DETERMINATION OF CURRENT DISTRIBUTION PATTERNS IN ELECTRICALLY STIMULATED TISSUE SPECIMENS

by

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I. INTRODUCTION

1.1 Description of the Problem

The use of current is widespread in both diagnostic and therapeutic medical applications. All diagnostic techniques involving measurement of impedance changes suffer from a limited ability to calibrate these variations with associated physiological events. The therapeutic application of current, electrical anesthesia, and electrical stimulation as an aid to tissue regeneration are also limited by a lack of information on current distributions [1].

Knowledge of the distribution of current in any application where current is introduced into biological tissue is necessary for a complete understanding of tissue response to stimulation [2]. The determination of the characteristics of these currents is complicated by the varying resistivities and spatial arrangements of different tissues encountered between the source electrodes. Nevertheless, an understanding of the relative spatial distribution of current in biological tissues is an important aid in many areas of research today.

In recent years, meat researchers have noted beneficial effects from electrical stimulation on the quality of their products. These benefits include increased tenderness, reduced muscle shortening during cooling, and improved color qualities [3]. Attempts to determine the optimum electrical parameters and methods of stimulation have been hindered by the lack of understanding, as well as the limited methods of measuring, the current distributions which occur in stimulated biological tissue.

1.2 General Current Density Measurements

A description of current distribution generally includes current density, which is defined as the amount of current flowing in a given area measured perpendicularly to the direction of current flow. The general units for biological studies are mA/cm^2 [2].

The simplest possible conditions for determining current density are those of uniform current density in a homogeneous, isotropic medium of simple geometry such as a rectangle. Isotropic, homogeneous media simplify the determination of current density because physical parameters of these substances are constant throughout and independent of the direction of measurement.

One physical property (parameter) which is important in the determination of current distribution is resistivity. Resistivity, ρ, is a continuous parameter and is related to resistance, a lumped parameter, by the equation

$$R = (\rho L)/A$$

where R is the resistance, L is the length of the material, A is the cross-sectional area, and ρ (rho) is the resistivity of the substance. Materials having similar dimensions of length and cross-sectional area have varying resistances to current depending on their varying resistivities. If ρ is constant, then R is constant for given values of length and area. Ohm's Law relates voltage (potential) and current by the relationship potential equals current times resistance. Current and potential are thus directly related, the constant of proportionality being the resistance of the conductive medium.

In Fig. 1.1, the total current is considered to form "current tubes" with even distribution [2]. Current tubes containing equal

currents are geometrically similar. In addition, the sides of the current tubes follow electric field lines. Lines of equipotential are perpendicular to these electric field lines and therefore perpendicular to the sides of the current tubes. The electric field lines and the equipotential lines form squares (cubes) of equal resistance. Constant current density is indicated by the fact that the squares are all of the same size.

In some applications the current is not evenly distributed in the homogeneous medium as shown in Fig. 1.2. The current density is altered by the physical contact of the electrodes with the substance, or by some irregular geometry which distorts the current distribution. In this case, the current tubes do not maintain a constant shape throughout their length. In any case where the medium is homogeneous, a knowledge of the potential distribution is sufficient to determine the current distribution.

Nonhomogeneous materials complicate the determination of current density. A substance having resistivities ρ_1 and ρ_2 in parallel is shown in Fig. 1.3. The resistivities are related by the equation $\rho_1=2\rho_2$. If current tubes containing equal currents, and selected lines of equipotential are drawn, the result is illustrated in Fig. 1.4. The medium with resistivity ρ_2 has twice the current density as the substance with resistivity ρ_1. Of importance is the fact that the measurement of potential alone does not indicate current density. In the case of nonhomogeneous substances, it is necessary to determine the resistance of the material at the point of interest in order to accurately determine the associated current density.

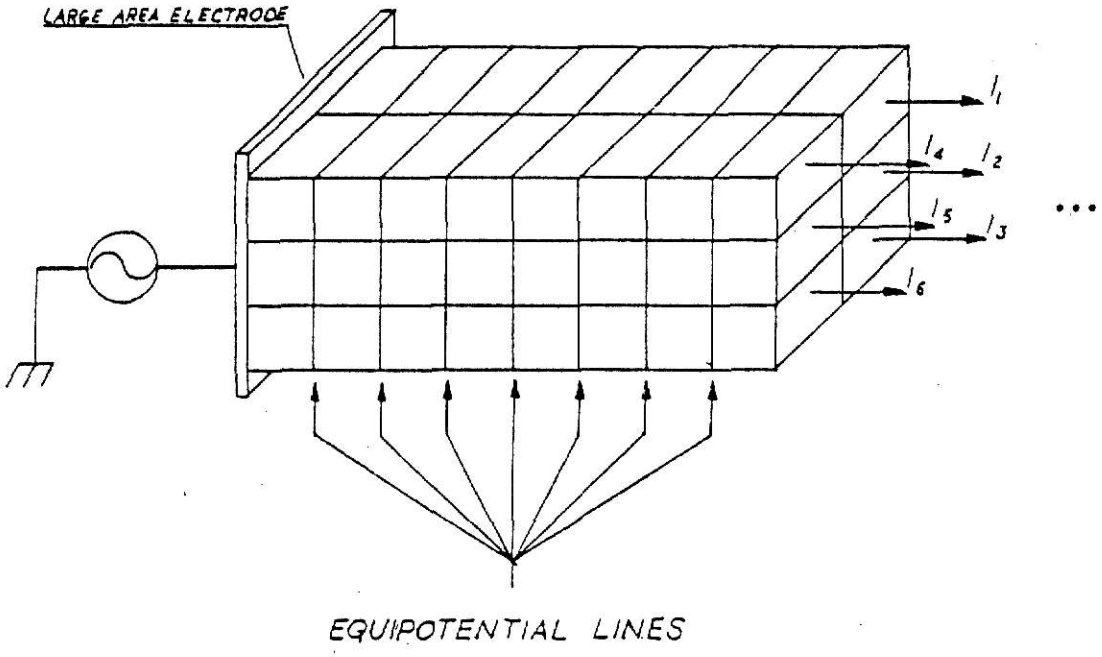


Fig. 1.1 Uniform Current Distribution in a Homogeneous Medium

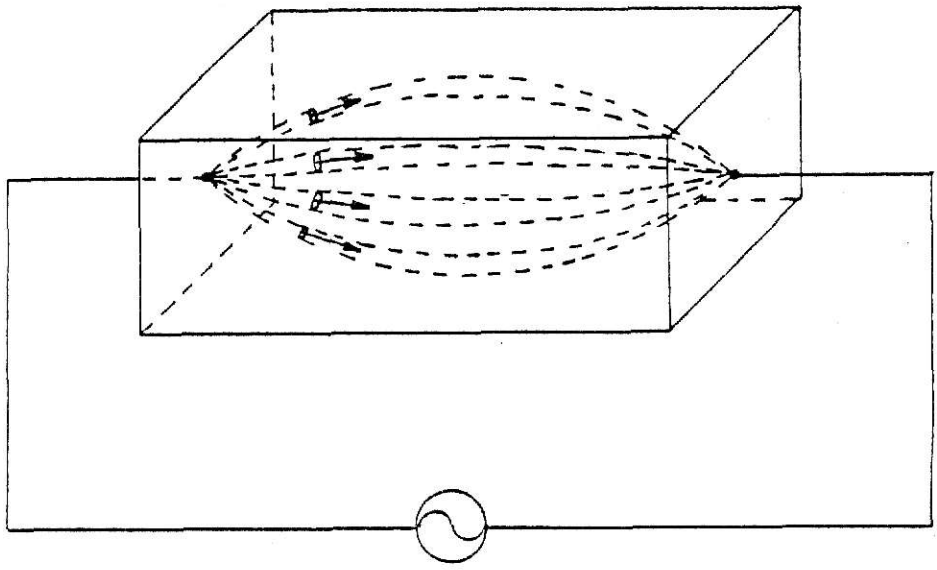


Fig. 1.2 Nonuniform Current Distribution in a Homogeneous Medium

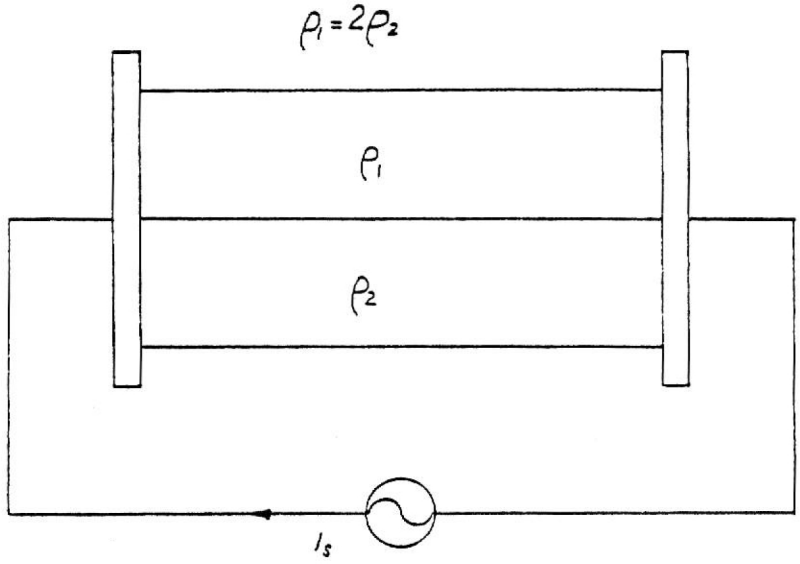


Fig. 1.3 Resistivity Distribution in a Nonhomogeneous Medium

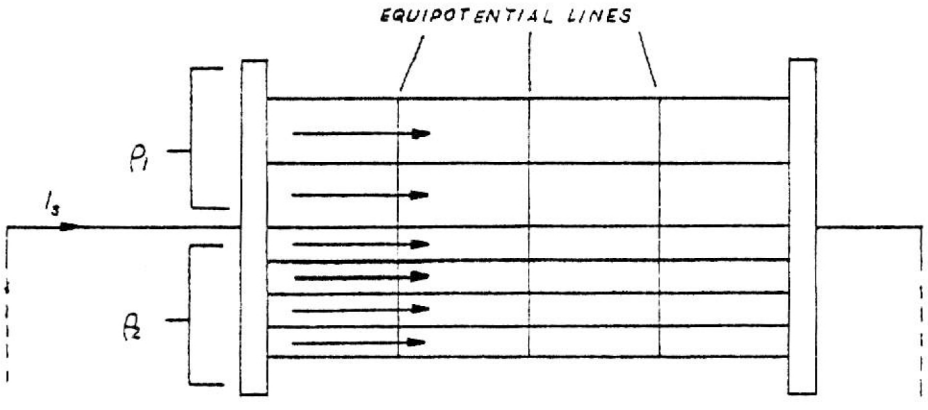


Fig. 1.4 Nonuniform Current Distribution in a Nonhomogeneous Medium

1.3 Literature Search

The investigation of electrical stimulation of muscle has a long history dating back to 1663 when Swammerdam demonstrated the contraction of frog muscles stimulated using bimetallic wire [4]. Luigi Galvani described much the same result in 1791 [5], and from that time on studies on the electrical stimulation of nerves and muscles flourished.

The first recorded observations of the beneficial effects on meat due to electrical stimulation came from Benjamin Franklin in 1749 [6] while experimenting with turkeys. Aside from the use of electrical shock as a method of stunning livestock, though, the use of electricity in the meat industry lay dormant until Harsham and Deatherage illustrated the effects of accelerated glycolytic rate and tenderization on post-slaughter beef carcasses in 1951 [7]. The use of electrical stimulation was ignored again until 1973 [3] when meat scientists from New Zealand began studies on the electrical stimulation of lamb carcasses in an attempt to solve the problem of meat toughening caused by cold shortening. At present, there are numerous studies being conducted on the effects of various electrical parameters and methods of stimulation on meat quality. These include such topics as electrode carcass contact, stimulation voltage, pulse rate, pulse duration, pulse shape, and time of stimulation postmortem [8].

H. J. Swatland has conducted numerous studies on the changes of electrical parameters as a function of time and pH in postmortem carcasses [9,10,11]. Generally his findings indicated that resistivity and capacitance decreased as pH decreased and as time postmortem increased.

In addition, Swatland conducted a study on the anisotropic nature of skeletal muscle in carcasses [11] from which the resistivity of muscle was found to be anisotropic, having minimum resistivity along the myofiber axis and maximum resistivity perpendicular to the myofiber axis. Data collected from studies using rats subjected to varying current magnitudes indicated that resistance decreased in a nonlinear fashion as current magnitude increased [12].

Bendall [13] reported data on voltage drop in different regions of electrically stimulated, undressed carcasses. Using a stimulating voltage of 600 V (peak), a voltage drop of 2.2 V/cm was noted in the mid-lumbar area of the Longissimus dorsi muscle compared to 3.0 V/cm over the whole path from neck to Achilles tendon. In contrast, the Triceps brachii muscles of the forelimbs, not in the direct current pathway, registered only a .4 V/cm drop. J. P. C. Chalcroft and B. B. Chrystall [13] also studied the current distribution patterns during electrical stimulation. There is some evidence [14,15,] that the stimulus follows directly through nerve pathways in carcasses stimulated within 15 minutes postmortem. After 15 minutes, though, the nerves have been shown to be nonviable and stimulation of muscle tissue must rely on less efficient distribution paths.

Factors affecting current densities in tissues were found to include stimulation electrode size, tissue (volume conductor) shape, and homogeneity of the tissue [8]. A practical method of estimating current density distribution proposed by Geddes and Baker used a bipolar electrode to determine the potential gradient in a given region and then they measured the resistance of the material in the same region. A

compilation of the resistivities of certain biological materials has also been produced by Geddes and Baker [16].

1.4 Objectives

Considering the desire to determine and comprehend the current distribution patterns in electrically stimulated carcasses, four major goals were established.

1. Determine the general requirements of a system that could be used to ascertain the relative current distribution patterns in electrically stimulated tissues.
2. Study the effectiveness of an electrode quad arrangement, with which two differential voltage measurements could be taken at right angles to one another at a given tissue site, as an indicator of current direction and relative magnitude.
3. Design and construct a system with the capabilities and characteristics described in objectives 1 and 2.
4. Study distribution patterns in subject tissues and evaluate them.

II. GENERAL SYSTEM DESCRIPTION

2.1 Function and Operation

The requirements of a system to determine current distribution patterns in stimulated tissue vary greatly depending on whether a qualitative or quantitative study is desired. Obviously, the quantitative study requires a much more careful consideration of system components to ensure that the data obtained match the actual signals as closely as possible. In addition, the determination of both potential and impedance at sites of interest is necessary to determine current densities quantitatively. Qualitative measurements, on the other hand, do not require the determination of tissue impedance if a specific site is to be compared only with itself as certain stimulation parameters are varied. The elimination of impedance measurements greatly simplifies system complexity.

The instruments described below are rudimentary systems for the qualitative determination of current distributions of stimulated tissues. The original system consisted of no more than a 2" x 4" board with 48 nails driven into it. Each of forty-eight three foot wires was connected at one end to a nail and at the other end to a straight pin. The straight pins were arranged in squares containing 4 pins each. Differential voltage measurements were made manually using a laboratory voltmeter, and the results were recorded by hand. While crude, this system did allow for the determination of the signal magnitudes one could expect using available stimulating apparatus. Once signal magnitudes were established, a system was necessary which would provide:

1. Consistent orientation and spatial relation of electrodes
2. Differential voltage measurements
3. Rapid acquisition of data
4. Storage of large quantities of data

A system which satisfies these requirements has the following features:

1. Mechanically stable electrode system
2. Differential amplifier
3. Multiplexed input
4. Microprocessor or computer control and mass storage capabilities.

With the desire to have computer control and mass storage capabilities comes the necessity to convert the acquired analog signals to digital signals. The analog-to-digital (A/D) converter works most effectively when the signal amplitude can be varied to make maximum use of the range and, therefore, the accuracy of the converter. In order to accommodate this feature a variable gain amplifier is necessary, and it is useful to make the gain computer controllable. In addition to variable gain, an A/D converter requires two other devices. First, unless provided on board, the A/D requires a relatively stable clock to regulate its internal processes. Second, the converter requires a constant input signal for the short time it takes to acquire and convert the analog data. This may be done in two ways; first, using a sample and hold circuit, or, second, using an RMS-to-DC converter. Since an exact reproduction of the acquired signal is not necessary and acquisition time is not critical, the method of choice is RMS-to-DC conversion. The result of these design considerations is the system shown in Fig. 2.1.

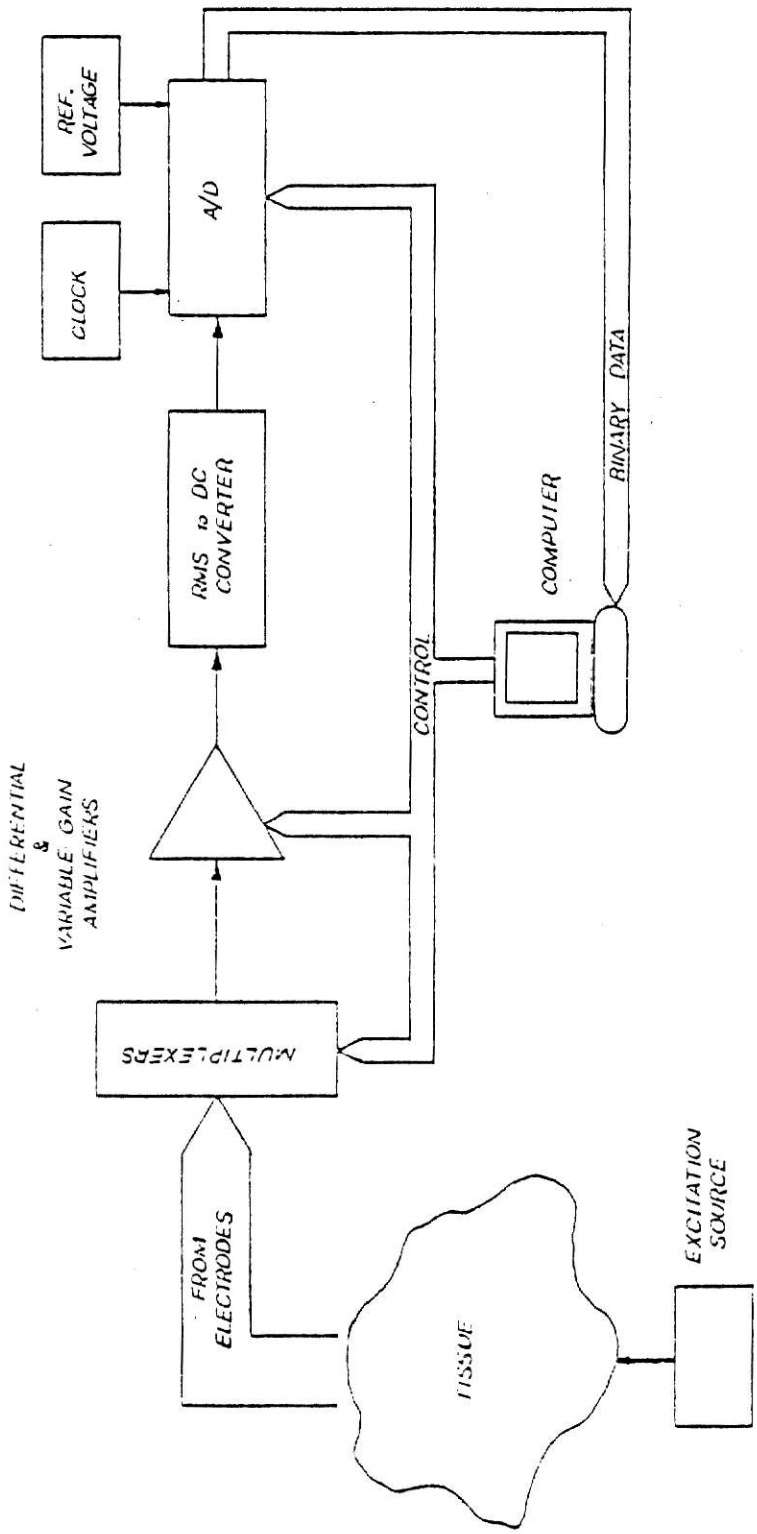


Fig. 2.1 General Block Diagram of Data Acquisition System

The stimulation of the subject tissue is provided by a commercial function generator (Tektronix FG501). This allows for signals with variable amplitude and frequency. In addition, power for the instrumentation, not including the computer, is provided by a commercial power supply (Tektronix PS503). The instrumentation system contains the electrodes, multiplexers, amplifiers, RMS-to-DC converter, and the A/D converter. The A/D converter also contains the necessary hardware to interface with the computer. The computer used is the Hewlett-Packard HP9835A. This was chosen for its availability, its ease of software development, flexibility, mass storage and interface capabilities.

2.2 Hardware

The following sections describe the electrodes and instrumentation system from a general viewpoint. Details of circuitry and software are found in Appendix I. The only inputs supplied to the instrumentation system other than power are control signals from the computer. Four bits are used to control the variable gain amplifier, five bits control the selection of multiplexer address lines, and two bits are used to control the A/D converter.

2.2.1 Electrodes

The electrode system for this project was required to meet several general constraints.

1. The electrodes were required to have relatively low impedance to avoid attenuation of the desired signal.
2. The electrodes were to limit noise acquisition as much as possible. This noise could range from 60 Hz power noise to RF (radio frequency) interference.

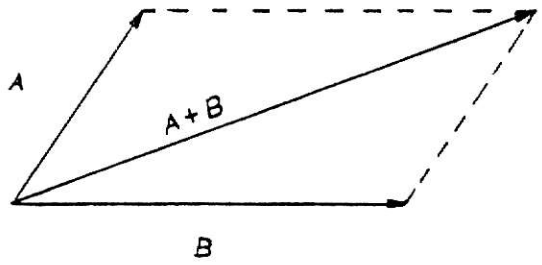
3. The electrodes were to be mechanically stable to avoid physical deformation upon insertion into the tissue.
4. The active portion of the electrode was to be conventionalized and limited so that the depth within the tissue at which data were obtained would be constant. This also minimizes the problem of acquiring data from several different muscle groups at once.
5. The electrode geometry was to aid in the determination of current direction.

