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3,483,286 METHOD FOR TANNING ABSORBABLE SURGICAL SUTURES

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5 Claims

ABSTRACT OF THE DISCLOSURE

Regenerated collagen sutures of unusual strength and surgically desirable characteristics are obtained by cleaning young beef tendons, grinding while frozen, treating with trypsin, washing in water, treating with the sodium salt of ethylenediamine tetracetic acid, washing with water, dispersing the collagen to form an aqueous gel, which may contain some ethanol, with hydrochloric acid, at a pH of about 2.3 to 3.3 to form a spinnable gel of about 0.86% solids, extruding into a dehydrating, coagulating ammoniacal solvent-water bath, pressure rolling to expel the solvent-water, and at least partially drying. The solvent may be ethanol or acetone. The ribbon is treated with pyrogallol, or other polyhydric phenol, the pyrogallol treated with hexavalent chromium, then the chromium is reduced further with a bisulfite, to the trivalent state, and the double tanned ribbon twisted and stretched and dried to form the suture. The unusual strength developed is protected by storing in a 3-4% water in isopropanol tubing fluid until ready for use. The double tan gives not only a high initial strength, but causes the suture, when used, to retain an adequate proportion of its initial strength for at least 15 days in muscular tissue. The smaller sizes hold until healing occurs in corneal tissue in eye operations.

BACKGROUND OF THE INVENTION

This invention relates to producing collagen sutures by regenerating collagen in a continuous extrusion process. In the past absorbable sutures have been produced from certain layers of the intestines, or gut, of sheep or beef or other animals. The process has been a comparatively long and expensive one because the layers are thin, they must be cleaned, they must be slit and even at best the non-uniformity of the animals used introduces a wider than desirable variation in the characteristics of the sutures produced.

For maximum strength, it has been well recognized that all materials other than the collagen itself are preferably removed and the suture when made must be stored under conditions which are adapted to the particular suture in question.

DESCRIPTION OF THE PRIOR ART

Patents and other literature show that many attempts have been made to prepare a regenerated collagen suture which is acceptable. The requirements are extremely rigorous in that a suture of the absorbable type (1) must be sterile when it is introduced into the body, (2) must be absorbable by body tissue in a reasonable length of time, preferably less than 90 days, (3) must retain strength for at least about 15 days, and (4) must not cause undesirable foreign body reactions in the tissue.

Desirably, the suture is produced at a minimum cost, is easily handled by the surgeon using the suture, and possesses many qualities best classified as surgically acceptable handling characteristics. These include such properties as the stiffness of the suture; run-down, that

is how easily it is frayed when a knot is run-down as by crossing two strands of the suture over themselves to form half of a square knot and running half a square knot down to the surface, on which the suture is to be emplaced; throwability, that is a characteristic of the suture being sufficiently pliable so that it may be placed by the surgeon and remain in place without being wiry; flexibility, so that knots may be tied; knot strength; and straight pull strength. Sutures are listed in the Pharmacopeia's of many countries and some of these qualities, particularly diameter and minimum tensile strength are specified and must be met if the sutures are to be marketed in these countries.

Even though a large number of attempts have been made to form the sutures from regenerated collagen or by other process, the vast majority of absorbable sutures in use today are still made from the gut of beef or sheep.

Certain processing steps are well recognized, for example, a patent to Sharp, 1,999,641, Apr. 30, 1935, Strand for Suture and Other Purposes and Method of Making the Same, discloses a suture strand of substantially pure collagen fibers and particularly on page 2, column 1, lines 21 to 25, teaches the use of enzymes, including specifically trypsin, to dissolve out impurities by selective digestive action from the shredded tendon of young animals. A method of spinning an acidified collagen solution or suspension is disclosed in Cresswell, 2,637,321, May 5, 1953, Shaped Article and Method of Producing It. A patent to Salo et al., 2,598,608, May 27, 1952, Preparation of Collagenous Materials, discloses solubilizing tendon slices in aqueous acetic acid with filtration to remove impurities and precipitating the collagen from a sol at about 0.1% collagen. The collagen is washed and fluffed and then redissolved to form a gel, using malonic acid, and spinning into an acetone dehydrating bath.

