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A STUDY OF THE DIELECTRIC CONSTANT AND FORCE-SHRINKAGE BEHAVIOR OF SKIN AS FUNCTIONS OF TANNAGE AND MOISTURE CONTENT

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by

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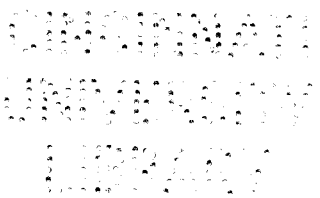

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A STUDY OF THE DIELECTRIC CONSTANT AND FORCE-SHRINKAGE BEHAVIOR OF SKIN AS FUNCTIONS OF TANNAGE AND MOISTURE CONTENT

Abstract

Two of the properties of skin collagen which are of fundamental interest to the biochemist, and of practical concern to the tanner, are the water-holding power of the protein, and the shrinkage temperature phenomenon. It was felt that the most worthwhile results could best be obtained by employing the most modern instrumental methods. In consequence a large part of the work was devoted to the development of electronic instrumentation, to aid in obtaining the desired information. The versatile high frequency oscillator titrimeter was adapted for the dielectric constant work on the water relationships of collagen, and an electrical strain gauge, and associated high-gain electronic amplifier, was developed to study the force shrinkage behavior of the collagen.

Any discussion of protein-water relationships leads inevitably to a consideration of "bound" water. This aspect has received a great deal of attention in the literature, and it is encountered in all natural fibrous materials, and is of considerable biological importance. The theoretical background and possible mechanisms are discussed.

To obtain an accurate quantitative picture, it is necessary to have a reliable method for the determination of total moisture. The difficulty lies in the fact that it is not always possible to remove the last increments of moisture, except under conditions so arduous as to denature or disaggregate the protein. In this study, however, it was found possible to obtain an accurate picture of the total water removable under a variety of conditions. The technique employed was the solvent distillation procedure; by the use of a number of solvents, whose boiling points covered a wide range, equilibrium values for moisture removable under the specified conditions were obtained. The amount of degradation of the protein (raw and chrome-tanned) was studied concurrently. The attainment of equilibrium values for each set of conditions leads to the conclusion that there is such a phenomenon as bound water in the physical chemistry of collagen, and that its quantitative relationship is that of a continuous function, rather than a discrete stoichiometric behavior. The chrome tanned skin yielded results which were identical to those of the untanned collagen.

To further investigate the bound water question, and to add to the available information on the knowledge of the mechanism of chrome tannage, the high frequency dielectric constants of raw and chrome tanned calfskin were studied as functions of their moisture contents. Inasmuch as a porous,

fibrous material such as skin or leather is about 2/3 free space, a direct measurement of the dielectric constant in a parallel plate condenser yields only an apparent dielectric constant. An immersion technique was used to measure the true dielectric constants. This involved placing the skin pieces in solvent mixtures of known dielectric constant values, and then measuring the positive or negative changes in instrument reading produced by the addition of the skin pieces to the solvent. The dielectric constant of the solvent whose instrument reading was not changed by the addition of the skin could then be determined graphically. This value was taken as equal to the dielectric constant of the skin or leather.

The method requires solvents which will not react with the protein, and which are immiscible in water. This limits the immersion technique; in practice, immersion measurements on samples containing as high as 25-30% moisture were made. The apparent readings were made on samples containing as high as 50% moisture. An empirical relationship was derived betweem the true and apparent readings, which permits estimation of the true values to be made up to 50% moisture content. The method of apparent readings, with suitable calibration has proved to be useful as a potential practical plant method for rapid determination of the moisture content of leather.

The results of this phase of the work are further evidence that the water binding power of the protein is a continuous function. The raw and chrome tanned skin exhibit identical dielectric constant values with changes in moisture content.

The past work on the shrinkage temperature of collagen is reviewed and discussed. The existing technique for studying this phenomenon was found to be inadequate for increasing our understanding of the mechanism; therefore the electronic shrink meter was constructed. The changes in the wet shrinkage temperature of raw and chrome-tanned calfskin as a function of time and temperature of heating were studied; the results correlate with other studies which suggest that tannage, in some aspects at least, does disorient the molecular organization of collagen.

The dry shrinkage temperature was studied by heating the samples in an inert heat transfer medium. The results showed that chrome tannage does not change the dry shrinkage temperature of the skin, although it does raise the wet shrinkage temperature by 40°C. and more.

These studies led to the following conclusions:

1 - A portion of the water that is capable of being held by the skin protein is "bound". The firmness of binding decreases continuously with increasing moisture content. Added increments of water above a moisture content of about 33% are in a relatively free state.

2 - The water relationships, the dry shrinkage behavior, and all the available quantitative chemical data

point to the fact that chrome tanning does not alter to a significant extent, many of the basic functional properties of the skin protein. This is taken to be further evidence that the theory of the physical deposition of the 2/3 basic chrome sulfate in and around the collagen fibers, is the most likely mechanism of chrome tanning.

A STUDY OF THE DIELECTRIC CONSTANT AND FORCE SHRINKAGE BEHAVIOR OF THE SKIN AS FUNCTIONS OF TANNAGE AND MOISTURE CONTENT

I - HISTORICAL AND THEORETICAL DISCUSSION

A - Introduction

The conversion of the collagen* of animal skin into leather is accomplished by a number of materials of widely differing chemical composition. In no case is the actual mechanism of this change satisfactorily understood. In practice the chief accomplishment of tanning is the conversion of the raw skin or hide into a material which is imputrescible when wet, and which can dry out from the wet state without acquiring a horniness and harshness due to fiber adhesions such as ensue when raw skin is treated in similar fashion.

* - The term "collagen" as used in this paper, refers, except where otherwise indicated, to cattle skin or hide which has undergone the beamhouse phases of tannery process, and is in a condition where it is ready to receive the tanning material. The isoelectric point of so-called "native" bovine hide collagen had been determined by Highberger (1) and Beek and Sookne (2) to be in the range of pH 7-8. The isoelectric point of collagen after it has been through the beamhouse processes is about pH 5. This is thought to be due to the loss of some of the acid amide groups of glutamine and aspargin in the alkaline liming treatment which is used to remove the hair.

The immense importance of the relationship between the skin protein and water to the practical tanner is thus immediately apparent. This relationship is also of fundamental interest to the biochemist in his attempt to understand protein structure and reactivity. There is an important corollary concept that stems from this protein-water relationship, which may be generalized: in all natural fibrous materials, and in many other classes of organic and inorganic substances, some portion of the water which is associated with the parent substance is held by that material in such a manner as to prevent the water from fully displaying the properties it exhibits in a completely free state. The terminology applied to this water is "bound" water, and any discussion of protein structure and reactivity must take this phenomenon into account. This aspect will be discussed more completely a little later.

The principal protein of the skin or hide, collagen, possesses a property which is unique among natural fibrous proteins. When it is placed in water, and the temperature of the water is gradually elevated, there occurs, in the region about 60°C. a relatively sharp shrinkage of the skin. If heating is continued, the collagen will be solubilized and converted to gelatin.

Tannage of collagen produces changes in the temperature at which shrinkage occurs. Certain chrome tanning procedures produce a piece of skin which is practically shrinkproof in boiling water for several minutes; almost any acceptable chrome tannage produces a leather whose shrinkage temperature is above 90°C. Chamois (oil-tanned) leather, on the other hand, exhibits a lower shrinkage temperature than untanned skin, although it is a very stable leather.

One aspect of this shrinkage temperature phenomenon has been almost entirely neglected in previous work. That is the dry shrinkage behavior, under conditions where little or no water is present. Investigation in this direction is capable of yielding worthwhile results concerning the denaturing effect of heat on collagen, and may also contribute to a further understanding of protein structure. In addition such data are necessary to complete the factual foundation that is required for any consideration of the meaning of the shrinkage temperature behavior.

The shrinkage temperature phenomenon, in addition to its significance to the tanner, has been the subject of a great deal of interest and speculation by protein chemists and physicists who are interested in the molecular organization of collagen and other fibrous proteins. The interest and the significance of the protein-water relationship, the

bound water concept, and the shrinkage temperature effect, are undeniable. The accuracy of the techniques hitherto employed to gather facts about these matters, and the information which is available is incomplete, and in many instances inconclusive and contradictory.

Therefore, it was felt that any study which would attempt to gain more information about these matters, and an inproved insight into the tanning mechanism, would require new and improved techniques. More careful control of experimental conditions, and a knowledge of the effects of changes in these conditions, were also required.

Progress in the application of electronic instrumentation to physical chemistry has made great strides in recent years. Two of the more recent developments were a wire resistance strain gauge, and a high frequency oscillator (3,4) for use in titrations and various chemical control procedures. An electrical strain gauge and an associated high gain electronic amplifier was developed and constructed for a study of the shrinkage temperature behavior. A high frequency oscillator titrimeter was developed and constructed, and then adapted for dielectric constant studies of skin and leather as functions of moisture content. These instruments and the techniques in which they were employed will be fully des-

cribed in the experimental section of this thesis.

A study of the nature of shrinkage temperature and bound water as they pertain to collagen behavior, and as they are affected by tannage, necessarily yields information which bears on the mechanism of tannage. In this study, the type of tannage which was chosen was that produced by chrome sulfate compounds. Chrome tannage was selected for the following reasons:

1) It is capable, when used alone, of producing a complete and satisfactory tannage. A very large percentage of commercial leather is chrome tanned.
2) A great deal of quantitative information regarding the fixation of hide and skin by chrome tanning materials is available in the literature. This is also true of the composition of chrome tanning materials. Such data enhances the possibility of accurate control of experimental conditions, and greatly aids in interpretation of results.
3) Chrome tannage produces the greatest elevation of the wet shrinkage temperature of any of the conventional methods of tannage, and thus permits

a wider latitude in a study of the shrinkage temperature phenomenon.

B - The Bound Water Concept

The subject of bound water has been the source of very great and diverse interest. Consequently there is a tremendous literature about this subject, and it contains a great deal of experimental data and theoretical speculation. An excellent and comprehensive review of the subject is to be found in the work of Gortner (5). In his textbook he discusses its biological significance, the techniques and methods which have been employed, and the possible mechanisms of binding that have been proposed.

From the biological point of view, the significance of bound water is exemplified by such diverse phenomena as the frost resistance of winter wheats, the existence of minimum moisture levels for the growth of microorganisms, and the possible role of changes of the bound water-free water equilibrium as a cause of fever.

The techniques which have been employed to study the bound water phenomena attempt to show, by a great variety of methods, that certain properties of the water associated with the substrate under consideration are of a magnitude which is significantly different from an equal amount of free

water. Among the physical factors which have been measured are: heats of fusion by calorimetric techniques, freezing point depressions, changes in volume by dilatometric means, osmotic pressure, specific heats, changes in solution concentration by polarimetric techniques, dielectric constant studies, heats of wetting and adsorption, x-ray diffraction and infra-red absorption spectra. From all these considerations, Gortner and others (5,6,7) conclude that there is very definite evidence for the existence of bound water in biological systems. In a great many instances, the quantitative data indicate that the uptake of water follows typical adsorption isotherms, rather than stepwise stoichimetric relations such as are to be found in the case of the hydrates of copper sulfate. This means that at very low levels of moisture content, the water is relatively firmly bound. Additional increments of water are progressively less firmly bound; while the exact amount of bound water is almost impossible to define sharply, the methods of measurement do indicate the approximate range where additional increments of water will exist in an essentially free state.

The only serious objection to the concept of bound water to be found in the literature is that of Blanchard (8).

Actually his objection is based only on the results obtained from the use of the cryoscopic technique. He postulates that supercooling is possible, and would produce results which could mistakenly be interpreted on the basis of the existence of bound water. Odd and extreme cases of supercooling are cited in support of this position. Whatever the validity of his objection to the cryoscopic technique, the rejection of the existence of bound water in its entirety has relatively little validity in view of the great mass of evidence, obtained from many different methods, which is available to support the existence of bound water.

Bull (10) studied the water vapor adsorption of several proteins, including collagen. He found that they obeyed the behavior predicted by the Brunauer-Emmett-Teller equations for adsorption isotherms.

