AMENDED SPECIFICATION

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PATENT SPECIFICATION

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The inventors of this invention in the sense of being the actual devisers thereof within the meaning of Section 16 of the Patents Act 1949 are KNUD HALLAS-MOLLER, of 80 Dronninggardsalle, Holte, Denmark, KARL PETERSEN of 176 Bellahojvej, Copenhagen, Denmark, and JORGEN SCHLICHTKRULL of 20, Ronnebærvej, Holte, Denmark, all subjects of the King of Denmark.

COMPLETE SPECIFICATION

Improved process for producing Insulin preparations with Protracted Effect

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:—

This invention relates to a process for producing vegetable insulin preparations with protracted effect.

The protracted effect of the ordinary depot-creating insulin preparations is due to the presence or formation of solid insulin in a slightly soluble or insoluble condition at the pH of the blood. The insolubility of the insulin is attained by means of foreign substances with a basic reaction, such as protamine, globin and bis-2-methyl-4-amino-quinolyl-6 carbamide, which form slightly soluble compounds with the insulin at neutral reaction conditions.

Thus, while in the production of depot-creating insulin preparations it was formerly considered necessary to utilize processes which require the employment of special auxiliary substances, it has according to the present invention been found that it is unnecessary to employ special auxiliary substances, which complicate the production and the clinical utilization of the preparations.

Hence, according to the present invention, it has been found that it is possible to obtain injectable insulin preparations of protracted action by employment of insulin in crystalline state, when a suspension of insulin crystals is produced, which has per litre a total content of one or more of the metals, zinc, cobalt, nickel, cadmium and copper, of at least $13 \times A \times 10^{-3}$ milliequivalents, where "A" indicates the number of international units of insulin per millilitre of the suspension.

It is true that Fisher and Scott have formerly, vide Journal of Pharmacology, 58, 93 — 104 (1936), in connection with scientific investigations concerning the importance of the presence of zinc in protamine-insulin-preparations, produced a suspension of insulin crystals in distilled water, but they found by subcutaneous injection on rabbits of the suspension of insulin crystals so produced that the effect of the suspension was not substantially different from the effect of dissolved insulin.

The reason therefore is assumed to be that ordinary insulin crystals dissolve comparatively easily at the pH value (7.2) prevailing in blood and fluids of the tissues. Thus, if commonly known insulin crystals are suspended in distilled water and the suspension is then adjusted to pH 7, for example with caustic soda, the crystals will dissolve completely or for the greater part. Consequently, suspensions of insulin crystals in distilled water have found no clinical use.

Biological and clinical experiments carried out by the inventors have now proved that it is possible by employment of suspensions of insulin crystals produced according to the invention to obtain by subcutaneous or intramuscular injection a degree of protracted

effect, which fully corresponds to, indeed may even be made considerably greater than, the protracted degree of the hitherto known insulin preparations.

This is illustrated in the following example:

A solution of crystalline insulin in a concentration of 40 international units per millilitre, to which have been added 4 milligrams zinc (as chloride) per 100 millilitres does not possess any noticeable protracted effect. On the other hand the same solution will possess a considerable protracted effect extending over more than 24 hours after the solution has been adjusted to pH 5 and allowed to stand, thereby causing crystallization of the precipitated insulin so that a crystal suspension is obtained.

The reason for the observed protracted effect of the preparations produced according to the invention has not been accounted for with certainty, but is presumably due to the insulin being present in crystalline state and the crystals in the suspension being slightly soluble or insoluble at the pH of the blood.

Thus, if known insulin crystals are suspended, for example in an amount of 40 international units per millilitre, in a zinc chloride solution, adjusted to a pH value of about 7, which for example contains 0.008% of zinc, dissolved insulin will not be traceable in the suspension medium.

If the suspension medium of the preparations produced according to the invention has a pH value of approximately 7 or is adjusted to this pH value, the surprising observation has been made by analyzing the separated insulin crystals that the crystals show an increased metal content, i.e., a content larger than the original metal content, with regard to zinc above approximately 0.4%.