A Canadian Patent to Yen, Vitucci and Valentine, 733,021, Apr. 26, 1966, Collagen Sutures, with a U.S. priority date of Feb. 17, 1958 describes preparing regenerated collagen sutures by extrusion of 0.5 to 1.5% solids aqueous hydrochloric acid collagen gel, prepared from ground cattle tendons, spun into an ammoniacal ethanol dehydrating bath to form a filament which is compressed into a flat ribbon which is dried, then tanned and twisted and stretched to form a suture. A similar process is described in U.S. Patent 3,098,696, Ball and Vitucci, July 23, 1963, Manufacture of Sterile Surgical Suture Collagen. These last two patents disclose much of the detail of the preparation of the collagen gel and its spinning used in the present process and are incorporated herein by reference. Said U.S. Patent 3,098,696 discloses the use of β -propiolactone as a sterilizing agent in the manufacture of sutures. β -propiolactone is one of the materials which may be used to sterilize the sutures of the present invention. As β -propiolactone is a lachrymator, other sterilizing processes are often more convenient.

A variety of tanning methods have been disclosed, including some such as 3,189,401, Griset, June 15, 1965, Simultaneous Aldehyde, Chrome and Aromatic Alcohol or Quinone Tannage of Spun Collagen Fiber.

A group of United States patents to Arthur Cresswell show various aspects of collagen spinning, including:

- 2,475,129, July 5, 1949, Method of Spinning Collagen Solutions;
- 2,475,697, July 12, 1949, Treatment of Collagen Strands;
- 2,485,957, Oct. 25, 1949, Apparatus for Treating Strands;
- 2,485,958, Oct. 25, 1949, Method of Spinning Collagen Filaments;

2,515,697, July 18, 1950, Treatment of Spinnerettes;
2,576,576, Nov. 27, 1951, Lubricated Thread;
2,637,321, May 5, 1953, Shaped Articles and Method
of Producing It; and

2,648,496, Aug. 11, 1953, Centrifugal Apparatus for
Separating Gases From Liquids.

U.S. Patent 3,036,341, A. S. Taylor, Centrifugal Casting of Collagen To Produce Films and Ribbons, and 3,285,903, Taylor, Collagen Film, describe casting of collagen gel to form a film, which may be twisted to form a suture.

With small sizes of sutures, the extrusion rate may be so small that a single gear pump tends to give a pulsating flow. One pump supplying a manifold, with a uniform pressure drop to each nozzle, can feed several nozzles. Such spinning equipment for collagen is described in Patent 2,220,226, Freudenberg et al., Nov. 5, 1940, Apparatus for the Manufacture of Threads, Bands, Films, Flexible Tubes, and the Like.

Not only is there an interaction between the tanning procedure and the method of preparing the regenerated collagen, but there is an interaction with the tubing fluids in which sutures are stored until time for use. U.S. Patent 2,524,772, Davis and Tringali, Oct. 10, 1950, Sutures, shows a tubing fluid for non-boilable absorbable sutures having a minimum of 70% of a propyl alcohol and at least 5% water. This patent teaches that the use of at least 5% water reduces the swelling while giving adequate pliability. This patent is working primarily with catgut sutures. Another patent, 2,519,404, Rynkiewicz, Aug. 22, 1950, Sutures, shows a boilable suture with a limit of 2% water in propyl or isopropyl alcohol.

There is a sharp distinction between boilable and non-boilable sutures. The boilable catgut sutures are those which have so little water in them that the sutures may be sterilized in boiling water, or more usually the glass tube in which the suture is stored may be sterilized in boiling water without damaging the suture. This can only be accomplished if the suture is comparatively dry; and such boilable sutures are so wiry that it is necessary to remoisten the suture, as for example, by immersion in sterile water or physiological saline for brief periods, usually a minute or less, the longest times being required for sutures of the largest diameter. After immersion, if not immediately used, the sutures may be stored between damp towels to maintain flexibility. Too long immersion will weaken the suture and soaking therefore creates some risk if not properly controlled. For this reason, non-boilable catgut sutures in which sufficient water or alcohol is incorporated into the suture from the storage fluid (usually called tubing fluid) to impart suitable flexibility were developed, and are preferred. Coincidentally, if such a suture is heated to boiling the collagen degenerates so that the suture becomes too weak to be useful.

Another disadvantage of moisture being present is that if too much water is present the suture swells, the maximum strength for a particular suture is obtained with a tubing fluid containing more water than that for the maximum strength per unit of cross-section. This occurs because as the suture swells it gains somewhat in strength, but the cross section goes up more rapidly than the strength so that the strength expressed in pounds per square inch may be dropping, even though the strength of the particular strand is rising. Inasmuch as the sutures are classed narrowly in size ranges, such a swollen suture may go over into the next size so that in effect the maximum strength for a particular size of suture necessarily occurs at a smaller water content.

