PATENT SPECIFICATION

DRAWINGS ATTACHED

835,638

Date of Application and filing Complete Specification: Nov. 15, 1957.

No. 35737/57

Application made in Denmark on Dec. 1, 1956.

Complete Specification Published : May 25, 1960.

Index at Acceptance:—Class 81(1), B2(B2 : C : D : G : H : S : Z), B12A.

International Classification:—A61k.

The inventor of this invention in the sense of being the actual deviser thereof within the meaning of Section 16 of the Patents Act 1949 is JØRGEN SCHLICHTKRULL, a Danish subject of 28B Ved Bellahøj, Brønshøj, Denmark.

COMPLETE SPECIFICATION

Insulin Crystal Suspensions having a Protracted Effect

We, NOVO TERAPEUTISK LABORATORIUM A/S, a Danish Joint Stock Company, 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:—

This invention relates to injectable insulin crystal suspensions having a protracted effect.

It is known to cause insulin to crystallize from an aqueous medium containing a crystallization-promoting metal, preferably zinc, by adjusting the aqueous medium to a pH-value within the range of 5 to 7. In order to fix the pH-value during the crystallization use is made of a buffer substance, usually sodium phosphate, sodium citrate, ammonium acetate or sodium acetate. Sometimes use has also been made of an addition of organic solvents able to prevent precipitation of amorphous insulin. As examples may be mentioned acetone, propyl, butyl and amyl alcohol.

The insulin crystals produced by these processes contain ions of crystallization-promoting metals chemically bound within the crystal structure itself, and the amount thereof varies depending on the conditions of crystallization employed. The amount of ions of crystallization-promoting metals may be expressed as the number of metal atoms per unit cell, a unit cell being defined as the crystallographic unit containing 390 atoms of protein nitrogen. It may also be expressed as the percentage of metal in the dried crystals or as the number of milliequivalents of metal per gram of the dried crystals. By dried crystals is to be understood crystals having such a water content that they show a content of protein nitrogen of about 14.2%.

When use is made of sodium citrate buffer, crystals will be obtained containing 2 metal atoms per unit cell corresponding to 0.34% of zinc. When the crystallization is effected in the presence of sodium phosphate buffer and recrystallization is effected in the presence of ammonium acetate buffer without any addition of zinc ions, recrystallized crystals will be obtained containing 3 metal atoms per unit cell of the crystals corresponding to 0.52% of zinc.

When the insulin is crystallized from a solution in the absence of zinc-precipitating substances, such as phosphate buffers, or in the absence of substances, such as citrate buffers, forming strongly complex zinc compounds, use being for instance made of sodium acetate buffer, and when use is made of a sufficiently high content of ions of crystallization-promoting metals in the crystallization medium, crystals will be obtained containing up to 5 metal atoms per unit cell, which in the case of zinc corresponds to a zinc content of about 0.8%.

If the crystallization medium has such a high ion concentration of crystallization-promoting metals that an insulin crystal after having been suspended therein will contain more than about 6 metal atoms per unit cell, insulin crystals will only be formed very slowly or not at all.

The insulin crystals produced by the processes described above are of rhombohedral structure but will not appear very often in the form of sharp-edged rhombohedra bounded by plane crystal faces. If the insulin contains impurities prior to the crystallization the resultant crystals will appear as rounded crystal bodies, when insulin derived from pork pancreas glands has been used as starting material. When insulin derived from bovine pancreas glands is used, the resultant crystals will have the form of twins, "stars" or de-

[*Price 3s. 6d.*]

formed rhombohedra, even after recrystallization. On the other hand, if the crystallization is effected as described in British Patent Specification No. 733,740, or is effected in the presence of triethanolamine hydrochloride, urea or formamide, insulin derived from bovine pancreas glands will also crystallize in the form of sharp-edged rhombohedra bounded by plane crystal faces.

When insulin crystals containing 0.52% of zinc and produced by crystallization from a sodium phosphate buffer solution and recrystallized from an ammonium acetate buffer solution without addition of extra zinc, are suspended in distilled water and injected into rabbits, the obtained biological action can hardly be distinguished from the quick and short action of an insulin crystal solution (ordinary insulin). On the other hand, if a water-soluble salt of a crystallization-promoting metal is added to the said insulin crystal suspension a strongly protracted effect will be obtained in that the suspended crystals at neutral reaction (the pH of the blood) will combine with the ions of crystallization-promoting metals under formation of a slightly soluble and slowly resorbable insulin-metal compound showing an increased metal content which will depend on the insulin concentration and the concentration of the crystallization-promoting metal ions added, vide British Patent Specification No. 709,927 according to which the suspended insulin crystals contain at least 0.25 milliequivalent of the crystallization-promoting metals zinc, cadmium, nickel, cobalt or copper, respectively, per gram of the crystals at pH 7 of the suspension medium, corresponding to about 5 atoms per unit cell of crystallization-promoting metals. A similar protracted effect is obtained when instead of the insulin crystals containing 0.52% of zinc insulin crystals are used containing about 0.7% of zinc and having the form of sharp-edged rhombohedra limited by plane crystal faces.