The ascertained protracted effect of the preparations produced according to the invention may therefore also be due to the presence or formation of insulin crystals with increased metal content. Thus, it has been found that the protracted effect aimed at may also be attained by suspending the insulin crystals with an increased metal content in a suspension medium not containing in itself the metal of which the crystals show an increased content.

The insulin crystals with increased metal content may contain one or more metals in addition to the metal, if any, present in the original insulin.

In order to obtain the protracted effect aimed at; it is at any rate sufficient that such conditions be present in the suspension medium that crystals exhibiting an increased content of the metal in question, exist at the pH value prevailing in blood and fluids of the tissue.

In accordance herewith the process according to the present invention is further characterised in that the suspension medium is of such a composition that the suspended insulin crystals, provided the suspension medium has a pH value of about 7 or is given this pH value, show an increased content of the metal in question.

When starting from the known insulin crystals containing about 0.4% zinc, or a corresponding amount of the other above-mentioned metals, the suspension medium must show such a content of the mentioned metals, that the suspended insulin crystals at pH 7 of the medium possess a metal content of at least 0.25 milliequivalents per gram of the crystals, which means approximately 0.80% zinc where zinc is concerned presuming that the insulin has been brought into the crystalline state by means of zinc. With a zinc content in the crystals of for example approximately 1% the produced preparations will be able to exhibit a protracted effect, which is not inferior to the protracted effect of the known protamine-zinc-insulin preparations. With a more increased zinc content it will be possible to obtain a greater protracted effect.

It is according to the invention preferred to give the suspension medium such a content of the mentioned metals that the suspended insulin crystals at pH 7 of the suspension medium show a metal content between about 0.25 and 0.83 milli-equivalent per gram of crystals.

With regard to the metal content in the aqueous suspension medium which may possibly be required it should first be noted, that an increasing metal content in the suspension medium with increasing insulin content is required for the formation of insulin crystals with a predetermined, increased metal content. If the suspension medium is thus caused to contain 40 international units of insulin per millilitre, insulin crystals with a zinc content of approximately 2.3% will be obtained at pH 7 of the medium — adjusted by means of alkali hydroxide — when the suspension medium contains zinc in the amount of 0.008%, while crystals with a zinc content of about 2.6% will be obtained when the medium contains 10 international units of insulin per millilitre, and with an insulin content of 80 international units per millilitre, insulin crystals with a zinc content of about 1.8% will be obtained, all provided that the crystals contained about 0.4% zinc prior to the suspension. With a predetermined amount of insulin crystals per unit of volume of suspension medium, increasing metal content in the medium will produce increasing metal content in the crystals.

If the suspension medium has a pH value of about 7 or is adjusted to this pH value under normal conditions, up to about 2.7% may be bound where zinc is concerned, which corresponds to the number of acid groups in the insulin molecule. Similar conditions apply to the other metals.

With the insulin concentration of 40 international units per millilitre most frequently employed in practice, and a zinc-containing suspension medium containing 0.002%, and with insulin crystals with a zinc content of

about 0.4%, the suspended insulin crystals with pH 7 of the suspension medium will contain about 1.1% zinc. At a zinc content of the suspension medium of 0.004% the crystals will contain about 1.7% zinc, while a zinc content in the suspension medium of 0.008% will impart a zinc content of about 2.2% to the insulin crystals.

If it is desired that the suspended insulin crystals should be saturated with zinc, i.e. contain about 2.7% zinc, the suspension medium should, under the above-mentioned conditions, contain considerably more zinc, viz. about 0.03% at an insulin concentration of 40 international units per millilitre.

The pH value of the suspension medium is one of the factors which has a deciding influence on the solubility of the insulin crystals in the medium and on the increased metal content of the crystals. Provided that the medium does not contain substances, for example inorganic salts, which have a specific ability to suppress the solubility of the crystals, it will not be possible with preparations for practical use to prevent all of the insulin crystals dissolving when suspended in a medium with a pH value of above 9. Besides, above this pH value it will be difficult to produce preparations of a sufficient durability. Nor will it, under normal conditions, be possible to prevent the crystals dissolving if the pH value of the suspension medium is below 3—4. Consequently, if the suspension medium does not contain substances which have a quite specific ability of reducing the solubility of the insulin, it is necessary to keep the pH value of the medium within the mentioned limits.