SUMMARY OF THE INVENTION

A wide variety of collagenous raw materials are suitable for the process of this invention. Mammalian tendon, such as pork, sheep, and beef tendon, are satisfactory sources of collagen. The preferred collagen raw material is tendon from the hooves of 2 to 3 year old beef cattle.

The Y-shaped tendon is conveniently frozen for storage and shipment from the abattoir, whereupon it is thawed, cleaned of extraneous matter such as the outer sheath, fat, and connective tissues; then cut up into approximately ¾ inch pieces and refrozen. These refrozen pieces are then granulated to produce a shredded collagenous material. Solid carbon dioxide is added during the shredding to keep the tendon frozen.

The shredded collagen is then thawed and subjected to a pretreatment to remove elastin which binds the collagen fibers, and also fats, lipids, non-collagenous protein and other extraneous matter. The elastin and other non-collagen protein is removed by treating the shredded collagen with an alkaline aqueous solution of a proteolytic enzyme such as trypsin, while other extraneous matter is removed by a subsequent bath of an aqueous solution of the sodium salt of ethylenediamine tetracetic acid.

The thus pretreated collagen consists of substantially pure collagen which is swollen and dispersed to form a gel. A pH of 2.5 to 4.5 using an acid such as hydrochloric or acetic is used as aqueous, alcoholic or aqueous-alcoholic swelling solutions. The preferred swelling is in an acidified aqueous-alkanol solution, more specifically an aqueous ethanolic solution containing sufficient hydrochloric acid to maintain the pH of the gel at about 2.5 and up to about 50% ethanol. The incorporation of the alcohol in the gel reduces the amount of water which must subsequently be removed, and thereby reduces the consumption of the solvent later in the process where a solvent such as alcohol or acetone is employed as a dehydrating agent. Furthermore, while increasing solids contents in aqueous gels produce an exponential rise in viscosity, it has been found that if there is an alcohol content in the gel, the viscosity increase occasioned by the increasing solids content is less, thereby permitting gels with higher solids contents to be conveniently handled.

The solids content of the gel can vary from about 0.5% to 2%. The preferred solids content is about 0.86%. After the gel is initially formed, it is passed through a colloid mill at a temperature below 25° C. several times until a uniform gel is produced. The gel is then filtered and de-aerated prior to extrusion. The temperature is kept below 25° C., and preferably about 0° C. to 10° C. to avoid denaturation at all times.

The gel is conveniently forced through a series of nozzles fed from a manifold, as disclosed in Freudenberg 2,220,226. The inner diameter of the nozzles controls the size noodle extruded. Larger diameter nozzles are required for preparing noodles for 000 sutures than for 6/0 sutures. The gel is extruded at room temperature under pressures of 20-40 p.s.i.g. (pounds per square inch, gage) onto a conveyor belt which is immersed in an ammoniacal aqueous solvent solution. The solvent may be acetone or a lower alcohol. About 80% ethanol is preferred. The belt speed is adjusted in accordance with the extrusion rate to provide approximately a 30 to 40% stretch. The ammoniacal aqueous ethanol as a dehydrating bath removes a large portion of the water in the extruded strand, while the ammonia neutralizes the acid of the gel. As the neutralization proceeds, the extruded noodle is regenerated into an opaque solid strand. The residence time in the bath is set to effect complete regeneration of the strand before it exits the bath. This time will vary depending on the size of the strands, the ammonia concentration in the bath, etc. 4 minutes to 30 seconds usually gives good results.

The regenerated strand is then passed through a series of unheated pressure rolls wherein the strand is flattened to a ribbon. Much of the solvent-water content is expressed. The ribbon is passed over a series of heated rolls which reduce the moisture content to about 10%. The ribbon may be either collected on a storage drum for later processing or processed directly.

If a chromacized suture is to be produced, the ribbon is next passed through a room temperature aqueous solu-

tion of a polyhydric phenol of not more than four rings, a pyrogallol or logwood solution is preferred. Depending on the degree of tanning desired, the concentration ranges from 0.2–0.8%. A 0.5% aqueous solution of pyrogallol is preferred. The ribbon is thereupon passed through a room temperature aqueous ammonium dichromate bath. Other soluble dichromates may be used. Again, depending on the degree of tanning required, the dichromate concentration as Cr_2O_3 may range from 1–4%, with a preferred concentration of 3%. The ribbon is then passed through a room temperature bath of an aqueous solution of about 20% sodium bisulfite to insure complete reduction of the hexavalent chromium. A water wash follows.

