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WATER CONTENT OF STRATUM CORNEUM IN VIVO.(U)
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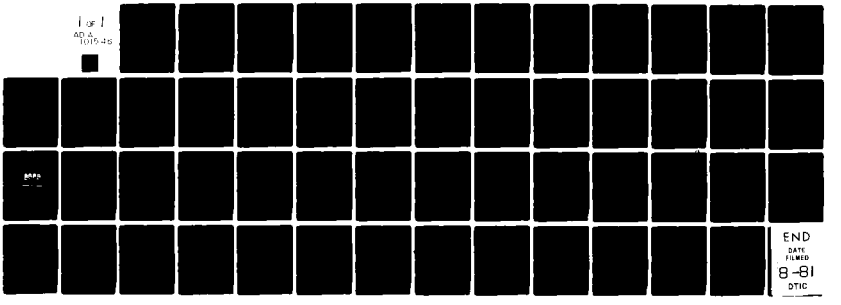

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WATER CONTENT OF STRATUM CORNEUM

IN VIVO, (U)

FINAL REPORT

by

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SUMMARY

We have developed a method to measure the water content of the stratum corneum of intact skin. In its present form the technique is a transfer method. Like many other transfer measurements in medicine the technique is most accurate when individually calibrated with samples of the subjects own tissue. Electrical resistivity is accurately measured *in vivo* during the course of experimental procedures using a four-fingered micro-electrode probe developed for this purpose. Calibration *in vitro* on samples taken from the same site is made by comparing resistivity vs. total water content measure gravimetrically. Differences in conduction properties of different samples are not now predictable, and can cause large estimate errors if a single calibration curve is used for all subjects. The transfer method eliminates this source of error. Present limitations on the accuracy of the transfer method arise from uncertainties in regional water distribution during calibrations.

The method is restricted in theory to stratum corneum thicker than the spacing of the electrode fingers. With present electrodes this theoretical restriction limits the use to the palms and soles. Future empirical testing of these electrodes and the manufacture of smaller electrodes may permit application of the method to areas of thin stratum corneum as well.

Procedures and sample data are presented to demonstrate the application of the method on human skin. Complete documentation of electronic devices constructed for Lettermann Army Institute of Research for field studies is provided.

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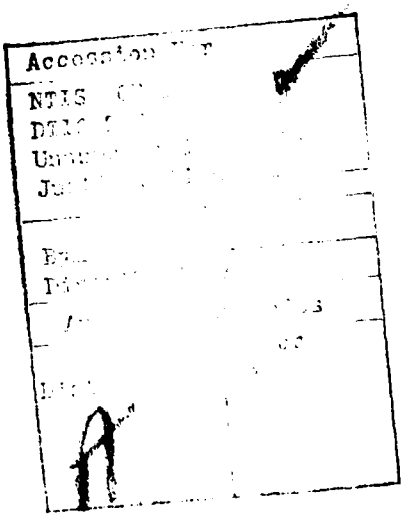
For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45CFR46.



I. INTRODUCTION

Prolonged exposure to water or the wearing of waterproof foot and leg wear can result in the production of incapacitating immersion disease, often complicated by infection (1-3). Under suitable conditions immersion disease can reach epidemic proportions in combat troups. Immersion diseases, dermatophyte infections, miliaria, and friction blisters, represent occupational hazards unique to the combat foot soldier, and thus uniquely military medical problems.

The pathophysiology of water immersion injury and other water or occlusion related diseases is not well understood, but one important factor may be the breakdown of the skin barrier layer, the *stratum corneum*. The integrity and mechanical properties of the stratum corneum are dependent upon many factors, a major one being the correct level of hydration (4,5,6). At this time, we do not know what this hydration level is *in vivo*, nor has there been devised a method for accurately measuring water content in the stratum corneum of intact skin.

The goal of these studies is to develop methodology for this purpose which could be used by researchers at LAIR or elsewhere (1) to better understand the pathophysiology of these diseases, and (2) to develop protective measures for maintaining hydration levels of stratum corneum consistent with optimal barrier function.

II. RESEARCH PROGRESS TO DATE

A. Criteria for a Method

At the outset of these studies we set up certain criteria for the selection of a potential measurement method. First, the method had to be noninvasive, that is somehow measuring the water content of the underlying stratum corneum from the surface of the skin. Secondly, the method had to clearly "see" the water content of the surface keratin layer and not be influenced by the well-hydrated viable epidermis. Thirdly, the method would have to be relatively inexpensive and simple to operate, or at least have a potential for becoming so, in order that such measurements could eventually be made by less experienced personnel or outside of a laboratory.

It was first necessary to choose some physical property of water which was well-related to the amount of water, and could be measured externally. The problem was complicated by the three practical criteria listed above, and by two additional factors. First, the stratum corneum is complicated and chemically a poorly defined matrix of insoluble protein, lipid, and low molecular weight solubles. Water does not enter and leave this matrix simply; some water is trapped or bound, while other molecules of water are freer to move in and out. Differences in the makeup of the matrix material between individuals could result in dif-

ferent distributions of bound and unbound water. Measured physical properties of water would probably be affected differently by bound and unbound water fractions.

Secondly, there existed no other quantitative method which could be applied as a comparison standard for measuring water in intact skin. Even for removed samples of stratum corneum which could be processed in the laboratory, the quantitative relationship between water content and most physical properties has not been determined.

Thus, at the outset it was clear that (a) a physical property would have to be chosen which met the practical measurement criteria, and (b) in all likelihood the quantitative relationship between this physical property and water content would have to be established by us, taking into account the variations in the relationship which might be brought about by variations in the matrix material and water binding, and (c) whatever method was finally developed would have to be calibrated *in vitro* and water content from *in vivo* measurements would have to be inferred by reference to these *in vitro* calibrations. Our primary standard for changes in water content in the calibration specimens would be changes in weight, a direct measure of total tissue water, as this parameter was of primary concern to LAIR. We were also aware that much of the work would have to be done on areas where the stratum corneum is thick, the palms and the soles, so that samples of pure stratum corneum could be directly removed from the *in vivo* test site for subsequent *in vitro* calibration.

B. Thermal Constants

Our first approach was to examine the feasibility of using measurements of heat conduction constants of skin as a measure of its water content. Work by one of us had indicated that the thermal conductivity of the surface skin layers could be measured with a nonpenetrating method in which the skin surface temperature rise was recorded in response to a surface heat flux transient. Hydrated stratum corneum had a higher thermal conductivity than did dry stratum corneum (8) (Figure 1a). A feasibility study showed that the potential for this method was low (9), for the following reasons. First of all, the entire range of thermal conductivity from the dry to the moist state varied only threefold, and the variability was great (Figure 1b). Secondly, calibration measurements were slow, making it difficult to determine the *in vitro* relationship between water content and thermal conductivity. Thirdly, the simplified measurement scheme that we were using showed that there was as much dependence upon the dry mass or volume of the sample as there was upon water content. This problem, although correctable, would have resulted in a system of great expense and poorly adapted to *in vivo* measurement.

C. Electrical Characteristics

Measurement of electrical characteristics has proven to be

a more effective approach than the measurement of thermal constants. The measurement of electrical characteristics has been used industrially to determine moisture levels in powders, grain, and timber. Some basic work has been done on AC electrical characteristics of purified globular and fiberous protein powders in various states of hydration (10). Several investigators have approached the problem of inferring stratum corneum moisture levels by measuring changes in its electrical properties, but none have proposed a truly quantitative assay method (11,12).

1. Instrument Development

To simplify data analysis, we chose to measure surface electrical resistivity at a single very low sinusoidal frequency (1 Hz). Using a sinusoidal waveform partly overcomes electrode polarization effects, which are troublesome to DC measurements. To eliminate problems of contacting resistance, a four electrode method was chosen instead of the conventional two electrode design. In the four electrode method (long used in semiconductor research) the current source is connected to the outer fingers and the voltage-drop is independently measured across the inner two fingers with an extremely high input impedance buffer amplifier (10^{12} Ω). In this way the voltage drop accumulating across the current-carrying fingers because of AC polarization and contact problems does not result in an error in estimating the sample potential drop.

To insure that the resistivity (ρ) measurement would uniquely reflect only the resistance of the stratum corneum and not the underlying tissue, it was necessary to design the electrode arrangement so that the distance between the current-carrying electrodes, (the outer two fingers), was less than the thickness of stratum corneum layer on which the probe would be used.

To meet all of these requirements, a state-of-the-art microelectrode was developed in conjunction the Microtechnology Laboratory at the University of Washington using microphotographic and etching techniques similar to those used in manufacturing integrated circuits. The probe consists of gold fingers evaporated onto a glass substrate (Figure 2). At the time of development, the best probe line resolution that could be obtained was 25 micrometer wide fingers spaced 35 micrometers apart, so that the applicability (in theory) was limited to specimens at least a quarter of a millimeter thick. This has suited our purpose to date, as we have needed to make measurements on sites of thickened stratum corneum which could then be removed for intensive *in vitro* study. Extention of these techniques to thinner stratum corneum may require the development of probes with smaller fingers and closer spacing.

Figures 3 and 4a show the electronic circuitry developed for use with the probe. The current source is of the constant current type with a selectable output in the nano-amp - micro-amp range. Extremely low current levels are used because of the close probe spacing. In addition, the voltage across the current carrying fingers is kept as low as possible as there has been some suggestion that the stratum corneum may exhibit non-linear restance chacteristics at different applied voltages (13).

Since the applied current (I) is constant, the measured resistance (R) can be determined from a strip chart recording of the potential drop (V) across the inner two fingers. The material property of interest is *resistivity* (ρ) which is equal to the measured R divided by a probe constant k. The value of k depends upon the width and spacing of the fingers. For our current probe design, the value of k is 4.53 mm. Our analysis has shown that k is also important in determining the theoretical minimum thickness of the sample upon which a valid measurement can be obtained. For the probes currently in use this thickness is 0.35 mm.

2. Accuracy of the Probe and Electronics

Special high accuracy resistors are appropriately inner-connected and attached to the current source and voltage amplifier to simulate a four electrode measurement on materials varying in resistivity from 1 MΩ-mm to 1,000 MΩ-mm. Periodic calibration in this manner has shown the electronics to give accurate and reproducable results. In use, care must be taken to see that the constant current source is set to correct level. If the resistivity of the stratum corneum is extremely high because of low moisture levels, and the current source is set at too great of a level, then the current source pushes the limits of the power supply and clipping of the sine wave results. Under these conditions, of course, it is not possible to obtain a valid measurement unless the current level is decreased. Under these conditions decreasing the current level causes an additional problem, in that the potential drop V is decreased and the signal to noise ratio deteriorates, making it difficult to obtain an accurate measurement of V. With the addition of appropriate filtering and care in interpretation, we have found that the instrument can give valid information from about 10^5 to 4-5 x $10^8\Omega$. Resistances greater than $10^8\Omega$ are measured with less certainty.

Direct verification of a microelectrode probe have been and continues to be a problem. We have not been able to identify substitute materials for stratum corneum whose resistivity is in the 100 - 1000 MΩ range and accurately known, and which would present the same surface characteristics as skin to the electrode fingers. Therefore, direct calibration of the entire system in terms of accuracy of resistance measurement is not now possible.

We do not view this as a serious problem. The microprobe is simply a mechanical extension of the electronics, wherein the real accuracy lies. The greatest potential problem with the four fingered probe would lie in mechanical inapplicability of the four-electrode method to this measurement set-up, i.e. four fine thin film lines applied to a fairly rough test site. If such mechanical problems existed they should show up as inability to obtain the same resistance values while repeatedly moving the probe on and off the same skin site. In addition, there should be a change in measured resistance with changes in application pressure of the probe. In our experience, however, these problems have not appeared. Identical values for resistance are obtained by successively moving the probe on and off the same site, and we have found that pressure loading past the point of making skin contact does not alter the measured resistance.

3. Quantitative Relationships Between Resistivity, Total Water Content, and Other Factors

At the outset of these studies there existed no quantitative data on the relationship between water content and resistivity for stratum corneum. The first step in developing a useful method was to determine how total water and other factors could affect the resistivity of stratum corneum material.

a. Test Methods

Samples of stratum corneum for *in vitro* study are obtained from the planter surface of the feet of normal volunteer subjects using a motor driven epidermal keratome fitted with a 0.4 mm thickness gauge. This procedure yields slices of pure stratum corneum approximately 1 cm x 1 cm x 0.3-0.4 mm thick. Sample dry weight is obtained after 48 hours of vacuum desiccation. At this point, the resistivity is extremely high, far higher than our instrumentation can measure. The samples are then reconstituted to different water contents and measurements are made at a test station consisting of a four-point probe mounted on a micromanipulator situated over a platform on the pan of an analytical balance (Figure 4b). Measurement of sample weight and resistivity can be made in rapid succession, and the probe can be applied with a uniform force measured with the pan balance. Total water content is calculated from the difference in weight at the time of the measurement and the weight after vacuum desiccation.

Two methods have been used for producing variable water contents in the specimens. The first is a modification of the desorption method described by Scheuplein and Morgan (14). In this method the dried samples are rehydrated by exposure for a fixed time period (24 hours) in a 100% RH chamber at room temperature. After this rehydration the sample is put on the balance pan and alternate weight and resistance measurements are

made as the sample dries out on the pan. Approximately four hours are required for the water content to decrease from a weight fraction of 0.7 (233% dry weight) to a weight fraction of 0.2 (25% dry weight), after which time the resistance has increased out of the measurement range.

The second is the equilibrium method, as described by Middleton, Spencer and others (15,16,17). Samples are equilibrated for 48 hours in sealed containers containing saturated solutions of various inorganic salts. These salts generate the following relative humidities: 60%, 75%, 83%, 90%, 93%, and "pure" water, about 98%. After equilibration, samples are put on the balance pan and a single measurement of weight and R is made; the sample is then put into another relative humidity chamber for re-equilibration to a different water content.

Both methods have advantages and disadvantages. The desorption method has the advantage of being more controlled, i.e. all resistance measurements are made on precisely the same small spot of the sample surface, and all are made within the same four hour time period. The desorption method has the disadvantage of generating changing gradients of water concentration through the sample as water is lost from the surface by evaporation, so that the water content by weight represents, at best, an average figure for the entire sample thickness and not necessarily the water content in the small volume of tissue under the electrode fingers.

The advantage of the equilibrium method is that theoretically it generates a uniform concentration of water throughout the sample, although this has never been validated. The disadvantages of the equilibrium method are that measurements are interspersed over many equilibration days, and there is much sample handling. It is not possible to precisely align the samples so that exactly the same microarea is seen by the probe fingers during each test.

b. Results With Normal Corneum

Both methods show a large reduction in R with increasing water content from a maximum R$\sim$ 1,000 MΩ at a weight fraction of 0.25 (33% dry weight) to a minimum R$\sim$ 5 MΩ at a weight fraction of 0.7 (233% dry weight).

Both methods show inter and intrasubject variability (Figures 5a, b). It is instructive to examine this variability. The well delineated curve patterns define by changes resistivity during desorption (Figure 5a) is partly due to the fact that there are many more data points during desorption than during equilibrium calibration. But also these well defined curve patterns indicate the high dgreee of precision with which the measurements

can be made (see esp. EM-21 and J-2), and show that for a single spot under the electrode fingers, there is a regular well-defined relationship between the total sample water and the resistance of that spot. Large discontinuities during a desorption experiment (i.e. A-2) can usually be traced to mechanical factors such as the sample sticking to the probe, movement on the pan, or changes in shape during the drying process.

On the other hand, variability between samples from the same or from different subjects appears much greater with the desorption method than with the equilibrium method. This may be due to real sample differences in the resistivity vs. water content relationship, but it is just as likely that the regional water content vs. total water content relationship was different during desorption in the several samples.