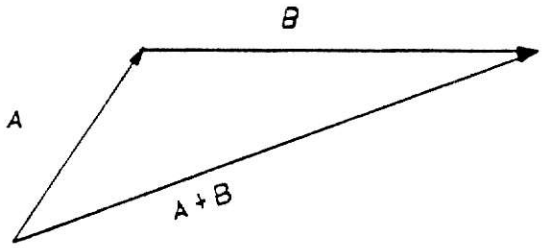
The electrode system designed fulfills the requirements in the following ways. The electrodes are composed of bronze rod which has a resistivity at 0 degrees centigrade of 9.6 Ω cm [17]. This compares fairly well with pure copper, having a resistivity of 1.6 Ω cm. The electrode impedance itself is fairly low; however, there are no available data on the impedance of the metal-electrolyte interface at any frequency so this remains an unknown quantity. In order to limit noise, the lead wires are shielded and the shields are connected to chassis ground. The electrodes are potted in a fiberglass resin which prevents them from moving and acts as an electrical insulator for the lead wire-electrode interface. In addition, the electrodes are coated with a thin layer of Varathane (registered trademark) liquid plastic along their entire exposed length except for one small region about 5 mm long. This prevents conduction along the electrode except in this region and standardizes the depth at which signals are acquired. In addition, the plastic coating prevents conduction at the tips of the electrodes. The electrode tips create field distortions which alter current flow in their immediate area if allowed to conduct. Finally, the geometry of

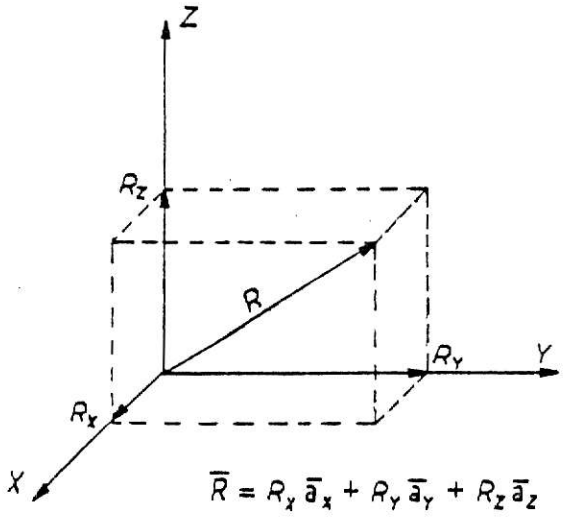
the electrodes is designed to allow the user to determine current directions through the vector addition of the magnitudes of two differential voltage measurements.

Vectors are described by two parameters; magnitude and orientation. In addition, vectors are governed mathematically by a set of rules called vector algebra. Two vectors may be added graphically either by drawing both vectors from a common point and completing the parallelogram, as in the top diagram of Fig. 2.2, or by beginning the second vector at the head of the first and completing the triangle illustrated in the center diagram of Fig. 2.2. Vectors may be added analytically as well. Any vector may be broken down into components which correspond to the magnitudes of the projections of the vector along two or three mutually perpendicular axes, e.g., the Cartesian coordinate system. The vector sum of the components must add to the original vector. The bottom diagram of Fig. 2.2 illustrates the components Rx, Ry, and Rz of the vector R. The small "a"'s represent unit vectors having magnitudes of 1 and directed along the coordinate axes. The magnitude of the resultant is shown to be the square root of the sum of the squares. The simplest vectors to add are those which lie along, or parallel to, the axes since these do not require the separation of their components.

The electrode system devised uses the concept of the addition of perpendicular vectors to determine, at least qualitatively, the current directions at chosen sites. The electrodes are divided into groups of four and arranged in squares. The four electrodes of each quad are given a letter designation (A,B,C,D) and are further subdivided into pairs (A-C, B-D) formed between diagonal electrodes. A differential voltage is measured across each pair of a given quad, and, since the







$$\overline{R} = R_X \overline{a}_X + R_Y \overline{a}_Y + R_Z \overline{a}_Z$$

$$|R| = \sqrt{R_X^2 + R_Y^2 + R_Z^2}$$

Fig. 2.2 Vector Algebra

measurements are across the diagonals of a square, they are perpendicular. The differential voltages are considered vectors and are added using the previously described method. The resultant vector is an indication of the current direction. Sixteen such electrode quads (64 total electrodes) were constructed for this project.

The electrode lead wires were purchased from a commercial manufacturer (Cooner Sales Co., Inc.). The wires are four conductor, 30 gauge, shielded, and are cut to lengths of 1 meter. The wires are soldered to edge card connector terminals and covered with heat shrink to provide insulation. The shields from each cable are connected to one terminal of the edge card connector. The corresponding terminal on the card is connected to chassis ground. The specifics of electrode connections to the instrumentation system are found in Appendix I. Details of the electrode system are shown in Fig. 2.3.

2.2.2 Instrumentation

The instrumentation is considered that portion of the system responsible for the processing of data prior to its storage in the computer. The processing includes conversion of differential voltages to a single voltage referenced to some ground, any signal gains desired, and conversion of analog data to its digital form.

2.2.2.1 Multiplexers

The signals acquired by the electrodes are scanned two at a time using the computer to vary the input lines selected. The two lines chosen make up one diagonal pair of an electrode quad. In order to select 2 lines from 64, eight 1-of-8 decoders and one dual 1-of-4 decoder are required. The multiplexers are digitally controlled analog switches having low "ON" impedances to limit attenuation of signals.

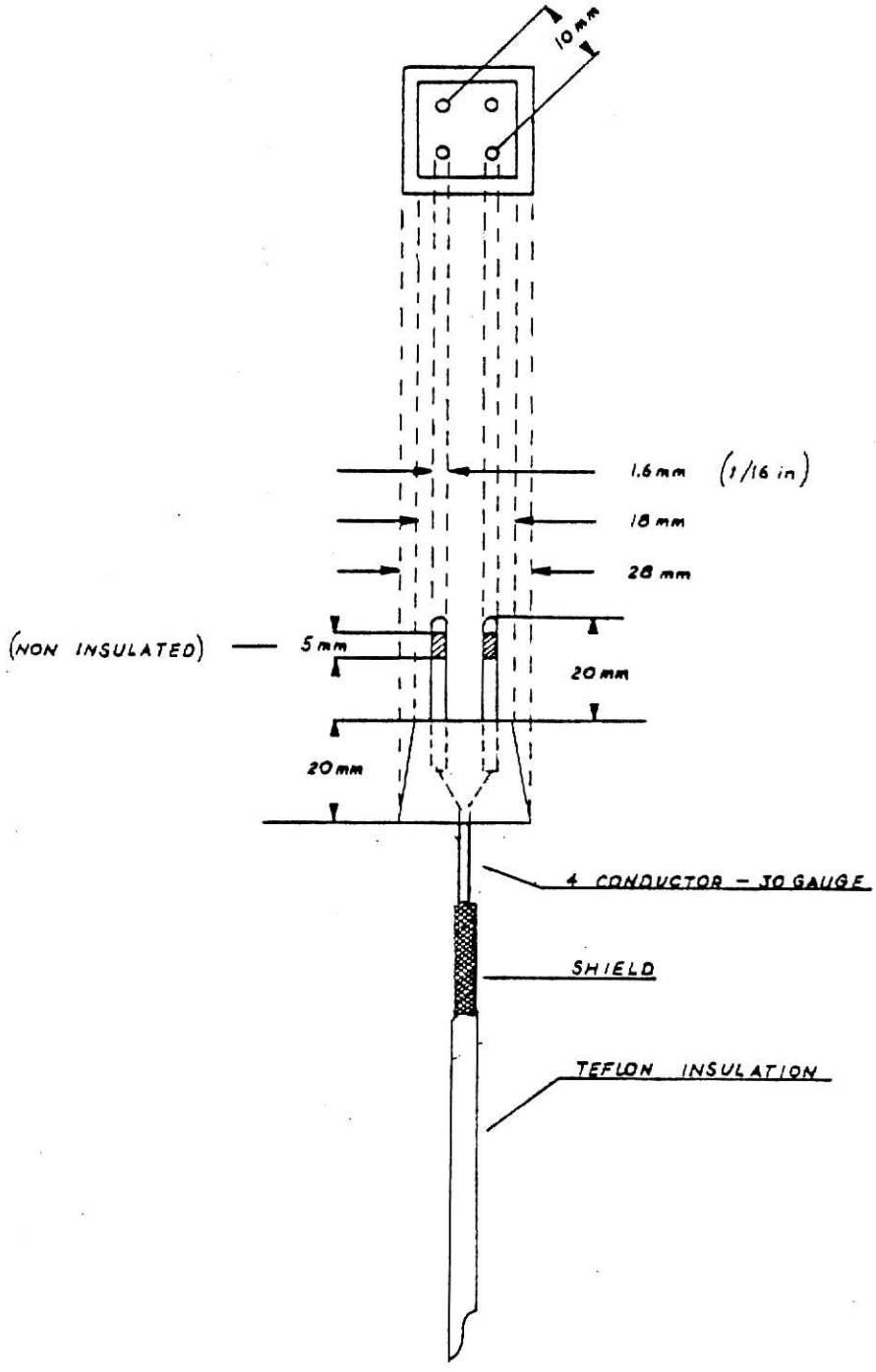


Fig. 2.3 Electrode Quad Details

2.2.2.2 Amplifiers

The two voltage signals selected are DC coupled to the inputs of a monolithic IC instrumentation amplifier. A monolithic instrumentation amplifier was chosen because of the optimized performance and limited drift variation among circuit components. An instrumentation amplifier was chosen for several reasons.

1. Low offset and drift
2. Low non-linearity
3. Stable gain
4. High input impedance
5. High CMRR
6. Low output impedance

The output of the differential amplifier is AC-coupled to the non-inverting input of a general purpose operational amplifier using a poly-styrene capacitor. The operational amplifier is used in a simple non-inverting amplifier circuit in which the feedback path may be connected, using computer controlled switches, to one of four potentiometers. These potentiometers are set for the desired range of gains expected to allow utilization of the full resolution of the A/D converter. To allow for computer control of the gain, an addressable latch and a quad bilateral switch are used. For more information on the control of the adjustable gain amplifier see Section 2.3.

2.2.2.3 RMS-to-DC Converter

The amplified output of the variable gain circuit passes directly to the true RMS-to-DC converter. Like the instrumentation amplifier, this is a monolithic integrated circuit and, as such, optimizes system performance and limits drift variability among components. This circuit

computes the true root-mean-square value of any complex AC, or AC plus DC input signal and gives an equivalent DC output level.

2.2.2.4 A/D Converter

Upon converting the RMS value of the analog signal to its DC equivalent, the voltage is sent to the analog-to-digital converter. The input signal is converted to its binary equivalent relative to some reference voltage. This voltage is provided by a precision IC voltage reference to prevent variations due to power supply fluctuations. In addition, the A/D converter requires an external clock to control the conversion process. To provide adequate clock stability, a crystal oscillator and supporting logic gates are used. Control signals from the computer initiate the conversions, and, once completed, the resulting binary data is sent, in parallel, back to the computer for storage.

2.3 Computer Control and Software

The computer has two primary responsibilities in the data acquisition process: to provide the control logic necessary to automatically scan through the electrodes, including adjusting the gain, and controlling the A/D converter; and, to handle the data storage and retrieval. The following paragraphs consider these two major functions.

2.3.1 Control Requirements

The first device which requires a control input from the computer is the adjustable gain circuit of the amplifier. The desired gain is selected by the user and input to the computer. Switching among the four resistances requires four control lines. All four control signals are inputs to an addressable latch, two are address selects, one is for enable (bar), and one is for clear. The computer sends out signals to

clear the latch and then the address of the desired resistance (gain) is transmitted. The address controls a constant signal (HI) to be transferred to a control line of a quad bilateral switch. The selected channel connects the feedback path of the non-inverting operational amplifier with the desired gain-controlling resistance. It should be noted that a latch is not required for computers having adequate numbers of control lines to allow dedication to a single control signal. A latch is included to allow for control by a microprocessor or other computer having limited I/O capabilities.

The second function controlled by the computer is the initialization of the A/D converter. The A/D converter requires two inputs; one to control the channel selected to receive incoming data, and the other to initiate the conversion of the data to its digital value.

The first control signal is toggled to latch in the address of the desired input channel. A multichannel A/D converter was chosen simply because of its availability and cost; however, only one channel is used for input. The second signal is pulsed to initiate the A/D's conversion process. Both signals are toggled for each electrode pair that is scanned.

In order to allow for the rapid scanning of the electrode array, the computer must control the multiplexer address lines. The eight 1-of-8 decoders require 3 control lines (n control lines for 2^n input lines) while the one dual 1-of-4 decoder requires 2 lines of control.

Once the amplifier gain is set and the channel select is set on the A/D converter, the computer selects the desired electrode pair by sending a number from 0 to 32 (binary) to the control lines of the multiplexers. While all of the electrodes are continuously active, only the

two selected have their data passed to the remaining instrumentation system. After the data of each electrode pair have been stored in the computer, the A/D's channel select line is pulsed and the multiplexer control signal is incremented and sent to the multiplexers. This process continues until all 32 electrode pairs have been selected.

2.3.2 Mass Storage Requirements

After the differential voltage of each electrode pair has been processed and converted to its digital equivalent, this value is stored in a vector (n x 1 matrix). This process is repeated for each of the 32 electrode pairs, and all electrode pairs are scanned twice during each run. Upon completion of all runs and subsequent storage of all data in the main data array, the data are ready to be stored on tape. Originally, each run's data were stored as a separate file on the tape, but this required too many listings on the tape's directory so this was changed to the present method of storing the data from all the runs of a given experiment in a single file.

The final requirement of the computer is to access the data once it is stored on tape. The process is essentially the reverse of storing the data. Once data is accessed it may be evaluated in some manner, plotted, or merely printed out in its raw form.

A flowchart of the Data Acquisition Program (DAP) is shown in Fig. 2.4.

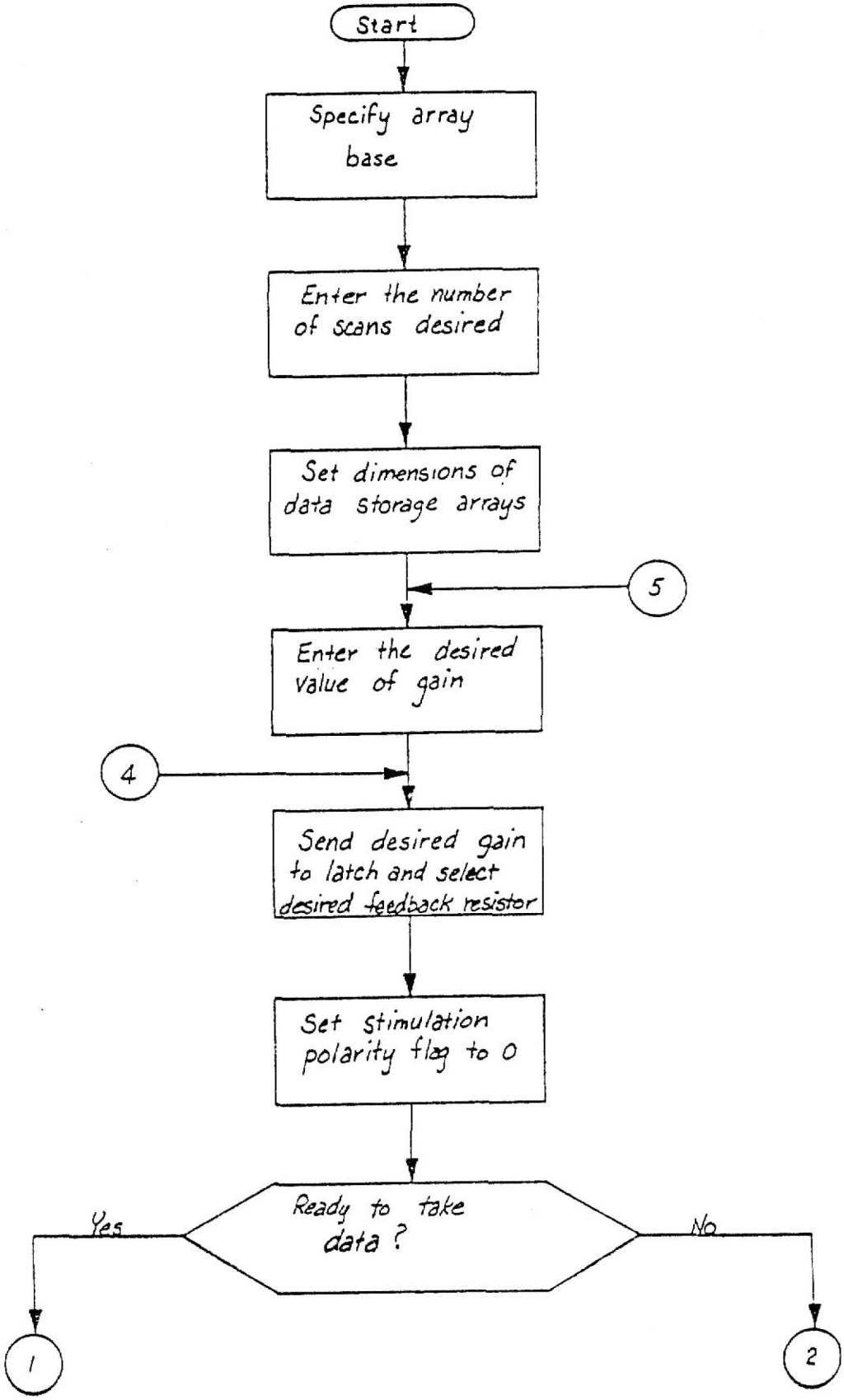


Fig. 2.4 Flowchart of Data Acquisition Program (DAP)

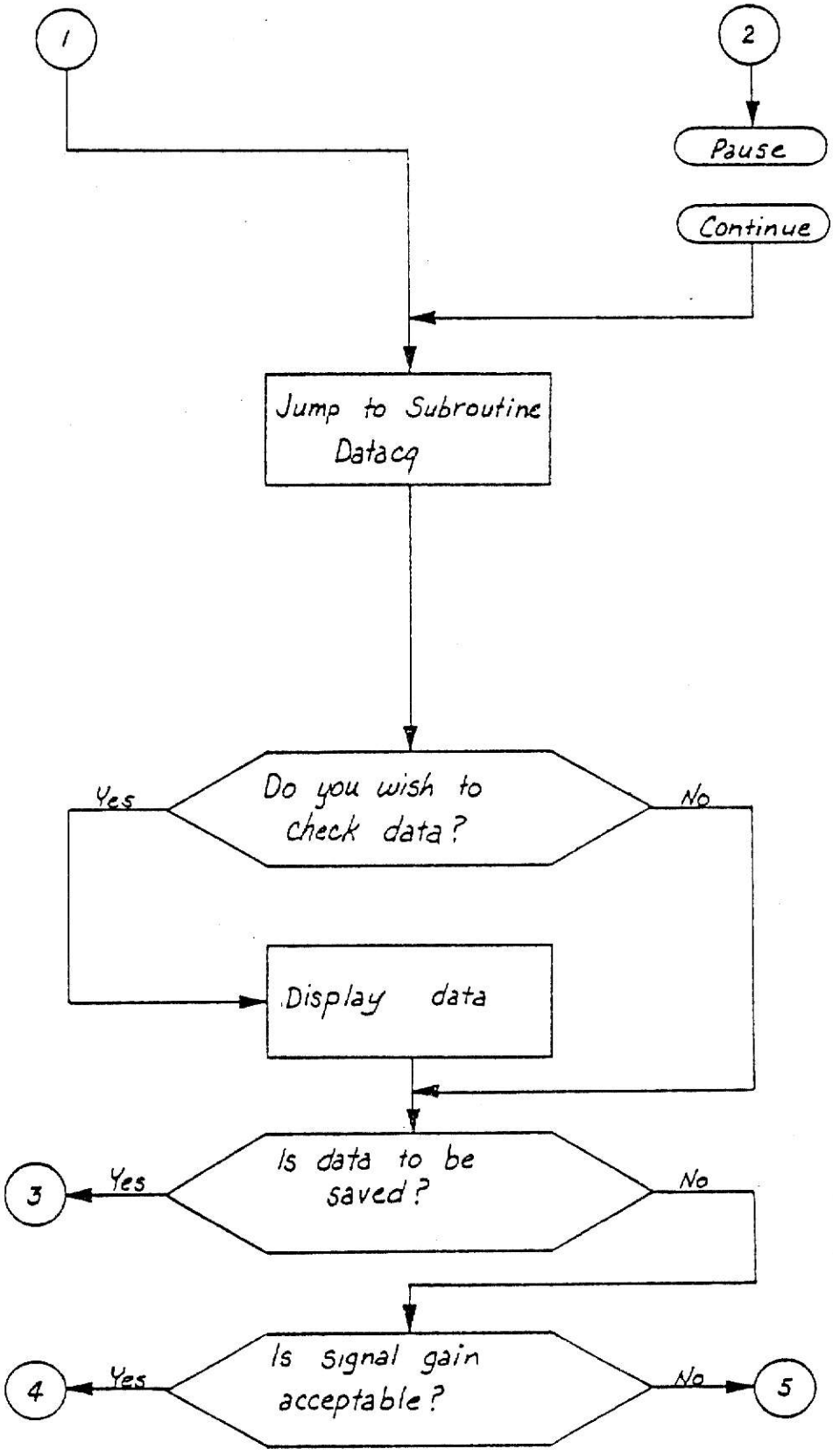
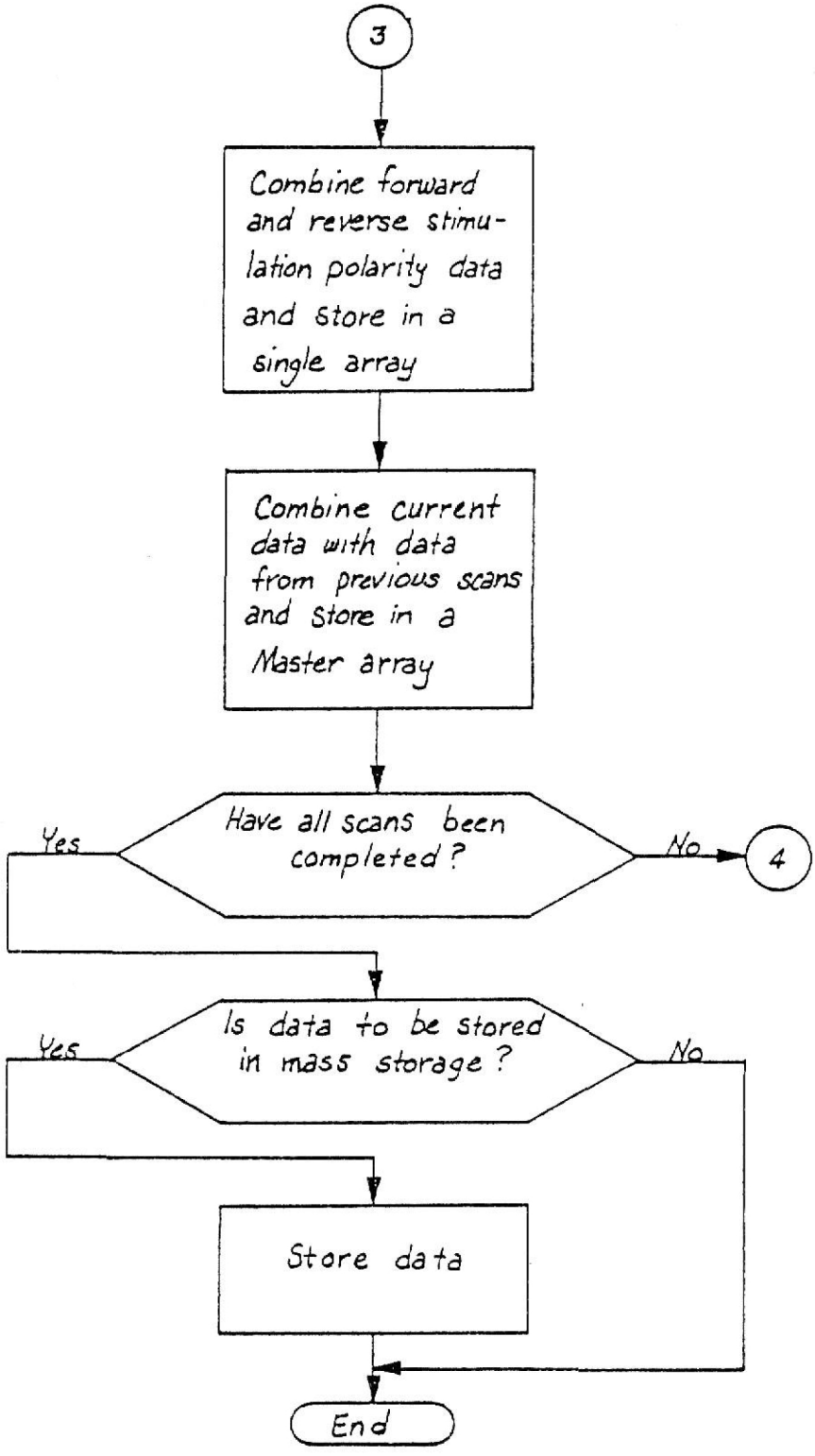