If non-chromacized suture is to be produced, the ribbon is passed through the pyrogallol or logwood bath and then into an aqueous ammonium sulfate solution to prevent swelling of the ribbon. The ammonium sulfate concentration may vary from 0.2–0.8% with a preferred concentration of 0.5%. The strand is then directly twisted followed by a water wash.

The pyrogallol and/or logwood treatments are considered as a first tanning treatment. This first tanning treatment is very important since ordinarily when a regenerated collagen strand contacts water it swells and elongates, thereby destroying the molecular orientation which had been previously imparted to the strand and from which it derives its strength. The decreased strength makes further processing difficult. Furthermore, the elongation will produce sags in the strand creating additional processing problems. The pyrogallol or logwood treatment is sufficient to minimize the swelling and elongation so that the orientation is not lost to any significant degree, thereby permitting ready processing of the strand.

The pyrogallol or logwood treatment also slows down the rate of digestion of the suture in tissue. Plain regenerated collagen sutures absorb in the body more rapidly than plain catgut sutures and for most surgical uses require treatment to slow down this absorption. For chromacized sutures, after the first tanning in a polyhydric phenol such as the pyrogallol or logwood, the ribbons are treated in a dichromate solution, which acts as a treating agent to fix the said phenol in position. Probably at least a partial oxidation occurs, changing the phenol to an insoluble form, of complex and unknown structure. Some of the chromium is partially reduced from the hexavalent state to the trivalent state, and this trivalent chromium bonds with the oxidized polyhydric phenol and with the collagen as well, giving a synergistic tanning action. Since the said phenol in its unoxidized form is dispersed uniformly throughout the cross-section of the collagen ribbon, the tanning action is uniform. The chrome containing ribbon is then passed to the bisulfite bath which reduces the chromium not previously reduced in situ. At this time all of the chromium is present in the trivalent state. Because the chromium is evenly distributed before reduction, the penetration is even throughout the cross-section of the ribbon. Hexavalent chromium has little or no tanning action, but the trivalent chromium rapidly reacts with and tans the collagen. Because the chromium and said phenol are both evenly distributed through the ribbon, the suture formed therefrom is evenly absorbed in body tissue. On examining the suture under a microscope, the color and characteristics are uniform throughout the cross-section. If the suture is tanned with trivalent chromium as such, the outer surfaces contact and fix chrome more rapidly, and are more tanned, so the absorption characteristics vary throughout the cross-section, which gives an uneven absorption rate, and erratic strength results in living tissue. A microscopic inspection of the suture shows the uneven tanning by variation in coloration.

For even penetration, the chromium must be added in the hexavalent form, but for tanning it must be present in the trivalent form. The combination of pyrogallol or other polyhydric phenol and the trivalent chrome tanning

gives desired absorption and characteristics in living tissue. If chrome tanning alone is used, either the suture absorbs undesirably fast, or the suture becomes brittle from too much tanning, and has poor knot strength, poor rundown, and fraying. If the chrome has such concentration as to give the preferred handling, the tissue strength after 7 or 15 days is undesirably low.

Following the tanning, the ribbons are fed to a twisting machine, where, depending on the size of the suture being prepared, a twisting action occurs which preferably is such as to give maximum density and strength to the suture. For example, the twist preferred is 7.3 turns per inch for a 000 suture, and 19.7 turns per inch for a size 6/0 suture and 23.7 turns per inch with a 7/0 suture. Other suture sizes, or non-standard sizes, or sizes in other size classifications get analogous turns per unit of length. Either too many or too few turns per inch give lower density, and lower strength per unit of cross-section.

A small amount of stretching (2–4%) occurs during the twisting. The twisted strands are given a final stretch of about 5% to 6% at 75° F. and 65% relative humidity. Following the stretching, the suture is dried under the same conditions, and stored for subsequent processing. Conveniently, the sutures are wound on a plastic drum of about 20 inches diameter, with convolutions not touching for the drying step, and after the drying, kept on reels or cut to desired lengths for needling, winding, packaging, sterilizing, etc.

The regenerated collagen sutures are packaged in accordance with conventional practice, preferably in a plastic envelope containing a tubing fluid which gives a preferred water content and flexibility to the final suture, and protects the suture from contamination from manufacture to use.