Examples of calibration data using the equilibrium method (Figure 5b) but on different samples, and different subjects than the desorption method show much less variation. Again, this could be due to a more homogeneous group of samples in terms of resistivity vs. water content relationship, but on the other hand such consistency would be expected if there was a more uniform state of hydration throughout the sample (which the equilibrium method is supposed to give). We are presently undertaking experiments in which both methods are used on the same samples. The data will give us some idea about the variability from sample to sample to be expected because of differences in regional vs. total water content during desorption. These experiments will not assure us however, of uniformity in hydration during equilibrium experiments.

c. Results With Pretreated Corneum

Other factors which could alter the relationship between resistivity and total water content might relate to the water binding characteristics of the stratum corneum matrix (22). Water which is tightly bound cannot fully participate in the conduction mechanisms as free water can. To test the influence of water binding and electrolyte concentration on the empirical relationship between resistivity and water content, the number of samples were twice tested, and before the second test were either (1) exposed formaldehyde vapor, which increases cross linking between stratum corneum proteins and therefore would increase from normal the ratio of the free water fraction to the bound water fraction at any given total water content (18), or presoaked in urea solution and then rehydrated. Urea supposedly increases water binding by its osmotic and keratolytical effects (19), and thus would decrease the ratio of free water to bound water at any given total water content.

The results are shown in Figures 6a and 6b. Formaldehyde exposure markedly altered the relationship between resistivity and water content by reducing the resistivity for any given water content. It was also evident that the rate of change of resistivity with changes in water content is much greater after formaldehyde treatment, and the total water pickup in 24 hours is less. (The desorption test method was used in these experiments). With urea pretreatment the relationship is altered by increasing the measured value of resistivity at each water content.

Lipid extraction and chloroform methanol solution followed by soaking in distilled water supposedly disrupts the structure of the cellular membranes and leaches out intracellular solubles (9,20). Samples soaked in physiological saline subsequent to this harsh chemical treatment (Figure 6c), dried and put through the standard desorption test, showed a depressed resistivity vs. water content characteristic relative to pretreatment. Lipid extraction is apparently crucial to this effect; soaking in distilled water or physiological saline without prior lipid extraction produces little effect.

d. Summary

Total water content appears to be by far the largest contributor to variations in resistivity in stratum corneum. Other factors contribute as well, but to a lesser degree. Physical chemical factors causing differences in water binding capacity between samples from different subjects may contribute to variations in resistivity. Variations in electrolyte distribution can also be a contributing factor.

Factors not related to the conduction mechanism may also be important. Differences in regional water content under the probe fingers may not always correspond with changes in total sample water content, particularly in the unsteady state desorption experiments. On the other hand, while the equilibrium experiments probably give more uniform results because regional water content is probably more closely related to total sample water content, the desorption studies may more closely parallel the *in vivo* situation since a gradient of water concentration is generated throughout the sample.

4. Measurement of Resistivity of Intact Stratum Corneum

The probe is small enough so that it is possible to fasten it almost anywhere on the body surface, although our measurements to date have limited to the sole of the foot. Although the voltage probe amplifier has a high common mode rejection ratio it also has an extremely high input impedance so that noise pickup and stray capacitance can be a problem. For this reason each of the four electrode wires is connected by a separate shielded cable to the appropriate points in the constant current source and voltage probe amplifier. The shields are not grounded but driven to the same potential as the inner conductor; this procedure minimizes stray input capacitance.

It is essential in the operation of probe that the subject remain electrically "floating" at the time of measurement, and not be in contact with earth, the DC power supply, or any large metal objects.

Large muscle movements can sometimes set up enough EMF to distort the waveform but this is usually of a very transient nature and in our application has not posed a serious problem.

Measurements can be made as soon as the probe is in contact with the skin; the only electrical settling time for the instrumentation is the one second time lapse for a complete one hertz waveform. The occlusive nature of the probe allows us to alter the water content of the stratum corneum during measurement of resistivity by simply leaving the probe on the skin surface. Because the stratum corneum is relatively impermeable to the transport of water vapor, initially the concentration gradient of water across the surface layer is extremely steep resulting in a very dry skin surface under normal condtions. When the probe is put on the skin surface the concentration of water in the surface layers immediately begins to change rapidly, but it is several seconds before concentration in these surface layers increases to a point where the resistivity change can be measured (weight fraction equal to 0.2, 25% dry weight). A mathematical model of this diffusion boundary value problem has been presented elsewhere (21,22). Eventually, of course, the water content under the probe will come into equilibrium with the underlying tissues. The time constant of this equilibration process is τ, and the value for τ can be computed from:

$$\tau = \frac{4\ell^2}{\pi^2 D} \qquad \text{(Equation 1)}$$

ℓ is the thickness of the stratum corneum layer, D is the diffusion coefficient for water in stratum corneum. Figure 6d is a plot from the solution of this boundary value problem using a value of τ equal to 16 minutes. τ was calculated on the basis of ℓ = 0.15 mm and D = 9.3×10^{-8}-sec^{-1} (23). With occlusion, changes in water concentration are much more rapid at the surface than any where else in the stratum corneum. They occur most rapidly during the initial stages of occlusion. Coupled with this is the fact that incremental changes in water content produce much larger changes in resistance at low water contents than at high water contents. Therefore, upon application of the four fingered electrode to the intact skin surface, one would expect a very rapid decrease in resistance during the first minutes of occlusion and that the change in resistance would gradually become much less at extended times as equilibrium is approached. (At this point, it is worth while to point out that Figure 6d with different values for ℓ probably is a fair representation of changes in concentration of water seen during the desorption experiments except that during desorption, zero depth indicates

the center of the hydrated sample and water concentration gradients would extend from the center to both sides.)

Figure 7 shows the result from a typical evaluation experiment where a small skin area on the bottom of the foot was first conditioned by exposure to a dry nitrogen gas stream at 2 l/min. for 20 minutes. Then the probe was applied to this area and left on for an additional 40 minutes. As predicted, initial resistivities decreased quite rapidly from about 130 MΩ after 2 minute of probe occlusion to about 16 MΩ at 20 minutes. Thereafter the rate of decrease was considerably less falling to only about 12 MΩ by 40 minutes. It should be emphasized that the range of resistivity obtained over this 40 minute period reflects the degree of dryness at the start and the change with transepidermal water loss over the 40 minute period. If the skin were prehydrated, by occlusion or by soaking one would not expect to see this large change in resistance.

5. Proposed Application Method for Inferring *in vivo* Water Content From *in vivo* Measurements of Resistivity

There are a number of factors which rationally prevent the application of a *general* calibration curve to a *specific* measurement of resistivity *in vivo* as shown in Figure 7. These factors are (1) a lack of complete understanding of the mechanisms of electrical conduction, and factors which affect these mechanisms, as they might vary from sample to sample and subject to subject, and (2) uncertainty about how the total water content of a sample during *in vitro* calibration reflects the water content of the areas seen by the four-fingered electrode.

In spite of this, we feel that the four fingered electrode method can now be used with reasonable accuracy in studies to measure the *in vivo* water content of stratum corneum if the following procedures are followed.

(1) The measurements must be confined to the thickened stratum corneum of the palms or the soles.

(2) The *in vivo* area on the palm or sole in which it is of experimental interest to measure water content or changes in water content must be carefully marked beforehand and the four fingered microelectrode applied within this marked area during the experimental procedures. This procedure will allow removal of the same test area for subsequent calibration. This procedure should assure that differences in the elctrical conduction characteristcs because of differences in water binding capacity or electrolyte concentrations will be

minimized. Each sample will serve as its own control.

(3) At the conclusion of *in vivo* experimentation the test site must be removed for individual calibration. To do this we use a Storz type epidermal keratome fitted with a 0.4 mm thickness gauge. We have found it necessary to attach a more powerful motor to the keratome to cut plantar corneum than was originally supplied with the instrument. At this point the sample can be dried and stored until such time as *in vitro* calibration is possible.

(4) At the time of *in vitro* calibration the sample should be cut so that the marked area of measurement is in the center and excess material is trimmed away. Exactly how large the remaining calibration sample should be is a technical question whose answer will depend on methods available to the calibration laboratory. Making the sample very small has the advantage of making regional water content, under the area seen by the probe, nearly the same as total water content in the sample. Making the sample too small will make water losses from the perimeter more significant, a problem which is not encountered *in vivo* and which can be ignored when the total sample area is much greater than the thickness. Practical limitations are imposed by the accuracy and sensitivity of weight measuring apparatus and the ability to process and handle extemely small fragments of tissue without damage.

(5) The advantages and disadvantages of the disorption method and equilibrium method for obtaining calibration curves of resistivity vs. water content has been discussed. While the desorption method has the advantgaes of a closer similarity to the *in vivo* water concentration gradient and giving many more data points than the equilibrium method it has the theoretical disadvantage of showing a poorer relationship between regional water content and the total water content of the sample measured experimentally. The water concentration gradient from the surface to the maximum depth of measurement of the electrode is nearly flat for a large number of water concentrations anyway (Figure 6d). It is probably wise at this time, if the sample is large, to choose the equilibrium method. Although the equilibrium method takes a number of days to secure

completion of the calibration, a large number of samples can be processed at the same time so that the total technician time is considerably less than for the desorption method. We are currently conducting direct comparison of the two calibration methods on the same samples. This information should help us arrive at a more definitive recommendation in the future.

Calibration data so obtained should provide the user with a method of reasonable accuracy for translating measurements of *in vivo* resistivity made during experimental procedures to estimates of total water content of that spot during the procedures. In this method resistivity is not used as a *direct* measure of water content *in vivo*, because of differences in conduction mechanisms, water binding, and electrolyte concentration which might exist between subjects. Rather, resistivity is used as a way to transfer information between the *in vivo* situation of unknown water content and the *in vitro* situation of controlled water content. In this respect we have shown that resistivity is an easily measured, accurate and reproducible *transfer measurement*. The assumptions required are (1) that in the process of transfer of the tissue from the *in vivo* to *in vitro* situation the relationship between resistivity and the physical chemical properties of the stratum corneum do not change, and (2) that the changes in total water content of the sample as measured by weight change reflect the changes in regional water content under the probe. The first assumption is one of long standing in investigative dermatology; nearly all of the published work relies on the assumption that removal of this outer dead keratin layer in no way affects its physical chemical properties (4,5,7,8,14,15,16,17,18). The second problem is one on which there is no direct evidence, as it is an emperical problem, related only to the interests of this study, and one which deserves more exploration.

6. Results From Human Experiments Illustrating the Use of the Proposed Application Methods

We conducted a number of experiments to illustrate the application of this method and to define problems encountered in its use. These are ongoing studies and not yet concluded; we present here the results to date.

In these experiments we were interested in seeing the range of change in resistivity and subsequent water content which should be obtained during a forty minute period of occlusion of transepidermal water loss from the skin surface. Five male and three female adult volunteers were used as subjects. The same area on the sole of the forefoot was used on all subjects. The four fingered microelectrode was mounted on a plunger inside of

a cup through which dry nitrogen flowed at 2 l/minute. Pushing the plunger brought the microelectrode in contact with the test site. Each test occlusion and resistivity measurement period lasted 40 minutes; these 40 minute periods were interspaced with 20 minutes of nitrogen dry-down with the plunger pulled back from the skin surface. Three dry down periods and three measurement periods constituted an experiment for an individual subject lasting 180 minutes. At the conclusion of the *in vivo* experimentation, the test site was removed with the epidermal keratome using a 0.4 mm thickness guage. Samples were trimmed, desiccated and the dry weight obtained. Calibration of the test site *in vitro* was obtained with the equilibrium method at 60%, 75%, 83%, 90%, 93%, 97%, and 100% relative humidity. The results are shown in Figures 8 - 15. All of the subjects showed essentially the same kind of response by the second or third test period, i.e., a very rapid initial decrease in resistivity followed by a slower change in resistivity toward the end of the 40 minute occlusion period. More variation was seen in the response during the first occlusion period, and this may be related to initial prehydration effects from foot wear which were not fully compensated for by the initial 20 minute dry down period. Six of the eight subjects showed remarkably linear individual calibration curves of log resistivity vs. total water content. Data from two of the equilibration relative humidities, 60% and 75%, never appeared in the final calibration curves as the water pickup at these relative humidities (10 and 12% dry weight respectively) was too low to produce any measureable change in resistivity. Of the remaining relative humidities, 83% produced an average water content of 29% dry weight and a resistance never lower than 600 MΩ, while 100% relative humidity yielded an average water content of 288% dry weight and the resistivity which was never greater than 10ΩM . The first *in vivo* observations were made one minute after placement of the probe, so that the initial very rapid phase of falling resistance is not shown. In practice we attempt to connect the calibration points with a best fit line over the range of resistances observed in the *in vivo* experiments. This was no particular problem in 6 of the 8 subjects where a straight line could easily be drawn. For the other two subjects curve fitting was more difficult, and we chose to connect the equilibration points rather than draw a straight line fit through them. No technical reason could be found for discounting the data of these two equilibration experiments, and at this time there is no theoretical justification for assuming that all subjects from all samples should show a straight line relationship. For this reason, we recommend that the individual calibration procedure be followed.

7. Construction of Workable Devices for Use in Scientific Studies at Letterman Army Institute of Research

In the last year of this contract we undertook the task of designing and constructing devices which could be used in field studies at LAIR. These devices were constructed and

delivered to LAIR personnel.

The devices are battery operated and equipped with a digital display of both time and the log of skin resistance to eliminate the necessity of using a chart recorder. A number of other electronic logical additions make the units easy to operate under field conditions. These devices are entirely the novel design of Dr. Eugen Schibli and are not available elsewhere. A detailed description of the circuitry follows.

A. DEVICE DESIGN

These circuits measure the magnitude of the electrical resistance of stratum corneum at 1 Hz by the four-line method, where a known current is forced to flow between the outer two microelectrodes and the voltage measured between the two inner electrodes. This voltage is proportional to the stratum corneum impedance. Our studies indicated resistance values on the order of 1000 MΩ or more for fairly dry skin, but when the occlusive 4-line probe is applied for some minutes, the resistance decreases rapidly with time, probably due to the accumulation of moisture under the probe and in the superficial skin layers. At 1 Hz the impedance is essentially resistive.

The constant source current was selected as small as possible in order to avoid non-linear electrical behavior of the stratum corneum and to prolong probe life in presence of the electrochemical processes at the probe-corneum interface. However, when the source current is made too small, the voltage between the inner probes is more difficult to analyze. Our device automatically selects the appropriate source current strength in decade steps from 50 pA to 50 nA, and computes and displays the logarithm of the resistance value. The ubiquitous 60 Hz interference in the *in vivo* measurements is reduced by a technique skin to the right-leg driver used in ECG work, i.e., by feedback. The amount of feedback supplied is operator controlled.

Very large d.c. offsets occur on very dry skin *in vivo* and tend to saturate the amplifiers. We have not investigated the origin of these offsets, but counteract them by another d.c. feedback loop internal to the current source. Typically, the device is then no longer saturated after a one minute lag period.

Our 4-line microcircuit probe and the associated voltage buffers are mounted on a hand-held probe that is connected to the instrument by a 6 foot section of flexible ribbon cable (the stiffness of coaxial shielded cables used in earlier designs is objectionable for *in vivo* work). Calibration resistors of 1000 MΩ and of 10 MΩ are also mounted on the probe and can be switched into the circuit through CMOS logic switches. The slight additional capacitance introduced by the switches has shown no adverse effects in our pilot measurements.

The display is a 3½ digit liquid crystal display (LCD) type digital voltmeter (DVM). By multiplexing the analog voltage input to the DVM we are able to alternately display time in seconds and the log of resistance on the same display. Each is displayed for 2 seconds, but this can be varied. Indicator lights (light-emitting diodes)on the front panel inform the operator of the status of the measurements. In the AUTORANGING MODE, the selected decade is indicated. A SATURATION light indicates when the current source amplifier output approaches the supply voltage limit. An EXCESSIVE DISTORTION light usually results from uncompensated 60 Hz interference, while HIGH and LOW CONVERTER INPUT lights announce an impending resistance range-switching, if distortion and supply voltage limitations permit. The OVERTIME signal effectively extends the timer to twice the display's 1999 seconds range.

