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THE INFLUENCE OF VARIOUS CHEMICAL TREATMENTS ON THE MECHANICAL PROPERTIES OF COLLAGEN

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by

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ABSTRACT

The technique of wet tension testing is applied to a study of some reactions of collagen, including conventional tanning agents. In a presentation of the theoretical background of the method, the mechanism of rupture of protein chains, the theories of fiber structure and of protein structure, and the types of intermolecular forces that may exist in the protein chain, are discussed.

The technique involves the application of a lead holder to kangaroo tail tendon collagen fiber aggregates. These lead pieces are held in turn by the jaws of an inclined-plane tensile tester (a constant rate-of-loading type of tester), fitted with a trough into which the wet fibers are placed. A stream of water pours down the trough, keeping the fibers wet during testing. The tensile strength is calculated as "mean breaking length", i.e., that length of fiber which, if suspended from one end, would break under its own length.

The use of water-swollen fibers in this type of testing facilitates interpretation of results in terms of some of the stronger of the inter-molecular forces. Data are presented on vegetable, chrome, and formaldehyde tannages, and on a cross-linking reaction, and a side-chain-specific protein reaction. Using statistical methods to test the significance of the data, it is shown that conventional tannages decrease the wet

strength of collagen fibers. However, an increase in the hydrophobicity of the protein may, in some cases, lead to an apparent increase in wet strength. It is shown that side-chain-specific reactions can decrease the strength of the fibers. Cross-linking of the protein chains results in an increase in strength.

The conventional interpretation of tannage as a covalent cross-linkage of collagen chains by tanning agents is incompatible with the data presented. The results obtained in this investigation require either that this theory be discarded, or that a different interpretation of the theory be made.

A hypothesis to explain the decrease in strength, due to tannage, is advanced.

A method for the preparation of 2,4-dinitrofluorobenzene is described.

HISTORICAL

The ever-increasing application of science to the art of tanning animal skins to make leather has resulted in an increased interest in the mechanism of this tannage. Many of these studies have taken the form of attempts to discover what, if any, stoichiometrical relationship may exist between the skin protein and the take-up of tanning material. In a different approach, Tu (43) recently studied the effect of the structure of some synthetic tanning materials closely related to the vegetable tannins on tannage.

This dissertation represents the report of an investigation of the mechanism of tannage by a still different approach: that of studying the mechanism of tannage by a study of the relative forces involved in the process. By way of establishing the theoretical basis for this investigation, a review is presented on past attempts at evaluation of these forces, the mechanism of rupture of protein fibers, the fine structure of skin protein, the forces between adjacent protein chains, and the application of tension testing in evaluating changes in these forces.

In a recent paper on the structural stability of collagen, Highberger (24) has discussed various theories of the mechanism of tannage, with particular reference to the mechanism of formaldehyde tannage. He reviews the currently favored theory of cross-bridging from the standpoint of the hydrothermal shrink-

age phenomenon, and the mechanical stability as evidenced by the determination of tensile strength of parallel disposed fiber bundles of collagen.

If one is to accept the claim of Theis (42) that the hydrothermal shrinkage temperature is a measure of the structural strength of the collagen fiber, he is led to the conclusion that tannage increases the inter- and intra-molecular forces in the collagen "molecule". According to this author, the increase in forces is probably effected through cross-bridging between adjacent peptide chains by the tannin molecule. (Most, if not all, of the commercially practiced tanning procedures raise the shrinkage temperature in water of hide substance from about 62°C. to higher temperatures, depending on the tannage, going as high as the boil temperature in the case of many chrome tannages.)

It is not the purpose of this dissertation to settle the question of the mechanism of hydrothermal shrinkage. The factors involved in shrinkage are probably too numerous to be readily settled. Nevertheless, it seems reasonable to assume that the forces between polypeptide chains may well be one of the factors involved, as well as such less definable factors as increased hydrophobicity of the collagen due to tannage. On the other hand, tensile strength measurements of parallel-arranged fiber bundles seem to be largely, if not exclusively,

a function of the forces between the polypeptide chains, or of the chain strength itself.

As Highberger (24) points out, the rupture of air-dry collagen fiber bundles occurs at a load corresponding to a tensile strength of about one-thirtieth of the load that would be theoretically required if the mechanism of rupture were the breaking of covalent linkages along the chain. He therefore concludes that the mechanism of rupture is the breaking of lateral cohesive forces between the chains, allowing the adjacent chains to slip over one another. This view is shared by leading fiber chemists, dealing with wool (21) and other fibrous materials (29).

From a logical viewpoint, this mechanism of rupture is the most likely explanation, because the point at which rupture will occur will be the weakest of the various links in the chain, and, as will be pointed out, it is these forces between chains that are weaker than the forces along the polypeptide backbone.

Harris (23), paraphrasing a discussion of Mark (29), gives a concise statement of the modern viewpoint of fiber structure: "If the polymer molecules fit poorly into a lattice and the intermolecular attractions are weak, the material should have rubber-like properties. In contrast, if the structure of the molecules permits easy fitting into a lattice

and the forces between molecules are strong, the material is a fiber. In intermediate cases, plastic systems result."

Any discussion of fibrous protein structure assumes long, relatively straight-chain, polypeptides, in a three-dimensional array, roughly similar to a bundle of sticks. As a qualitative picture, there seems to be little reason to question this hypothesis. In recent years, various investigators have attempted to give a more quantitative picture of the fine structure of the fibrous proteins. Of these, the investigations of Astbury (1, 2, 3, 4, 5, 6, 7) have justifiably received the greatest attention.

Astbury derived most of his ideas from an interpretation of the x-ray diffraction patterns of the fibrous proteins. He has attempted to describe the fine structure in such respects as: (1) average length of residues along the chain, (2) backbone spacing, (3) side chain spacing, (4) number of residues in a "molecule", (5) arrangement of amino acids along the chain, and (6) the degree of "kinking" of the chain.

Astbury's quantitative picture is no longer the popular conception, because of the work of Schmitt and co-workers (19, 35) and Bear (9, 10), who demonstrated by electron microscopy and low angle diffraction studies that a 640 Ångstrom repeating unit exists in collagen. This spacing is inconsistent with Astbury's picture, as originally formulated, of the number of residues in the chain. Further, the periodicity

theory (11) of the arrangement of amino acids in collagen has been shown to be inconsistent with later, and probably more accurate, proline determinations (12, 13).

In another criticism of the Astbury theory of collagen structure, Huggins (26) has suggested an alternative structure. He proposes a spiral structure with the side chains alternately above and below the main chain. Proper arrangement of spiral layers would give close-packing of the polypeptide chains.