A proper tubing fluid is an important aspect of this invention, as not only must the suture be formed correctly, but it must be stored correctly in order for the suture to be surgically acceptable at time of use. The tubing fluid found preferable for the regenerated sutures, particularly in the smaller sizes, is uniquely useful with the regenerated collagen sutures. An isopropyl alcohol-water tubing fluid with about 3 to about 4% water gives optimum knot and tensile strengths, as well as a minimal swelling. If the water content is above this range, the suture swells somewhat, so that either the suture is made smaller, or it swells to the next size bracket. The maximum strength expressed in pounds per square inch measured at the time of test peaks at a lower water content than does the strength of a particular suture, as the strength increases somewhat as the suture swells, but not as fast as does the cross-section area. Beyond a water content of around 6% even the strength of a suture, independent of the degree of swelling begins to diminish.

A somewhat larger water content may be used with larger sutures, as the small size of 6/0 and 7/0 permits sufficient flexibility even though the water content is lower than would be desired for larger sutures.

The sutures may be sterilized by several processes. The completed sutures, in final packages may be readily sterilized with Gamma radiation, from a cobalt-60 source, such as described in Canadian Patent 699,145, Yarrow and Cresswell, Sterilization of Sutures. The sutures may be sterilized in processing with a sterilant such as β -propiolactone, Patent 3,098,696, supra, or by ethylene oxide, either as a gas, or in liquid solution, as for example in the tubing fluid, or by penetration of the seals, or other method of addition. The sutures may be sterilized by heat, after thorough drying, with the tubing fluid later supplying sufficient water to flexibilize the suture, or by other means which have been used to sterilize catgut sutures.

As used in this specification, sizes are expressed in accordance with the sizes set forth in the U.S. Pharmacopeia, the current issue being XVII.

The standard sizes there given are:

U.S.P. XVII Size	Diameter	
	Min.	Max.
0-----	0.013	0.016
00-----	0.010	0.013
000-----	0.008	0.010
4/0-----	0.006	0.008
5/0-----	0.004	0.006
6/0-----	0.003	0.004
7/0-----	0.002	0.003

Where used in this specification, the term polyhydric phenol of not more than four rings means a structure with at least some aromatic rings, either fused or separate, which in the reduced form has at least two hydroxyl groups in such relationship that at least some can be converted to a quinone type of double bonded oxygen—although usually the products on oxidation are somewhat complex and may be of unknown structure. Included are such compounds as pyrogallol, logwood (hematein), resorcinol, hydroquinone, 1,2-naphthoquinone-4-sulfonic acid, as the sodium salt, and toluquinone. Others of the same general characteristics, or with non-interfering substituents are identifiable by chemists, and as such become available, can be substituted. Either pyrogallol or logwood are presently the cheapest, and most available commercially.

Many tests have been suggested for absorbability of collagen in sutures. Heat shrink temperature, papain digestion time, or trypsin or pepsin digesting time, and others. While such tests are frequently of great value, and rapid, and very useful particularly in comparing sutures from a particular process, the tests must be standardized against actual absorption time in living tissue to be of value. Comparisons between sutures produced by different processes are of dubious value. For instance, the trypsin absorption test works well with denatured collagen, as for example after heat treatment. Pepsin digests the raw undenatured collagen, but is much slower in attack on a heat treated collagen. Heat shrinkage is a means of comparing the degree of tanning, particularly to a water resistant stage.

The attack in tissue does not follow any of these exactly. With chrome tanned sutures, there seems to be a chelating attack on the chrome, followed by enzymatic attack on the collagen.

A preferred test for strength in tissues is actual emplacement in living tissue, with recovery and testing for strength after standardized times. An excellent animal, based on ease of care, and convenient size is the white New Zealand female rabbit. Using an animal of about 5 pounds weight, the animal is anesthetized, and using sterile surgical technique, has implanted in the anterior, lateral ventral tissue, under the skin of the animal about ½ inch below the lateral costal margin, and carried across the mid-line, ½ inch below the xiphoid process to the contralateral side. Preferably about 5 to 6 inch pieces of suture are emplaced. A series can be implanted about ½ inch apart in the same animal.

The sutures are removed from sacrificed animals, and stored in physiological saline until tested for strength. Conveniently, a group of animals can be sacrificed in the morning and the strength tests run in the afternoon. A standard Scott plane tester (U.S.P. XVII) is used for tests. Tests are conveniently run at 7 and 15 days for strength retention; and at 40 to 90 days for digestion characteristics.

