CHITIN AND CHITIN DERIVATIVES FOR PROMOTING WOUND HEALING

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Field of Search ................. 424/28, 95, 180, 154

References Cited

UNITED STATES PATENTS

2,040,879 5/1936 Rigby.......................... 260/54
2,795,579 6/1957 Doczi.......................... 260/211

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ABSTRACT

Wound healing compositions and the process of healing wounds with such compositions are described, the compositions containing chitin, partially depolymerized chitin or a chitin derivative.

9 Claims, No Drawings
CHITIN AND CHITIN DERIVATIVES FOR PROMOTING WOUND HEALING

This application is a division of my copending application Ser. No. 704,538 filed Feb. 12, 1968 now U.S. Pat. No. 3,632,754 and a continuation-in-part of copending application Ser. No. 619,007 filed Feb. 27, 1967 now abandoned.

This invention relates to methods of promoting the healing of wounds and compositions therefor comprising chitin, and/or chitin derivatives and/or partially depolymerized chitin.

Medicine has long been interested in improving the healing of wounds. Patients suffering from diabetes or undergoing extensive cortisone treatment show extremely slow rates of healing of any wounds which they receive. Thus, surgery on such patients involves additional risks not present with other patients. Moreover, rapid healing of wounds is particularly desired for patients in tropical countries where the risk of infection is high. Rapid healing is also desired in the case of soldiers who have been wounded in a battle zone and cannot easily and quickly be removed therefrom. Acceleration of wound healing is highly desirable in the case of patients who cannot readily be immobilized, such as farm animals.

In evaluating the utility of a material to promote wound healing, a reproducible test is necessary to give comparative data. Such a test method has been described by Prudden et al. in: "The Acceleration of Wound Healing with Cartilage", Surgery, Gynecology and Obstetrics, 105:283 (1957). In this method, rats are tested in pairs, each pair receiving an identical surgical incision, only the one rat of the pair receiving a measured dose of the material whose wound healing properties is to be determined. The pair is then kept in the same cage and the tensile strength of the wounds in the two rats is determined in millimeters of mercury. The difference in the tensile strengths between the treated rat and the control rat is expressed as the percentage improvement obtained. Considering biological variance it is believed that only differences of about 10% or more are significant.

There have been several recent developments reported concerning materials which promote wound healing. In this connection U.S. Pat. No. 3,232,836 describes the parenteral administration of N-acetylg glucosamine as a wound healing material. Utilizing the test method of Prudden et al referred to in the preceding paragraph, N-acetylg glucosamine showed improvement in tensile strength of only about 10% whereas Prudden and his co-workers have reported significantly larger increases in wound healing by the use of cartilage preparations from various animals. Depending on the age and species of animal and the fineness of the cartilage powder, improvements ranging from 20 to 40% in wound healing tensile strength have been reported by Prudden.

Now it has been discovered that finely divided chitin, partially depolymerized chitin, and chitin derivatives possess the ability to promote the healing of wounds.

Accordingly, one aspect of the present invention relates to novel methods of promoting and assisting the healing of wounds as, for example, damaged mammalian tissue, open ulcers, etc., and to compositions therefor.

Another aspect of the invention relates to significant improvements in wound healing strength achieved by the administration of finely divided chitin, partially depolymerized chitin or chitin derivatives to a patient.

These and other aspects of the present invention will be apparent from the following description.

Chitin is a polysaccharide, believed to be poly(N-acetylg glucosamine) which forms the cell walls of fungi and the hard shell of insects and crustaceans. As used herein, the term "chitin" embraces naturally occurring chitin synthetic chitin, as well as poly (N-acetylg glucosamine) and its epimer poly (N-acetylgalactosamine). The N-acetylated partially depolymerized chitin, e.g. chitotriose, chitobiase, is a substance which retains its polymeric nature but has undergone a reduction in molecular weight (i.e. chain length) as a result of (1) enzymatic action such as by a chitinase enzyme, (2) chemical treatment such as acid hydrolysis or alkaline treatment, and (3) physical treatment. These materials are known in the art and procedures for their preparation may be found in "Advances in Carbohydrate Chemistry" Vol. 15, Pages 380 to 384, Academic Press, New York 1960, the disclosure of which is incorporated herein by reference. Thus, the molecular length is in the range from n=1 in which n corresponds to the number of repeating units in chitin to n=0 which is acetylated chitobiose.