Another idea of fibrous protein structure has been suggested by Schmitt and Denues (36) who indicate that "many reactive fibrous proteins consist of globular molecules in linear array and ... their physical properties depend on a reversible globule-fiber transformation sensitive to pH, ionic environment and enzymes". Most of the evidence advanced pertains to the muscle protein, which is unlike collagen, and to fibered globular proteins, such as insulin and virus particles. However, Farrant, Rees, and Mercer (17) have considered keratin as a linear array of globular particles, although, as Schmitt and Denues point out, this may be an artifact. The evidence for this consists of electron photomicrographs of gold shadowed specimens; gold tends to migrate and form aggregates, which may explain the evidence without assuming arrays of globular particles. On the other hand, Schmitt and Gross (37), in a review of the electron microscopy of collagen, suggest

"that the periodically contoured structure may result from loss of water from interband regions during drying. Shadowed replicas of moist fibrils show little intraperiod contouring. The fibril is thought to be composed of longitudinally disposed polypeptide chains which tend to aggregate in bundles.." (aggregates which form fibrils).

A further discussion of the formulation of collagen is given by Highberger (in Monograph of McLaughlin and Theis, "The Chemistry of Leather Manufacture") (30). The exact details are not especially relevant to the purposes of this paper. However, the postulates of collagen structure are generally consistent with Mark's (29) theories of fibrous structure, and we may consider the fiber to consist of three-dimensional arrays of polypeptide chains, presumably parallel. As will be shown later, the inter-molecular forces holding the collagen chains together are most probably hydrogen bonds, salt bridges, and van der Waals forces.

In the discussion of the nature of the fibrous state, it was noted that there is a strong emphasis on the forces between molecules, or to modify for protein fibers, the forces between polypeptide chains. The forces which might exist between peptide chains may be classified as follows:

1. Covalent bridges, i.e. bridges between adjacent polypeptide chains, linked to the main chain by a covalent type of

linkage. An excellent example of these linkages is the disulfide bridge between adjacent polypeptide chains in wool and hair keratin. This bridge has been demonstrated by a number of investigators, and the procedures involve tensile testing, in addition to chemical evidence (see, for example, Speakman (39)). In essence, the procedure involves wet tension testing (to reduce weaker forces) of the wool fiber. When these disulfide bridges are broken (e.g. by reduction of the disulfide link), the strength and resistance to elongation of the fibers decrease considerably. When these bridges are reformed, (see Speakman (39), and Harris, et al. (18)) by reaction with organic dihalides, the fibers are considerably strengthened.

Due to the low cystine content of collagen, 0.0 (8, 13, 31), no cross-bridging from disulfide bridges would be expected. Further, there is no other amino acid known that has two amino and two carboxyl groups, and therefore it would not be expected that covalent cross-linking of any kind would be found in collagen.

2. Hydrogen bonding, also known as strong van der Waals forces. These may occur between adjacent peptide links, as well as between side chain groups. Many chemists who have studied the structural chemistry of collagen (24, 27) believe these hydrogen bonds are one of the most important, if not the

most important cross-linking forces existing in the fiber.

3. Salt bridges. These are coulombic forces, represented by oppositely charged groups (esp. NH_3^+ and COO^-), and hold the adjacent chains together by electrostatic attraction. The importance of this force in collagen fibers is believed by many (24, 27) to be a significant factor, especially in the explanation of the hydrothermal shrinkage phenomenon. However, the relative amount of free amino and free carboxyl groups in collagen is small, especially in native untreated collagen, where presumably all acid amide groups are still intact. It would therefore seem that salt bridges are of secondary importance in a consideration of the structural stability of the collagen fiber.

The 10 Ångstrom spacing in vacuum-dry collagen is generally believed to be due to the salt bridge--side chain spacing (24). This spacing increases with water content of the fiber, and it therefore seems reasonable to assume that this force decreases in a water swollen fiber. (Coulombic forces are inversely proportional to the square of the distance between the two charged groups).

The above discussion involves coulombic attractive forces, but it is conceivable that coulombic repulsive forces might also exist in a protein fiber, if the adjacent charged groups were of the same sign. There seems to be no evidence on which

to base an estimate of the relative importance of these coulombic forces, although most investigators in the field place much stress on the attractive forces. This stress is quite probably due to a misplaced emphasis on attractive forces, to the exclusion of proper consideration of repulsive forces.

4. Van der Waals forces.[++] In an excellent discussion of these forces, Rice and Teller (33) point out their various components: (a) dipole-polarizability interaction, (b) dipole-dipole interaction, (c) temperature effect, and (d) polarizability-polarizability interaction. The magnitude of the force is inversely proportional to the n th power of the distance between the groups, where n varies, depending on the specific component of the force, and having values from 3 to 8 (28). In a water-swollen fiber, therefore, the van der Waals forces should be decreased even more than the coulombic forces, and can be considered to be practically negligible.

In addition to the van der Waals attractive forces, mentioned above, there are also stronger van der Waals repulsive

[++]There is some confusion existing regarding the terminology of "van der Waals forces". Some authors include the hydrogen bond as one component of these forces. In this paper, however, whenever the term "van der Waals forces" is used, hydrogen bonds are not included as one of the components. Thus, what is here called "van der Waals forces" are called by some authors as "weak van der Waals forces". These authors sometimes call hydrogen bonds "strong van der Waals forces".

forces. There is no evidence on which to base an estimate of these replusive forces. It would seem, however, that because of the small distance over which they are effective, that they would be negligible in swollen collagen, although possibly of importance in the dry fiber.

While the covalent forces are the strongest of these forces, and hydrogen bonds the next strongest, the number of bonds must be taken into account. Thus, van der Waals forces, weak though they be, may contribute more to the stability of a fiber (due to greater number) than a stronger force less prevalent.

Harris and Brown (23) list some of the various fibrous materials, with a discussion of their molecular structure and properties, and discuss two broad classes of fibers: the silk model and the wool model. Silk possesses high strength and low elasticity; wool, on the other hand, has low strength but has a long-range elasticity. These authors attribute the high strength and low elasticity of silk to high amounts of low-molecular-weight amino acids, viz., glycine, alanine, and serine, yielding polypeptide chains that are relatively bare, with few bulky side chains. Thus, close-packing of the chains would be possible, with considerably inter-chain hydrogen bonding. Wool, however, has a low proportion of low-molecular-weight amino acids, and large bulky side chains would make

close-packing more difficult, with inter-chain hydrogen bonding less likely. Thus, the low strength of wool can be explained by the small amount of inter-chain bonding. The disulfide bridges present are shown to give the wool what little strength it has. In addition, the lack of close-packing would be expected to indicate a smaller van der Waals attraction in wool than in silk.

Collagen has a relatively high strength, but a low stretch, so it might be expected to fit into the silk pattern. The large amount of glycine (roughly one-third of the amino acid residues) (13) and proline and hydroxyproline (about one-fourth of the residues) (13) and alanine (about one-tenth) (13), would be expected to contribute to close-packing and inter-chain hydrogen bonding. The size of the proline molecule might prevent as much close-packing as would exist in the silk fiber, and might alter the size of the chain, and the degree of kinking. Thus, while the proline (and hydroxyproline) residues should account for some differences from the silk fiber, collagen should be, and is, roughly similar to silk in its properties.