The instrument is powered by a large 12 V battery and 3 1½ volt D cells. The conditon of the voltage supplies can be read from the DVM, and these voltages are also made available at the front panel. The resistance readings under manual range control may be in error by multiples of ten, however, proper values are deduced easily with the aid of the built-in calibration resistors.

B. Detailed Circuit Description

The 1 Hz oscillator consists of a 1 Hz 2-pole low-pass filter followed by an integrator. Two matched diodes at the integrator output stabilize the oscillator amplitude in presence of a varying supply voltage. The oscillator frequency is matched to the notch frequency of a subsequent 1 Hz reject filter by means of the fine-tuning potentiometer P6 (see diagrams). The sinewave has an amplitude of a few hundred millivolt peak-to-peak, and is amplified from 0.025 times to 25 times, approximately, in the subsequent switched-gain stage C1/14.*) Gain control may be manual or automatic and is achieved by selecting the appropriate resistors in the amplifier's feedback network. The maximum gain resistor of 1 MΩ remains in the circuit, and its presence is accounted for by a slightly increased feedback resistance in the next branch. The 0.022 µF capacitor eliminates spurious high frequency signals in the circuit.

Autorange control is through the signals labelled 0, 1, and 2, that are derived from the decoder circuit at F4. Manual gain control is from a front-panel switch via the lines G10/10,6,11. The manual gain control lines are pulled low in the autorange-mode, by the 470 kΩ resistors at B1/14,15,16. Offset compensation for our switched-gain stage involves separate adjustments for low (P8) and for high gain (P1). Other compensation schemes are

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* Components are identified by location on the circuit board (Fig. 15). The letter designating row, and the first number the column of the socket position. The number after the slash refers to the pin-number on the socket. The socket pin-number is not necessarily identical with the accepted pin-numbering

possible and perhaps simpler, especially when the oscillator output has no d.c. component as might be caused e.g., by imperfectly balanced diodes. The interaction between the oscillator level adjustment P12 and the offset control for low gain P8 makes the adjustment procedure slightly more involved.

The 308-type operational current source amplifier D1/9 drives a current through the specimen in series with a 20 MΩ current sensing resistor. The voltage drop across the latter provides the feedback signal to the inverting input of the source amplifier, and equals the sinusoidal driving signal at the non-inverting input. The gain of the current source amplifier is rolled off at a few Hertz by the 0.01 uF capacitor.

As previously mentioned, major d.c. components were sometimes observed in our *in vivo* studies on rather dry skin. A separate d.c. feedback loop that is active in the maximum resistance range only, remedies this situation. This loop includes the current source amplifier, a 2-pole 0.016 Hz low-pass filter G3/14 and the minimum-gain feedback branch C0/16 in the switched gain amplifier. The gain of this loop is roughly a few times the stratum corneum resistance divided by 2000 MΩ, and is constant up to the filter's 0.016 Hz cut-off frequency. The d.c. loop gain is adjusted by potentiometer P15 for optimum response at a stratum corneum resistance of several thousand MΩ. The d.c. loop gain might then approach unity, while the 1 Hz gain of this loop is expected to be about 3500 times less than that, assuming a frequency independent stratum corneum resistance. By opening the loop we confirmed that the d.c. feedback has negligible effect on the resistance readings at 1 Hz.

The output voltage of the current source amplifier is further monitored by an overload detector circuit and by a 60 Hz high-pass feedback loop filter (C1/7,8). The overvoltage sensors C1/8,7 activate the overload indicator when the output voltage exceeds 55% of the supply voltage. Provision is also made for an additional overload indicator light on the handheld probe for operator convenience. The 55% limit in effect maintains a minimum of 2 volts between the operational amplifier's output and a 9 volt supply. In practice, the overload indicator will light more frequently as the voltage of the instrument's nominal 12 volt battery decreases below 9 volt. Upon an overload indication the source current is reduced automatically via D2/6 by a factor of ten.

C. 60 Hz Interference Reduction

Low level high impedance measurements in the laboratory are made difficult by the 60 Hz line frequency interference. We found this to be especially true in the *in vivo* studies because the large volume of the body facilitates the capacitive coupling of the 60 Hz into the sample region. We have devised a feedback circuit that provides an effective shunt path for the 60 Hz to

ground thus bypassing the probe region. Distortion in the current source output voltage at D1/9 at frequencies of 60 Hz and higher, is detected by the 60 Hz 2-pole high-pass filter C5/8. The filter output is attenuated by a panel-mounted 10-turn helipot, and drives an RCA operational transconductance amplifier (OTA) at A6/14. The OTA feeds a current to the subject's body via a large-area electrode, so that the high frequency distortion vanishes. The OTA in our circuit has an output impedance of several thousand MΩ, so that the system requirement of an electrically floating specimen is still closely approximated. The maximum output current of the OTA increases with decreasing output resistance; we found that about 0.7 μA is sufficient in most cases to effectively counteract 60 Hz interference that is coupled capacitively to the subject's body. If not properly adjusted, excessive feedback causes this feedback circuit to oscillate at 4-5 Hz. This is made apparent by the rapid flickering of the overload indicator.

D. The Handheld Probe

Restrictions on subject movement during long experiments are minimized by the use of highly flexible ribbon cable between the actual 4-line probe electronics and the instrument box. The unshielded ribbon conductors are driven from low impedance sources: three RCA 3140 CMOS operational amplifier circuits act as voltage followers on the handheld probe.

The 4-line microcircuit is on a 1 cm^2 microscope slide and is mechanically fastened to the handheld probe in a matching slot by the simple expedient of double-sided cellophane tape. The 4-line probe is electrically connected to the buffer inputs by short sections of 30 gauge wire that are soldered to the contact pads on the probe. In parallel with the 4-line probe, we have connected a switch-resistor network for calibration. In the CALIBRATE mode, the switches D5,6,12 connect a 1000 MΩ resistor between the voltage probes and connect the latter to the current probe lines through 10 MΩ contact resistors. An additional 10 MΩ resistor may also be connected in parallel with the 1000 MΩ calibration resistor through switch D13, and provides a second calibration point. Two 100 kΩ pulldown resistors keep the switches off during a measurement, and they may be placed inside the instrument box to save some space on the handheld probe. The compensation capacitors C1,2,3 are not needed in this application. The power-supply-bypasss-capacitor (0.22 μF) suppresses any supply voltage oscillations at the probe-end of the 6 foot long ribbon cable.

E. Voltage Measurement

The voltage V between the inner two probe lines is determined by the difference amplifier C1/1, whose common mode gain at 1 Hz is minimized by the CMR potentiometer adjustment P2. The 60 Hz reject filter removes any line interference from the rather small

signal at this point. The signal at the output G1/8 of the 60 Hz notch filter contains the desired 1 Hz component that is proportional to the product of source current times stratum corneum impedance; however, it may also contain harmonics of the 1 Hz signal, if the source amplifier saturates, or if the stratum corneum response is nonlinear. The subsequent 1.6 Hz low-pass filter E3/14 removes these harmonics, and peak and bottom values of the remaining 1 Hz component are determined by separate rectifier circuits. The two rectified voltages are stored on 0.33 μF capacitors at H3/2 and H3/9, that do not discharge towards zero, but rather towards zero difference. The output voltage of the difference amplifier E3/7 is proportional to the stratum corneum resistance. This peak-to-bottom measurement operates satisfactorily even in the presence of large d.c. components as occur frequently at the beginning of a measurement, and allows data collection before the transients have did out. This type of amplitude measurement represents a significant advance over our earlier designs.

F. Distortion

The definition of impedance magnitude as the amplitude ratio of voltage (V) to current (I) requires that these quantities be sinusoidal. Our circuit detects when large deviations from a sinusoidal shape occur and signals the operator. Distortion is determined not as total harmonic distortion in the usual engineering sense, but rather by a fixed-frequency filter and rectifier technique. The first filter stage is a 1 Hz notch, with the oscillator frequency trimmed to match the filter's notch. The following 2 Hz high-pass filter G2/10 removes the d.c. component and any residual 1 Hz component, so that the simple positive peak detector H3/3 provides a measure for the distortion. Typically, a DISTORTION indication occurs when the distortion voltage exceeds some preset percentage of the Hz signla voltage. The percentage level is set by adjusting the gain of amplifier G2/14 via P4 according to subjective operator criteria.

G. Logarithmic Conversion

The peak-to-bottom voltage at E3/7 is sampled once per second at the positive-going zero crossing of the oscillator sinewave output. The sampling period of 16 milliseconds further suppresses spurious 60 Hz components at this point in the circuit. The sampled voltage of magnitude M is stored on a 1 μF capacitor and buffered by the 3140 voltage follower A2/14.

The long-converter circuit uses two transistors of the RCA array CA3096 as the log-converting elements. The remaining transistors in the array are unused to avoid possible thermal effects under changing load conditions. The offset-null network at B4/12 cancels the offset of the preceeding 3140 follower as well as that of the B4/12. This log-converter is useful over

at least two decades of input voltage, while in actual use in the autorange-mode the voltage magnitude M usually varies by less than 20:1. However, under manual operation, or when autoranging is inhibited for some reason, the signal M may fall below the range of the low-converter, resulting in a LOW CONVERTER INPUT indication. In the autorange mode, the summing amplifier C5/14 adds 1 Volt/decade to the log(M) output. This addition is inhibited in the manual mode, so that proper log-resistance readings occur only in the maximum resistance position. In manual operation, the range should be selected so that the resistance display is in the -1000 to -1500 region, i.e., 100 MΩ to 1000 MΩ . The true resistance is then between 10 MΩ and 100 MΩ in the 100 MΩ manual range, and between 1 MΩ and 10 MΩ in the 10 MΩ manual range. In the manual mode, the range indicator lights are off. The output voltage at C5/14 is offset via P9 so that 0 volt output indicates 10 MΩ resistance (autorange), and it is also made available at the front panel.

H. Autorange Control

The circuits attempt to maintain a probe voltage between 0.05 and 0.05 volt peak-to-peak. If the processed and rectified probe voltage at E3/7 is less than 60 millivolt (signal B high), or greater than 940 millivolt (signal A high), the 4029 counter E4 will advance of decrease, respectively, such that a more appropriate current range may be selected via the 4028 decoder circuit at F4. In order to minimize transients, the current range switching occurs at the zero crossing of the oscillator's sinewave. The current range in use is identified in the autorange mode by the LED range indicators. These indicators are disabled in the manual mode.

The decision to increase the source current is also partly based on the distortion in the output voltage of the current source. The signal increases tenfold in response to a current range increase while the distortion consisting mostly of 60 Hz pick-up would remain the same. The circuit anticipates possible saturation of the current source after switching by comparing the sum of the distortion voltage and a voltage approximately 10x the 1 Hz component with the saturation limits U_L and L_L which are fractions of the supply voltage. Thus when either one of the rectifier voltages C7/7 and C7/1 exceeds its respective limit, the counter E4 advance is inhibited, while a downcount is still possible. In the manual mode, range select decisions are made by the operator.

I. Voltage References

A reasonably stable voltage is needed (1) for amplifier offset compensation, (2) as a reference in the log-converter, (3) in the decade summing amplifier, and (4) in the voltmeter display, even though the stratum corneum resistivity measurements are low precision. The effects of the residual reference voltage variations are expected to cancel at least partially, because

the same reference is used throughout the circuit. Amplifier offset nulling, however, is adversely effected by reference variations.

Reference variations in our simple diode-feedback circuit are mainly due to battery voltage changes. A battery voltage decrease of 25% from the nominal 12 volt to 9 volt, is expected to cause a 2.5% decrease of the reference voltage.

J. Timer

A resettable built-in timer, A9, counts the number of 1 Hz oscillator cycles. An Analog Devices AD7520 10-bit digital-to-analog converter B9 translates the cycle count into an analog voltage. The maximum timer display is adjusted by potentiometer P16 to be 1999 seconds. An OVERTIME indication, derived from the most significant bit of the A9/1 counter, in effect doubles the time display's 1999 seconds range.

K. Display

Our display unit is an Intersil liquid crystal display (LCD) digital voltmeter (DVM) kit, that displays alternately the log of the resistance (C9/6 enabled) and the elapsed time (C9/12 enabled) in seconds. A display time of two seconds were judged adequate for reading. To distinguish between resistance and time, we have prefaced the resistance readings by a minus sign. The analog signals for log resistance and time are multiplexed asynchronously to the DVM's operation. Consequently, an incorrect value may be displayed just after a multiplex operation. The 12 volt supply voltage may be monitored by enabling C9/13, and the 4.5 volt LED supply by enabling both C9/5 and C9/13. The respective switches are enabling both C9/5 and C9/13. The respective switches are enabled via front panel control.

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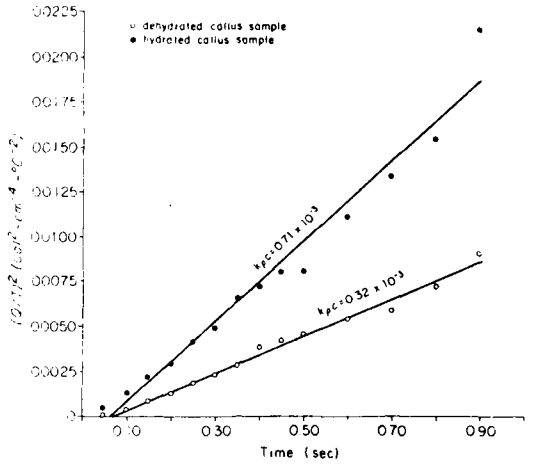


FIGURE 1a: Initial observations on kpc in dry and hydrated callus.

