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TOMIO NISHIHARA
SOLUBILIZATION OF INSOLUBLE COLLAGEN
FIBERS AND RECONSTITUTION THEREOF

3,034,852

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2 Sheets-Sheet 1

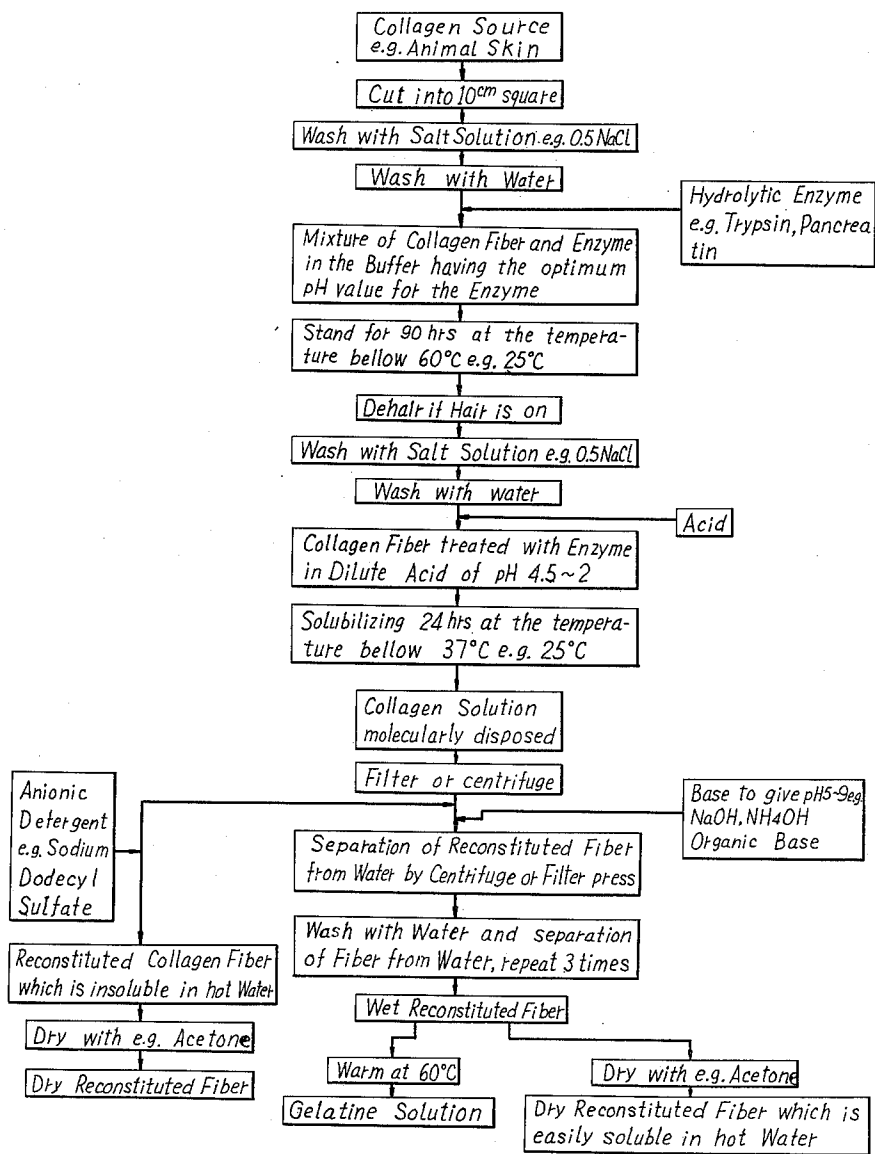


Fig. 1.

TOMIO NISHIHARA - INVENTOR.

