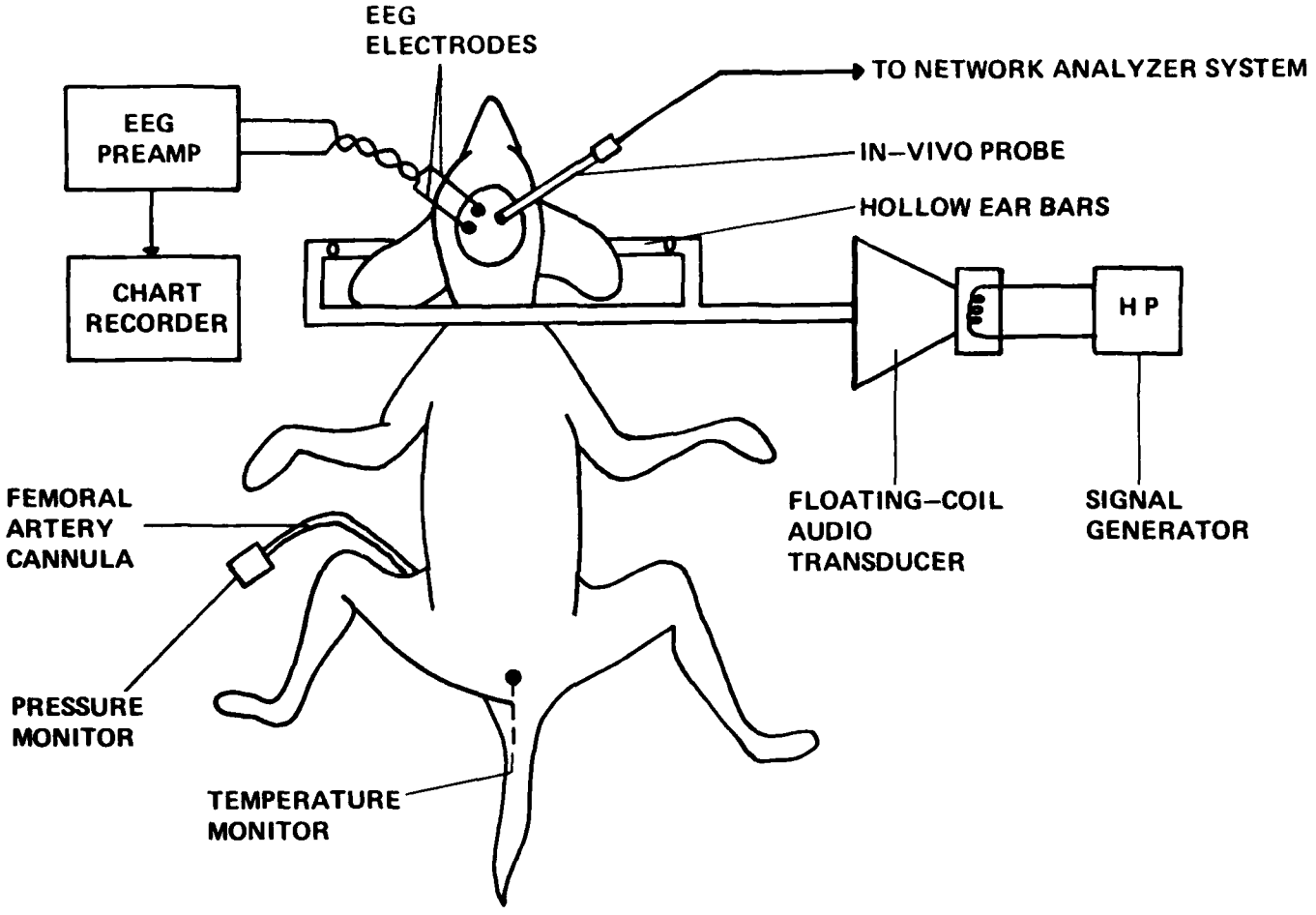


Figure 12. Experimental setup used during acoustical stimulation experiments for recording in-situ dielectric properties and cortical EEG from canine brain.

The surgical procedure for exposing the brain was similar to that described in previous subsections of this report. When auditory stimulation was to be used, however, a 3/4 inch hole was trephined in the skull approximately 4 cm lateral to the midline and halfway between the orbit and the lamboidal ridge. The hole was then carefully enlarged with rongeurs, the goal being to expose the ectosylvian gyrus, on which, as reported by Ruch and Patton [15], the auditory cortex of the dog brain is mapped.

Hollow ear bars were used in the stereotaxic apparatus, and a single frequency tone of 1 KHz was directed through the hollow bars into the dog's ears. The in-vivo probe was used to record dielectric properties, while the EEG was monitored with two Ag/AgCl ball electrodes to determine whether or not the dog was actually cognizant of the sound. The Ag/AgCl ball electrodes were fabricated from thin silver wire with the tip melted to form a 0.5 mm ball, which was then chlorided in a 0.1 M HCl solution using a platimum cathode.

A total of three dogs were investigated for auditory effects. No correlation between dielectric properties and auditory scimulation was established, however. The first two dogs were heavily anesthetized with pentobarbital sodium; the third was anesthetized lightly with pentobarbital and supplemented with α- chloralose. Although no EEG was recorded in the first experiment, the EEG's of the following two dogs were monitored but failed to indicate any auditory response. This may have been due to several factors. First of all, pentobarbital acts as a general depressant on the CNS, and thus very likely interfered with the dogs' ability to hear the stimulus. It was for this reason that α-chloralose, was used in later experiments. Although α-chloralose also acts as a general CNS depressant, its effect is limited to the higer centers of the CNS. The data indicate, however, that any general CNS depressant may be inappropriate for the auditory stimulation experiments. Secondly, one of the subject dogs appeared to be comatose. Thus, the dog's ability to respond to auditory stimuli may have been impaired. Finally, the stimulus paradigm may have been inappropriate. The auditory cortex of the canine brain is tonotopically organized as shown in Figure 13; that is, specific regions