Fig. 2.4 Continued



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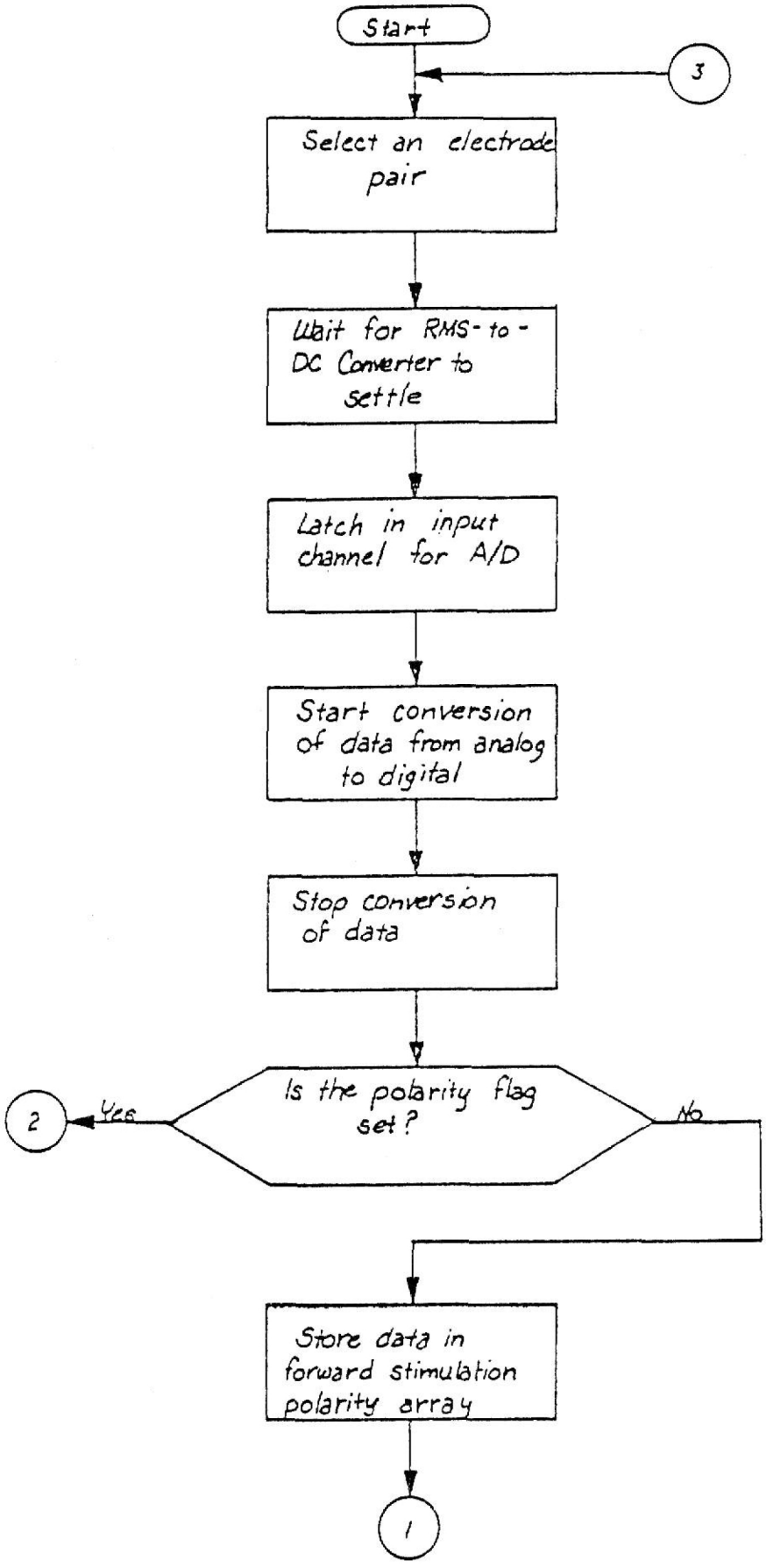

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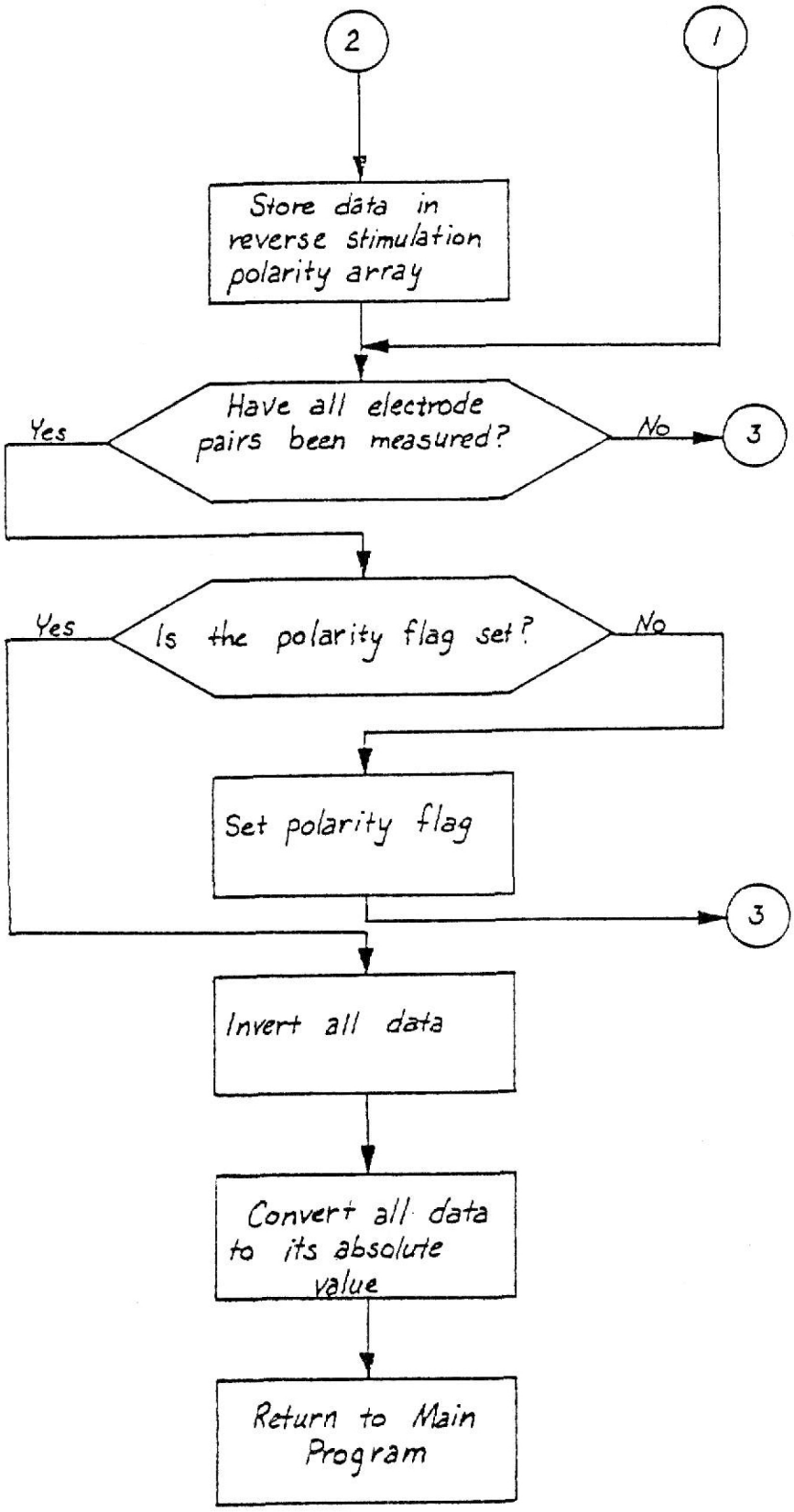

Fig. 2.4 Continued

III. EXPERIMENTAL PROCEDURES

The original impetus for this research was to determine the current patterns in whole, electrically stimulated carcasses. Because of this objective, the original experiments were conducted using whole carcasses. The size and complexity of whole carcasses created concerns about the ability to evaluate both the system and the data. With only 16 electrode quads available, it was difficult to adequately blanket the entire carcass. In addition, it was difficult to determine whether the instrumentation was functioning properly since there are so many current paths available in a whole carcass. As a result, experiments were conducted on individual muscle specimens. Single muscles, in addition to being more homogeneous, limited the available current pathways for a better determination of system performance and allowed better electrode coverage of the tissue.

3.1 Whole Carcass Experiments

All of the whole carcass experiments used dressed, post-rigor lambs having an average carcass weight of about 27.2 Kg. The carcasses were chilled for 24 to 48 hours postmortem in a cooler maintained at a temperature of approximately 4° C [18]. Just prior to the beginning of the experiment, the carcasses were removed from the cooler and allowed to warm towards room temperature (about 12.8°C) as the experiment progressed. No measurements were taken of carcass temperature at any time during the tests.

The stimulation and data acquisition electrodes were placed in the carcass at the locations shown in Fig. 3.1. The muscles in which the

electrodes were placed are noted in Table 3.1. All electrode quads were oriented so that electrode pair A-C was perpendicular to the floor. The two stimulating electrodes were stainless steel pins (dead locks) approximately 15 cm long and .8 mm (1/32") in diameter. One stimulation electrode was inserted transversely in the left rear leg (gastrocnemius muscle) about 6 cm below the proximal attachment of the achilles tendon, while the second was inserted at the medial line of the neck-shoulder junction, parallel to the dorsal spinous process of the vertebrae [19].

Only two experiments were conducted using whole carcasses. In the first, a single frequency of stimulation was used - a 1 kHz sinewave with an open circuit amplitude of 4.46 V RMS. The second set of tests used the same electrode locations but stimulated with a sinewave at frequencies of 10, 100, 1k, 10k, and 50 kHz. The open circuit stimulation voltage was maintained at 5.92 V RMS. In both experiments only 14 of the 16 electrode quads were used (1 through 14).

After all the electrode pairs were scanned in a given run, the polarity of the stimulation electrodes was reversed and the electrodes were scanned again. This procedure was followed to study the anisotropic characteristics of the carcass tissue. Reversal of stimulation polarity was part of the experimental procedure in all the experiments including those using individual muscles.

3.2 Individual Muscle Experiments

The individual muscle used in all such experiments was porcine longissimus dorsi. All of the muscles were approximately 30 cm long, 11 cm wide, and 4 cm thick. The muscles had been chilled or frozen for

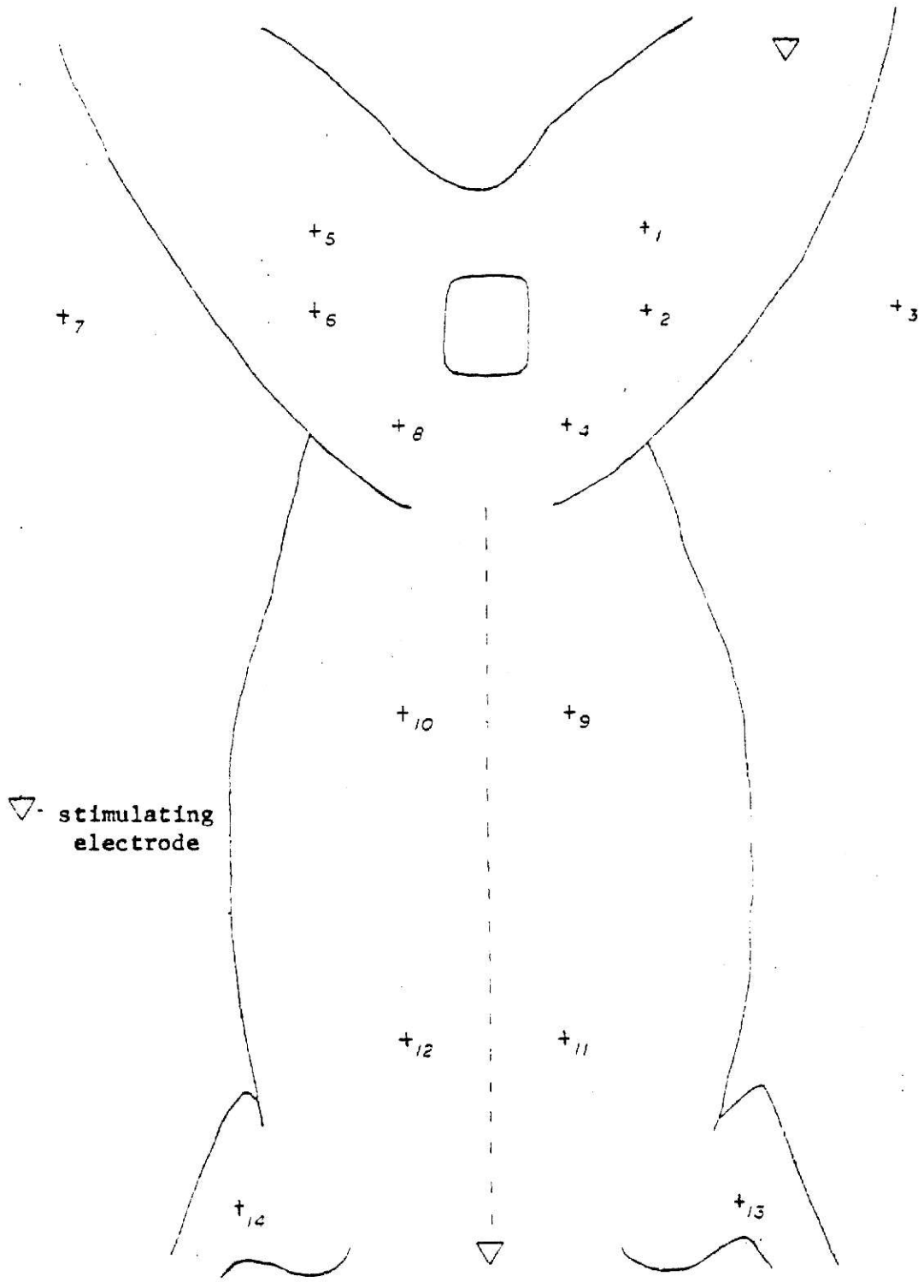

Fig. 3.1 Whole Carcass Electrode Locations

Table 3.1 Whole Carcass Electrode Quad -- Muscle Correspondence

Electrode Quad	Quad Muscle Location
1	Semitendinosus (left leg)
2	Biceps femoris (left leg)
3	Quadriceps femoris (left leg)
4	Gluteus medius (left leg)
5	Semitendinosus (right leg)
6	Biceps femoris (right leg)
7	Quadriceps femoris (right leg)
8	Gluteus medius (right leg)
9	Longissimus dorsi (lumbar, left)
10	Longissimus dorsi (lumbar, right)
11	Longissimus dorsi (thoracic, left)
12	Longissimus dorsi (thoracic, right)
13	Triceps Brachii (left forelimb)
14	Triceps Brachii (right forelimb)
15	NOT USED
16	NOT USED

periods of from 48 hours to 3 weeks before use. Muscles were allowed to thaw for a period of 2 to 3 hours and, like the whole carcasses, no temperature measurements were made on the specimens during the experiments.

The first study used 14 electrodes which were placed in a regular pattern with constant orientation as shown in Fig. 3.2. The remaining experiments conducted on single muscles used only 8 of the 16 electrodes as this provided adequate coverage. The electrode placement is shown in Fig. 3.3 The stimulation electrodes were constructed of 18 gauge solid copper wire cut approximately 15 cm long and placed on each end of the muscle perpendicular to the muscle fibers.

The sample muscles were stimulated with a sinewave at frequencies of 10, 100, 500, 1 k, 5 k, 10 k, 30 k, and 50 kHz. The tissues were stimulated at these frequencies with both constant voltage (4.65 V RMS) and constant current (10 mA). The polarity of stimulation was reversed after each scan of the electrodes for each frequency.

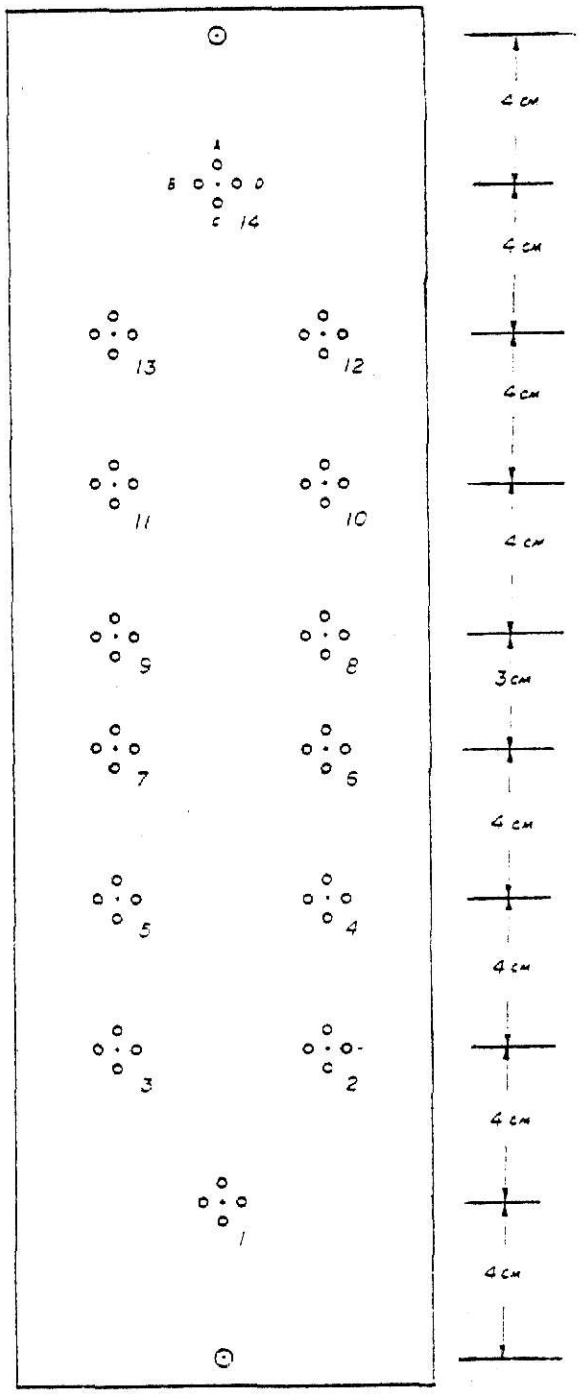

Fig. 3.2 Single Muscle Electrode Locations-
Experiment 1

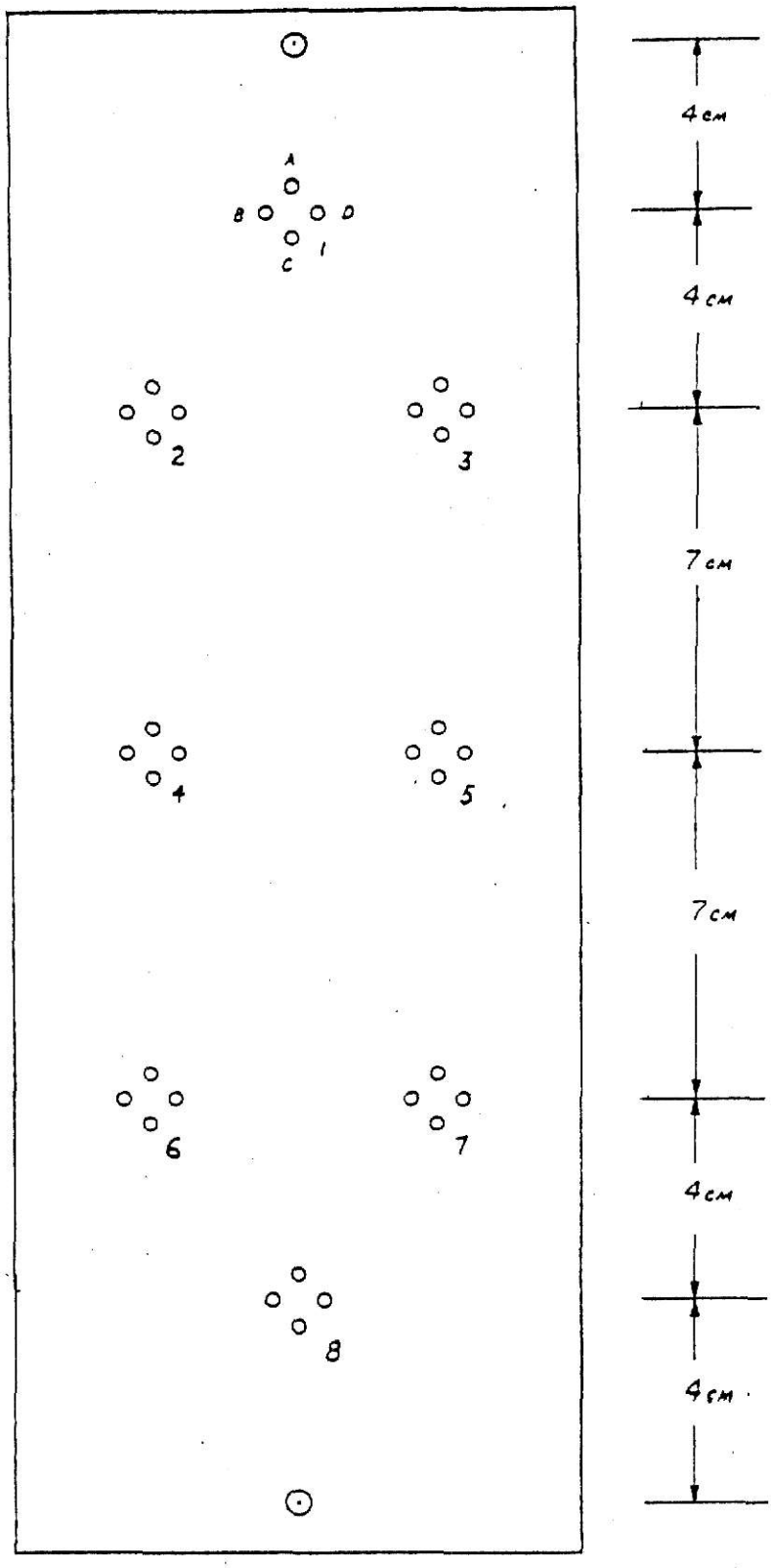

Fig. 3.3 Single Muscle Electrode Locations-
Experiments 2-5

IV. RESULTS

The differential voltage data obtained in the experiments conducted on both whole carcasses and individual muscle specimens are stored as binary values on tape. However, when displayed by the computer or hard-copy printer the binary data are listed as the decimal equivalent of their stored values. These values are not adjusted to offset the signal gain or scaled to reflect the A/D's conversion of the signals to binary values. Thus, the values are not to be taken as actual differential voltages, only as indicators of the relative magnitudes of the differential voltages measured in a given experiment.