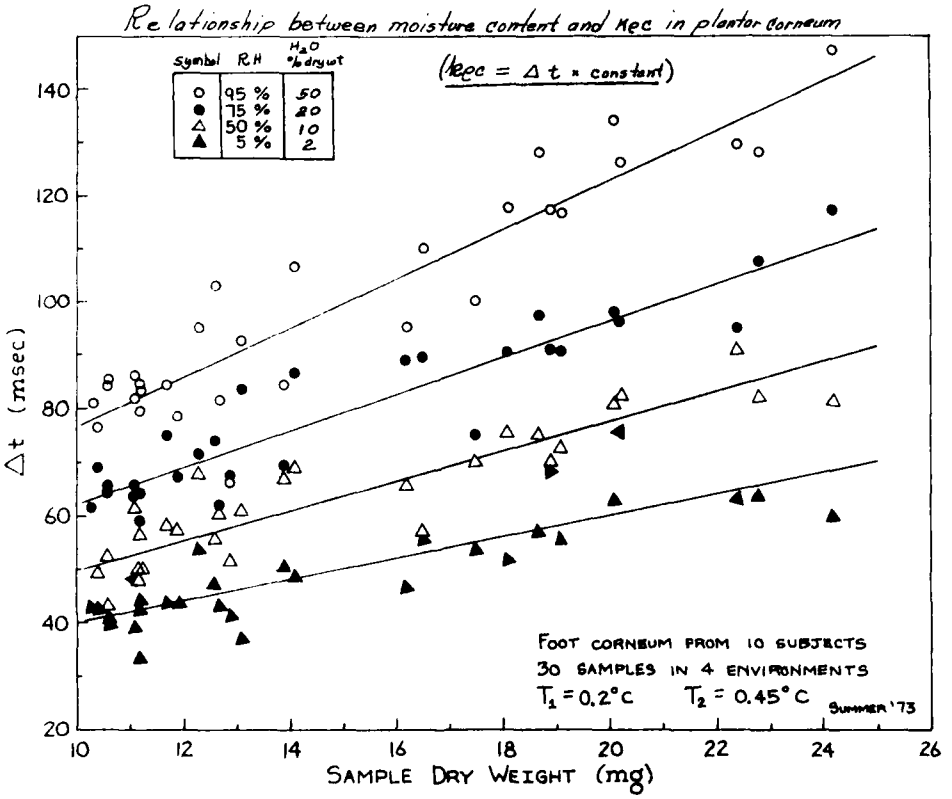


FIGURE 1b: Results of pilot studies to quantitate relationship between water content and kpc

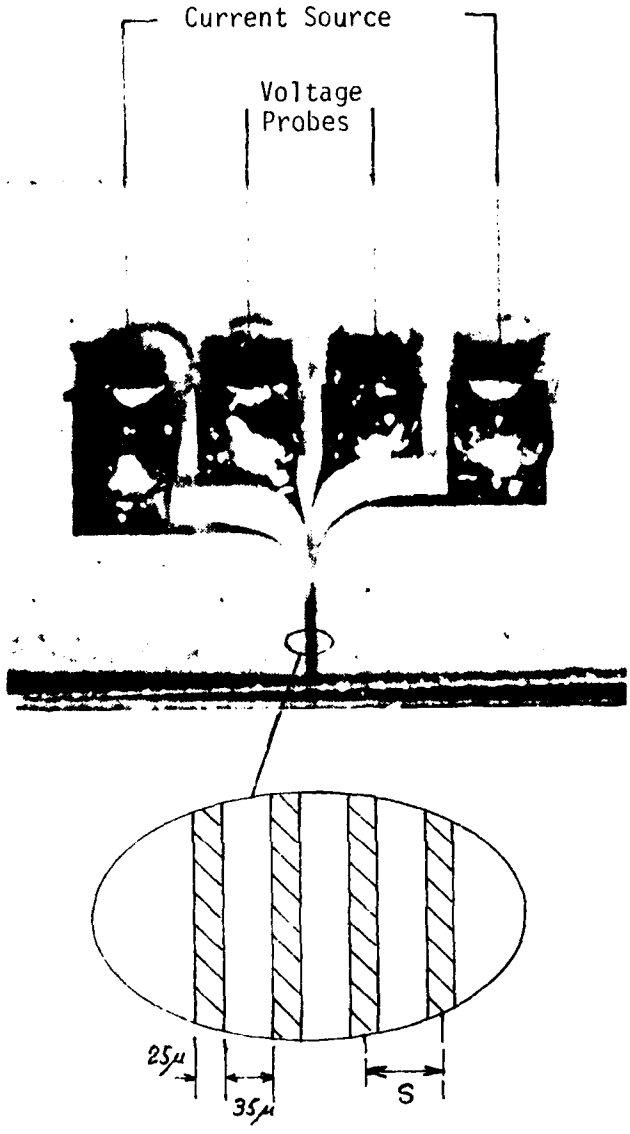


Figure 2. Four-line microcircuit electrode. The 4 large rectangles are contact pads for wire attachment.

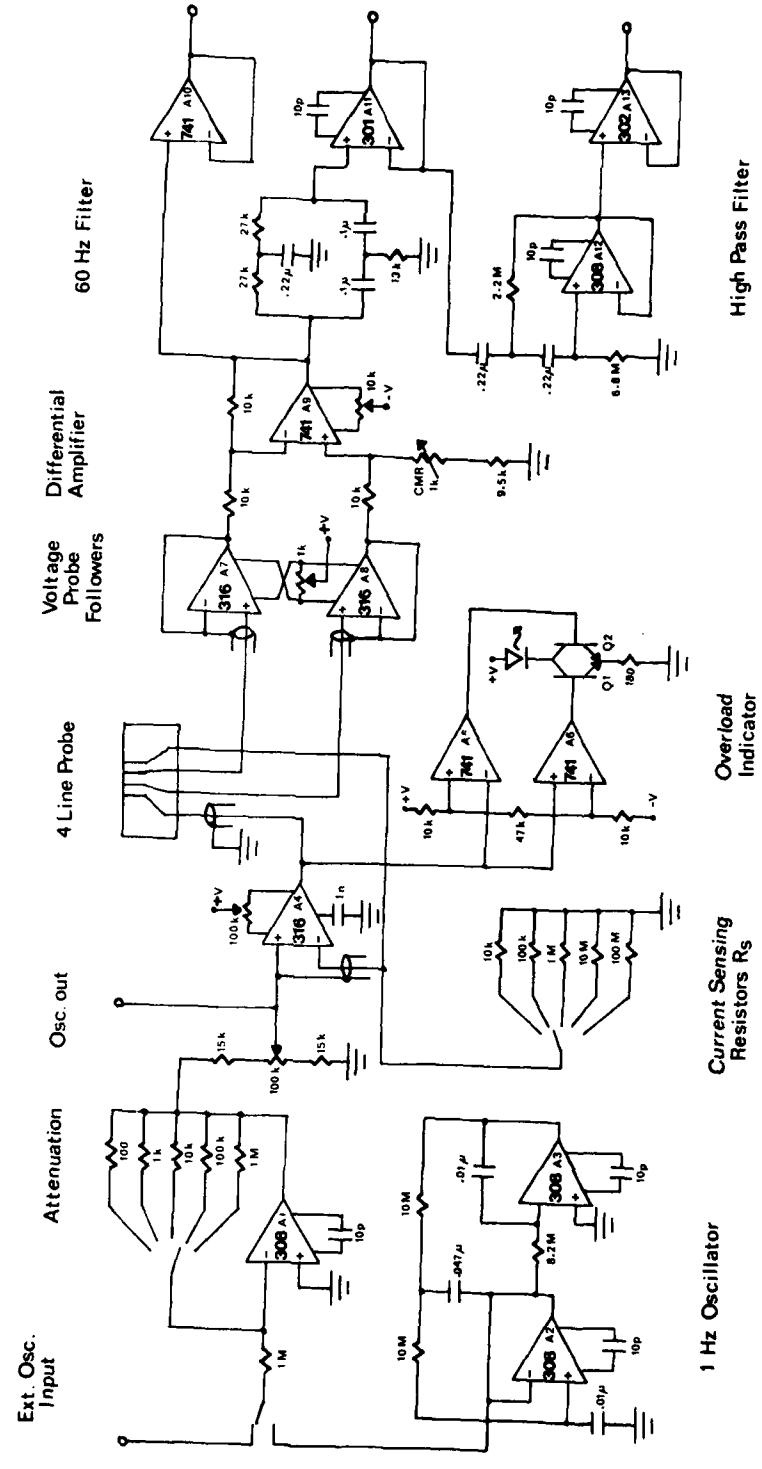


Figure 3. 4-Line Probe Circuitry

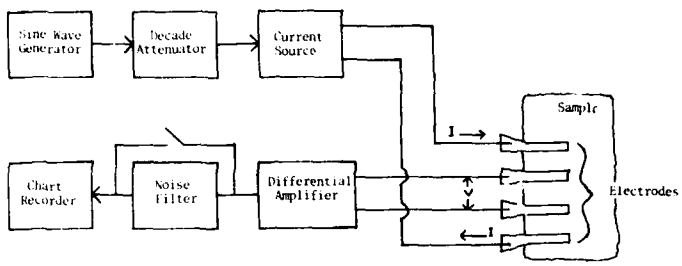


FIGURE 4a: Block diagram of probe electronics

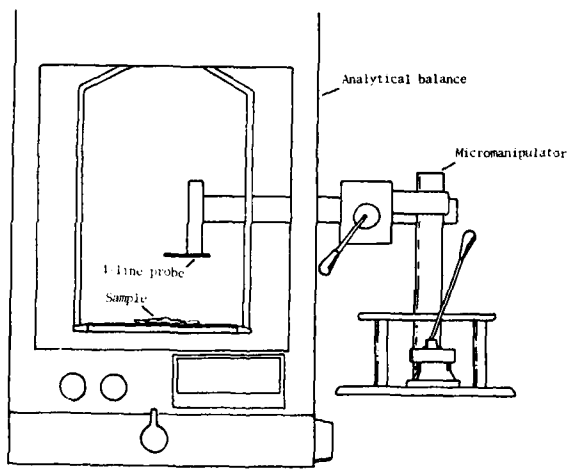


FIGURE 4b: In vitro test station

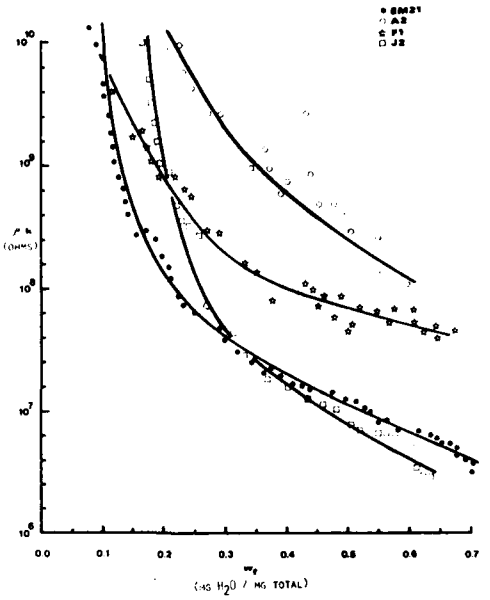


FIGURE 5a: In vitro test results from five subjects using desorption method

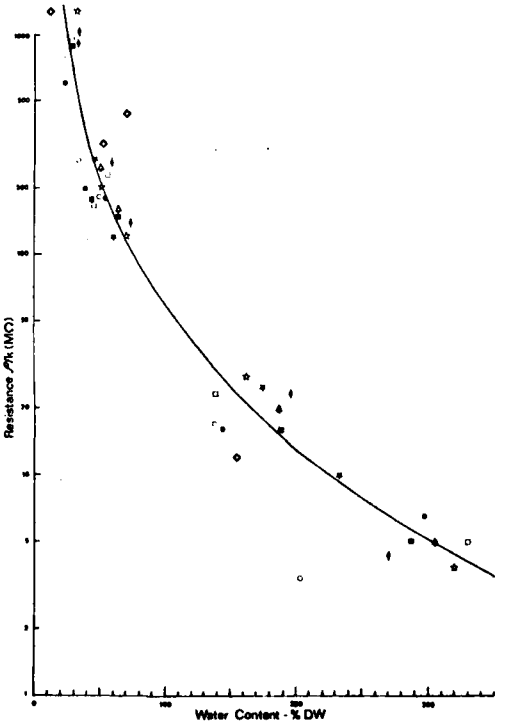


FIGURE 5b: In vitro test results from nine subjects using equilibrium method

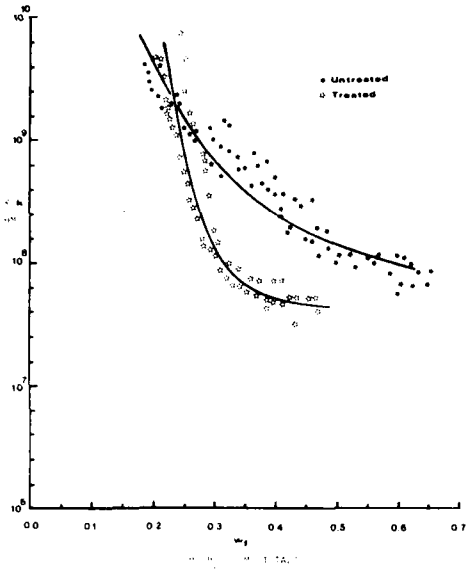


FIGURE 6a: Resistivty vs water content before and after formaldehyde treatment

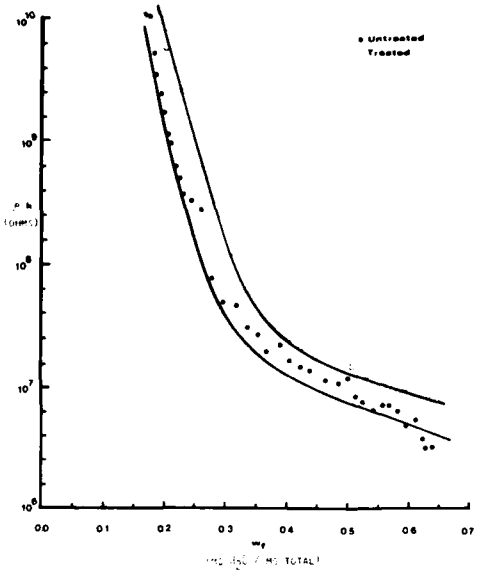


FIGURE 6b: Resistivity vs water content before and after urea treatment

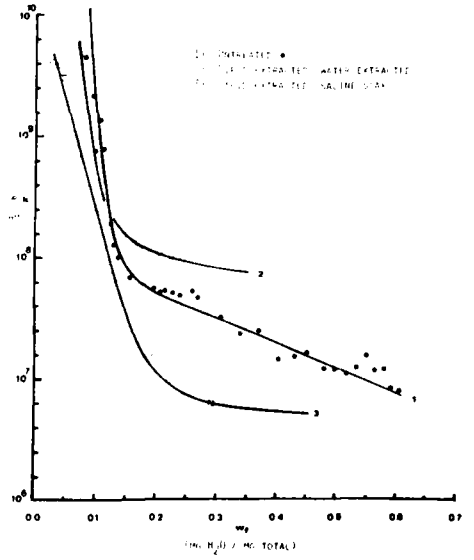


FIGURE 6c: Resistivity vs water content before and after lipid extraction followed by distilled water and saline soak

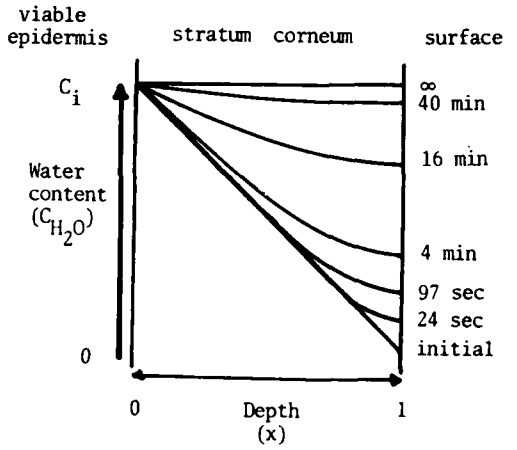


FIGURE 6d: Calculated effect of continued surface occlusion on relative changes in the water concentration gradient with time in intact stratum corneum. Assumed l=0.15 mm, D= 9.3 x 10^{-8} cm^2-sec^{-1}, τ =16 minutes.