The chitin derivatives contemplated are materials such as ethers formed with pharmaceutically-acceptable radicals and esters or salts with pharmaceutically-acceptable acids. Examples of suitable derivatives include hydroxy lower alkyl chitin such as hydroxyethyl chitin, carboxy alkyl chitin such as carboxymethyl chitin, salts of carboxy lower alkyl chitin such as the zine salt, lower alkyl chitin such as methyl chitin and ethyl chitin, chitin acetate, chitin nitrate, chitin citrate, chitin phosphate, N-acyl derivatives derived from monocarboxylic aliphatic acids such as N-formyl, N-acetyl, N-propionyl, N-caproyl, etc.

It is preferred to use natural chitin as the wound healing accelerator. The naturally occurring chitin is preferably chitin of fungal origin, both by reason of its ready availability and its high degree of effectiveness.

The degree of improvement in wound healing obtained with the chitin materials is at least equal to and in many instances greater than that derived from the cartilage materials of the prior art. The substantial improvement in rate of healing which is obtained from the use of poly (N-acetylg glucosamine), i.e., chitin, as compared to monomeric N-acetylg glucosamine is particularly surprising. As compared to the great variability in cartilage depending on the animal, its age and the method of collecting the cartilage, chitin, particularly chitin of fungal origin, is a relatively uniform and easily obtained material.

The compositions of the present invention are applied using the same techniques and processes developed for cartilage, and N-acetylg glucosamine. Thus, it is preferred to topically apply finely divided chitin directly to the wound surface. However, tablets, capsules or pellets of chitin may be prepared from mixtures of chitin, partially depolymerized chitin or chitin derivatives with well-known pharmaceutical excipients such as starch, sugar, certain forms of clay, etc. Such tablets, capsules or pellets may be taken orally or implanted.
near the situs of the wound. Alternatively, a colloidal solution may be prepared from chitin, preferably in isotonic saline, or a water-soluble derivative of chitin may be dissolved preferably in isotonic saline solution, and the solution administered intramuscularly, parenterally or intravenously.

A powder or solution of chitin or of a chitin derivative may also be used to impregnate a surgical gauze or pad which is applied to the wound. Chitin may also be dissolved as the alkaline xanthate, spun into fibers and regenerated as the virtually undegraded polymer in accordance with the procedures described in the prior art by Thor et al. Partially deacetylated chitin filaments and fibers may be prepared in accordance with the procedures described in U.S. Pat. No. 2,040,880. These chitin fibers may then be used as surgical sutures or included in bandages or other support base for surgical dressings either in a woven or nonwoven fabric structure in the manner described in U.S. Pat. 3,196,075. Chitin or chitin derivative may also be made up into an ointment or salve. The use of nonactive carriers for the chitin is not preferred as the presence of extraneous matter in a wound frequently tends to interfere with the healing process due to the interposition effect.

As previously stated, where the chitin is to be applied by injection, i.e., either intramuscularly, parenterally or intravenously, it is first necessary to prepare a dispersion or a solution of the material in a pharmaceutically acceptable liquid. Colloidal solutions of chitin may be prepared by dispersing chitin in water-soluble or water dispersible media. Colloidal solutions of chitin, for example, may be prepared by dissolving chitin in isotonic saline, or a water-soluble derivative of chitin may be prepared. It is preferred to administer the compound, in isotonic solution, such as isotonic saline, intravenously, it is preferred to administer the compound, in isotonic solution, such as isotonic saline, intramuscularly, parenterally or intravenously, as it is first necessary to prepare a dispersion or a solution of the material in a pharmaceutically acceptable liquid. Colloidal solutions of chitin may be prepared by dispersing chitin in water-soluble or water dispersible media. Colloidal solutions of chitin, for example, may be prepared by dissolving chitin in isotonic saline, or a water-soluble derivative of chitin may be prepared. It is preferred to administer the compound, in isotonic solution, such as isotonic saline, intravenously, or as an aerosol.

In the following examples, the wound healing efficiency of the various chitinaceous materials is determined by using the method of Prudden et al as described above. In general, at least 10 pairs of rats are used to obtain a meaningful average for each material tested. In each of these examples a powder insufflator is used to apply 2 to 10 mg/cm² of wound surface of the material tested.

**EXAMPLE 1**

Commercial lobster shell chitin is ground to a fine powder in a laboratory four-quart size porcelain jar mill loaded with one-inch size (average) flint pebbles in a weight ratio of 1:1 to 2:1 pebbles. Dry ice is then put on top of the mill charge and the mill is kept open for 5 minutes to allow the CO₂ to displace the air in the mill. The lid of the mill is then clamped on tight and the grinding carried out for 96 hours. Approximately 50% of the powdered chitin passed through a 40 micron screen.