4.1 Whole Carcass

The whole carcasses were stimulated at frequencies varying from 10 Hz to 50 kHz with constant voltage (open circuit). Fig. 4.1 shows the results of the experiment with the electrode pairs' (1,4,9,11) "A-C" decimal-equivalent differential voltages plotted versus frequency. Fig. 4.2 illustrates the equivalent values for the electrode pairs 1,4,9,11 "B-D". In both plots electrode 11 was closest to the positive stimulation electrode.

Figs. 4.3, 4.4, and 4.5 are vector representations of the data at 10 Hz, 1 kHz, and 50 kHz respectively. Note that the dotted lines seen in Fig. 4.3 and 4.4 indicate vector magnitudes less than 50. The following explanation describes the method used for determining the vector representations from the raw data.

With the carcasses hung vertically by their hindlimbs, the electrode quads were oriented so that the diagonal lines joining

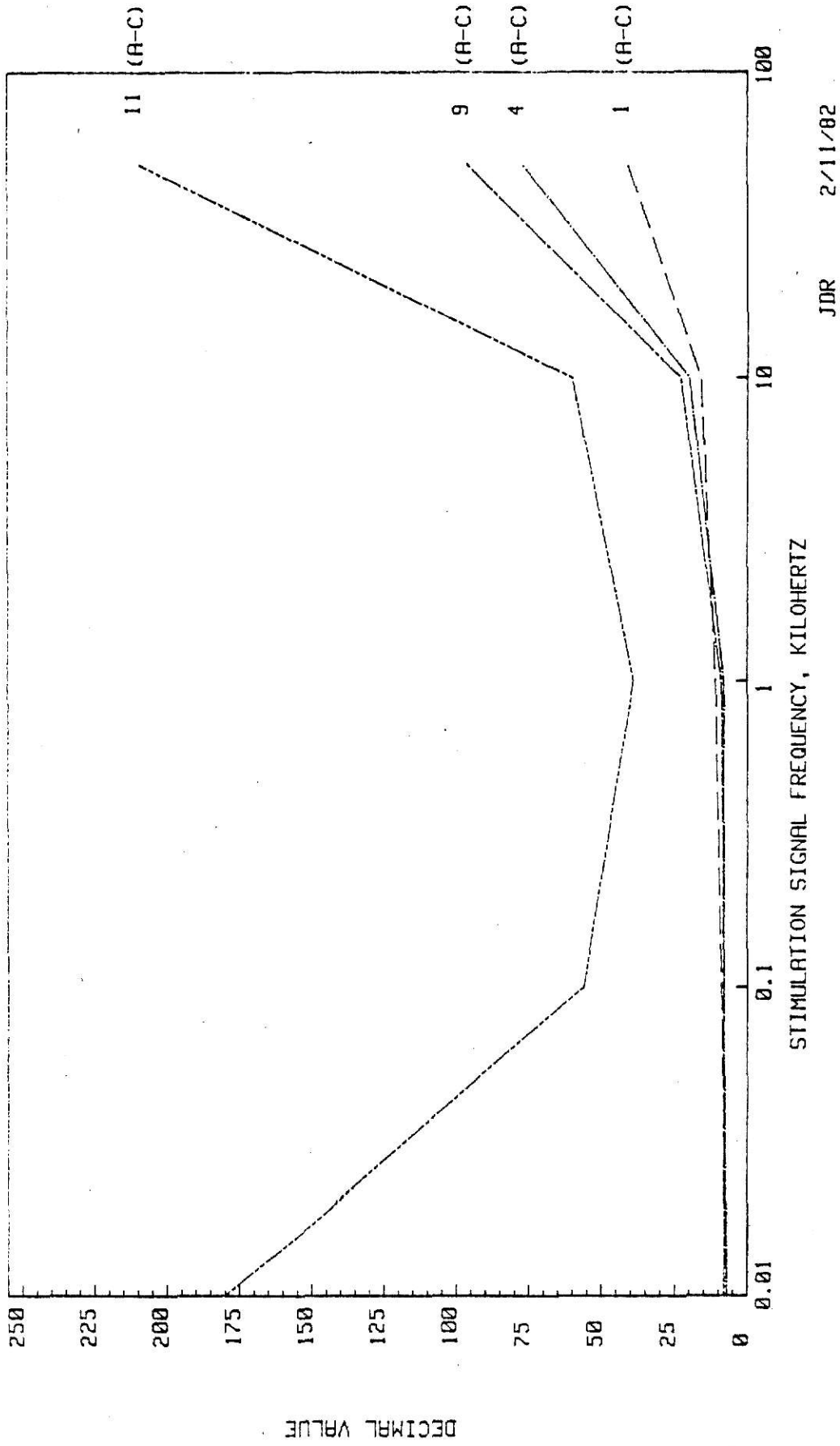

Fig. 4.1 Whole Carcass Differential Voltage
Decimal Values (Pairs A-C)

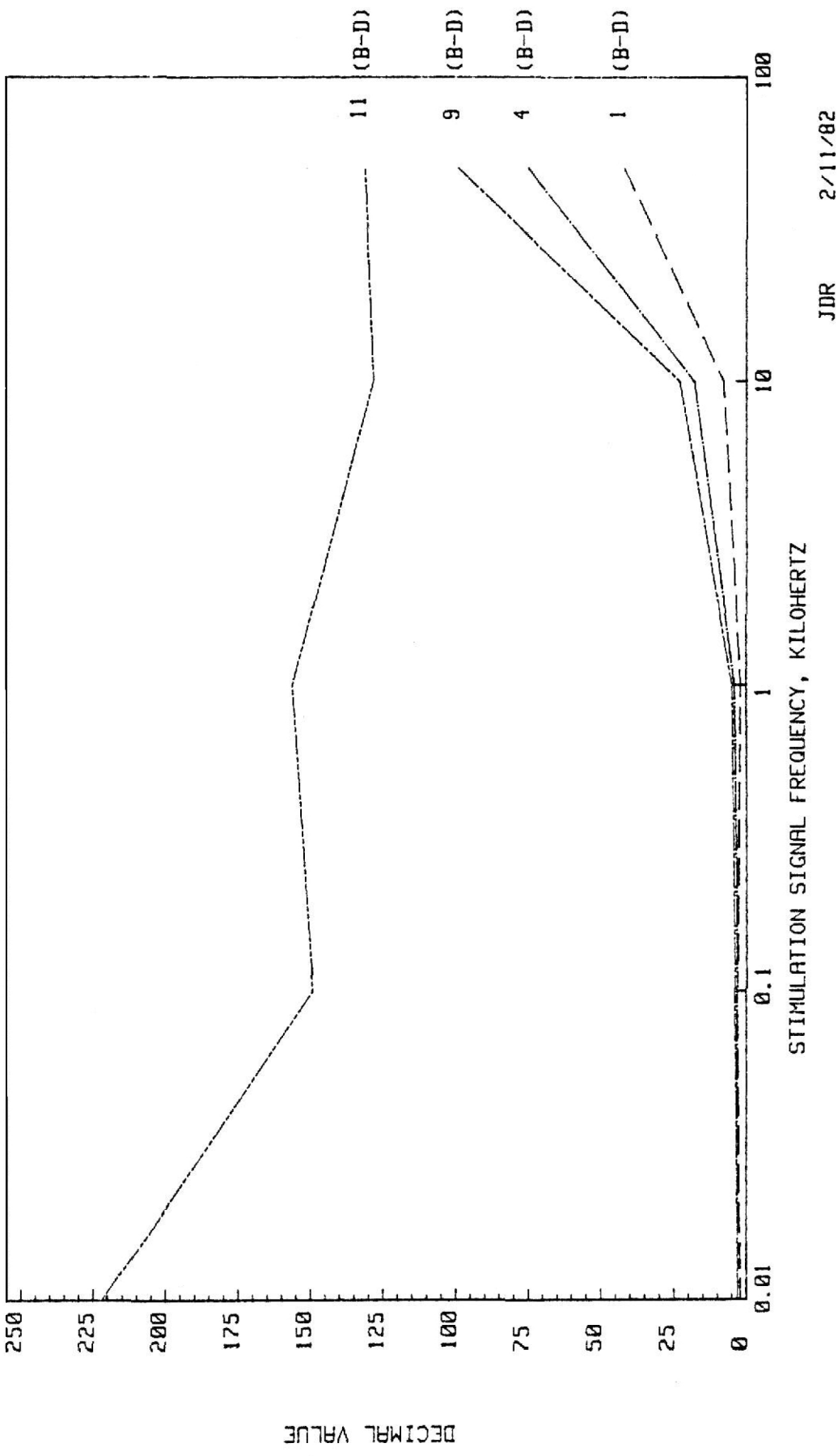

Fig. 4.2 Whole Carcass Differential Voltage
Decimal Values (Pairs B-D)

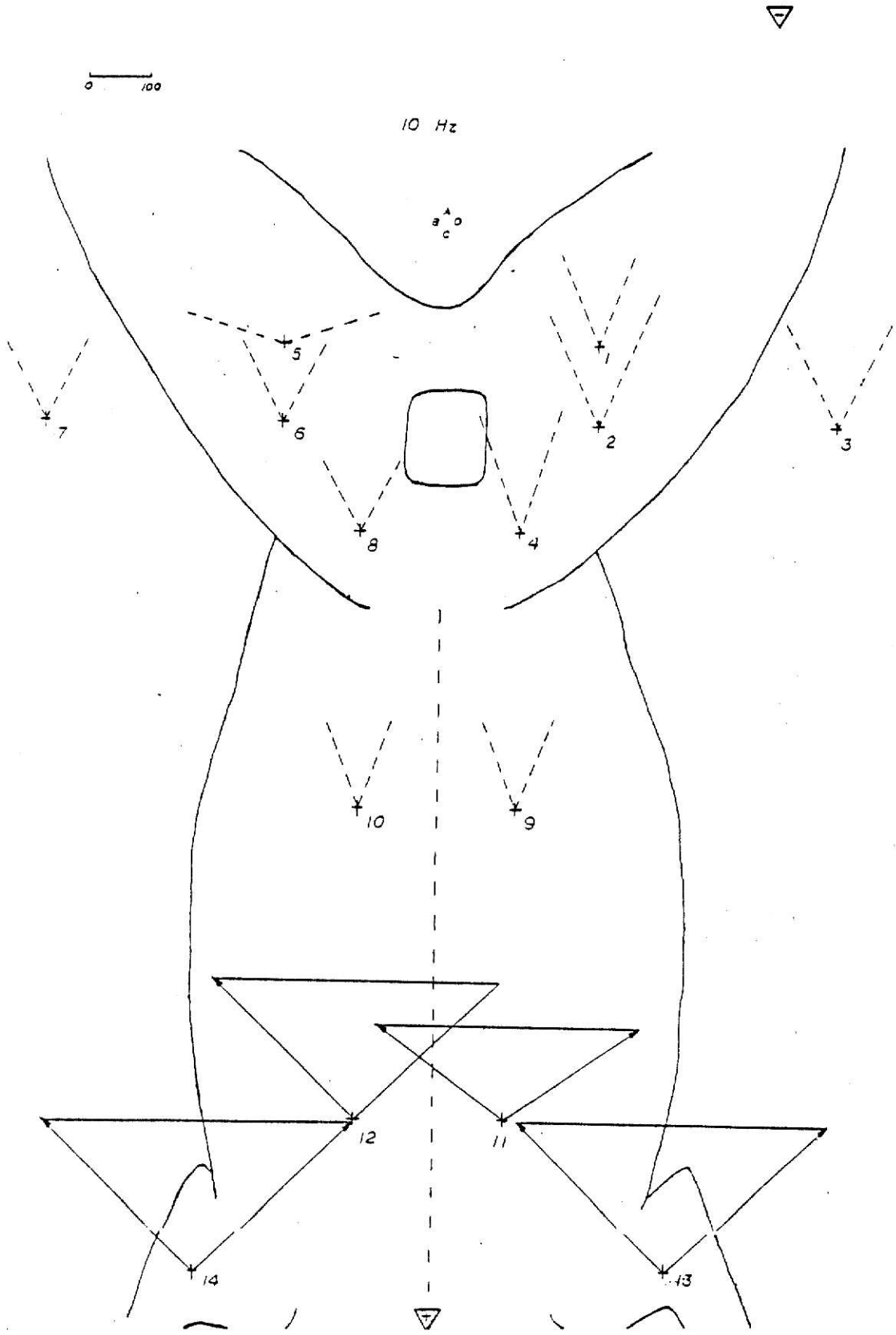

Fig. 4.3 Whole Carcass Vector Representation (10 Hz)

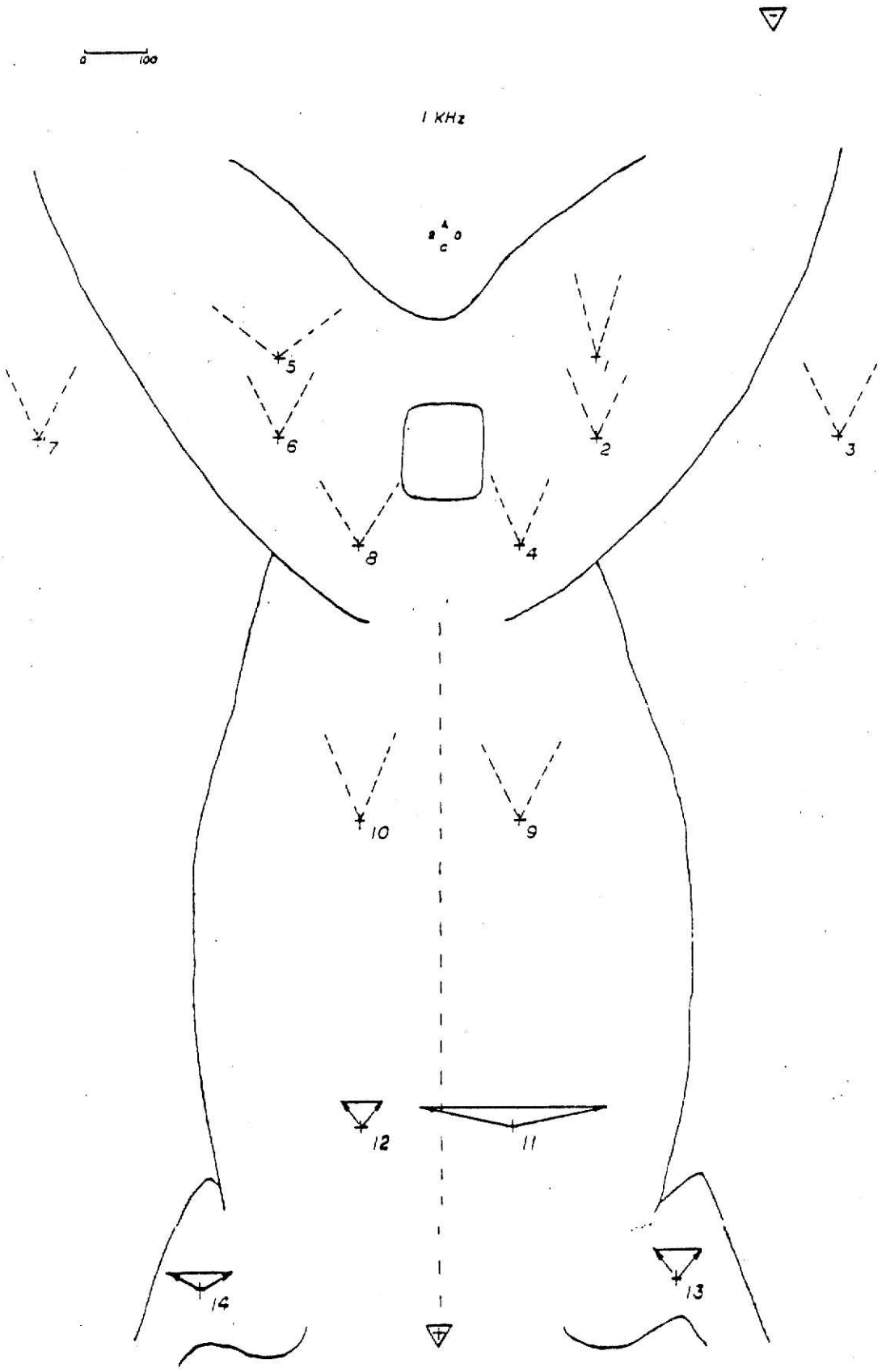

Fig. 4.4 Whole Carcass Vector Representation (1 kHz)

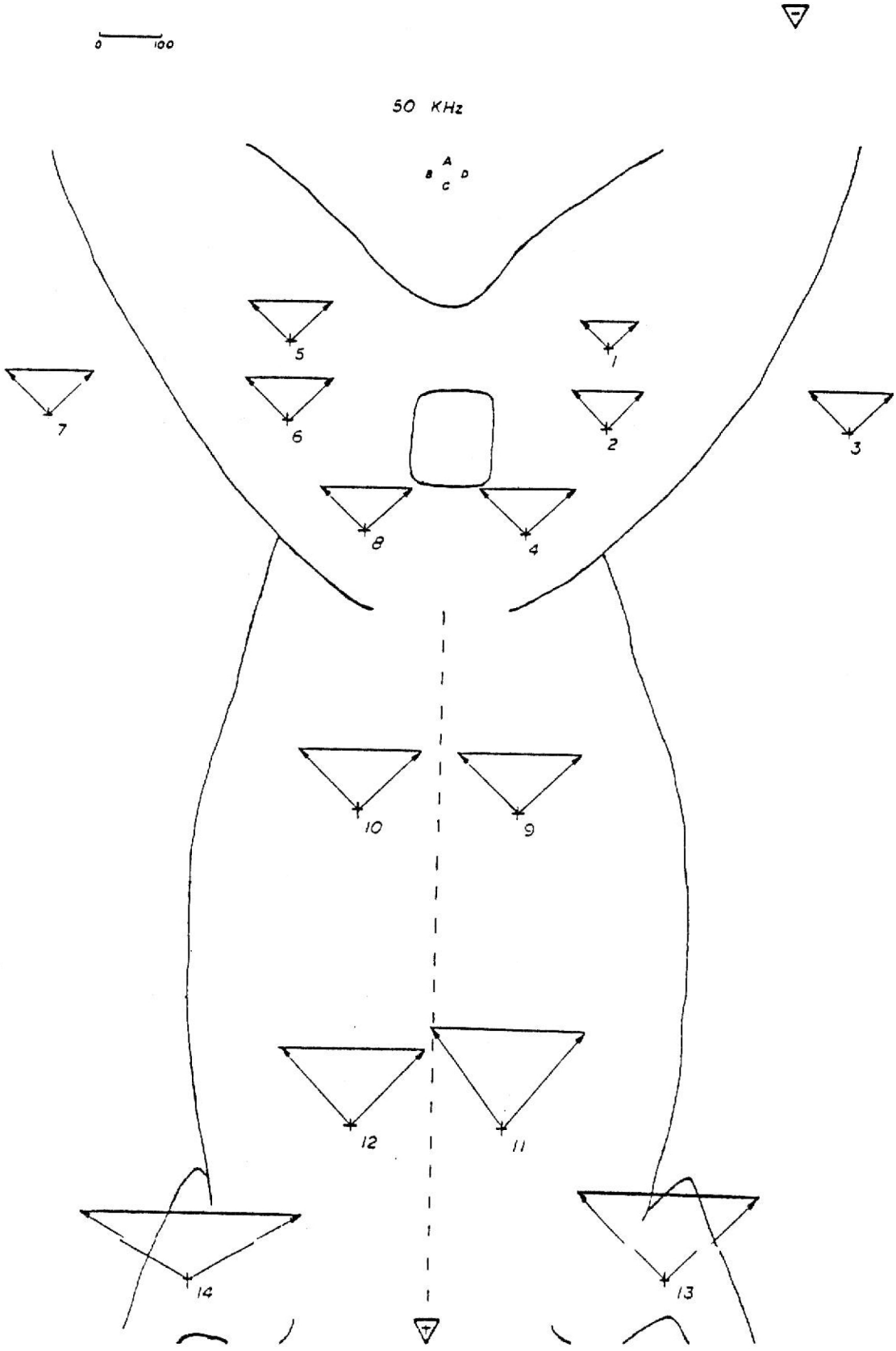

Fig. 4.5 Whole Carcass Vector Representation (50 kHz)

electrodes A and C were perpendicular to the floor and the diagonal lines joining electrodes B and D were parallel to the floor.

The differential voltage measured across electrodes A-C at a given site and frequency were added vectorially to the differential voltage measured across electrodes B-D at that same site and frequency. The direction of the vector A-C was assumed from the polarity of the stimulation (positive vector orientation coincided with the positive to negative stimulation direction). The direction of the vector B-D was not assumed, allowing for two possible orientations differing by 180 degrees. Two resultant vectors were produced using the assumed A-C vector as one component and using the two B-D vectors, one for each resultant vector, as the second component. The resultant vectors were defined by the following two equations:

$$\text{Magnitude of resultant} = \sqrt{[\text{value of (A-C)}]^2 + [\text{value of (B-D)}]^2}$$

$$\text{Angle of resultant} = \text{Arctan}[(\text{B-D})/(\text{A-C})]$$

$$\text{or} = -\text{Arctan}[(\text{B-D})/(\text{A-C})]$$

The vector representation was used to illustrate the changes in current diffusion as the frequency of stimulation was varied. Current diffusion is a representation of the directedness of the signal path through the medium.