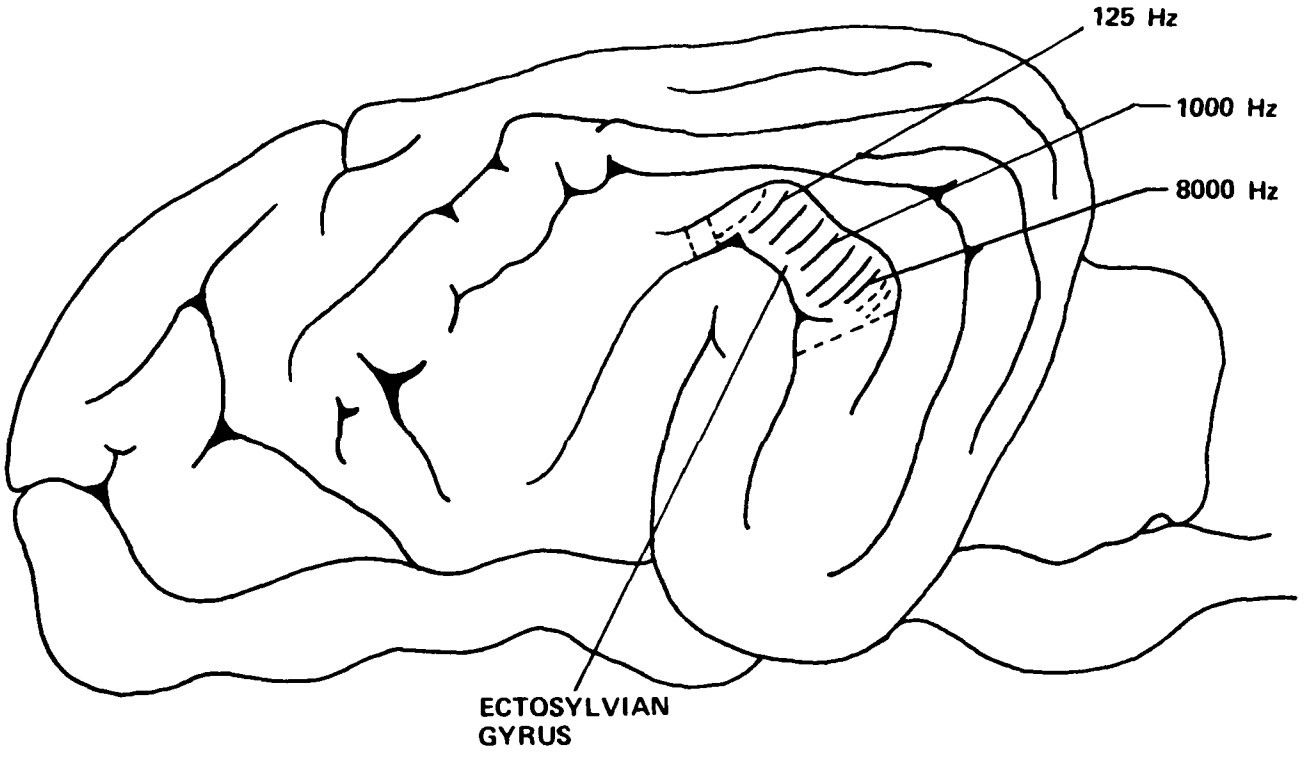


Figure 13. Drawing of canine brain cortex showing tonotopic organization and location of the auditory cortex on the ectosylvian gyrus.

of the cortex respond to specific frequencies. Since the stimulus was a single frequency tone, it is possible that the appropriate area of the brain was not monitored.

In future experiments involving auditory stimulation, an anesthetic will be used which does not interfere with the hearing process (e.g., N_2O + Halothane). In addition, the stimulus will not be a single tone, but will consist of either several frequencies simultaneously or large audible clicks in order to affect a larger area of the brain, or possibly a single tone of variable frequency which may be adjusted while maintaining the electrodes in a constant position. It is hoped that these changes will be sufficient to enable us to detect the expected auditory response.

D. Renal Flow Studies

Results obtained from measurements of the brain indicated that blood flow could have a significant effect on the dielectric properties of tissues. To further investigate this phenomenon, renal dielectric properties were measured under varying flow conditions. Dielectric properties of kidneys in the first few dogs used for this investigation were measured in-vivo with the renal capsule intact; later the kidneys were isolated and put on an external perfusion circuit, and portions of the renal capsule were removed.

The in-vivo measurements were performed with the dog supine and anesthetized with pentobarbital sodium. After injecting 2-3 cc heparin intravenously (in later dogs, 5 cc of anticoagulant citrate dextrose (ACD) [16] were also used), and allowing the anticoagulant to mix in the bloodstream, the right femoral artery was cannulated to provide a controlled source of blood for the kidney. The output from the femoral artery was directed to the perfusion circuit illustrated in Figure 14. From the circuit, heated, oxygenated blood could be supplied to the kidney at a rate easily controlled by adjusting the pump speed. Next the left kidney was exposed and the renal artery isolated. This was accomplished by first making a diagonal incision 8-10 cm in length just below the bottom rib on the dog's left side with an electrosurgical unit (see Figure 14). The subdermal fascia and fatty tissue, as well as the several layers of

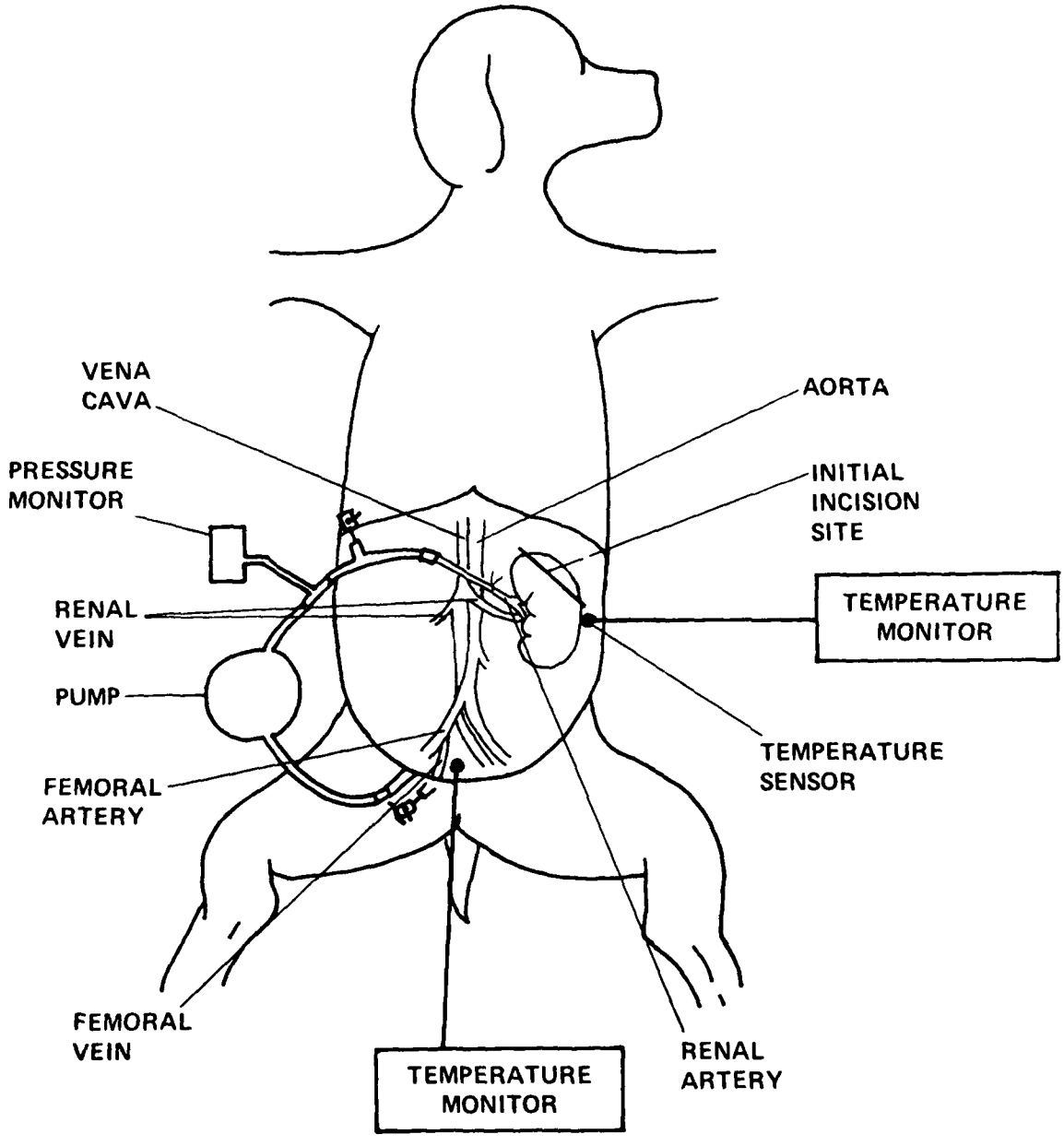


Figure 14. Diagram of extracorporeal perfusion circuit used for control of renal blood flow during in-situ renal dielectric property measurements. Note site of initial surgical incision.