Tension testing has been used as a method of qualitative evaluation of inter-chain forces in wool (39). Compton (14), using "teased" fiber aggregates from the kangaroo tail tendon (because they are mono-directional) studied the effect of

tannage on the tensile strength of collagen. He used dry fibers (equilibriated to 65% relative humidity at 70°F.), and measured the "mean breaking length" of the fibers. This is the length of fiber that would break under its own weight if suspended from one end. Because of the difficulty of accurately measuring cross-sectional area of the fibers, it was felt that mean breaking length was a more accurate measure of tensile strength. Showing his results to be statistically significant, Compton concluded that "jaw breaks", i.e. fiber aggregates which break at the jaw of the inclined-plane tensile tester, could be included in the data without effect on the error of the determination. He also found little apparent lateral or longitudinal non-uniformity of the fiber bundles used.

Compton's study included formaldehyde and chrome tannage. He found a decrease in tensile strength in the case of both tannages, the chrome tanned specimens being the stronger of the two. Increasing formaldehyde concentration in the collagen led to a progressive decrease in strength, although the shrinkage temperatures were the same. Increasing chrome concentration in the collagen had little effect on strength. However, he suggests that if less chrome had been applied, a more pronounced range of strength might have been observed. As in the case of formaldehyde tannage, the effect of chrome tannage on

the mean breaking length appeared to be independent of shrinkage temperature.

Compton suggested his technique as representing a possible method for the evaluation of tannage, and made no claims for the method as being of theoretical importance. Harris (20) has suggested a modification of the dry tensile test, viz., wet tensile testing, as a means of obtaining results of theorectical importance. Harris' idea is that by working with a fiber swollen in water, the chains are separated to an extent so that van der Waals forces (and probably also coulombic forces, i.e. salt bridges) are reduced so that tension testing gives an estimate of the role played by covalent bonds and hydrogen bonds. In Harris' work (22,23) primary emphasis has been placed on the stretchiness of the fiber (wool); a decrease in stretchiness for a given load means an increase in the interchain forces, and conversely, an increase in stretchiness means a decrease in these forces. Harris has placed great importance on the hysteresis "loop", i.e. the area between the curve of stretch (upon the application of load) and the curve of recovery (upon release of the load), as being indicative of this stretchiness, and therefore of the inter-chain forces. It should be noted, however, that Harris' work, and also Speakman's (39), and others who have used this technique, is on wool, which is quite a stretchy fiber. Collagen, on the

other hand, is a stronger and less stretchy fiber, and a quantitative measure of stretch and hysteresis "loop" would be more difficult.

Stubbings and Theis (41) have discussed the work of Harris, and suggested that similar work be conducted on collagen. They point out that if tanning forms a bridge between collagen chains, the wet strength should be increased over that of untanned collagen. "It is this distinction between wet and dry strength that should be investigated rather than a comparison between tanned and untanned dry strengths alone."

However, these authors also mention that because of the lack of cystine in collagen, collagen should have a low wet strength compared to wool. This does not give proper emphasis to the greater hydrogen bonding in collagen than in wool. Further, inactivation of polar groups, rendering them non-polar, should reduce coulombic and van der Waals attractive forces, thus decreasing the dry strength, but probably not affecting the wet strength. Stubbings and Theis claim that if polar groups are rendered non-polar, the wet strength of the fiber should increase, without suggesting the mechanism of this increase.

It is the purpose of this dissertation to advance experimental evidence which may be helpful in interpreting the effect of tanning materials on the mechanical stability of tanned

collagen. The experimental approach will involve a study of the effect of various protein modifications upon the wet strength of collagen fibers.

EXPERIMENTAL METHODS

For the purposes of tension testing, it was desired to use samples of collagen consisting of parallel-arranged fiber bundles. Highberger (24) has suggested the use of kangaroo tail tendons for the purpose, pointing out the difficulty of isolating fibers from skin in sufficient quantity, and the lack of sufficient dimensional uniformity of fibers++ prepared from cattle heel tendon. Kangaroo tail tendon fibers were also used by Compton (14) in his study, previously discussed.

Longitudinal sections were made and examined in the stained condition. The collagen fibers, while arranged in a parallel manner, were wavy in appearance. A small amount of elastic tissue was found present.

Neuman (31) has made a study of the amino acid composition of collagen from a number of different sources. On the basis of these analyses he has shown that collagen from the kangaroo tail tendon has the same amino acid composition as that of many skin collagens.

++To simplify the discussion, the terminology of textile workers is used, and these fiber aggregates are called simply fibers. It should be borne in mind, however, that these "fibers" are macro units, not micro units.

The identity of collagen from kangaroo tail tendon with that of skin collagen has not been demonstrated other than by the amino acid composition, although Highberger (24) states that the kangaroo tail tendons are "composed of nearly pure collagen, and give a very sharp X-ray fiber pattern identical with that given by other collagenous tissues".

The tendons were received from two sources: The Armour Laboratories, and The Ethicon Suture Division of Johnson and Johnson. They would normally have been processed and sold as surgical suture material. They were untreated except for drying and splitting. The identity of the materials from the two sources was demonstrated by a comparison of their mean breaking lengths (see "Discussion of Results" section).

The fibers, taken as received from the suture supply house, were cut into lengths of 7 inches, and weighed. 150 to 175 of these fibers (weighing less than 0.1 gram) were randomized into groups of 25 each, such that each group had the same weight distribution, and therefore the same distribution of cross-sectional area.

After randomization, each group of 25 fibers was soaked for one day in 10 per cent sodium chloride solution. They were then soaked in successive changes of distilled water, until the water that had been in contact with the tendons for at least 12 hours failed to give a positive chloride test with a one per cent solution of silver nitrate. They were then

allowed to dry in air, and were re-wet in distilled water prior to treatment.

After the chemical treatments, the fibers were allowed to dry slowly, by first placing them in a chamber where the relative humidity was maintained at 100 per cent for a day. They were then put into a 50 per cent relative humidity chamber. The fibers were then "leaded" (see below). Following this, they were re-soaked in water for at least a day prior to a hysteresis test. This hysteresis test was made with a 10 lb. total load, and was done to show what, if any, changes in hysteresis, i.e. area between upstroke (application of load) and downstroke (removal of load), were produced by tannage. The fibers were allowed to "relax" in water for one day before being given a breaking test, at 25 lbs. total load.