BY *Otto John Mung*
ATTORNEY

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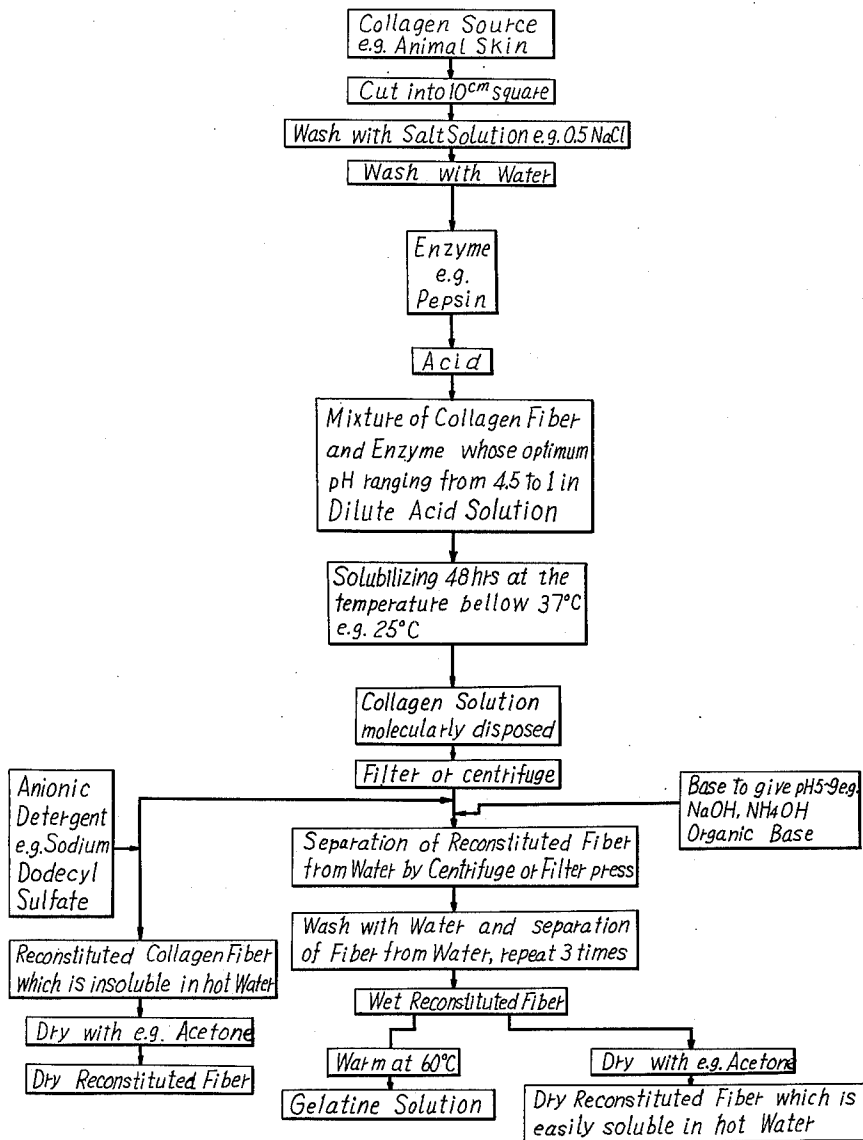


fig. 2.

TOMIO NISHIHARA - INVENTOR.

BY *Otto John Mung.*
ATTORNEY

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SOLUBILIZATION OF INSOLUBLE COLLAGEN FIBERS AND RECONSTITUTION THEREOF

Tomio Nishihara, Tokyo, Japan, assignor to The Japan Leather Mfg. Co., Ltd., Tokyo, Japan, a corporation of Japan

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9 Claims. (Cl. 18—54)

The present invention relates to the solubilization of collagen fibers previously considered insoluble. More particularly, the present invention relates to solubilization of collagen fibers, a fibrous protein which constitutes the principal element of connective tissue in animals. Previously, it was considered impossible to form a solution of such collagen without either converting it to a gelatin or else altering its inherent molecular structure from a helical, rigid rod type molecule into a foldable random coil structure which was accomplished either by heating at a temperature above 50° C. or by the use of a chemical denaturing agent, e.g. potassium thiocyanide, calcium chloride, urea, etc. But since 1927 it has been known that a small percentage of a given quantity of collagen can be dissolved in a solution of dilute acid or alkali or neutral salt without altering the inherent structure of the collagen, that is, the helical structure of the rigid rod type, and that the original fiber can be reconstituted from said solution by any suitable method. Collagen in this state has been named "soluble collagen." However, such soluble collagen makes up only a small percentage of the total amount, the exact percentage varying slightly depending on the age, the portion of body, or the kind of animal, and the greater part of the collagen has up to now been considered as insoluble.

