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PREPARATION OF COLLAGENOUS MATERIALS

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Fig. 1

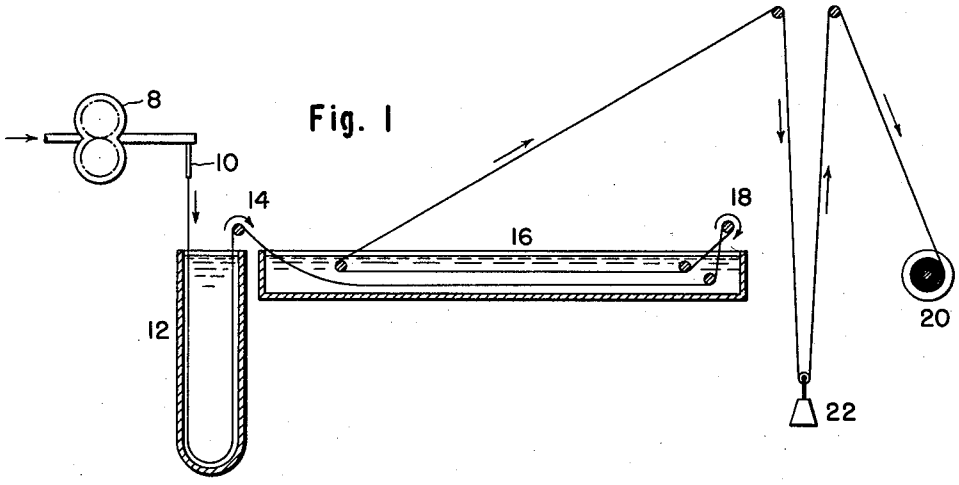


Fig. 2

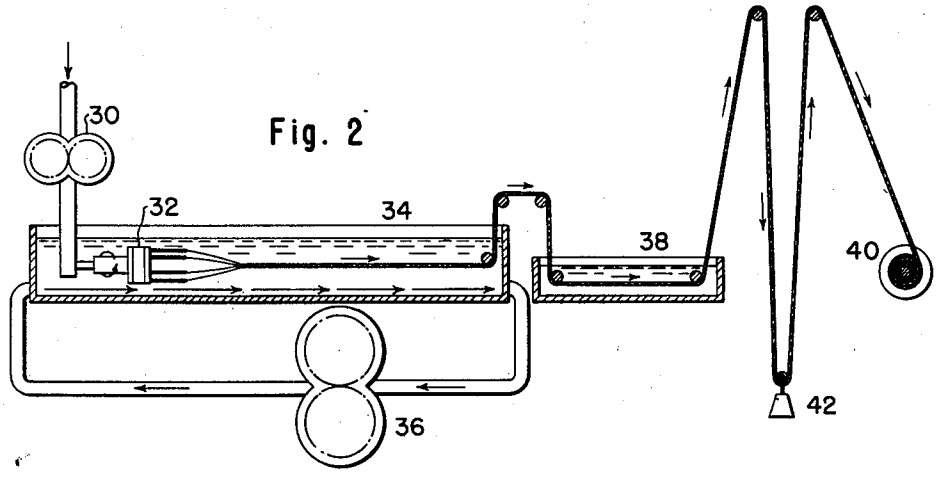


Fig. 3

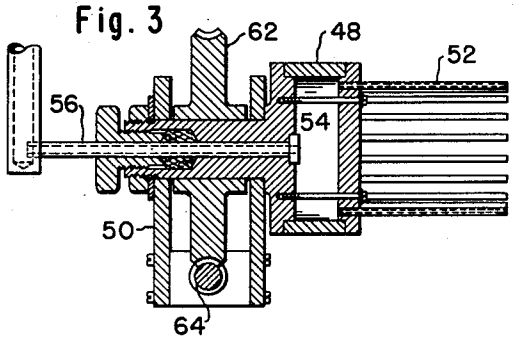
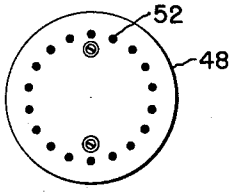


Fig. 4



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PREPARATION OF COLLAGENOUS MATERIALS

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The present invention relates to collagenous articles and their preparation, with particular emphasis on the preparation of articles of substantially pure collagen for surgical uses and the repair of wounds.