In surgical usage, absorption is desired in not more than 90 days, with shorter times being preferable; and the strength of the suture should be at least 10% of original after 15 days in muscular tissue. A high strength up to 15 days is usually desirable, and as rapid digestion as is consistent with 15 day strength is desired. The requirements are somewhat inconsistent, as strength loss is relative to time, and it is difficult to get a good 15 day strength, and yet sufficiently rapid digestion thereafter.

The rate of digestion and strength loss varies somewhat with the type of tissue in which the suture is placed. The rates are higher in areas with good blood supply. The rates are somewhat slower in bone or tendon. As a standard, the rate of digestion in muscular tissue is used as a criterion—such as in the rabbits above described—and the sutures are found to be acceptable where used.

In the following examples and claims, percentages are expressed by weight unless otherwise clearly stated. Where weights are given for the collagen, the weight is given on a bone dry basis—for instance drying at 100° C. to constant weight.

Conveniently, but not necessarily, ethanol is the U.S. Formula 2B, which is a convenient form of denatured alcohol. Pure alcohol is equally satisfactory, but tax problems arise. Where given in percentages, the percentage unless otherwise stated is on the basis of 100% 2B ethanol. A commercial grade of 96% ethanol, or other available grade may be used, with appropriate corrections for the water content introduced with the ethanol.

Example 1.—Shredding and pretreatment of collagen raw material

Whole frozen tendon from the hooves of 2 to 3 year old cattle as received from the abattoir is thawed in tap water. The outer sheath, fat and connective tissue are thereupon manually removed. The Y-shaped tendons are then cut into four sections and are refrozen in single layers. The cleaned frozen tendon, as required, is granulated in with an equal weight of Dry Ice to prevent denaturation by overheating. After three passes through the mill the average particle size is 0.03 inch in the smallest dimension.

A cylindrical vessel of approximately 4½ liter capacity is charged with 200 grams of frozen granulated tendon, bone dry basis (approximately 600 grams of frozen granulated tendon). Demineralized water is added to a total weight of tendon plus water of 4,145 grams. 4.80 grams of trypsin powder (1:110) is added to the mixture and distributed by gentle agitation. The pH is adjusted to 8.0 with 3 Normal sodium hydroxide. The temperature of the jar is raised to 30° with continuous gentle agitation, using a water bath. The vessel is sealed and is subjected to gentle rolling agitation for 5 hours at an ambient temperature of 37.5° centigrade. The treating liquid is separated from the tendon with the aid of a 16 mesh screen and is discarded. The treated mass is then subjected to overflow washing with filtered tap water. Agitation is effected by introducing water at high velocity, directed at the mass, and overflowing at the rate of approximately 2 liters per minute. After one hour of washing the tendon is returned to the treatment vessel and is recharged with demineralized water to a combined weight for tendon and water of 4,145 grams. Ninety milliliters of a 40% aqueous solution of the tetrasodium salt of ethylenediamine tetracetic acid, are then stirred into the mixture. The mixture is again rolled at an ambient temperature of 37.5°, for 16 hours; the tendon is separated from the treatment liquor, and is washed; by the procedures described above.

Example 2.—Preparation of collagen gel

9,000 grams of ethanol (99% by weight) are cooled in a tared vessel to approximately 5°. The pretreated tendon from Example 1 is strained and demineralized water added to a total weight of water plus tendon of 12,000 grams. While the alcohol is being agitated, water is added by slowly decanting from the tendon. The alcohol water mixture is agitated to dissipate the heat of solution and cooled to 10° centigrade. Sixty-six milliliters of 3.67 Normal hydrochloric acid are mixed in, and the tendon is added in small portions while continuing agitation. The vessel is covered and the blending is continued for one hour. A highly viscous translucent envelope swells about each of the granular particles. The suspension is transferred to a conical hopper tank which supplies a colloid

mill by gravity feed. Shear forces developed in the mill, strip the viscous envelope of gel from each of the particles and disperse it throughout the medium, thereby exposing fresh unswollen surfaces to the medium.

Tabulated below is a typical behavior pattern for alternating milling and agitated cooling. Changes in throughput time are indicative of increasing viscosity and homogeneity of the dispersion for the second and third passes. The faster throughput rate on the fourth pass is interpreted as signifying a point beyond which no benefits are likely.