abdominal wall muscle, were separated by blunt dissection until the peritoneal cavity was exposed. Then the left kidney was located manually and separated from the surrounding peritoneal fascia and fatty tissue. The renal artery was located where it entered the renal pelvis and traced back to its junction with the aorta. Both vessels were tied loosely with ligatures so as not to restrict blood flow. The output of the perfusion circuit was delivered to the kidney via a Swann-Ganz catheter, which was used to cannulate the left femoral artery. The catheter tip was guided up the femoral artery and aorta to the junction with the renal artery. The tip was manually guided into the renal artery, and after the catheter was hooked to the output of the perfusion circuit, the balloon tip was inflated to secure the catheter in place and prevent additional blood flow (above that controlled by the pump) to the kidney. The pump was then immediately turned on to establish flow rate at near normal renal blood flow.

At this point the perfused kidney was allowed to stabilize for approximately ten minutes. Flow rate and perfusion pressure were constantly monitored via the manometer in the perfusion circuit and the pump speed gauge. A chart shown in Figure 15 correlates pump speed and flow rate for various perfusion pressures in this circuit.

After the perfusion pressure and flow had stabilized, a dielectric measurement probe was placed on the renal capsule. Flow rate was varied by changing the pump speed, and each condition of reduced flow was maintained for 1-5 minutes. Several measurements were recorded under each flow condition.

Despite the use of heparin, clotting was a major problem in the initial in-vivo renal flow experiment with an external perfusion circuit. However, the use of ACD as an additional anticoagulant seemed to eliminate this problem [16]. In a later experiment, flow rates were varied from 80 ml/min to negligible flow. No identifiable changes in dielectric properties were measured, although pressure at the kidney in excess of 200-mm Hg indicated possible renal shutdown. The properties measured were a relative dielectric constant of 52.9, which is in the expected range for in-vivo renal cortex, and a conductivity of 17.3, which is between expected in-vivo and in-vitro values.

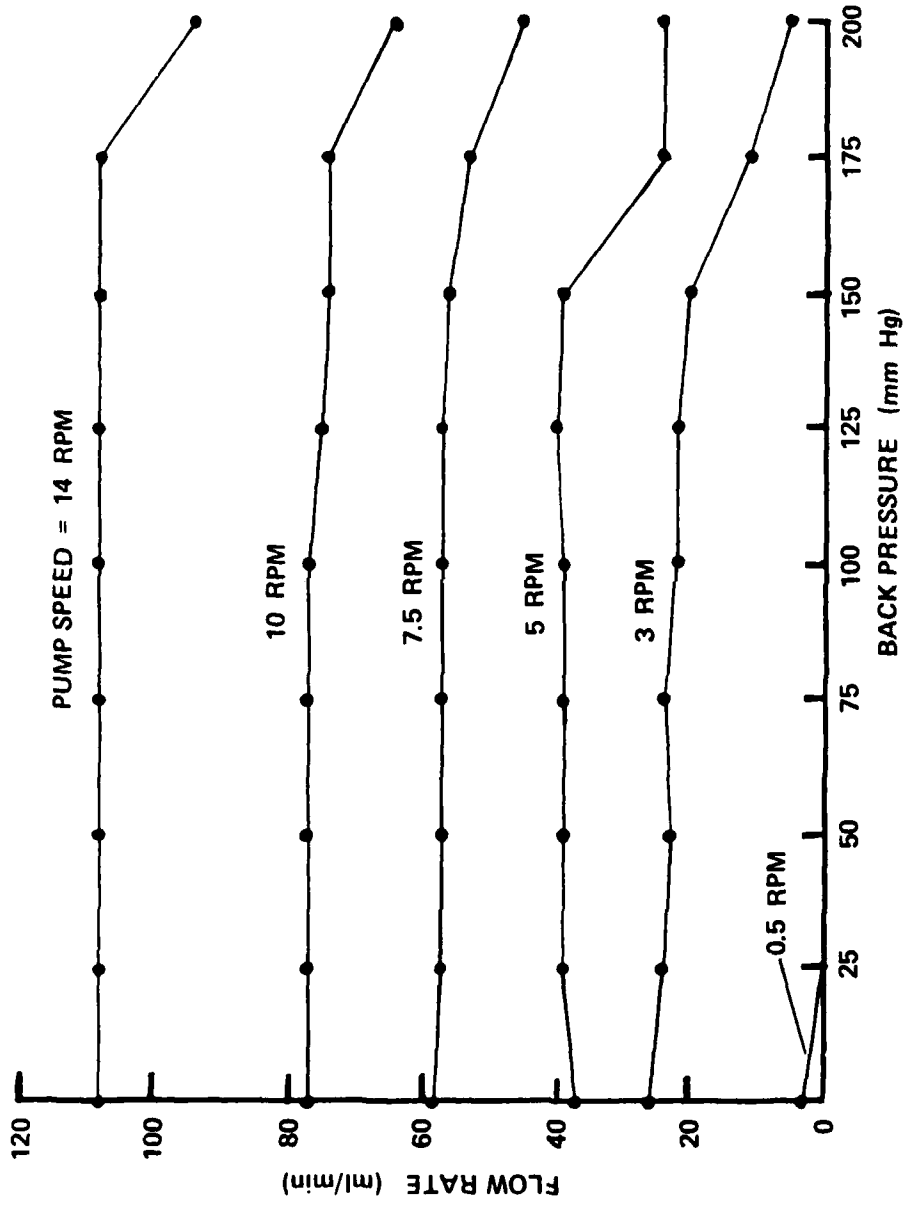


Figure 15. Pressure-Flow Rate relationships measured under calibration conditions for various pump speeds (RPM) used for renal perfusion in-situ and in-vitro.

In order to isolate the kidney from all uncontrolled in-vivo influences (neurological, hormonal, etc.) it was decided to excise a kidney and measure it in-vitro. Figure 16 illustrates the perfusion circuit used for the in-vitro experiments. The surgical procedure for exposing the kidney was the same as the in-vivo experiments. After this was accomplished, the kidney was manually separated from the peritoneal fascia, and the renal artery, vein, and ureter were isolated. The renal artery was tied at its junction with the aorta,and immediately afterwards,the renal vein was tied and both blood vessels and ureter were cut. The free kidney was then promptly moved to the organ chamber and the renal artery cannulated, so that perfusion could begin. The perfusate used in-vitro was a salt solution known as "Solution A", each liter of which contains 96.2 mM NaCl, 40.3 mM KCl, 11.9 mM $NaHCO_3$, 1.7 mM $CaCl_2$, 12.5 mM $MgSO_4$, 11.1 mM dextrose, 2000 units Heparin, and 10 mg isoxsuprine HCl (Vasodilan, Mead Johnson). The solution was filtered through Millipore® paper before use in the perfusion circuit. Once the kidney was being perfused, its temperature, pressure, and flow rate were monitored. Three distinct regions of the kidney were measured: the surface, with a small section of the capsule removed; the interior cortex, approximately 5 mm below the surface; and the medulla, 10-15 mm below the surface. Typical kidney cross-sections showing the regions of the kidney and probe positions in the interior are illustrated in Figure 17.