Gelatin gels have been extensively studied from the viewpoint of bound water (9). A great many of the conclusions obtained have been applied to collagen. Actual studied of the bound water behavior of collagen and leather are less numerous, but there are studies which are worthy of mention. Green (11) studied the water vapor adsorption of chrome tanned hide powder, and came to the conclusion that within the range of water uptake which is possible from the vapor phase, the

affinity of water for collagen is not diminished by chrome tannage.

Eilers and Labout (12) studied what they termed the "non-solvent" water in hides. Essentially their technique consisted of immersing the hides in solutions of various sugars, and then determining by polarimetric techniques the changes in sugar concentrations at equilibrium. A rise in the concentration of sugar in the external solution was interpreted as being due to the preferential binding of the water by the hide substance. Eilers and Labout used this method to study the water-binding characteristics of hides through tannery processes. They came to the conclusion that "the percentage of non-solvent water in hide is not much affected by the various operations preparatory to tanning". They did believe that chrome tanning increased the non-solvent water, while vegetable tanning decreased it. They did admit however that it was difficult to distinguish between water held by the hide substance, and water held by the tanning materials.

The authors also ran into difficulties in interpreting the variations in their results which were due to the influences of the differing molecular weights and functional groups of the sugars and alcohols which they used as

auxiliary materials. On the basis of extrapolated values, to eliminate the influence of these variables, they concluded that the non-solvent water bound to the hide substance was 20 per cent, and that there was an additional amount of water held in the interspaces of the hide structure.

Cheshire and Holmes (13) employed a number of different techniques to study bound water in vegetable tanned leather. Their methods included:

1) Attainment of equilibrium with air at various values of relative humidity.
2) Changes in color upon moistening and drying.
3) Minimum moisture requirements for mold growth.
4) Contraction of the leather water system.
5) Direct current conductivity at room temperature and at elevated temperatures.
6) Freezing methods.

From all of these methods a "probable true value" of 51.2 per cent of bound water on the hide substance was calculated. It must be noted that there was some variation in the values achieved by the individual methods, and that the value of 51.2 per cent is an average value. All of the work was done on a leather which contained only 31.2 per cent hide substance. Some attempt was made to correct for water held

by the tanning materials, but it must be realized that the assumptions on which these attempts are based have little or no experimental basis.

Compton (14) employed the dielectric constant method to study the retained water of tanned and bated calfskin. He concluded that tannage with quebracho extract significantly lowered the water-binding capacity of the calfskin. His data showed that chrome tannage lowered it by an insignificant amount, if at all. He concluded that the bated hide could bind between 44 and 47 per cent of its weight of water.

Any consideration of Compton's results must take cognizance of his methods and instrumentation. He determined the dielectric constants of skin squares at various moisture levels between the plates of a parallel plate condenser. The investigation was made at a frequency of 1000 cycles per second. The actual moisture range in which measurements were made was from about 14 to 26 per cent.

The technique of a direct measurement does not take cognizance of the voids which account for approximately 2/3 of the apparent volume of leather in the air-dry state (15,16). At higher moisture levels where a good part of the voids may be filled by water, this error may not be too serious. On the other hand, the low frequency which was employed means

that errors in measurement due to conductance may be large, and this may well account for some of the extremely high values of dielectric constant that were reported at higher moisture levels.

Nevertheless, Compton did obtain a measure of the change in electrical properties of collagen as a function of varying moisture content, and his results may well have been proportional to the "true" dielectric constant values. In that case his conclusions may be essentially correct.

The dielectric constant techniques which are described in the experimental section of this paper represent an attempt to overcome some of the objections and limitations to Compton's work that have been noted. The dielectric constant method is capable of providing information which may permit an estimation of the amount of bound water. It may also provide information as to the character of this entity. That is to say, it can reveal the presence of bound water, and also provide some appraisal of the strength of the binding under different conditions. A more detailed discussion of this feature will be found in the section on discussion of the results of this thesis.

The mechanism of the binding of water has been the subject of considerable speculation. There is substantial

agreement that the binding is due to hydrogen bonds (5,6,7). The only direct evidence of the existence of such a link in a protein water system, is that presented by Sponsler and co-workers (18). They studied the infra-red absorption spectra of gelatin-water systems, and came to the conclusion that hydrogen bonds existed between the protein and the water.

They also postulated that there are two types of hydrophilic groups capable of binding water in proteins. These are the polar side chains, and the oxygen and nitrogen atoms of the peptide links. Pauling (19) studied the data of Bull (10) and concluded that the peptide groups had little water binding power. This was based on the data for nylon, which has no polar side chains, and also has little or no power of water vapor adsorption. A referee who studied the paper, pointed out however, that the close packing of the chains in nylon may be responsible for the failure of the water to penetrate. Pauling, using the best available amino acid analyses and the water vapor adsorption data of several proteins, found good agreement for his theory that the polar groups were responsible for the water binding of proteins. However, collagen and gelatin were practically unique, in that they did not show agreement on this basis.

Pauling points out that these two proteins are also almost uniquely high in proline and hydroxy proline content, and that the existence of such large amounts of bulky side chains may liberate many peptide groups, and allow greater permeability to water. He does concede that the peptide groups, if not bonded to each other, are probably capable of binding water. The work of Mellon, Korn, and Hoover (20) with casein and polypeptides is further evidence for this belief.

C - The Shrinkage Temperature of Collagen and Leather and the Mechanism of Tanning

Reference has already been made to the unique property of shrinkage which collagen possesses under certain conditions, and the extent to which this property is altered by tannage. The bulk of the literature on the subject, and its entire practical application, are concerned with the shrinkage of collagen in hot water. Fairly complete statements of the existing literature and viewpoints on this subject are to be found in the books and papers of Powarnin (21), McLaughlin and Theis (22), Stiasny (23), Kunzel (24), Hobbs (25), Balfe and Humphreys (26), and Astbury (27).

The chrome tanner does make a practical use of the shrinkage temperature; it is strictly a control procedure for an established process. It provides the tanner with an index which he can correlate with his experience, to arrive at an estimate of the quality of his final product. The recent work of Bowes and co-workers (28) provides laboratory evidence which confirms the validity of this long established practice. They studied the effect of increasing chrome content of leather on the shrinkage temperature, and they found that there is an exponential relationship; a plot of the shrinkage temperature against the log of the chrome content yields a straight line.

There is a tendency among research workers (22a, 29) to use the shrinkage temperature as a universal criterion of leather quality, and to base all deductions of the tanning mechanism on the effects of experimental variables on the shrinkage temperature. There are several objections to this practice. In the first place the method of measurement is not a precise or fully reproducible procedure by any means, and its sensitivity is not established. The work of Weir (30) suggests that this phenomenon is a rate process of such a nature that small changes in conditions may significantly affect the final measured result. The work of Theis (22b)

and others (31) indicates the profound effects of such experimental variables as salts, acids, and bases, various organic materials, and thoroughness of wetting on the measured value of the shrinkage temperature.

A further objection to the use of the shrinkage temperature as a universal criterion is the fact that experience has shown that elevation of the shrinkage temperature alone is not sufficient to guarantee good leather quality. Examples of this are to be found in the depression of shrinkage temperature produced by oil tanning, and in the production of rather acceptable leathers (32) whose shrinkage temperatures were not appreciably higher than that of raw skin. Vegetable tanned leathers of good quality may have an appreciably lower shrinkage temperature than a poorly tanned piece of chrome leather. In general there is a range of shrinkage temperature which is characteristic of the class of tanning materials used.

The effects of dry heating on collagen and leather, and shrinkage behavior under dry conditions or in non-aqueous media has received little study. The general subject of dry heating and denaturation of proteins other than collagen has received more attention, and an excellent treatment of this subject is to be found in the work of Mecham and Olcott (33).

A comparison of their results, and those reported for collagen in this paper, will be found in the section of this thesis which deals with the experimental results.

Powarnin and Sapagenin (34) studied the influence of temperature on the tensile strength of dry leather. They concluded that dry, untanned skin is most stable to heating, and that chrome tanned leather is the least stable. Green (11), on the basis of a change in the color of chrome tanned leather after heating, concluded that it was not heat stable in the vicinity of 100°C. He assumed that the chrome complex had been altered in some manner. Kanagy (35) studied the evolution of CO_2 and water from vegetable tanned leather at elevated temperatures. His conclusions regarding vegetable tanned leather are obscured by the inability to differentiate between protein degradation and tannin destruction. The results of a control experiment indicate that untanned hide powder is practically unattacked at 100°C. and only slightly attacked at 140°C. Balfe and Humphreys (26a) mention the effects of dry heating on leather, and the shrinkage temperature in oil. No data is presented; unpublished data and private communications are cited as references. As a consequence the significance of their data cannot be evaluated at this time. Lloyd and Garrod (31) mention that collagen

has a shrinkage temperature of about 150°C. in glycerine. Their experimental conditions are not described.

Experience at this laboratory has indicated that the use of the conventional measuring device (22c) for skin and leather which is in the dry state, or has been degraded to some extent, is very unsatisfactory. The changes are in many cases extremely slow and of a magnitude which is below the sensitivity of the instrument. Use of the conventional technique under such circumstances will reflect only gross changes, and the existence of changes of lesser magnitude, but nevertheless significant importance, may well be overlooked. The development of an electronic instrument for performing this measurement with greater sensitivity, and the results obtained thereby under wet and dry conditions is fully described in the experimental section of this thesis.

The mechanism of shrinkage temperature, and its relationship to the mechanism of tannage has been the subject of considerable speculation in the leather literature. Astbury (27) and other authors postulate that the polypeptide chains of collagen are in an extended state, and that they are so held by cross links of various kinds. The penetration and rupture of these cross links by the combined effect of heat

and water is then supposed to result in a collapse of the chains, the gross manifestation of which is the shrinkage temperature. Spiers (36) on the basis of an idea originally propounded by Meyer and Mark (37) offered the theory that in tanning, the tannin molecules penetrate between the chains and form additional cross links which are more difficult to rupture. Hence, the observed increase in shrinkage temperature.

This theory, while plausible and attractive, and acceptable to many workers in the field (22d), is not yet on a secure factual basis. The work of McLaughlin and his associates (38) on chrome tannage provides strong factual support for an entirely different theory of the mechanism of chrome tanning. They postulate tanning by chrome sulfate compounds as being caused by the deposition in and around the fibers of the insoluble 2/3 basic chrome sulfate salt as a result of acid fixation by the skin. The production of acceptable leathers with depressed or unchanged shrinkage temperatures is not explained by the bridge theory. The work of Jacobson (39) on the mechanical properties of tanned collagen fibers has yielded results which do not support the bridge theory.

Humphreys (40) and Balfe (41) regard tanning as a process of replacing the bound water which exists between

polypeptide chains with cross links of tanning material, whose strength is greater. Their views are essentially a more detailed and explicit version of the bridge theory, and no significant amount of experimental evidence is cited to support their viewpoints.

In the experimental section of this thesis, data will be offered which will provide a further basis for interpretation of the various phenomena which have been considered in this discussion of bound water, shrinkage temperature, and the mechanism of tanning.

II - EXPERIMENTAL METHODS

The experimental investigation of the subjects which have been previously discussed required the development of three types of methods and instrumentation. A detailed description of the methods and the instruments will be found in this section.

A - Determination of Total Water in Skin and Leather

A necessary requirement for any method of investigation of the water relationships of a system, is a method for determining the total amount of water which is present. Proteins, however, are very susceptible to degradation by the action of heat and other methods of desiccation. Thus, it is not always possible to remove all of the water which is not water of constitution, without damaging the protein.

Many methods have been developed, and are in current use, to measure the amount of water in a protein sample. In general they are empirical procedures which serve a practical purpose for industrial and laboratory control work. If the stated conditions are meticulously observed, reproducible results may be obtained. Complete removal of water is not necessarily accomplished, nor is the protein unaltered by the procedures in all cases. Bull (10), Mecham and Olcott (33), and Gortner (5) have discussed and illustrated the limitations of some of the existing reference methods.

