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A STUDY OF THE MECHANISM OF BATING

A dissertation submitted to the

Graduate School of Arts and Sciences
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in partial fulfillment of the
requirements for the degree of

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by

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One of the important steps in the making of leather is the process known as bating or puering. This operation consists in treating the limed, unhaired skins with materials containing a proteolytic enzyme absorbed on an inert material, plus a deliming salt such as ammonium sulfate. The object of the process is to reduce some of the alkalinity of the skin and remove, from the grain surface particularly, any substance which would interfere with subsequent tanning operations.

The mode of action of the bating materials used by the tanner is not well understood. This is due in part to the lack of suitable methods for measurement of the characteristics of the skin which are used by the tanner to indicate the completion of the operation. Previous studies by other workers have dealt with the action of bates on individual skin constituents and other protein systems, but the correlation and interpretation of their results in terms of the whole skin has not been too satisfactory.

The object of this study is to develop reproducible measurements of the so-called "bated characteristics" of skin and correlate these measurements with physical, chemical, and histological observations. This goal involves the establishment of a method for measuring "fall" and "smooth, silky feel" in a bated skin. The fall of a skin is reflected by its permanent distortion after release of pressure, while the smooth silky feel is a reflection of the coefficient of friction. The measurement of these two factors represents a new approach to the problem presented in the study of the bating process.

Experimental

The experiments included in this thesis deal with the following points.

1. The establishment of a laboratory controlled bating procedure.

2. The measurement of the physical properties of friction and distortion on wet calf skin.

3. The correlation of these measurements with histological examinations.

4. The correlation of these measurements with the amount of protein nitrogen removed from the skin as a result of the bating process.

5. The comparison of the action of varying percentages of different bate materials and ammonium sulfate under controlled conditions.

Results

The results of the experiments performed indicate the following:

1. The ratio of the coefficient of friction of a water control to the coefficient of friction of the bate treated pieces is a measure of the degree of enzyme action on the skin.

2. The permanent distortion of the bated samples is higher than the water or ammonium sulfate controls. This difference is not great, however, and may not be statistically significant.

3. The pancreatic bate produces higher values for the ratio of the coefficients of friction than either the bacterial or fungal bates. This factor may be statistically significant.

4. Histological examination of micro-sections demonstrated that the action of the enzymes changes the hair pocket angle, as well as the arrangement of the elastic tissue structure, and the amount of coagulable proteins in the grain area is reduced. The fibroblasts lose their staining ability and further, the pattern of the lime distribution in the skin is changed.

5. There is a direct correlation between the amount of bate used and the degree of histological changes in the sections.

6. Under the conditions of our experiments the amount of protein nitrogen (precipitated by tri-chloracetic acid) recovered from the liquors of the pancreatic and bacterial bates is somewhat less than the amount recovered from the liquors of the ammonium sulfate and the fungal bates. This difference is not great and may not be statistically significant. The trend which does exist seems to bear further investigation.

Conclusions

The results of the experiments indicate that under controlled conditions for the bating operation, the coefficient of friction may be used as a measure of enzyme action upon the skin. The factor of permanent distortion probably measures only the action of the deliming salts, since the results obtained cannot be considered statistically significant on this group of samples. Enzyme action is associated with changes in the internal structure of the skin rendering it closer to the structures found in the fresh skin and removing interfibrillary material. On the basis of our evidence there seems to be no direct correlation between the amount of protein nitrogen removed from the skins as a result of the bating process and the type of enzyme material used, but this should be investigated further.

A STUDY OF THE MECHANISM OF BATING

Introduction

There are several reasons why leather making is often referred to as "The Art of Tanning" and probably one of the most important of these arises from the process known as bating. Many centuries ago the tanners found that if the swollen skins, taken from the lime solutions used for unhairing the skins, were treated with an aqueous infusion of dog or fowl dung, certain desirable characteristics were imparted to the finished leather. This process, known to the tanner as bating or puering, was first systematically studied by Wood (25) in 1898, who demonstrated that the important constituents in these infusions were proteolytic enzymes and organic acids. As a result of these studies and their practical development and application by Röhm (20) and others, the bating of skins in the modern tannery consists of a treatment by commercial preparations containing enzyme material and ammonium salts, instead of the "filthy, disgusting operation" of Wood's time. The composition of the bating materials used by the tanner is essentially a mixture of a proteolytic enzyme absorbed on an inert material such as wood flour or sawdust, and a deliming salt such as ammonium sulfate or chloride. The proteolytic enzyme is obtained from one of three sources; tryptic enzyme, extracted from the pancreas of animals, bacterial or fungal enzymes, extracted from cultures of selected microorganisms. Even though progress has been made toward greater uniformity of product, the mode of action of these bates on the various skin constituents and the skin as a whole still is not completely understood.

The complexity of the bating mechanism, as well as its importance in leather manufacture, has stimulated many workers to study the process, yet no complete knowledge is available at the present time. This is easily understood when one realizes the difficulties besetting such studies. One of the stumbling blocks to understanding the bating mechanism has been the lack of methods for measuring those characteristics of the skin which are observed by the experienced tanner and used by him as the criteria for the control of the bating process.

The present work was undertaken as a foundation for a long term program aimed at a more complete understanding of the entire bating mechanism. Reproducible measurements of the skin characteristics associated with proper bating, correlated with physical, chemical and histological procedures, are unquestionably essential to any program seeking a more complete understanding of the bating mechanism. The development and correlation of methods for such measurements is the purpose of the present undertaking.

The bating process as used in the modern tannery consists of the following steps. After the skins have been unhaired and freed from extraneous tissues, they may be given a preliminary deliming treatment to remove any traces of calcium carbonate on the surface of the skin. The skins are then transferred to a paddle vat containing water at the temperature selected by experience as suitable for the type of skins, the particular tannery conditions and the kind of leather desired. In some tanneries, sufficient acid delimer is added to the warm water to lower the pH of the surface of the skin to a value below that necessary

to remove traces of calcium carbonate, and to aid in adjusting the pH to a point near the optimum for enzyme activity. This degree of deliming varies with the type of skin as well as in the particular tannery while some tanners depend upon the ammonium salts contained in the bate for all the pH adjustment in the process. The skins are brought to the desired temperature and the bate is added. The maintenance of the temperature, throughout the bating period as well as the agitation of the skins and length of time the skins are bated, depends upon the type of skin and the characteristics desired in the leather. The "bate master" determines the completion of the operation by the "feel" of the skins.

At the end of the operation, the previously swollen, turgid skin is now flaccid, the grain has a soft silky feel, the impression of the fingers is retained if the skin is squeezed, and it is said to be somewhat permeable to air. The ammonium salts contained in the bate have reacted with the lime of the skin, thus lowering the pH of the entire system to approximately 8.0-9.0. This change in the lime picture contributes to the so-called "fallen" condition of the skin as well as changing the pH of the skin to the optimum necessary for the activity of the enzymes.

In previous studies, attempts have been made to explain the entire bating mechanism on the basis of the action of bates on individual constituents of the skin. Other studies have been concerned with the mechanism of the action of proteolytic enzymes on such substrates as casein, hide powder and gelatin. The interpretation of these studies

in terms of their relation to the skin as a whole is somewhat hazardous.

Publications dealing with the theories (4, 10, 23) of the bating mechanism have considered the effect of bating on:

1. Skin collagen
2. Elastic tissue
3. Reticular tissue
4. Muscle tissue
5. Degraded keratinous substances present in hair follicles
6. Coaguable proteins (albumins and globulins)
7. Mucoids

For instance, one of the more complete studies on this subject was that of McLaughlin et al (9) who concluded on the basis of their results that:

a. The changes in hydration of involuntary and voluntary muscle tissue are not effected by the action of the bate enzymes.

b. Removal of keratinous substances is not an enzyme function but can be carried out by simple deliming agents.

c. Removal of interfibrillary matter is probably the prime function of the bate enzyme.

The methods proposed for testing the various preparations which may be used by the tanner for bating have been numerous. They consist briefly in permitting the enzyme to act under a set of standard conditions, on a given protein, i.e. casein, gelatin or hide powder, and then measuring the amount or type of change which has taken place. One deviation from these methods was suggested by McLaughlin and Wilson (11) in the use of 1" limed calf skin squares. This work compared the total amount of soluble nitrogen obtained from 100 grams of limed calf skin squares after treatment with various enzyme preparations under controlled conditions. They pointed out that the method could be used for control work in the tannery, but in the form reported was not applicable for a standard method.

McLaughlin (*10*) also recommended that any significant study of the bating process should be performed with the skin itself, since investigations employing other protein systems bear no direct relation to the conditions found in the complex skin system. When we consider that limed animal skin contains not only collagen but also muscle tissue, reticular tissue, elastic tissue, coaguable proteins, fats, water and salts, we realize that this observation is a pertinent one. All these materials are present in variable quantities and in various degrees of denaturation and hydration. Also, the physical structure of the skin varies with the position of the sample taken, the age and type of animal, and any previous treatment given to the skin. Any attempt to measure the characteristics of the smoothness and fall of a skin as a result of bating, must take all of these factors into consideration.

The research covered by this thesis extends the early work of this laboratory and utilized recent developments and knowledge accumulated since that time. We have attempted herein, to develop measurements of those characteristics observed by the experienced tanner as indications of proper bating and correlate these measurements with physical, chemical and histological measurements on 3 x 6 inch samples of limed calf skins. The characteristics studied are "the soft silky feel" typical of a bated skin and the "fall" or lack of resilience of the bated skin after pressure has been applied and then released. The soft silky feel of the skin is a reflection of the physical characteristic of friction; while the fall reflects the porosity of the skin and state of turgor of the various constituents

and their relation to each other.

Recently the supply of tryptic enzyme has been sharply curtailed due to the demand for animal pancreas for use in the preparation of insulin and protein hydrolysates for human consumption. There exists among tanners a controversy as to the effectiveness of the substitution of the products obtained from microorganisms for use in the bating process. Without adequate methods for comparing activity of these preparations under controlled conditions on the whole skin, there still remains a doubt as to the complete interchangeability of the several types of enzyme bates. One portion of the work deals with the application of our methods in the comparison of bating materials containing pancreatic, baterial and fungal enzymes.

The methods used in this study are as follows:

1. Determination of "Fall" or percent distortion.

The characteristic of fall of a bated skin represents the lack of recovery of the skin after it has been subjected to pressure, i.e., its distortion. In the work reported, this factor is expressed as the per cent loss in thickness of the skin after recovery from a standard load and is calculated on the basis of the thickness of the limed skin after washing and before any deliming or bating is done. The determination of this loss is made by means of the Schiefer compressometer (Figure 1) with certain modifications. A detailed description of the actual test is given on page 16 later in this report.

2. Determination of smoothness or "Bate Index".

The smoothness of any material is a function of its coefficient

of friction and may be conveniently measured by means of the inclined plane. Thus, the tangent of the angle at which a given object begins to slide over the inclined surface of the substance to be evaluated, represents the coefficient of friction between the object and substance. In attempting to apply this law to the soft wet surface of a bated skin many difficulties become apparent. If the determination is done in air, the surface of the skin dries and changing values are obtained. If the sliding object is too heavy or has sharp edges it will dig into the soft surface of the skin and also produce false results.

In order to overcome these errors, the following apparatus was assembled (Figure 2). It consists briefly of an ordinary glass aquarium $7\frac{1}{2}$" x 11" x 8" filled with buffer solution maintained at a constant temperature and constant pH. The height of the liquid was also kept constant throughout the experiment. The piece of skin to be treated was fastened lengthwise on a glass plate by means of rubber bands, care being taken to eliminate wrinkles in the skin. One end of the plate rested against the bottom edge at one end of the vessel while the other was attached by means of a clamp with a thread strung through a pulley to a winding mechanism. An ordinary fishing reel was employed with success.

The sliding object consisted of two pieces of coarse grained frosted glass 3" x 1" cemented together and with one end bevelled sufficiently to keep the slider from digging into the surface of the skin. All other edges were rounded to eliminate sharp angles but not to as great a degree as was the leading edge. The total weight of the slider was 36 grams.

A large protractor covering the entire area of the back wall of the aquarium was mounted on the outside. A detailed description of the test is given on page 15 later in this report.

After preliminary experiments, it was observed that the tangent of the angle determined could not be used for comparisons from one skin to another, due to the wide variations in the degree of smoothness of the original skins before treatment. Accordingly all comparisons were made between treated samples and a water control taken from the same skin, the water control sample receiving the same mechanical treatment as the bated pieces, i.e., shaking in plain water in a closed jar for the same time and at the same temperature. Results are then expressed as a "Bate Index", i.e. the ratio of the coefficient of friction of the water control to the coefficient of friction of the treated sample:

$$\frac{\text{Tangent of angle of water control}}{\text{Tangent of angle of treated piece}} = \text{Bate Index}$$

3. Determination of Protein Nitrogen.

The determination of protein nitrogen was carried out on aliquots taken from the combined spent bate liquor and bate wash according to the standard A.L.C.A. procedures (26).