A major problem in this investigation was to find a suitable method of holding wet fibers in the jaws of the tensile tester, without allowing the fibers to slip, or to disorient them to any great extent. A number of procedures were tried, such as knots on sticks and wires, fastening with glues, etc. The method finally adopted was to pinch the end of the fiber with a lead fishing sinker (Pflueger "Clincher", No. 2080, Size 2), and then pressing the lead repeatedly in a vise, until the lead[++] "flowed" around the fiber. Even with this method,

[++]The fiber was originally 7 inches long. The lead sinker held about one inch of the fiber on each side, leaving approximately 5 inches of fiber free for tension testing.

a few cases of slippage of the fiber from the clamp occurred, but these results were discarded in the calculations.

The fiber aggregates used were too small to allow convenient methods of cross-sectional area measurement, and microscopic methods were shown to be too inaccurate. Consequently, the method of "Mean Breaking Length" as a substitute for conventional tensile strength measurements was used. This method has previously been described by Highberger (24) and Compton (14). The fibers, after breaking, were allowed to "rest" a day in distilled water. The part of the fiber not held by the sinker was cut away, and its length measured with a centimeter rule. The fibers were placed in individual test tubes, and dehydrated with acetone for one-half hour. The acetone was then poured off, and the tubes were allowed to sit overnight. They were then placed in an oven at 75°C., in vacuum, for four hours. After cooling, they were weighed to 0.1 mg. on an analytical balance. The results were calculated from the formula --

$$\text{Mean Breaking Length, in Km.} = 4.54 \times 10^{-3} \frac{BL}{W}$$

B = Breaking load, in lbs.
L = Length, in cm.
W = Weight, in gms.
4.54×10^{-3} = Conversion factor for units used.

Compton (14) found that the occurrence of "jaw breaks", i.e. the breaking of the fiber at the jaw of the tester, does not affect the results, and no contradiction of this conclusion

was found in this investigation. A majority of the fibers tested broke at the jaws.

The tensile tester used was a Scott IP-4 incline-plane Serigraph, a constant rate-of-loading instrument. In hysteresis tests (at 10 lbs. total load), a rate of loading of 0.6 lbs. per second was used; in the breaking test, a rate of loading of 1.5 lbs. per second was used.

The tester was modified such that a small trough extended between the two jaws, with a water inlet near one jaw (such that when the plane became inclined, the water poured from the upper jaw). Thus, the fiber, when clamped between the jaws, was held in the trough, but not touching the trough at any point. A slow stream of water poured down the trough during the test, keeping the previously soaked fiber wet.

The tester automatically recorded the load-stretch curve, giving stretch directly; the load at any point could be calculated, knowing the load on the carriage of the instrument. When the fiber ruptured, a sharp break occurred in the load-stretch curve, and the breaking load corresponding to this point on the load axis was calculated.

The tanning materials used for tanning the fibers, quebracho, wattle, and chrome, were all commercially available tanning materials. The chrome was the commercial product known as Tanolin R, a sugar-reduced chromium complex, known

as one-third basic chrome sulfate.

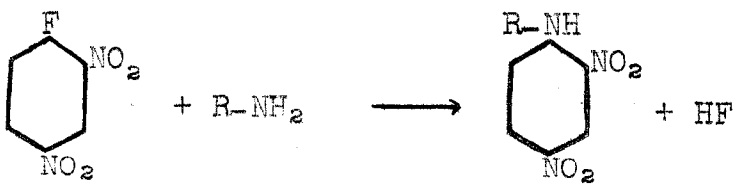
It was desired to put a large side chain in the protein molecule. Sanger (34) has suggested the usefulness of 2,4-dinitrofluorobenzene (DNFB) as a protein reactant. A major advantage of this material is that the reaction with protein occurs at room temperature.

The DNFB was prepared by a procedure analogous to the preparation of 2,4-dinitrochlorobenzene (15). 180 ml. of fuming nitric acid (sp. gr. 1.5) were mixed with 57 ml. of concentrated sulfuric acid (sp. gr. 1.84), in a three-necked liter round bottom flask in an ice-salt bath. 96 grams of fluorobenzene were placed in a dropping funnel, and added dropwise to the cooled acid mixture. A mechanical stirrer kept the mixture well mixed. The fluorobenzene was added dropwise so that the temperature of the mixture would be below 5°C. After the fluorobenzene had been completely added, the temperature was raised to 5-10°C., and maintained there with stirring for one hour. Then the temperature was raised to 50°C., and maintained for an hour with stirring. 195 ml. of concentrated sulfuric acid (sp. gr. 1.84) were then added dropwise. The mixture was finally heated to 115°C., and held there for one hour, with stirring. The mixture was cooled and then poured into a beaker containing cracked ice. The DNFB crystallized out. After standing overnight, the mixture

was quickly filtered with suction, and washed well with cold water. The precipitate was dissolved in benzene, and anhydrous sodium sulfate was added to dehydrate the solution. The benzene solution was separated from the sodium sulfate layer, and the benzene evaporated off on the steam bath. The remaining benzene was distilled off under vacuum.

The DNFB was then fractionated by distillation in vacuo. Melting point 26-27°C. (Uncorr.). Proof of the identity of the compound was made by a nitrogen analysis, (using glucose to reduce the nitro groups), and refluxing with sodium carbonate to produce the dinitrophenol. This derivative melted at 111-114°C.; the literature value for 2,4-dinitrophenol is 114°C.

Dinitrophenyl derivatives of several amino acids were prepared in order to study the reaction, and Sanger's (34) postulated reaction was confirmed by nitrogen analyses. Sanger believes that this reaction occurs:



Sodium bicarbonate (in amount equivalent to the concentration of the HF expected to be yielded) is present in the solution,

to neutralize the HF.

Since the collagen used in this investigation was unlimed, it was of interest to see if DNFB would react with amide groups. Dinitrophenyl-asparagine was prepared, and nitrogen analyses confirmed that the amide group does react.

In the reaction of DNFB with collagen, 25 of the fibers[++] were soaked in two liters of a solution of 10 g. of sodium bicarbonate. They were then placed in a cylinder containing 600 ml. of water, and 0.76 g. of sodium bicarbonate was added, followed by the addition of a solution of 1.5 g. DNFB in 50 ml. of acetone. The reaction was given gentle stirring by a magnetic stirrer, and was allowed to proceed for three and one-half hours (when the evolution of no more carbon dioxide bubbles was observed). The fibers were then soaked in an aqueous acetone solution, then in water, and were then put in a chamber of 100 per cent relative humidity.

For the purpose of introducing a cross-link in the protein fiber, hexamethylenediisocyanate was reacted with some fibers. The material was obtained from the DuPont de Nemours and Co., and was redistilled in vacuo. The fibers were dehydrated through acetone into anhydrous ether. 0.7 g. of the redistilled

[++]The weight of 25 air-dry fibers is approximately 1.8 g. in all cases.

diisocyanate, in sufficient ether to dissolve it, was added to the cylinder containing 600 ml. of ether and the fibers. The reaction proceeded with gentle stirring (with a magnetic stirrer) for five hours. The fibers were then wet back through acetone, aqueous acetone, and finally into water, before they were carried through testing.

