PATENT SPECIFICATION

NO DRAWINGS

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The inventor of this invention in the sense of being the actual deviser thereof within the meaning of Section 16 of the Patent Act 1949 is Jorgen Schlichtkrull, a Danish subject of 115 Fuglebakkevej, Copenhagen, Denmark.

Improvements in or relating to insulin preparations.

COMPLETE SPECIFICATION

We, NOVO TERAPEUTISK LABORATORIUM A/S, a Danish Body Corporate, of 115 Fuglebakkevej, Copenhagen, Denmark, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to injectable insulin preparations having a protracted effect and to a process for producing such preparations.

It is known that insulin may be crystallized from an aqueous medium containing ions, hereinafter called M-ions, of one or more of the crystallization-promoting metals (zinc, cadmium, cobalt, nickel and copper), preferably zinc, and having a pH value of between 5.5 and 7. In the crystallization use has been made of a buffer substance, usually sodium phosphate, sodium citrate, ammonium acetate or sodium acetate. Sometimes use is also made of an addition of solvents to prevent precipitation of amorphous insulin. Examples of such solvents are acetone and propyl, butyl and amyl alcohols.

The insulin crystals thus produced contain M-ions chemically bound within the crystal structure and the amount thereof varies, depending on the crystallization conditions employed. It may be expressed as the percentage of metal in the dried crystals or as the amount of metal in gram-equivalents per gram of the dried crystals. By dried crystals is to be understood crystals having such a water content that they exhibit a content of protein nitrogen of about 14.2 per cent.

When the crystallization is effected in the presence of citrate buffer, the crystals produced will be found to contain about 0.1 milliequivalent of the crystallization-promoting metal per gram of the dried crystals, corresponding to 0.34 per cent zinc. When crystallization is effected in the presence of sodium phosphate buffer and recrystallization is effected in the presence of ammonium acetate buffer without addition of zinc ions, the recrystallized crystals will contain about 0.16 milliequivalent of the crystallization-promoting metal per gram of the dried crystals, corresponding to 0.52 per cent of zinc.

If the insulin is crystallized from a solution in the absence of zinc-precipitating substances, such as phosphate ions, or substances forming complex compounds with zinc, such as citrate ions, use being made for example of sodium acetate buffer, the crystals produced will, when use is made of a sufficiently high content of M-ions in the crystallization medium, be found to contain up to about 0.25 milliequivalent of the crystallization-promoting metal per gram of the dried crystals, corresponding to a zinc content of about 0.8 per cent.

If the crystallization medium shows such a high M-ion concentration that an insulin crystal when suspended therein will be found to contain more than about 0.32 milliequivalent of the crystallization-promoting metal per gram of the dried crystals, corresponding to about 1% of zinc, insulin crystals will only be formed very slowly or not at all.

The insulin crystals produced in these ways have a rhombohedral structure and the form of twin crystals, "stars" or deformed rhombohedrons when the insulin originates from ox or calf pancreas glands.

It should be noticed that, when insulin originating from ox or calf pancreas glands is employed, insulin crystals may be obtained having the form of sharp-edged rhombohedrons with plane crystal faces if the crystallization medium contains halogen ions, for example in the form of added sodium chloride, at a suitably high concentration (see British Patent Specification No.

(Price 3s. 6d.)

733,740). Crystals of this kind are not considered here.

Insulin crystals having a zinc content of 0.52 per cent have been suspended in distilled water and injected into rabbits (see *J. Pharm.*, 58, 93-104 (1936)), and it has been found that the effect can hardly be distinguished from the quick-acting and brief effect of a solution of the crystals. If on the other hand M-ions are added to the crystal suspension in such a proportion that the suspenson shows a content of the crystallization-promoting metal of at least $13 \times A \times 10^{-3}$ milliequivalents per liter, A being the number of international units of insulin per millilitre of the suspension, the later will show a useful prolonged insulin action when injected parenterally, and the suspended insulin crystals will, at neutral reaction of the suspension medium show a metal content of at least 0.25 milliequivalent per gram of the dried crystals, *vide* British Patent Sepecification No. 709,927.

It has now been shown that it is not an essential condition for obtaining a highly protracted effect of insulin crystal suspensions, that absorption of crystallization-promoting metals by the insulin crystals from the suspension medium takes place at pH 7.

The present invention is based on the following discoveries.