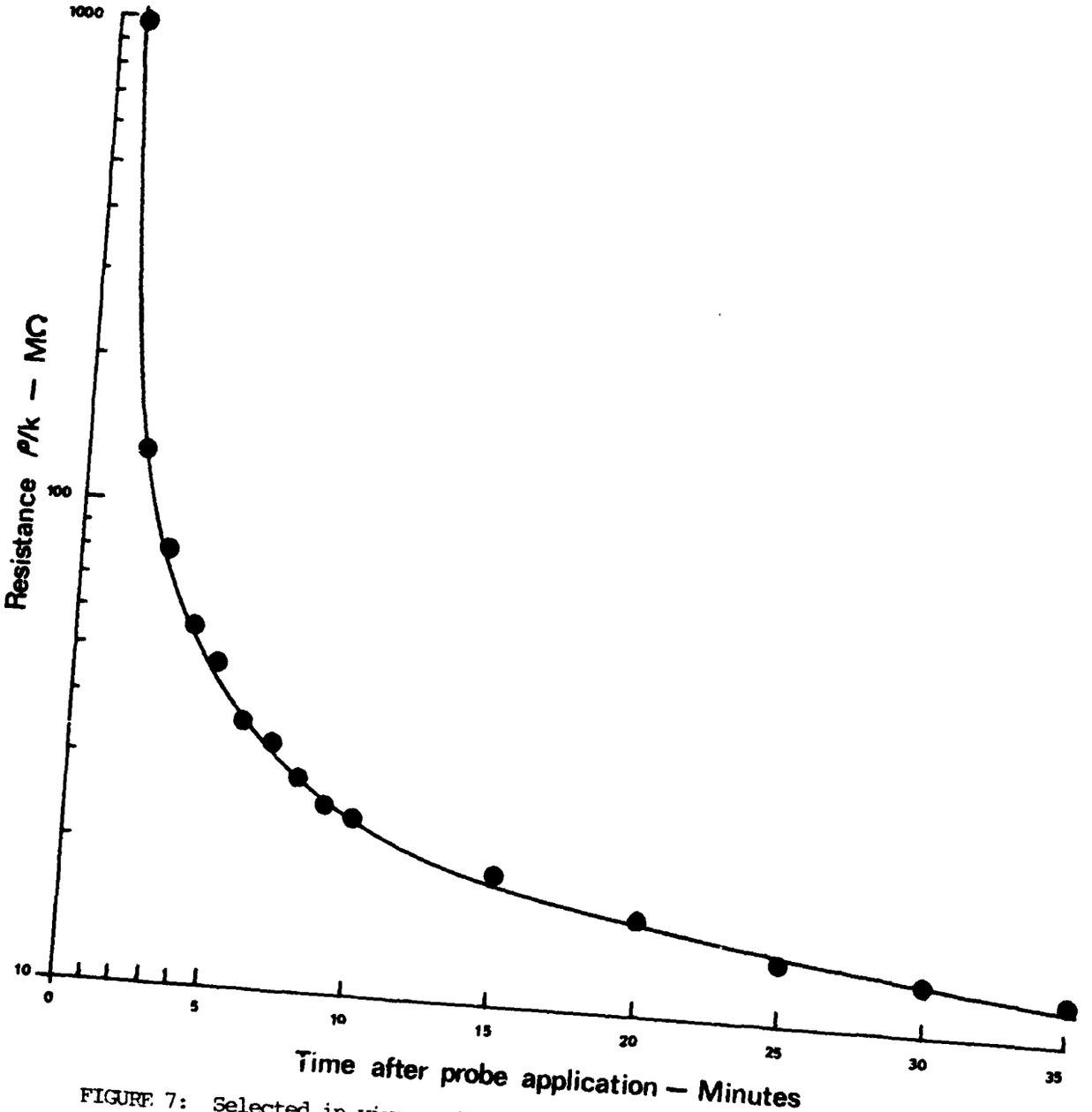


FIGURE 7: Selected in vivo application curve to show occlusive effects of probe on electrical resistivity

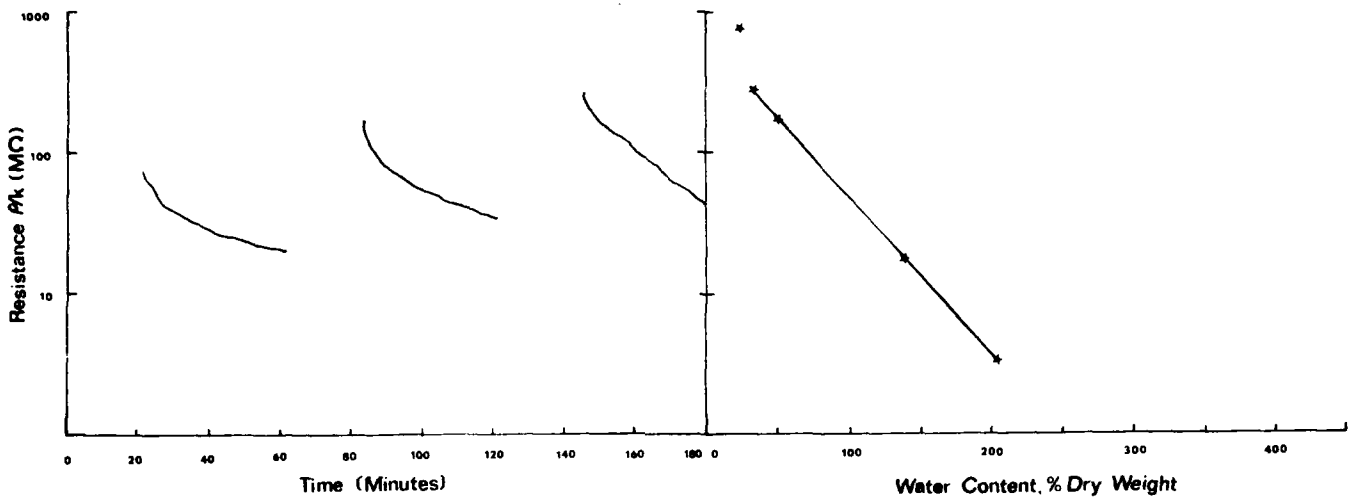
LEGEND FOR FIGURES 8-15

Figures 8-15 are the results of studies on eight different subjects to illustrate the use of the resistivity transfer method for estimating water content *in vivo*. There were three periods of probe application each lasting forty minutes. Prior to each measurement period the test site was dried for a twenty minute period with a flow of dry nitrogen gas at 2 L/min. Following the study, a 0.4 mm thick section of the test site was removed for calibration with the equilibrium method.

UPPER LEFT: Time course of resistivity changes at test site during *in vivo* study

UPPER RIGHT: Relationship between resistivity and water content at test site during *in vitro* calibration experiments

LOWER: Derived time course of water content during *in vivo* study


1000
100
10
Resistance Rk (MΩ)
0
20
40
60
80
100
120
140
160
180
Time (Minutes)
0
100
200
300
400
Water Content, % Dry Weight

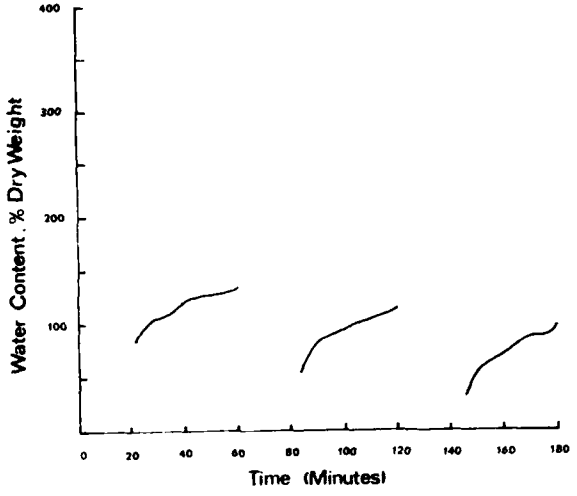
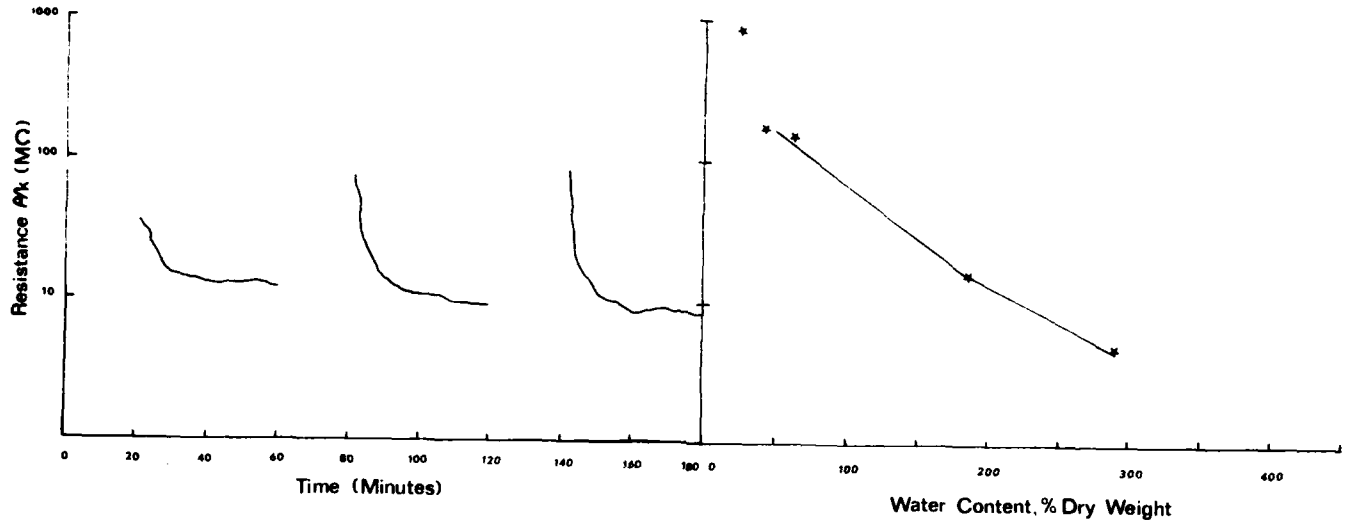

400
300
200
100
Water Content, % Dry Weight
0
20
40
60
80
100
120
140
160
180
Time (Minutes)

Fig 8


Resistance Rx (MΩ)
1000
100
10
0
20
40
60
80
100
120
140
160
180
Time (Minutes)
0
100
200
300
400
Water Content, % Dry Weight

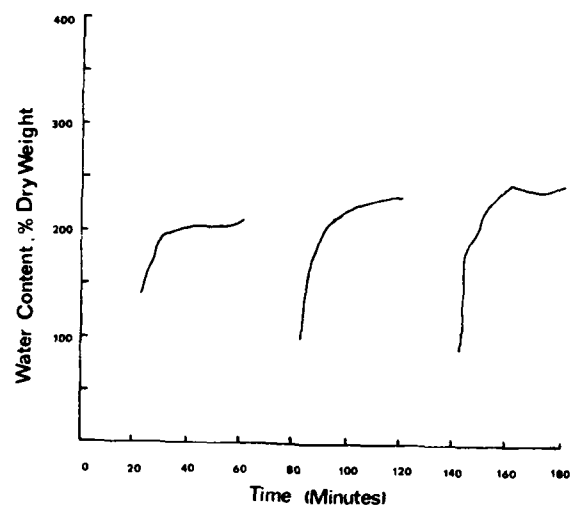
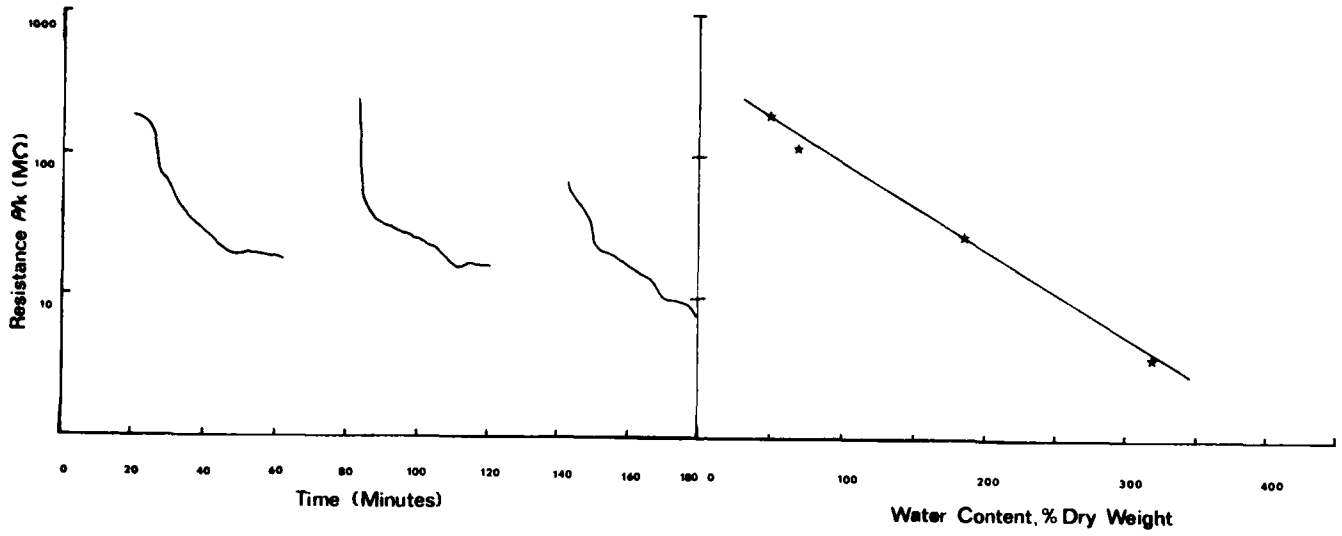

Water Content, % Dry Weight
400
300
200
100
0
20
40
60
80
100
120
140
160
180
Time (Minutes)

Fig. 9


1000
100
10
Resistance Rk (MΩ)
0
20
40
60
80
100
120
140
160
180
Time (Minutes)
0
100
200
300
400
Water Content, % Dry Weight

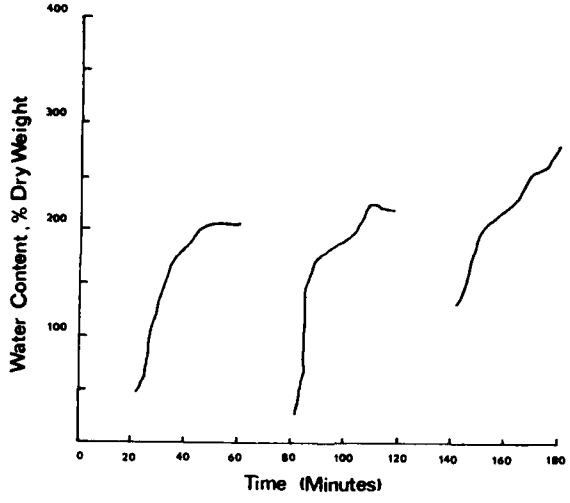
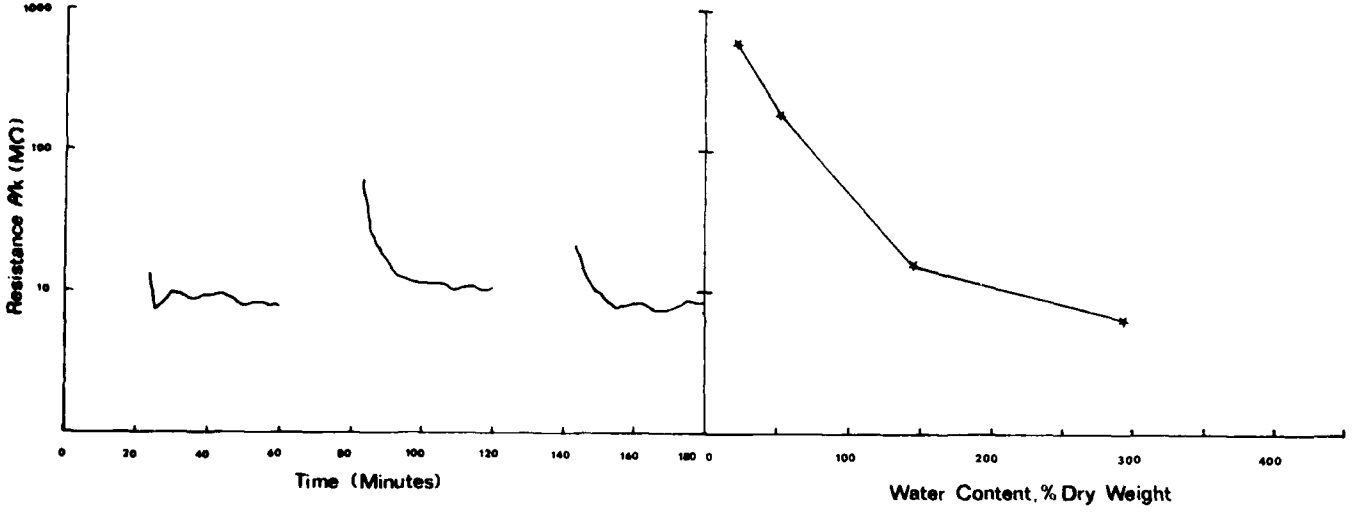
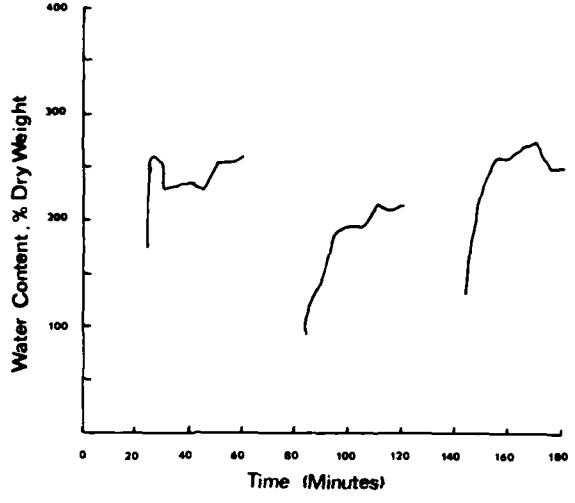