It has now been found that without creating such conditions in the aqueous medium that the suspended insulin crystals show a content of the specified metals of at least about 5 atoms per unit cell when the aqueous medium has a pH-value of 7, it is possible to obtain a reliable protracted effect of clinical value, provided use is made of the known insulin crystals derived from bovine pancreas glands and having the form of sharp-edged rhombohedra limited by plane crystal faces and the aqueous insulin crystal suspension satisfies quite specific conditions.

The injectable insulin preparation of the present invention comprises a suspension of insulin crystals derived from bovine pancreas glands and having the form of sharp-edged rhombohedra limited by plane crystal faces in an injectable aqueous medium, said suspension having a pH-value of 6.5 to 7.5 and said suspension medium showing such a content of crystallization-promoting metal ions that the suspended insulin crystals contain chemically bound within the crystal lattice less than 5 atoms per unit cell of crystallization-promoting metals.

On the background of what was known in the art it could not be foreseen that such a suspension possesses a clinically valuable prolonged insulin action and that it was a condition for the suspension to be stable, i.e. to maintain the same therapeutic effect even after long storage at a temperature of 25°C or more, that the suspension has a pH-value of about 7.

According to the present invention there is also provided a process for producing the injectable insulin preparations having a protracted effect. In this process insulin crystals having the form of sharp-edged rhombohedra limited by plane crystal faces are suspended in an injectable aqueous medium, and the characteristic features of the process comprise using insulin crystals derived from bovine pancreas glands, adjusting the pH-value of the suspension to 6.5 to 7.5 and giving the aqueous suspension medium such a content of crystallization-promoting metal ions that the suspended insulin crystals contain chemically bound within the crystal lattice less than 5 atoms per unit cell of crystallization-promoting metals.

When the bovine insulin crystals are produced as mentioned in the foregoing, they will show a content of ions of crystallization-promoting metals chemically bound within the crystal lattice, of at least 4 metal atoms per unit cell corresponding to a zinc content of about 0.7%. However, the metal content may be removed after the crystals have been formed so that crystals may be prepared which do not show any metal ion content. Thus, if insulin crystals containing 4 metal atoms per unit cell are suspended in water containing sodium citrate buffer (pH 6 to 7) the suspended crystals will only contain 2 metal atoms per unit cell, and if the crystals are washed with an acidified 20% sodium chloride solution (pH 2.5) all the metal ions will be removed from the crystals.

From a clinical view point it is advantageous to have at one's disposal insulin preparations with different timing of action. It is to be understood that "the time of action" and "the duration of action" are the period of time in which the preparation in question shows any blood sugar-lowering effect, while the terms "timing of action" and "biological timing" mean the manner in which the preparation in question lowers the blood sugar level during its action. Thus, two preparations may show the same "duration of action" and different "timing of action" in that one of the preparations might show a comparatively big initial effect while the other preparation may have no such effect. A different timing of action may according to the present invention be obtained in various ways.

Firstly, the timing of action of the produced suspensions may be changed by the employment of insulin crystals of different origin, it being such that suspensions of insulin crystals derived from pork pancreas glands and even having the form of sharp-edged rhombohedra bounded by plane crystal faces show a relatively short duration of action. Thus, it is possible to obtain a desired initial effect by employing insulin crystals a predetermined percentage of which originate from swine pancreas. By varying the weight proportion in the crystals between insulin originating from swine pancreas and insulin originating from ox pancreas it is possible within broad limits to vary the biological timing of action of the final suspension.

Secondly, the duration of action of the produced crystal suspensions increases with increasing crystal size. When using small crystals, e.g. about 3μ, and larger crystals, e.g. 30μ, in varying weight proportions it is possible to vary the rate of resorption and hence the biological timing of the suspensions which is the longer the larger are the crystals. Consequently, it is convenient to use insulin crystals having a predetermined size distribution.

Thus, by means of the present invention it is possible in an extremely simple way using only insulin crystals as such without the use of additional auxiliary substances, to produce insulin preparations having a polyphasic effect, i.e. containing both quick-acting and slow-acting components. Actually, by means of the present invention one has for the first time without additional substances of any kind succeeded in placing at the disposal of diabetes therapy insulin preparations consisting of an insulin crystal suspension having a protracted effect and being mixable in any proportion with a quick-acting insulin crystal suspension and with dissolved insulin.