If insulin crystals originating from ox or calf pancreas glands and having the form of twin crystals, "stars" or deformed rhombohedrons are suspended in an aqueous medium having a pH value of 5.5 and having such a composition that the content of free M-ions in the suspension is so low that at pH 7 of the medium the insulin crystals will contain less than 0.25 milliequivalent of the crystallization-promoting metal per gram of the dried crystal it will be found that this suspension has no appreciable protracted effect. If on the other hand the suspension is given pH 7, the effect of the suspension will surprisingly prove to be appreciably prolonged. This is all the more surprising when compared with the properties of insulin crystals produced from pig pancreas glands. If the suspension is given the same composition as that referred to above except that insulin crystals originating from pig pancreas glands are used, and the suspension is given a pH of 5.5, this suspension will not possess any protracted effect, nor will it do so if the pH value is changed to 7, the insulin crystals thereby going into solution.

Further, it has turned out that the time of action of aqueous suspensions of insulin crystals originating from ox or calf pancreas glands will be increased if these suspensions are stored for an extended period of time at room temperature or at more elevated temperatures and if they have a pH value appreciably below 7, while there will be no change of the time of action of practical importance if the suspensions have a pH value of about 7 (6.5 to 7.5). An insulin preparation is required to be stable, and tests performed have shown that the stability of these suspensions will be obtained at a pH value of about 7(6.5 to 7.5) and only within this pH range.

In accordance with the above observations the insulin preparations of the invention comprise a suspension of insulin crystals in an injectable aqueous medium, said suspension containing ions of at least one of the crystallization-promoting metals zinc, cadmium, cobalt, nickel and copper, preferably zinc, said insulin crystals in suspension showing a content of the metals referred to below 0.25 milliequivalent per gram of the dried crystals at pH 7.0 of the suspension medium, and the characteristic features of the preparations are that the insulin crystals originate from ox or calf pancreas glands and have a rhombohedral structure and the form of twin crystals, "stars" or deformed rhombohedrons, and that the suspension has a pH value of about 7.

According to the process for producing these insulin preparations insulin crystals originating from ox or calf pancreas glands and having a rhombohedral structure and the form of twin crystals, "stars" or deformed rhombohedrons are suspended in an injectable aqueous medium, and the suspension is given a pH value of about 7 and is adjusted on such content of at least one of the crystallization-promoting meals zinc, cadmium, cobalt, nickel and copper, prefereably zinc, that the suspended insulin crystals contain less than 0.25 milliequivalent of the said metals per gram of the dried crystals at pH 7.0 of the suspension medium.

It is possible to carry out the process by suspending pre-prepared insulin crystals in an injectable aqueous medium. In such case it is preferred to use insulin crystals that have not beforehand been subjected to any drying as a drying process has proved to decrease the time of action of the suspensions.

It is, however, more appropriate to carry out the crystallization of the insulin under sterile conditions and to give the crystallization medium such a composition that it may be made injectable so that according to the invention the mother liquor of the crystals is used as suspension medium, to which are added substances, such as water, isotonics and preserving agents, making the medium suitable for injection.

It is preferred to use an aqueous medium not containing substances, such as buffer substances, forming complexes or slightly soluble compounds with the free M-ions, in which case the total content of M-ions in the

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suspension produced is not allowed to reach $13 \times A \times 10^{-3}$ milliequivalents per litre, A being the number of international insulin units per millilitre.

The insulin crystal suspensions according to the invention posses an initial effect superior to that obtainable with the known aqueous insulin crystal suspensions which contain in addition amorphous insulin in suspension and which have been given such higher content of zinc ions that the suspended insulin crystals at pH 7 of the suspension medium will contain about 2.3 per cent of zinc.

If it is desired to obtain a more pronounced initial effect from the insulin crystal suspensions according to the invention, use may be made of the addition of a solution of insulin originating from pig pancreas glands, said solution having the same pH value. Such a solution is disclosed and claimed in our co-pending Application No. 22705/58 (Serial No. 840870). The stability of the suspensions is not influenced thereby under storage.

The present invention will be further illustrated by the following specific Example.

EXAMPLE

3.48 grams of ox insulin crystals having the form of twin crystals, "stars" and deformed rhombohedrons and containing 0.78 per cent of zinc are suspended in 100 millilitres of water, and dissolved by the addition of 4 millilitres of N-hydrochloric acid. The solution is sterile-filtered and diluted to 150 millilitres with sterile water. To this solution are added 50 millilitres 0.4 M sodium acetate containing so much sodium hydroxide that the mixture attains a pH of 5.5. The mixture is then slowly stirred for about 24 hours, during which time the insulin crystallizes, whereafter the suspension is diluted by the addition of 1800 millilitres of methyl *p* hydroxybenzoate solution containing 0.11 per cent by weight of methyl *p*-hydroxybenzoate and 14 grams of sodium chloride and so much sodium hydroxide that the pH value of the final suspension is 7.3 to 7.4.

Instead of using sodium acetate buffer, as in the above Example, it is possible to use sodium phosphate or sodium citrate buffer. It is also possible to use ox insulin crystals having a lower zinc content or ox insulin crystals having a content of other crystallization-promoting metals than zinc.