One object of this invention is to provide a method for obtaining a good yield, in soluble form, of that collagen previously considered insoluble without denaturing the collagen (i.e. without destroying the helical, rigid rod molecular structure) and in such form that the original fiber can be reconstituted in good yield. Thus, the object of this invention is to prepare a soluble collagen on an industrial scale. Collagen films, collagen fibers, collagen fabrics and collagen sponges can be obtained from the collagen solution thus obtained. Furthermore, if the collagen fiber which has been solubilized and then reconstituted is suspended in water and heated at a temperature of 5 to 70° C., so as to dissolve it therein, one obtains in 100% yield a homogeneous gelatin having higher purity and higher freezing and melting points than prior art collagen gelatins. Moreover, this gelatin forms far more quickly than do those of the prior art.

I have discovered that so-called "insoluble" collagen can be dissolved without denaturation to form a solution of uniform molecular weight, by reacting the "insoluble" collagen with a hydrolytic enzyme at a temperature below 60° C., its shrinkage temperature, and then extracting the collagen with a dilute acid solution at a temperature below 37° C., its denaturation temperature. Moreover, the original fiber can be reconstituted in 100% yield from the collagen solution so obtained, using any known method, e.g. neutralization, dialysis, ion exchange, addition of a surface-active agent, or extraction with an organic solvent such as acetone or alcohol. The hydrolytic enzymes utilized in accordance with the present invention are the proteolytic enzymes, that is, pancreatin, trypsin and pepsin and their specific employment is shown in the following examples.

This collagen solution of uniform molecular weight in which the protein retains its inherent molecular struc-

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ture can be obtained only by extracting the hydrolytic enzyme-treated collagen with a dilute acid at a temperature below 37° C. Some of the prior art methods of reconstituting the fiber, e.g., dialysis of a water or disodium hydrogen phosphate solution, or addition of a sodium salt such as the chloride, citrate, acetate, etc., are inconvenient in that they require an extended time both for reconstituting the fiber and for washing the reconstituted fiber. By contrast, if a surface active agent is used, these inconveniences are avoided and the fibers are simultaneously reconstituted and purified.

In the accompanying drawings FIGURES 1 and 2 are representations in flow sheet form of embodiments of the invention hereinafter described.

The present invention is further illustrated by the following examples:

Example 1

3 kg. of cowhides (moisture content: approximately 70%) from which either the soluble protein has been removed by treating with a 5% aqueous solution of sodium chloride and then washing with water, or else the hair has been removed by liming, neutralizing with hydrochloric acid and then washing with water, are immersed in 3 l. of aqueous solution containing trypsin (6 g.) and allowed to stand at the temperature of 25° C. with occasional stirring for 90 hours. Then the hydrogen ion concentration of the enzyme solution is adjusted to pH 8 with caustic soda or boric acid buffer solution. The same result is obtained by using pancreatin instead of trypsin. The amount of enzyme used is from about 0.5 to 2.0% of the substrate in accordance with its activity. During the treatment with enzyme, the collagen does not dissolve at all and also no remarkable change is observed in its appearance. After removing the trypsin by sufficient rinsing in running water, the cowhide thus treated with enzyme is immersed in 100 l. of water and the aqueous solution is adjusted to pH 2-3 at equilibrium by adding hydrochloric acid with stirring. For this purpose about 35 cc. of 12 N concentrated hydrochloric acid is required. By stirring at a temperature of 20-25° C. for about 24 hours, the cowhide is 100% dissolved to form a viscous solution similar to gluten. If a mineral acid such as sulfuric acid, phosphoric acid etc. or an organic acid such as acetic acid, citric acid etc., is used instead of hydrochloric acid the same result is obtained. The viscous solution is filtered through a filter press using cloth and non-fat cotton as a filter. The filtrate is neutralized by adding about 56 cc. of 30% caustic soda solution thereto, the hydrogen ion concentration is adjusted to pH 5-8, and the solution is allowed to stand for several hours, and a fibrous white precipitate is produced. This precipitate is washed thoroughly with water, collected by filtration or centrifugation and dried in air. Approximately 700 g. of snow white collagen is obtained. Since the nitrogen content of the supernatant liquid is nearly zero, it is obvious that the collagen fiber has been completely reconstituted. And also, such collagen fiber can be reconstituted in 100% yield by dialysis of a 0.02 M disodium hydrogen phosphate solution or by adding an organic solvent such as acetone or alcohol in an amount sufficient to render the concentration of the mixture about 30%, as well as by the above neutralization process solubilized. The soluble collagen fiber thus obtained has the same physico-chemical properties—viscosity, birefringent flow, specific rotation, denaturation temperature, shrinkage temperature, sedimentation constant etc.—as the soluble collagen well known in the prior art. That is, my solubilized collagen fiber has a molecular structure of rigid rod type, an intrinsic viscosity of 15, a uniform molecular length and diameter of 3,000 A. and 13.6 A. respectively, a specific rotation of -415°, a sedimenta-