Fig. 4.6 shows a plot of signal diffusion for selected electrode sites versus frequency. The decimal value given for a particular electrode quad at a particular frequency was determined by the equation

$$\text{Decimal value} = .5[(\text{value of A-C})(\text{value of B-D})]$$

which is the equation for the area of a right triangle. The three vectors; component A-C, component B-D, and the resultant vector comprise

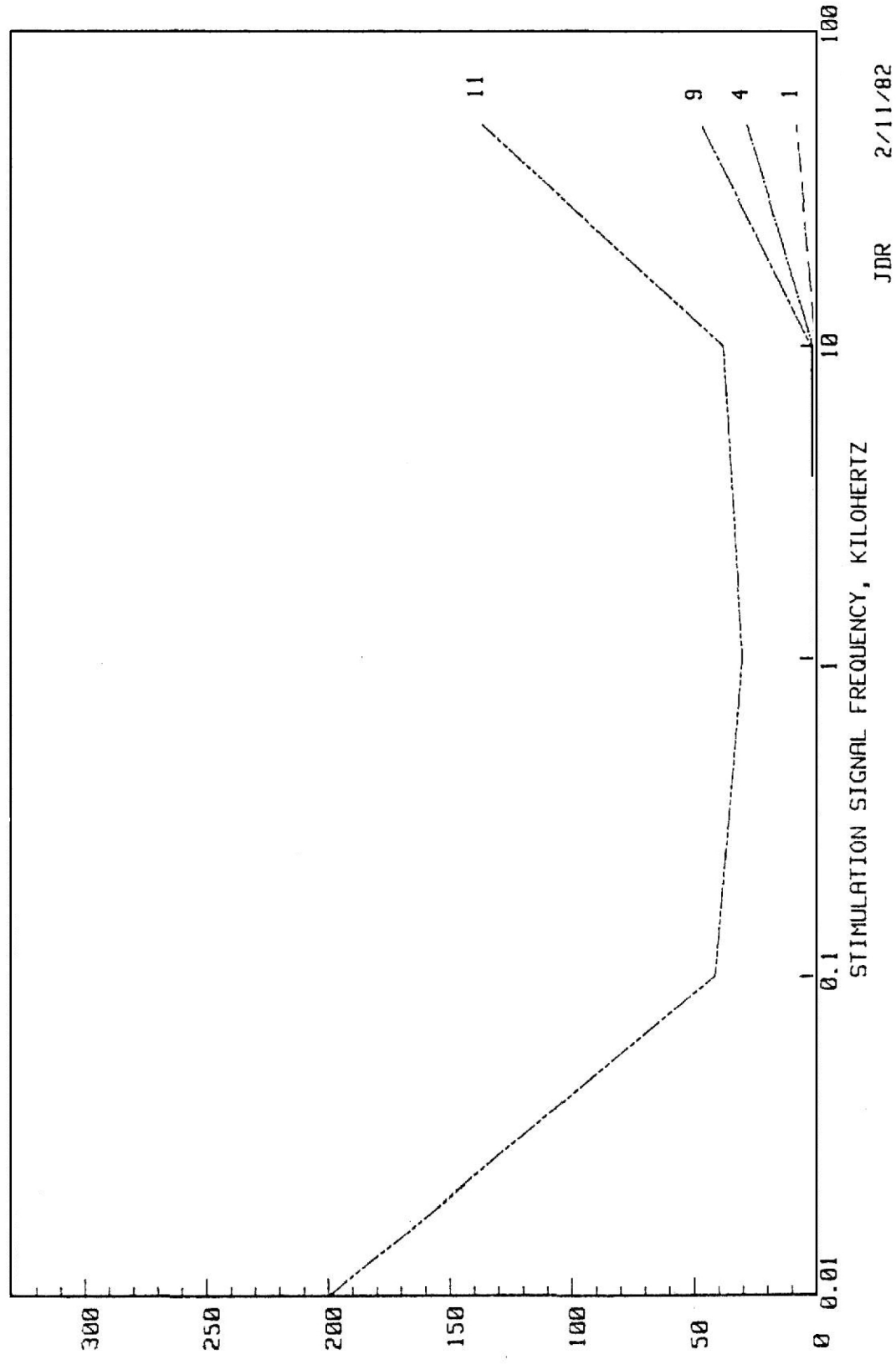

Fig. 4.6 Whole Carcass Signal Diffusion

the three sides of the right triangle. This plot was also used to illustrate changes in current diffusion as stimulation frequency was varied. As in the decimal value plots quad 11 was nearest the positive stimulation electrode.

4.2 Individual Muscle

The data obtained from individual muscle specimens are displayed in much the same way as that for the whole carcass data. For most of the experiments conducted using individual muscle specimens both constant current and constant voltage stimulation were used. Figures 4.7 through 4.10 illustrate representative differential voltage values plotted versus frequency. The notations "cc" and "cv" after the electrode quad and pair designations indicate constant current and constant voltage stimulation data respectively. Fig. 4.7 and 4.8 show data for constant current stimulation. Fig. 4.9 and 4.10 illustrate similar data for constant voltage stimulation. In all plots electrode 8 was nearest the positive stimulation source.

Figs. 4.11 through 4.14 illustrate vector drawings of data for an individual muscle group with a positive stimulation electrode nearest electrode quad 1. The determination of the vectors was accomplished in the same fashion as that used with whole carcass data. Four representative frequencies of stimulation were selected to illustrate the data; 10 Hz, 1 kHz, 10 kHz, and 50 kHz.

In a manner similar to the signal diffusion plots produced using whole carcass data, Figs. 4.15 though 4.18 display signal diffusion versus frequency of single muscle data. Figs. 4.15 and 4.17 represent data where electrode quad 1 was nearest the positive stimulation source.

Figs. 4.16 and 4.18 present data where quad 8 was nearest the positive source.

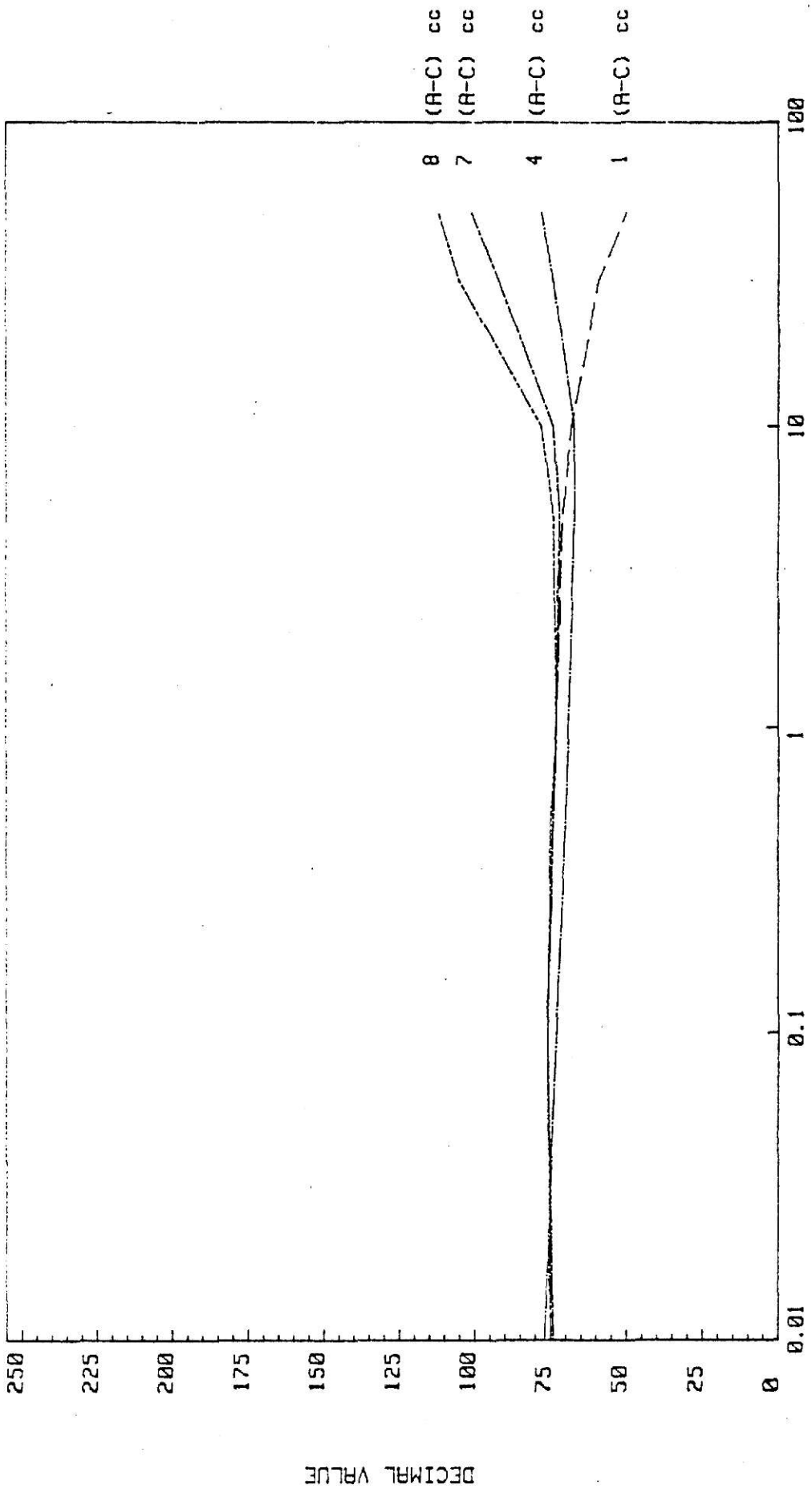

Fig. 4.7 Single Muscle Differential Voltage Decimal Values
Constant Current Stimulation (Pairs A-C)

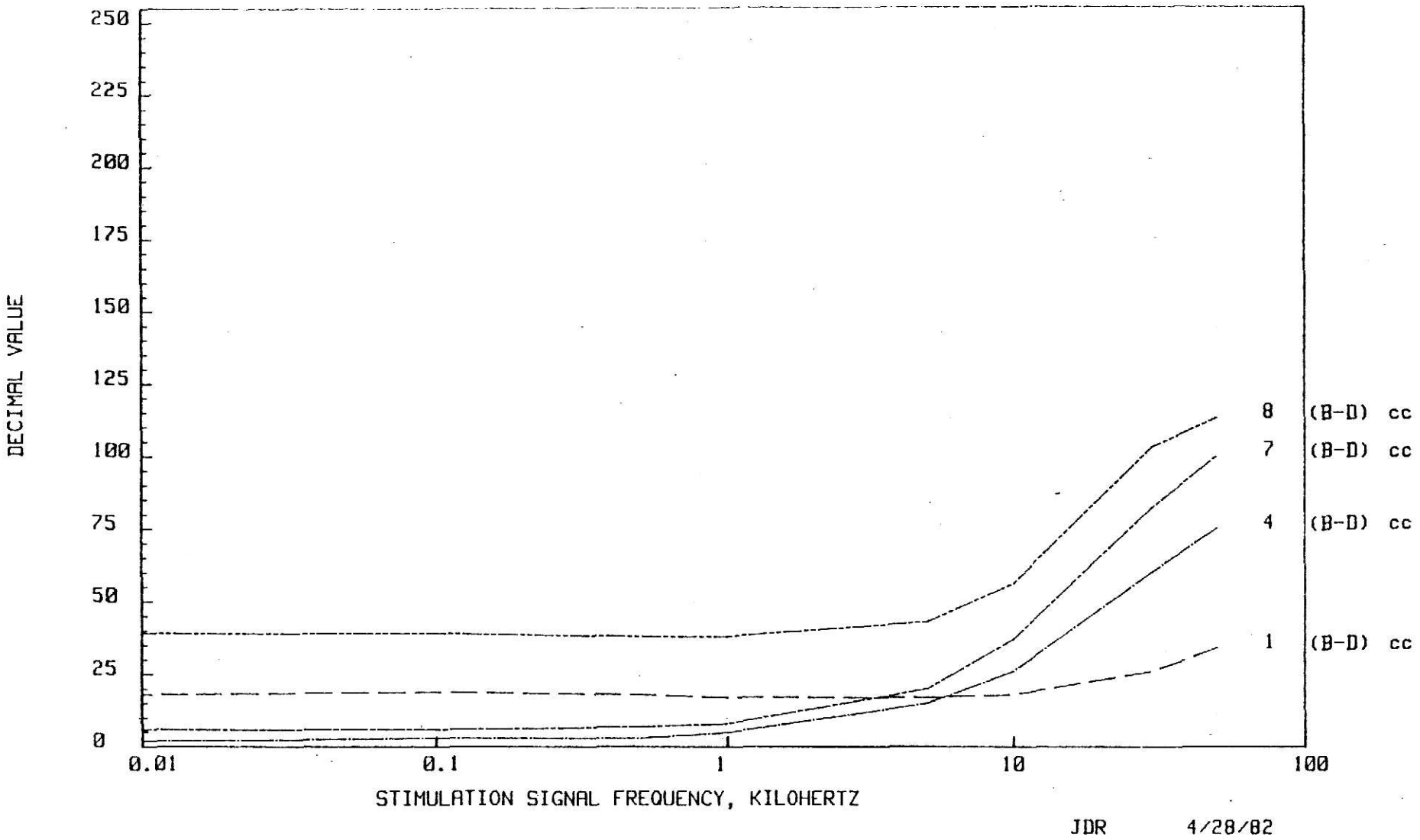

Fig. 4.8 Single Muscle Differential Voltage Decimal Values
Constant Current Stimulation (Pairs B-D)

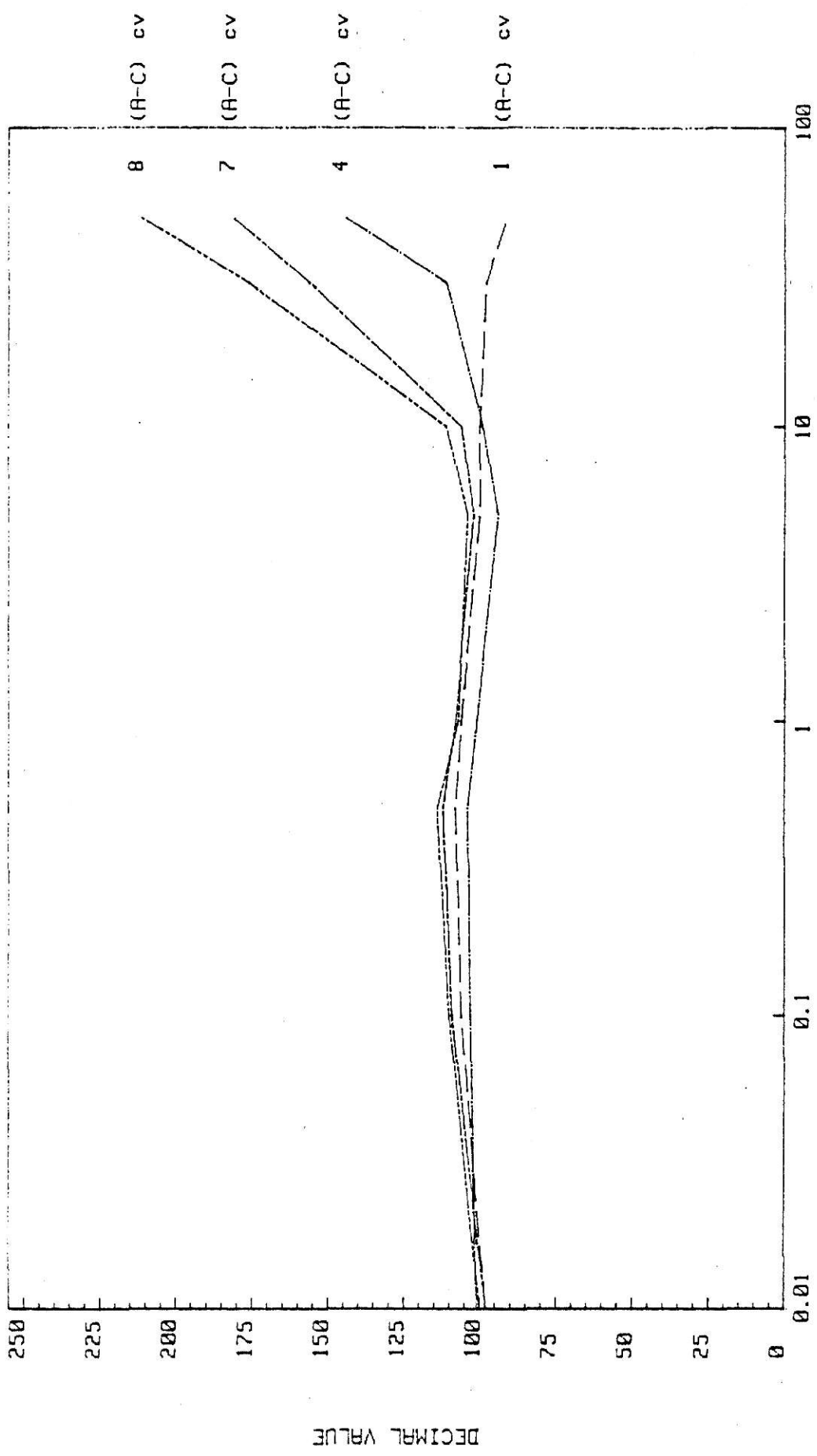

Fig. 4.9 Single Muscle Differential Voltage Decimal Values
Constant Voltage Stimulation (Pairs A-C)

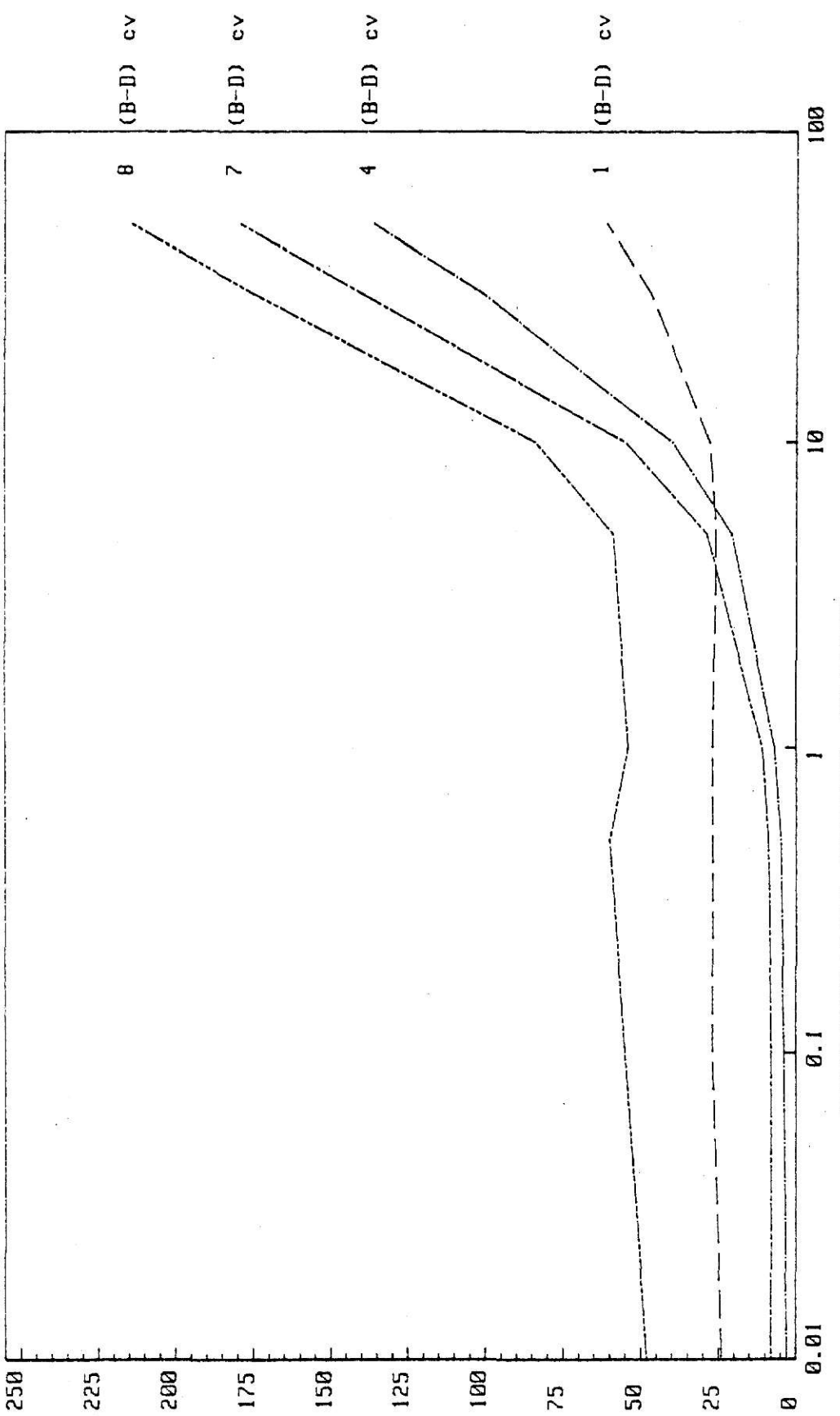

Fig. 4.10 Single Muscle Differential Voltage Decimal Values
Constant Voltage Stimulation (Pairs B-D)

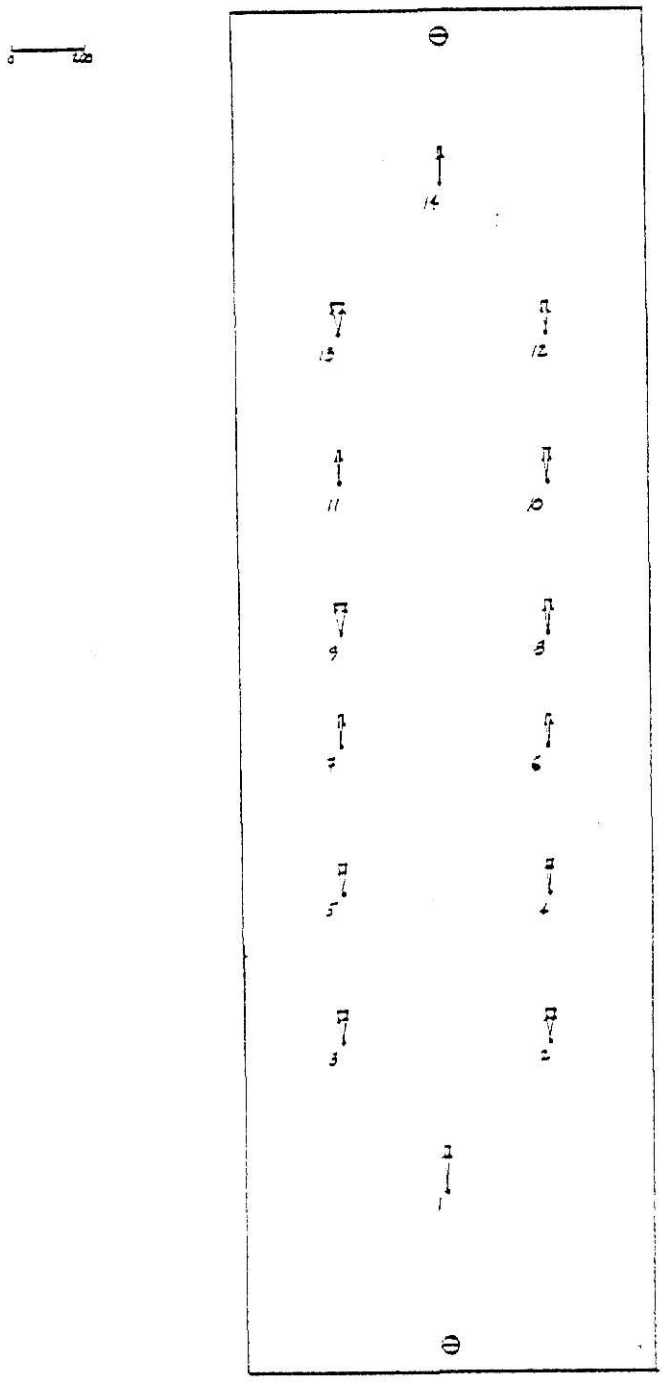

Fig. 4.11 Single Muscle Vector Representation (10 Hz)