The protein collagen is found in animal tendons, skin, and in the supporting and integrating fibrous material of all tissues. It is distinctive, as compared with other proteins, in its slight tendency to excite foreign body reaction when carried from one animal to the tissues of another. Furthermore, it is relatively slow to yield to proteolytic enzymes, such as are found in the tissues and in the digestive tract.

The possibility that collagen might be separated from animal tissues and then reconstituted to form articles such as sheets or filaments of collagen has been of considerable interest for some time. A number of attempts have heretofore been made to devise techniques to accomplish this, but only limited success has been reported. In general, these attempts have been directed along markedly different lines. According to one prior technique, the collagenous tissues are subjected to treatment which hydrolyzes the collagen to gelatine, so that the collagen is present in a molecular sense only. The gelatine is then subjected to processing to yield filaments or the like. The products, however, are usually found to be seriously lacking in strength. In another type of approach, collagenous tissues may be shredded to produce fibers or strands that are thereafter woven or spun in the manner of textile fibers. This method obviously does not permit the production of articles of collagen alone.

Not only do these prior techniques fail to provide the requisite strength and uniformity of product, but they lack the essential purity for implantation in human tissues. Impurities such as elastin (protein of elastic tissues), the muco proteins or mucins associated with collagen, and the blood constituents which contaminate any extirpated tissue, must be effectively removed if the final collagen product is not to cause undue tissue reaction. Furthermore, any non-homogeneity, whether due to collagenous or to non-collagenous substances, if not removed in the course of the process, causes discontinuities in the final collagen structure. Such discontinuities seriously impair the strength of the finished articles, particularly those of a filamentary origin such as sutures.

We have found that collagenous tissues, when subjected to suitable treatment hereinafter described in detail, may be formed into filaments, sheets, and tubes of substantially pure collagen

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exhibiting high and uniform strength and possessing high purity. Such products are ideally suited for surgical uses.

The present process is characterized by the preparation of a collagen gel, in which the collagen is present in highly purified, homogeneous condition and in the form of fibrils rather than in the molecular sense. The gel is thus in the nature of highly solvated collagen fibrils rather than one in which the original fibrous structure has been wholly destroyed by grinding or by hydrolysis. From this gel of solvated fibrils, long filaments and strands may be formed by appropriate techniques to be described, which involve dehydration of the gel to leave the substantially pure collagen structure.

It is one of the several objects of the invention to provide a process for the production of a collagen gel having such properties that collagenous articles of high and uniform strength may be made therefrom.

More specifically, it is an object to produce a gel from which collagen filaments may be spun that are well adapted for surgical sutures, being superior to catgut in uniformity, wet strength, and non-antigenicity.

Another object of the invention is to provide an improved process for the production of artificial sutures of substantially pure collagen, wherein the sutures are formed of a plurality of fine collagen filaments to provide a strong and flexible suture of predetermined uniform properties.

The production of the collagen gel requires, as has already been pointed out, that particular care be taken to eliminate contaminating materials which, if allowed to remain, would deleteriously affect the chemical and physical characteristics of the collagenous articles produced from the gel. It is likewise important to attain a high degree of homogeneity with respect to the collagen itself in the gel, if the formation of filaments of high and uniform strength is to be successful. While the gel making process to be described follows, in its broader aspects, known techniques and principles, it is distinguished from prior processes in its inclusion of steps which are important to the production of what may be termed, for want of a better expression, a homogeneous, pellicle-free gel of highly purified collagen, wherein the collagen is in a fibrillar and not a molecular dispersion.

In the accompanying drawings, Fig. 1 illustrates the manner of forming single collagen filaments from the prepared gel, Fig. 2 illustrates the

manner of forming a multi-filament strand, and Figs. 3 and 4 are detail views of the extrusion nozzle unit for multi-filament spinning.

In the gel making process to be described, certain of the steps are concerned with purification of the collagen, while other steps involve the provision of a homogeneous fibrillar dispersion without excessive disintegration of the individual fibrils. It will be understood that the invention is not to be rigidly limited to the identical steps set forth, as equivalent procedures may be substituted.