The problem of determining all of the water associated with a protein, without causing alteration of the protein, may be fundamentally incapable of solution, as the complete removal of bound water may leave the protein in a thermodynamically unstable state. A knowledge of the effects of experimental conditions and the determination of the extent of protein denaturation is therefore the best alternative. The investigation of this problem as it pertained to collagen and leather was therefore undertaken.

There are three principal methods for the determination of moisture which are employed in the analysis of almost all types of materials. The first method and probably the most common of these is the use of oven drying procedures. These procedures may be refined by the use of vacuum ovens to minimize oxidation. Temperature and time are the variables which must be standardized. The underlying assumption is that the loss of sample weight which is observed is due entirely to volatilization of water.

A second method is the distillation procedure of Bidwell and Sterling (42) and various refinements thereof (43). The material is immersed in a boiling inert solvent, generally an aromatic hydrocarbon. Solvent and water are then distilled and condensed in a continuous extraction, the water which is removed is collected in a calibrated receiving tube,

and the amount may be read directly.

The third method, known as the Karl Fischer technique (44), has recently been applied to protein analysis. In this procedure the water is extracted from the sample by the use of anhydrous methanol. The addition of the Karl Fischer reagent causes an oxidation - reduction reaction to occur which is specific for water, and involves the liberation of iodine which may be determined by a suitable titrimeter procedure. McComb has recently published (45) a study of the applicability of this procedure to the analysis of several proteins. She points out, that in addition to the manipulative difficulties the completeness of the methanol extraction is a significant variable which must be thoroughly studied and controlled before precise results may be obtained.

The solvent distillation procedure was adopted as the principal method for studying the removal of moisture from collagen and leather. Vacuum oven procedures were also employed to check the results. The solvent distillation procedure was selected for the following reasons:

1) The presence of the inert hydrocarbon atmosphere at all times, practically precludes errors due to oxidation.

2) Accurate and automatic control of temperature is maintained by the choice of a solvent whose boiling point is suitable.

3) The method determines only the amount of water which is removed. Any weight loss other than water which may occur simultaneously cannot cause an error. Loss of water of constitution will of course be reported along with water from any other source.

4) If the moisture removal is studied as a function of time, frequent readings may be made by simply reading the calibrated receiving tube. An oven procedure requires removal of the sample from the oven, cooling in a desiccator, and then a weighing operation. After samples are returned to the oven there is a lag period before thermal equilibrium is reestablished.

5) The only disadvantage of the solvent distillation method is that it requires relatively large samples (10-15 gms.) for most accurate results. This was not a problem in the work at hand.

B - Measurement of Shrinkage Temperature Characteristics

The difficulty of measuring the shrinkage temperature behavior of skin and leather pieces which have been subjected to possible degradation has been previously mentioned and discussed (P.18). The study of the changes in the wet shrinkage temperature which are produced by dry heating required a method which possessed a greater sensitivity than the existing instrument.

A wire resistance strain gauge and a suitable electronic amplifier were constructed for the purpose of improving this measurement. A block diagram of the instrument is shown in Figure 2. The instrument operates on the principle that certain types of resistance wire undergo changes in electrical resistivity as a function of the mechanical force which is applied in the form of stretch or tension. The gauges are so constructed that the wire is wound in the form of a Wheatstone's Bridge. A constant current is applied to the bridge. Any change in the resistance of the wire which is caused by the mechanical force applied causes the bridge to become unbalanced, and this unbalance is reflected in voltage changes which may be measured at the indicated reference points on the bridge. The changes in voltage are a linear function of the applied force, within specified limits.

A commercial gauge was used in the instrument, and its response was linear in the range of 20-1300 gms. Changes of the order of magnitude of one gram were detectable. Known weights were suspended from the gauge, and it is thereby possible to calibrate the microammeter dial in grams or any other suitable units of force. Electronic controls were incorporated into the amplifier circuit which permitted the balance position and calibration to be rapidly and conveniently checked during operation. In practice, little or no drift was observed if the instrument had been well warmed-up prior to use.

The instrument was equipped with a built-in electric stirrer and an immersion heater. The strain gauge was attached to the frame by means of a shaft which had a screw thread. One jaw of the sample holder was attached to the lower end of the gauge. The other jaw was rigidly fixed to the frame of the instrument. After the sample was fastened between the jaws, the screw thread was adjusted so that any desired amount of initial tension could be applied to the sample. This was done in order to compensate for the elongation of the sample and consequent relaxation of tension that occurs as the temperature of the water is raised.

Samples were cut from pieces which had been split to a

uniform thickness (generally about 0.060"). The dimensions of the samples were 1/2" x 1". The samples were thoroughly soaked and wetted prior to the determinations. For samples of this size, it was found that an initial tension of 100 gms. was sufficient to maintain a positive stress on the samples at all times, and this tension was used for all the determinations. This initial tension did not appear to have any bearing on the final results. The other experimental condition which was standardized was the rate of heating, which was set at 3°C./min. starting at room temperature in most cases. In the case of severely degraded samples, the samples were hydrated in an ice and water bath, and the temperature at the start of the determination was between 2 and 5°C.

A typical curve of the data which is obtained by this procedure is shown in Figure 3. The data appear in Table V. A study of the curve for untanned skin shows several phases of shrinkage temperature behavior which are of interest:

1) There is a relaxation of the initial tension, which gradually decreases to a relatively constant value.
2) The tension remains at a relatively constant value for several degrees, and then gradually

begins to increase. The temperature at which the first evidence of increase is noted, is defined as the shrinkage temperature.

3) Shrinkage, as evidenced by increasing tension, occurs at a repidly accelerating rate for an interval of several degrees, and then the rate of increase diminishes, and the tension again becomes fairly constant for an interval of 2-4 degrees; then a gradually accelerating relaxation is observed. The final relaxation marks the onset of gelatinization of the collagen. Gelatinization of chrome tanned pieces is not obtained under the experimental conditions which are used in this determination.

C - Measurement of the Dielectric Constants of Skin and Leather

The study of the dielectric constants of systems which contain water has yielded results of fundamental interest and value, and in some cases it has provided methods whereby the moisture content of a material could be determined in a rapid and reasonably accurate manner (46). The only instance where this technique has been applied to collagen and leather has been the work of Compton (14), which has

been mentioned earlier.

Recent advances in the field of electronic instrumentation provided the means of constructing an instrument which would be capable of greater accuracy, reproducibility, and less subject to errors caused by polarization (47) and conductance. The construction of this particular instrument and its operating characteristics has been described (4). In the series of experiments which will be described, all measurements of the dielectric constants were made at a frequency of 11.184 m.c.s. A simplified schematic circuit diagram is shown in Figure 4.

Condenser C-2 is equipped with a vernier dial which permitted changes in capacity of the order of 0.1 micro-microfarad to be accurately measured. The circuit is initially tuned by appropriate setting of the variable condenser C-3. A minimum reading on the tuning meter indicates that the circuit is tuned to resonance. The material to be studied is placed in condenser C-3. The lower portion of Figure 4 shows a diagramatic representation of the construction of C-3. The diameter of the upper plate of the condenser was 7/8 inch. Condenser C-2 was also equipped with a micrometer to allow for accurate adjustment of the distance between the condenser plates. The design of the condenser also permitted

the dielectric constants of liquid samples to be studied.

After the sample is placed in C-3, a change in the total capacity between points 1 and 2 results. The circuit may now be restored to resonance by changing the capacity of C-2 until the total capacity between points 1 and 2 is restored to the initial value. The vernier dial on C-2 permits this capacity change to be accurately measured, and the dielectric constant of the unknown material may then be calculated.

In practice, the measurement of the dielectric constant of a porous, fibrous material such as leather presents a special problem. Actually the apparent volume of leather is much greater than its true volume. Measurements of the true density of leather (15,16) have indicated that about two-thirds of the volume of leather is empty space. Any method of measurement of the dielectric constant must consider the fact that the dielectric constant of this air space is unity. In an effort to obtain a true measurement of the dielectric constant, the so-called "immersion" technique was adopted. A description of this technique, and its application to the measurement of the dielectric constants of natural and synthetic fibers may be found in the work of Errera and Sack (48).

The immersion technique utilizes the following procedure: a number of solvents and mixtures of solvents are prepared. Pure solvents whose dielectric constants have been reliably determined, and the values of which are unquestioned in the literature, are placed in the measuring condenser, C-3. The dial readings on C-2 which results after the circuit is retuned are noted. A plot of dial readings against known dielectric constants of the solvents may then be made. The upper part of Figure 5 is an example of such a calibration curve. (The data for this curve are to be found in Table VII). This calibration curve then permits the dielectric constants of solvent mixtures to be calculated from the dial readings which they give when they are placed in the instrument.

Samples of skin or leather are prepared by splitting to a uniform thickness, and then cutting into circular discs whose diameter is equal to that of the upper plate of the measuring condenser. The samples are immersed for 48 hours in the various solvents in order to insure that complete penetration and filling of the interspaces has occurred. The sample is then placed between the plates of C-3, and the condenser is filled with solvent to a definite level, which is constant for all determinations. The circuit is

retuned, and the reading of C-2 is noted. The difference in the readings of C-2 which are caused by the addition of the sample to the solvent may be calculated. This difference is plotted against the known dielectric constant of the solvent or solvent mixture. If solvents, having a suitable range of dielectric constants are chosen, both positive and negative differences are obtained. Therefore the curve must cross the axis (the line where the change is zero). The value of the dielectric constant at the point where the curve crosses the axis is taken as the true dielectric constant of the sample. The reason is that under such conditions the dielectric constant of the skin and the solvent must be identical, as no change is produced in the instrument response by the addition of the sample. The data for a typical determination are to be found in Table VII. The results are plotted in the lower portion of Figure 5. They also show that the method is independent of the nature of the solvents that are used.

This technique has certain limitations. The solvents must be immiscible with water, insofar as practical, and incapable of reacting with the protein. This greatly limits the choice of solvents and the span of the dielectric constant range which may be studied. The solvents which met the

requirements to the greatest extent was the chlorinated paraffin compounds, carbon tetrachloride, chloroform, and ethylene dichloride. Intermediate values of dielectric constant were obtained by mixing these solvents in suitable proportions. The highest dielectric constant obtainable (that of pure ethylene dichloride) was 10.6, which limited the use of the immersion technique to the moisture range of 0-30 per cent approximately.

It was also found to be possible to obtain an estimation of the true dielectric constants at moisture values above 30 per cent, and as high as 50 per cent. The method which was used was as follows: pieces were placed in the measuring condenser, without solvent, and the readings thus obtained were noted. From these readings, and a calibration curve of liquids of known dielectric constant, an arbitrary value, termed an apparent dielectric constant was calculated for the sample. This procedure is limited only by the tuning range of C-2 and the loading capacity of the oscillator. In practice, this permitted measurements on pieces having moisture contents as high as 50-55 per cent.

The specific ways in which these methods and instruments have been applied, and the results which have been obtained are fully described in the following section.

III - EXPERIMENTAL RESULTS

The solvent distillation procedure which has been previously described was applied to the determination of moisture in standard hide powder, untanned and chrome tanned calfskin pieces*. Samples were conditioned for one week or more at 70°F. and 65 per cent R.H.; they were then weighed into flasks and immediately covered with the various solvents. The solvents which were used had boiling points which ranged from 50 to 204°C. The determinations were run continuously for periods of 4 to 5 days, and readings were made at intervals of a few hours during each day. The results obtained for hide powder are shown in Table I and Figures 1A and 1B. The results obtained with chrome tanned and untanned skin pieces are shown in Table II and Figure 1B. Table III shows a comparison of results obtained by the solvent procedure, with those obtained by the use of vacuum ovens which were set at the same temperatures as the boiling

* - The untanned pieces were obtained from commercial calfskins which had been limed, bated, and pickled. They were then washed free of acids and salts, and dehydrated by the use of alcohol and acetone(38). Half of each skin obtained in this fashion was thoroughly soaked in water and chrome tanned in a drum. Sulfur dioxide reduced liquors were used whose basicity ranged from 27 to 33 per cent. The analytical data for these skins are to be found in Tables VIII and IX.

points of the solvents which were used. The results which were obtained led to conclusions concerning the nature and extent of bound water. Their significance is discussed in the subsequent section which deals with the significance of results.