Results from three different dogs are illustrated in Figures 18 through 20. Measurements from the first dog were largely exploratory,and the flow was not adjusted according to a predetermined schedule. Therefore, results in Figure 18 have been organized to show flow rates and resulting dielectric properties in a time progression corresponding to the order in which they were measured. Note that information about absolute and percentage changes in flow rate and resulting effects on dielectric properties have thus been preserved.

The sign correlation between flow rate changes and dielectric property changes is excellent. That is, an increased flow rate caused a decrease in the values of the dielectric constant and the conductivity; a decreased flow rate caused an increase in the value of these properties. Figure 18

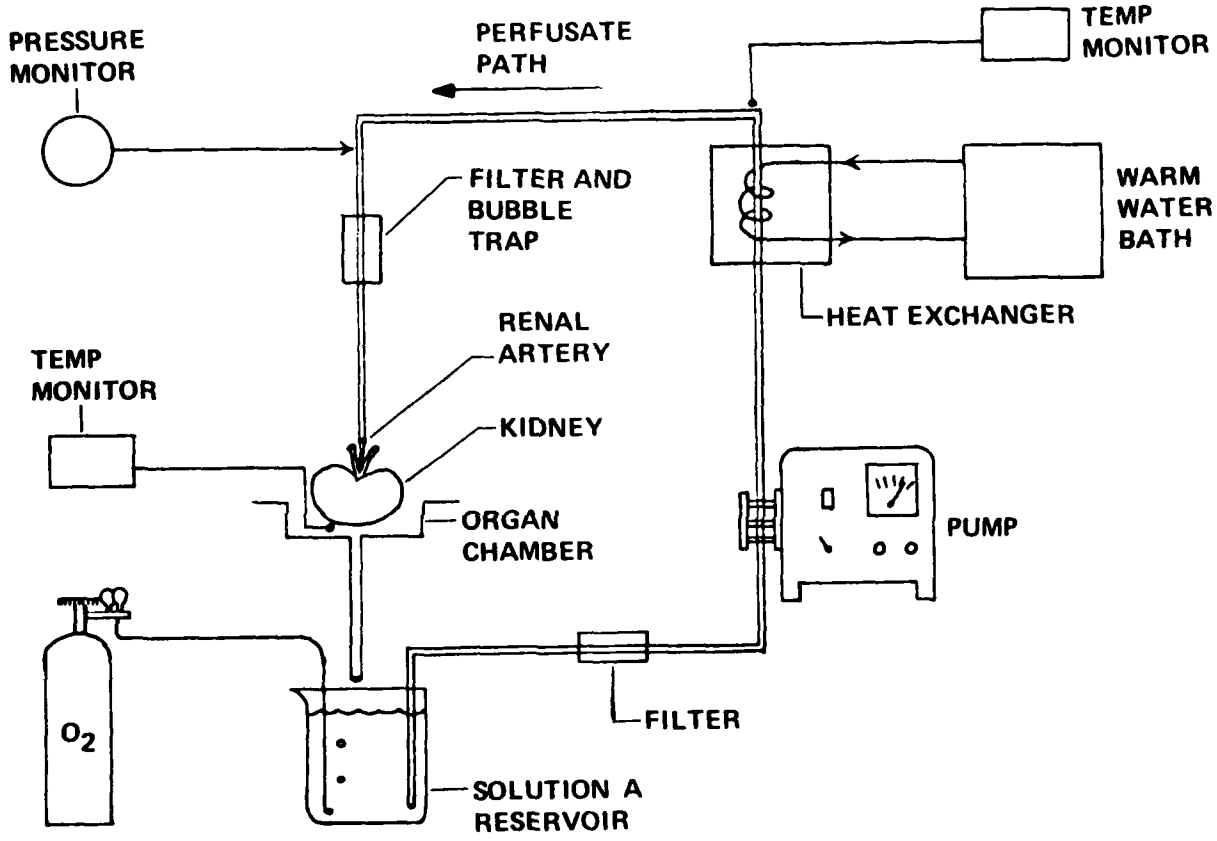
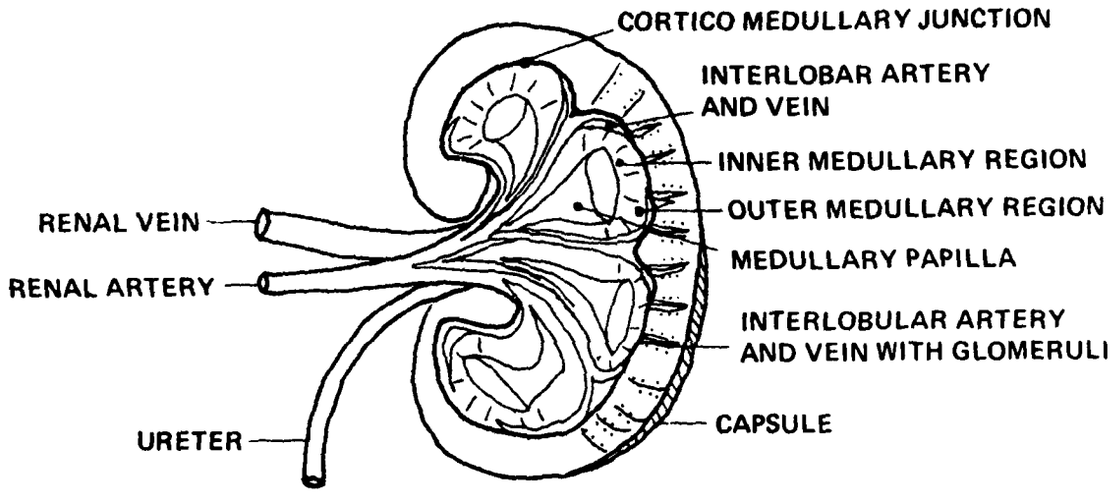
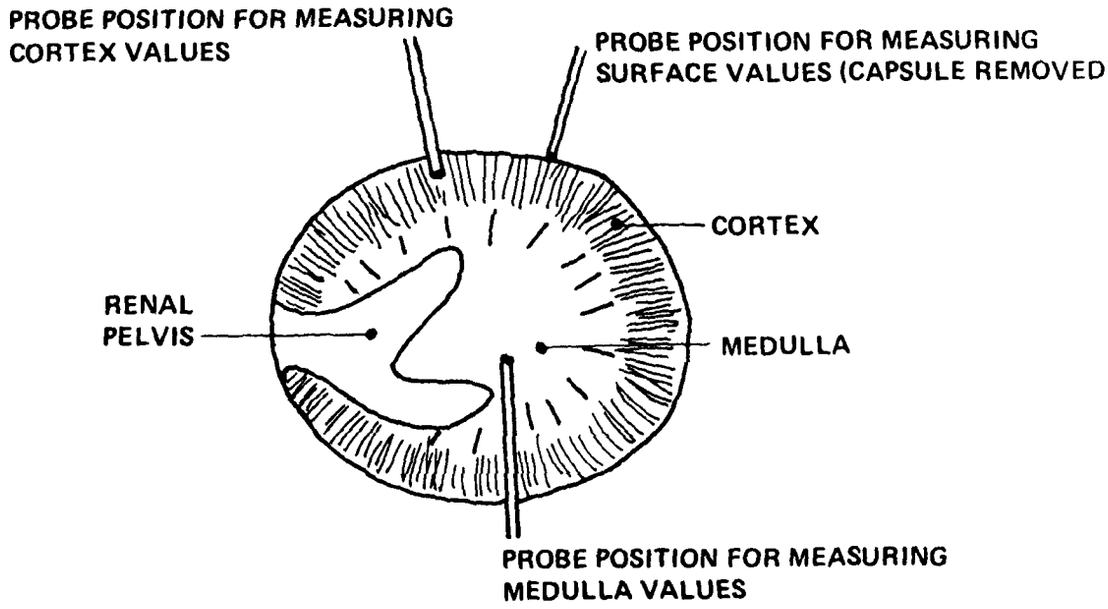