400
300
200
100
Water Content, % Dry Weight
0
20
40
60
80
100
120
140
160
180
Time (Minutes)

Fig. 10


Resistance Ak (MΩ)
1000
100
10
0
20
40
60
80
100
120
140
160
180
Time (Minutes)
0
100
200
300
400
Water Content, % Dry Weight


Water Content, % Dry Weight
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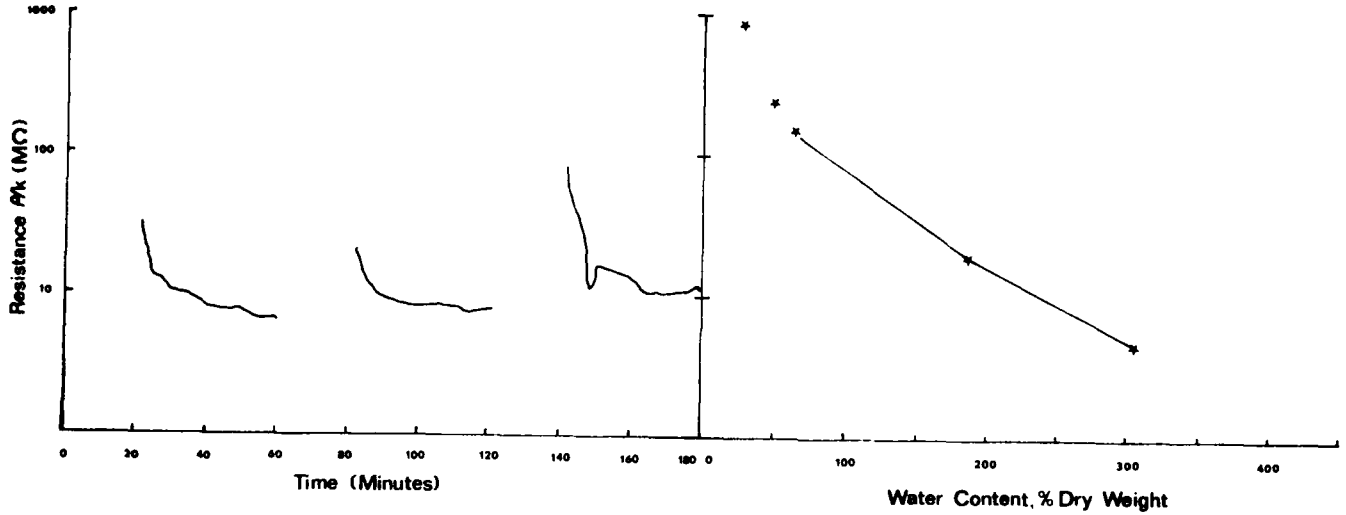
300
200
100
0
20
40
60
80
100
120
140
160
180
Time (Minutes)

Fig. 11


Resistance Rx (MΩ)
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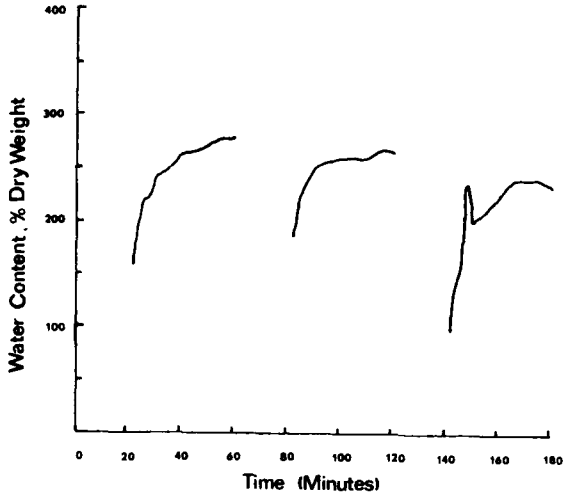
100
10
0
20
40
60
80
100
120
140
160
180
Time (Minutes)
0
100
200
300
400
Water Content, % Dry Weight


Water Content, % Dry Weight
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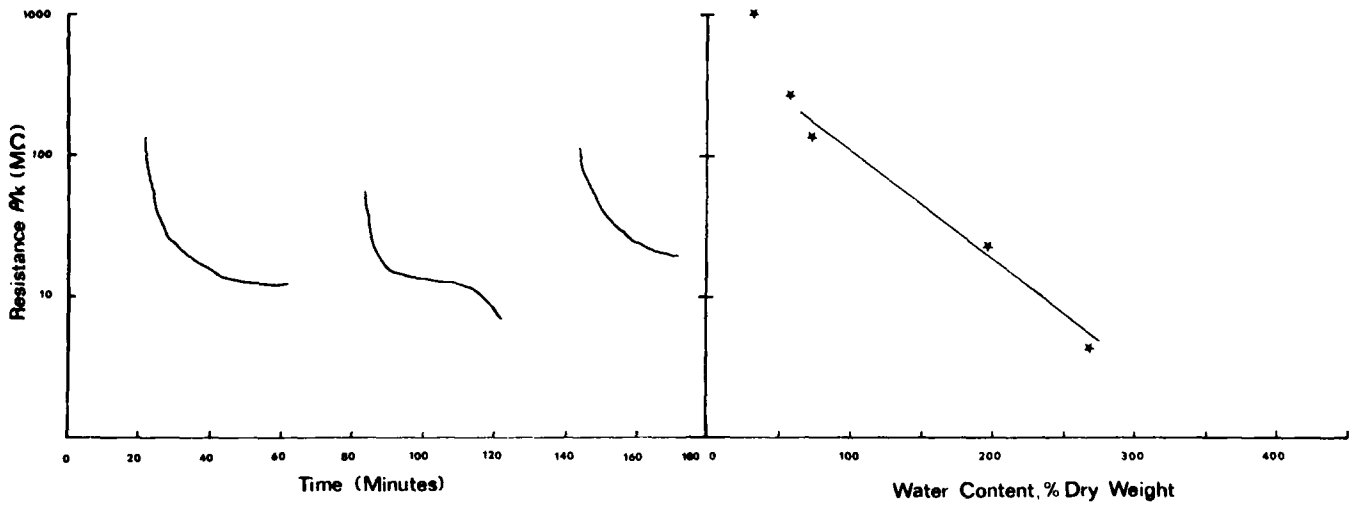
300
200
100
0
20
40
60
80
100
120
140
160
180
Time (Minutes)

Fig. 12


1000
100
10
Resistance Rk (MΩ)
0
20
40
60
80
100
120
140
160
180
Time (Minutes)
0
100
200
300
400
Water Content, % Dry Weight

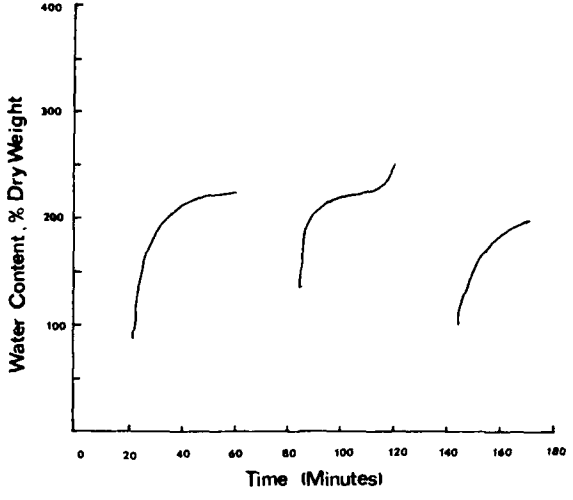
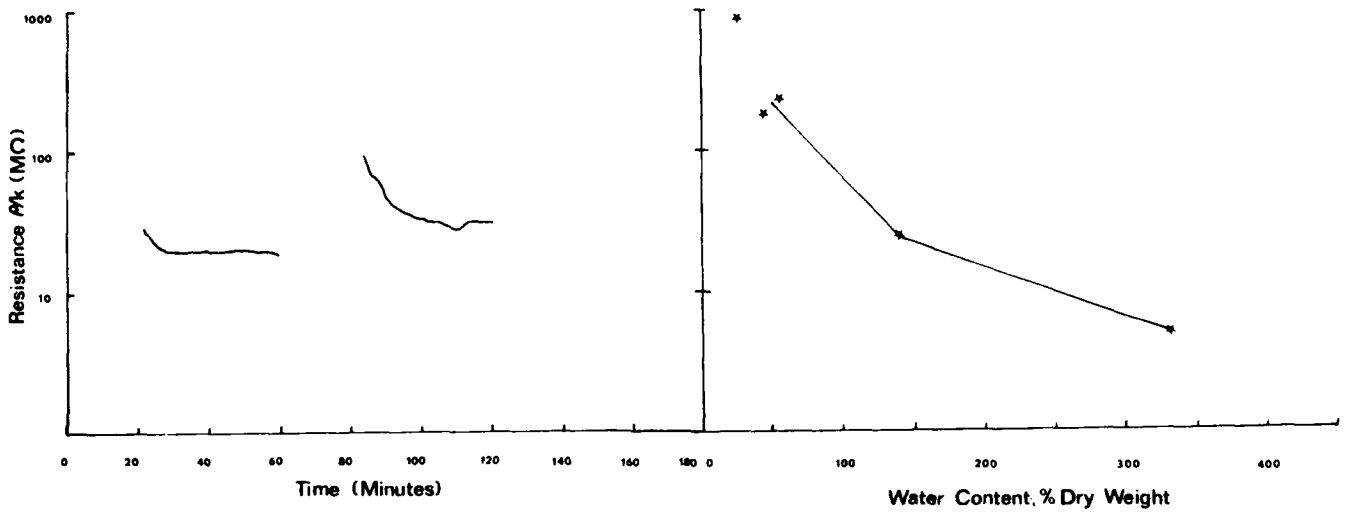

400
300
200
100
Water Content, % Dry Weight
0
20
40
60
80
100
120
140
160
180
Time (Minutes)

Fig. 13


1000
100
10
Resistance Rk (MΩ)
0
20
40
60
80
100
120
140
160
180
Time (Minutes)
0
100
200
300
400
Water Content, % Dry Weight

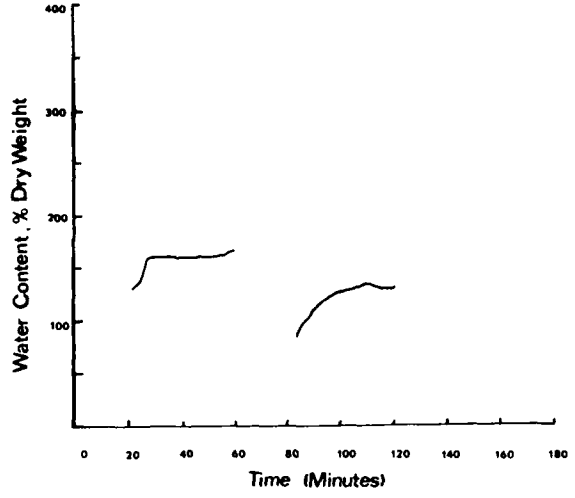
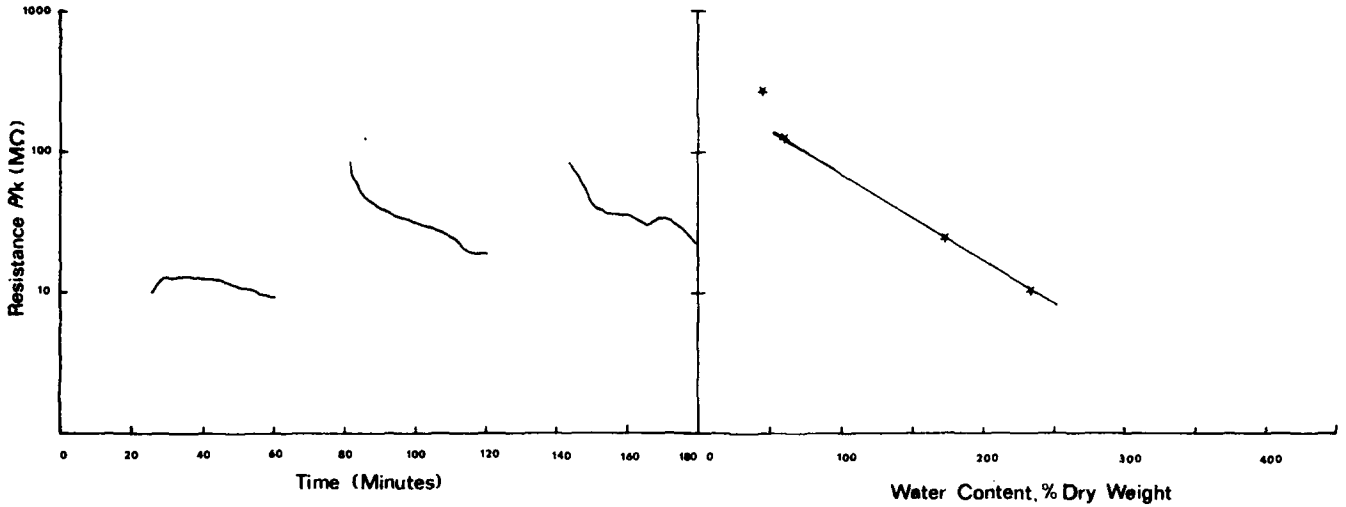

400
300
200
100
Water Content, % Dry Weight
0
20
40
60
80
100
120
140
160
180
Time (Minutes)

Fig. 14


1000
100
10
Resistance Rk (MΩ)
0
20
40
60
80
100
120
140
160
180
Time (Minutes)
0
100
200
300
400
Water Content, % Dry Weight

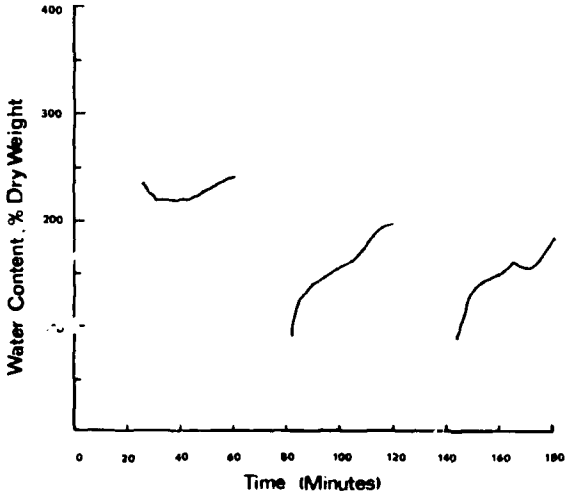

400
300
200
Water Content, % Dry Weight
0
20
40
60
80
100
120
140
160
180
Time (Minutes)