As a starting material for the source of collagen, beef tendon has been found particularly suitable, the tendons from freshly killed steers being preferred. The tendons are stripped of their sheaths and trimmed free of fat and other extraneous tissues. The cleaned tendon will be found to have a shank portion of "soft" tendon, and branching portions of "hard" tendon. While both portions contain collagen, the soft portions are somewhat more amenable to the processing treatment hereinafter described. The hard portion may be retained temporarily to serve as a handle in the preliminary operations, or it may be subjected to separate treatment in suitable swelling reagents. Following a thorough soaking and washing of the tendons to remove soluble impurities such as blood, they are frozen to permit slicing, only the soft parts being sliced and retained. It has been found that slicing to a thickness of the order of 1 mm. is of aid in securing a uniform gel. It is likewise considered advantageous to allow no drying of the tendon material at any stage prior to the final dehydration in the formation of the finished product.

The tendon slices, after soaking and washing, preferably in distilled water, are caused to swell by the action of acid to obtan a jelly-like mass. Acetic acid has been found satisfactory, using about 20 volumes of 2.5 to 5% acid. This generally will reduce the tendon slices to the desired swollen state in a three-hour period, although an appreciably longer period may be used.

To permit the effective removal of unswollen particles by a subsequent operation, the mass of swollen tendon is then blended with distilled water to form a dilute sol, using roughly eight volumes of water to one volume of the swollen tendon. The dispersion should be so carried out as to provide a longitudinal shearing action which separates the swollen tendon units into uniform solvated fibers. Extreme shearing action should be carefuly avoided, however, as should heat, since the collagen is heat sensitive. The blending may be effected in conventional blending equipment, such as a Waring blendor, or the requisite dispersion may be provided by extruding the tendon mass through a perforated plate and the water added thereafter. In either event, it is desirable to screen or filter the dilute sol to remove any large unblended tendon pieces. Filtration through a twenty mesh stainless steel screen is suitable for this purpose.

The dilute sol which passes through the screen still contains unswollen particles, including non-collagenous substances such as mucoprotein, which if permitted to remain in the gel would seriously interfere with the success of subsequent processing. Particularly in the case of formation of filaments by extrusion, these unswollen particles or non-homogeneities in the gel, even if small enough to pass freely through the extrusion nozzles, result in discontinuities in the filament structure which markedly reduce the ultimate strength of the fiber.

To remove these unswollen particles, the dilute sol may be passed through a centrifuge, the most efficient method being to employ the so-called super-centrifuge. Alternatively, the particles may be removed by passing the sol through suitable filters. Whatever the actual manner of removal, the formation of a dilute sol appears to be a prerequisite to successful removal of the unswollen matter.

The blending and centrifugation steps cause a large amount of air to be trapped in the sol. This must be removed to prevent floating of precipitated collagen during subsequent steps. Removal of air is readily effected by subjecting the sol to evacuation, or by allowing the sol to stand for sufficient time.

The deaerated sol at this stage will contain approximately 0.1% collagen. Since this is too dilute to permit spinning into filaments, the sol must be concentrated to a gel. This is effectively carried out by precipitating the collagen, which step likewise permits additional purification to be accomplished. The precipitation is effected by adding normal ammonium hydroxide to the sol until a pH of substantially 7.0 is reached. Not only does a pH of 7.0 provide a high collagen concentration, but it results in neutralization of all the free acid and alkali of the dilute sol. In adding the base to the sol, it is preferable to add roughly three-fourths of the required amount of hydroxide rapidly while slowly stirring the sol. The remaining ammonium hydroxide is then added slowly, but with rapid stirring, until the desired pH value is reached.

The precipitated collagen is now thoroughly washed in distilled water to remove all traces of salts, the collagen being fluffed up in the liquid to permit free diffusion. Several changes of liquid may be necessary before the wash water shows a negligible amount of electrolyte.

The thoroughly washed precipitate is converted to a gel by the addition of an organic acid, such as malonic acid. For satisfactory spinning properties, the final gel should contain approximately 1.8% collagen and 0.6% acid by weight. After stirring until all unswollen or "ropy" strands have disappeared, the gel is smoothed or homogenized by conventional techniques and apparatus to give a uniform and homogeneous gel of collagen fibrils. The smoothing should be so carried out as to produce a uniform dispersion of the collagen fibrils without appreciably affecting the fibrillar units themselves. Following deaeration, the gel is ready for extrusion to form filaments, or for formation into other types of product. The gel, if stored in sterile containers kept at a temperature of the order of 4° C., will remain usable for two to three weeks following its preparation, but it is preferable to use the gel as soon as possible after it has been made.