As will appear from what is stated above, the suspension contains insulin crystals with increased metal content, when the suspension has a pH value of about 7, still provided that the medium does not contain substances which have a greater affinity to the mentioned metals than the insulin crystals. The increased metal content decreases with a falling pH value and is not obtainable under normal conditions at pH 4—5 or below.

It is not necessary in order to obtain the protracted effect aimed at, that the ready made suspension *in vitro* contains insulin crystals with increased metal content. It is sufficient that the increased metal content be obtained by changing the pH value of the suspension to 7. The reason is presumably that changing of the pH value to about 7 takes place by injection of such a suspension, whereby insulin crystals with increased metal content are formed *in vivo*. Thus, clinical tests have shown that two otherwise identically composed suspensions of which one is adjusted to pH 4.5 and the other to pH 7, produce the same protracted effect. Analyses of the metal content of the suspended insulin crystals in the two suspensions show that insulin crystals in the suspension with pH 4.5 contain the original metal content, while crystals in the suspension with pH 7 contain an increased metal content.

Consequently, a further characteristic feature of the present invention consists in that the aqueous suspension medium shows an acid reaction or is given such reaction.

Adjustment of the suspension medium to the desired pH value may according to the invention be effected by means of a buffer substance or a mixture of buffer substances. Examples hereof will be given later.

In choosing buffer substances and other additions to the aqueous suspension medium, another fact should be taken into consideration. Thus it has been found that the presence of certain anions in the suspension medium influences the protracted effect of the insulin preparations, presumably on account of the influence of the anions on the solubility of the insulin crystals in the medium, as well as on the ability of the crystals to absorb metal from the suspension medium or to maintain an increased metal content. Thus, if for instance, ordinary insulin crystals are suspended in an aqueous solution containing 0.008% zinc (as chloride) and 1/20-molar sodium phosphate and adjusted to pH 5, and the suspension medium is then adjusted to pH 7, it has been found that the crystals dissolve completely or partly without binding zinc. However, if nickel is employed instead of zinc and a phosphate buffer is employed the crystals will not dissolve by changing pH to 7 but will absorb nickel from the suspension medium.

It is presumed that the phosphate ions have greater affinity for the zinc ions than the insulin crystals and thus bind the zinc ions by neutral reaction, while the phosphate ions are unable to bind the nickel ions in such a way that they are not at disposal for the insulin crystals.

Therefore, if the greatest possible protracted effect of the produced suspensions is desired, and use is made of a buffer substance for adjustment of the pH value of the suspension medium, a buffer substance should be employed, the anion of which does not bind the metal in question at neutral reaction.

On the other hand, advantage may be taken of a certain solubility of the insulin crystals, as this makes it possible to produce insulin preparations which show, partly an initial insulin effect and partly a protracted effect. It will be possible to give the suspension medium such a composition and acidity that the medium contains or will contain insulin in dissolved or precipitated amorphous state. The presence of dissolved or precipitated amorphous insulin may be procured in advance or by allowing part of the insulin crystals to dissolve and to be precipitated in amorphous state, if desired.

It is in accordance herewith a further characteristic feature of the invention that a suspension medium is employed which in advance contains dissolved or precipitated amorphous

insulin, or that such a content is provided by means of the added insulin crystals.

By varying the content of the suspension medium of zinc or similar metals, the pH value of the medium, its content of anions and its content of dissolved or precipitated amorphous insulin, it will thus be possible, according to the invention, to provide insulin preparations which, at the same time as they produce a protracted effect of a predetermined duration, also to a predetermined degree the same effect as insulin in dissolved state.