Fig. 15

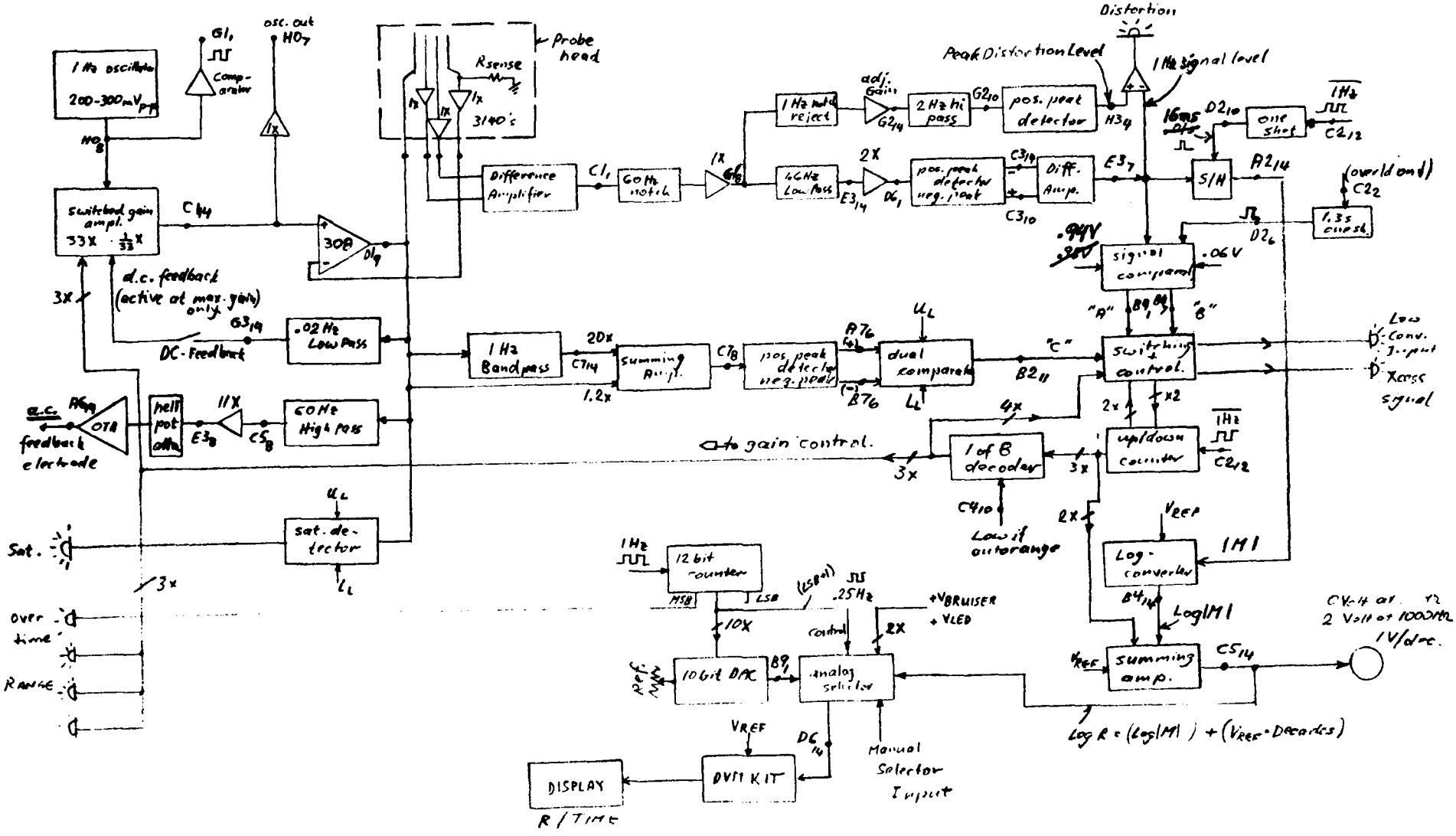


FIGURE 16: BLOCK DIAGRAM OF DEVICE TO MEASURE STRATUM CORNEUM RESISTANCE

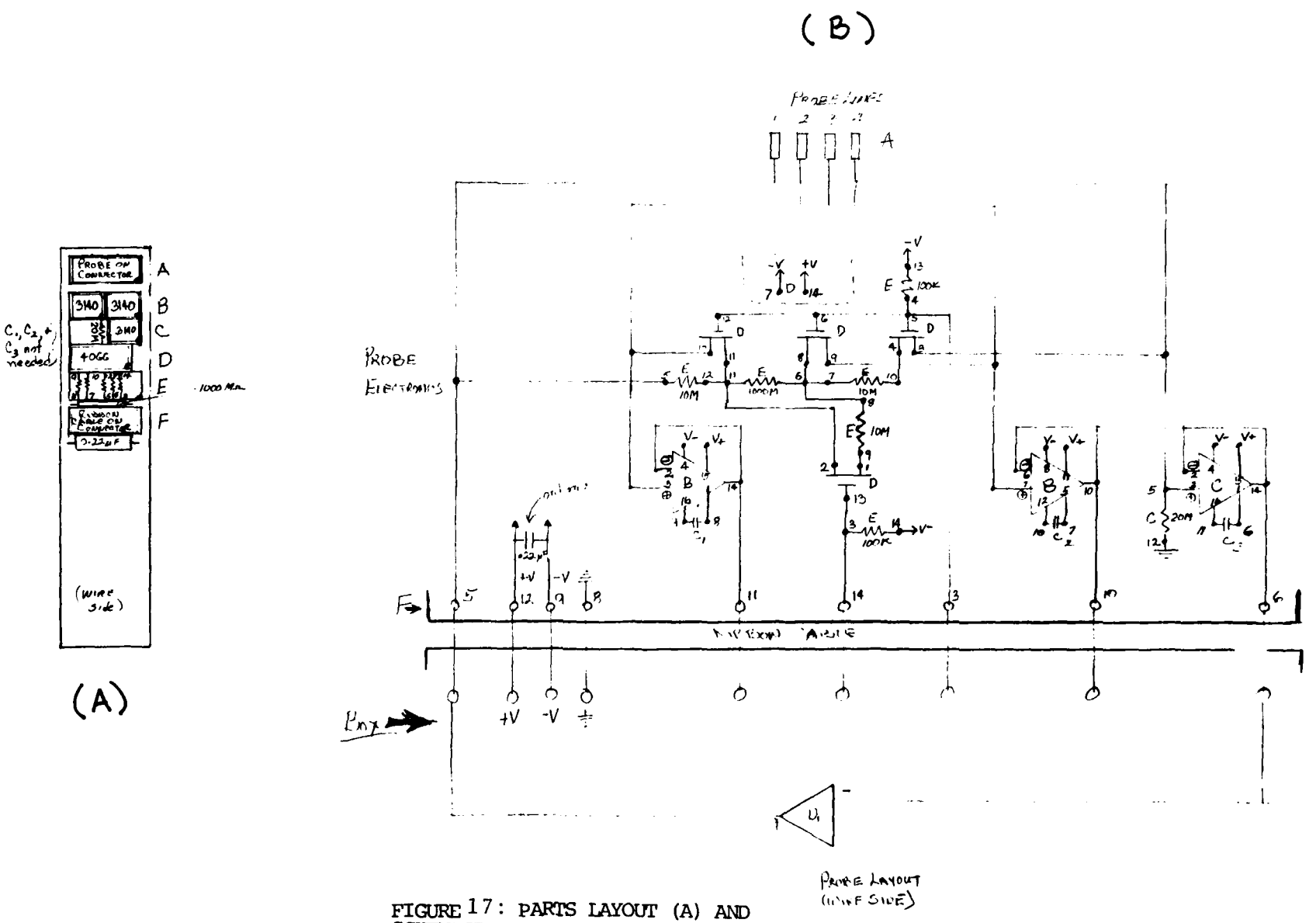


FIGURE 17: PARTS LAYOUT (A) AND SCHEMATIC DIAGRAM (B) OF PROBE HEAD

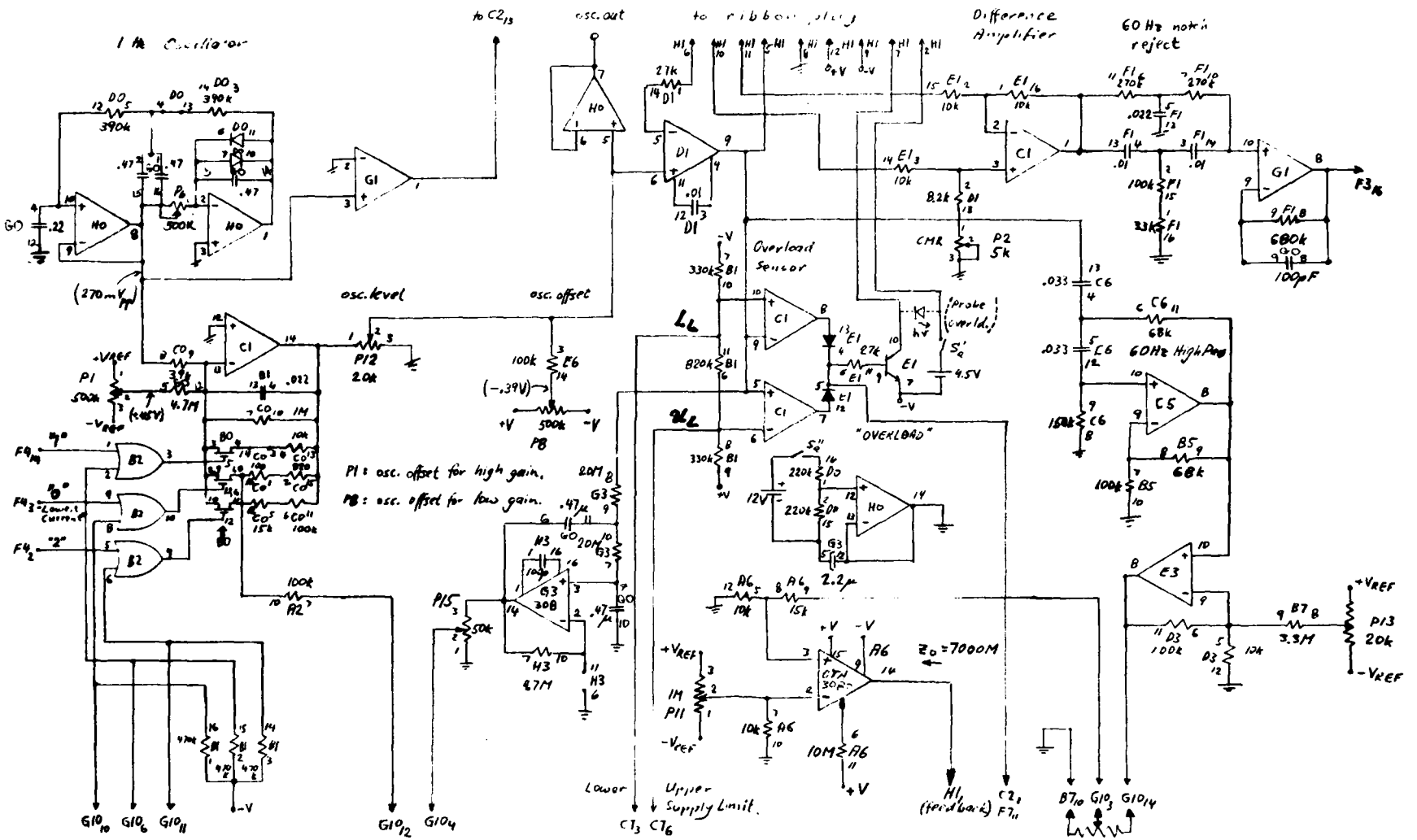


FIGURE 18: RESISTANCE DEVICE SCHEMATIC
PART 1

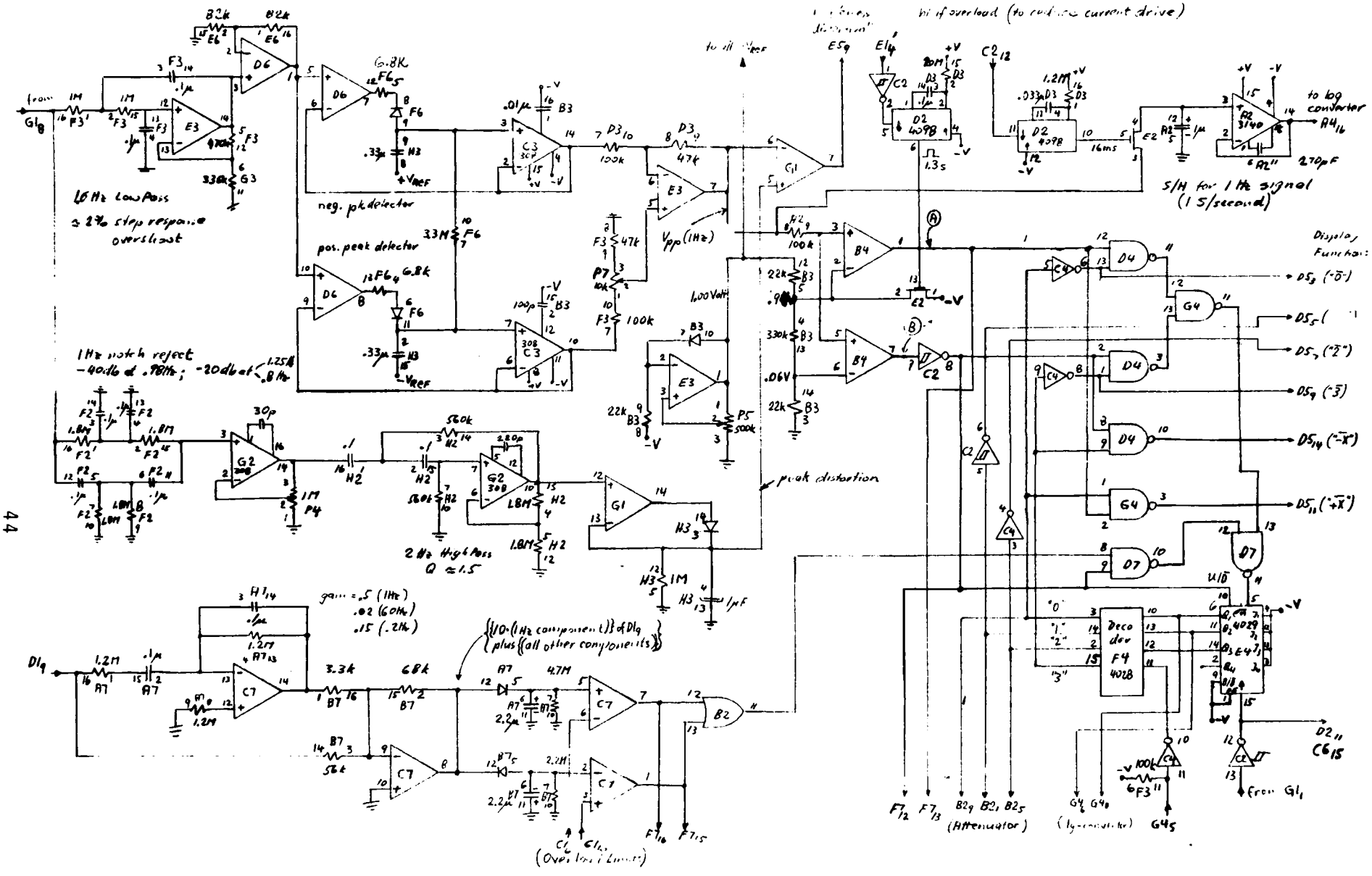


FIGURE 19. RESISTANCE DEVICE SCHEMATIC
PART 2

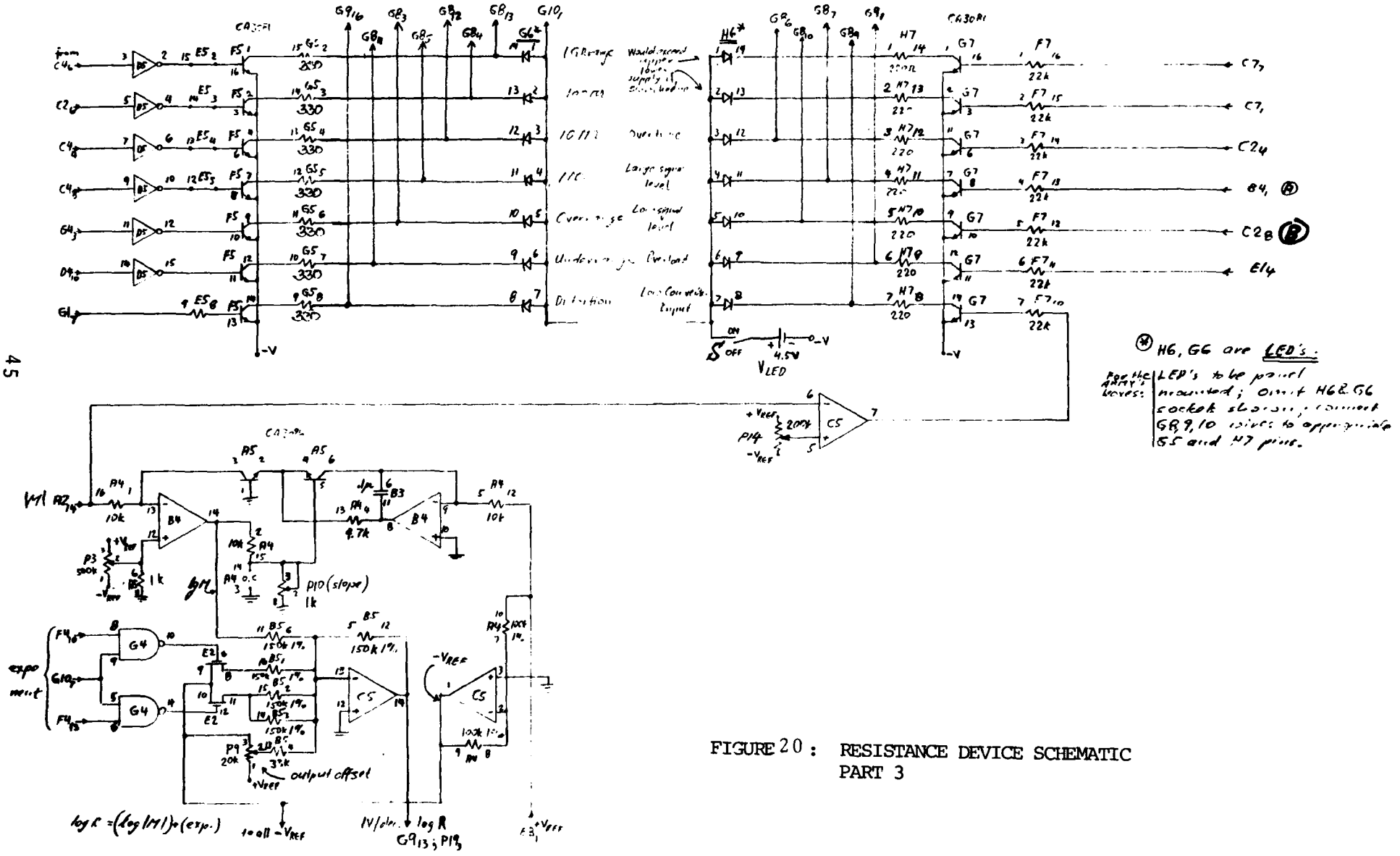


FIGURE 20 : RESISTANCE DEVICE SCHEMATIC
PART 3

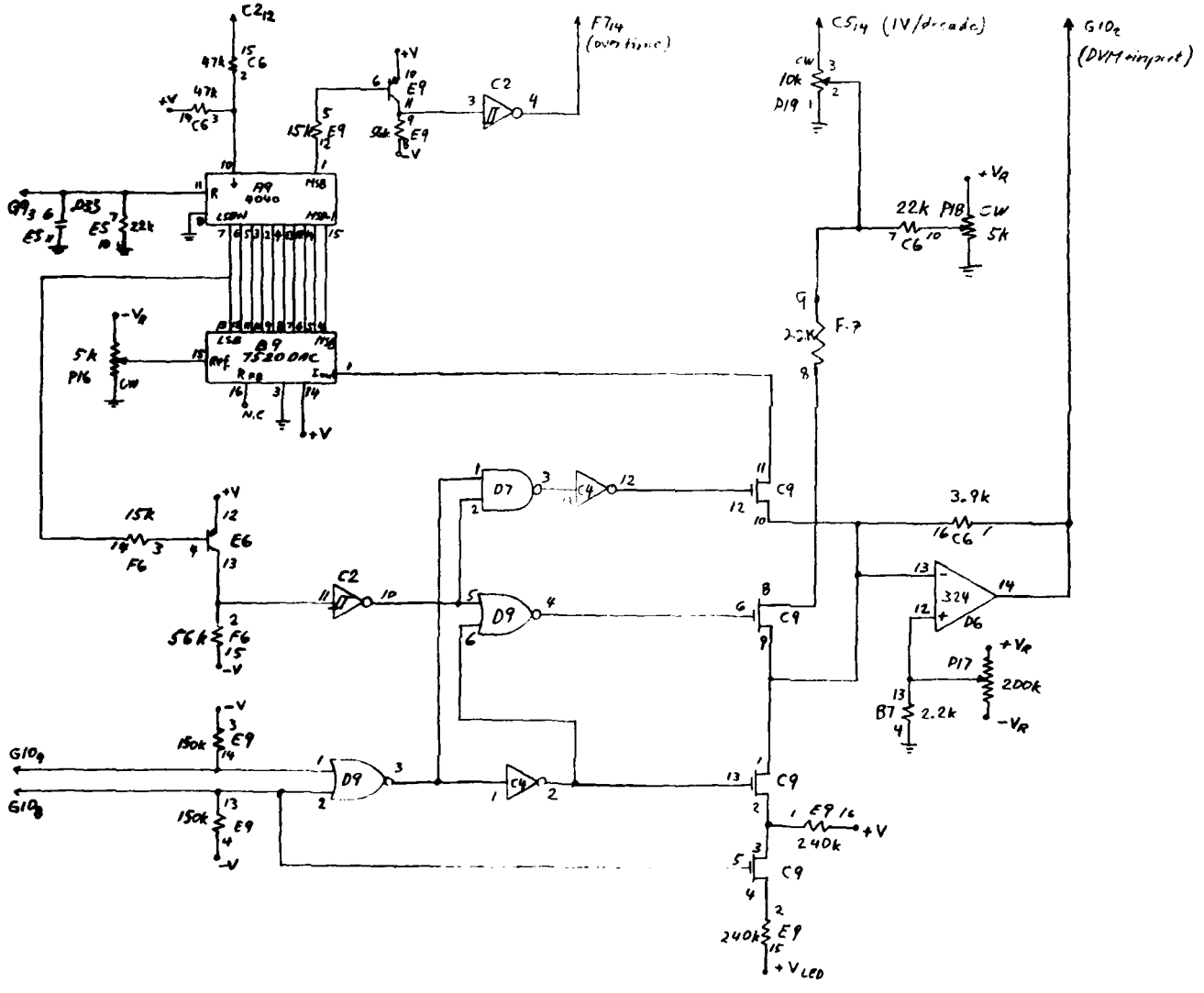
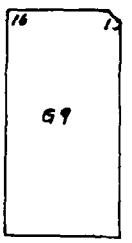


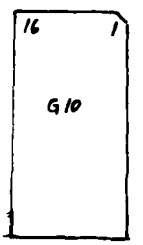
FIGURE 21. RESISTANCE DEVICE SCHEMATIC
PART 4

Ribbon Cable Connectors to Chassis Box.

pin #	function	is connected to
16	current source electrode	$H1_5$
1	potential probe #1	$H1_{11}$
15	" " #2	$H1_{10}$
2	current sink electrode	$H1_6$
14	ground →	$G3_{12}$
3	LED #1 : overrange	$G6_{10}$
13	2 : 1000 MΩ range	$G6_{14}$
4	3 : 100 "	$G6_{13}$
12	4 : 10 "	$G6_{12}$
5	5 : 1 "	$G6_{11}$
11	6 : underrange	$G6_9$
6	7 : overtimer	$H6_{12}$
10	8 : Low signal level	$H6_{10}$
7	9 : Excess " "	$H6_{11}$
9	10 : Low Converter Input	$H6_8$
8	neg. supply terminal (-V)	$F5_5$



16	LED #12 : Distortion	$G6_8$
1	11 : Saturation	$H6_9$
15	{calibration range switch} →	$H1_{14}$
2		$H1_3$
14	calibration/run/reset	$H1_3$
3	selector switch	$F7_9$
13	Testpoint #1 : Log R[V/dec.]	$C5_{14}$
4	2 : not defined	
12	3 : "	
5	4 : $+V_{REG}$ (1Volt)	$E3_1$
11	} not connected	
6		
10		
7		
9		
8		



16	positive supply voltage (+V)	$E3_4$
1	LED supply voltage ($+V_{LED}$)	$G6_1$
15	neg. DVM Input (→)	$C5_{12}$
2	pos. DVM "	$D6_{14}$
14	#3 terminals of	$E3_8$
3	#2 10k helipot	$A6_9$
13	#1 for feedback	$B7_{10}$
4	d.c. feedback switch →	to slider of 50k pot. (P15)
12		$A2_7$
5	Manual / MΩ range	N.C.
11	" 10 "	$B2_6$
6	" 100 "	$B2_2$
10	" 1000 "	$B2_8$
7	Autorange	$G4_5$
9	Battery test (4V)	$E9_{14}$
8	" " ($+V_{LED}$)	$E9_{13}$

FIGURE 22. CONNECTIONS TO RIBBON CABLES

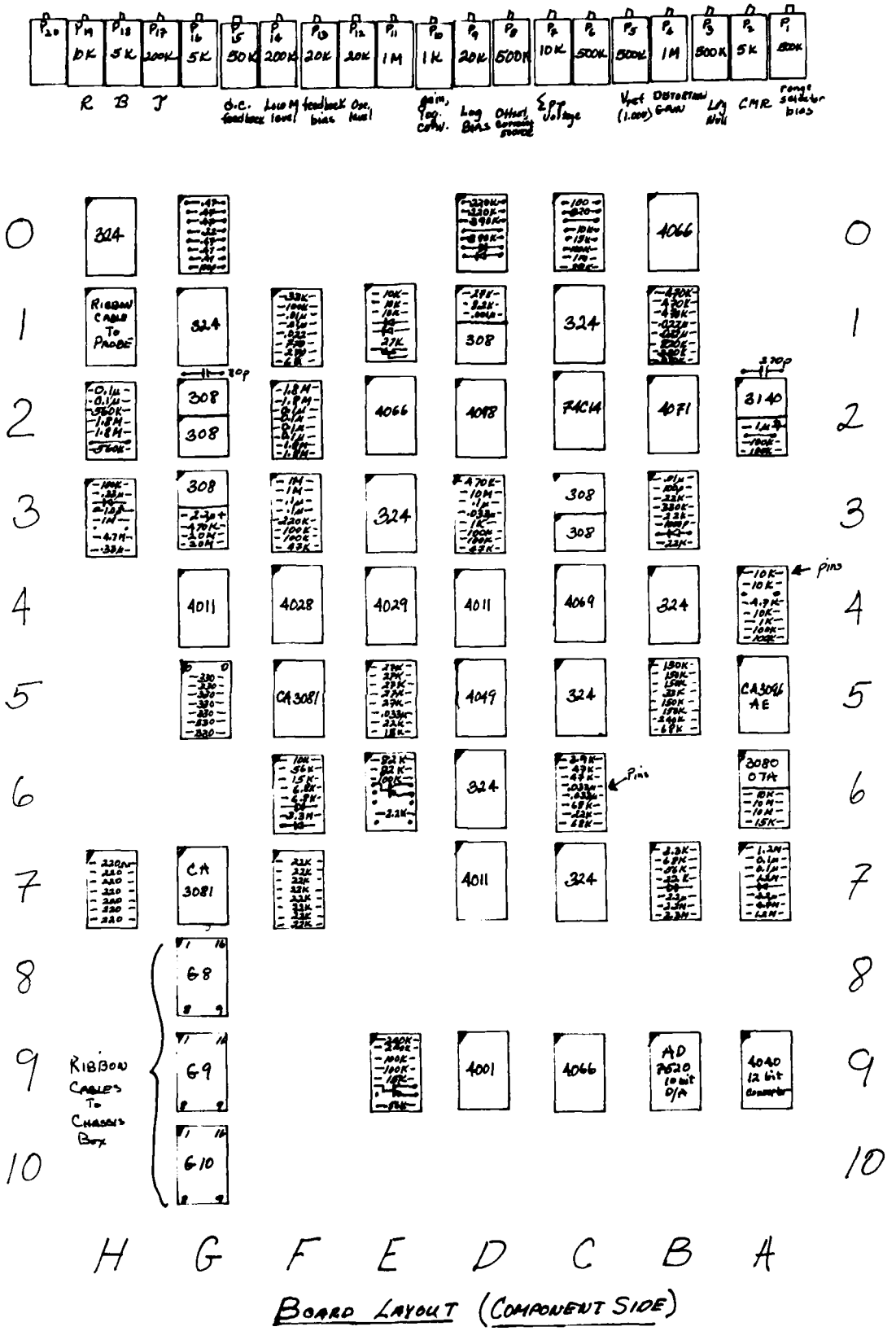


FIGURE 23. COMPONENT LAYOUT: MAIN CIRCUIT BOARD

V. BIBLIOGRAPHY

Publications Arising From This Project

Kraning KK, II: Heat conduction in blackened skin accompanying pulsatile heating with a xenon flash lamp. J. Appl. Physiol. 35:281-287, 1973.