As will be shown later, this attempt to introduce a covalent cross-link with diisocyanate was unsuccessful, confirming the conclusions of Noerr and Hees (32). They claim that diisocyanate does not cross-link collagen.

In another attempt to cross-link the protein, mercuric acetate was used. Speakman (39,40) has claimed that "the most effective cross-linking agent for animal fibers is mercuric acetate. Amino, guanido, carboxyl and, possibly, imino groups are involved in cross-linkage formation..." The fibers to be treated with the mercuric acetate were soaked overnight in one liter of a solution 0.1M with respect to sodium acetate and 0.1M with respect to acetic acid. Then 300 ml. of a solution 0.1M with respect to sodium acetate, 0.1M with respect to acetic acid, and 0.2M with respect to mercuric acetate, was added, in 50 ml. portions, over a period of 7 hours. The fibers were then soaked in several changes of a solution 0.1M with respect to sodium acetate and 0.1M with respect to acetic acid. Following this, they were soaked in several changes of

distilled water, and then carried through the routine processes, previously mentioned, before testing.

The tannages were carried out in either of two general procedures. In order to obtain a lesser amount of combined tannin, a rocker-type apparatus was used. For tannages where it was desired to obtain a higher degree of combined tannin, a drum-type tannage was used. The fibers and tanning materials were put in a test tube, which was slowly rotated.

In the rocker-type tannage, the fibers were strung on a frame, and this frame was dipped up and down in a battery jar. In order to keep solution to protein ratio low, soaked hide powder was added to this type of tannage, in an amount sufficient to maintain approximately a 10 to 1 solution to protein ratio (the dry weight of the fibers was approximately 1.8 grams).

In chrome tannage A, 25 grams of Tanolin R were dissolved in 400 ml. of water, and added to the rocker-type tanning bath in 4 equal portions, twenty minutes apart. The rocking was continued for a total of 24 hours; the fibers were then processed for testing.

In chrome tannage B, the fibers were pickled in a solution of 5 per cent NaCl, brought to pH 2 with H_2SO_4. 10 g. of Tanolin R were dissolved in 250 ml. of water, and adjusted to pH 2.5 with H_2SO_4. A drum-type tannage was given the fibers; 50 ml. of water and 5 ml. of the tannin solution were put in

the "drum", and three more 5 ml. portions of the chrome tanning solution were added at half-hour intervals. Several hours later, in the evening, 5 ml. more of the solution were added, 5 ml. more the following morning, and 5 ml. more that evening. The following afternoon, two days after the start of the tannage, 10 ml. more were added, and the solution allowed to sit in the test tube, without drumming, for another day. The solution was neutralized to pH 3.8 with borax, and then processed for testing.

In the rocker-type vegetable tannage (quebracho), a total of 75 grams of quebracho was added, over a period of two days, to the tanning bath. This was somewhat over 100 per cent of the amount of wet protein (fibers and hide powder), but the hide powder appeared to take up most of the tannin. The tanning was carried out in 0.1M acetate buffer, adjusted to pH 4.9.

In the drum-type tannage (wattle), the fibers were soaked over-night in a solution of 5 grams of wattle in one liter of water, de-tanned with hide powder, according to the A.L.C.A. tanning analysis method. Increasing amounts of wattle solution (about 30 per cent wattle) were added till 23 ml. of the solution had been added to the original de-tanned solution, over a period of one week. The volume was kept at about 50 ml. (All wattle solutions were in 0.1M acetate buffer, at pH 5.0). The outsides of the fibers were then bleached slightly by

soaking in a one-half per cent sodium bicarbonate solution for one hour. The fibers were then drummed for five more days in a solution of about 12 per cent wattle. The pH of the tanning solution was then lowered to 4.0, and the drumming continued for four days more. The tanning was then discontinued, and the fibers carried through pre-testing processes, and then tested.

In soaking the vegetable tanned fibers prior to testing, water was not used, because of the bleeding effect. Instead, they were kept wet in a 0.5 per cent aqueous solution of the vegetable tannin. In drying the fibers prior to weighing, they were not soaked in acetone, but rather were air dried for several days prior to oven-drying them.

The rocker-type tannage was used for the formaldehyde tannage. 2500 ml. of a 0.1M solution of Na_2HPO_4 were brought to pH 8.0, and the hide powder and fibers soaked in this solution. A total of 34 grams of commercial formalin were added in 6 portions at intervals of one-half hour. The rocking was continued for a total of 24 hours.

All analytical methods not otherwise noted are those of the A.L.C.A. The analysis for chromium was carried out by a micro-modification of the Provisional A.L.C.A. method. The lipid analyses were carried out by the A.L.C.A. method, but with 5 g. samples of ground tendon, and a chloroform extraction was done rather than a petroleum ether extraction. The ash

was determined on the chloroform-extracted material.

Samples prepared for analysis of chromium, nitrogen, etc., were dried by heating in an oven at 75°C. for 18 hours in vacuum.

The statistical methods as described by Deming and Birge (16), Hoel (25) and Snedecor (38) were used in interpreting the results. Any results which came from fibers that pulled from the lead holder, rather than breaking, or broke at an obviously weak spot, or for any other known reason were obviously incorrect, were disregarded in the final computations. The arithmetic mean of the individual values was computed. The variance and standard deviation were also computed, and the F test applied to test the assumption that the variances were equal. Student's test was applied to indicate the level of significance of the assumption that chemically treated tendons were different from untreated tendons.

To demonstrate the identity of the tendons from Armour Laboratories, and from Ethicon, a number of fibers from each source were processed for testing, and broken, and the results calculated. As will be shown in the discussion of results, these fibers are from the same population. They were therefore averaged together, and the cumulative average used for statistical comparison with treated fibers.

Shrinkage temperatures were obtained with an apparatus essentially the same as the Theis Shrinkage Meter, modified

to hold fibers. Water was used as the shrinkage medium in each case.

DISCUSSION OF RESULTS

It has previously been pointed out that workers using the technique of wet tension testing on keratin fibers have placed considerable emphasis on the stretch and hysteresis characteristics of the fibers. It was attempted to use these characteristics as one criterion of changes in collagen fibers due to chemical treatment. However, collagen has such a low stretch that very little hysteresis is shown by these tendon fibers. The error of measurements of these small "hysteresis loops" is so great that it was deemed inadvisable to base conclusions on whatever small changes might be introduced by chemical treatment.