The preparation of the crystal suspensions takes place under aseptic conditions. It is preferred to make the suspension medium isotonic, e.g. by means of sodium chloride, and a preserving agent may be added, e.g. methyl *p*-hydroxybenzoate and, if desired, a buffer substance, e.g. an acetate, phosphate or citrate buffer. As mentioned above, the insulin crystal suspensions are given a pH-value of 6.5 to 7.5, because the suspensions are most stable within this limited pH-range. The crystals employed are preferably produced with the employment of zinc as crystallization-promoting metal. Together with or instead of zinc it is, however, also possible to use other crystallization-promoting metals, such as nickel, cadmium, cobalt and copper.

Following is a description, by way of example, of processes in accordance with the present invention.

EXAMPLE 1

A sterile mixture is produced containing 1.7% of insulin from ox pancreas, 0.013% of zinc in the form of zinc chloride, 0.1 M sodium acetate and 7% of sodium chloride together with so much sterile hydrochloric acid that the pH-value of the mixture will be 5.5. The crystals produced in this medium have the form of sharp-edged rhombohedrons with plane crystal faces and contain about 0.7% of zinc corresponding to about 0.21 milli-equivalent of zinc per gram, and have a biological activity of about 23 international units per mg.

One part of the insulin crystal suspension thus produced is diluted with 9 parts of sterile water containing 0.11% of methyl *p*-hydroxybenzoate, whereafter the pH-value of the suspension is changed to 7.0 by the addition of 1 N sodium hydroxide. The insulin crystals in this final suspension will show a zinc content of about 0.7%.

EXAMPLE 2

A sterile mixture is produced containing 1.7% of insulin from ox pancreas, 0.13% of zinc in the form of zinc chloride, 0.05 M sodium citrate and 7% of sodium chloride, and from this mixture insulin is crystallized by adding so much sterile hydrochloric acid that the pH-value of the mixture will be 6. The crystals produced from this mixture have the form of sharp-edged rhombohedrons with plane crystal faces and contain about 0.7% of zinc corresponding to about 0.21 milli-equivalent of zinc per gram.

The insulin crystal suspension thus produced is diluted with so much sterile water containing 0.11% of methyl *p*-hydroxybenzoate that the final crystal suspension will contain 40 international units of insulin per millilitre after the suspension has been adjusted to pH 7 by the addition of 1 N sodium hydroxide. Because of the content of citrate buffer in the crystallization medium the zinc content of the suspended crystals will not be increased when the suspension is adjusted to pH 7.

The insulin crystal suspension thus produced has a protracted effect corresponding to that of the suspension produced according to Example 1.

EXAMPLE 3

A suspension of insulin crystals is prepared in the same manner as in the first paragraph of Example 1, and the suspension produced is diluted with sterile water containing so much zinc in the form of zinc chloride and so much methyl *p*-hydroxybenzoate that the final suspension after having been adjusted to pH 7.4 contains 8.6 mgs of zinc per 100 millilitres and 0.1% of methyl *p*-hydroxybenzoate. When the suspension is adjusted to pH 7.4 the insulin crystals will take up zinc from the medium so that they will contain about 2.3% of zinc corresponding to about 0.7 milli-equivalent per gram. Then the crystals are removed by suction and dried whereafter 174 mgs thereof are suspended in 100 millilitres of

sterile water containing 0.7% of sodium chloride, 1/100 M sodium phosphate and 0.1% of methyl *p*-hydroxybenzoate. Because of the presence of the zinc-binding phosphate buffer the zinc content of the crystals will decrease to below 0.8% without the crystals going into solution. The final suspension has a protracted degree corresponding to that of the suspension produced according to Example 1.

In order to further illustrate the invention reference is made to the accompanying drawing in which:—

Figure 1 shows on an enlarged scale an insulin crystal employed according to the invention; and

Figure 2 blood-sugar curves on fasting rabbits which have been given an injection of different insulin crystal suspensions, and ordinary insulin, i.e. crystalline insulin in solution.

The crystal shown in Figure 1 is produced as indicated in Example 1. The crystal appears as a sharp-edged rhombohedron limited by plane crystal faces. The obtuse angle in each of the rhombs of the crystal is 114 1/2°.

The blood-sugar curves shown in Figure 2 are average curves from a series of experiments in which fasting rabbits have been given a subcutaneous injection of the same insulin dose (2 units) of different insulin preparations.

Curve 1 is obtained by injecting a solution of crystalline insulin. This solution is a hydrochloric insulin solution (pH about 3) with a content of 2.7 mgs of zinc per 100 millilitres and with a glucose content of 5%.