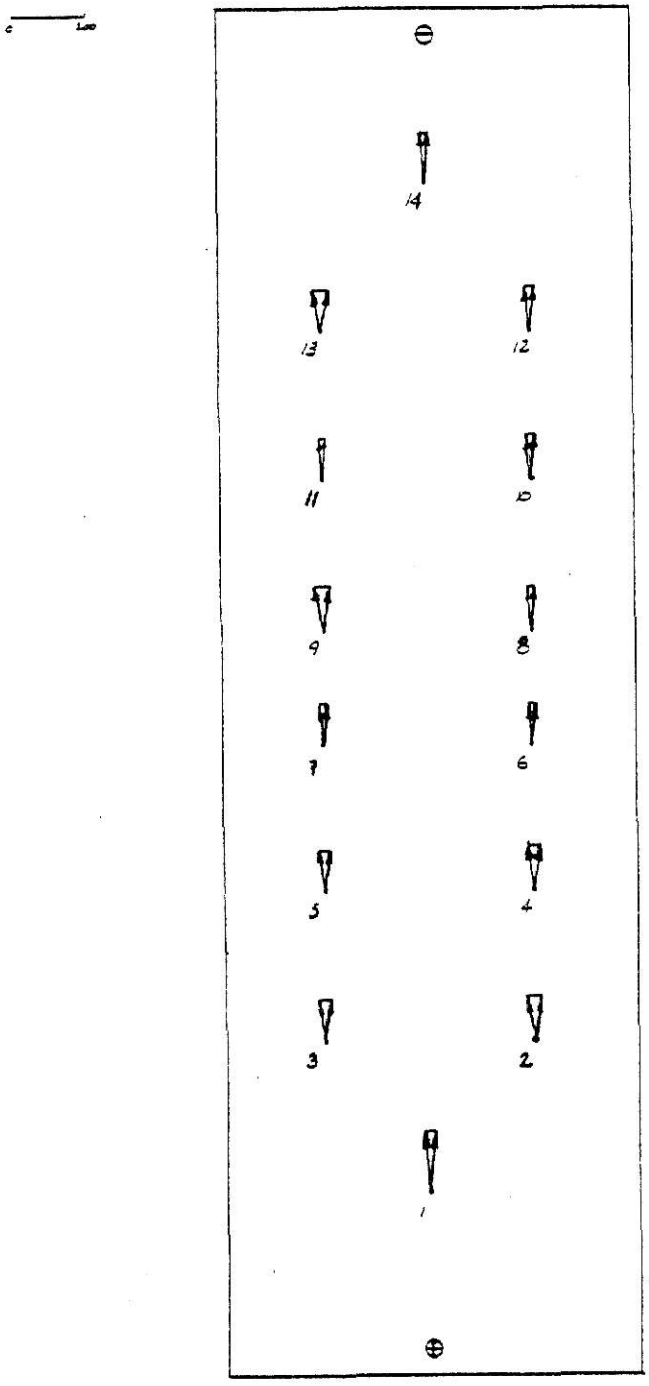

Fig. 4.12 Single Muscle Vector Representation (1 kHz)

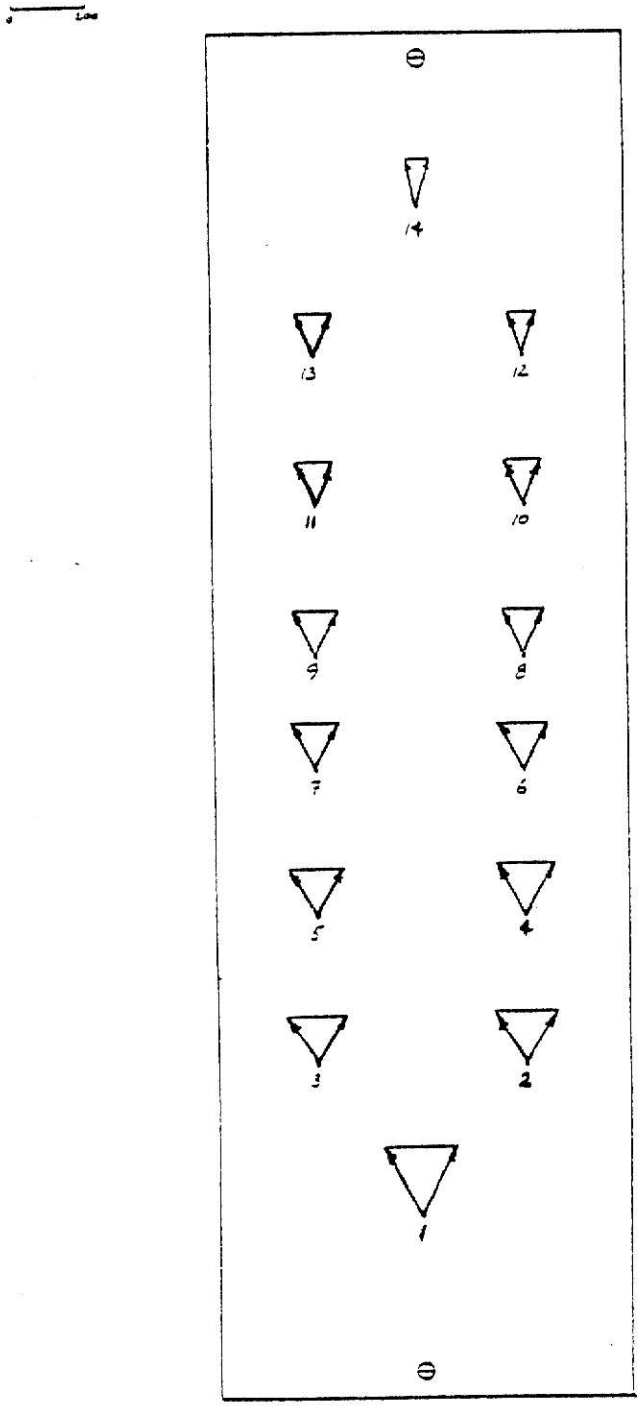

Fig. 4.13 Single Muscle Vector Representation (10 kHz)

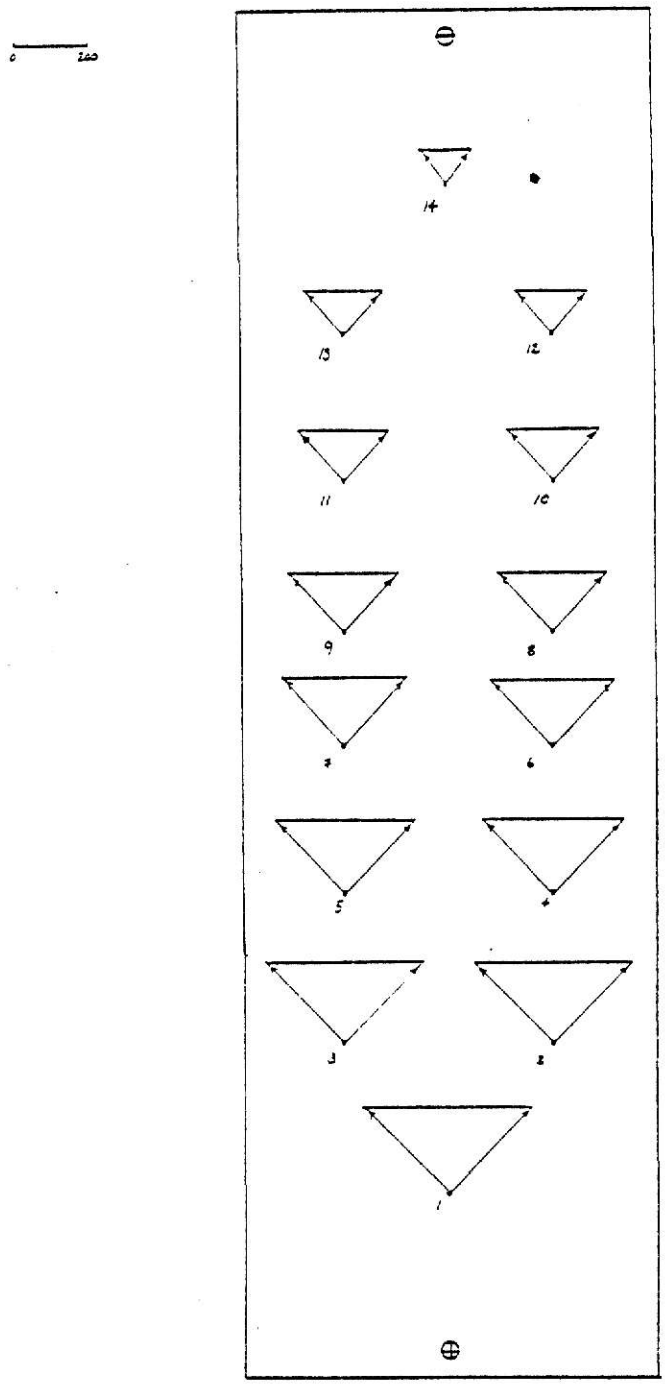

Fig. 4.14 Single Muscle Vector Representation (50 kHz)

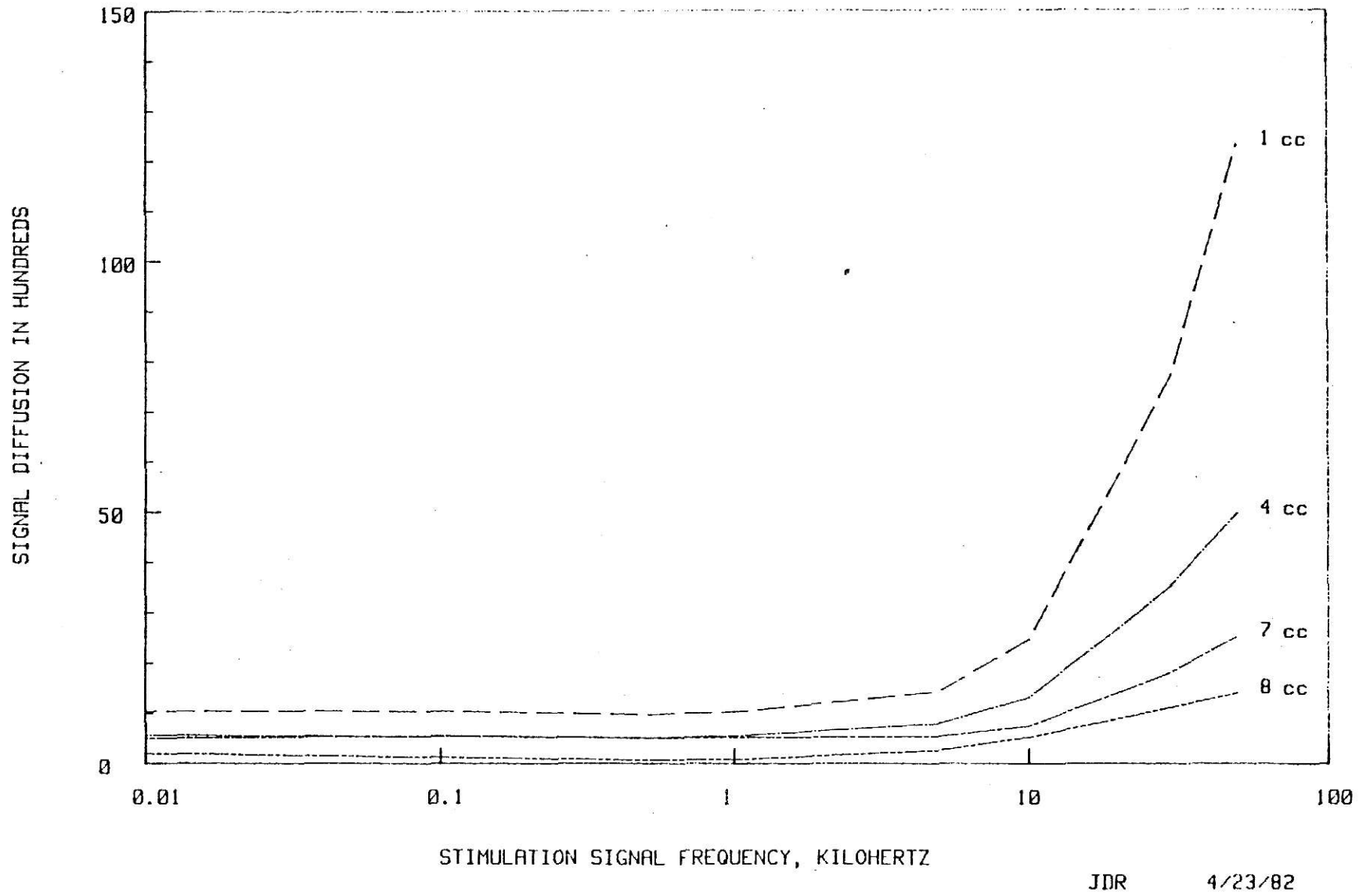

Fig. 4.15 Single Muscle Signal Diffusion
Constant Current, Forward Stimulation

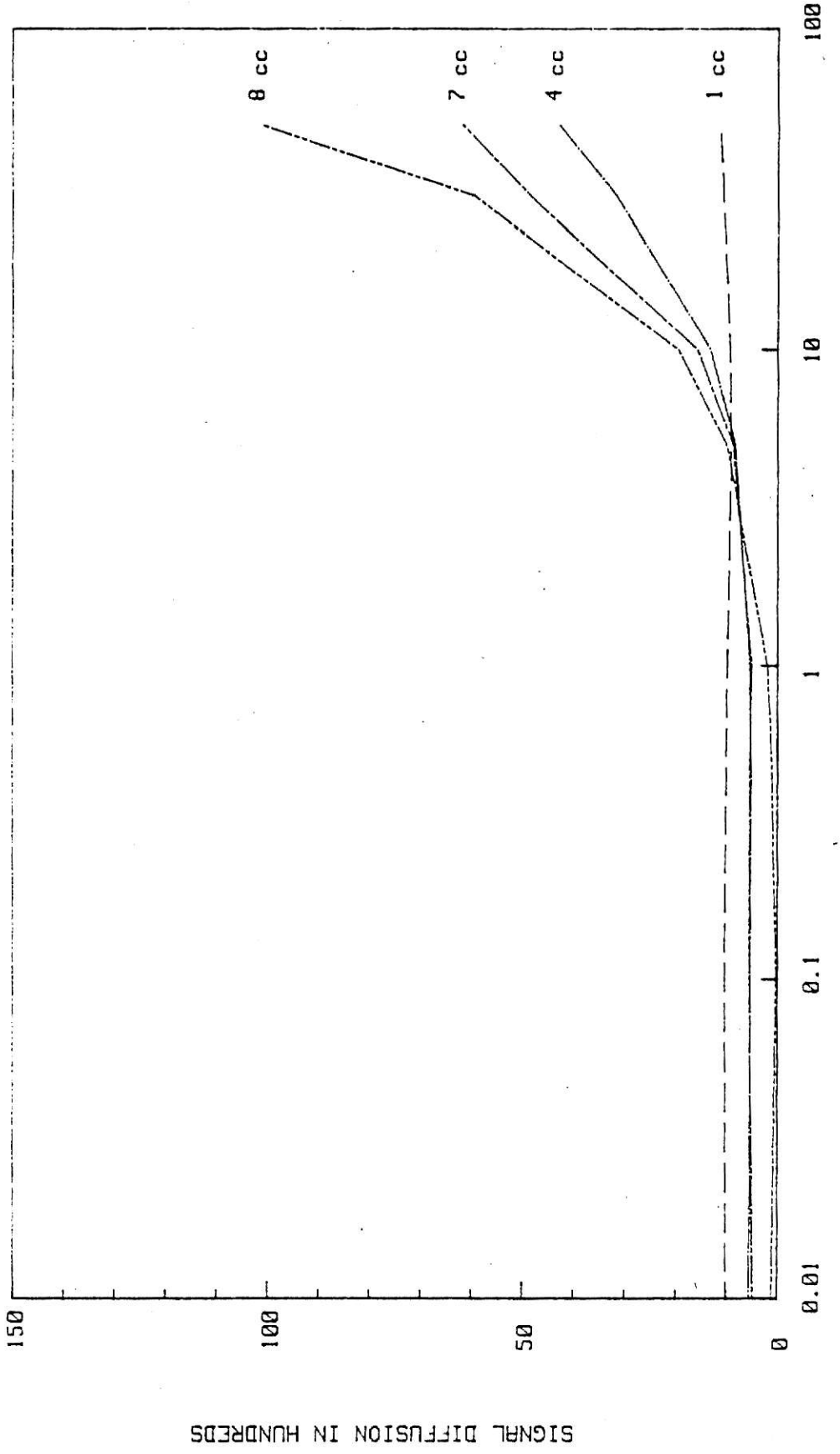

Fig. 4.16 Single Muscle Signal Diffusion
Constant Current, Reverse Stimulation

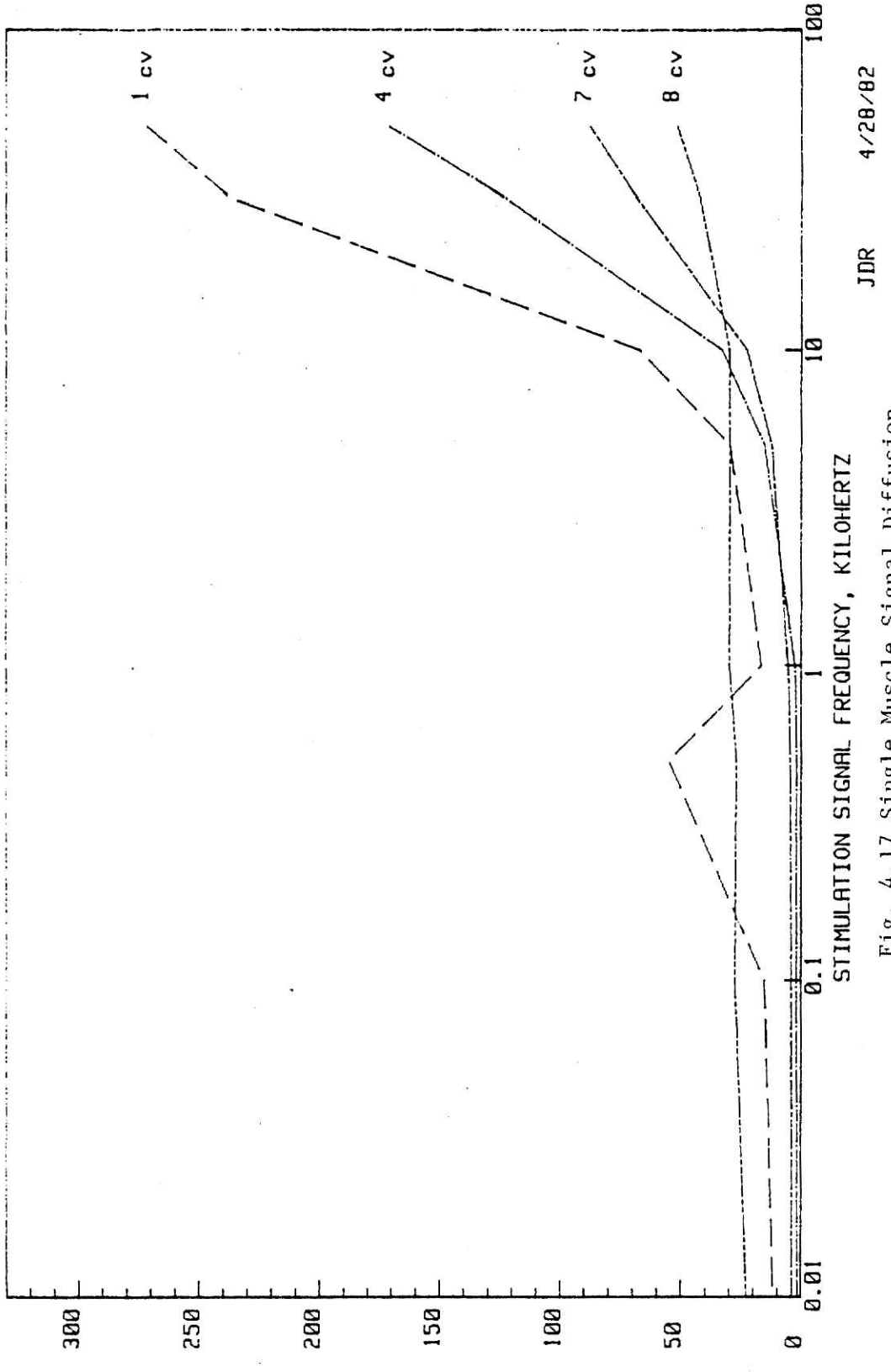

Fig. 4.17 Single Muscle Signal Diffusion
Constant Voltage, Forward Stimulation

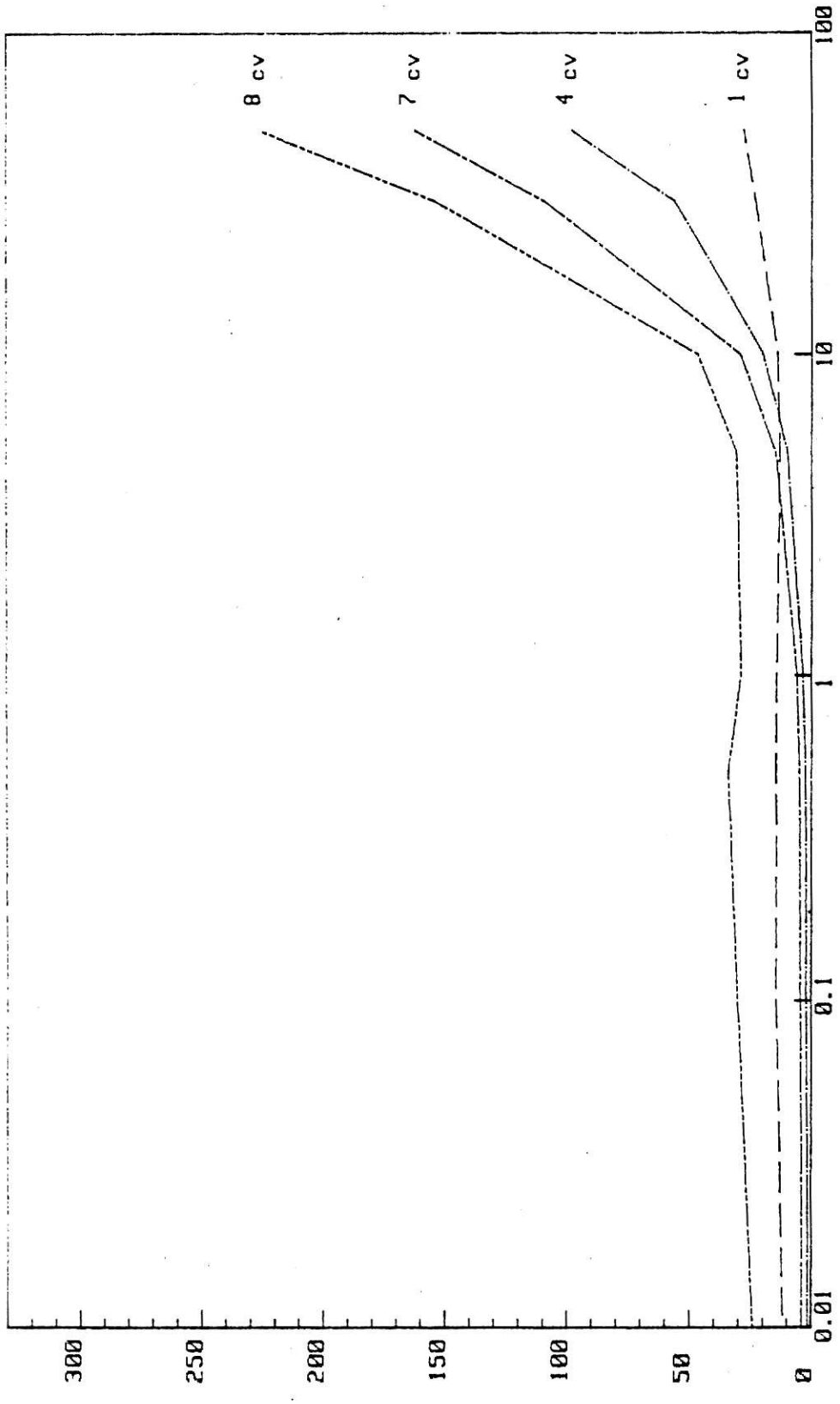

Fig. 4.18 Single Muscle Signal Diffusion
Constant Voltage, Reverse Stimulation

V. DISCUSSION OF RESULTS

5.1 Instrumentation System Evaluation

In general, the instrumentation system that was produced satisfied the project's requirements. The primary difficulties encountered were packaging problems, especially the interfacing of the electrode leads with the remainder of the instrumentation circuitry. The original materials selected for circuit layout; experimenter breadboard (ProtoBoard) and vector board, allowed for rapid hardware development while surrendering reliability and, especially in the case of the vectorboard, serviceability. Ultimately, the vectorboard gave way to the universal circuit cards with edge connectors.