To form collagen fibers from the gel, an extrusion technique is preferably employed. If high strength and, in the case of fibers for surgical uses, resistance to enzymatic digestion are to be attained, it is necessary to insure proper orientation and longitudinal alignment of the individual fibrils. These fibrils, submicroscopic in size, are in various states of coiling and tangling in the gel, and, as heretofore indicated, account for between 1.5 and 2.0% of the weight of the gel.

Such a gel is relatively stringy and cohesive, but only slightly elastic. When discharged from a suitable nozzle that is directed generally in a downward direction, the gel column will remain coherent for a free drop of the order of forty centimeters. The weight of the lower portions of the column gives rise to tension in the region adjacent the jet, and this tension causes the gel column to contract or "neck down." Along with the "necking down," an appreciable increase in elasticity may be observed, indicative of a progressive orientation of the fibrils from their initial random and tangled arrangement into a more or less orderly longitudinal alignment.

The importance of securing and maintaining the maximum degree of longitudinal orientation of the fibrils in the extruded filament is due to the belief that the ultimate strength of the finished fiber is not so much a function of the tensile strength of the individual fibrils as it is dependent on the lateral bonds between fibrils. On this theory, breaking under tension occurs because of disruption of these lateral bonds, with subsequent slippage of the fibrils past one another. Tangles of fibrils, and fibrils at an angle to the longitudinal axis of the main fiber thus reduce the number of lateral bonds available to carry a load in tension.

To aid in attaining the desired orientation of fibrils, it is advantageous to employ as the nozzle or jet, through which the gel is extruded, a relatively long tube or capillary. By way of example, a tube ten to twenty cm. in length is appropriate. In general, the jet diameter, or tube bore, is preferably within the range of 0.6 mm. to 1.5 mm.

In carrying out the extrusion of the gel into filaments and fibers, it is desirable to provide for careful control of the elongation at the various stages of the process. A procedure appropriate to the extrusion of single filaments is illustrated in Fig. 1 of the drawings.

The gel, carefully prepared according to the previously described technique to minimize local differences in the gel characteristics, is supplied by a metering pump 8 to downwardly directed nozzle 10. The nozzle has a length many times its internal diameter, and is some distance above the surface of a tank 12 to bring about stretching of the discharged gel by the action of gravity. Heights greater than about twenty cm., and up to thirty to forty cm. will generally be found to provide adequate stretch, and can satisfactorily be employed with a properly prepared gel.

The tank into which the gel is discharged from the jet contains a liquid for dehydrating the gel. Acetone has been found preferable for this purpose. It has been established that during the initial stages of dehydration the fiber becomes weaker than the original gel column, before it increases in strength. Accordingly, the loop of fiber in the first bath is so adjusted that the "weak spot" occurs near the bottom of the loop. By the time the fiber reaches pulley 14, the fiber has gained considerable strength.

Additional stretch is provided in a second dehydrating bath 16. The pulley 18 is arranged to provide a predetermined stretch between it and pulley 14. In general a stretch of not over 15% is sufficient in this region. The fiber is returned to the bath for still further dehydration, and then taken up on a winding reel 20. A constant tension is maintained by means of the weight 22.

The fiber as it comes from the acetone is, in effect, a loose network of collagen fibrils containing some residual acid. Not only is it necessary to remove the acid, but also the fibrils must be further oriented and bonded to one another. Washing is preferably carried out by passing the fiber into distilled water, or into a solution containing a dilute buffer at pH 9.1. A tension of 1.2 to 1.5 g. tension is advisable, and the subsequent drying should also be carried out with tension maintained.

To provide collagen strands of greater effective cross section than can conveniently be extruded from a single nozzle, a multi-filament extrusion process may be employed. In carrying out this method, a rotating nozzle head having a plurality of nozzles is arranged to discharge multiple gel streams into the coagulating bath. By reason of the nozzle rotation, the individual filaments are twisted into a strand, and in the event a filament breaks, the loose end is automatically picked up.

In carrying out the multi-filament spinning according to Fig. 2, the gel is supplied by pump 30 to the rotating nozzle assembly 32 shown in detail in Fig. 3. The multiple gel filaments are discharged into a bath 34 of flowing acetone to impart stretch for purposes of fibril orientation and to cause more rapid initial dehydration. The acetone is caused to flow continuously from one end of the bath to the other by means of a circulating pump 36. Further dehydration and stretching is carried out in the second bath 38, after which the strand is wound at 40 under the tension afforded by weight 42. Washing and drying may be carried out in the same fashion as for the mono-filament.