Campbell SC, Kraning KK, Schibli EG, Momii ST: Hydration characteristics and electrical resistivity of stratum corneum using a noninvasive four-point microelectrode method. J. Invest. Derm. 69:290-295, 1977.

Kraning KK, Schibli EG, Momii ST, Merrick EA: A gas-stream heater to control local skin temperature under evaporative sweat capsules. J. Appl. Physiol. 44:981-985, 1978.

Personnel Supported in Part by this Project

Kenneth K. Kraning, Sc.D., Principal Investigator
Eugen G. Schibli, Ph.D., Associate Investigator
Steven C. Campbell, Ph.D., Research Associate

Graduate Degrees Supported by this Project

Steven C. Campbell, M.S. E.E.,
University of Washington, 1978.

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Stratum Corneum	Resistivity
Water Content	Measurement

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

We have developed a method to measure the water content of the stratum corneum of intact skin. In its present form the technique is a transfer method. Like many other transfer measurements in medicine the technique is most accurate when individually calibrated with samples of the subjects own tissue. Electrical resistivity is accurately measured *in vivo* during the

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course of experimental procedures using a four-fingered micro-electrode probe developed for this purpose. Calibration *in vitro* on samples taken from the same site is made by comparing resistivity vs. total water content measure gravimetrically. Differences in conduction properties of different samples are not now predictable, and can cause large estimate errors if a single calibration curve is used for all subjects. The transfer method eliminates this source of error. Present limitations on the accuracy of the transfer method arise from uncertainties in regional water distribution during calibrations.

The method is restricted in theory to stratum corneum thicker than the spacing of the electrode fingers. With present electrodes this theoretical restriction limits the use to the palms and soles. Future emperical testing of these electrodes and the manufacture of smaller electrodes may permit application of the method to areas of thin stratum corneum as well.

Procedures and sample data are presented to demonstrate the application of the method on human skin. Complete documentation of electronic devices constructed for Lettermann Army Institute of Research for field studies is provided.