The present invention is furthermore based on the observation that the protracted effect of the produced suspensions of insulin crystals is dependent on the size of the suspended crystals. Biological tests performed according to the invention on depancreatised dogs have shown that insulin crystals having a size of about 10μ in zinc-containing suspension under comparable conditions show a protracted effect which is of the same magnitude as that of protamine-zinc-insulin, while insulin crystals having a size of 25—100μ produce a still more protracted effect.

This should be taken into consideration by employing crystalline insulin particles of a predetermined size. The desired size may be provided by a suitable regulation of the condition of crystallization and/or by grinding or crushing the produced crystals.

Insulin crystals produced by employment of any method of crystallization whatsoever, which gives useful practical results, may be employed in producing the new insulin preparations. Amongst the hitherto known methods the so called citrate method is preferred, according to which the crystallization is effected in the presence of substances liberating citrate ions, in that citrate buffers are used instead of phosphate or acetate buffers. Of other available methods may be mentioned, for example, the method developed by Scott, *vide* Biochemical J. 28, 1592 (1934), and later methods derived therefrom. Ordinary commercial insulin crystals contain about 0.4% zinc, but according to the known crystallization methods it is possible to produce insulin crystals containing a corresponding amount of cobalt, nickel or cadmium. It is also possible to produce insulin crystals containing no metals. All sorts of insulin crystals are suitable for the purpose of the present invention.

For the production of a suspension medium showing a content of the mentioned metals, distilled water may *per se* be used with an addition of one of the mentioned metals, for example in form of their water soluble salts. For clinical reasons it is however more advantageous to employ isotonic suspension media. Substances such as glucose, sodium chloride or glycerine may be employed to make make aqueous media isotonic. These substances are however only to be regarded as examples. Other examples will appear from the literature available in this field.

In consideration of the durability of the insulin preparations it is advantageous to add one or more of the preserving agents usually employed for injection fluids. Examples of such are phenol and phenol derivatives, methyl-p-hydroxybenzoate and propyl-p-hydroxybenzoate and phenyl mercuric acetate.

Various examples of aqueous suspension media suitable for production of insulin preparations according to the invention, and showing a content of the mentioned metals will be given below.

EXAMPLE 1

A suspension medium consisting of an isotonic solution to which a preserving agent, and one of the formerly mentioned metals have been added, may have the following composition:

0.9% sodium chloride
0.1% methyl-*p*-hydroxybenzoate
0.004% zinc (as chloride)

By adjustment of this suspension medium to a pH value between 6 and 7 ordinary insulin crystals will be even more insoluble herein than in pure water, and will, after having been suspended in the medium, show an increased zinc content at pH about 7 of the medium. If the crystals prior to suspension contain about 0.4% zinc, and they are added in such an amount that the suspension medium will contain 40 international units per millilitre, the suspended crystals will contain about 1.7% zinc.

By employment of insulin crystals which are appreciably larger than 10μ, it is advantageous to add a stabilizer to the medium, the stabilizer increasing the viscosity of the suspension medium to such a degree that the crystals are kept suspended a suitable time after shaking of the ampoule.

As suspension stabilizers, for example, high molecular polysaccharides such as dextran may be employed, but the invention is not limited to the employment thereof. Other known stabilizers which, under the circumstances in question, are inert to the insulin crystals and the other constituents of the suspension medium may also be employed.

EXAMPLE 2

A suspension agent consisting of an aqueous isotonic solution to which solution a preserving agent, one of the mentioned metals and a suspension stabilizer have been added, may have the following composition:

0.9% sodium chloride
0.2% methyl-*p*-hydroxybenzoate
0.005% nickel (as chloride)
5% dextran.

The pH value is adjusted to 6—7 with caustic soda.

If insulin crystals which do not contain nickel but which contain 0.4% zinc or no metal at all, are suspended herein in such an

amount that the suspension medium will contain 40 international units per millilitre, the suspended crystals will contain nickel.

It has previously been mentioned that an aqueous suspension medium may contain a buffer substance or a mixture of buffer substances for regulation and maintenance of the pH value of the medium. As examples of employable buffer substances may be mentioned acetate buffer, borate buffer, diethyl barbiturate buffer, phosphate buffer, malate buffer or mixtures thereof.