The next phase of the problem was to ascertain insofar as possible the extent of protein change which had occurred as a result of the drying procedure. It was noted in the course of the experiments that the untanned skin and hide powder tended to darken in color at temperatures above 100°C. This browning reaction was appreciable at the boiling point of cumene (153°C.). Furthermore, significant decomposition and solubilization occurred at temperatures above 165°C. and the odor of nitrogen containing degradation products was very pronounced. Increasing amounts of area shrinkage were also observed as the temperature was raised above 100°C.

A procedure was adopted to determine the amounts of nitrogen lost by the pieces in those cases where the decomposition was not immediately apparent to the eyes and nose. Duplicate samples were run in conjunction with the moisture removal experiments. At intervals, pieces (which had been previously weighed and coded by punching a pattern of holes) were removed from the flasks. The pieces were then immersed in absolute ethanol to remove the hydrocarbon; the alcohol

was allowed to evaporate, and the total amount of nitrogen in the pieces was determined by the Kjeldahl procedure. The results obtained were compared with those obtained with unheated control pieces. The results of this procedure are shown in Tables II, III, and IV.

In addition to the study of the chemical decomposition of the protein which may be induced by heating, it was deemed advisable to obtain information about the physical changes that may have occurred. The characteristic which was selected for study was the change in the wet shrinkage temperature which was produced by dry heating. The procedure which was employed was essentially the same as that used to study the nitrogen losses. Pieces were removed from the flasks at intervals, immersed in absolute alcohol to remove the hydrocarbon, and then soaked in water until they were thoroughly rewet. Their wet shrinkage temperatures were then determined on the instrument which has already been described. The results are shown in Table VI.

It was also deemed worthwhile to obtain information regarding the shrinkage behavior of skin and leather under anhydrous conditions. A USP grade of Paraffin Oil (100 second viscosity) was used as the heat transfer medium. The pieces were in the air dry state. Some determinations were made

on dry samples which had been brought to about 2 per cent moisture in the vacuum desiccator. The results were the same as those obtained for the air dry samples. Ethylene glycol was used as the heat transfer medium in one instance to check on the validity of the results obtained with the paraffin oil. Determinations were made on the untanned and chrome tanned calfskin pieces which were used for the previous experiments, on a commercially chrome tanned mechanical leather containing more than 10 per cent chrome oxide on the dry basis, and on raw and chrome tanned kangaroo tail tendon fibers. All determinations were made with the electronic force shrinkage-meter. The results are noted below:

COMPARISON OF WET AND DRY SHRINKAGE TEMPERATURES

Sample	Shrinkage Temperature (°C.)		
	Water	Mineral Oil	Ethylene Glycol
Untanned calfskin	60	92	--
Chrome tanned calfskin	93	92	--
Chrome tanned mechanical leather	Above 100	92	92
Untanned tendon fiber	54	52	--
Chrome tanned tendon fiber	91	51	--

The measurements of the dielectric constants of untanned skin and leather was adopted as a procedure which would extend the information on bound water to higher moisture ranges, and to provide additional information on the effect of tannage

on the water binding power of the skin. The instrumental techniques have been described and the differentiation between the immersion method and the direct method has been defined. These procedures were then applied to a study of the dielectric constants of untanned and chrome tanned calfskin at various moisture levels.

The results which were obtained are shown in Tables VIII and IX. A log-log plot of the data, with suitable empirical constants added to obtain rectification is shown in Figure 6. The relationship between the immersion and apparent dielectric constants is also demonstrated in Figure 6. It is thus possible to estimate by graphical means, the true dielectric constant from the measured value of the apparent dielectric constant.

The use of the technique for obtaining apparent dielectric constants has provided a means of determining the moisture content of leather in a rapid and simple manner, and it may have a definite practical application. In Figure 7 there is a plot of the known moisture contents of skin pieces against the instrument readings which are obtained. This curve may be used as a calibration curve, from which it is possible to predict the moisture content of samples from the instrument reading that they cause. This has proved to be possible. A sample set of data of this type may be found

in Table X. The comparison of the predicted values with the actual values is definitely within the range of practical utility.

IV - SIGNIFICANCE OF RESULTS

The results obtained from the use of the solvent distillation method (see Tables I, II, III and IV) have served to provide information about the effects of heat, time, and chrome tannage on the removal of moisture from hide substance. A study of Figure 1A shows that when the determinations were carried out for a sufficient length of time (4 to 5 days) an equilibrium condition is reached at any one value of temperature as determined by the boiling point of the solvent. The amount of water which is removed at this equilibrium time is a function of the temperature which prevails. The higher the temperature, the greater is the amount of water which is removed. Similar behavior has been observed for other proteins (49).

Figure 1B graphically relates the amounts of water which were removed at equilibrium time with the temperature of removal. The data indicate that bound water exists at the low levels of moisture under consideration (i.e.-the final 1 to 2 per cent water which remains in the skin after conventional drying procedures at 75-100°C. overnight). The curves indicate that there are gradations in the strength of the binding, as additional increments of energy are required to remove additional amounts of water. The similarity of

the shape of all three curves, and in particular the similarity of the curves for untanned and chrome tanned skin pieces, suggests that this is a characteristic property of the hide substance, and that it has not been affected by chrome tannage.

Additional confirmation of such an interpretation is available from the data which are presented in Table III. Comparisons were made of the amounts of moisture which were removed by the solvent distillation procedure with the amounts removed by the use of vacuum ovens. The vacuum ovens were maintained at the same temperature as the boiling points of the solvents. The observed behavior was similar with regard to the effects of increased temperature. However, in all cases, slightly larger amounts of water were removed by the vacuum oven than by the solvent at the same temperature. This is probably due to the fact that the vacuum oven combines a temperature effect with a pressure differential, with the result that effectively more energy is supplied for the water removal mechanism.

The validity of the above conclusions is dependent on the fact that any water which is removed is not water of constitution, but discrete molecular water only, and which is associated in some manner with the hide substance. It is reasonable to believe that protein degradation which

would liberate the elements of water would also be accompanied by a loss of nitrogen. Tables II, III, and IV contain data concerning the amounts of nitrogen which are present at all stages of the various heating procedures. In no instance was there any loss of nitrogen observed. The data show results for temperatures as high as 153°C.; and actually there is no significant loss of nitrogen up to temperatures as high as 160-165°C. At temperatures in the range of 175-200°C. there is obvious decomposition, with profuse volatilization of the degradation products of nitrogen. The work of Kanagy (35), who studied the loss of carbon from hide powder, indicates that there is little loss of carbon at temperatures as high as 140°C. Further confirmation may be found in the work of Mecham and Olcott (33). They studied the effects of dry heating on a number of proteins (collagen and gelatin were not included) and found no loss of nitrogen up to 153°C.

The results which have been presented, and the literature that has been cited, substantiate the conclusion that the moisture which was removed from the hide substance was not water of constitution. However, the hide protein was not unchanged by the heating procedures. At temperatures above 100°C., area shrinkage was observed, and it appeared to increase with rising temperature. At temperatures in

the region of 140°C. a certain amount of browning is observed in the hide powder and untanned skin pieces (the color of the leather would mask any such manifestation in the chrome tanned pieces). At higher temperatures, particularly above 175°C., this browning is very pronounced.

The procedure which was used to study the extent of the changes that were observed, other than chemical decomposition, was the measurement of the wet shrinkage temperatures. The results in Table VI, secured by the techniques already described, compare the values of the wet shrinkage temperature of skin pieces; the variables are time, temperature, and chrome tannage. The salient features of these data are:

1) Chrome tannage decreases the stability of hide substance to thermal changes. Chrome tanned pieces exhibit reductions in shrinkage temperature when heated at temperatures as low as 69°C. Raw pieces are relatively stable at 100°C. At all temperatures up to 140°C. the wet shrinkage temperatures of chrome tanned pieces are lowered more rapidly and to a greater extent than those of the untanned pieces. The unstability of chrome tanned leather to dry heat which was observed is in agreement with

the conclusions of Powarnin (34) and Green (11) which have been mentioned previously.

2) The action of heat on the untanned hide substance seems to produce a resistance to gelatinization. The results noted in the column entitled "Temp. of Max. Force" (the temperature at which gelatinization commences) show that as the temperature of heating is raised, the untanned skin requires progressively higher temperatures of gelatinization. At any one temperature, increased time of heating also serves to raise the temperature of gelatinization. This is definitely the case of temperatures up to 140°C. The results for cumene (B.Pt.-153°C.) suggest that a maximum value for this effect may have been reached.

3) The results for the actual magnitude of the forces present during the determination, and at gelatinization, are inconclusive. Although an attempt was made to standardize the weight and dimensions of the samples, the data do not warrant any conclusions regarding the significance of the magnitudes of the forces.

The results of the determinations of the shrinkage temperatures in non-aqueous media are shown in the tabulation on P.37. The wet shrinkage results are also shown for purposes of comparison. The significance of these results lies in the fact that chrome tannage has produced no change in the dry shrinkage temperature. In a few instances other workers have measured shrinkage temperatures under non-aqueous conditions. The results of Meyer (50) and his associates with tendon collagen indicates that the dry shrinkage temperature is in the neighborhood of 120°C. Lloyd (31) mentions that the shrinkage temperature in glycerin is in the region of 150°C. The lower values of the results in this paper are very probably due to the greatly increased sensitivity of the electronic instrument which was used for this work. In all instances where a comparison of the shrinkage temperatures obtained by the use of the official instrument (22c) has been made with those obtained by the new electronic instrument, the results have been appreciably lower in the case of the electronic instrument. The differences varied in magnitude, depending on the type of material and the medium in which the measurement was made.

In any event the important factor is the comparison between untanned and tanned materials, and between skin

collagen and tendon collagen. Inasmuch as the same instrument was used for all materials, the comparisons on the basis of changes due to tannage and different media are nonetheless valid. In the absence of additional evidence from other methods of judgment it is impossible to completely and finally reconcile, in terms of the mechanism of tannage, the wet shrinkage temperature behavior with the dry shrinkage temperature behavior. A reasonable hypothesis may however be offered.

An increasing hydration, which finally results in a probable rupture of forces holding polypeptide chains in a certain configuration, accompanies the shrinkage temperature effect. This hydration results in a swelling and elongation of the collagen which is quite noticeable just prior to shrinkage. When the determination is carried out under inert non-aqueous conditions, the shrinkage which is observed is due to purely thermal effects. These facts, and the results obtained, suggest that the shrinkage which is observed in water is actually a net effect of two or more individual effects which are occurring simultaneously at different rates. In the case of untanned skin hydration sufficient to rupture the forces linking the polypeptide chains and permit reordering of the chains occurs at a temperature at which the reordering due to purely thermal effects is occurring at a

negligible rate.

Baker (52) has discussed this reordering of chains in systems of synthetic polyamides, and presents X-ray evidence which contrasts the relative rates of reordering with paraffin oil and water at 100°C. The effect of the water is very much greater. In the case of the chrome tanned skins the hydration which precedes reordering presumably occurs at a lower rate than that of raw skin, and a considerably higher temperature is required to hydrate the leather to the point where rupture and subsequent shrinkage will occur. If the reordering due to purely thermal effects is unchanged and proceeding at a constant rate, its effect will be overshadowed by the swelling and elongation which is occurring simultaneously. This hypothesis therefore accounts for the apparent anomalies between the wet and dry shrinkage temperatures of untanned and chrome tanned skin.

There still remains to be explained the fact that the wet shrinkage of chrome tanned skin is so much greater than that of untanned skin. Lloyd and Garrod (51), on the basis of Speakman's criteria for evaluating relative porosity at molecular dimensions, conclude that collagen has a relatively open structure. McLaughlin, Cameron, and Adams (38) have postulated that chrome tannage is due to the physical deposition of the 2/3 basic chrome sulfate in and around

the collagen fibers. From these considerations it may be postulated that one of the actions of tannage is to reduce the porosity, and thus greatly reduce and hinder hydration. This would explain why higher temperatures and longer times are required to hydrate chrome tanned skin to the point of interchain rupture.