4. Histological observations.

The effect of bating on the skins are observed microscopically by preparing frozen cross-sections both before and after treatment of the samples. After staining and mounting the following observations were made:

A. The cross-sections stained with Weigert's Elastic Tissue Stain.

1. Width of elastic tissue pattern.
2. Width of total section.
3. Measurement of angle of the hair pockets (the angle between a line parallel to the grain surface and the hair pocket).
4. Appearance of fiber bundles.

B. Cross-sections stained with Nile Blue Sulfate Stain.

1. Presence of fibroblasts (stained)

C. Incinerated sections.

1. Changes in the deposition pattern of the inorganic salts.

Experimental

Preliminary experiments were carried out to establish those conditions which would give reproducible readings of the friction and distortion measurements. The wide variations in texture, architecture, and chemistry which are found from skin to skin, as well as those encountered due to the position of the sample taken had to be kept at a minimum. For instance, it is known that areas near the neck and on the flanks have a looser internal structure and may compress and recover differently, while those close to the tail and along the center of the back bone are more compact. The following experiment was designed to deal with these variations.

1. Position of the sample.

To determine the influence of sample location, limed calf skin was cut lengthwise from head to tail into 3" wide strips. These strips were bated with a pancreatic bate, using 1% of the bate calculated on the basis of the limed weight of the skin. The time of the bating was 2 hours and the temperature was 85°F. At the end of this treatment distortion measurements were made. Table I shows the results. It may be seen from the table that distortion figures for the center cuts (Figure 3) were more uniform than those for other parts of the skin.

2. Skin thickness.

It was pointed out by Schieffer (27) that wide variations in the thickness of a given material would cause errors in the values obtained where the compressometer was used to evaluate the resilience of fabrics. In considering the wet skin, we realize that this observation

would apply to our work. In addition the practical tanner has observed that heavy skins do not seem to bate as well as lighter skins for a given concentration of bate and time and it is obvious that this factor must be controlled.

Pieces of skin of varying thickness were washed and bated. Table 2 shows the effect of wide variations in skin thickness. It may be seen that minimum variations occur when the skin thickness ranges between 90 and 130 thousandths of an inch as measured on the Schieffer compressometer. Later experiments demonstrated that other factors such as surface pH and wash procedure tended to remove some of these objections.

3. Previous treatment of the skins.

McLaughlin et al (//) observed that "skin fibers which are covered with coagulated and denatured protein material, require more drastic soaking, liming, and bating than if less of such materials are present".

Table 3 shows the effect on distortion and bate evaluation samples undergoing the following treatment:

1. Long lime periods
2. Holding in saturated lime solution
3. Holding at ice box temperatures
4. Quick freezing and holding in the frozen state.

It may be observed that samples of calf skin which had undergone prolonged liming or were held in saturated lime for 2 weeks after unhairing or held in the limed state in the refrigerator resulted in skin which is difficult to bate. Those samples which had received shorter lime periods and were quick frozen retained their ability to bate easily and results were more uniform.

Since it was desirable to use calf skin which had been limed, unhaired and scudded in the tannery, the method of quick freezing and holding in the frozen state in sealed water-proof cellophane bags was adopted and used throughout the work reported.

4. Control of wash procedure.

In order to obtain reproducible results a standard wash procedure was adopted after preliminary trials. This procedure had to take into account the amount of lime remaining in the skin and on its surface, the starting pH and temperature of the skin. The procedure adopted is described in detail on page /3.

The preliminary experiments which were used to establish this washing procedure are summarized in Table 4. It may be seen that samples which had been washed in water only, thus bringing the surface of the skin to a pH of 10-10.5, as evidenced by the pH of the wash liquor, gave lower values for the Bate Index than those samples which had been given the slight deliming in ammonium sulfate thus changing the pH of the surface of the skin to 8.8 or 9.0 as it went into the bate.

After the preliminary experiments had been completed, the following specifications and procedures were adopted as those most likely to give reproducibility of conditions and results for experimental work.

Specifications for the Standard Experimental Procedure.

1. Skin

A. Position of samples.

Samples were taken from tannery processed skins and from the area designated in Figure No. 3, care being exercised to eliminate any areas which contained wrinkles.

B. Thickness of the sample.

The thickness of the sample should lie between 90 and 130 thousandths of an inch as measured on the compressometer, this measurement to be taken after the first preliminary wash and before any deliming or bating treatment. Limed calf skins weighing approximately 10-11 pounds were found to fall within this range.

C. Storage of samples.

Samples may be stored in the freezer after rapid freezing for as long as 2 months without appreciable change. Care must be taken that no wrinkles are present on the grain surface during freezing and storage and they must be protected against dehydration both during freezing and subsequent thawing.

D. Thawing of samples.

Samples are removed from the freezer approximately 12 hours before use and thawed at room temperature in their original sealed containers. Longer periods may be used but lower temperatures are necessary.

2. Wash Procedure.

A. First Wash.

Samples which were to be tested were given the following wash treatment. After thawing, the sample was removed from the cellophane bag, weighed and properly labelled and all the samples for one experiment placed in a large container. Cool distilled water was added in the ratio of 4-1 and the samples shaken on the mechanical shaker for 10 minutes. The

skin was removed from the wash water at once, histological samples taken and distortion measurements ascertained.

B. Second Wash.

Each sample was then tied with thread (end to end with the grain out) and placed in individual mason jars. To each jar sufficient ammonium sulfate solution was added to bring the pH of the wash liquor after washing to 8.8-9.0. The amount of the salt to be added must be determined for each skin used. This is most conveniently done by using scraps taken from the same skin and adding varying amounts of ammonium sulfate, shaking for 10 minutes and then determining the pH of the resulting liquor. It was found that for most of the skins which we received from the tannery that 1/2 per cent based on the limed weight of the skin sample was sufficient to produce the desired result.

The temperature of the ammonium sulfate solution was 85°F. and was the same as that used in the subsequent bating procedure. In this manner, the temperature of the skins was raised to the proper level before it was added to the bate solution.

The jars were then placed in the launderometer (set for 85°F.) and shaken for 10 minutes. In the case of the water controls, no ammonium sulfate was added to this wash, plain distilled water being substituted.

3. Bating Procedure.

After the second wash the samples were transferred immediately to

another series of jars containing the bate solutions under study. The temperature of these solutions was 85°F. The concentration of the bate was calculated on the limed weight of the skin. The ratio of water to skin was 4-1.

The jars were then placed in the launderometer for the required length of time under study. At the end of this time the samples were removed from the spent bate liquor and transferred at once to fresh distilled water at 85°F. They were then returned to the launderometer for 20 minutes.

After the bate wash, the samples were removed from the liquor, histological samples taken, and friction and distortion measurements determined. Care was taken that the grain portion of the skin was not exposed to rapid changes in temperature in order to avoid the roughening effect which is known to occur.

4. Determination of the "Bate Index".

The samples were fastened to the glass plate of the apparatus previously described (Figure 2) and the plate immersed in the bath. The temperature of the bath was 85-86°C. (or the temperature used in the bating process under study) and contained M/20 borate buffer of a pH 8.8-9.0. The height of the liquid in the container was kept constant throughout the experiments.

The glass plate was set at a random approximate angle and the slider gently placed on the surface of the skin and any movement observed. Then the angle of the plate was changed, either up or down as necessary, each time carefully placing the slide so that no settling occurred or air bubbles collected between the slider and

skin surface. That angle at which the slider just started to move downward was recorded. Then the plate was moved to a slightly higher angle (usually 5°) and then to a lower angle in order to check the accuracy of the original reading.

5. Determination of Per Cent Distortion.

The per cent distortion was determined after the first wash and after the bating procedure. All calculations were made on the basis of the original thickness of the skin after the first wash. The determinations were made as soon as possible after each operation in order to eliminate any possible changes in the skin. An attempt was made to standardize the position of the point at which the pressure was applied by choosing the center of the skin sample each time. This choice of position also eliminates any edge effects and thus greater accuracy may be expected.

The piece of skin was placed between two thin rubber sheets (dental dam) and then under the foot of the instrument. The reading of the lower dial was allowed to come to an equilibrium for 1 minute (measured by a stop watch) and the thickness recorded (A). Pressure (2 lb./sq.in.) was then applied and maintained at this level for a period of two minutes. After release of the pressure a second thickness reading (B) was taken. The per cent distortion was calculated as follows:

$$\frac{\text{Reading A - Reading B}}{\text{Reading A}} \times 100 = \% \text{ Distortion}$$

6. Determination of pH of Liquors.

As soon as possible after the various liquors were available, their

pH was determined by means of the glass electrode. This procedure was advisable particularly in the case of the bate liquor which contained free ammonia.

7. Determination of Protein Nitrogen.

The combined spent bate and bate wash liquors were measured and an aliquot taken. Tri-chlor acetic acid reagent was added and the mixture allowed to stand overnight. The precipitate was filtered off and the amount of nitrogen determined by the Kjeldahl analysis. The ammonia was distilled into excess standard acid and back titrated with standard alkali, using a Fisher titrimeter to determine the end point. The amount of protein present was then calculated on the basis of the original limed weight of the sample. The precipitation of the protein nitrogen was carried out not longer than 3-5 hours after the completion of the bating period.

8. Histological Observations.

Samples were taken from each skin sample for histological examination after the first wash and the bate wash. In both cases they were placed in 10% formalin solution at once and later frozen cross-sections were made. The cross-sections were stained, mounted and examined microscopically. Staining methods developed in this laboratory were used throughout. (12, 15, 19).

A summary of the procedures discussed above is given in flow sheet form in Figure 4.

The experimental work was all based upon these standard procedures for the bating of 3" x 6" pieces of calf skin. Studies were made wherein the temperature, time and pH were kept constant and the concentration

and type of enzyme was varied. Throughout most of the experiments a temperature of 85°F. and a bating time of two hours were used. There were a few experiments in which the time was one half and six hours. These latter experiments considered only a few samples and cannot be regarded as significant as those where the two hour period was used and more samples examined.

The percentage of bates employed was 0.25, 1, 2, 3, and 4 per cent based upon the original limed weight of the skin. The types of bates used were Oropon CS (Pancreatic), Oropon CS2 (Bacterial) and Oropon CSO (Fungus). Ammonium sulfate was also used in the same percentages in order that a comparison could be made between simple deliming and the action of the bates which contain both deliming salt (ammonium sulfate) and the active enzyme preparation. One small series of samples was done with inactive bates, the enzymes being inactivated by heating for 1 hour at 194°F. The results and summaries are presented in the tables accompanying this report.

Discussion of Results

Tables 5-10 are a detailed record of the results of the experiments in which a 2 hour bating period was used. They show the values obtained for the per cent distortion of both limed and bated samples, the Bate Index, the pH of the spent liquors and the amount of protein nitrogen recovered from the spent bate liquors and bate wash. In considering these detailed results, it may appear that variations in the values obtained were rather large, but definite trends seemed to exist. Accordingly, these values were examined by statistical methods to determine whether these trends were real or only represented variations in the skins themselves or the methods used. Tables 11-15 give summaries of these results in the form of mean values and standard deviations. Figures 5-10 are the same results presented graphically.

It may be seen from Table 11 and Figure 5 that;

1. In all cases where active bates were used, the mean value for the Bate Index is higher than that found for the ammonium sulfate treated samples.

2. The mean Bate Index values for the pancreatic bates are higher than either the bacterial or the fungal bates at all percentages tested (Figure 5).

3. If, however, we observe the standard deviations for each point determined, we find that there is an overlapping of areas for the low values of the pancreatic bates and the high values for the bacterial bates (Figures 6,7,8.) while the Bate Index for the fungus bates shows

no overlapping with the exception of the area between 3.5 and 4 per cent.

4. In all cases, the Bate Index for the ammonium sulfate treated samples was lower than the active enzyme treated samples. (Figure 6, lower lines).

Examination of Table 12 demonstrates that the ammonium sulfate and the active bates show higher mean values for the per cent distortion measurements of the bated pieces than those measurements taken of the water controls. If, however, we examine the effect of the standard deviations of the various points, this difference seems to drop out and there is no significant difference apparent. The mean value for all the per cent distortion figures for all the limed skin values was 14.03 with a standard deviation of $\pm$ 1.67.

Table 13 shows the values for the "permanent distortion", i.e., the per cent distortion of the bated samples minus the mean of the percent distortion of the limed sample. It may be observed that again, the water control shows a lower value than the treated samples. If the figures for each of the percentages are compared in this table against the percentage of permanent distortion of the water control it may be seen that there is no regular order established for any of the concentrations reported.

If the values for this "permanent distortion" are averaged for each bate material, regardless of the concentration of bate used we can show that the materials fall in the order of water controls (low), ammonium sulfate, bacterial, pancreatic and fungus (highest), (Table 13). The differences are so small, however, that they appear to be without significance.

Table 14 and Figure 9 tabulate the mean values for the protein nitrogen contained in the combined spent bate and bate wash liquors. The results are expressed in milligrams of protein nitrogen per 100 grams of limed skin sample. From the table and figure, it may be seen that:

1. There is a drop in the values in the ammonium sulfate and bate materials at the one per cent level. Subsequently, the values rise until a maximum is reached at the 4 per cent concentration. This point lies above the value found for the water controls.