Figure 16. Diagrammatic illustration of experimental setup used for perfusion during in-vitro renal dielectric measurements.



(a) Renal anatomy in longitudinal cross-section



(b) Transverse cross-sectional view of dielectric property measurement sites

Figure 17. Two anatomical views of kidney. Sites where dielectric property measurements were performed are shown on transverse view of kidney.

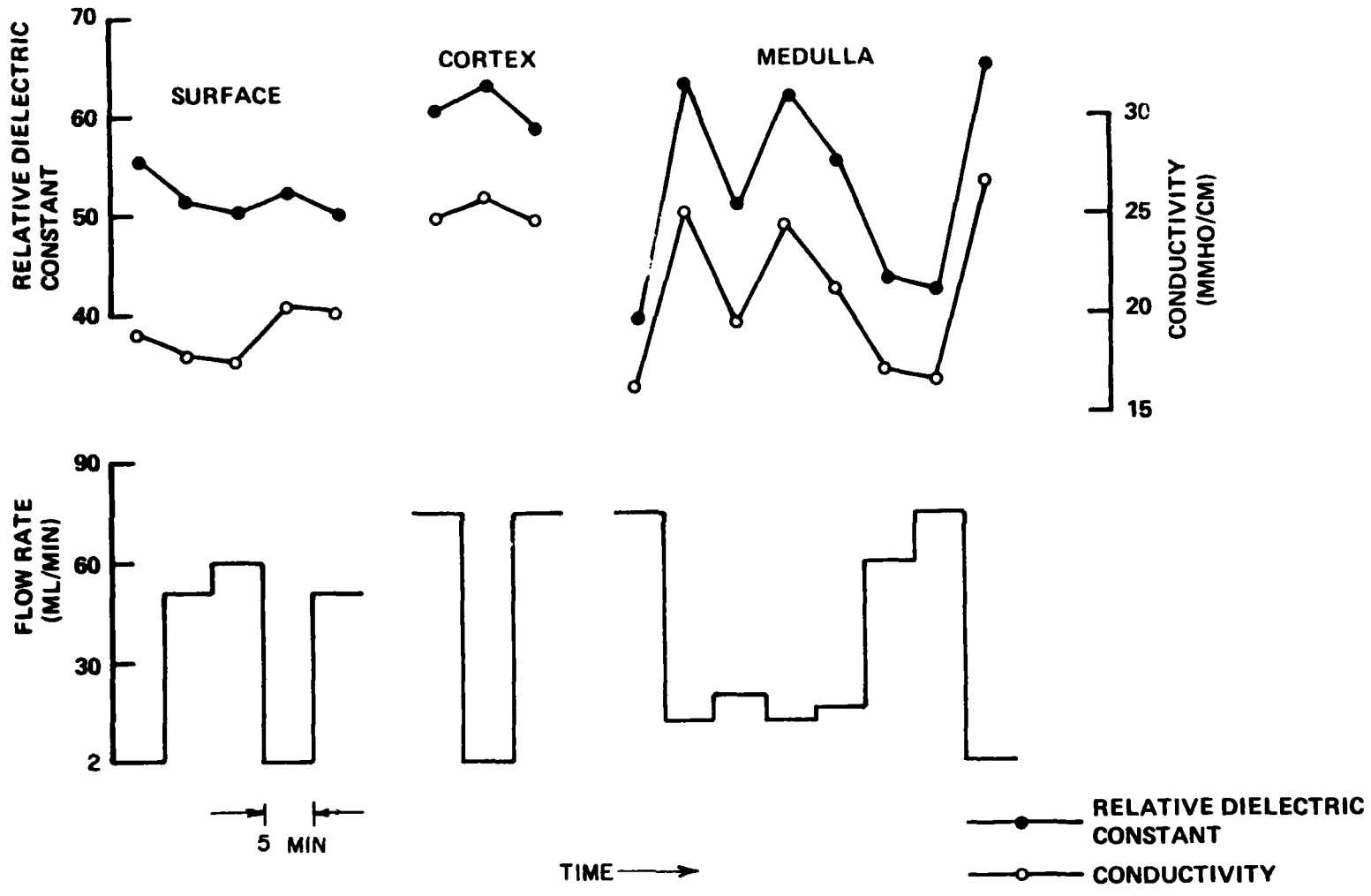


Figure 18. Time progression of one renal dielectric measurement experiment showing correlation between changes in renal flow and changes in measured renal dielectric properties.