There is implicit in the foregoing considerations and conclusions the belief that shrinkage temperature is a rate phenomenon. Everyday experience in the plant and the laboratory where shrinkage temperatures are determined supports this view. More formal support is available in the literature in the papers of Weir (30). All the results which have been presented in this paper were obtained under conditions where the rate of heating was standardized in order to remove any variations due to this factor.

The work on the measurement of the dielectric constants of skin and leather has also served to provide an insight into the effect of chrome tannage. The results of the measurements of the true or immersion values of the dielectric constants of two sets of untanned and chrome tanned skins are shown in Tables VIII and IX. When the dielectric constant values are plotted against the moisture content on a hide substance basis, the values for the chrome tanned and untanned skins may be plotted on the same line. Direct

measurements yielded the same results. The fact that the results obtained by both methods could be plotted on the same line, is taken as evidence that chrome tannage did not significantly alter the water binding properties of the hide protein in the moisture range (0-50%) which was studied.

The results noted in Table VII and Figure 5, in which a variety of solvents were used are proof that the immersion method is independent of the nature of the solvent which is used. This fact, coupled with the reproducibility of the procedure that is indicated by the data for the two sets in Tables VIII and IX and Figure 6, supports the validity of the results obtained by the immersion method. The same data are evidence that uniform and complete penetration of the immersion solvent was achieved. Observations of the behavior of the pieces in solvents of varying specific gravity, were correlated with the values reported (15,16) for the true density of skin and leather. Good agreement was found, which was taken as further evidence of complete penetration. The value of the dielectric constant of the dry skin was found to be about 4. This is a reasonable value, and it is in excellent agreement with the results reported by Errera and Sack (48) for other fibrous proteins.

The curve shown in Figure 7 is typical of the results obtained for both the immersion and direct readings when

dielectric constant is plotted against moisture content. Of course, measurements by the immersion method cannot be made when the moisture content exceeds 25 to 30 per cent, but the general shape of the curve up to that region is the same. There is a relatively small increase in dielectric constant per unit moisture increase at levels below 30 per cent. Above 35 per cent moisture, the increase in dielectric constant per unit increase in moisture is relatively large. This suggests that if there is a transition from a region where bound water predominates, to one where free water predominates, it occurs in the range of 30 to 35 per cent moisture, or in the region of 0.4-0.5 gm. water per gram of hide substance. This is generally in accord with the values for collagen and gelatin which are available in the literature (9,13,14).

All of the experimental measurements which have been made in this work have indicated that chrome tannage has produced no significant alteration in the functional groups of the hide protein, whether they be polar or peptide groups. The results cannot be reconciled with a theory of tannage by bridging or bound water replacement. They are not inconsistent with a theory of physical deposition in the manner postulated by McLaughlin (38). This also applies to the observed thermal instability of the chrome tanned skin

below 100°C. Changes at such a low temperature are unlikely to be due to changes in the chrome complex. They suggest some disaggregation or disordering of the collagen. Again, that is not inconsistent with a theory of physical deposition, nor with the considerations of porosity which have been discussed.

V - SUMMARY

Methods and instrumentation have been developed for the study of the effects of heating and moisture removal on the chemical and physical properties of untanned and tanned hide substance. Methods have also been developed for studying the high frequency dielectric constants of skin and leather, and the influence of moisture and chrome tannage on this property has been evaluated.

The experimental results may be summarized as follows:

1) The conditions and effects surrounding the removal of water from hide substance by the action of heat have been demonstrated.
2) The existence and nature of bound water under specified conditions has been demonstrated.
3) The true dielectric constants of skin and leather have been measured for the first time, both in the dry state, and in various stages of moisture content.
4) Shrinkage temperature studies have indicated the relative heat stability of untanned and chrome tanned skin. Dry

shrinkage temperature studies have demonstrated that chrome tannage does not alter the collagen in this respect.

5) The bearing of the results on possible mechanisms of shrinkage temperature and chrome tannage has been discussed. A hypothesis has been offered to explain the results that have been obtained. This hypothesis accepts the physical deposition theory of McLaughlin (38), which was based on stoichiometric considerations concerning the fixation of the chrome tanning compound by the hide substance.

TABLE I

PER CENT WATER REMOVED FROM STANDARD HIDE POWDER BY VARIOUS HYDROCARBON SOLVENTS OVER A 4-5 DAY PERIOD

Solvent:	Cyclopentane		Hexane (n)		Methyl Cyclohexane		Ethyl Cyclohexane		Xylene		Mesitylene	
B. Pt.:	50°C.		69°C.		100°C.		131°C.		137°C.		164°C.	
	Time (Hrs.)	% Water Removed	Time (Hrs.)	% Water Removed	Time (Hrs.)	% Water Removed	Time (Hrs.)	% Water Removed	Time (Hrs.)	% Water Removed	Time (Hrs.)	% Water Removed
	3	5.95	1	7.44	1/4	6.58	1/6	8.57	1/6	9.08	1/6	9.33
	5	6.43	2½	8.65	½	7.98	½	9.74	½	10.21	1/4	10.0
	6½	7.63	4	9.42	1	9.33	5/6	10.07	1 1/3	10.69	1	10.91
	9	7.69	6	9.82	1½	9.75	1 1/6	10.17	2½	10.86	2	11.03
	12	7.69	7½	10.03	2	10.0	1½	10.27	4	10.99	4½	11.09
	23	8.05	16	10.63	3	10.37	3	10.43	6	11.1	5½	11.22
	26½	8.29	30	10.63	18½	10.73	3½	10.50	7½	11.28	7½	11.33
	31	8.35	43	10.72	20½	10.73	5	10.54	18	11.28	18	11.59
	47½	8.77	51	10.98	23	10.73	6½	10.59	20	11.28	19½	11.59
	49	8.77	67	11.03	25½	10.82	10	10.71	30	11.33	25	11.59
	53	8.98	74½	11.03	27	10.86	12	10.71	43	11.40	43	11.83
	55	9.13	79½	11.06	30	10.98	23	10.71	51	11.49	50½	11.94
	71	9.66	90½	11.08	32	10.98	26½	10.76	67	11.57	67	12.07
	76	9.72			44	11.10	27½	10.97	71	11.57	71	12.07
	79	9.72			47	11.10	31	11.19	75	11.57	74	12.07
	84	9.85			51½	11.1	47½	11.31	80	11.61	80	12.07
	95	9.85			68	11.21	49	11.31	90	11.63	90	12.13
	125	9.85			70	11.21	53	11.35				
					74	11.25	55	11.41				
					76	11.25	71	11.56				
					92	11.41	76	11.56				
					96½	11.41	79	11.56				
					99½	11.41	84	11.59				
							95	11.59				

TABLE II

Moisture Removed From Untanned and Chrome Tanned Calfskin Pieces After Boiling in Various Hydrocarbon Solvents

Equilibrium Attained After 4-5 Days. Initial and Final Nitrogen Content is Also Listed, In Those Instances Where They Were Determined

Initial Nitrogen Content			Untanned Skin 149.7 mgm/gm.		Chrome Tanned Skin 136.0 mgm/gm.	
Solvent	B.Pt.(°C)	Time	$\%H_2O$	Final N Content	$\%H_2O$	Final
n-Hexane	69	120	20.10	----	19.35	----
Benzene	80	120	20.35	----	19.60	----
Methyl Cyclo-hexane	100	100	20.40	147.5	19.83	135.8
Toluene	111	96	20.41	----	19.80	----
Xylene	137	96	20.58	----	19.88	----
Cumene	153	100	21.00	149.0	20.30	134.6

TABLE III

Moisture Removal, Shrinkage Temperature and Nitrogen Checks As Function of Varying Drying Conditions - Calfskin Squares

Drying Conditions	Time (Hours)	% Water Removed	Nitrogen (mgm/gm) Initial	Final
Cyclopentane (B.Pt. 50°C.)	94	12.65	147.1	147.1
Vacuum Oven (50°C.)	96	12.91	147.9	147.2
Methylcyco-hexane (B.Pt. 100°C.)	96	14.6	147.3	147.7
Vac. Oven (100°C.)	96	15.4	147.8	148.9
Cumene (B.Pt. 153°C.)	96	15.8	146.0	145.0
Vac. Oven (150°C.)	96	16.2	145.3	146.4

TABLE IV

% WATER REMOVED FROM STANDARD HIDE POWDER BY OVEN DRYING

Drying Conditions

70°C. Vac.		100° Air		140° Vac.	
Time Hrs.	% Water Removed	Time Hrs.	% Water Removed	Time Hrs.	% Water Removed
11	10.22	11	10.3	11	11.35
19	10.20	18	10.52	19	11.42
35	10.58	34	10.63	35	11.78
42	10.54	42	10.67	41	11.78
59	10.58	58	11.0	58	11.80
89	10.58	65	11.0	88	11.84
		82	11.0		
		112	11.0		

NITROGEN DETERMINATIONS TO CHECK ON

DECOMPOSITION BY LOSS OF NITROGEN FROM HIDE POWDER

UNDER VARIOUS DRYING CONDITIONS

Initial Nitrogen Content (mgm./gm.; as-is basis) - 161

	Drying Conditions	Final Nitrogen Content (mgm./gm.)
Boiling Hexane	24 Hrs.	162
" "	48 Hrs.	162
" "	72 Hrs.	160
" Xylene	24 Hrs.	160
" "	48 Hrs.	163
" "	72 Hrs.	161
Air Oven - 100°C.	130 Hrs.	162
Vac. Oven - 70°C.	107 Hrs.	160
Vac. Oven - 140°C.	106 Hrs.	161
A.L.C.A. Evaporator	26 Hrs.	160

TABLE V

TYPICAL DATA OBTAINED WITH ELECTRONIC FORCE-SHRINKAGE METER

Untanned Skin			Chrome-Tanned Skin		
Time (min.)	Temperature (°C.)	Force (gm.)	Time (min.)	Temperature (°C.)	Force (gm.)
0	29	100	0	27	100
2	36	75	2	32	85
4	41	65	4	36.5	75
6	46	50	6	41	68
7	49	45	8	44	63
7.5	50	43	10	50	52
8	52	42	12	56	45
8.5	53	40	14	62	40
9	54	36	16	68	32
9.5	56	35	18	74	30
10	57.5	35	20	79	25
10.5	58.5	35	21	82	25
11	60	35	22	85	25
11.5	61	39	23	86.5	25
12	62	85	24	89	25
12.5	63.5	130	25	91	25
13	65	150	26	92	25
13.5	67	175	26.5	93	26
14	68.5	250	27	94	30
			27.5	95	35
15	70.5	300	28	96	40
16	73	340	28.5	97	47
17	76	365	29	98	65
18	79	380	29.5	99	110
18.5	80	385	30	Boil	325
19	81	395	31	"	750
19.5	82	390	32	"	800
20	84	375	33	"	950
21	86	370	34	"	1075
22	88	350	35	"	1150
23	90	335	36	"	1200
24	91	325			

TABLE VI

CHANGES IN THE WET SHRINKAGE TEMPERATURE OF UNTANNED AND CHROME TANNED PIECES, PRODUCED BY HEATING IN VARIOUS HYDROCARBON SOLVENTS AT THE BOILING POINT FOR PERIODS OF 4-5 DAYS

	Untanned			Chrome-Tanned*
	Shrink Temp.-Control-60°			Shrink. Temp.-Control - 93°
Time (Hrs.)	Shrink. Temp. (°C.)	Temp. of Max. Force (°C.)	Max. Force (gm.)	Shrink. Temp. (°C.)
	n-Hexane - B.Pt. - 69°C.			
2	59.5	82	57.5	91
5	60	85	800	92
22	59	85-89	1000	88
46	59	86-88	1240	88
70	59	84-87	425	88
94	60	85-88	850	84
	Benzene - B.Pt. - 80°C.			
2	60	81	395	87
5	60	86	750	87
22	59	85	510	77
46	60	89	1320	79
70	59	83-Boil	1270	87
94	58	92	595	79
	Methyl Cyclohexane - B.Pt. - 100°C.			
1	60	82-88	900	77
3	60	84	745	74
19	56	86-88	535	69
43	56	**		66
68	55	**		64
100	55	**		

*No maximum ever observed in water for Chrome Tanned Skin
**No maximum reached up to B.Pt. of H_2O

(Con'd. on next page)

TABLE VI - (Con't.)