2. The values for the pancreatic and water controls are lower for all points than the values for the fungal bates.

3. The values for the fungal bates are higher than the ammonium sulfate samples in all cases except at the 3 per cent level, while the values for the pancreatic and bacterial bates are higher than the ammonium sulfate values only at the 0.25 and 1 per cent levels, and again approach the figure for the ammonium sulfate at the 4 per cent concentration.

Table 15 and Figure 10 record a further analysis of these results. It was noticed that the amount of protein nitrogen removed from the various skins in the water controls varied over wide ranges (3.5-13.2) and it seemed possible that if the nitrogen figures could be calculated on the basis of the amount of protein nitrogen from the particular skin from which the sample was taken, significant trends might be established. On the basis of this table it may be seen that the fungal bates show higher values for protein nitrogen than either the ammonium sulfate or the pancreatic and bacterial bates, except at the 1 per cent level.

The bacterial bates also give higher values for protein nitrogen recovered than do the pancreatic bates. It is interesting to note that the trend in this analysis is the same as in the previous one.

Table 15 shows a summary of the histological changes observed in the cross-sections. When the work was started it was hoped that actual numerical values could be assigned for hair pocket angles and the change in width of the elastic tissue pattern. This aim was not realized for this group of samples because of the wide variations encountered in the different skins. Microscopic examination of cross-sections magnifies the difference of these variations from skin to skin and to obtain results which may be considered statistically significant it is necessary to examine many cross-sections for each variable before the results may be considered definite and reliable.

The figures reported in Table 16 thus are trends and averages of the several hundred cross-sections examined during this study. It should also be pointed out that these readings were made by three trained workers and three untrained workers and there was good correlation between the factors observed by the different workers. It may be seen in Table 16 that:

1. Definite changes in the staining ability of the fibroblasts were apparent only in the case of the pancreatic bates. The failure of the fibroblasts to stain accompanies the removal of the simpler proteins of the skin corium (7,18) Roddy, et al. There was some slight change noted at the 4 per cent levels for the ammonium sulfate, the bacterial, and the fungal bates.

2. The hair pocket angle gradually increases from the low angle for the limed skin (24°) and ammonium sulfate treated samples (25°) to a higher angle (33°) for the bated samples. This higher angle is in the direction of the angle found in fresh skin (58°). Cross-sections were made of a few samples which had received a 6 hour bate with 4 per cent concentration and these angles (50°) in general were much closer to those found in fresh skin.

3. The average width of the elastic tissue pattern in the cross-section from the grain surface to near the base of the hair shaft, increases from a width of 197 microns for limed skins to 220 microns for the ammonium sulfate treated samples and 281 microns for the bated samples. This increase in the width of the elastic tissue pattern followed the rise in concentration of the bate materials used in the treatment.

The results of the examination of the fiber bundle appearance also followed the trend shown in the outer histological observations, i.e. the limed cross-sections contained fiber bundles which were greatly swollen and distinct fibers were not apparent while in the bated samples the fiber bundles had some of the swelling removed and the fibers were more distinct. The ammonium sulfate treated samples were intermediate between the two.

Limed micro-incinerated cross-sections showed the lime to be deposited throughout the cross-section seemingly being mainly at the interspace around the fiber bundles through the corium proper. The water controls showed a more even distribution and less ash through the corium proper.

The change in the deposition pattern of the lime ash in the incinerated cross-sections of bated stock indicated removal of lime and a much finer ash pattern than observed in the water controls. The redistribution of the residual lime during bating was an indication of the bate activity for the greater the concentration of bate the greater the change in ash pattern.

Table 17 gives a summary of the few experiments where the bating material was inactive pancreatic bate. The enzymes were inactivated by heating at 194°F. for 1 hour. The results indicate that there is little change in the Bate Index when inactive pancreatic bates are used in one and two per cent concentrations while in the three and four per cent concentrations there was some evidence of change. It should be noted, however, that this is a very small group of samples and the average deviation is equivalent to 40 per cent of the mean value.

Tables 18 and 19 give a summary of the results from the few experiments where the time was one half and six hours. It may be seen in Table 18 that the Bate Index for all of the bated pieces is higher than that of the ammonium sulfate controls. These results are in accordance with the previous experiments.

Table 19 presents the same data for the six hour bate periods and demonstrates that the Bate Index for the pancreatic bates are higher than either the bacterial or the fungal bates. The number of samples tested in this series is too small for conclusions on the basis of statistical analysis but it is interesting to note that the same trends are present although the actual values are greater, except in the case of the distortion measurements.

Summary

Enzyme bates bring about certain changes in the skin; some are associated with the action of the deliming salts and others are associated with the activity of the proteolytic enzymes. According to our present knowledge these changes include the following:

1. A lowering of the pH of the skin system.
2. A change in the degree of swelling of the various tissues which make up the whole skin.
3. The removal of certain constituents which are either soluble in the dilute salt solution or which are produced by the enzyme by the conversion of complex materials to simple soluble constituents.

In attempting to correlate and interpret the results of any bating experiments, these points must be kept in mind. It was desirable that the action of the deliming salts be separated from the action of the enzymes and each set of measurements were considered with this in mind. The controlled conditions under which our experiments were performed were such that direct comparisons between simple deliming and active enzyme bates may be made.

1. Correlation of Bate Index and enzyme activity.

Examination of the results shows that there is a definite correlation between the Bate Index and the presence of active enzymes for all of the types of bating materials used. In all samples containing active enzymes the Bate Index was high while in those samples of inactive bate and ammonium sulfate the Bate Index was low. We may consider, therefore, that one of the functions of the enzymes is to act upon the surface of the skin to render it smoother than an untreated

skin or a skin which has been simply delimed by a deliming salt.

We also observe that in these experiments, the pancreatic bates produce a higher Bate Index than either the bacterial or the fungal bates. In considering this point it should be mentioned that the optimum pH for pancreatic activity lies between 8.0 and 9.0 and it is possible that the activity of the bacterial and fungal bates lies within a different pH range. Since all of our experiments were carried out between the range of pH 8.0 and 9.0 this difference between the action of the three types of enzymes may not represent actual differences in their action on the skin. If pH conditions were changed, the bacterial and fungal bates may produce an equal change in the skin. This topic requires further experiments and will be considered in future work.

2. Correlation of per cent distortion and enzyme activity.

The measurement of the per cent distortion and the permanent distortion does not give as clear cut a demonstration of the difference between enzyme activity and deliming, as does the Bate Index. The trend however is still there although the differences are small. It is possible that the effect of the deliming comprises such a large proportion of the total fall of the skin that it masks any effect of the enzyme activity. The small differences in the permanent distortion shown between the enzyme preparations and the water and ammonium sulfate controls may be accounted for by the activity of the enzymes. In order to establish this point, further work is necessary. This should include enough determinations for adequate statistical analysis. In all probability if this were done certain low and high points on all samples would be eliminated and significant trends could be established.

3. Correlation between the protein nitrogen removed and enzyme activity.

The correlation between the protein nitrogen removed by active enzyme preparations and by simple deliming salts or inactive bate preparations is confused by the action of the enzymes themselves on the protein materials contained in the skin, as well as the method of analysis. The degree to which enzymes may break down the proteins of the skin is unknown at the present time and the tri-chlor acetic acid reagent may not completely precipitate all of the end products of the reaction. Ammonium sulfate on the other hand, probably removes the protein material from the skin in a denatured state and thus precipitation by the tri-chlor acetic acid reagent represents a better picture of the deliming salt action on the skin. Further, the quantities involved are quite small and error in the determination is undoubtedly magnified.

It should also be pointed out, that when we attempted to determine total nitrogen in the spent liquors the presence of free ammonia caused wide variations due to loss of ammonia during the bating operation and subsequent handling of the samples. Unless this total nitrogen determination figure is corrected for ammonia loss, any total nitrogen determination done in bating studies is meaningless. This error has not been considered by any of the previous workers. In future work we expect to use this procedure for all bating studies in order to eliminate these errors which have presented themselves as a result of our present studies.

If we correlate the amount of protein nitrogen removed by the pancreatic bates with the histological picture of the change in staining

ability of the fibroblasts it appears that the pancreatic enzymes remove simple proteins with greater efficiency than the ammonium sulfate, bacterial or fungal bates.

4. Correlation between histological changes and enzyme activity.

The changes in the overall histological picture between the delimed and bated skins, while not at this stage statistically significant are nevertheless marked. Differences between the delimed and bated samples are striking when high per cents of bate are used for long time periods. Even for a two hour bating period, the change in appearance of the cross-section was quite obvious when compared with either the water controls or the delimed samples.

It appears that one of the functions of bating is to change the internal architecture of the skin so that it resembles that found in fresh untreated skin. Bating also changes the chemical constitution of the skin so that a purer collagen is available to the tanner for subsequent operations.

Conclusions

A controlled bating procedure for use in laboratory bating studies has been developed. Under these conditions studies have been made which measured the changes in the characteristics of calf skin which are the result of the bating procedure. The experiments show that:

1. The Bate Index or smoothness of the skin is a measure of the enzyme action on the skin.

2. Under the conditions of our experiments pancreatic bates produce a higher Bate Index than the bacterial bates, the fungal bates or ammonium sulfate.

3. The factor of distortion probably measures only the action of the deliming salts contained in the bate materials. A trend exists however, which though not statistically significant for the number of samples studied, may be significant when larger groups of samples have been tested.

4. The protein nitrogen recovered from the spent liquors show no direct correlation with enzyme activity, although a trend has been observed. This should be qualified by pointing out that the method of analysis used does not completely represent the protein material removed from the skin. Further investigations are necessary which take into account the total nitrogen minus the variable amounts of ammonia present.

5. Enzyme activity of the bates shows a direct correlation between concentration of the enzyme and changes in the architecture and chemical constitution of the skin.

Table 1

The Effect of Position of Sample on the Distortion Measurements of Limed Calf Skin

Position No.	Percent Loss	Orig. Thickness
1	23.7	177
	39.4	195
	14.4	181
	19.7	218
	19.4	192
	32.9	225
2	13.6	140
	21.1	161
	10.1	129
	12.7	150
	11.8	157
3	13.6	140
	13.9	144
	18.0	155
	12.7	150
	15.7	140
	11.8	157
	21.1	161
4	15.7	140
	14.5	130
	13.8	130
	14.3	140
	13.7	124
	13.5	132
	13.7	131
	13.7	133
5	13.3	180
	11.8	152
	10.5	143
	8.6	132
	8.5	142

Table 1 (Continued)

Position No.	Percent Loss	Orig. Thickness
6	14.2	183
	12.8	187
	17.5	207
	11.0	191
	16.6	211
7	14.1	163
	12.9	163
	9.1	153
	9.4	160
	13.4	149
8	10.8	130
	10.6	123
	12.0	133
	11.1	135
	10.4	125
	10.5	141

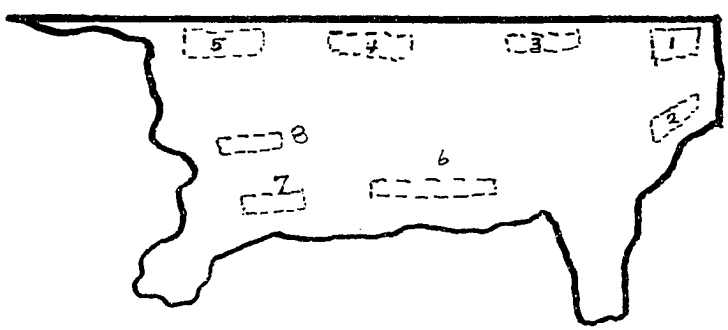


Table 2

The Effect of Skin Thickness on the Bate Index

% Bate	Original Thickness	Bate Index
1	65	1.73
	74	2.40
	83	3.20
	108	3.00
	112	3.00
	117	2.87
2	76	7.28
	77	6.10
	114	4.84
	108	4.92
	150	2.61

Table 3

The Effect of Types of Holding Procedures on the Bating of Limed Calf Skin

Date Test	Distortion after Bate		Feel		Holding Procedure
	Active	Inactive	Active	Inactive	
10/27/47	42	28	Smooth	Rough	Used at once
11/7/47	20	14	Rough	Rough	Ice box in lime
11/11/47	17	15	Rough	Rough	Ice box in lime
11/11/47	39	28	Smooth	Rough	Frozen 10/27/47

Bate - 1% Oropon C

Temperature - 85°F.