EXAMPLE 3

A suspension medium consisting of an aqueous isotonic solution to which have been added a preserving agent, one of the mentioned metals and a buffer substance for maintenance of the pH value of the medium, may have the following compositions:

0.8% sodium chloride
0.1% methyl-*p*-hydroxybenzoate
0.008% zinc (as chloride)
1/120 molar sodium acetate

The pH value is adjusted to about 5 with HCl.

Ordinary insulin crystals exhibit the same slight solubility in this suspension medium as in a suspension medium according to example 1. If insulin crystals, which contain about 0.4% zinc, are suspended herein in such an amount that the suspension medium will contain 40 international units per millilitre, and if the pH value of the suspension medium is changed to 7, for example with NaOH, the suspended crystals will contain about 2.2% zinc.

EXAMPLE 4

An aqueous suspension medium, which has the same acidity as the fluid of the tissues in the human body and is isotonic with the blood, may have the following composition:

1.3 vol.-% glycerine
0.1% methyl-*p*-hydroxybenzoate
0.002% zinc (as chloride)
1/120 molar diethyl barbituric acid.

The pH value is adjusted to about 7.2 with NaOH.

Less than 2 international units of insulin per millilitre will go into solution in this suspension medium. If insulin crystals containing about 0.4% zinc are suspended herein in such an amount that the suspension medium will contain 40 international units per millilitre, the suspended crystals will contain about 1% zinc.

EXAMPLE 5

An aqueous suspension medium with weak alkaline reaction may have the following composition:

1.3 vol.-% glycerine
0.1% methyl-*p*-hydroxybenzoate
0.016% zinc (as chloride)
1/120 molar boric acid.

The pH value is adjusted to about 8 with NaOH.

Also in this suspension medium less than 2 international units of insulin per millilitre will go into solution. If insulin crystals containing about 0.4% zinc is suspended herein in such an amount that the suspension medium will contain 40 international units per millilitre, and if then the suspension medium is adjusted to pH 7, for example with HCl, the insulin crystals will contain about 2.5% zinc.

EXAMPLE 6

There is produced an aqueous suspension medium containing:

0.8% sodium chloride
0.1% methyl-*p*-hydroxybenzoate
0.001% zinc (as chloride)
1/120 molar sodium acetate

and the pH value is adjusted to 5 with HCl.

If insulin crystals with a zinc content of about 0.4% are suspended in this suspension medium in such an amount that the suspension medium will contain 40 international units per millilitre, the suspended crystals will contain about 0.8% zinc, provided the suspension medium is adjusted to pH 7 with NaOH.

EXAMPLE 7

There is produced an aqueous solution containing:

0.8% sodium chloride
0.1% methyl-*p*-hydroxybenzoate
0.008% nickel (as chloride)
1/120 molar sodium phosphate,

and the solution is adjusted to pH 7 with HCl.

If insulin crystals with a nickel content of about 0.3% are suspended in this solution in such an amount that the suspension will contain 40 international units per millilitre, the suspended insulin crystals will exhibit an increased nickel content.

EXAMPLE 8

There is produced an aqueous solution containing:

1.3 vol.-% glycerine
0.2% methyl-*p*-hydroxybenzoate
0.014% cadium (as chloride)
1/120 molar sodium acetate,

and the solution is adjusted to pH 5 with HCl.

If insulin crystals with a zinc content of about 0.4% are suspended herein in such an amount that the suspension will contain 40 international units per millilitre, the insulin crystals will contain about 3% Cd, provided the pH-value of the suspension is changed to 7 with NaOH.

EXAMPLE 9

There is produced an aqueous solution containing:

0.8% sodium chloride
0.1% methyl-*p*-hydroxybenzoate
0.006% copper (as chloride)
1/20 molar sodium acetate,

and the pH value is adjusted to 6 with HCl.

If insulin crystals with a zinc content of about 0.4% are suspended herein in such an

amount that 40 international units per millilitre will be present in the suspension, the suspended insulin crystals will contain copper.