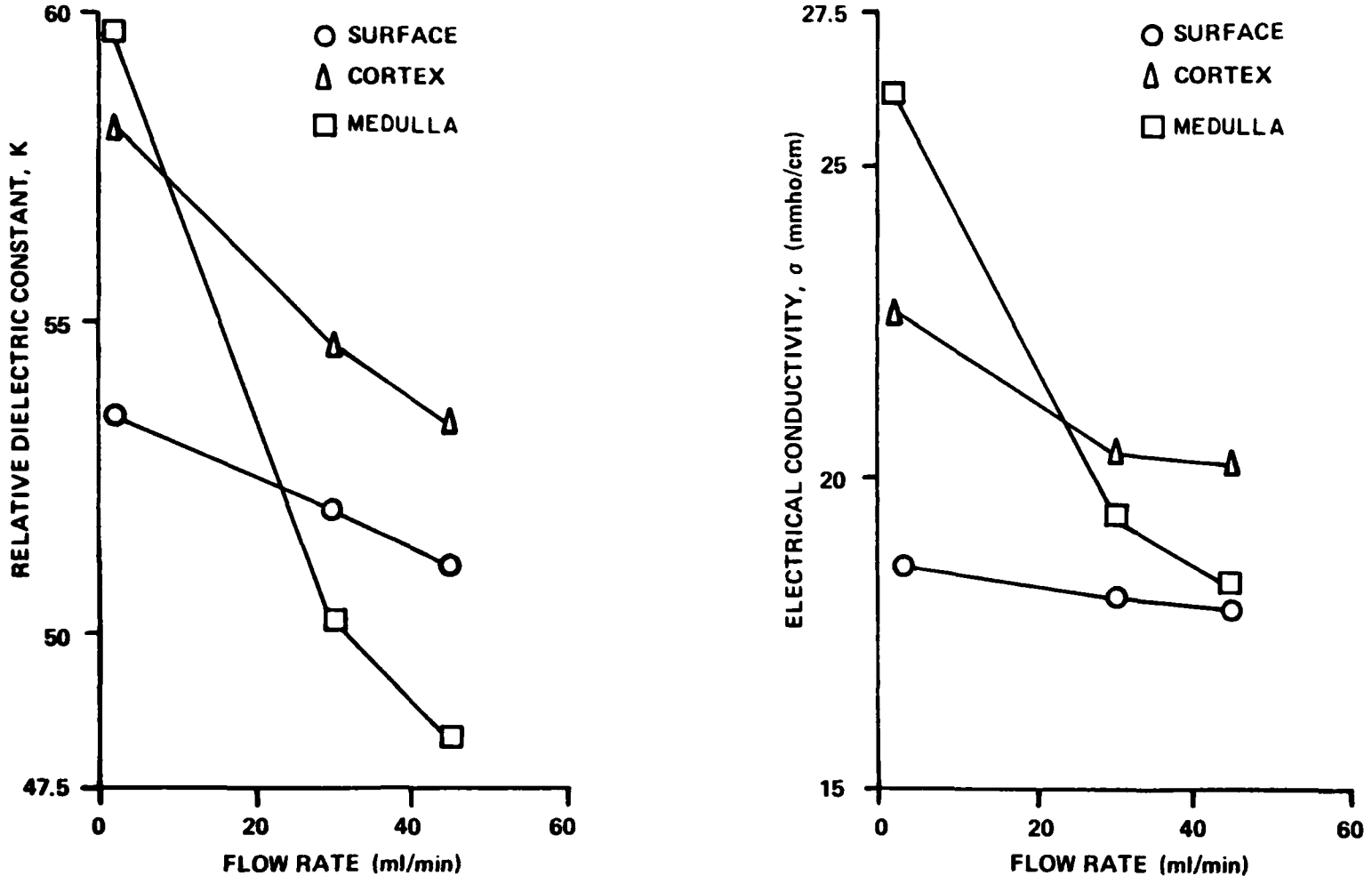


Figure 19. Changes in dielectric properties of canine kidney surface, cortex, and medulla corresponding to different total renal blood flow rates. These measurements were performed at 2.45 GHz and 37°C on one experimental animal.

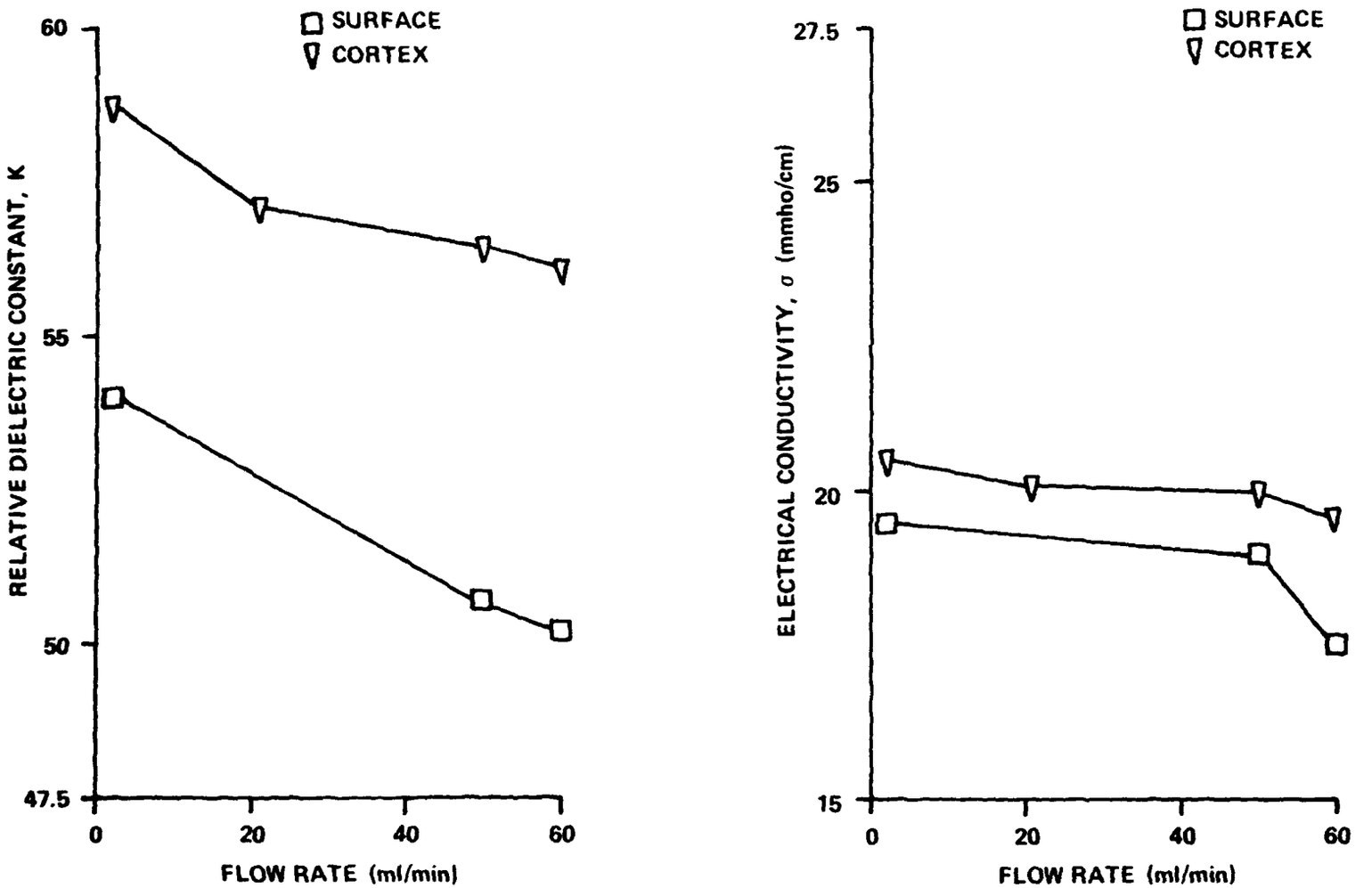


Figure 20. Changes in dielectric properties of canine kidney surface (capsule removed) and cortex corresponding to different total renal blood flow rates. These measurements were performed at 2.45 GHz and 37°C on one experimental animal.

is a comparison of flow rate changes and the resulting property changes. It can be seen that the correlation between absolute changes is not as good, but the general relationship between changing flow rate coupled with a concommittant change in electrical properties in the opposite direction is apparent.

The information from two other dogs illustrates the trend once more. In the first of these experiments, renal flow was adjusted between three discrete rates of approximately 45 ml/min, 30 ml/min,and 2 ml/min. It was decided not to increase flow above 45 ml/min, since pressures approaching 200 mm Hg were developed at higher flow rates (indicating the kidney may already have partially shut down). The results, illustrated in Figure 19 agree with the trend established from the previous dog: as the flow rate is increased the values of dielectric constant and conductivity decrease. Cortical surface measurements of dielectric properties revealed relative dielectric constants between 50 and 55 units and conductivities between 17 and 20 mmho/cm. The cortical dielectric constant values were lower than those obtained for the dog of Figure 18, although both sets of measurements showed increases over the surface values. Measurements of the medulla again showed reasonably good agreement between the two dogs, with low flow-rate values near 60 for the dielectric constant and near 25 mmho/cm for the conductivity. Kidney dielectric measurements from the second dog did not exhibit changes of as large a magnitude as those seen in the first dog, which was probably symptomatic of a partial renal shutdown in the second case.