Colloid milling	Temperature/rise (°C.)	Throughput time (min.)	Time to recool to 10° C. (min.)
1st.....	5	2.7	-----
2nd.....	4	2.2	75
3rd.....	5	3.6	55
4th.....	4	3.2	30

After a final 30 minute blending, the dispersion is refrigerated overnight, or for a maximum of 3 days before further processing.

At this point, the solids of the gel range from 0.88 to 0.93 weight percent (the spread reflects slight variations in treatment and handling losses). Anhydrous 2B alcohol is blended into the gel to yield an adjusted solids of 0.86 weight percent.

Filterable impurities and air bubbles, which could produce weak points at subsequent stages of processing are removed from the gel. The dispersion, as prepared above, is removed from refrigeration to a vertical blender where, with the aid of an external water bath maintained at 25° C. the temperature of the dispersion is raised over the course of an hour to 20° C. The material is then delivered by air pressure to a plate and frame filter press using paper and a pressure differential across the filter of 8 lbs. per square inch.

The filtered gel is then delivered by a gear pump to a deaeration nozzle mounted in the top of an inclined receiving vessel. Thereby, gel is delivered under a pressure of approximately 20 p.s.i.g. through a cluster of 4 capillary tubes approximately .021 inch in diameter and ¾ inch long into the receiver, which is maintained at an absolute pressure of approximately 13 millimeters of mercury. With the rapid expansion of the small air bubbles and controlled evaporation of some of the solvent, the thin film of gel surrounding each bubble is burst and the deaerated gel travels down the side wall of the vessel. After admission of the entire batch to the receiver, the gel is maintained under vacuum for approximately 30 minutes. Air is slowly admitted over the gel and the batch is allowed to remain at 23° C. overnight or for a maximum of three days. Gel characteristics at this point are pH 2.6; viscosity, 3500 centipoises at 23° C. and 2.5-1 seconds, and solids content on a weight-weight basis of 0.86%.

Example 3.—Extrusion and regeneration

Filtered, deaerated gel, as prepared in Example 2, is delivered to a manifold supplying a number of gear pumps, under a pressure of approximately 40 pounds per square inch. The extrusion pumps used are Zenith size ½" Type B-3491. Each extrusion pump delivers gel through a length of flexible tubing to a 10 inch long nozzle of Type 304, or 316 stainless steel. Typical nozzle sizes are:

Suture size:	Inside diameter of nozzle (inches)
000	0.1875
4/0	0.1425
5/0	0.1050
6/0	0.071
7/10	0.049

The inner surfaces of the tubes are smooth and square-edged. The gel emerges from each tube as a continuous, cylindrical noodle and settles through an alcoholic bath onto a moving belt, the speed of which is controlled to provide 30-40% stretch. The composition of the extrusion bath is 80% ethanol and 20% demineralized water on a weight-weight basis, and 0.06% ammonia on a weight-volume basis. As the gel is gradually neutralized, the surface becomes progressively more opaque. After approximately 4.6 minutes in the bath the noodles exit the extrusion bath and are passed to a drum type ribbon dryer. Differential speeds of the two units effect stretch of the noodle. Stretch tends to orient the fibrils for improved tensile strength. In addition, stretch serves to express water from the noodle structure. As a result of de-watering, the solids content of the noodle increases from 0.86% solids in the gel to over 1.5% solids. The noodles follow in sequence through pairs of compression rolls, which may be covered with absorbent papermakers' felt, and are then threaded through a series of rolls graduated from ambient temperature to a maximum of 112° F., which dry to about 10% moisture. The dried ribbons are wound under gentle tension on individual reels.

Example 4.—Tanning

Dried ribbon, as prepared in Example 3, is conveyed through a series of four baths with a controlled elongation of about 20%. The first bath consists of 0.5 weight percent pyrogallol in demineralized water. The second bath consists of ammonium dichromate, at a concentration of 3 weight percent Cr₂O₃. The third bath consists of 20 percent sodium bisulfite. The fourth bath is an overflow water wash.

Example 5.—Twisting, stretching and final drying

Freshly tanned wet ribbon, as prepared in Example 4, is fed to a restraining roll-cluster, which effects a small further elongation, thence to the planetary carrier member of the twisting head of a conventional twisting apparatus, and then inward to a receiving bobbin. The proper combination of twisting and elongation rolls one edge of the ribbon to the center of the resulting cylindrical structure; the other edge makes a uniform helical pattern on the exterior surface.