The suspension media for the insulin crystals in the above-mentioned examples have such a composition that the crystals are practically insoluble in the media. The insulin preparations thus produced will in consequence first and foremost be distinguished by their protracted effect. However, preparations which partly produce a protracted effect, partly an initial insulin effect may also, as previously pointed out, be produced.

EXAMPLE 10

There is produced a solution containing:

1.3 vol.-% glycerine
0.1% methyl-*p*-hydroxybenzoate
0.008% zinc (as chloride)
1/120 molar sodium acetate,

and pH is adjusted to 4 with HCl.

If insulin crystals with a zinc content of about 0.4% are suspended herein in an amount of 40 international units per millilitre, about 1/3 of the insulin crystals will go into solution, with the result that the suspension medium will contain 12—14 international units of dissolved insulin per millilitre. By subcutaneous or intramuscular injection of the resulting suspension, there will be obtained partly an initial effect due to the content of dissolved insulin, partly a protracted effect due to the content of the insulin crystals in suspended state.

EXAMPLE 11

A hydrochloric, aqueous solution is produced, which solution contains:

1.3 vol.-% glycerine
0.1% methyl-*p*-hydroxybenzoate
0.008% zinc (as chloride)
1/120 molar diethyl barbituric acid
0.087% insulin (23 international units per milligram) dissolved with HCl.

The pH of the solution is adjusted to about 7 with NaOH, whereby the dissolved insulin is precipitated quantitatively in amorphous condition (20 international units per millilitre). If insulin crystals are suspended in the resulting medium, an initial effect due to the presence of amorphous insulin as well as a depot effect due to the presence of the insulin crystals, will be obtained by subcutaneous or intramuscular injection of the suspension.

In the above-mentioned illustrative examples, the metals dealt with have been added in the form of their chlorides. However, there is nothing against adding them in the form of other inorganic or organic compounds, such as sulphates, nitrates, acetates, hydroxides, oxides or complex metal compounds, or in the form of the metal itself, if only the metal or the metal compound employed is present in such a condition during the condition prevailing in the medium that the metal may be taken up by the insulin crystals.

It should furthermore be noted that the change of the pH value after suspending the insulin crystals in the medium as mentioned in some of the examples, is only to be regarded as an analytic auxiliary. The suspension media are suitable for employment with the pH value to which they are adjusted when produced.

In the previous Examples the insulin crystals have been produced separately under employment of aseptic crystallization, and the crystals are then added to a ready made, sterile, aqueous suspension medium by employment of aseptic conditions.

According to the invention it is also possible to make the insulin crystallize in the ready suspension medium, for example as described in the following Examples:

EXAMPLE 12

1.74 milligrams of pure amorphous insulin are dissolved in 20 millilitres of 0.01 *n* hydrochloric acid, and in the indicated succession are added:

20 millilitres of a 4% sodium chloride solution
10 millilitres of a 1/12 molar sodium acetate solution
50 millilitres of a solution containing 0.004% zinc
0.2% methyl-*p*-hydroxybenzoate

The pH value is then decreased to above 5 with hydrochloric acid, and the resulting suspension may be filled in an ampoule. When allowed to stand the precipitated insulin becomes crystalline.

According to the invention the mother liquid of the insulin crystals may also be used as a suspension medium, the mother liquid being supplemented with any missing substances, such as isotonics, zinc or similar metals, suspension stabilizers, and pH correcting substances.

EXAMPLE 13

1.74 milligrams of crystalline insulin containing about 0.4% zinc are dissolved in 20 millilitres of 0.01 *n* hydrochloric acid, whereupon 20 millilitres of a 4% sodium chloride solution and 10 millilitres of 1/12 molar sodium acetate solution are added.