To demonstrate the identity of the tendons from the two sources (Armour and Ethicon), the mean breaking length of randomly selected fibers from each source were compared (see Table I). The two groups of Armour fibers each gave the same mean breaking length, 24.4 km. The Ethicon fibers gave a mean breaking length of 25.3 km. Statistically, these must be from the same population (by use of Student's "t" test). Therefore, all data for untanned fibers were averaged together, giving a mean of 24.7 km. The comparisons of the mean breaking lengths of chemically treated fibers were made with this figure (24.7 km.). The cumulative standard deviation of the

untanned fibers was calculated from the following formula:

$$s^2_{cum} = \frac{\text{sum of squares of deviations}}{\text{total number of samples - number of groups}}$$

$$= \frac{162.94 + 251.71 + 160.64}{18 + 20 + 20 - 3}$$

$$= \frac{575.29}{55} = 10.46$$

where $s^2_{cum.}$ is the cumulative variance (the square of the cumulative standard deviation).

TABLE I

COMPARISON OF TENDON FIBERS FROM ARMOUR AND ETHICON

Fiber Group	n	MBL	$\Sigma\delta^2$	s^2	s
Armour A	18	24.4	162.94	9.58	3.1
Armour B	20	24.4	251.71	13.25	3.6
Ethicon	20	25.3	160.64	8.47	2.9
Cumulative	58	24.7	575.29	10.47	3.2

variance = $s^2 = \frac{\Sigma\delta^2}{n - 1}$ δ = deviation, and $\Sigma\delta^2$ = sum of squares of deviations.

standard deviation = s = square root of variance.

MBL = Mean Breaking Length, in kilometers.

TABLE II

LIPID AND ASH ANALYSIS OF SALT-EXTRACTED ARMOUR AND ETHICON FIBERS

Fiber type	Per Cent Chloroform Extract	Per Cent Ash
Armour	0.29 0.30	0.10 0.13
Ethicon	0.17 0.17	0.04 0.04

The data for the individual tensile strengths are given in a series of tables in the Appendix. The summation of the date on tensile strengths, and on hide substance (per cent nitrogen X 5.62), and for shrinkage temperature are given in Table III.

The F test, which tests the assumption that the variances are equal, was not satisfied in the case of the formaldehyde and wattle treated fibers. From this, it can definitely be stated that these fibers are from different populations. But the difference might be due to either the difference in variance, or to the difference in the mean. Intuitively, it would not seem possible to ascribe the dissimilarity between the wattle tanned and the untanned fibers as due to the difference in the means, inasmuch as they differ by only one kilometer. The difference would therefore seem to be due to the difference in variances, and no significance could be

TABLE III

SUMMARY OF TENSION TESTS, CHEMICAL TESTS, AND STATISTICAL DATA

	number of samples	mean breaking length, km.	sum of squares of deviations	variance	standard deviation	F value, calculated	F value, for .95 level	t value, calculated	t value, for .99 level	level of significance, per cent	per cent hide substance	shrinkage temperature
Cumulative untanned	58	24.7	575.29	10.47	3.2	----	----	----	----	----	98.5	62
Rocker-type chrome[+]	22	19.8	139.40	6.64	2.6	1.58	1.93	6.46	2.33	>99.5	93.6	84
Drum-type chrome[++]	21	22.0	346.50	17.32	4.2	1.65	1.93	3.06	2.33	>99.5	81.5	>100
Quebracho	22	19.6	201.25	9.58	3.1	1.09	1.96	6.45	2.33	>99.5	90.7	70
Wattle	11	23.7	470.95	47.09	6.9	4.50	2.00	----	----	----	77	73
Formaldehyde	22	7.8	34.88	1.66	1.3	6.31	1.96	----	----	----	97.8	79
DNFB	18	22.8	100.36	5.90	2.4	1.78	2.08	2.32	2.33	99	97.0	62
Diisocyanate	18	23.2	284.83	16.75	4.1	1.60	1.81	1.63	2.33	95	97.0	62
Mercuric acetate	15	26.9	135.44	9.67	3.1	1.08	2.18	2.40	2.33	99	91.7	73

[+]1.8 per cent Cr_2O_3

[++]7.6 per cent Cr_2O_3

$$F = \frac{\text{larger variance}}{\text{smaller variance}}$$

$$t = X_1 - X_2 \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{(n_1 + n_2)\, SX^2}}$$

X_1 = mean of sample 1
X_2 = mean of sample 2
n_1 = number of samples of 1
n_2 = number of samples of 2
SX^2 = sum of squared deviations of 1 and 2

attached to the assumption that wattle tannage decreases the strength of collagen fibers appreciably. However, there is no increase in wet strength due to wattle tannage.

In the case of the formaldehyde treated fibers, however, the difference in means (between the tanned and untanned fibers) was so great that it can be stated intuitively that the difference is highly significant. Further, if the coefficients of variation, i.e. the ratio of variance to arithmetic mean, are compared, the F test is satisfied, and the "t" test shows a high degree of significance to the assumption that the means are different. Since the variance would reasonably be expected to be a function of the mean, it seems fair to use this coefficient of variation in such cases.

In the case of the drum-type chrome tannage (7.6 per cent Cr_2O_3), with a mean breaking length of 22.0 km., while the result is shown to be highly significant, the increase in mean breaking length over the rocker-type tannage (1.8 per cent Cr_2O_3) (19.8 km.) is difficult to explain. Comparing Compton's (14) data on air-dry strength with these data, it would seem that the chrome tannage with the high chrome level is more hydrophobic than the other chrome tannage and the untanned fibers. This would mean that less swelling would occur, and therefore that dry testing is being approached. If this argument is correct, it would be likely, therefore, that the true wet strength of the 7.6 per cent chrome tannage would be

less than the reported figure.

This same argument would seem applicable to the comparison of the two vegetable tannages -- wattle and quebracho. There is a complicating factor, here, in that there are two different tanning materials, and information is not available on which to assess the difference in strengths that might be attributed to such a difference in tanning materials.

In the case of all tannages, there is a decrease in strength due to tannage. The formaldehyde treatment gives the lowest wet strength, which is similar to Compton's (14) data on dry strength. The extent of this decrease is difficult to explain on the basis of side-chain inactivation as suggested by Highberger (24). The decrease might possibly be explained, however, if reaction at peptide bonds, with resultant breaking of peptide hydrogen bonds, occurred.

The mean breaking lengths shown are not corrected for hide substance decrease due to dilution caused by treatment. Were this to be done, it would show some increase in wet strength over the untanned fibers in the case of the drum-type chrome (i.e. 7.6 per cent Cr_2O_3) and wattle tannages. It has been previously argued that these figures are unduly high due to the increase in hydrophobicity; therefore such an increase in hide-substance-corrected values would be an artifact.

Without regard to the effect of this correction in the

case of these two tannages, the reason for not making this correction is more fundamental. The mean breaking lengths reported are those for the system studied, and the correction would give an artificial estimate of the strength of the system.

To restate the hypothesis on which this investigation is based: If there is an increase in the number of inter-chain linkages (hydrogen bonds and/or covalent linkages) due to chemical treatment, the wet strength of the treated fibers will be greater than the untreated fibers. If there is a decrease in these linkages, the wet strength of the treated fibers will be lesser than the untreated fibers.