A 2% elongation occurs during the twisting operation, and 19.2 turns per inch of wet twisted suture are imparted for size 6/0. The number of turns per inch depends upon the size suture. Size 000 suture has about 7.3 turns per inch, 7/0 suture, 23.7 turns per inch.

In order to prevent premature drying of the twisted suture, the area in the immediate vicinity of the twisting head is maintained at high humidity.

The twisted strand is stretched and given a final drying, at 75° F. and 65% relative humidity. The wet twisted suture material is threaded through a cluster of restraining rolls, which, by differential surface speed imparts an increment of stretch of 1% over that on the twisting bobbin. From the restraining rolls it is threaded to a rotating stretching tube which increases the total elongation from wet twisted suture to approximately 5.5%. The suture is taken up in a single layer on a stretch tube. After drying overnight, the suture material may be transferred to a storage reel as a continuous length, or it may be cut. The suture is sterilized, needled and packed.

Example 6.—Tubing fluid composition

Chromacized 3/0 regenerated collagen sutures, prepared as above, were heat treated at 125° C. for one hour and then packaged in water-isopropanol solutions containing from 0-15% water using packaging materials and sterilizing methods shown by U.S. Patents 2,917,878, Carnarius and Kaufman. The sterilized packaged sutures were incubated at 52° C. for a period of two weeks for

accelerated aging. Suture diameters, straight pull, and knot pull were measured, as shown:

Storage fluid		Dia. (mils)	Tensile strength		Knot strength	
Wt. percent water	Wt. percent isopropanol		Lb.	P.s.i.	Lb.	P.s.i.
0-----	100	11.13	6.65	68,300	2.87	29,500
1-----	99	11.22	6.84	69,200	2.99	30,200
3-----	97	11.32	7.22	71,700	3.15	31,300
5-----	95	11.44	7.19	70,000	3.13	30,500
7-----	93	11.89	7.58	68,300	3.06	27,600
10-----	90	12.25	7.35	62,400	2.99	25,400
15-----	85	12.41	7.04	58,200	2.93	24,200

Example 7

Similar chromacized regenerated collagen sutures are produced following the procedures of Examples 1-5 except that the pyrogallol in Example 4 is replaced by logwood, such as that obtained as Adcelane Crystals number 75 from American Dyewood Company.

Excellent sutures are obtained having similar properties, except the sutures are blacker.

Example 8.—Preparation of non-chromacized sutures

Plain (i.e. no chrome) regenerated collagen sutures are prepared by passing the dried ribbon, as prepared in Examples 1-3, through a series of two baths. The first bath contains an aqueous pyrogallol solution, 0.5 weight percent. The second bath contains an aqueous ferrous ammonium sulfate solution, 0.5 weight percent. The ribbon is twisted directly upon leaving the last bath with no interruption in the processing sequence. Subsequent to twisting it is water washed and then stretched and dried in accordance with the stretching and final drying procedures outlined in Example 5. Good sutures are obtained.

We claim:

1. In the process of forming a surgically acceptable absorbable regenerated collagen suture by extruding a dilute acidic aqueous gel of collagen in an alkaline dehydrating coagulating bath, compressing to form a thin ribbon, and twisting a suture from at least one ribbon, the improvement comprising the combination therewith of the steps of tanning first with a solution of a polyhydric phenol having not more than four rings, treating and at least partially oxidizing said polyhydric phenol by treatment with hexavalent chromium, thereby fixing said polyhydric phenol and reducing part of said chromium

to the trivalent state, and then reducing the remainder of the chromium to the trivalent state, which trivalent

chromium reacts with and partially tans the collagen; thereby forming a suture which has a good straight pull strength, good knot pull strength, good rundown, and surgically acceptable handlability, and which retains at least about 10% of its strength in living muscular tissue for 15 days, and which is absorbed by muscular tissue within 90 days.

2. The process of claim 1 in which the dilute aqueous acidic gel of collagen contains up to about 50% of ethanol.

3. The process of claim 2 in which the dehydrating bath contains from about 60% to about 95% aqueous ethanol, and the ethanol from the dehydrating bath, diluted by water in dehydrating is recovered and reused to form the aqueous acidic solution and the dehydrating bath.

4. The process of claim 1 in which the thin ribbon is tanned with said polyhydric phenol, treated with hexavalent chromium, and the chromium reduced to the trivalent state all before the ribbon is twisted to form the suture.

5. The process of claim 4 in which a plurality of ribbons are twisted together to form a suture.

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8-94.11, 127.5; 128-335.5