Time - 2 hours

Table 4

The Effect of Various Wash Procedures on Distortion and Bate Index Measurements

Type Wash	Distortion Wash	Distortion Bate	pH Wash No. 2	pH Spent Bate	Bate Index	Remarks
1x20 min. water	13.5	47	10.9	9.1	2.47	Samples held 1 hour at 85°F. before bating
	15.0	47	10.9	9.2	3.00	
2x10 min. water	12.6	39.6		9.2	2.47	
	14.3	45		9.3	2.70	
	12.0	48	10.8	9.0	2.47	
	14.0	47	10.8	9.0	2.70	
	14.0	50	10.8	9.0	3.00	
	14.2	50	10.8	9.0	3.00	
2x10 min. water	11.5	39		9.8	1.44	Samples bated at once
	12.4	45		9.0	1.73	
	16.0	48	10.5	9.0	1.44	
	14.0	44	10.5	9.0	1.90	
	11.6	38	11.1	9.1	1.90	
2x10 min. M/10 borate buffer	29.0	50	9.1	8.2	2.70	
	18.8	48	9.1	8.2	3.00	
	14.0	48	9.5	8.7	2.70	
	29.0	48	9.1	8.6	2.70	
1x10 min. water 1x10 min. buffer	14.8	47	9.0	8.9	6.45	Samples held in buffer 1 hour. Bated in buffer.
	13.0	45	9.0	9.0	5.50	
1x10 min. water 1x10 min. ammonium sulfate*	18.4	53	9.1	8.5	3.61	Samples bated at once
	19.0	52	9.1	8.5	2.88	
	11.2	44	9.1	8.8	2.87	
	10.8	43	9.1	8.8	2.87	
	25.0	51	8.8	8.7	3.00	
	14.6	45	8.9	8.8	3.46	

*0.5 percent based on limed weight of sample.

Table 4 (Continued)

The Effect of Various Wash Procedures on Distortion and Bate Index Measurements

Type Wash	Distortion Wash	Distortion Bate	pH Wash No. 2	pH Spent Bate	Bate Index	Remarks
Borate buffer control	14.6	47	9.2	9.0	1.37	
	11.4	34	9.1	9.0	1.37	
Ammonium sulfate control	15.0	43	9.0	10.8	1.37	
	14.0	48	8.9	10.7	1.37	

Table 5

Detailed Results of Measurements for Water Controls

Treating Temperature 85°F.
Time - 2 Hours

Distortion Lime	Distortion Treated	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
14.0	30.0			11.4
15.0	43.0	82.7	10.8	10.6
14.0	48.0	131.5	10.7	10.4
12.0	39.0	93.0	10.9	10.3
12.8	36.0	88.6	10.8	10.8
17.0	48.0	90.0	10.2	10.3
16.2	45.0	82.0	10.2	10.6
13.8	31.0	64.5	11.0	10.6
7.3	38.0	50.9	11.0	10.9
16.0	40.0	106.0	11.1	11.0
11.0	36.0	91.0	11.1	11.0
7.4	46.0		9.0	9.4
10.0	48.0		9.0	9.8
21.0	47.0		10.6	9.8
20.0	47.0		10.1	10.7
15.6	43.0	53.2	10.8	10.5
13.0	34.0	62.0	10.5	10.8
11.5	41.5	46.5	10.6	10.6
14.4	32.5	52.6	10.6	10.6
12.9	46.0	63.7	9.7	10.3
11.2	38.9	51.3	10.0	10.4
16.6	45.0	71.5	10.3	10.1
14.6	45.0	63.8	10.3	10.1
17.9	47.1	56.2	9.7	10.4
16.8	40.6	48.7	10.1	10.7
14.4	43.0	40.0	10.4	10.4
8.4	32.8	41.3	10.5	10.4
19.0	56.0	137.0	10.3	10.3
14.4	54.5	107.0	10.4	10.2
18.6	51.0	56.0	10.6	10.6
13.3	36.0	34.6	10.5	10.6
16.3	38.4	44.7	10.0	10.7
13.6	43.5	42.7		
10.4	45.5	26.6	10.6	10.9
15.5	41.0	76.6	10.8	10.9
12.0	38.0	61.0	10.7	10.1

Table 5 (Continued)

Detailed Results of Measurements for Water Controls

Treating Temperature 85°F.
Time - 2 Hours

Distortion Lime	Distortion Treated	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
10.0	34.5	56.5	10.5	10.2
8.6	25.0	40.5	10.8	10.7
10.9	18.8	42.5	10.8	10.6
11.5	38.0	52.0	10.3	10.85
17.2	25.6	55.3	10.3	10.75
13.3	36.1		10.1	10.5
9.1	34.0	52.5	10.1	10.6
11.9	19.0	40.7	10.7	10.7
10.5	25.4	60.0	10.6	10.6

Table 6

Detailed Results of Measurements for One-fourth Percent Bates Concentrations

Bating Temperature 85°F.
Bating Time - 2 Hours

Material	Distortion Lime	Distortion Bate	Bate Index	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
¼% $(NH_4)_2SO_4$	14.3	43.7	1.0	24.7	8.9	9.2
	15.4	47.0	1.0	35.4	8.9	9.3
	12.3	40.0	1.0	31.9	8.8	9.1
	19.8	46.0	1.0	31.2	8.8	9.1
	11.3	40.7	1.21	53.1	9.0	9.5
	11.3	42.0	1.21	43.0	8.9	9.5
	8.1	43.5	1.32	80.0	8.9	9.5
	12.8	54.3	1.14	38.4	8.9	9.5
	11.7	33.0	1.00	42.0	8.7	9.3
	13.3	46.0	1.00	56.8	8.9	9.35
¼% Pancreatic Oropon CS	14.0	47.0	1.44	112		
	14.0	44.0	1.44	74		
	18.3	51.0	1.75	56.2	9.2	9.2
	17.0	52.0	1.55	43.7	9.1	9.0
	13.2	48.0	1.00			
	21.0	49.0	1.00			
	18.3	52.0	1.55	28	8.9	9.3
	12.3	53.0	1.55	21	8.9	9.3
	12.7	47.0	1.32	46	8.9	9.7
	13.0	51.0	1.88	109	8.9	9.35
¼% Bacterial Oropon CS_2	8.8	48.0	3.60	135	9.0	9.4
	12.5	50.0	3.60	122	9.1	9.4
	20.0	55.0	1.00	32.2	8.9	9.2
	18.9	50.0	1.00	29.4	8.8	9.3
	23.1	42.0	1.00	30.6	8.9	9.2
	20.9	43.6	1.00	73.2	8.8	9.2
	13.2	43.0	1.00	28.2	8.9	9.2
	17.8	47.2	1.00	24.6	8.9	9.1

Table 6 (Continued)

Detailed Results of Measurements for One-fourth Percent Bates Concentrations

Bating Temperature 85°F.
Bating Time - 2 Hours

Material	Distortion Lime	Distortion Bate	Bate Index	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
¼% Fungus Oropon CSO	13.0	57.0	3.60	132	9.1	9.4
	16.0	52.0	2.38	132	9.0	9.4
	14.3	49.5	1.50	48.0	8.7	9.3
	11.5	45.5	1.21	35.8	8.7	9.3
	17.1	50.0	1.09	24.8	8.8	9.2
	15.7	51.0	1.09	141.0	8.8	9.2
	10.7	37.8	1.80	61.5	8.9	9.4
	11.5	39.2	1.80	47.4	8.9	9.5
	9.3	38.0	1.45	35.6	8.9	9.4
	10.6	24.8	1.45	35.0	8.9	9.6

Table 7

Detailed Results of Measurements for One Percent Bates

Bating Temperature - 85°F.
Bating Time - 2 Hours

Material	Distortion Lime	Distortion Bate	Bate Index	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
$(NH_4)_2SO_4$	13.4	42.0	1.00	30	8.9	8.8
	12.2	40.0	1.00	47.6	8.9	8.7
	16.0	41.0	W	75.8	8.9	8.4
	16.0	44.0	W	59.3	8.9	8.4
	16.0	45.0	1.08		9.1	8.7
	20.0	45.0	1.08	77.8	9.0	8.7
	22.0	46.0	1.30	40.6	8.9	8.8
	15.0	40.0	1.30	36.0	8.8	8.8
	13.5	35.6	1.00	25.0	8.8	8.9
	11.3	36.0	1.00	19.6	8.8	8.9
Pancreatic Oropon CS	13.0	41.0	2.47	21.5		9.4
	13.0	41.0	3.00	12.7		9.6
	12.0	48.0	2.47		10.8	9.0
	14.0	47.0	3.00			9.0
	14.2	50.0	3.00		10.8	9.0
	11.1	50.0	3.00		10.8	9.0
	13.0	57.0	2.60	48.2	10.5	9.0
	15.8	56.0	3.20	72.9	10.5	9.0
	13.0	44.0	2.87	126.0	9.0	8.5
	14.0	49.0	2.87	37.5	8.9	8.6
	16.2	50.0	2.38	48.1	10.8	9.0
	15.4	48.0	2.02	36.8	10.8	9.0
	18.4	53.0	3.61	36.9	9.1	8.5
	19.0	52.0	2.88	31.5	9.1	8.5
	11.2	44.0	2.87	13.2	9.1	8.8
	10.8	43.0	2.87	40.0	9.1	8.8
	15.0	48.0	2.87		9.0	
	13.8	44.0	2.34		9.0	
	15.0	43.0	3.60	54.6	9.0	8.7
	12.0	40.0	2.38	41.2	9.0	8.7
	25.0	51.0	3.00	24.9	8.8	8.7
	14.6	45.0	3.46	43.5	8.9	8.8

Table 7 (Continued)

Detailed Results of Measurements for One Percent Bates

Bating Temperature - 85°F.
Bating Time - 2 Hours

Material	Distortion Lime	Distortion Bate	Bate Index	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
Bacterial						
Oropon CS2	16.4	53.0	3.61		10.9	8.8
	14.0	53.0	2.87	83.5	10.8	8.9
	18.2	48.0	W		8.9	8.7
	16.0	49.0	2.38	-	8.9	8.5
	16.3	44.0	1.76	27.6	8.8	8.8
	14.3	45.0	2.52	43.3	8.8	8.8
	20.0	43.6	1.21	28.4	8.8	8.9
	20.0	40.0	1.21	33.6	8.8	8.8
	13.0	42.0	1.75	33.7	8.8	8.8
	9.3	41.3	2.02	37.6	8.8	8.8
	19.2	46.0	1.37	32.4	8.9	8.8
	18.9	46.0	1.22	24.0	8.8	8.8
Fungus						
Oropon CSO	18.0	55.0	2.38	81.8	10.7	8.8
	20.0	48.0	2.38	109.0	10.7	9.0
	12.2	51.0	2.02		9.0	8.5
	14.2	52.0	2.38		8.9	8.55
	14.9	36.8	1.39	45.8	8.7	8.9
	21.0	45.5	1.39	51.7	8.7	8.8
	10.1	45.8	1.37	32.2	8.7	8.7
	8.6	41.8	1.50	31.2	8.7	8.7
	15.0	46.0	1.61	27.0	8.8	8.6
	17.2	48.5	1.45	28.0	8.9	8.6
	14.5	47.5	2.01	36.7	8.75	8.7
	17.9	48.0	2.01	33.4	8.7	8.7

Table 8

Detailed Results of Measurements for Two Percent Bates

Bating Temperature - 85°F.
Bating Time - 2 Hours

Material	Distortion Lime	Distortion Bate	Bate Index	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
$(NH_4)_2SO_4$	18.0	41.0	1.08	66.0	9.0	8.3
	20.0	42.0	1.08	51.4	9.0	8.3
	9.7	34.0	1.09	38.4	8.7	8.3
	18.0	43.0	1.09	44.8	8.7	8.3
	12.3	41.0	1.32	59.5	8.8	8.5
	12.1	36.4	1.32	62.0	8.8	8.5
	13.1	39.2	1.55	52.3	8.8	8.5
	11.1	38.0	1.32	61.5	8.8	8.5
	10.6	26.0	1.32	67.0	8.8	8.5
	12.8	49.5	1.55	65.5	8.8	8.5
Pancreatic Oropon CS	9.4	39.0	7.28	73.2	9.2	8.6
	6.6	40.0	6.10	57.0	9.2	8.6
	10.0	39.0	7.30		9.0	8.5
	10.0	49.0	7.30		9.0	8.4
	15.2	43.0	3.60	58.3	8.9	8.4
	13.6	39.0	2.38	33.0	8.9	8.4
	16.0	41.0	4.84	88.0	9.0	8.2
	16.0	45.5	4.84	45.1	9.1	8.4
	18.0	44.0	3.46	42.3	8.8	8.6
	15.6	42.0	4.92	32.6	8.9	8.7
	15.4	49.5	2.61	28.6	8.5	8.5
	13.0	42.0	2.61	25.8	8.5	8.45
Bacterial Oropon CS2	12.2	50.5	3.42		8.9	8.4
	11.7	49.5	3.42	39.5	8.8	8.3
	19.6	45.7	1.97	42.2	8.8	8.6
	15.2	43.5	2.52	48.0	8.8	8.6
	15.3	46.0	2.38	42.8	8.8	8.4
	11.5	45.0	2.38	39.1	8.8	8.4
	15.7	47.2	2.02	34.6	8.9	8.9
	12.4	41.3	2.02	55.5	8.9	8.7
	10.0	39.4	2.01	36.0	8.8	8.7
	11.5	31.0	1.45		8.8	8.7

Table 8 (Continued)

Detailed Results of Measurements for Two Percent Bates

Bating Temperature - 85°F.
Bating Time - 2 Hours

Material	Distortion Lime	Distortion Bate	Bate Index	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
Fungus Oropon CSO	14.3	42.0	1.59	26.3	8.8	8.6
	16.0	46.8	1.83	45.1	8.8	8.4
	11.4	42.3	1.69	43.0	8.7	8.4
	10.9	43.0	2.61	42.4	8.7	8.3
	6.3	38.8	2.61	39.2	8.7	8.3
	13.1	47.7	2.61	43.0	8.7	8.3
	24.1	56.0	2.01	47.5	8.8	8.3
	14.0	46.0	2.31	45.0	8.8	8.2
	17.0	52.8	2.66	66.1	8.7	8.4
	13.8	47.5	3.13	56.4	8.7	8.4
	9.1	39.4	2.35	73.0	8.8	8.5
	9.0	47.0	1.88	73.0	8.8	8.4