The nozzle assembly for multiple filament extrusion comprises a head 48 rotatably mounted in a support 50. A plurality of nozzles 52 are arranged parallel to the axis of rotation of the head and opening into the chamber 54 within the head. These nozzles are preferably similar to those employed for single filament extrusion, having a bore of 0.6 mm. to 1.5 mm. and a length of 10 cm. to 20 cm. The gel is supplied through an axial tube 56 to the chamber 54, a rotatable seal being provided by a suitable stuffing box. The head is rotated during extrusion by means of a worm gear 62 that is driven by worm 64 from a suitable driving motor. The extrusion rate and the speed of rotation are coordinated to provide the desired twist in the finished collagen strand.

It is likewise possible to provide collagen strands which are made up of mono-filaments or small multi-filaments, braided together to form a strand of the desired effective cross-section. Such a strand offers great flexibility and is particularly effective for surgical uses, since the flexibility aids in tying small but secure knots.

The pure collagen strands and fibers formed according to the techniques hereinbefore described, when immersed in water or implanted in tissue, swell substantially. Along with the swelling, there is a considerable loss in strength. In the case of fibers implanted in tissue, absorption occurs relatively rapidly.

To minimize swelling and to increase the wet strength of the collagen strands, it is desirable to subject the strands to appropriate tanning treatment. Such treatment likewise increases the resistance to enzymatic digestion.

It has been found that chrome tanning techniques analogous to those employed in the treatment of leather are effective in minimizing absorption of water and in increasing resistance to

enzyme digestion. Chrome alum solutions containing sodium adipate or sodium adipate and sodium lactate may be used to advantage, employing a considerable excess of chrome liquor in the tannage.

Following the tanning, a heat sterilization procedure is necessary where the collagen strands are to have surgical uses, as in sutures or the like. Heating at approximately 130° for three to six hours may be considered typical.

The fibers produced in accordance with the invention exhibit substantial tensile strength. By way of illustration, a dry strength of more than 40 kilograms per square millimeter of fiber cross section may readily be attained, and wet strengths of the order of 30 kilograms per square millimeter. Furthermore, these fibers when implanted as sutures exhibit substantial resistance to enzymatic digestion, so that over 50% of the initial wet strength may be realized at the end of a seven day period in tissue. Not only do such strengths compare favorably with catgut sutures, but they are materially greater than are afforded by processes wherein the collagen is converted to gelatine rather than retained as fibrillar units.

In addition to formation into fibers and strands, it will be understood that the collagen gel may be formed into sheets, tubes, and other shapes by appropriate dehydrating techniques.

We have described as our invention a procedure for the preparation of highly purified and homogeneous collagen gels, and the formation of articles, particularly fibers and strands, that are well adapted for surgical uses. In the case of fibers and strands for sutures, a technique has been evolved that permits the production, in continuous lengths measured in thousands of feet, of strong, uniform, and extremely pure collagen filaments, both single and multiple, that are well qualified to replace the usual catgut.

We claim:

1. In the process of converting a swollen mass of tendon pieces to a gel of solvated fibrils of substantially pure collagen from which filaments may be spun, the steps which comprise blending the swollen mass with a water to form a dilute sol and centrifuging the dilute sol prior to conversion to a gel for spinning.

2. In the process of converting a swollen mass of tendon pieces to a gel of solvated fibrils of substantially pure collagen from which filaments may be spun, the steps which comprise blending the swollen mass with a water to form a dilute sol, centrifuging the dilute sol to remove unswollen particles therefrom, precipitating the collagen and washing the precipitate, prior to conversion of the collagen to a gel for spinning.

3. In the process of converting a swollen mass of tendon pieces to a gel of solvated fibrils of substantially pure collagen from which filaments may be spun, the steps which comprise blending the swollen mass with a water to form a dilute sol containing less than approximately 0.5% collagen by weight, centrifuging the dilute sol to remove unswollen particles therefrom, and precipitating the collagen from the sol, prior to conversion of the collagen to a gel for spinning.

4. In the process of converting a swollen mass of tendon pieces to a gel of solvated fibrils of substantially pure collagen from which filaments may be spun, the steps which comprise blending the mass with water to form a dilute sol containing approximately 0.1% collagen by weight, treating the dilute sol by centrifuging to remove unswollen hard particles therefrom, and precipitating the collagen from the treated sol, prior to conversion of the collagen to a gel for spinning.

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