Time (Hrs.)	Untanned: Shrink. Temp. (°C.)	Untanned: Temp. of Max. Force (°C.)	Untanned: Max. Force (gm.)	Chrome-Tanned: Shrink. Temp. (°C.)
		Toluene - B.Pt. - 111°C.		
1	58	80	510	82
4	58	91	1025	74
12	58	93	825	66
28	56	**		64
52	53	**		59
72	52	**		42
96	52	**		42
		Xylene - B.Pt. - 137°C.		
1	56	90-98	850	64
4	50	**		44
12	48	**		46
27	31	**		37
52	23	**		22
72	22	**		22
96	14	**		22
		Cumene - B.Pt. - 153°C.		
1	52	**		42
3	44	**		22
8	32	**		27
19	21	99	830	19
43	9	Boil	1065	15
68	4	**		12
96	4	95-Boil	700	17

TABLE VII

EXAMPLE OF IMMERSION READINGS ON DRY CHROME TANNED CALFSKIN

Solution	Dielectric Constant	Dial Reading Solution	Dial Reading Solution and Skin	Change in Dial Reading (ΔP)
$n\text{-}C_6H_{12}$	1.89	83.9	82.7	-1.2
CCl_4	2.24	83.0	81.9	-1.1
C_6H_6	2.28	83.0	82.0	-1.0
A - 1	3.0	81.0	80.2	-0.8
B - 1	3.1	80.7	80.0	-0.7
A - 2	4.0	78.4	78.2	-0.2
$(C_2H_5)_2O$	4.34	77.5	78.4	+0.9
$CHCl_3$	4.95	75.9	76.5	+0.6
A - 3	5.0	75.7	76.2	+0.5
B - 2	4.9	76.0	76.7	+0.7
C_6H_5Cl	5.6	74.0	75.3	+1.3
A - 4	5.9	73.1	74.9	+1.8
B - 3	5.9	73.4	75.0	+1.6
A - 5	6.7	71.3	73.5	+2.2
B - 4	6.7	71.1	73.2	+2.1

Series A - mixture of CCl_4 and $CH_3CHClCH_2Cl$

Series B - mixture of $n\text{-}C_6H_{12}$ and $1,2\text{-}C_6H_4CH_3NO_2$

Dielectric Constant of Series A and B determined from Calibration Curve

Dielectric Constant of the solvents are taken from the literature.

Interplate Distance - 0.048"
Frequency - 11.184 m.c.s.
Series Condenser - 130 mmfd.
(to block D.C., bypass R.F. - large enough to maintain linearity in range studied)

TABLE VIII

CHEMICAL ANALYSES AND DIELECTRIC CONSTANT MEASUREMENTS

Series 1

Chemical Analysis (Dry-Basis)

Untanned	Chrome-Tanned
Lipid - Trace	Trace
Ash - Trace	4.87%
Hide Substance - 100%	88%
Chrome oxide - 0	4.51
Shrink. Temp. - 54°C.	88°C.
pH - 4.8	2.95

Thickness - 0.050"
Frequency - 11.184 m.c.s.
Series Condenser - 2500 mmfd.

Untanned		Chrome-Tanned	
gm. H_2O per gm. H.S.	Dielectric Constant (Immersion)	gms. H_2O per gm. H.S.	Dielectric Constant (Immersion)
0.00	3.6	0	3.9
.089	4.5	.121	5.0
.124	5.2	.168	6.0
.182	6.3	.208	6.7
.227	6.8	.237	6.9
.284	7.5	.314	8.0
.302	7.6		

TABLE IX

CHEMICAL ANALYSES AND DIELECTRIC CONSTANT MEASUREMENTS

Series 2

Chemical Analysis - (Dry Basis)

	Untanned	Chrome-Tanned
Lipid	Trace	Trace
Ash	"	3.48
Hide Substance	100%	92.5%
Chrome Oxide	0	3.23
Shrink. Temp.	60°C.	95°C.
pH	5.55	3.36

Thickness - 0.060"
Frequency - 11.184 m.c.s.
Series Condenser - 2500 mmfd.

Untanned			Chrome-Tanned		
gm. H_2O per gm. H.S.	Dielectric Constant (Immersion)	Dielectric Constant (Direct)	gm. H_2O per gm. H.S.	Dielectric Constant (Immersion)	Dielectric Constant (Direct)
0	4.2	1.32	0	4.6	1.34
0.098	4.95	1.39	.115	5.5	1.50
0.130	5.2	1.55	.150	6.3	1.62
0.147	5.5	1.65	.178	6.6	1.80
0.199	6.4	1.72	.233	7.2	1.98
0.284	6.8	2.35			
.286	7.2	2.48	.314	7.7	2.85
.385	--	3.1	.316	7.7	2.85
.77	--	14	.475	--	2.9
			.84	--	17

TABLE X

PART I - DATA USED TO PREPARE A CALIBRATION CURVE FOR THE DETERMINATION OF MOISTURE IN LEATHER*

Per Cent Water	Change in Dial Reading (ΔP)
55.3	31.7
52.2	31.2
45.9	17.2
38.5	11.7
30.5	5.7
29.7	5.0
15.7	1.5
12.7	1.2
9.1	1.05
0	0.75

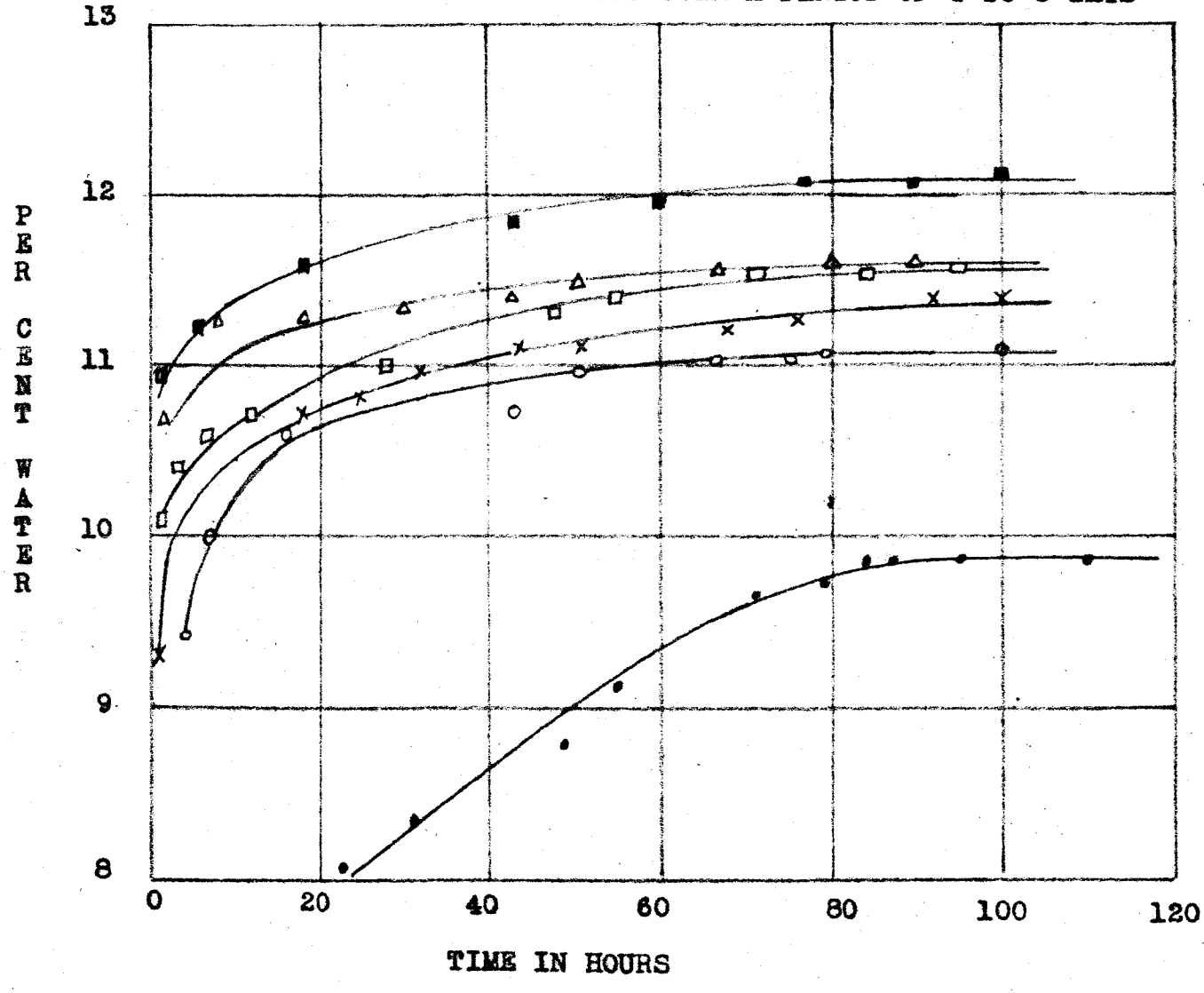
COMPARISON OF RESULTS PREDICTED FROM OSCILLATOR READINGS AND CALIBRATION CURVE WITH THOSE OBTAINED BY OVEN DRYING OF SAME PIECES

Predicted	Found	Error	Predicted	Found	Error
2%	2.8	-0.8	30.3	29.7	+0.6
11	9.7	+2.3	31.0	33.6	-2.6
15.5	12.1	+3.4	36.3	37.0	-0.7
17.0	13.7	+3.3	36.7	33.7	+3.0
18.2	19.2	-1.0	38.5	40.6	-2.1
19.3	18.5	+0.8	45.5	44.8	+0.7
21.0	18.7	+2.3	47.8	47.2	+0.6
22.0	21.1	+0.9	54.2	51.9	+2.3
25.8	23.8	+0.2	54.7	54.8	-0.1
27.2	27.7	-0.5			

*Commercial chrome-tanned calfskin. Light vegetable topping, fat-liquored, and dyed.