In a third experiment, renal flow rate was adjusted to 60 ml/min, 50 ml/min, 20 ml/min, and 2 ml/min. The results of this experiment are presented in Figure 20. No medullary recordings were made in this experiment. Once again, the same relationship between renal dielectric properties and vascular flow was observed, although the magnitude of the measured dielectric changes was less than in the two previous cases.

SECTION IV
CONCLUSIONS AND RECOMMENDATIONS

The research efforts performed during the second year of this four-year research program have been successfully completed. The primary second-year objectives of (1) development of a method for maintaining uniform probe contact pressure on tissue during in-situ measurements, (2) examination of time-locking data sampling to respiration, (3) evaluation of data recording methods, (4) measurements of antemortem/postmortem dielectric characteristics of dog brain, (5) studying effects of changing renal blood blow rate on renal dielectric properties, and (6) initial measurements of potential dielectric changes of the auditory cortex in dogs during acoustical stimulation were achieved. In this section, conclusions based on results of the second year's efforts are first discussed, followed by recommendations for the third and fourth year efforts. The tasks reflected in these recommendations are those included in our renewal proposal for the third and fourth year investigations.

A. Conclusions from Results

The dielectric probe measurement technique was further improved during the second year efforts by the development of a software filter for reducing noise present in the measured phase angle of small-magnitude reflection coefficients and by the development of a spring-loaded probe holder for maintaining more uniform contact pressure between the probe and the tissue under study. The software filter significantly reduced the phase noise to well within the instrument measurement accuracy. The spring-loaded probe holder reduced probe contact related errors from 4-6% to approximately 1% of the measured dielectric constant and conductivity values.

The importance of maintaining good electrical contact between the dielectric measurement probe and the sample being measured was amply demonstrated. However, efforts to maintain in-vivo tissue samples in a stationary position are complicated by such basic physiological processes as pulse and respiration. The spring-loaded probe holder (SLPH) allowed

the probe to track minor tissue movements while maintaining good electrical contact with the time. Furthermore, the movement of the probe when the tissue moved prevented any major interference by the probe with the tissue's blood supply.

Future use of the probe, especially in deep body work such as in-vivo kidney measurements, may involve much more pronounced movements from factors such as respiration. This will necessitate redesign of the SLPH to allow even greater range of movement.

Data were obtained in both analog and digital form. Disadvantanges were associated with each form. The problem with the analog form is that it required a great deal of manual processing before it was usable. This was a slow (and tedious) process which was a very inefficient use of manpower. The digital data acquistion system did a great deal of the data processing automatically. It's major drawback was a slow sampling rate. Physiological processes which involved rapid change (especially $CaCl_2$ sacrifice) were not accurately recorded by the digital system. The increased sampling rate of up to a maximum of one sample/5 sec will suffice for most of our needs. However, the auditory stimulation experiments are a very possible exception. It seems likely that blood flow changes associated with stimulation of the auditory cortex will probably occur faster than could be characterized by a 0.4 GHz signal (equivalent to one sample/5 sec). We also do not know how these blood flow patterns are maintained after the initial auditory stimulation. For instance, if the response to the stimulus involved a surge of blood to the auditory cortex followed by a rapid decrease to a lower quiescent level, it is very likely our digital system could not detect such a fast change. It is planned to continue to record both analog and digital results while improving the frequency response of the digital system.

Various alternative data recording techniques were also evaluated. These included analog and digital methods. From the experimental investigations, it was determined that a need existed to increase the sampling rate in order to follow rapid changes (both dielectric and physiological) which took place at the onset of termination and cessation of blood flow. The optimal solution would be the use of a digital recording system with a high data sampling rate that is gated on and off via software control. Such a system would also be automatically gated "on" at the onset of physiological change.

Results of measurements from the K^+ and Ca^{2+} sacrifices, as well as brain blood flow experiments, indicate that varying blood flow in a tissue can be detected by the probe technique. In these cases, higher volume of water present corresponded to higher values of K and σ, as expected. Results from measurements on the kidney were contrary to this simple correlation. However, this could mean that the response of the kidney to increased flow is not the simple swelling apparently encountered with brain tissue. One might anticipate a more complicated effect in the kidneys because of the complex flow-volume-pressure relationships operating during autoregulation.

The inverse dielectric property/flow relationship observed during our in-vitro measurements of dog kidneys appears contrary to what one would expect from simple bulk water effects. That is, if increased renal flow caused an increased presence of bulk water, one would expect a corresponding rise in the dielectric properties rather than the decrease which actually occurred. Thus, these results obtained using the probe measurement technique suggest that the pressure-flow-volume relationship in the kidney is not a simple one, and that redistribution of flow within the renal vasculature would likely influence local renal dielectric properties. Further, bulk water shifts may not be the only influencing factor in the measured dielectric changes. The use of the probe measurement technique offers the advantage that measurements could be performed at both HF and microwave frequencies, possibly elucidating effects due to changes in bound water or shifts in local density of proteins.

Another case of a non-bulk-water effect detected by the probe appears to be the gradual decrease in value of dielectric properties postmortem in value of electrical properties postmortem in brain tissue. Since the decrease continues after the blood has had a chance to drain, the changes are probably due to metabolic changes associated with death. It would be interesting to check this by elevating the subjects body for one of the postmortem experiments so that blood does not drain from the head, and then checking for electrical changes associated with metabolic changes alone.

The results of dielectric property measurements and on the pia during the presence or absence of a non-noxious auditory stimulus indicate little or no effect by the acoustic stimuli on the dielectric properties. For the two stimulus frequencies used, 200 Hz and 1 kHz, similar results indicating no change were obtained at both frequencies. However, the lack of positive results from these experiments involving auditory stimuli could have been due to two factors: (1) no cortical EEG response was observed which could have been due either to the anesthetic agent used or the stimulus used, and (2) the stimulus level to the dog's cochlea was unknown and may have not been significant. Use of a gaseous anesthetic agent (N_2O + halothane) and a different stimulus paradigm (large audible clicks or multiple frequency tone) should alleviate the problem.

The in-vivo dielectric property measurement system, procedures for data acquisition and processing, and data itself presented in this report represent an important advancement in the ability to define the dielectric characteristics of tissue in-situ and in the ability to reliably detect reasonably small changes in dielectric properties due to physiological changes. In many cases of measured dielectric results reported herein, these data represent the only available reported results of their type.

B. Recommended Future Efforts

Although major advancements in the realm of dielectric measurements were accomplished during the second year of this program, a number of questions of physiological significance remain. Further, the results raise additional questions that should be investigated to provide

significant information to the Army in terms of EM field interaction with living tissues.

During the discussions between the Army Contracting Officer's Technical Representative and project personnel from Georgia Tech, additional tasks were identified which would require additional resources and time to accomplish. Because of the detailed experimental nature of these investigations, it is recommended that resources be allocated to perform additional tasks in third- and fourth-year efforts. Specifically, the following additional tasks are recommended:

Task 1. Investigate Further Signal Processing Methods

Investigate and develop signal processing methods to obtain increased sensitivity and resolution for the probe measurement technique. Methods investigated would include signal integration, spectral analysis, and statistical analysis of measured data. The feasibility of a dual-frequency probe measurement approach to enhance the technique's ability to discern physiological changes would be examined.