The whole powdered chitin so produced is then applied to the 45 test rats of 45 pairs of rats used in the Prudden et al assay method described above. The percent of wound healing for the treated rats, stating the control rats as 100%, is 122%, i.e., the use of chitin results in an average 22% increase in wound healing activity.

**EXAMPLES 2-5**

Various fungi are grown on either brain-heart infusion (200 gm. calf brain, 250 gm. beef heart, 10 gm. proteose peptone, 2 gm. dextrose, 5 gm. sodium chloride and 2.5 gm. disodium phosphate) called “BHI” or on Sabouraud’s broth (40 gm. dextrose and 10 gm. bac-to-peptone) called “SAB”. The cultures are grown in shallow layers of media contained in flasks and held stationary until good growth and extensive sporulation occurs. Prior to collection of the growth mats, the cultures are killed by placing the flasks into a closed oven under CO₂ at 127°C. for three hours. The flasks are then cooled in the oven for an additional one hour and fifteen minutes. Culture broths are removed by filtration through Buchner funnels and the growth mats washed with distilled water. The mats are then frozen and lyophilized and the dry products ground in a mortar with a pestle under CO₂. No attempt is made to purify the chitin. Twelve pairs of rats are used for each test. Some inflammation is observed on all treated wounds and infection on several. The increase in wound healing obtained may be all the more significant in view of those adverse factors.
<table>
<thead>
<tr>
<th>Ex.</th>
<th>Fungus</th>
<th>Me-</th>
<th>%Wound Healing (Control=100)</th>
<th>%Improvement</th>
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<td>Cryptococcus</td>
<td>BHI</td>
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</tr>
</tbody>
</table>

**EXAMPLE 6**

100 grams of dried fungus material (obtained from Penicillium fungus of Example 4, cultured on a BHI medium, sterilized by boiling the fungus with the medium and then filtering, washing with distilled water and drying the fungus material) is defatted by extracting the solvent-soluble fatty materials with 1000 ml. chloroform at room temperature. The chloroform is removed by filtering and then drying at reduced pressure in a vacuum desiccator.

The defatted fungus material is treated with 2000 ml. 1.0 N—NaOH solution for 18 hours at room temperature. The material is then acidified with HCl. Thereafter the material is dialyzed in distilled water until the wash water is free from chlorine ions. This procedure is repeated until a substantially purified material is obtained. The material is dried in vacuum below 50°C and is a gray, friable mass.

The dried material is ground in a laboratory mortar and screened through a 400 mesh standard screen. When the screened material is applied to 20 test rats of 20 pairs of rats there is obtained an average of about 25% increase in the wound healing of the treated rats over the untreated control rats.

**EXAMPLE 7**

Lobster shell chitin is purified by first slurring it in 10% aqueous NaOH for 5 minutes at 80°C, then it is washed, drained and slurried in 10% HCl for 5 minutes at 80°C, drained, slurried in water, the pH of the water adjusted to 6 with dilute aqueous NaOH, and finally drained and dried.

The dried chitin material is pulverized to a fineness of about 40 microns. The material shows an average 25% increase in the wound healing over the untreated control rats.

Although the present invention has been described in conjunction with preferred embodiments, it is to be understood that modifications and variations may be resorted to without departing from the spirit and scope thereof, as those skilled in the art will readily understand.

What is claimed is:

1. A process for facilitating healing of a wound in a mammal which comprises applying as a wound healing aid at the situs of the wound a wound-healing amount of a woven fabric structure including fibers selected from the group consisting of chitin and an N-acetylated partially depolymerized chitin.

2. A process according to claim 1 wherein said wound healing aid is in the form of a bandage including chitin fibers.

3. A process according to claim 1 wherein said fibers are used in the form of sutures.

4. A process according to claim 1, wherein said wound healing aid is in the form of a dressing including chitin fibers.

5. A process according to claim 1, wherein said wound healing aid is in the form of a bandage including N-acetylated partially depolymerized chitin fibers.

6. A process for facilitating healing of a wound in a mammal which comprises applying as a wound healing aid at the situs of the wound a wound-healing amount of a non-woven fabric structure including fibers selected from the group consisting of chitin and N-acetylated partially depolymerized chitin.

7. A process according to claim 6, wherein said wound healing aid is in the form of a bandage including N-acetylated partially depolymerized chitin fibers.

8. A process according to claim 6, wherein said wound healing aid is in the form of a dressing including chitin fibers.

9. A process according to claim 6, wherein said wound healing aid is in the form of a dressing including N-acetylated partially depolymerized chitin fibers.

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