FIGURE 1-A

PER CENT WATER REMOVED FROM STANDARD HIDE POWDER BY VARIOUS HYDROCARBONS OVER A PERIOD OF 4 TO 5 DAYS



SYMBOL	SOLVENT	B.Pt.(°C)
●	Cyclopentane	50
○	n-Hexane	69
×	Methyl Cyclohexane	100
□	Ethyl Cyclohexane	132
△	Xylene	137
■	Mesitylene	164

FIGURE 1-B

PER CENT WATER REMOVED AT EQUILIBRIUM BY SOLVENT DISTILLATION METHOD FROM HIDE POWDER, UNTANNED AND CHROME TANNED SKIN AT VARIOUS TEMPERATURES

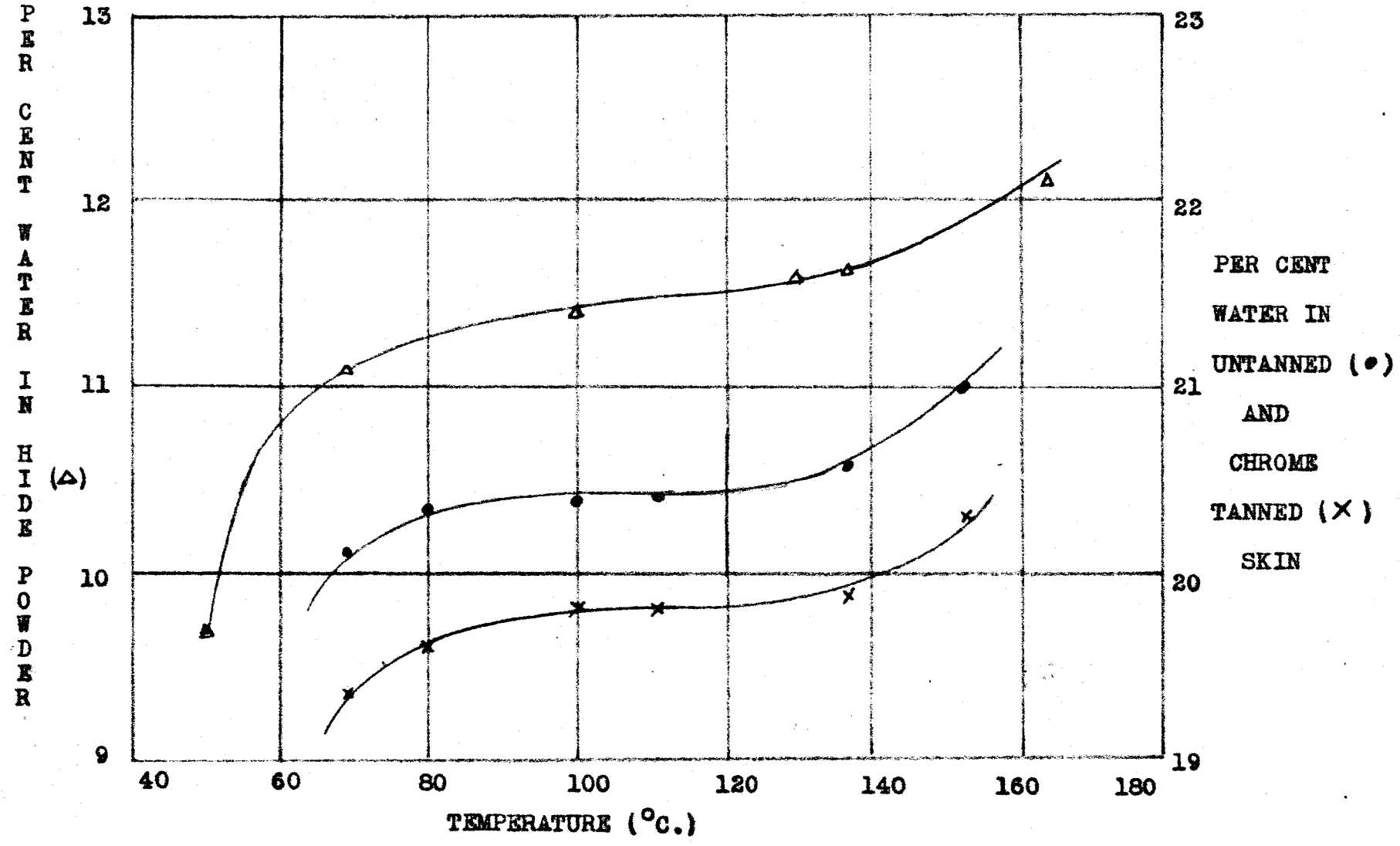


FIGURE 2

BLOCK DIAGRAM

WIRE RESISTANCE STRAIN GAUGE AND AMPLIFIER

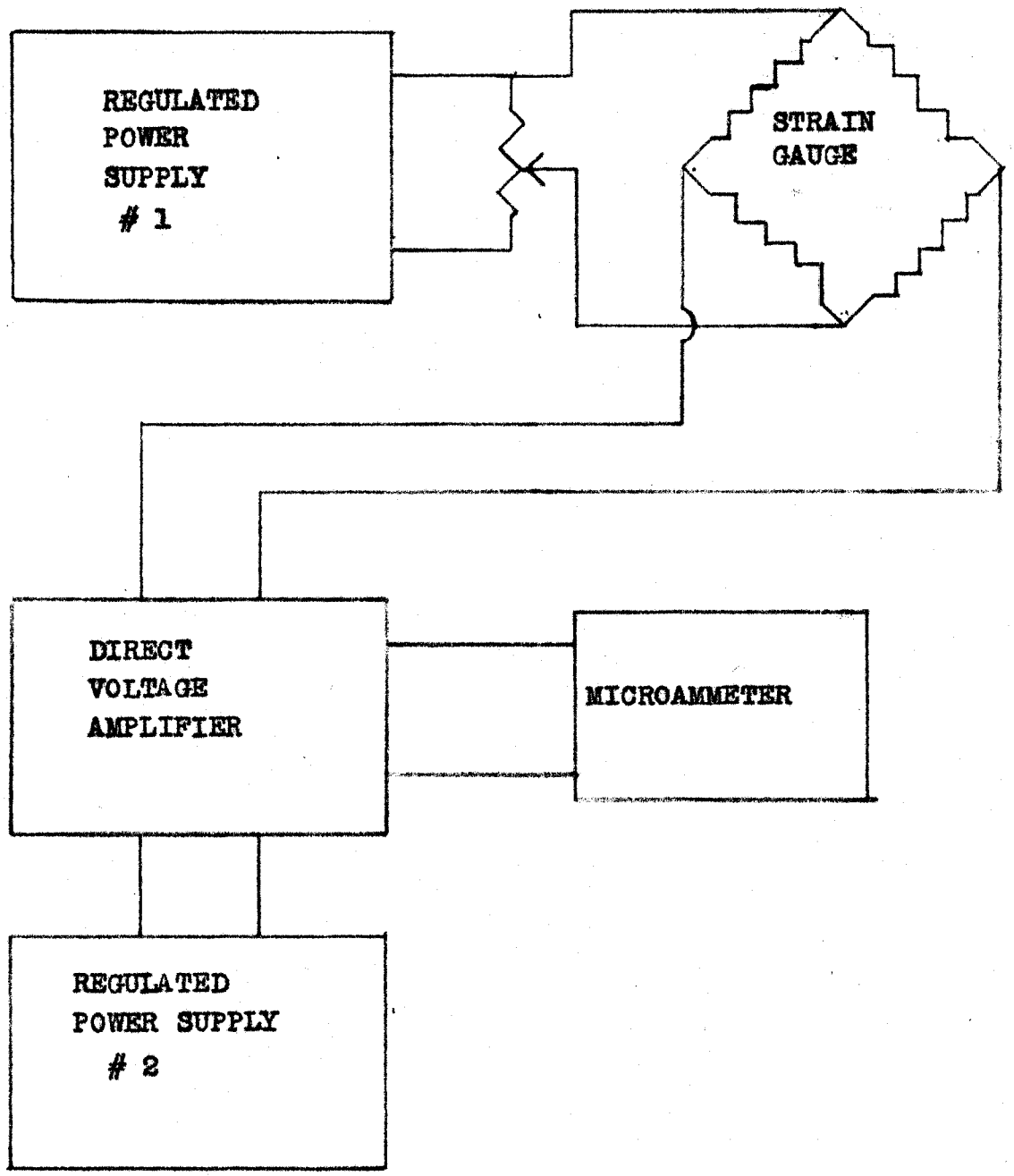


FIGURE 3

TYPICAL FORCE-SHRINKAGE CURVES IN WATER

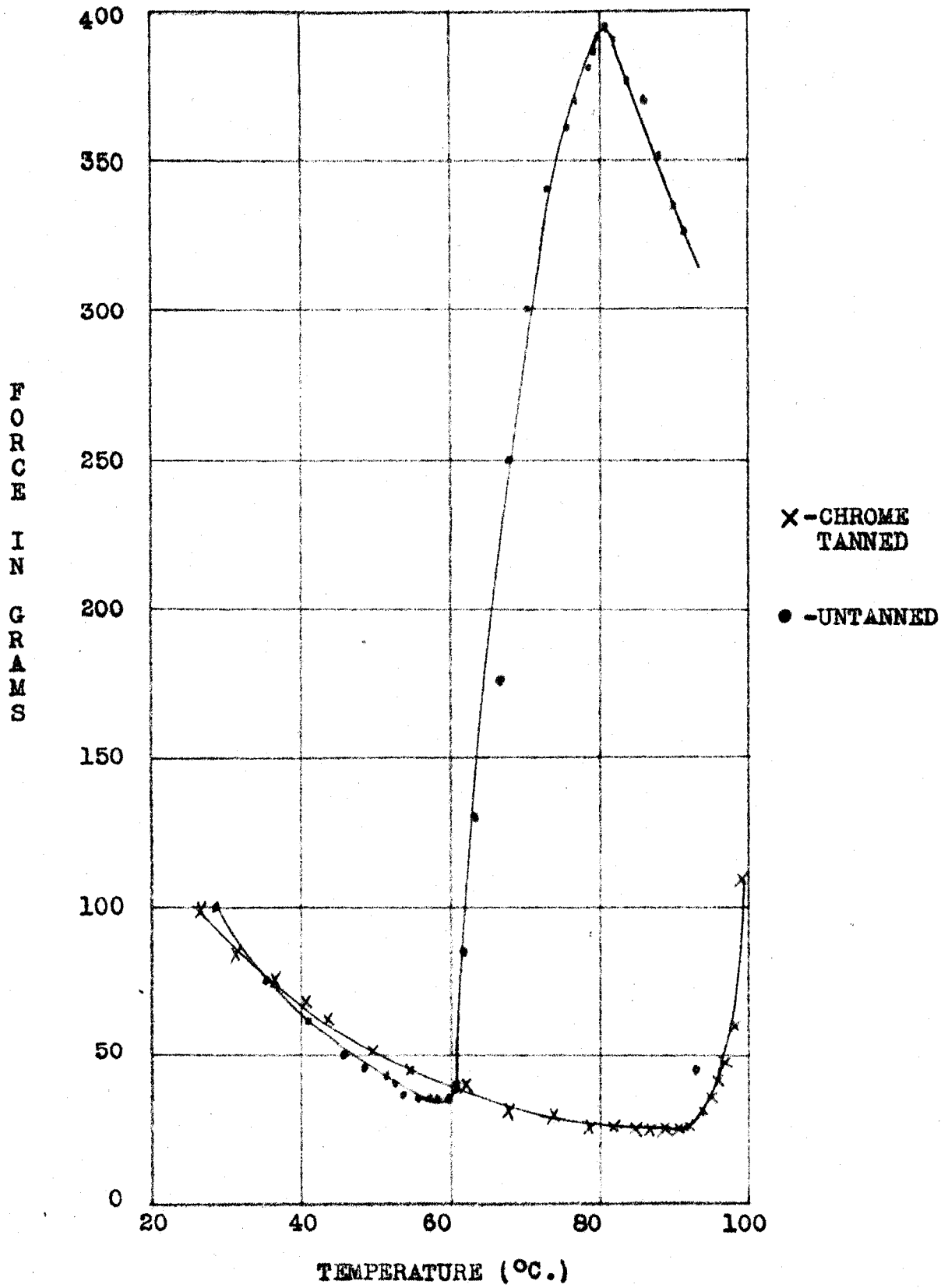
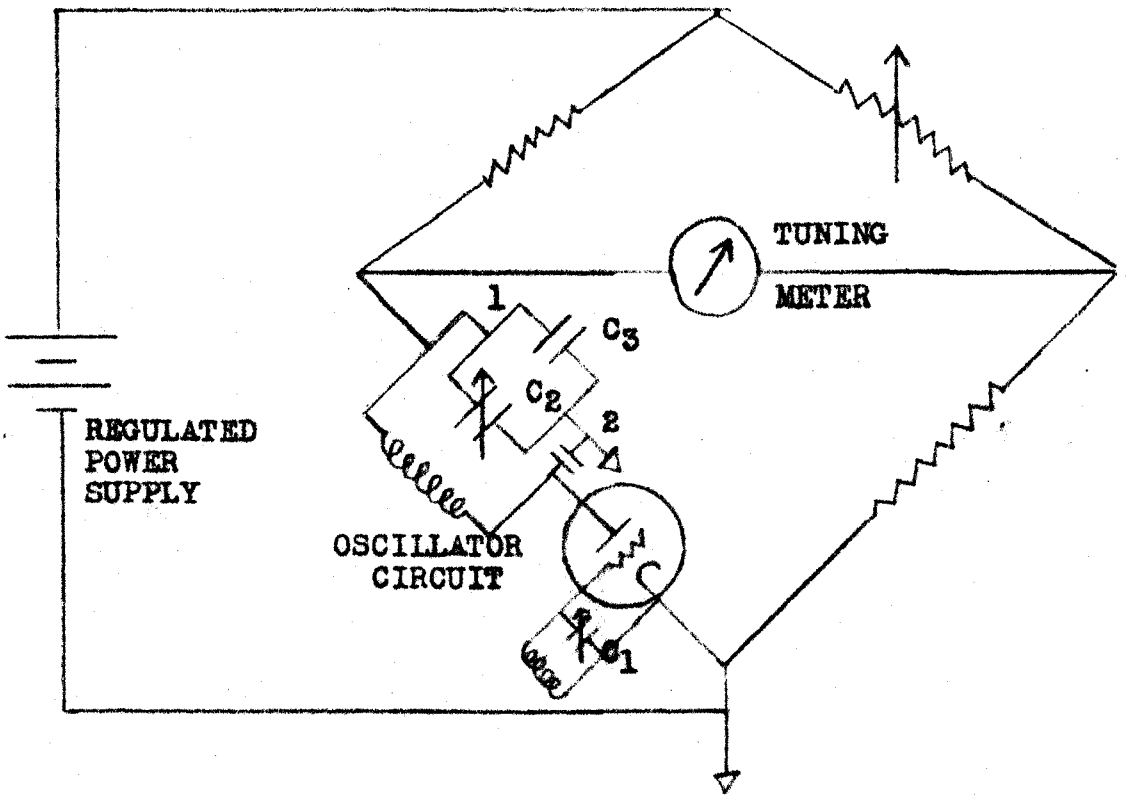