Table 9

Detailed Results of Measurements for Three Percent Bates

Bating Temperature - 85°F.
Bating Time - 2 Hours

Material	Distortion Lime	Distortion Bate	Bate Index	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
$(NH_4)_2SO_4$	12.2	40.5	1.44	49.5	8.8	8.4
	15.3	46.0	1.44	45.1	8.8	8.2
	11.4	30.5	1.00	45.5	8.8	8.5
	12.3	30.0	1.00	36.0	8.8	8.4
	14.4	50.5	1.11	82.5	9.0	8.5
	17.2	45.5	1.26	68.2	8.9	8.5
	15.6	49.5	1.26	70.5	8.9	8.5
	10.2	47.0	1.26	78.0	8.8	8.6
	7.2	47.0	1.65	61.2	8.9	8.6
	13.2	41.0	2.34	92.5	8.8	8.5
Pancreatic Oropon CS	10.8	40.0	3.61	49.5	9.2	8.4
	10.0	42.0	3.61	61.0	9.2	8.4
	15.4	45.0	4.84		9.1	8.3
	13.2	46.0	4.84		9.0	8.3
	12.0	44.0	4.92	39.4	8.8	8.45
	13.6	43.0	4.92	40.3	8.9	8.4
	13.3	47.4	3.16	35.5	8.5	8.4
	8.95	36.0	3.97	42.0	8.5	8.4
	12.10	48.4	3.97	37.8	8.5	8.35
	7.85	34.0	3.97	42.0	8.5	8.4
Bacterial Oropon CS2	20.4	48.5	4.10	43.9	8.8	8.3
	15.5	40.0	4.10	60.9	8.8	8.4
	16.7	41.6	2.52	56.5	8.8	8.4
	16.0	43.0	2.52	67.7	8.8	8.4
	10.0	44.0	3.60	70.0	8.8	8.4
	12.0	43.5	3.60	63.7	8.8	8.5
	17.4	50.3	2.38	46.0	8.8	8.5
	13.1	44.0	2.38	51.5	8.8	8.5
	9.6	30.4	2.66	60.0	8.8	8.5
	9.1	29.2	2.66	41.0	8.8	8.5

Table 9 (Continued)

Detailed Results of Measurements for Three Percent Bates

Bating Temperature - 85°F.
Bating Time - 2 Hours

Material	Distortion Lime	Distortion Bate	Bate Index	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
Fungus Oropon CSO	14.9	45.8	3.27	73.2	8.8	8.5
	13.3	43.0	3.27	54.3	8.8	8.3
	9.5	48.5	2.61	59.5	8.7	8.2
	8.1	43.5	2.61	60.5	8.7	8.2
	9.2	39.0	2.61	53.0	8.7	8.2
	11.7	44.5	2.61	59.0	8.7	8.2
	17.5	53.5	3.78	70.5	8.8	8.1
	18.5	55.5	3.13	36.4	8.8	8.1
	17.4	45.0	3.78	78.5	8.8	8.3
	16.2	49.5	3.78	76.0	8.8	8.3
	11.7	40.0	2.31	41.3	8.9	8.5
	11.9	38.6	2.31	71.3	8.9	8.5

Table 10

Detailed Results of Measurements for Four Percent Bates

Bating Temperature - 85°F.
Bating Time - 2 Hours

Material	Distortion Lime	Distortion Bate	Bate Index	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
$(NH_4)_2SO_4$	16.3	42.0	1.24	88.2	8.9	8.5
	17.8	44.4	1.24	78.2	8.8	8.2
	21.1	45.5	1.20	43.5	8.8	8.0
	23.8	47.8	1.31	34.4	8.8	8.1
	11.2	41.3	1.32	107.	8.9	8.0
	13.4	45.0	1.14	74.5	8.9	8.0
	18.1	37.0	1.32	114.	8.8	8.0
	10.3	38.4	1.14	111.	8.8	8.0
	14.4	42.5	1.14	61.0	8.7	7.8
	13.3	46.5	1.32	65.5	8.9	8.0
Pancreatic Oropon CS	16.0	45.0	5.5	36.		8.7
	16.0	49.0	6.5	90.		8.7
	13.4	47.0	4.84	115.0	9.0	8.3
	10.0	48.0	4.84	119.0	9.0	8.3
	12.6	50.0	3.61	93.0	9.2	8.2
	8.4	47.0	3.61	81.0	9.2	8.1
	13.6	43.0	3.60		9.0	8.2
	12.0	48.0	3.60		9.0	8.2
	13.6	47.0	7.50		8.7	8.2
	11.0	44.0	7.50	53.6	8.8	8.3
	7.85	39.0	5.32	51.3	8.5	8.35
	9.20	48.0	5.32	57.6	8.5	8.35
Bacterial Oropon CS2	9.5	46.0	4.84	128.	9.1	8.4
	9.6	43.0	3.60	102.	9.1	8.3
	11.7	42.5	5.19	69.7	8.8	8.25
	10.5	42.5	5.19	68.7	8.8	8.2
	13.1	43.0	3.60	62.5	8.8	8.5
	14.4	48.5	3.60	70.6	8.6	8.45
	12.3	34.2	3.13	52.0	8.8	8.5
	6.4	23.2	3.13	44.3	8.8	8.4
	11.1	29.0	2.31	45.0	8.8	8.5
	12.5	29.0	2.31	61.5	8.8	8.4

Table 10 (Continued)

Detailed Results of Measurements for Four Percent Bates

Bating Temperature - 85°F.
Bating Time - 2 Hours

Material	Distortion Lime	Distortion Bate	Bate Index	Protein Nitrogen	pH Wash No. 2	pH Spent Bate
Fungus Oropon CSO	14.6	47.0	3.60	148.	9.2	8.4
	12.0	49.0	3.60	112.	9.0	8.3
	11.2	45.0	3.27	55.6	8.8	8.4
	13.6	44.5	3.27	74.7	8.8	8.3
	4.3	37.8	3.97	51.5	8.7	8.1
	9.3	42.0	3.97	61.7	8.7	8.1
	23.9	53.0	2.61	57.6	8.8	8.1
	18.3	49.5	3.16	81.0	8.8	8.2
	18.5	46.2	3.13	96.5	10.1	8.3
	16.0	44.5	4.76	112.	8.8	8.15
	10.9	44.5	4.60	93.5	8.9	8.0
	11.4	40.0	2.35	93.	8.9	8.0
	26.8	60.5	3.14	112.	8.8	7.95
	15.4	50.0	4.60	114	8.8	8.0
	15.2	43.0	4.60	98.0	8.8	8.0
	14.5	46.0	4.60	92.5	8.8	8.0

Table 11

The Mean Values and Standard Deviation of Bate Index Measurements

Water Controls 1.00

Material	0.25%	1%	2%	3%	4%
Ammonium sulfate	1.09	1.09	1.27	1.38	1.24
Std. deviation	0.03	0.05	0.28	0.40	0.08
Pancreatic enzyme	1.45	2.85	4.77	4.18	5.14
Std. deviation	0.90	0.40	1.90	0.20	1.40
Bacterial enzyme	1.20	2.02	2.36	3.05	3.69
Std. deviation	0.00	0.58	0.85	0.67	0.81
Fungal enzyme	1.42	1.82	2.27	3.01	3.79
Std. deviation	0.91	0.42	0.50	0.58	0.77

Bating temperature 85° F.

Bating time 2 hours

Table 12

The Mean Values and Standard Deviation of Percent Distortion Measurements

Mean for all limes - 14.0 1.67 Water treated controls - 37.2 8.5

Material	0.25%		1%		2%		3%		4%	
	Lime	Bate	Lime	Bate	Lime	Bate	Lime	Bate	Lime	Bate
Ammonium sulfate	13.0	43.6	15.5	41.5	13.8	39.0	12.9	42.8	16.0	43.0
Std. deviation		±4.5		± 3.8		±6.2		±7.0		±3.3
Pancreatic enzyme	15.4	49.4	14.5	47.4	13.2	42.7	11.7	42.6	12.0	46.3
Std. deviation		±4.3		± 4.7		±1.0		±4.7		±2.8
Bacterial enzyme	17.4	48.4	16.3	45.9	13.5	43.9	14.0	41.5	11.1	38.1
Std. deviation		±4.4		± 4.2		±5.6		±7.4		±8.5
Fungal enzyme	13.0	44.5	15.3	47.2	13.3	45.6	13.3	45.4	15.4	46.4
Std. deviation		±9.5		± 4.8		±5.1		±4.9		±3.6

Bating temperature 85° F.

Bating time 2 hours

Table 13

The Mean Values of "Permanent Distortion". Permanent distortion = Mean Bate Distortion - Mean of all Lime Distortions.

Water Controls 23.1

Material	0.25%	1%	2%	3%	4%
Ammonium sulfate	29.6	27.4	25.0	28.7	29.0
Pancreatic enzyme	34.4	33.4	28.7	28.6	32.2
Bacterial enzyme	34.4	31.9	29.9	27.4	24.0
Fungal enzyme	30.5	33.2	31.2	31.4	32.4

The Mean of All "Permanent Distortion" Values Regardless of Concentration Used

Material	Mean	Std. Deviation	Maximum	Minimum
Water control	23.1	+8.5	31.6	15.0
Ammonium sulfate	27.9	+4.96	32.9	25.0
Pancreatic enzyme	31.4	+3.50	34.9	27.9
Bacterial enzyme	29.5	+6.0	35.5	23.0
Fungal enzyme	31.7	+5.6	37.3	26.1

Bating temperature 85° F.

Bating time 2 hours

Table 14

The Mean Values of Protein Nitrogen Removed in Spent Bate and Bate Wash Expressed as Milligrams Protein Nitrogen per 100 grams Limed Skin

Water controls vary from 3.5 to 13.2 - mean 6.5

Material	0.25%	1%	2%	3%	4%
Ammonium sulfate	4.4	3.6	5.7	6.3	7.8
Pancreatic enzyme	6.2	4.3	4.8	4.3	7.7
Bacterial enzyme	5.7	3.8	4.2	5.6	7.0
Fungal enzyme	6.9	4.8	6.0	6.1	9.1

Bating temperature 85° F.

Bating time 2 hours

Table 15

The Percent Protein Nitrogen Recovered

$$\frac{\text{Prot. N. in sample}}{\text{Prot. N. in same skin water control}} \times 100 = \% \text{ protein N. recovered}$$

Material	0.25%	1%	2%	3%	4%
Ammonium sulfate	67	75	58	88	159
	64	62	85	86	83
	102			164	137
	75				
	83				
Mean	78	68	72	113	126
Pancreatic enzyme	82	136	76	64	107
	84	31	65	80	152
	46	31	67	76	134
		59		96	
Mean	74	64	69	79	107
Bacterial enzyme	45	48	74	98	103
	78	55	87	119	130
	82	83	65	86	99
		80	76	106	106
		65			
Mean	65	66	75	102	131
Fungal enzyme	50	46	66	122	118
	115	60	103	166	125
	108	72	113	160	138
	73	108	114	150	185
					158
Mean	86	71	102	149	145

Each figure represents the mean of a minimum of 2 samples per skin.

Table 16

The Histological Changes Observed in Stained Cross-sections

Loss in Staining Ability of Fibroblasts

Water Control - No Change

Material	0.25%	1%	2%	3%	4%
Ammonium sulfate	No. chg.	No. chg.	No. chg.	No. chg.	Slight
Pancreatic enzyme	Slight	Change	Change	Change	Change
Bacterial enzyme	No. chg.	No. chg.	No. chg.	No. chg.	Slight
Fungal enzyme	No. chg.	No. chg.	No. chg.	No. chg.	Slight

Average Angle of Hair Pocket

Fresh skin	58°
Limed skin	24°
Amonium sulfate treated	25°
Bated	35°
Bated 6 hours with 4% bate present	55°

Average Width of Elastic Tissue Pattern

Limed skin	197 microns
Ammonium sulfate treated	220 "
Bated	281 "
Bated 6 hours with 4% bate present	426 "

Table 17

The Distortion, Bate Index, pH of the Spent Liquors and Protein Nitrogen for Inactive Pancreatic Bate

Conc.	Distortion Lime	Distortion Bate	Bate Index	pH Wash No. 2	pH Spent Bate	pH Bate Wash	Prot.* Nitrogen
1%	13.1	47.3	1.43	8.8	9.0	8.7	4.30
	13.4	45.5	1.74	8.5	8.8	8.6	7.45
	10.5	43	1.48	8.75	8.8	8.6	5.22
Mean		45	1.16				
2%	12.8	40.5	1.26	8.8	8.8	8.5	4.85
	12.4	48.0	1.94	8.9	8.7	8.5	6.10
	10.1	38.2	1.74	8.7	8.7	8.4	
	13.3	39.2	1.28	8.7	8.7	8.4	
Mean		41.2	1.55				
3%	9.1	41	1.94	8.8	8.7	8.3	
	12.0	43.5	1.65	8.8	8.6	8.3	7.20
Mean		42	1.74				
4%	10.3	41	2.9	8.8	8.5	8.1	6.6
	10.7	42.5	2.9	8.8	8.5	8.1	9.9
	8.1	38.2	1.3	8.7	8.5	8.1	8.9
	8.6	28.2	1.3	8.7	8.4	8.1	9.4
Mean		37.5	2.1				

*Mg. per 100 g. limed skin
Bating Temperature - 85°F.
Bating Time - 2 hours

Table 18

The Distortion, Bate Index, pH of Spent Liquors and Protein Nitrogen for Samples Treated One-half Hour at 85°F.