On the basis of this hypothesis, it can be stated that the results shown in Table III, for chrome, vegetable, and formaldehyde tannages, indicate that there is a decrease in inter-chain linkages. These data and the conclusions therefrom are inconsistent with the covalent cross-linkage hypothesis of tannage. The method presents a more clear-cut method for interpreting the changes in forces due to tannage than has heretofore been possible. The results of this investigation require, therefore, that the cross-linkage hypothesis of tannage be disregarded, or that it be re-examined and re-interpreted. It is to be noted, however, that these results are consistent with the mechanism of tannage advanced by Tu (43).

In the strictly chemical treatments, i.e. the side-chain reaction and the cross-linking reaction, it is evident from the data that hexamethylenediisocyanate does not cross-link, because the wet strength of these fibers is not greater than the untanned fibers. As previously mentioned, this confirms the statement by Noerr and Mees (32) that cross-linkage of collagen does not occur due to the action of diisocyanate.

As shown in Table III, mercuric acetate caused an increase in the wet strength of the collagen fibers. Therefore, it must have cross-bridged the fibers. In further work, it was demonstrated qualitatively that more cross-linking by mercuric acetate could be obtained, and therefore a greater increase could be obtained. This would result in fibers stronger than the tensile tester could break, and for that reason no further data on mercuric acetate treatment can be reported.

The side-chain reaction, with DNFB, gives a slight decrease in the wet strength of the fibers. The fibers were soft and supple, and it is not likely that they represented a hydrophobic protein. The explanation of this slight decrease may lie in the possibility that the effect of salt bridges is not completely lost in wet testing, but this does not seem likely. DNFB does not react with the peptide link, and thus could not break hydrogen bonds directly. It is possible, however, that this bulky side-chain might lessen

close-packing of the chains, and therefore break a few hydrogen bonds. The significant factor, however, probably lies not in the reasons why DNFB gave a slight decrease to the fibers' wet strength, but rather to the fact that no increase in wet strength was shown.

SUMMARY

1. A review is presented on the theoretical background for a technique allowing interpretation of changes in the stronger forces between protein fibers due to chemical treatment.

2. A technique is described for measuring the mean breaking lengths (tensile strength) of collagen fiber aggregates.

3. A method for the preparation of 2,4-dinitrofluorobenzene is described.

4. Data are presented to show that tannage decreases the wet strength of collagen fiber aggregates. Interpretation of the data shows that tannage does not introduce covalent cross-linkages into the fiber. Thus, the cross-linking theory of tannage must be re-examined or discarded to be consistent with these data and their interpretations.

5. It is shown that a side-chain reaction does not increase the wet strength of these fibers. Mercuric acetate is demonstrated to introduce cross-linkages into the fiber.

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APPENDIX

TABLE IV

UN-TANNED ARMOUR FIBERS

Group A

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
13.0	.0440	15.7	21.3
13.0	.0455	18.2	23.6
13.0	.0431	17.0	23.3
13.6	.0661	22.0	20.5
13.3	.0454	19.7	26.1
13.5	.0320	14.5	27.7
13.0	.0354	16.1	26.8
13.8	.0488	22.5	28.8
13.7	.0446	18.6	25.9
13.8	.0250	11.0	27.6
12.3	.0168	8.6	28.6
8.7	.0350	21.5	24.3
12.4	.0506	21.2	23.6
13.4	.0533	17.5	19.9
13.4	.0410	13.0	19.6
12.7	.0371	14.7	22.9
13.1	.0507	22.4	26.3
13.1	.0393	15.8	23.9

Number of samples - 18
Arithmetic mean - 24.4
Variance - 9.58
Standard deviation - 3.1

APPENDIX

TABLE V

UN-TANNED ARMOUR FIBERS

Group B

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
13.5	.0361	15.2	25.8
11.6	.0388	19.2	26.1
12.1	.0424	15.3	19.8
13.0	.0353	12.4	20.7
12.3	.0462	21.6	26.1
12.4	.0442	16.7	21.3
13.1	.0571	19.8	20.6
13.6	.0311	13.0	25.8
12.7	.0297	13.5	26.2
12.3	.0515	22.4	24.3
12.8	.0540	19.2	20.6
12.3	.0411	18.7	25.4
13.0	.0371	13.4	21.3
13.2	.0431	15.5	21.6
12.5	.0501	22.5	25.4
12.4	.0439	23.5	30.2
13.4	.0264	14.0	32.2
12.8	.0382	18.2	27.7
12.9	.0466	22.5	28.1
13.1	.0437	14.0	19.1

Number of samples - 20
Arithmetic mean - 24.4
Variance - 13.25
Standard deviation - 3.6

APPENDIX

TABLE VI

UN-TANNED ETHICON FIBERS

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
12.5	.0466	17.2	20.9
11.9	.0407	20.6	27.3
12.1	.0459	22.6	27.0
11.5	.0404	17.2	22.2
12.2	.0447	23.6	29.2
12.3	.0363	15.1	23.2
12.1	.0467	24.5	28.8
12.2	.0264	11.5	31.4
11.9	.0505	24.0	25.7
11.8	.0294	12.5	22.7
11.9	.0497	23.4	25.4
11.0	.0388	17.9	26.4
12.6	.0433	18.4	24.3
11.7	.0595	25.0	22.3
12.3	.0340	15.7	25.8
12.4	.0272	12.2	25.2
12.1	.0569	23.0	22.2
11.3	.0442	22.5	26.1
9.5	.0269	17.8	28.5
9.0	.0358	18.6	21.2

Number of samples - 20
Arithmetic mean - 25.3
Variance - 8.47
Standard deviation - 2.9

APPENDIX

TABLE VII

CHROME TANNED ARMOUR FIBERS

Rocker-Type Tannage

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
13.4	.0443	13.4	18.4
12.4	.0312	11.8	21.3
12.2	.0535	20.2	20.9
12.5	.0582	20.4	19.9
13.4	.0491	12.7	15.7
12.8	.0449	17.7	22.9
12.8	.0604	18.2	17.5
12.7	.0610	23.8	22.5
12.4	.0622	23.2	21.0
13.0	.0501	15.0	17.7
12.7	.0495	15.7	14.8
12.8	.0380	14.0	21.4
12.2	.0371	14.5	21.7
12.9	.0430	12.8	17.6
13.6	.0552	16.2	18.1
12.6	.0379	15.0	22.6
13.3	.0497	15.6	19.0
13.0	.0528	17.1	19.1
12.6	.0549	17.5	18.2
12.3	.0526	21.1	22.2
12.6	.0484	14.9	17.6
12.4	.0480	21.4	25.1

Number of samples - 22
Arithmetic mean - 19.8
Variance - 6.64
Standard deviation - 2.6