Task 2. Validate Probe for Measurement of Blood Flow Changes

Validate the use of the probe technique as a method for measuring blood flow changes by comparing results obtained using the probe with results obtained using standard techniques (electromagnetic flowmeter, thermal dilution) in measurements of flow in perfused, isolated tissues (e.g., Kidney).

Task 3. Characterize Renal Electrical Properties as a Function of Flow Changes

Perform measurements of in-situ renal dielectric properties a function of renal blood flow (RBF) and arterial perfusion pressure. Relate dielectric changes to blood flow changes as determined by flow measurement techniques such as thermal dilution, clearance of radioactive microspheres, and electromagnetic flowmeter.

Task 4. Characterize Renal Electrical Properties with Changes in JGA, GFR, and Neural Activity

Measure in-situ renal dielectric properties as a function of induced changes in the juxtaglomerular apparatus, glomerular filtration rate, and renal sympathetic nerve activity.

Task 5. Investigate Dielectric Changes in Brain During Auditory Stimulation

Perform studies to determine the effects of auditory stimulation on in-situ dielectric properties of the auditory cortex (under halothane anesthesia). Correlate measured changes in dielectric properties with neural electrical recordings and with blood flow changes.

Task 6. Investigate Effects of Hypercapnia and Anoxia on Brain Dielectric Properties

Perform experiments under known hypercapnic and anoxic conditions (monitoring blood gases under halothane anesthesia) to determine effects of regional cerebral blood blow on in-situ dielectric properties of dog brain.

Task 7. Develop Truly Needle Probe for Tissue Mapping

Develop a truly needle probe to permit "mapping" in-situ tissue dielectric characteristics within an organ. this development will involve the investigation of the biocompatibility of materials for probe fabrication, the design of special coaxial probes, (with the outer conductor being effectively a hypodermic needle), and the design tooling, and fabrication of special connectors.

Task 8. Perform Dielectric Mapping of In-Situ Brain Tissues

Characterize brain tissue as a function of depth beneath the pial surface by performing dielectric measurements using the needle probe.

It is anticipated that the above eight tasks would be performed over a two-year period.

SECTION V

REFERENCES

1. H.A. Ecker, E.C. Burdette, F.L. Cain, and J. Seals, "In-Vivo Determination of Energy Absorption in Biological Tissue," Annual Technical Report, Project A-1755, U.S. Army Research Office Grant No. DAAG29-75-C-0182, July 1976.

2. F.L. Cain, E.C. Burdette, and J. Seals, "In-Vivo Determiation of Energy Absorption in Biological Tissue," Annual Technical Report, Project A-1755, U.S. Army Research Office Grant No. DAAG29-75-G-0182, July 1977.

3. E.C. Burdette, F.L. Cain, and J. Seals, "In-Vivo Determination of Energy Absorption in Bological Tissue," Final Technical Report, Project A-1755, U.S. Army Research Office Grant No. DAAG29-75-G-0182, January 1979.

4. E.C. Burdette, F.L. Cain, and J. Seals, "In-Vivo Probe Measurement Technique for Determining Dielectric Properties at VHF Through Microwave Frequencies," IEEE Trans. Microwave Theory Tech., Vol. 28, No. 4, pp. 414-428, 1980.

5. W.J.V. Osterbout, Injury, Recovery and Death in Relation to Conductivity and Permeability. Philadelphia, PA: Lippincott, 1922.

6. B. Rajewshy, Ultrakurzwellen, Ergebnisse der biophysikalischen Forschung, Bd. I. Leipzig, Germany: Geog Thieme, 1938.

7. E.C. Burdette, J. Seals, and S.P. Auda, "Dielectric and Resistive Characteristics of Sarcoma and Normal Muscle, Liver, and Brain in the Dog and the Rat," in preparation.

8. L.E. Larsen, J.H. Jacobi, and A.K. Krey, "Preliminary Observations with an Electromagnetic Method for the Noninvasive Analysis of Cell Suspension Physiology and Induced Pathophysiology," IEEE Trans. Microwave Theory Tech., Vol. MTT-26, pp. 581-595, 1978.

9. H.P. Schwan and K.R. Foster, "RF-field Interactions with Biological Systems: Electrical Properties and Biophysical Mechanisms," Proc. IEEE, Vol. 68, No. 1. pp. 104-113, 1980.

10. H.P. Schwan, "Electrical Properties of Tissue and Cell Suspensions," Advan. Biol. Med. Phys., Vol. 5, pp. 147-209, 1957.

11. E.C. Burdette, R.L. Seaman, J. Seals, and F.L. Cain, "In-Vivo Techniques for Measuring Electrical Properties of Tissues," Annual Technical Report No. 1, Project A-2171, U.S. Army Medical Research and Development Command, Contract No. DAMD17-78-C-8044, July 1979.

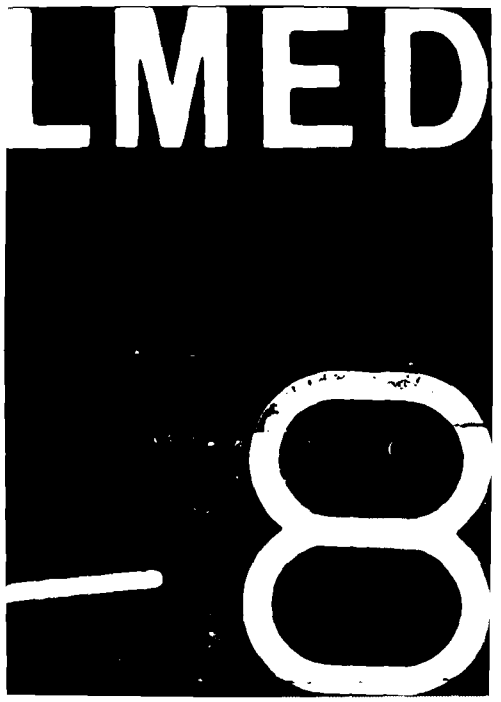
12. E.C. Burdette, F.L. Cain, and J. Seals, "In-Situ Permittivity Measurements: Perspective Techniques, Results," Electromagnetic Dosimetric Imagery Symposium, 1980 IEEE MTT-S International Symposium, Washington, D.C., May 1980 (IEEE Press, New York).

13. G.A. Deschamps, "Impedance of an Antenna in a Conducting Medium," IRE Transactions on Antennas and Propagation, AP-10, pp. 648-650, September 1962.

14. A. Goth, Medical Pharmacology, 9th Edition, C.V. Mosby Co., St. Louis, MO, 1968, pp. 347-357.

15. A.L. Towe, "Audition and the Auditory Pathway," Ch. 18 in Physiology and Biophysics, 19th Edition, T.C. Ruch and H.D. Patton, Eds., W.B. Saunders, Philadelphia, PA, 1965, pp. 379-400.

16. R. Aster, "Effect of Anticoagulant and ABO Incompatability on Recovery of Transfused Human Platelets," Blood, Vol. 26, pp. 732-743, 1965.


LMED
8