Material	Distortion Lime	Distortion Bate	Bate Index	pH Wash No. 2	pH Spent Bate	pH Bate Wash	Protein * Nitrogen
1%	10.8	45	1.74	8.9	8.5	8.5	
Pancreatic	11.4	39	1.74	8.9	8.5	8.5	
	14	43	1.75	9.0	8.5	8.6	3.68
	18	44	1.75	8.95	8.5	8.6	
1%	10.6	45	2.38	8.9	8.5	8.5	
Bacterial	16.3	52	2.02	8.9	8.5	8.5	
1%	14	46	1.75	8.95	8.5	8.45	
Fungus	15	46	2.02	9.0	8.5	8.5	
Ammonium	29	41.5	1.08	9.0	8.6	8.5	4.10
sulfate	16	46	1.08	9.3	8.6	8.5	

*Mg. per 100 g. limed skin

Table 19

The Distortion, Bate Index, pH of Spent Liquors and Protein Nitrogen for Samples Treated 6 Hours with 4% Bate at 85°F.

Material	Distortion Lime	Distortion Bate	Bate Index	pH Wash No. 2	pH Spent Bate	pH Bate Wash	Protein* Nitrogen
Water controls							
60	19	42					13.2
225	15.8	41		10.2	10.8	10.8	8.6
226	16.8	45.5		10.7	10.7	10.7	8.0
290	11.6	26.5		10.6	11.0	10.8	8.65
291	20	37		10.9	11.0	10.8	7.89
Ammonium sulfate							
84							
212	13.0	41	1.22	11.0	8.3	8.3	9.2
213	12.2	42	1.22	11.1	8.4	8.4	10.0
298	13.8	37	1.09	8.7	8.2	8.4	7.53
299	17.8	36.5	1.09	8.8	8.3	8.3	6.20
Mean			1.15				
Pancreatic							
17							
18							
232	13	43	7.3	9.0	8.1	8.1	11.1
233	13.2	45	7.3	9.0	8.1	8.1	12.1
292	16.8	43.5	6.39	8.8	8.3	8.3	9.43
293	18	44	6.39	8.8	8.3	8.3	8.59
Mean			6.84				
Bacterial							
234	18	45	4.84	9.0	8.1	8.1	14.0
235	15.4	44	4.84	9.0	8.2	8.2	15.1
294	15	44.5	6.39	8.8	8.3	8.3	12.8
293	14.4	39.5	6.39	8.8	8.2	8.2	13.2
Mean			5.61				
Fungus							
227	15.2	39	3.6	9.1	8.1	8.1	14.1
228	13.8	49	3.6	9.1	7.9	7.9	11.7
296	8.0	32	4.76	8.7	8.3	8.2	13.3
297	17	41	4.76	8.7	8.3	8.3	12.0
Mean			4.18				

Figure 1

AMERICAN INSTRUMENT AMINCO COMPANY, INCORPORATED

The COMPRESSOMETER*

For Measuring Thickness, Compressibility, and Compressional Resilience of Textiles and Similar Materials

Compressibility and compressional resilience of textiles are properties which very largely determine the quality and "feel" of the cloth. The instrument described here provides a precise means of comparing these properties in different materials, over a wide range of pressures and thicknesses. The results of tests on rug underlays, blankets, felts, on knit, woven and pile fabrics, and on sheet rubber and paper are given in the Bureau of Standards paper referred to below.

Operation

The operation of the instrument is simple. The specimen is placed upon the anvil without tension, with the circular foot of the lower micrometer dial resting upon it. The knob is now turned, pressing the foot down on the fabric, the pressure being applied through a calibrated spring in the barrel. When the upper dial, which is measuring the extension of the spring, reaches the point corresponding to the pressure at which the first reading is to be taken, both dials are read, and the readings are recorded. A series of readings are made, first with increasing pressure, then with decreasing.

Definitions

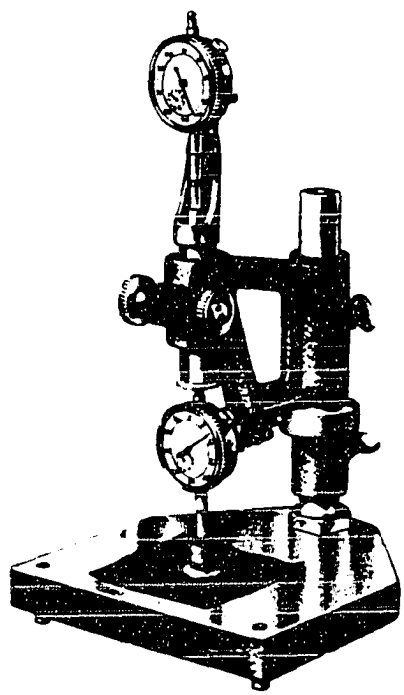
For convenience in comparing tests the following definitions are used:*

(1) The thickness of a specimen is the distance between the foot and the anvil when the pressure has been increased to a stated amount.

(2) The standard thickness is the thickness when the pressure has been increased to 1 lb. per sq. in.

(3) The compressibility of the specimen is the ratio of the rate of decrease in thickness at a pressure of 1 lb. per sq. in. to the standard thickness.

(4) The compressional resilience of the specimen is the amount of work recovered from the specimen when the pressure is decreased from 2.0 to 0.1 lb. per sq. in., expressed as a percentage of the work done on the specimen when the pressure is increased from 0.1 to 2.0 lb. per sq. in.



2010-10

Construction

The Compressometer is accurately and strongly constructed, and easy to use. The frame may be raised and lowered, to accommodate the instrument to specimens of various thicknesses up to 2 inches. The range of compression of the fabric is up to 0.75 inch, and the pressure range 0 to about 2.6 lb. per sq. in., although special springs for other ranges can be supplied. A calibration curve is furnished with the instrument.

The dials are graduated in steps of .001 inch, and readings are estimated to a tenth of that value.

2010-10 Fabric Compressometer . . . . . [redacted]

* U. S. Bureau of Standards Journal of Research, Vol. 10, No. 6, June, 1933: "The Compressometer, an Instrument for Evaluating the Thickness, Compressibility, and Compressional Resilience of Textiles and Similar Materials."—HERBERT F. SCHIEFER.