Curve 2 is obtained by injecting a suspension of star-shaped insulin crystals known *per se* in an aqueous medium without added sodium chloride, but with the employment of acetate buffer and at a pH-value of 5.5. The total zinc content of the suspension is 1.3 mgs per 100 millilitres. The crystals employed are produced from an aqueous crystallization medium containing 0.5% of insulin, 40 mgs of zinc (as chloride) per 100 millilitres, 0.05 M sodium citrate and 15% of acetone without the addition of sodium chloride. The crystallization is effected at pH 6.2, and the produced crystals are separated from the crystallization medium, washed and dried.

Curve 3 is obtained by injecting an insulin crystal suspension produced in the same manner as in Example 1 except that the suspension has pH 5.5 and that a content of 0.7% of sodium chloride instead of 7% has been used in the crystallization. As a consequence thereof the crystals produced will have a defective form and will not appear as sharp-edged rhombohedra with plane crystal faces.

It will be seen that there is no noticeable difference in the effect between the solution of crystalline insulin and the two insulin crystal suspensions of curves 2 and 3.

Curve 4 is obtained by injecting the insulin crystal suspension produced in Example 1, in which suspension the crystals appear as sharp-edged rhombohedra with plane crystal faces except that the suspension has pH 5.5 for the sake of comparison with the other insulin crystal suspensions. The effect is distinctly protracted, the blood-sugar values about 8 hours after the injection having only about half the size as before the injection.

WHAT WE CLAIM IS:—

1. An injectable insulin preparation having a protracted effect comprising a suspension of insulin crystals derived from bovine pancreas glands and having the form of sharp-edged rhombohedra limited by plane crystal faces in an injectable aqueous medium, the suspension having a pH-value of 6.5 to 7.5 and the suspension medium showing such a content of crystallization-promoting metal ions that the suspended insulin crystals contain chemically bound within the crystal lattice less than 5 atoms per unit cell of crystallization-promoting metals.

2. A process for producing insulin preparations as claimed in claim 1, in which insulin crystals having the form of sharp-edged rhombohedra limited by plane crystal faces are suspended in an injectable aqueous medium, characterized by using insulin crystals derived from bovine pancreas glands, adjusting the pH-value of the aqueous suspension to 6.5 to 7.5 and giving the aqueous suspension medium such a content of crystallization-promoting metal ions that the suspended insulin crystals contain chemically bound within the crystal lattice less than 5 atoms per unit cell of crystallization-promoting metals.

3. A process as claimed in claim 2, wherein a predetermined percentage of insulin crystals which originate from swine pancreas glands is employed.

4. A process as claimed in claim 2 or 3, wherein insulin crystals having a predetermined size distribution are employed.

5. A process as claimed in any one of claims 2 to 4, wherein the aqueous suspension medium is made isotonic and there is added a preserving agent with or without a buffer substance.

6. A process for the production of injectable insulin preparations having a protracted effect substantially as described in any one of the specific examples hereinbefore set forth.

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Chartered Patent Agents.

Printed for Her Majesty's Stationery Office by J. Looker Ltd., Poole, Dorset. 1960.
Published at The Patent Office, 25, Southampton Buildings, London, W.C.2, from which copies may be obtained.

sterile water containing 0.7% of sodium chloride, 1/100 M sodium phosphate and 0.1% of methyl *p*-hydroxybenzoate. Because of the presence of the zinc-binding phosphate buffer the zinc content of the crystals will decrease to below 0.8% without the crystals going into solution. The final suspension has a protracted degree corresponding to that of the suspension produced according to Example 1.

In order to further illustrate the invention reference is made to the accompanying drawing in which:—

Figure 1 shows on an enlarged scale an insulin crystal employed according to the invention; and

Figure 2 blood-sugar curves on fasting rabbits which have been given an injection of different insulin crystal suspensions, and ordinary insulin, i.e. crystalline insulin in solution.

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WHAT WE CLAIM IS:—

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2. A process for producing insulin preparations as claimed in claim 1, in which insulin crystals having the form of sharp-edged rhombohedra limited by plane crystal faces are suspended in an injectable aqueous medium, characterized by using insulin crystals derived from bovine pancreas glands, adjusting the pH-value of the aqueous suspension to 6.5 to 7.5 and giving the aqueous suspension medium such a content of crystallization-promoting metal ions that the suspended insulin crystals contain chemically bound within the crystal lattice less than 5 atoms per unit cell of crystallization-promoting metals.

3. A process as claimed in claim 2, wherein a predetermined percentage of insulin crystals which originate from swine pancreas glands is employed.

4. A process as claimed in claim 2 or 3, wherein insulin crystals having a predetermined size distribution are employed.

5. A process as claimed in any one of claims 2 to 4, wherein the aqueous suspension medium is made isotonic and there is added a preserving agent with or without a buffer substance.

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