APPENDIX

TABLE VIII

CHROME TANNED ARMOUR FIBERS

Drum-Type Tannage

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
13.5	.0485	16.5	20.8
13.4	.0616	23.0	22.7
11.7	.0610	14.0	12.2
13.2	.0676	23.3	20.7
13.1	.0685	23.8	20.6
12.9	.0683	20.0	17.1
12.1	.0638	24.5	21.1
12.7	.0491	16.4	19.3
13.5	.0560	17.0	18.6
12.2	.0361	19.5	29.9
13.4	.0374	15.6	25.4
12.4	.0499	19.8	22.3
12.6	.0539	22.5	23.9
13.3	.0483	21.0	26.2
14.0	.0444	20.0	28.6
13.5	.0700	23.1	20.2
13.2	.0583	16.9	17.3
11.5	.0273	12.9	24.6
12.6	.0472	22.2	26.9
12.3	.0478	20.4	23.8
13.2	.0560	18.9	20.2

Number of samples - 21
Arithmetic mean - 22.0
Variance - 17.32
Standard deviation - 4.2

APPENDIX

TABLE IX

QUEBRACHO TANNED ARMOUR FIBERS

Rocker-Type Tannage

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
13.1	.0370	11.8	19.0
13.6	.0472	12.1	15.9
12.2	.0490	20.2	22.8
12.2	.0332	13.7	22.8
13.2	.0465	12.2	15.7
13.5	.0663	18.7	17.3
13.4	.0655	22.5	20.9
12.8	.0553	21.5	22.6
13.5	.0638	21.8	20.9
12.7	.0570	19.1	19.3
12.8	.0633	17.0	15.0
12.8	.0498	19.6	22.8
12.9	.0530	18.2	20.1
13.1	.0505	19.0	22.4
13.3	.0406	14.5	21.6
13.0	.0500	15.0	17.7
13.1	.0516	22.8	26.3
13.2	.0438	8.3	11.7
12.6	.0534	19.5	20.9
12.3	.0553	19.8	20.0
12.6	.0556	18.5	19.1
12.5	.0411	12.6	17.4

Number of samples - 22
Arithmetic mean - 19.6
Variance - 9.58
Standard deviation - 3.1

APPENDIX

TABLE X

WATTLE TANNED ETHICON FIBERS

Drum-Type Tannage

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
11.2	.0388	24.2	31.7
12.8	.0532	13.5	14.7
11.9	.0441	18.7	22.9
10.7	.0541	24.5	22.0
12.6	.0340	16.1	27.1
12.1	.0379	22.3	32.1
12.1	.0612	18.2	16.3
13.0	.0439	19.2	25.8
12.9	.0438	25.0	33.4
13.3	.0636	19.9	18.9
12.5	.0468	13.0	15.7

Number of samples - 11
Arithmetic mean - 23.7
Variance - 47.09
Standard deviation - 6.9

APPENDIX

TABLE XI

FORMALDEHYDE TANNED ARMOUR FIBERS

Rocker-Type Tannage

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
13.0	.0414	4.6	6.6
12.5	.0425	5.9	7.9
12.3	.0374	6.2	9.3
12.0	.0426	5.0	6.4
12.5	.0347	4.9	8.1
12.3	.0445	4.9	6.2
11.6	.0442	7.8	9.3
13.0	.0512	7.5	8.7
12.7	.0305	5.8	11.0
11.8	.0509	8.2	8.7
12.4	.0448	5.3	6.7
12.4	.0579	7.4	7.2
12.7	.0424	4.5	6.2
12.9	.0555	8.4	8.9
12.0	.0545	9.0	9.0
12.4	.0602	6.9	6.5
12.3	.0351	4.4	7.1
13.0	.0630	7.5	7.0
12.9	.0504	6.3	7.3
12.7	.0491	7.8	9.2
12.7	.0396	4.8	7.0
12.0	.0486	6.9	7.7

Number of samples - 22
Arithmetic mean - 7.8
Variance - 1.66
Standard deviation - 1.3

APPENDIX

TABLE XII

2,4-DINITROFLUOROBENZENE-TREATED ARMOUR FIBERS

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
11.8	.0448	18.4	21.9
13.3	.0370	12.4	20.2
12.3	.0352	12.7	20.2
13.1	.0520	22.4	25.6
12.4	.0504	20.3	22.7
12.4	.0500	21.0	23.6
12.6	.0487	19.3	22.7
12.9	.0454	19.5	25.1
12.6	.0427	16.0	21.4
11.8	.0397	18.5	25.0
12.4	.0557	15.8	16.0
12.9	.0431	18.5	25.2
12.8	.0580	21.0	21.0
12.6	.0313	13.5	24.7
13.9	.0631	24.1	24.1
12.9	.0342	14.8	25.3
13.8	.0276	9.5	21.5
13.0	.0572	23.2	24.0

Number of samples - 18
Arithmetic mean - 22.8
Variance - 5.90
Standard deviation - 2.4

APPENDIX

TABLE XIII

HEXAMETHYLENEDIISOCYANATE-TREATED ARMOUR FIBERS

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
12.4	.0380	12.4	18.4
12.7	.0466	19.6	24.2
13.1	.0480	18.0	22.3
12.8	.0492	22.7	26.8
12.1	.0477	20.0	23.0
12.0	.0474	23.2	26.7
11.8	.0285	15.2	28.6
13.2	.0511	24.2	28.4
12.4	.0587	22.8	21.9
12.5	.0596	15.7	15.0
12.4	.0410	16.0	22.0
12.1	.0404	16.4	22.3
13.4	.0644	17.3	16.3
12.1	.0308	15.0	26.8
18.6	.0449	16.8	21.4
12.7	.0420	15.2	20.9
12.2	.0321	16.9	29.2
12.8	.0588	25.0	24.7

Number of samples - 18
Arithmetic mean - 23.2
Variance - 16.75
Standard Deviation - 4.1

APPENDIX

TABLE XIV

MERCURIC ACETATE TREATED ETHICON FIBERS

Length, in cm.	Weight, in gms.	Breaking Load, lbs.	Mean Breaking Length, Km.
13.0	.0530	21.4	23.8
12.4	.0375	20.8	31.2
12.8	.0542	22.5	24.1
12.5	.0276	14.0	28.8
12.7	.0470	19.0	23.3
12.6	.0491	23.5	27.4
12.5	.0342	16.8	27.9
12.0	.0294	14.0	25.9
12.6	.0384	14.8	22.0
12.2	.0503	20.2	22.3
11.6	.0394	22.4	30.0
12.9	.0440	22.1	29.4
12.0	.0443	24.2	29.8
11.8	.0402	20.5	27.4
12.7	.0465	24.5	30.4

Number of samples - 15
Arithmetic mean - 26.9
Variance - 9.67
Standard deviation - 3.1