Figure 2

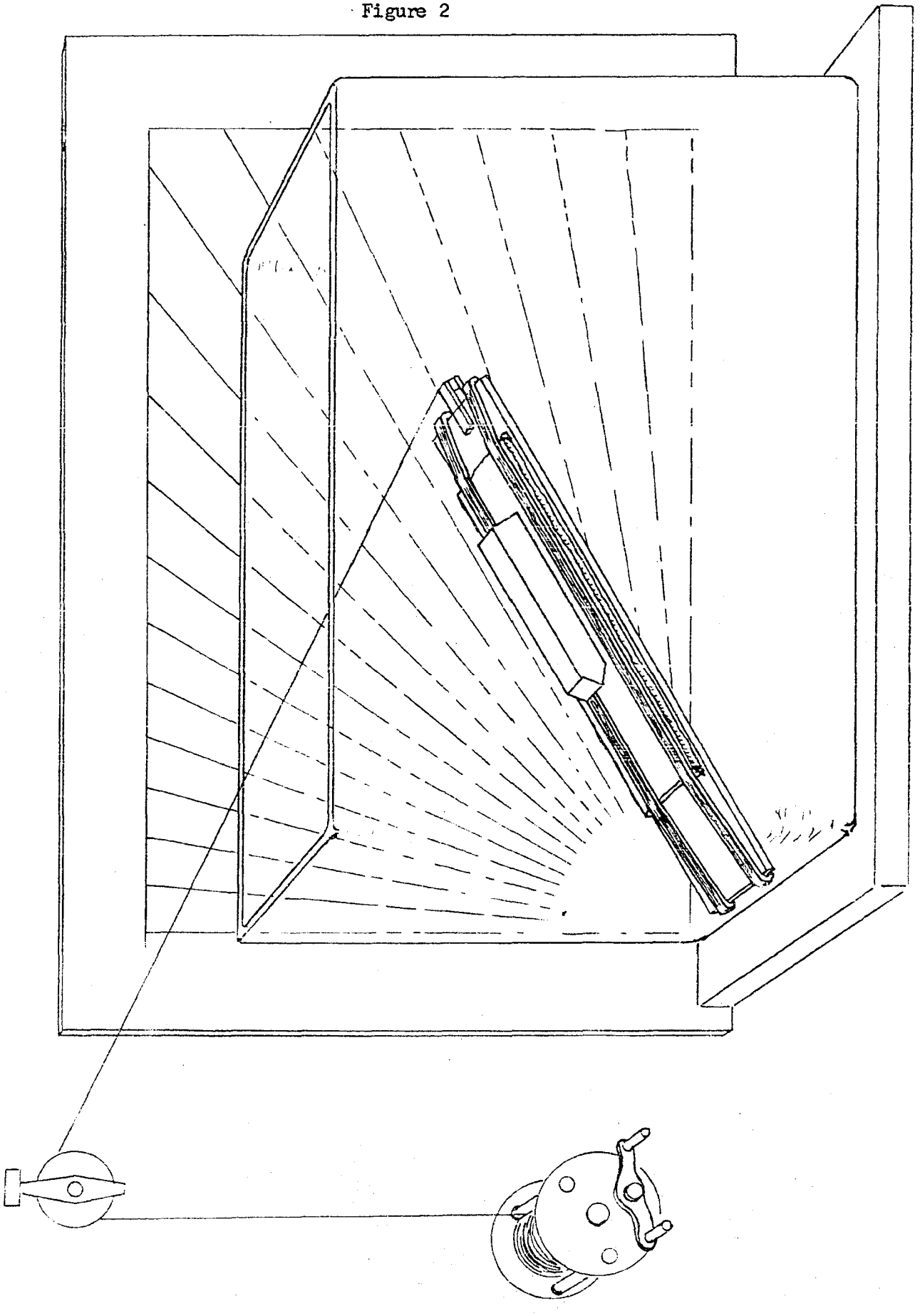


Figure 3

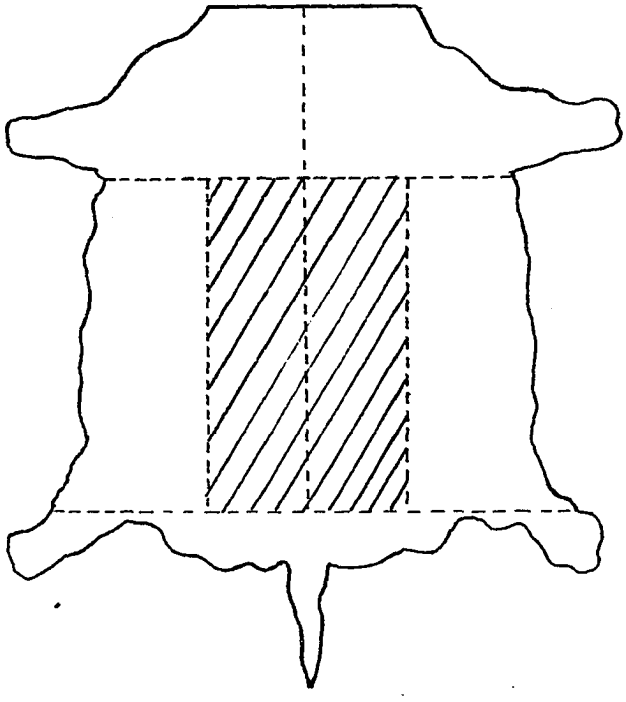


Figure 4

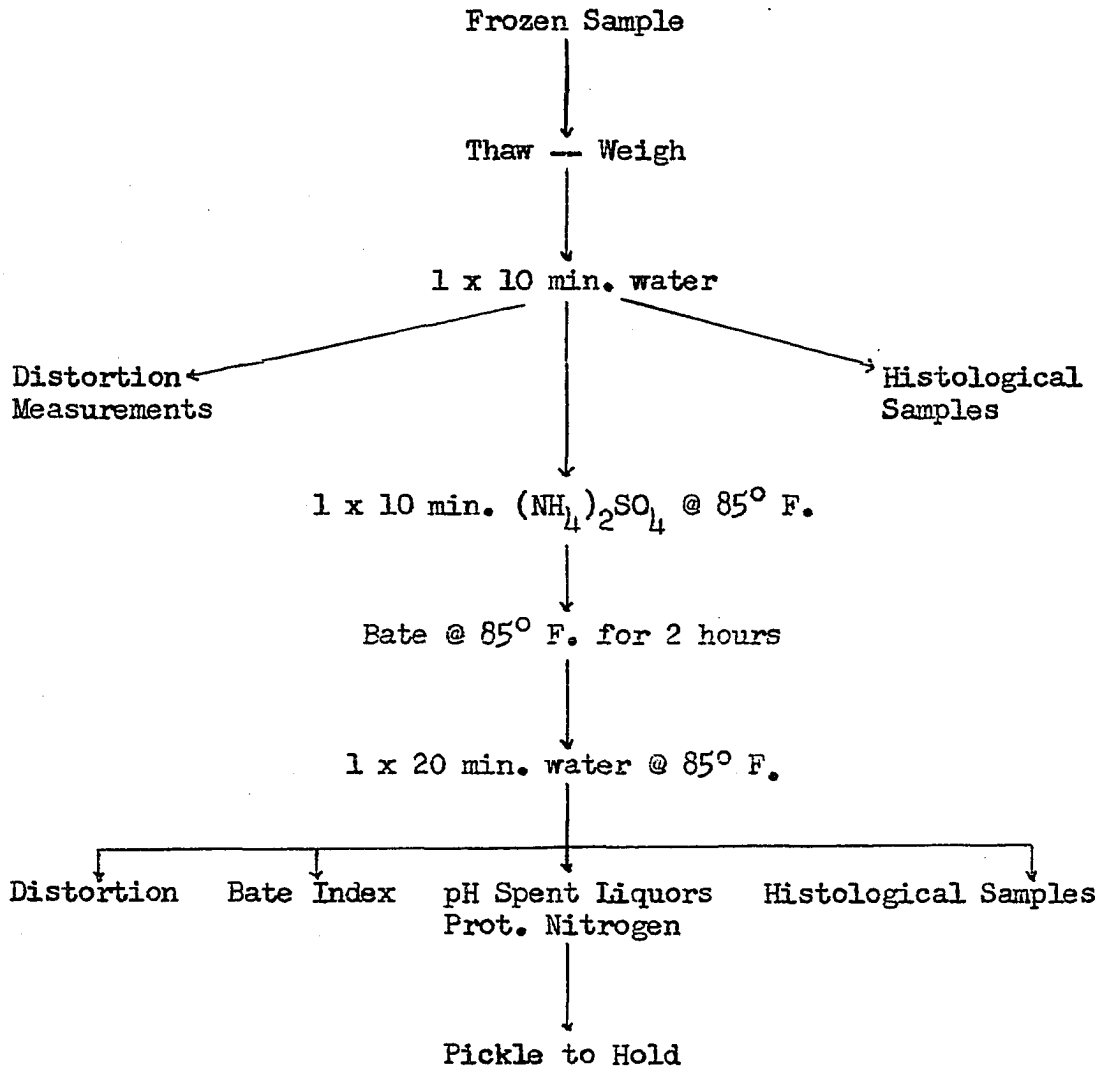


Figure 5

Mean Values of Bate Index vs Percent Bate

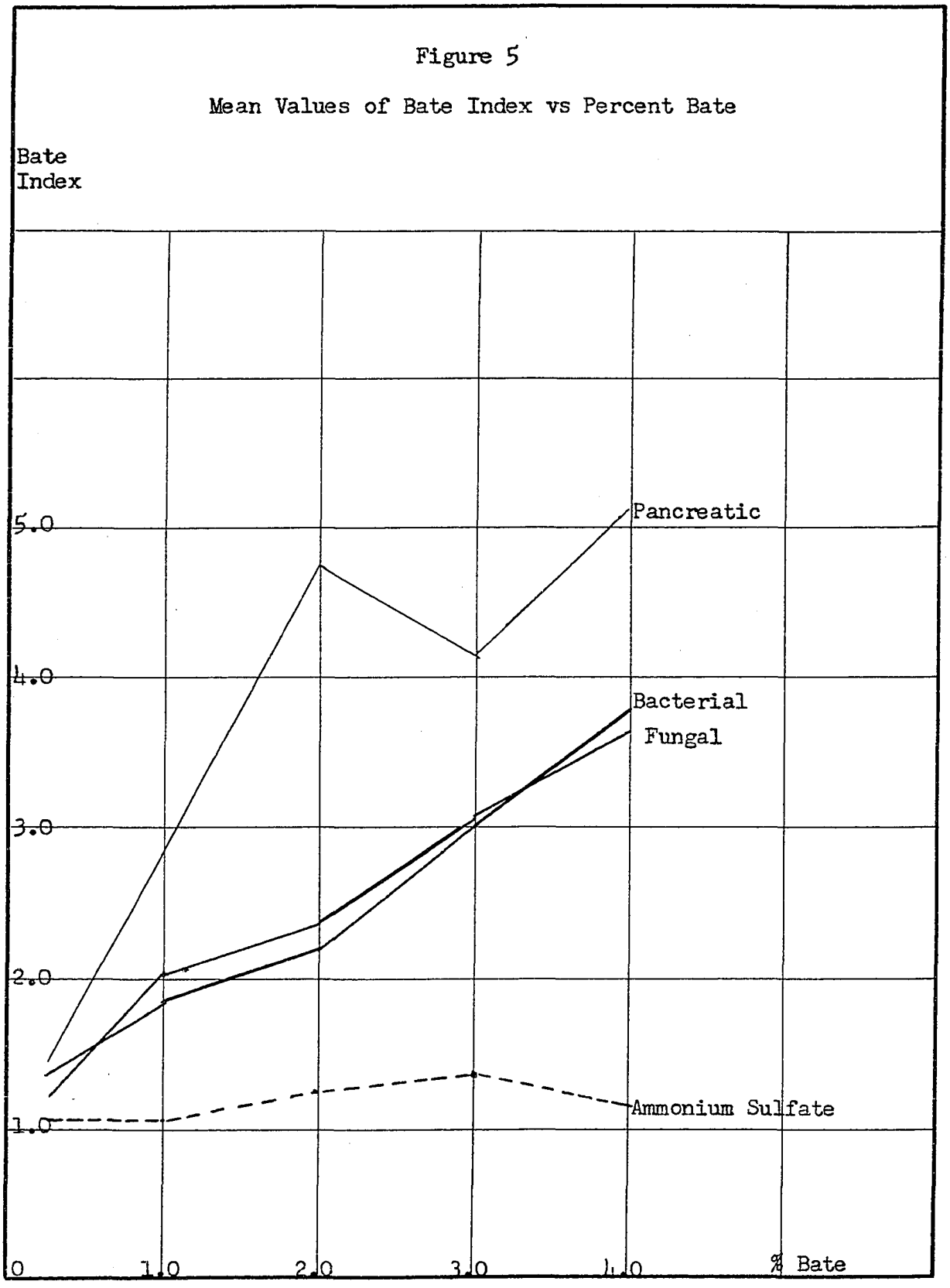


Figure 6

Mean Values and Standard Deviations vs Percent Bate

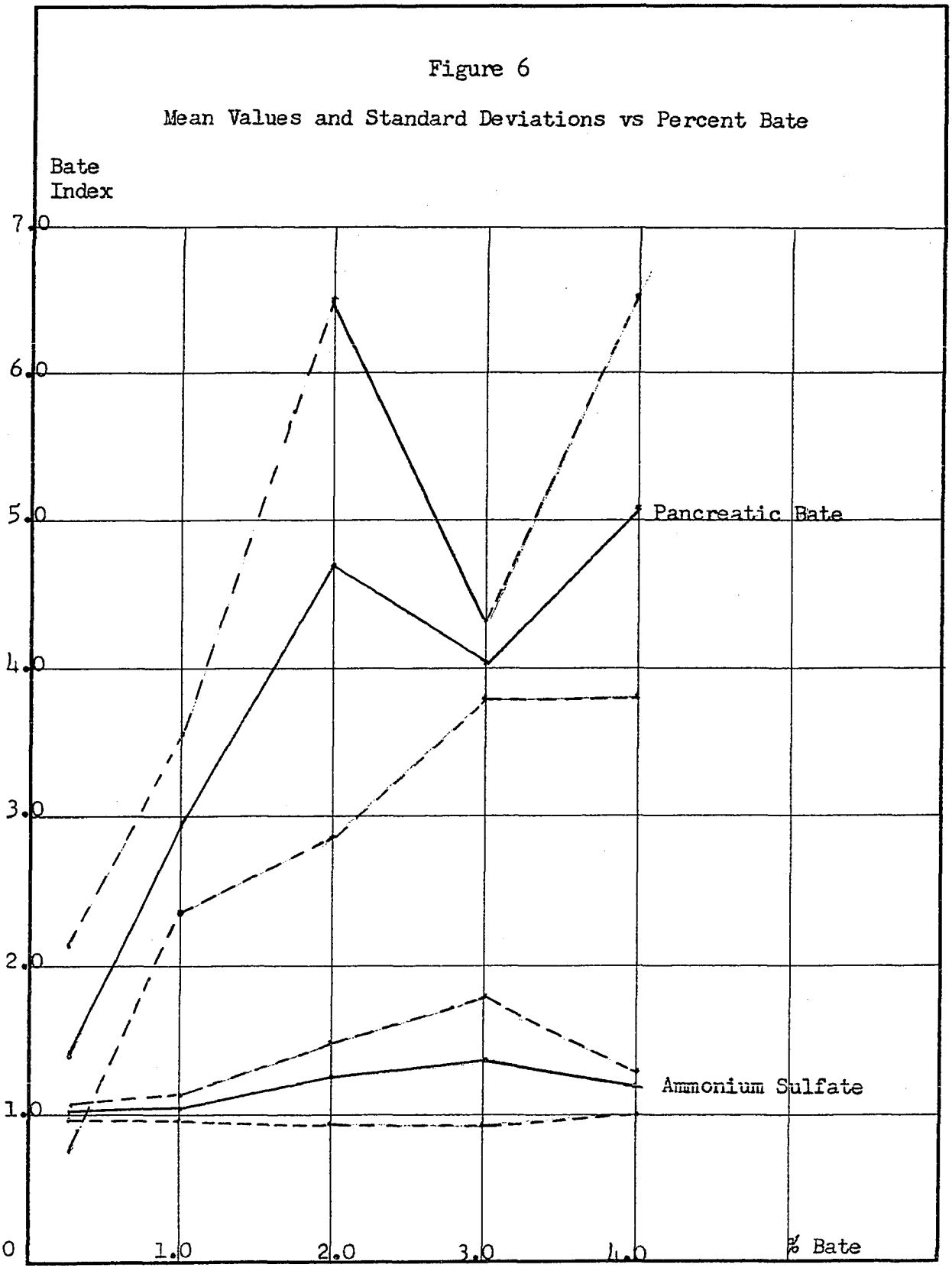


Figure 7

Mean Values and Standard Deviations vs Percent Bate

Bacterial Bates