tion constant of 30 (Svedberg unit), a denaturation temperature of 37° C. and a shrinkage temperature of 60° C. When the reconstituted collagen fiber is observed under an electron microscope, it is seen that the collagen fiber has a cross striated pattern having a period of 700 Å., which is also characteristic of the undissolved native collagen fiber.

Example 2

3 kg. of cowhides (moisture content, approximately 70%) which have been pretreated as described in Example 1 are added to 3 l. of aqueous solution containing pepsin (6 g.) and its hydrogen ion concentration is adjusted to pH 2.0-2.5 with hydrochloric acid and is maintained at the temperature of 25° C. with occasional stirring for 48 hours. Since the optimum pH of pepsin is about 2, the enzyme treatment of the insoluble collagen and its dissolution in dilute acid are here accomplished simultaneously. However, the solubility of collagen in dilute acid is only about 1%, so that the cowhides dissolve only partially. After the treatment with enzyme for 48 hours, the volume of the solution is increased to 100 l. by adding 0.005 N. hydrochloric acid and it is stirred constantly at the temperature of 25° C. for 24 hours, then all the collagen is dissolved. The same result is obtained by using another inorganic acid or an organic acid as described in Example 1, instead of hydrochloric acid. The method for reconstituting a fiber from the collagen solution thus obtained is the same as that described in Example 1. That is, by neutralizing the solution with caustic soda and letting it stand for several hours, the collagen is precipitated. After washing and drying the precipitate, about 700 g. of collagen fiber is obtained. The collagen fiber thus obtained by pepsin digestion is slightly different in its molecular properties from the soluble collagen obtained by the prior art and from the solubilized collagen obtained by trypsin digestion as described in Example 1. That is, this collagen fiber is the same as the other two in its denaturation temperature, shrinkage temperature, specific rotation, sedimentation constant, homogeneity of molecular weight, etc., and, also, it has the same helical structure of rigid rod type as the other two types; however, the intrinsic viscosity is 9.5, which is slightly less than in the other two and, also, the molecular length is shorter than the other two by approximately 200 Å. in accordance with the measurement of birefringence of flow. Thus, it is seen that this process cuts off a portion near the end of the natural collagen fiber molecule. When the soluble collagen thus obtained is suspended in water in the ratio of 1 part collagen to 2 parts water by weight, and heated at a temperature of 60-70° C., the collagen is denatured and dissolved in a few minutes. The collagen solution thus obtained is cooled to form a gel and then it is dried to produce a gelatin. As compared with the gelatin produced by the prior art, the gelatin thus obtained is superior in the following respects:

(1) As compared with the prior art liming method, only about one-fifth as many hours are required for making gelatin.

(2) The yield is almost 100%.

(3) The physical properties such as jelly strength, setting point, melting point etc. are better than the best quality of product produced in the prior art method, and the best quality product constituted only about 30% of the total product obtained in this prior art method.

(4) No concentration step is necessary, which cuts the heating cost in half; in the prior art method concentration after extraction is necessary.