The following paragraphs provide, in more detail, the evaluation of the instrumentation system including the electrodes.

5.1.1 Electrodes

The electrodes worked well with a few minor exceptions. The electrode quads were mechanically stable, maintaining a consistent orientation and spatial relation among the electrodes comprising the quads. The mechanical strength of the quads also enabled relatively easy insertion into the subject tissue.

The effectiveness of the insulation in limiting the signal conduction location was difficult to assess. It is safe to say that the insulation did work to some degree, although the application of the insulation was not completely even over the electrodes' surfaces. Also, in the course of using the electrodes, portions of the insulation were worn off.

Noise acquisition was the largest problem encountered. The primary

cause of this noise accretion was found to be the variations in resistance at the edge card/connector interface. This was determined by measuring the resistance from a noisy electrode to its corresponding multiplexer input terminal. The same measurement was taken on the other electrode pair member and the two were compared. Those electrode pairs that had registered considerable noise during signal-free scans were found to have a relatively large variation in resistance between the two electrode to multiplexer-input paths. This difference was on the order of from 2 to 10 ohms. Once the combined resistances were determined, the individual resistances from electrode to connector and from edge card terminal to multiplexer input were measured. In those electrodes considered to be noisy, no major discrepancies between the resistances of the separated signal paths were found. The conclusion was that variations in the impedance of the signal paths at the connector/edge card terminal interface were resulting in unbalanced voltage signals at the input terminals of the instrumentation amplifier. This conclusion was further validated by carefully re-seating the edge card connector onto the card terminal and noting the attenuation of noise signals from previously noisy electrode pairs.

Also, electrodes 1A and 5B were found to be short circuited to their respective shielding wires. This must have occurred accidentally during construction of the electrodes. The electrodes still functioned properly provided the shields for these two electrodes were not grounded.

The number of electrodes that were used for this system was adequate for small carcass and individual muscle tests. A greater number would be useful in larger carcass studies in order to better map the

current distribution throughout the increased conductor volume.

Finally, the electrodes could have been improved by using stainless steel rather than bronze rod as the electrode material. While it was quite highly conductive electrically, the bronze rod corroded when placed in the tissue.

5.1.2 Instrumentation

The instrumentation beyond the electrodes worked relatively well within the constraints of the design. Two primary limitations were identified for the system. The first restriction was the data acquisition rate limit which was determined by the AD536 RMS-to-DC converter. In order to minimize output ripple and DC offset error, the averaging capacitor had to be relatively large. However, a large averaging capacitor increased the settling time for a step change in input level. Settling time also increased for low signal levels. This problem was handled in software by incorporating a wait period of 750 milliseconds before the A/D began conversion. This required wait period was the major source of the data acquisition rate limit.

The second major constraint of the system was signal bandwidth. Frequency response was a function of the complex interactions and limitations of the instrumentation components. Using an oscilloscope (Tektronix 5403), a function generator (Tektronix FG503), and a universal counter/timer (Tektronix DC503A) the 3 dB cutoff frequency was found to be approximately 126 kHz with a reference sinewave input signal of .6 V peak-to-peak at 100 Hz.

There were few major problems, outside of design constraints, with the instrumentation circuitry. The most serious difficulty was produced by the A/D converter. During power-up, a transient voltage spike on the

A/D's +5 V power supply caused a latching action in the A/D resulting in a large current demand and eventual overheating. The solution to this problem was to include a low-pass filter in series between the power supply and the A/D's power supply input terminal. The filter removed the transient spike and, as a result, the large current demand.

Overall, the system provided data with very good repeatability. As a result, the data variability was primarily dependent on differences in the tissue specimens, the orientation and placement of electrodes, and stimulation parameters.

5.2 Data Evaluation

Both whole carcass data and single muscle data generally provided similar patterns of response. One consideration necessary when evaluating the data was that the active portions of the electrodes were all at approximately the same penetration depth in the tissue specimen. To a great degree, then, the data represented a view of a two-dimensional plane in a three-dimensional volume conductor.

One difference seen between the whole carcass data and data from single muscles was the large differential voltage values measured at electrodes in close proximity to the positive stimulation electrodes at low frequencies. This is most likely due to fewer low impedance paths in the areas where the stimulation electrodes were located. The muscle mass of the whole carcass in these areas was less than the excised muscle mass. The remaining tissue of the whole carcass; connective tissue, bone, etc., presented a higher impedance path, thus the locally smaller carcass muscle tissue was forced to conduct higher currents.

Data obtained during these studies suggest that as the frequency of stimulation increased the current pathways became more diffusive. At low frequencies, 10 Hz to 5 kHz, the magnitude of the differential voltages measured along the fibers were much greater than those voltages measured across the fibers. This is not surprising since the conductivity along pathways parallel with the fibers is much greater than the conductivity along pathways perpendicular to the fibers. As frequency increased, however, the magnitudes of the differential voltages for electrode pairs lying perpendicular to the fibers increased and approached values obtained from pairs parallel to the muscle striae. This is particularly well illustrated in the vector drawings of Chapter IV. The increased diffusion was assumed to be a result of the decreased impedance of pathways which do not lie along the fiber axis. The decreased impedance was most likely an indication of membrane capacitance between fibers since the impedance of a capacitor decreases with increasing frequencies.

The data also indicate that for frequencies below 1 kHz the differential voltages measured across electrode pairs both parallel and perpendicular to the muscle fibers remained fairly constant. Both constant current and constant voltage stimulation elicited this response although the baseline magnitudes were different. This suggests several possible explanations. First, the subject tissue's impedance may be independent of frequency below 1 kHz, implying a purely resistive response. Second, the tissue reacted in such a nonlinear fashion as to counteract changes in impedance and maintain constant differential voltage measurements. Finally, the current distribution may have altered so that the current density in the active regions of the electrodes in-

creased while tissue impedance decreased, thus maintaining differential voltages at electrode sites.

Above 1 kHz the differential voltage values measured perpendicular to the fiber axis began to increase even though tissue impedance was decreasing. The characteristic of decreased tissue impedance with increased signal frequency has been widely reported [1]. At frequencies of between 5 kHz and 10 kHz a similar response, increased differential voltage, was noted for electrode pairs parallel to the myofiber axis. This suggests that above 1 to 10 kHz the tissue either began to respond in some nonlinear fashion or the current distribution was shifting into the plane of the active region of the electrodes.

As can be seen, the data does not lend itself to easy explanations. In addition to the complexities of the tissues, the response of the data acquisition system may contribute to the "coloring" of the data.

VI. CONCLUSIONS

The results of this study suggest several general conclusions concerning both the system used to acquire the data and the data sets.

1. A data acquisition system was designed and constructed which fulfilled three basic requirements including the use of multiple electrodes, computer control and data storage and, analog-to-digital conversion.
2. The differential voltages obtained from the electrode quad arrangement was successful, in part, as an indicator of current direction and magnitude. However, the nonhomogeneous nature of the tissues studied prevented any quantitative comparison between electrode sites. Nevertheless, the use of perpendicular differential voltage measurements was effective in displaying variations in current pathways due to changes in stimulation parameters.
3. Currents in biological tissue, especially voluntary muscle, become less directed and more diffuse as stimulation frequencies increase. At low frequencies the current tends to flow along the muscle fibers, but as signal frequency increases current pathways extend across the muscle striae.
4. Overall, differential response voltages generally remained constant out to 1 kHz for constant current and constant voltage stimulation. Above 1 kHz differential voltages measured began to increase even though tissue impedance was decreasing. This suggests, especially in the case of constant current stimulation, that the tissue media is responding in a

nonlinear fashion or that current densitites are increasing in the plane of the active portion of the electrodes.

VII. FUTURE PROJECTS

There are a number of changes or additions to the hardware, software, or experiments that could clarify, quantify, or otherwise provide insight into the current distribution patterns of electrically stimulated tissue.

7.1 Electrodes

There are three major suggestions for improvement of electrodes:

1. Use of gold plated bronze or silver chloride instead of bronze for the active electrode segments.
2. The active portions of the electrodes could be set at varying depths. Perhaps whole groups of electrode quads could be set at different depths to provide tissue response for various planes. This may aid in the determination of changes in current distribution similar to skin effects found in conductors.
3. The method of connecting the electrode lead wires to the remaining instrumentation system should be changed to reduce the noise which occurs at the connector/edge-card terminal interface. Subminiature D connectors with wire-wrap contacts may be one solution.

7.2 Instrumentation

The following are several instrumentation additions and alterations to be considered.

1. Construction of the instrumentation system on printed circuit boards would improve overall system reliability, serviceability, and most probably signal quality. Separate signal and power return paths could be used, further reducing noise. In addition, separate ground references for analog and digital components could be provided.
2. A system for the determination of impedance at a given electrode site at various frequencies would allow, along with the differential voltage measurements, a quantitative determination of current magnitudes. This would most likely entail a constant current source capable of multiple frequencies which could be demultiplexed to the electrodes using computer control.
3. A circuit located prior to the instrumentation amplifier that would allow for the determination of the phase of the differential voltages. This would indicate current direction.
4. The addition of an on-board power supply would improve portability of the system.

7.3 Software

The inclusion of informative comments from a given experiment with the data would improve data correlation with experimental procedures. This pertinent information includes the date of the experiment; stimulation parameters-voltages; frequencies, currents; anomalies in experimental procedures; and details about the experimental procedure and tissue specimen.

7.4 Research

There are numerous additional experiments of interest that would hopefully provide insights into tissue response both from the viewpoint of the animal scientist and the bioengineer. Most of these studies would best be handled through an interdisciplinary approach.

The first steps include a continuation of frequency studies on individual muscles. The interest here is to extend the stimulation frequencies beyond 50 kHz. This would give an expanded view of the tissue response.

Another area that could be studied is the effect that various waveforms, including pulses, may have on current distribution in biological tissue. Most meat stimulators provide a pulsed voltage so this may provide useful data on the effectiveness of these devices.

In association with the Department of Animal Science, a study of frequency of stimulation versus rate of pH decline may yield valuable data. In addition, stimulation parameters such as current, waveform, and duty cycle may be considered.

Finally, with adequate empirical data, attempts at simple computer models for a single muscle system might be considered.

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APPENDIX I. DETAILED DESCRIPTION OF INSTRUMENT

A1.1 Hardware

The instrumentation system is constructed on three boards -- two universal circuit cards, each with 44 pin edge connectors and one breadboard. The cards and the breadboard are housed in a 12" x 17" x 3" aluminum box as shown in Fig. A1.1. The breadboard circuitry is wired using conventional breadboard wire while the card circuitry is wire-wrapped. Data signals from Board 3 to Board 1 are transferred through two twisted 26 gauge wires. Power and ground reference for the system is brought from the source (Tektronix PS503) through four 18 gauge conductors to the banana jacks provided on the breadboard (Board 1). The power and ground is transferred from the banana jacks to Board 2 via 22 gauge wires and from Board 2 to Board 3 through a 16 conductor ribbon cable with DIP terminals. The cable also transfers computer control signals and data outputs from the multiplexers on Board 2. Table A1.1 lists pin assignments for the ribbon cable interface between Boards 2 and 3.

The system requires three power sources, +7 V, -7 V, and +5 V. System ground is referenced to the common terminal of the power supply. The quiescent current demand of the system is 7.65 mA for the +7 V supply, 10.65 mA for the -7 V supply, and .29 mA for the +5 V supply.

The following paragraphs provide specifics on circuit hardware. A detailed circuit schematic of the instrumentation system is provided in Fig. A1.2. Discussions on particular circuitry are referenced to this schematic unless otherwise noted.

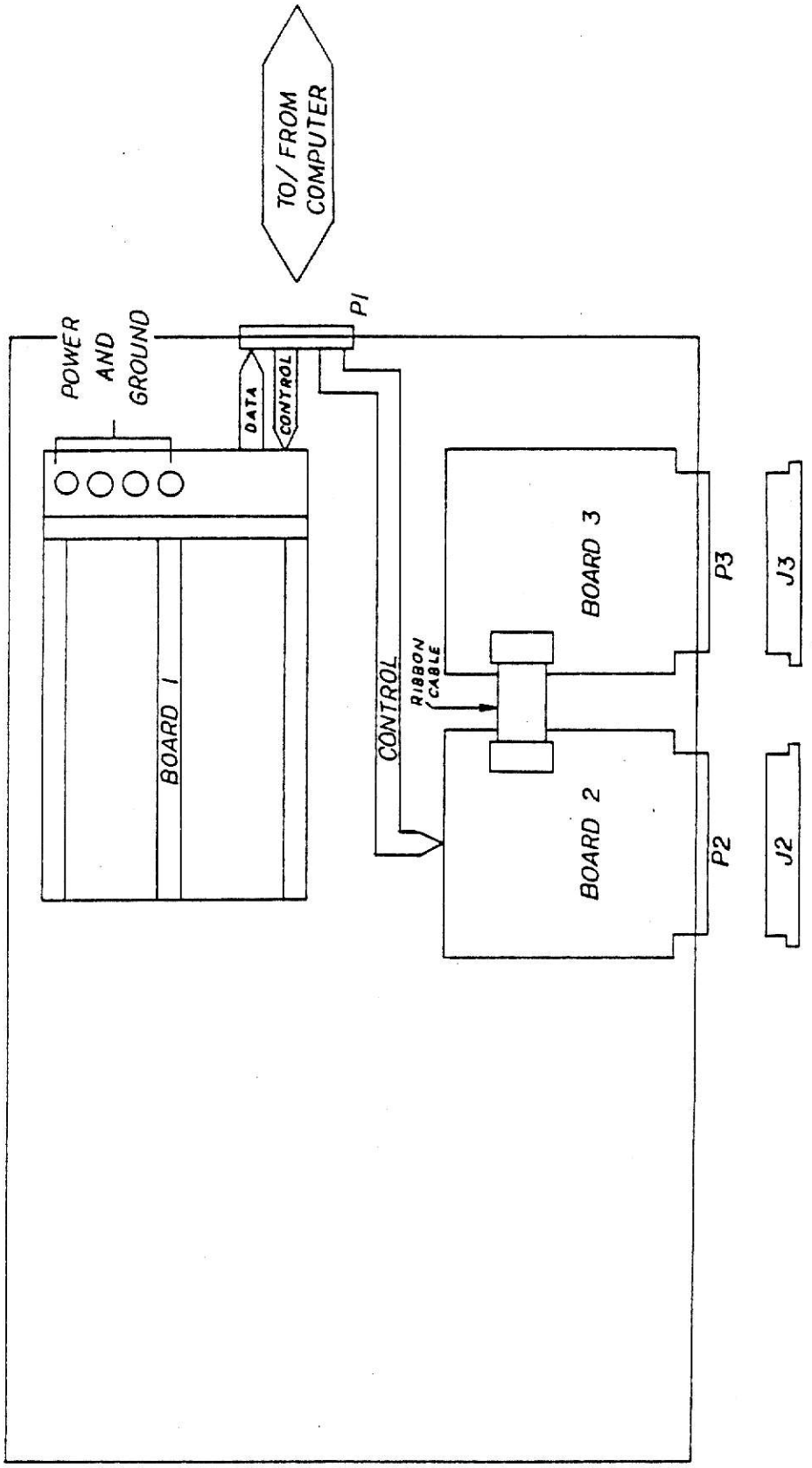

Fig. A1.1 Board Layout and Interconnections

A1.1.1 Interface and Interconnections

There are two major signal interconnections required by this system. These are the connection between the electrodes and the instrumentation and the connection between the instrumentation and the computer. In addition, the latter, specifically the control lines from the computer, requires an interface for proper signal levels.

Signals between the computer and the instrumentation system are transferred using the HP98032A 16-bit parallel interface. The pin assignments for the HP98032A are listed in Table A1.2. The HP98032A communicates with the instrumentation circuitry via 50-pin connector P1. I/O functional assignments for P1 are given in Table A1.3.

All eleven signals coming from the computer to the instrumentation system require pull-up resistors, R110-R115 and R201-R205. In addition, all but two signals, START CONVERSION and ADDRESS LATCH ENABLE which are used by the A/D converter, require additional adjustments of their levels. Two quad comparators, U101 and U205, and one general purpose FET operational amplifier, U206, and their accompanying pull-up resistors, R116-R119 and R206-R210, are used to accomplish the level changes. The signals from the computer are compared with a 2.5 volt reference provided by U111 and typically switched to either +7 V or -7V depending on the input signal level.

As was previously noted, the electrode lead wires are attached to two 44 pin edge-card connectors. There are 64 lead wires and these are divided into half between the two connectors, J2 and J3. Electrode quads 1 through 8 are attached to J2 and quads 9 through 16 to J3. The I/O pin assignments for J2 and J3 between electrode lead wires and edge card connector terminals are given in Table A1.4. The pin assignments

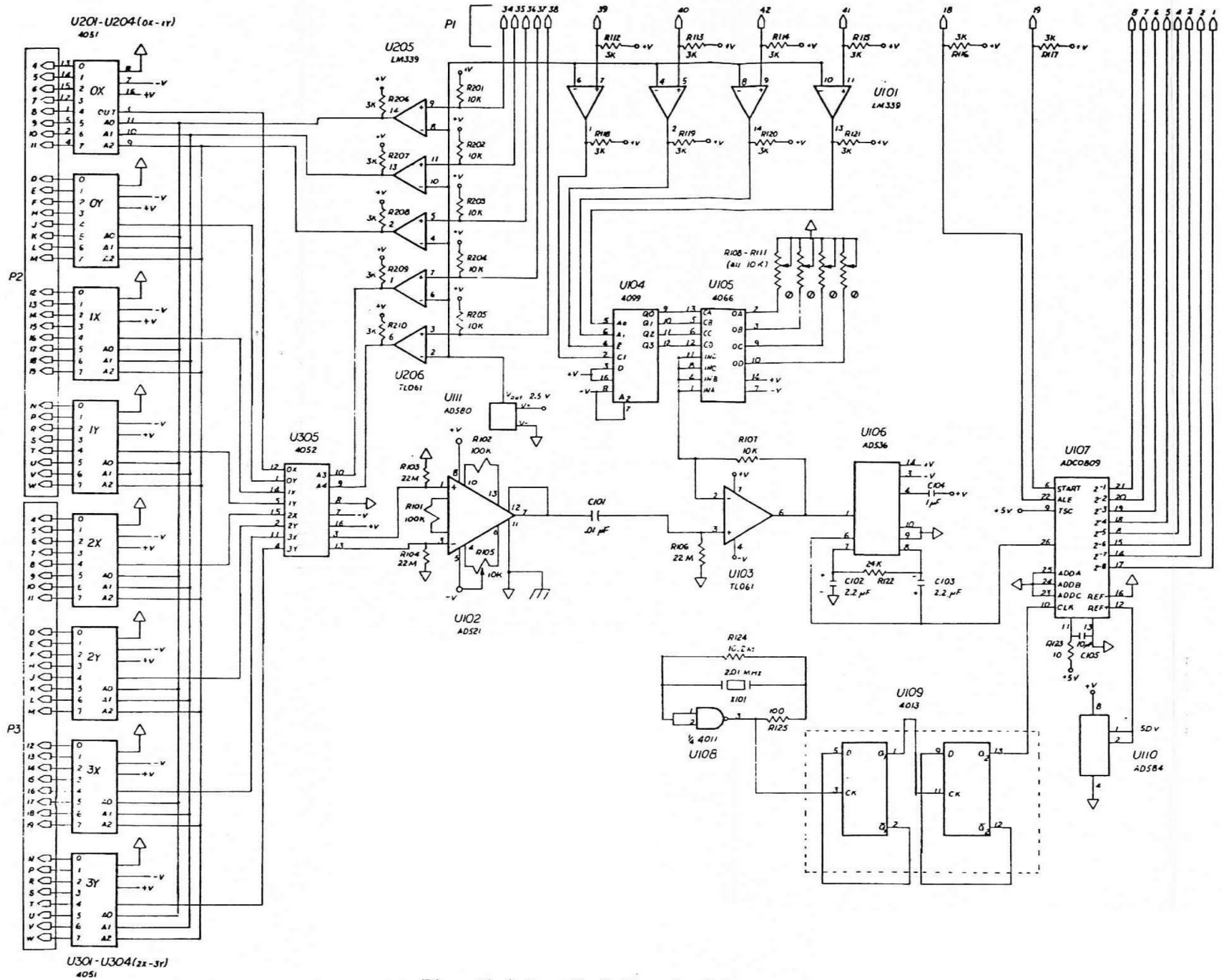

Fig. A1.2 Detailed Circuit Schematic

Table A1.1 Ribbon Cable Pin Assignments Between Boards 2 and 3

1 -- OX output
2 -- OY output
3 -- 1X output
4 -- 1Y output
5 -- +V
6 -- GND
7 -- -V
8
9
10 - A4
11 - A3
12 - A2
13 - A1
14 - A0
15
16

Table A1.2 HP98032A 16-Bit Parallel Interface 50-Pin Connector Assignments (P1)

Pin No.	98032A Signal
1	DI0
2	DI1
3	DI2
4	DI3
5	DI4
6	DI5
7	DI6
8	DI7
9	DI8
10	DI9
11	DI10
12	DI11
13	DI12
14	DI13
15	DI14
16	DI15
17	[NC]
18	CTL1
19	CTL0
20	STI1
21	STI0
22	PCTL
23	PFLG
24	RSTS
25	EIR
26	GND
27	PRESET
28	I/O
29	NC
30	GND
31	GND
32	GND
33	GND
34	DO0
35	DO1
36	DO2
37	DO3
38	DO4
39	DO5
40	DO6
41	DO7
42	DO8
43	DO9
44	DO10
45	DO11
46	DO12
47	DO13
48	DO14
49	DO15
50	DO16

Table A1.3 I/O Functional Assignments for 50-Pin Connector P1

INPUT		OUTPUT	
Name	Function	Name	Function
DI0	2^{-8}	CTL1	ADDRESS LATCH ENABLE
DI1	2^{-7}	CTL0	START CONVERT
DI2	2^{-6}	GND	GND
DI3	2^{-5}	DO0	MUX ADD 0
DI4	2^{-5}	DO1	MUX ADD 1
DI5	2^{-3}	DO2	MUX ADD 2
DI6	2^{-2}	DO3	MUX ADD 3
DI7	2^{-1}	DO4	MUX ADD 4
		DO5	LATCH CLEAR
		DO6	LATCH ENABLE [BAR]
		DO7	LATCH ADD 0
		DO8	LATCH ADD 1

Table A1.4 Jacks 2, 3 Edge-Card Connector Interconnections with Electrode Leads

	J2				J3			
	1	A			1	A		
	2	B			2	B		
Shield gnd	3	C		Shield gnd	3	C		
1A	4	D	1C	9A	4	D	9C	
1B	5	E	1D	9B	5	E	9D	
2A	6	F	2C	10A	6	F	10C	
2B	7	H	2D	10B	7	H	10D	
3A	8	J	3C	11A	8	J	11C	
3B	9	K	3D	11B	9	K	11D	
4A	10	L	4C	12A	10	L	12C	
4B	11	M	4D	12B	11	M	12D	
5A	12	N	5C	13A	12	N	13C	
5B	13	P	5D	13B	13	P	13D	
6A	14	R	6C	14A	14	R	14C	
6B	15	S	6D	14B	15	S	14D	
7A	16	T	7C	15A	16	T	15C	
7B	17	U	7D	15B	17	U	15D	
8A	18	V	8C	16A	18	V	16C	
8B	19	W	8D	16B	19	W	16D	
Shield gnd	20	X		Shield gnd	20	X		
	21	Y			21	Y		
	22	Z			22	Z		

for P2 and P3 between edge card terminals and multiplexer inputs are given in Table A1.5. It should be noted that the electrode quads can be connected to either of the multiplexer boards (2 and 3) which can cause errors in correlating data with electrode quads unless the user is cognizant of the computer selection scheme and corrects accordingly.

