

[54] **PROCESS FOR PROMOTING WOUND HEALING WITH CHITIN DERIVATIVES**

[58] **Field of Search**..... 424/180, 28, 95

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[56] **References Cited**

[*] **Notice:** The portion of the term of this patent subsequent to Jan. 4, 1989, has been disclaimed.

UNITED STATES PATENTS

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| 2,040,879 | 5/1936 | Rigby..... | 260/54 |
| 2,795,579 | 6/1957 | Doczi..... | 260/211 |
| 3,232,836 | 2/1966 | Carlozzi et al..... | 424/180 |
| 3,257,275 | 6/1966 | Weisberg et al..... | 424/330 |

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Related U.S. Application Data

[63] Continuation of Ser. No. 27,977, April 13, 1970, abandoned, which is a continuation-in-part of Ser. Nos. 619,007, Feb. 27, 1967, abandoned, which is a continuation of Ser. No. 704,538, Feb. 12, 1968, Pat. No. 3,632,754.

[57] **ABSTRACT**

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

[52] **U.S. Cl.** 424/180; 424/28; 424/95

4 Claims, No Drawings

[51] **Int. Cl.²**..... A61K 31/70

PROCESS FOR PROMOTING WOUND HEALING WITH CHITIN DERIVATIVES

This is a continuation of application Ser. No. 27,977, filed Apr. 13, 1970 and now abandoned, which in turn is a continuation-in-part of my copending application Ser. No. 619,007 filed Feb. 27, 1967, now abandoned, and a continuation of application Ser. No. 704,538 filed Feb. 12, 1968, now U.S. Pat. No. 3,632,754.

This invention relates to methods of promoting the healing of wounds and compositions therefor comprising chitin derivatives.

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-acetylglucosamine as a wound healing material. Utilizing the test method of Prudden et al referred to in the preceding paragraph, N-acetylglucosamine 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.

An additional aspect of the present invention is concerned with articles of manufacture such as surgical bandages, surgical sutures, etc., containing the wound healing materials of the present invention.

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

Chitin is a polysaccharide, believed to be poly (N-acetylglucosamine) 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-acetylglucosamine) and its epimer poly (N-acetylgalactosamine). The partially depolymerized chitin, e.g. chitotriose, chitobiose, 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.

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 zinc 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-acetylglucosamine), i.e., chitin, as compared to monomeric N-acetylglucosamine 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-acetylglucosamine. 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 alkali chitin 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 procedure 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. No. 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 using the method described by Lingappa and Lockwood in NATURE, 189, page 158 (1961). When administered intravenously it is preferred to administer the compound in isotonic solution such as isotonic saline.

The chitin or chitin derivatives may be used alone, in admixture with each other, with cartilage, or may be co-administered with other therapeutically effective agents such as ascorbic acid, ascorbyl palmitate, pharmaceutically acceptable zinc salts such as zinc oxide, zinc ascorbate, zinc sulfate and zinc stearate; antiseptics such as thimerosal and benzalkonium chloride; local anesthetics such as lidocaine and procaine; antibiotics such as chloramphenicol, sulfanilamide and ampicilline. Combinations of the therapeutically effective agents described above with chitin and/or chitin derivatives may be used.

Suitable sources of chitin are from lobsters, shrimp and other crustacea. To utilize chitin from such sources, it is necessary to reduce the chitin in particle size to less than about 150 microns and preferably less than about 50 microns. Due to the tough and rather fibrous nature of chitin from such sources, this grinding is difficult and expensive. Accordingly, it is preferred to use chitin of fungal origin. The cell walls of fungi are made of chitin. It has been found that it is not necessary to extract the chitin from the remaining cell material. Thus, if desired, after suitable sterilization as by heat or gas (i.e., ethylene oxide), the entire fungal mat produced by fermentation of a fungus in a suitable nutrient medium may be ground and used to promote healing of wounds. Preferably, however, the fungal mat is treated to remove the extraneous materials leaving only the chitin skeletons. Purifying the material in this manner eliminates the nonchitinous materials, thus substantially reducing the possibility of an allergic reaction and eliminating any interference with the healing process which might be caused by such materials.

Finely divided chitin or chitin derivatives may be applied topically by blowing a metered amount of the material onto the wound using a hand atomizer. Alternatively, it may be applied by dusting as from a hand shaker or may be placed together with an inert gas under increased pressure (i.e., above atmospheric pressure) in a pressure vessel. In this latter means of appli-

cation, termed "aerosol application," the finely divided chitin or chitin derivative, optionally with other medicaments as indicated, may be packaged as a dry aerosol powder as described in Dutch patent application No. 6,415,252, published July 5, 1965 (this patent application is directed to a medicament for bovine mastitis but the method of aerosol packaging described is applicable to powdered medicament having the described particle size) or as an aerosol foam.

In the following examples, the wound healing efficiency of the various chitinous 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 4-quart size porcelain jar mill loaded with 1-inch size (average) flint pebbles in a weight ratio of 1 chitin to 2 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. bacto-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 3 hours. The flasks are then cooled in the oven for an additional 1 hour and 15 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 increases in wound healing obtained may be all the more significant in view of those adverse factors.

| Example | Fungus | Me- dium | % Wound Healing (Control = 100) | % Improve- ment |
|---------|-------------------|-------------|--|--------------------|
| 2 | Mucor spinosus | SAB | 136 | 36 |
| 3 | Aspergillus niger | BHI | 118 | 18 |
| 4 | Penicillium | BHI | 146 | 46 |
| 5 | Cryptococcus | BHI | 128 | 28 |

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 1,000 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 2,000 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 topically administering a therapeutically effective dosage of a material selected from the group consisting of hydroxy lower alkyl chitin, carboxy lower alkyl chitin, lower alkyl chitin, chitin acetate, chitin phosphate, chitin nitrate, a salt of carboxy lower alkyl chitin and chitin citrate.

2. A process for accelerating the healing of a surgical wound in a mammal requiring healing which comprises administering to said wound a therapeutically effective dosage of a finely divided material selected from the group consisting of hydroxy lower alkyl chitin, carboxy lower alkyl chitin, lower alkyl chitin, chitin acetate, chitin phosphate, chitin nitrate, a salt of carboxy lower alkyl chitin and chitin citrate.

3. A process for facilitating healing of a wound in a mammal which comprises parenterally administering a therapeutically effective dosage of a material selected from the group consisting of hydroxy lower alkyl chitin, carboxy lower alkyl chitin, lower alkyl chitin, chitin acetate, chitin phosphate, chitin nitrate, a salt of carboxy lower alkyl chitin and chitin citrate.

4. A process according to claim 2 which comprises topically administering said finely divided material at the site of said wound.

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