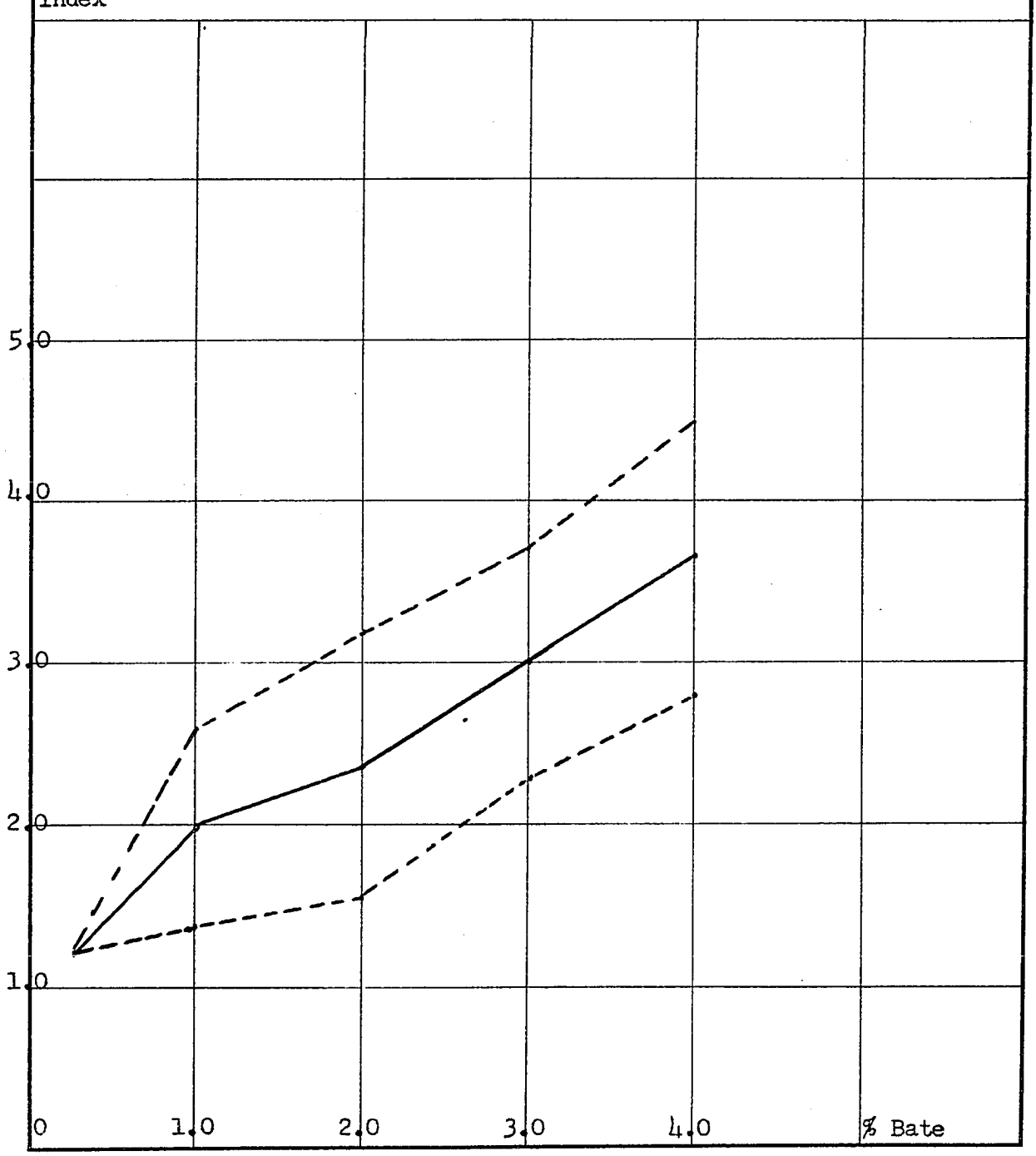


Figure 8

Mean Values and Standard Deviations vs Percent Bate

Fungal Bates

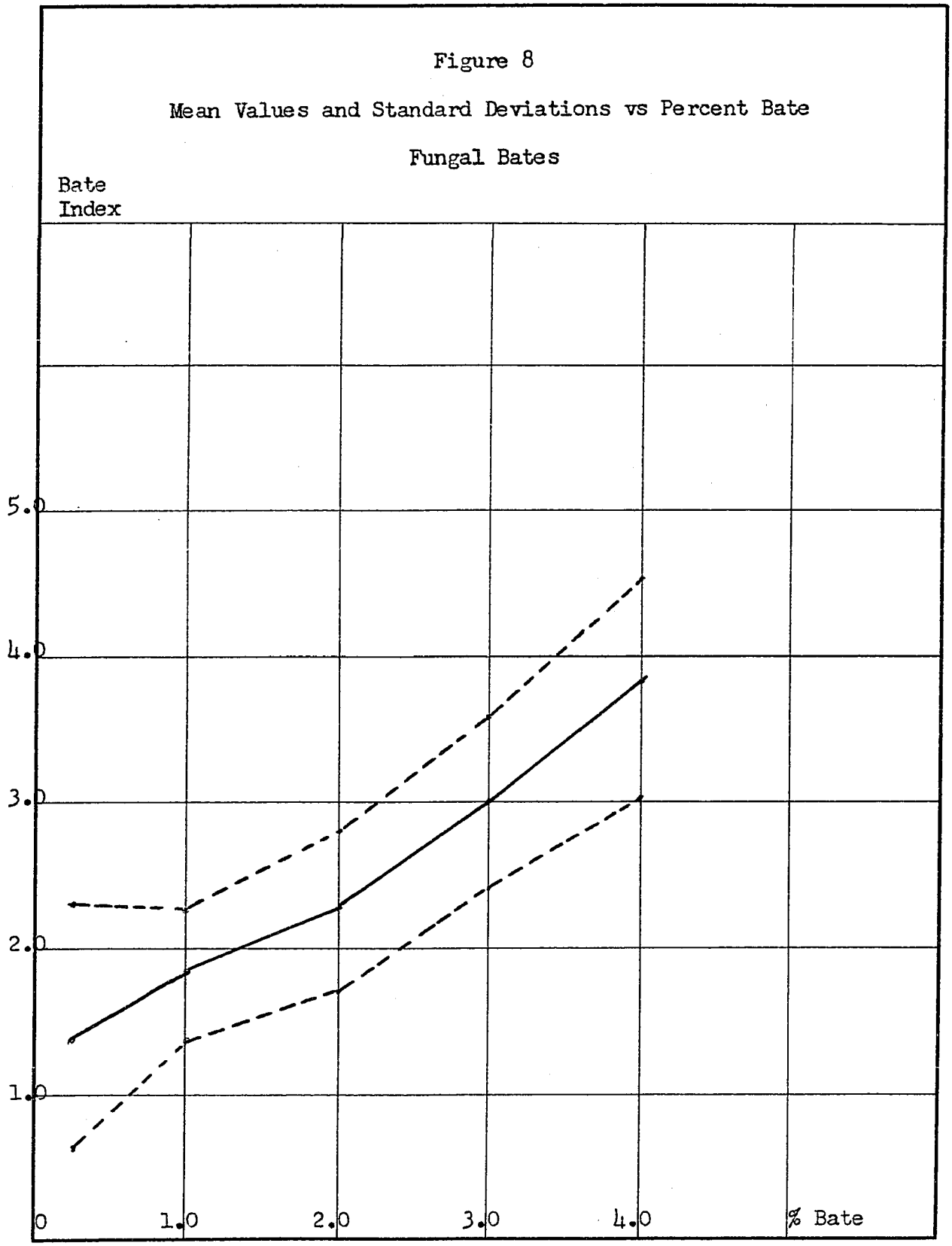


Figure 9

Milligrams of Protein Nitrogen per 100 Grams Limed Skin in Spent Bate Liquors vs Percent Bate

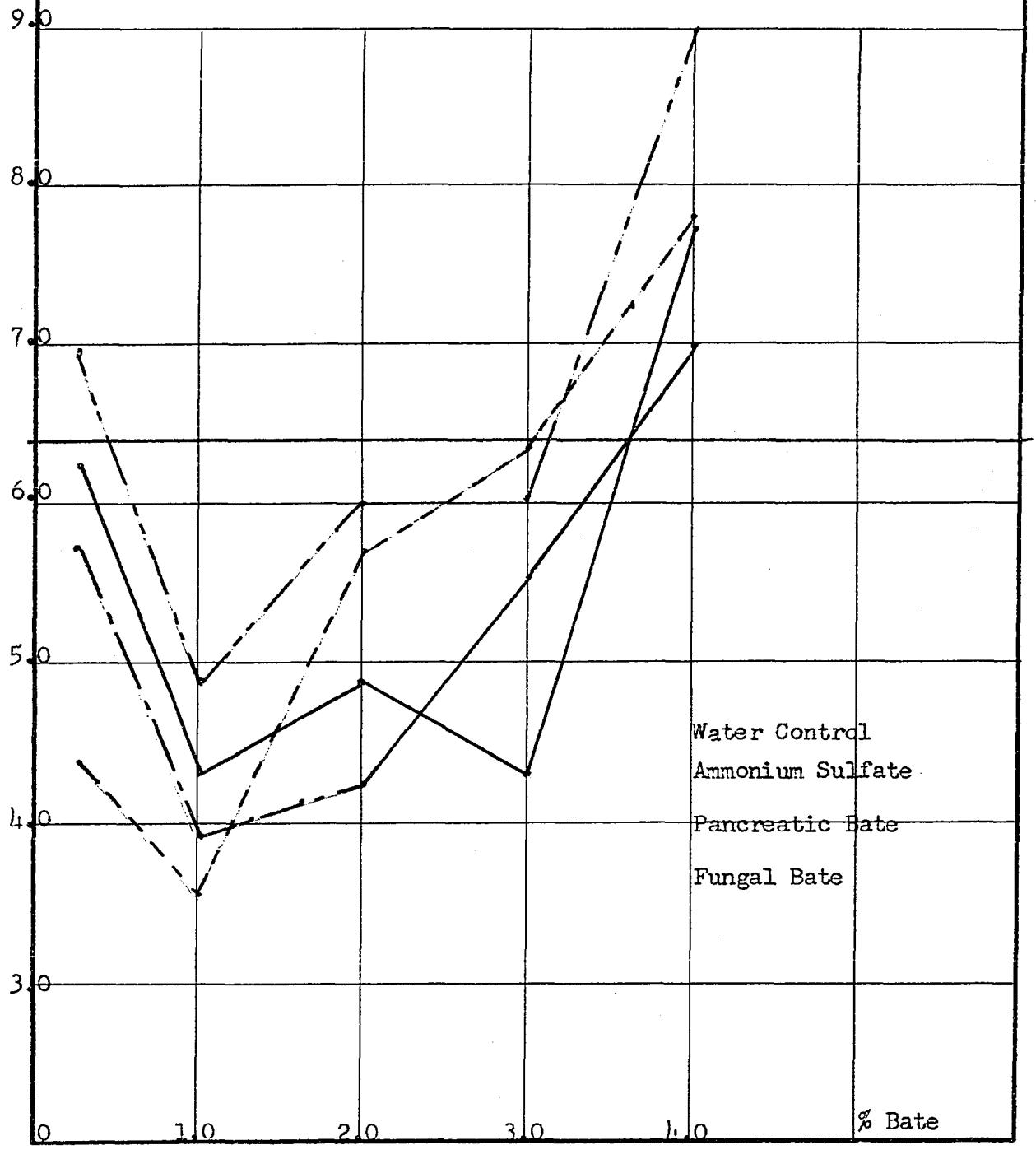
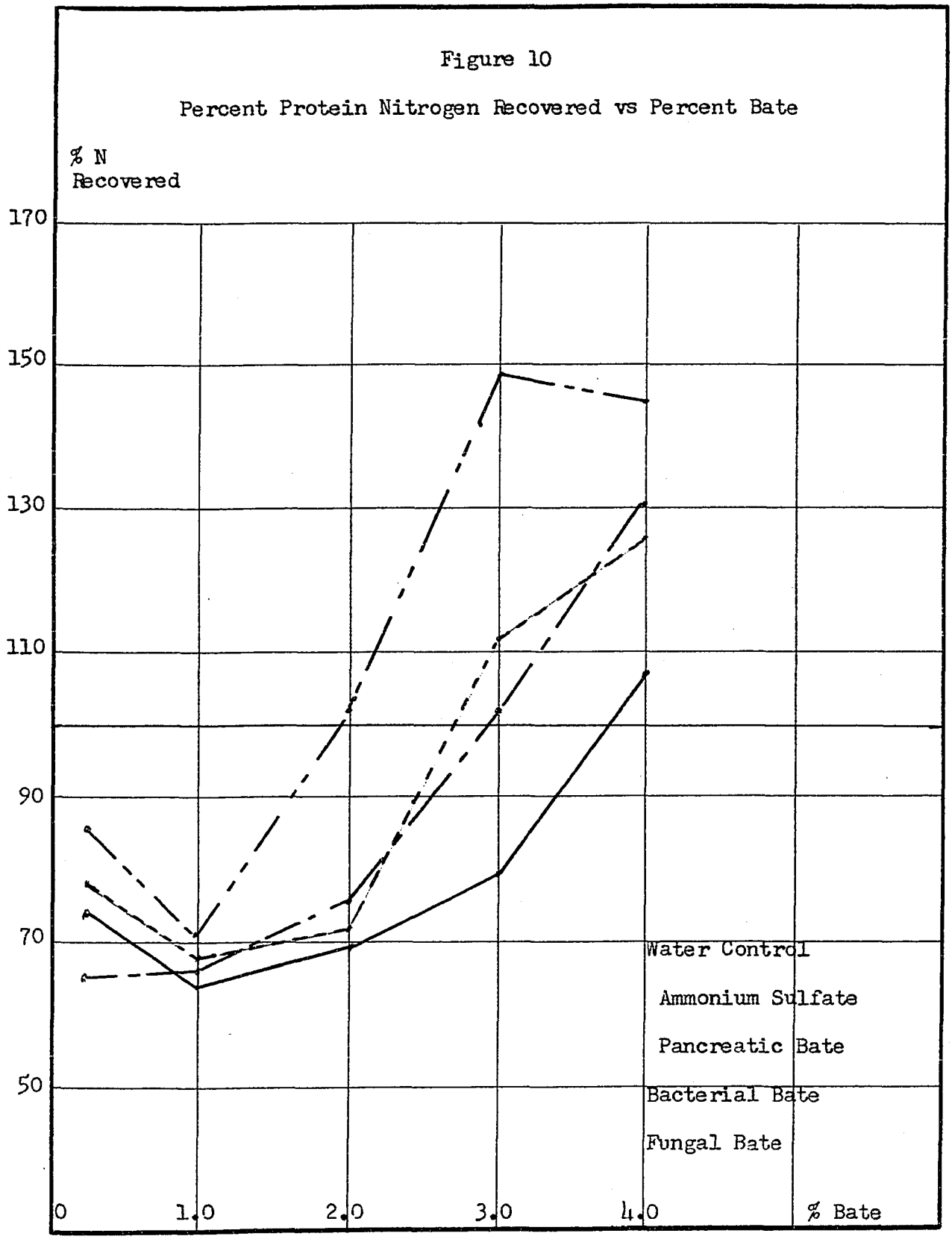


Figure 10

Percent Protein Nitrogen Recovered vs Percent Bate



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