A1.1.2 Multiplexers

The 64 electrodes are selected using 9 CMOS analog multiplexer/demultiplexers. Eight of the multiplexers are 1-of-8 decoders, U201-U204 and U301-U304, and one is a dual 1-of-4 decoder, U305. The 1-of-8 decoders are designated 0X through 3X and 0Y through 3Y. Units 0X, 1X, 0Y, and 1Y are located on Board 2 and units 2X, 2Y, 3X, and 3Y along with the dual 1-of-4 decoder are on Board 3. Under computer control, the input channels are selected by the binary signals present at the multiplexer control inputs. Two data input channels are selected at a time beginning with inputs 0 of 0X and 0Y and proceeding to inputs 7 of 3X and 3Y.

These switches can handle analog signals of up to 15 volts peak-to-peak and can be controlled by digital signal amplitudes of 3 to 15 volts. This is accomplished by adjusting the three supply voltages Vdd, Vss, and Vee. The voltage difference Vdd - Vee determines the range of analog signals that can be controlled while the difference Vdd - Vss determines the amplitudes of the digital signals required to select a particular channel.

A1.1.3 Instrumentation Amplifier

The two electrode signals selected are sent to the integrated circuit instrumentation amplifier U102. Resistors R101 and R102 determine the gain which can be set to any value between 0.1 and 1000.

Table A1.5 Boards 2, 3 Edge Card Terminal Interconnections with Multiplexer Inputs

	Board 2-P2						Board 3-P3				
	1	A					1	A			
	2	B					2	B			
	3	C					3	C			
	13	4	D	13			13	4	D	13	
	14	5	E	14			14	5	E	14	
	15	6	F	15			15	6	F	15	
0X	12	7	H	12	0Y	2X	12	7	H	12	2Y
	1	8	J	1			1	8	J	1	
	5	9	K	5			5	9	K	5	
	2	10	L	2			2	10	L	2	
	4	11	M	4			4	11	M	4	
	13	12	N	13			13	12	N	13	
	14	13	P	14			14	13	P	14	
	15	14	R	15			15	14	R	15	
1X	12	15	S	12	1Y	3X	12	15	S	12	3Y
	1	16	T	1			1	16	T	1	
	5	17	U	5			5	17	U	5	
	2	18	V	2			2	18	V	2	
	4	19	W	4			4	19	W	4	
		20	X					20	X		
		21	Y					21	Y		
		22	Z					22	Z		

The gain relation is given by

$$Vout/Vin = R102/R101.$$

R105 is a 15 turn, 10 kilohm potentiometer used to trim the output offset voltage. With both inputs grounded, R105 is adjusted to give a 0V DC output. Input offset voltages multiplied by any amplifier gain are also removed by this process.

Resistors R103 and R104 are necessary to provide a return path to ground for input bias currents. Without these return paths, bias currents will cause the output to saturate. The value of the resistor is determined by dividing the maximum allowable common mode voltage for the application by the bias current [A1].

Pin 11 of U102 can be used to provide a DC bias at the amplifier output. For this application no DC bias is desired so pin 11 is tied to chassis ground.

A1.1.4 Gain Circuitry

The signal from U102 is capacitively coupled via C101 to the gain circuitry consisting of the integrated circuits U103, U104, and U105; fixed resistors R106 and R107; and the 10 kilohm potentiometers R108 through R111. U103 is a general purpose FET-input operational amplifier configured in the non-inverting mode to prevent loading the output of the previous stage. The gain is given by

$$Vout/Vin = 1 + R2/R1$$

where R2 is R107 and R1 is one of the four gain potentiometers, R108-R111.

U104 is an 8-bit addressable latch and U105 is a quad bilateral switch. Both are used to select a potentiometer which is included in the feedback path of U103 and used to determine signal gain.

Control signals are sent from the computer to U104 which, in turn, latches them. The control signals select the channel which transfers a Data signal (pin 3) to one of the control pins of U105. The Data signal of U104 is always HI. The control pin of U105 which receives the HI signal allows a corresponding switch to conduct, enabling U103's feed-back current to flow through the potentiometer to ground.

A1.1.5 RMS-to-DC Converter

The amplified signal is next converted to the DC equivalent of its RMS value. Integrated circuit U106 is a true RMS-to-DC converter. The signal is rectified (absolute value), squared, divided by the fed-back output and finally filtered. This process approximates the RMS definition

$$Eo = Avg[Vin^2/Eo] = \sqrt{Avg(Vin)^2} \qquad [A2].$$

The easiest method of averaging involves a single-pole filter, using a filtering capacitance. As capacitance is increased the accuracy for low frequency RMS measurements is increased and output ripple is reduced. Unfortunately, settling time for DC step changes is increased. With the addition of the 2-pole "post" filter consisting of R122, C102, and C103 the settling time is minimized while maintaining minimum ripple and acceptable accuracy. The averaging capacitor, C104, is selected to produce a .1% DC error at approximately 50 Hz using data provided by the manufacturer [A3].

A1.1.6 A/D Converter

The DC output of U106 provides the input for U107 which is an 8-channel, 8-bit A/D converter with microprocessor compatible controller logic. Since only one input channel is necessary for this application,

the input multiplexer control lines (pins 23, 24, 25) are all tied to ground selecting pin 26 (IN0) as the input channel.

Before conversion of the data begins, the address of the selected channel is latched into the decoder on the low-to-high transition of the ADDRESS LATCH ENABLE signal. Conversion begins on the falling edge of the START CONVERSION pulse and continues from 1 to 8 clock cycles after the rising edge of START CONVERSION. Both the ADDRESS LATCH ENABLE and START CONVERSION signals are provided by the computer.

The positive reference used by the converter is provided by U110, a pin programmable precision voltage reference which is selected for 5.0 V. A separate voltage reference is supplied to prevent conversion errors due to power fluctuations. The negative reference of the A/D is tied to ground.

Clocking for the A/D is produced by U108, U109, R124, R125, and the crystal X101. The crystal provides a 2.01 MHz signal at pin 3 of U108, a quad 2-input NAND gate. U109, a Dual D Flip-Flop, is configured as a divide-by-4 circuit providing a 500 KHz output at pin 13.

Since the A/D converter is the only device sending data back to the computer, the three-state control line (pin 9) is tied high so that the output buffers are always in the low impedance state.

R123 and C105 act as a low pass filter from the +5 V power supply to prevent latch up caused by transients during power up.

A1.2 Software

The data acquisition program (DAP) which controls the instrumentation system and records the data is written entirely in BASIC. This language was chosen because it is resident on the HP9835A computer, it

is easy to assimilate and use, and speed of execution is not critical for this system.

All signals transferred to and from the computer via the HP98032A interface are inverted; therefore, the output signals are determined to correct for this and the input data is adjusted in the program.

The signals required to select gain values are listed in Table A1.6. The gains may be altered by changing the resistance values of the various pots. Current values of gain are shown.

A1.3 Program Listing

The following pages contain the BASIC source listing for the data acquisition program.

Table A1.6 Selection Values for Signal Gain

Selection Value	Gain	Potentiometer
-33	20	A
-161	10	B
-289	5	C
-417	2	D

The selection vlaues correspond to the following binary signals on the respective control lines of the addressable latch (U104).

A1	A0	E (Bar)	C1	
0	0	0	1	(-33)
0	1	0	1	(-161)
1	0	0	1	(-289)
1	1	0	1	(-417)

```
10   REM  Latest revision date: 8/20/82
20   REM  Program name: DAP
30   REM  This is a BASIC program which controls an instrumentation system
40   REM  designed to measure differential voltages across 64 electrodes, two
50   REM  at a time, and store them for further evaluation. The program also
60   REM  allows the user to select from one of four gains to control signal
70   REM  amplitude.
80   OPTION BASE 1
90   PRINT "The number of scans desired is calculated by determining the total"
100  PRINT "number of times the electrode array differential voltages are to be"
110  PRINT "measured. The electrode array differential voltages are measured"
120  PRINT "twice for each scan, once each for both forward and reverse stimu-"
130  PRINT "lation polarities."
140  INPUT "ENTER THE NUMBER OF SCANS DESIRED",Knt
150  PRINT LIN(1)
160  INTEGER Data1(32),Data2(32),Datar(64),Datam(1024)
170  PRINT "The following table lists the potentiometers, gain values currently"
180  PRINT "available, and values required to select each gain."
190  PRINT LIN(1)
200  PRINT USING 210;"POT","GAIN","SELECT VALUE"
210  IMAGE (K)5X
220  DATA A,20,-33,B,10,-161,C,5,-289,D,2,-417
230  FOR B=1 TO 4
240  READ A$,Gain,Select_value
250  PRINT USING 260;A$,Gain,Select_value
260  IMAGE X,A,5X,4D,10X,4D
270  NEXT B
280  INPUT "ENTER THE SELECT VALUE TO SET DESIRED GAIN",V
290  FOR I=1 TO Knt
300  WRITE IO 2,4;-97        !  Reset gain latches to 0 (0000000011100000)
310  WRITE IO 2,4;V          !  Send gain select value to latch
320                          !  Binary values depend on the gain selected
330                          !  0000000000100000(-33),0000000010100000(-161)
340                          !  0000000100100000(-289),0000000110100000(-417)
350  WRITE IO 2,4;V+32       !  Set gain of op amp (reset Bit 5)
360  WRITE IO 2,5;47         !  Initiate ALE to 0 (set CTL0 and CTL1 to 0)
370  Cnt=0                   !  Initialize loop counter
380  BEEP
390  INPUT "READY TO TAKE DATA?",R$
400  IF R$="Y" THEN 420
410  PAUSE
420  GOSUB Dataacq
430  BEEP
440  INPUT "DO YOU WISH TO CHECK THE DATA?",Y$
450  IF Y$="N" THEN 680
460  REM   This section displays the data taken during the current scan in the
470  REM   the decimal equivalent of their binary values
480  PRINTER IS 16
490  PRINT LIN(3)
500  GOSUB Dot_line
510  PRINT LIN(1)
520  PRINT USING 530;"Quadrature Number","Electrode Pair","Differential Voltage"
,"Reverse Voltage"
530  IMAGE (K)3X
540  GOSUB Dot_line
550  PRINT LIN(2)
560  Ct=0
570  FOR K=1 TO 32
580  IF Ct=0 THEN 630
590  El$="S - D"
600  Ct=Ct-1
610  Quad=K/2
```

```
620  GOTO 660
630  El$="A - C"
640  Ct=Ct+1
650  Quad=(K+1)/2
660  PRINT TAB(9),Quad;TAB(24),El$;TAB(45),Data1(K);TAB(68),Data2(K)
670  NEXT K
680  INPUT "DO YOU WISH TO SAVE THIS DATA?",A$
690  IF A$="Y" THEN 750
700  INPUT "DO YOU WISH TO CHANGE THE GAIN?",E$
710  IF E$="Y" THEN 280
720  GOTO 300
730  REM   This section combines the data from each run into a single file and
740  REM   after all scans are completed stores it on tape, if desired
750  FOR L=1 TO 32
760  Datar(2*L-1)=Data1(L)
770  Datar(2*L)=Data2(L)
780  NEXT L
790  FOR M=1 TO 64
800  Datam(M+64*(I-1))=Datar(M)
810  NEXT M
820  NEXT I
830  INPUT "DO YOU WISH TO STORE DATA ON TAPE?",V$
840  IF V$="N" THEN 900
850  INPUT "ENTER THE DATA FILE NAME",D$
860  CREATE D$,1,256*Knt
870  ASSIGN #1 TO D$
880  ON END #1 GOTO 900
890  PRINT #1,1;Datam(*)
900  PRINT "RUN COMPLETE"
910  END
920 Dot_line:   FOR L=1 TO 80
930  PRINT "-";
940  NEXT L
950  RETURN
960  REM   This subroutine selects two electrodes at a time from the 64 total
970  REM   and initiates the conversion of the differential signal from analog
980  REM   to digital form. The digital equivalent is stored in one of two
990  REM   arrays depending on the stimulation polarity
1000 Dataacq:    FOR N=1 TO 2
1010 FOR J=1 TO 32
1020 WRITE IO 2,4;V+33-J    !  Increment decoders
1030 WAIT 750               !  Wait 750 milliseconds for the AD536 to settle
1040 WRITE IO 2,5;45        !  Latch in address for ADC, raise ALE line
1050 WRITE IO 2,5;46        !  Raise start convert line, lower ALE line
1060 WRITE IO 2,5;47        !  Lower start convert line
1070 ON N GOTO 1080,1100
1080 READ IO 2,4;Data1(J)   !  Store the voltage data
1090 GOTO 1110
1100 READ IO 2,4;Data2(J)   !  Store the reverse voltage data
1110 NEXT J
1120 IF Cnt<>0 THEN 1130    !  If finished, go to next section
1130 Cnt=Cnt+1
1140 BEEP
1150 PRINT "READY TO REVERSE POLARITY OF INPUT SIGNAL"
1160 PAUSE
1170 NEXT N
1180 MAT Data1=Data1-(255)     !  Invert data to remove interface inversion
1190 MAT Data2=Data2-(255)
1200 MAT Data1=ABS(Data1)      !  Take the absolute value
1210 MAT Data2=ABS(Data2)
1220 BEEP
1230 RETURN
```

A1.4 Component Locations by Board and Function

Board 1

Instrumentation Amplifier

R101-105

U102

Gain Circuit

C101

R106-111

U103-105

RMS-to-DC Converter

C102-104

R122

U106

A/D Converter and Peripherals

C105

R123-125

U107-110

X101

Interface

R112-121

U101, 111

Board 2

Multiplexers

U201-204

Interface

R201-210

U205, 206

Board 3

Multiplexers

U301-U305

A1.5 Parts List

Capacitors

C101	.01µf Polystryene
C102-103	2.2µf Tant.
C104	1µf Tant.
C105	10µf Tant.

Resistors - all are 5%, 1/4 W unless otherwise indicated

R101-102	100KΩ
R103-104	22 MΩ
R105	10KΩ, 15 turn
R106	2.2 MΩ
R107	10 KΩ
R108-111	10 KΩ, 15 turn
R112-121	3 KΩ
R122	24 KΩ, 1%
R123	10 Ω
R124	10.2 MΩ
R125	100 Ω
R201-205	10 KΩ
R206-210	3 KΩ

Integrated Circuits

U101	LM339
U102	AD521
U103	TL061
U104	CD4099
U105	CD4066
U106	AD536
U107	ADC0809
U108	CD4011
U109	CD4013
U110	AD584
U201-204	CD4051
U205	LM339
U206	TL061
U301-304	CD4051
U305	CD4052

Miscellaneous Components

P1	50-Pin, Cannon Plug (DP-50-P)
J2, J3	22-Pin Double Contact Edge-Card Connector
X101	2.01 MHz Crystal

A1.6 References

[A1] Data Acquisition Components and Subsystems, Analgo Devices, p. 2-7 - 2-12, 1980.

[A2] ibid, p. 6-4 - 6-12.

[A3] ibid, p. 6-10, Fig. 5.

APPENDIX II INSTRUMENTATION OPERATION

To clarify operation of the instrument, a sample operational sequence is included. In order to conduct experiments using the instrumentation system the following devices are required:

1. Data Acquisition System (DAS) with electrodes
2. Hewlett-Packard HP9835A computer
3. Hewlett-Packard HP98032A 16-Bit Parallel Interface
4. Tektronix PS503 or equivalent power supply
5. Tektronix FG501 or equivalent variable frequency function generator, and
6. Tektronix DM501 or equivalent multimeter.

Operating Procedures:

1. Prepare carcass or muscle specimen. This may range from killing and dressing carcass to thawing a frozen sample and may require the assistance of someone knowledgeable in meat science.
2. Connect HP98032A parallel interface to the back connector of the HP9835A computer.
3. Connect the 50-pin cannon connector of the HP98032A to the corresponding connector on the DAS chassis.
4. Power should be off when connecting power to DAS. Ensure that power supply voltages are correct (+7 V, -7 V, +5V), adjusting supply as necessary, and connect them to their appropriate jack on Board 1 (ProtoBoard). Once connected, turn power source on.

5. Turn on the HP9835A computer and load the Data Acquisition Program (DAP) into computer memory. DAP is stored on the cassette tape labelled "DATA". The statement used to transfer this program into the computer is LOAD "DAP". Type the load statement and press the EXECUTE key on the computer keyboard to initiate transfer.

6. Connect each electrode group's edge-card connector to a card edge terminal. Electrodes 1 through 8 should be connected to Board 2 (see Fig. A1.1), and electrodes 9 to 16 connected to Board 3. This is to ensure that the electrode quad and the data assignments correspond. If connections are reversed the computer will assign electrode quad 9's data to electrode quad 1, etc.

7. Insert electrodes, both stimulating and acquisition, at the desired locations in the subject tissue. To aid in data evaluation it is best to establish a consistent orientation of the acquisition electrode quads. As an example, electrode A and C of each quad may be placed so that the line joining electrodes A and C is parallel to the line joining the stimulation electrodes.

8. Set the stimulation source for the desired waveform (generally a sinewave), and connect to the stimulation electrodes which are in the tissue.

9. Set the stimulation frequency to 50 kHz and turn stimulation source on.

10. Using a multimeter set desired stimulation output voltage or current. Measure voltage across the stimulation electrodes,

not open circuit. Measure current with subject connected. A range of 2-6 V RMS or 5 to 15 mA is suggested.

11. Press the key marked RUN on the HP9835A. This initiates the program DAP. The program asks for the number of scans desired. Before a gain value has been determined, any arbitrary value can be entered by typing in the value and pressing the CONTINUE key.
12. Choose one of the four designated gains (20, 10, 5, 2) and enter the corresponding value which selects that gain as prescribed by the table shown on the screen. Press CONTINUE. The computer will beep and display the prompt "READY TO TAKE DATA?" When ready to continue, enter "Y" and press CONTINUE.
13. The computer will scan the electrodes, beep, pause, and display a message to reverse stimulation polarity. Before signal gain is established, it is not necessary to reverse stimulation polarity, unless desired. Press CONTINUE.
14. Once scans are complete data may be displayed by responding "Y" to the prompt "DO YOU WISH TO CHECK DATA?". Press CONTINUE.
15. Determine if present gain setting allows for maximum utilization of the A/D converter. Data collected from electrodes nearest the positive stimulation electrode should have decimal values which approach 255. If most of the values are 255 the gain is set too high, and if all of the values are much less than 255 the gain is set too low. If the gain is correct, press the STOP key and go to step 16. If the gain is incorrect, enter "N" to the prompt "DO YOU WANT TO SAVE DATA?"

and "Y" to the prompt "DO YOU WISH TO CHANGE GAIN?". Repeat steps 12 through 15 until gain is correctly set.

16. Press RUN. Enter the number of scans desired (1 scan means two passes through the electrodes - forward and reverse polarity).
17. Select the correct gain and enter into the computer.
18. Adjust stimulation parameters (frequency, current, etc.) for first scan and begin experiment. After each pass the stimulation polarity is reversed. The computer will beep and display a prompt to reverse polarity. Once reversal is accomplished, press CONTINUE.
19. After each scan (2 passes) the data may be displayed, and, if desired, saved by entering "Y" to the prompt "DO YOU WISH TO SAVE THIS DATA?". If, for some reason, the data is unacceptable, respond "N" to the above prompt. Once the experiment has begun, it is usually desirable to maintain a constant signal gain. Therefore, respond "N" to the prompt "DO YOU WISH TO CHANGE THE GAIN?". This will allow the user to re-scan the subject with the same stimulation parameters.
20. After all data has been taken, data may be stored on tape by responding "Y" to the prompt "DO YOU WISH TO SAVE DATA ON TAPE?". Enter the name of the data file and press CONTINUE.
21. Once all data has been stored on tape, the computer will display RUN COMPLETE on the screen.

DETERMINATION OF CURRENT DISTRIBUTION PATTERNS IN ELECTRICALLY STIMULATED TISSUE SPECIMENS

by

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ABSTRACT

A knowledge of the current distribution patterns in electrically stimulated biological tissues can be useful in both medical (diagnostic and therapeutic) applications and in consumer meat processing. Complete understanding of the associated tissue responses to current applications is often hampered by a lack of such information.

This study examined a system to determine the current pathways in electrically stimulated animal tissues. An instrumentation system including multiple quad electrodes was designed and produced which would give a qualitative indication of current direction and magnitude at specific tissue sites. Current distribution patterns in both whole carcass and single muscle tissues were observed using this system.

The results of this study indicate that current paths become more dispersive as stimulation frequencies are increased. In addition, higher signal frequencies elicit complex, probably nonlinear, tissue responses.

The data also suggests additional studies; namely,

1. frequency studies using varying depth electrodes,
2. instrumentation changes to allow measurement of impedances at electrode sites, and
3. continued research at higher frequencies

which would provide greater insight into a more quantitative evaluation of current distribution in tissues.