FIGURE 4

SCHEMATIC CIRCUIT DIAGRAM AND MEASURING CONDENSER USED TO DETERMINE DIELECTRIC CONSTANTS OF SKIN AND LEATHER PIECES



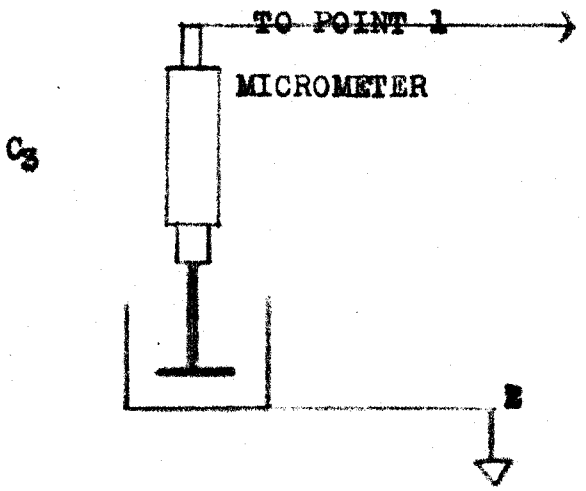
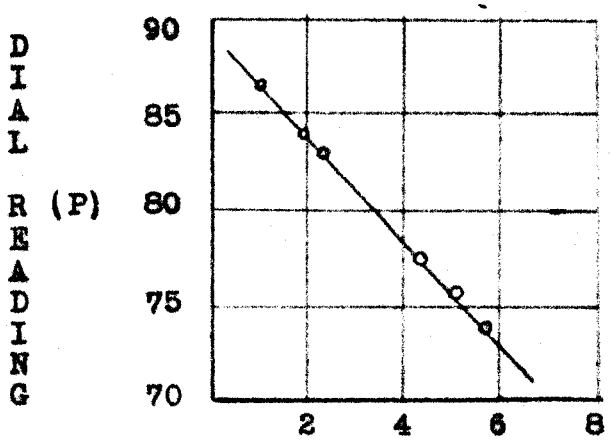


FIGURE 5

(DATA FROM TABLE 7)

CALIBRATION CURVE FOR DETERMINING

THE DIELECTRIC CONSTANTS OF LIQUIDS



PLOT OF IMMERSION READINGS

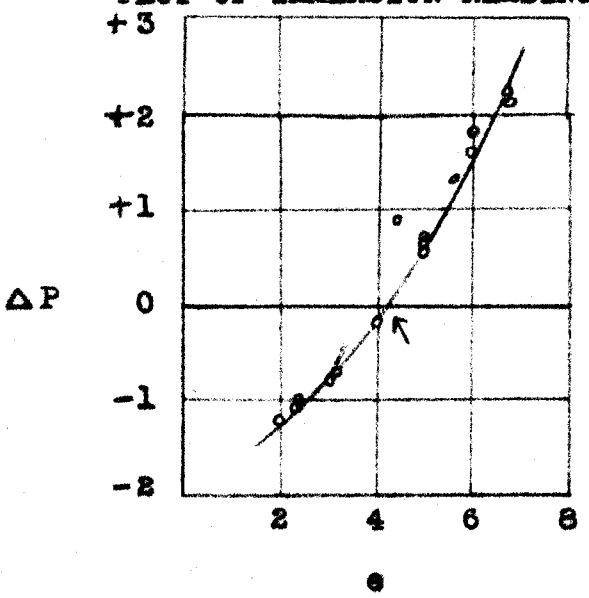


FIGURE 6

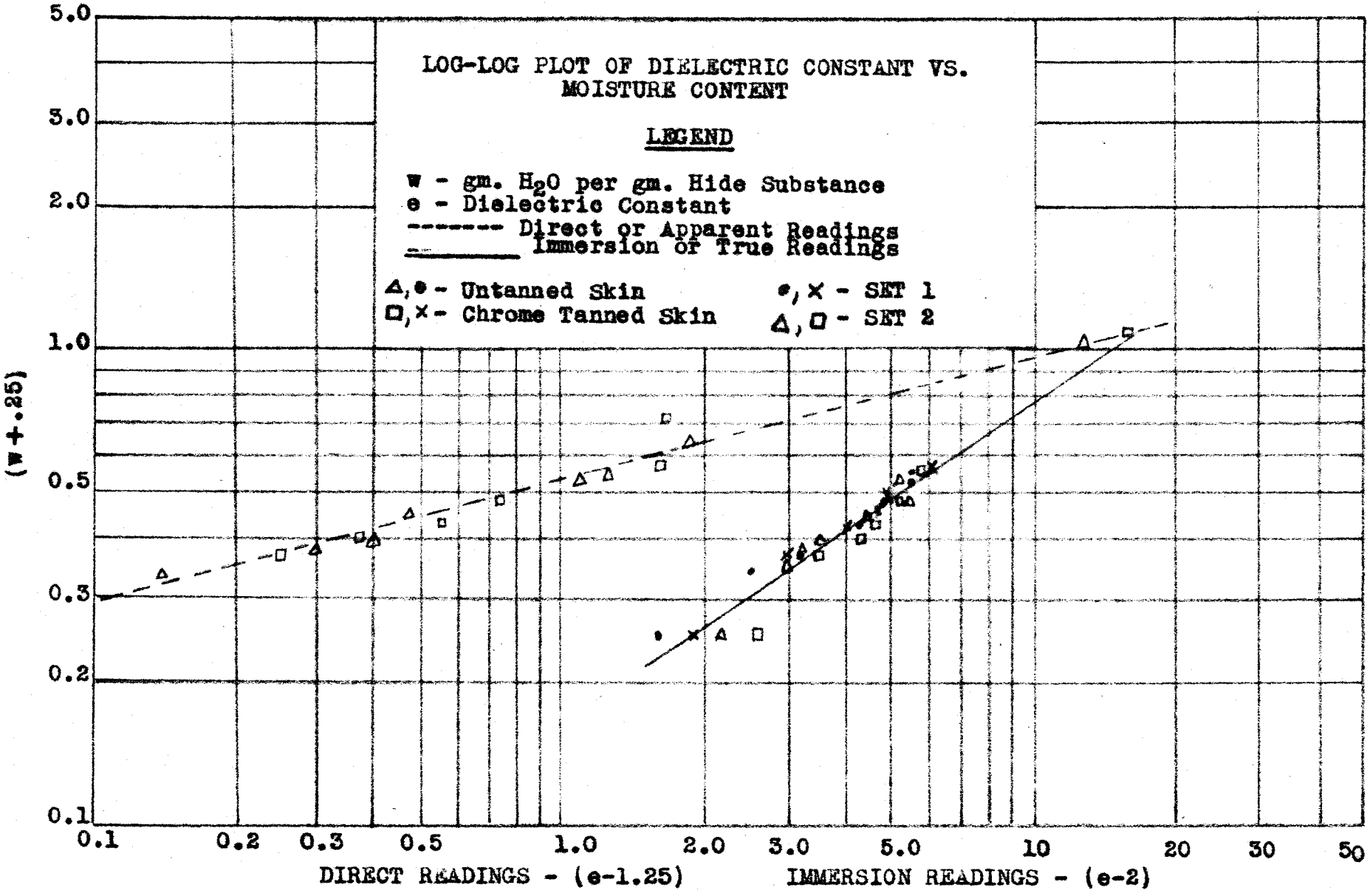
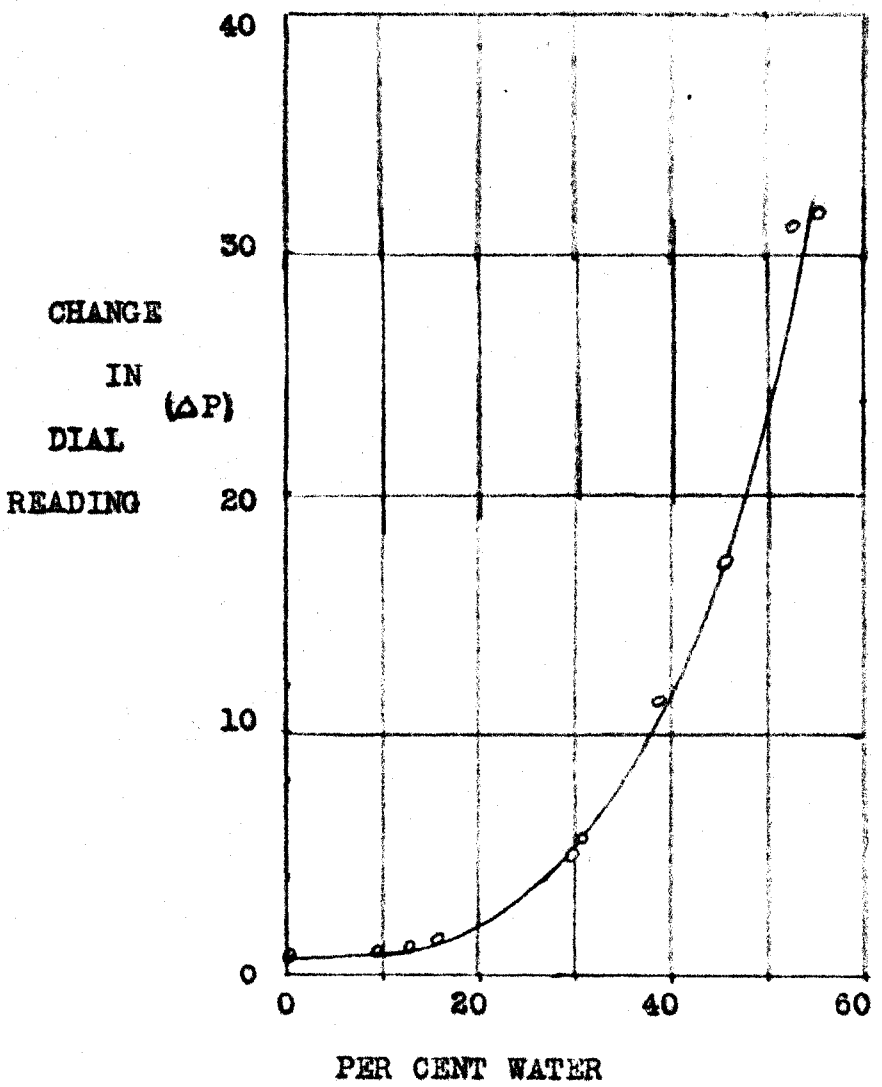


FIGURE 7

CALIBRATION CURVE FOR DETERMINING THE MOISTURE CONTENT OF LEATHER



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