Further, the aqueous suspension medium may be given an extra addition of M-ions, it being kept in mind, however, that the M-ion content may not be so high that the metal content of the suspended insulin crystals at pH 7.0 of the suspension medium is allowed to reach 0.25 milliequivalent per gram of the dried crystals.

WHAT WE CLAIM IS:—

1. An insulin prepartion having a protracted effect, which comprises a suspension of insulin crystals in an injectable aqueous medium, said suspension containing ions of at least one of the crystallization-promoting metals zinc, cadmium, cobalt, nickel and copper, preferably zinc, and said insulin crystals in suspension showing a content of the metals referred to below 0.25 milliequivalent per gram of the dried crystals at pH 7.0 of the suspension medium, characterized by the fact that the insulin crystals originate from ox or calf pancreas glands and have a rhombohedral structure and the form of twin crystals, "stars" or deformed rhombohedrons and that the suspension has a pH value of about 7.

2. An insulin preparation as claimed in claim 1, also containing dissolved insulin originating from pig pancreas glands.

3. A process for producing the insulin preparation claimed in claim 1, wherein insulin crystals originating from ox or calf pancreas glands and having a rhombohedral structure and the form of twin crystals, "stars" are deformed rhombohedrons are suspended in an injectable aqueous medium and the suspension is given a pH value of about 7 and is adjusted to such a content of at least one of the crystallization-promoting metals zinc, cadmium, cobalt, nickel and copper, preferably zinc, that the suspended insulin crystals contain less than 0.25 milliequivalent of the said metals per gram of the dried crystals at pH 7.0 of the suspension medium.

4. A process as claimed in claim 3, wherein use has been made of insulin crystals which have not beforehand been subjected to any drying process.

5. A process for producing the insulin preparation claimed in claim 1, wherein the crystals are formed in the suspension medium to which are added substances, such as water, isotonics and preserving agents, making the medium suitable for injection.

6. A process for the production of an insulin crystal suspension having a protracted effect, substantially as hereinbefore described with particular reference to the specific Example.

NOVO TERAPEUTISK LABORATORIUM A/S.
Per: BOULT, WADE & TENNANT,
111/112 Hatton Garden, London, E.C.1.
Chartered Patent Agents.

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suspension produced is not allowed to reach $13 \times A \times 10^{-3}$ milliequivalents per litre, A being the number of international insulin units per millilitre.

The insulin crystal suspensions according to the invention posses an initial effect superior to that obtainable with the known aqueous insulin crystal suspensions which contain in addition amorphous insulin in suspension and which have been given such higher content of zinc ions that the suspended insulin crystals at pH 7 of the suspension medium will contain about 2.3 per cent of zinc.

If it is desired to obtain a more pronounced initial effect from the insulin crystal suspensions according to the invention, use may be made of the addition of a solution of insulin originating from pig pancreas glands, said solution having the same pH value. Such a solution is disclosed and claimed in our co-pending Application No. 22705/58 (Serial No. 840870). The stability of the suspensions is not influenced thereby under storage.

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WHAT WE CLAIM IS:—

1. An insulin prepartion having a protracted effect, which comprises a suspension of insulin crystals in an injectable aqueous medium, said suspension containing ions of at least one of the crystallization-promoting metals zinc, cadmium, cobalt, nickel and copper, preferably zinc, and said insulin crystals in suspension showing a content of the metals referred to below 0.25 milliequivalent per gram of the dried crystals at pH 7.0 of the suspension medium, characterized by the fact that the insulin crystals originate from ox or calf pancreas glands and have a rhombohedral structure and the form of twin crystals, "stars" or deformed rhombohedrons and that the suspension has a pH value of about 7.

2. An insulin preparation as claimed in claim 1, also containing dissolved insulin originating from pig pancreas glands.

3. A process for producing the insulin preparation claimed in claim 1, wherein insulin crystals originating from ox or calf pancreas glands and having a rhombohedral structure and the form of twin crystals, "stars" are deformed rhombohedrons are suspended in an injectable aqueous medium and the suspension is given a pH value of about 7 and is adjusted to such a content of at least one of the crystallization-promoting metals zinc, cadmium, cobalt, nickel and copper, preferably zinc, that the suspended insulin crystals contain less than 0.25 milliequivalent of the said metals per gram of the dried crystals at pH 7.0 of the suspension medium.

4. A process as claimed in claim 3, wherein use has been made of insulin crystals which have not beforehand been subjected to any drying process.

5. A process for producing the insulin preparation claimed in claim 1, wherein the crystals are formed in the suspension medium to which are added substances, such as water, isotonics and preserving agents, making the medium suitable for injection.

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