(5) The molecular weight of the gelatin is fairly uniform, being in the neighborhood of 120,000, while the prior art gelatin contains molecules of widely distributed weights. The purity is much higher than in the gelatin of the prior art, since according to this invention the collagen fiber is dispersed in a molecular state as a solution and is then reconstituted as a fiber; that is, the manufac-

turing process includes recrystallization. Therefore, my gelatin is of especially high value for photographic purpose.

When the collagen solution obtained as described in Examples 1 and 2 is coated on a plastic plate and dried at room temperature, a clear film of collagen is obtained. This film is useful for medicine capsules, food casings, and the like.

When the collagen solution obtained as described in Example 1 is filtered, extruded through a nozzle into a 2 M sodium chloride solution at the temperature of 25° C. to be reconstituted into fibers, and water is removed with acetone, and the resulting product tanned in a mixture of 10% formalin and 0.02 M disodium hydrogen phosphate and dried in air or with acetone, a collagen yarn is obtained. Furthermore, if a solution consisting of the collagen solution to which has been added a gelatin obtained in the process described above is extruded through a nozzle into a mixture of 0.02 M disodium hydrogen phosphate, 10% formalin and 2 M sodium chloride to reconstitute fibers, a yarn having a moderate elasticity can be obtained. In reconstituted fibers made out of protein such as albumin, casein or silk by previous processes the molecular structure is changed in the course of manufacturing process; in contrast, the reconstituted fiber made out of collagen by this invention is characterized by having the same molecular structure as the original.

The invention has been described in detail with particular reference to preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention as described hereinabove and as defined in the appended claims.

What is claimed is:

1. A process for making a solution of undenatured collagen from a collagen which is insoluble in dilute acid, alkali, and neutral salt solutions, which comprises treating said insoluble collagen with a hydrolytic enzyme selected from the group consisting of trypsin and pepsin at a temperature below 60° C. and extracting the collagen with a dilute acid of about pH 1 to about pH 4 at a temperature below 37° C., whereby a collagen solution is produced.

2. The process of claim 1 in which the solution is then treated to reconstitute the collagen fiber.

3. The process of claim 2 in which the reconstituted fiber is suspended in water, heated to a temperature of about 60° C. to 70° C. whereby dissolution takes place, then cooled to form a gel, and the gel is dried to a gelatin.

4. The process of claim 3 in which the hydrolytic enzyme is pepsin.

5. The process of claim 2 in which the collagen solution is reconstituted by extruding it through a nozzle into a 2 M sodium chloride solution, water is removed with acetone, and the resultant fiber is tanned in a mixture of 10% formalin and 0.02 M disodium hydrogen phosphate and then dried to produce a yarn.

6. The process of claim 5 in which the gelatin is dissolved in water and admixed with a collagen solution prepared by treating a collagen insoluble in dilute acid, alkali and salt solutions with trypsin at a temperature below 60° C. and extracting the collagen with a dilute acid of about pH 1 to about pH 4 at a temperature below 37° C., and the mixed solution is extruded into a mixed solution of 2 M sodium chloride, 0.02 M disodium hydrogen phosphate and 10% formalin, and the resultant fiber is dried to form a yarn.

7. The process of claim 1 in which the solution is coated on a plastic plate and dried at room temperature to form a film.

8. A process for making a solution of undenatured collagen from a collagen which is insoluble in dilute acid, alkali, and neutral salt solutions which comprises treating said insoluble collagen with a proteolytic enzyme at a

temperature below 60° C. and extracting the collagen with a dilute acid of about pH 1 to about pH 4 at a temperature below 37° C., whereby a collagen solution is produced.

9. A process for making a solution of undenatured collagen from a collagen normally insoluble in dilute acid, alkali and neutral salt solutions, comprising the steps of treating said insoluble collagen with pancreatin at a temperature below the shrinkage temperature of said collagen; removing the trypsin; hydrolyzing the collagen; adjusting the aqueous solution to pH 1-4 with dilute acid

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and stirring at a temperature below the denaturation temperature of the collagen until a collagen solution is produced.

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