The pH value of the solution is then adjusted to about 5.5. When the crystallization has been finished, 50 millilitres of a zinc solution containing 0.016% zinc and 0.2% methyl-*p*-hydroxybenzoate are added. The pH value of the resulting suspension is regulated afterwards, if necessary. As will be apparent from what has previously been stated, the metal according to the invention may be added as well before as after suspending the insulin crystals in the medium. It is thus also possible, to start from aseptic produced insulin crystals, which are suspended in an aqueous medium, to which the desired metal is added later.

EXAMPLE 14

1.74 milligrams of insulin crystals containing about 0.4% zinc are suspended in 50 millilitres of an aqueous suspension medium which contains

1.6% sodium chloride
0.2% methyl-*p*-hydroxybenzoate
1/60 molar sodium acetate

and is given a pH value of 5.5 by addition of HCl. To this suspension are added 50 millilitres of a zinc solution of the same acidity and containing 0.016% zinc. A zinc solution of an acidity other than that of the suspension medium may also be employed, in which case a regulation of the pH value of the final suspension may have to be done afterwards, if desired. By changing the pH value of the suspension medium to 7, the suspended insulin crystals will contain about 2.2% zinc, provided the crystals originally contained about 0.4% zinc.

Likewise, the metal content of the suspension may completely or partly be provided by employment of insulin crystals, which in advance exhibit an increased metal content.

EXAMPLE 15

There is produced a solution containing:

1.3 vol.-% glycerine
0.1% methyl-*p*-hydroxybenzoate
1/120 molar sodium acetate,

and the pH value is adjusted to 5 by means of HCl.

If 1.7 grams of insulin crystals with a zinc content of about 2% are suspended in 1 litre of this aqueous medium, the crystals will remain undissolved, but part of the zinc will go into solution in the suspension medium with the result that it becomes zinc-containing.

In the above Example the metal in question is added to the suspension medium through the crystals, that is during the suspension. This may also occur by suspending crystals with a normal metal content while simultaneously adding the metal in question.

The Examples given above are only to be regarded as illustrating embodiments of the invention, the main characteristics of which consist in the provision of suspensions of insulin crystals, which exhibit a content of the metals zinc, cobalt, nickel, cadmium, copper, manganese and/or iron which is larger than the content mentioned in the preamble of the specification.

According to the invention it is preferred to give the insulin crystal suspension a total content of at least one of the metals referred to of from $13 \times A \times 10^{-3}$ to $127 \times A \times 10^{-3}$ milli-equivalents per litre, A indicating the number of international units of insulin per millilitre of the suspension. Within this metal range it is considered possible to obtain the degree of protraction which is desired for practical clinical purposes.

It is therefore within the scope of the invention to suspend the insulin crystals with increased content of one or more of the mentioned metals according to the present invention in a medium suitable for injection and not showing any content of the metals in question before the crystals have been suspended therein. As examples of such media may be mentioned distilled water and fatty oils, such as olive oil, sesame oil, and peanut oil.

It should finally be noted that the insulin preparations according to the invention may be put on the market in two ampoules of which one contains the suspension medium and the other the insulin crystals, which have beforehand been given an increased content of the mentioned metals, if desired.

WHAT WE CLAIM IS:—

1. An injectable insulin preparation having a protracted action comprising a liquid medium having insulin crystals suspended therein, the preparation containing at least $13 \times A \times 10^{-3}$ milliequivalents per litre of at least one of the metals zinc, cobalt, nickel, cadmium and copper, wherein "A" is the number of international units of insulin per millilitre of the preparation.

2. An insulin preparation for injection as claimed in claim 1 in which the liquid medium is an aqueous medium and in which the insulin crystals have a content of the metals specified of at least 0.25 milliequivalent per gram when the pH of the aqueous medium is, or is adjusted to, approximately 7.

3. An insulin preparation for injection as claimed in claim 1 or 2, having a total content of at least one of the metals specified of from $13 \times A \times 10^{-3}$ to $127 \times A \times 10^{-3}$ milliequivalents per litre, wherein "A" is the number of international units of insulin per millilitre of the preparation.

4. An insulin preparation for injection as claimed in any one of claims 1 to 3 having also amorphous insulin suspended in the liquid medium.

5. A process for producing an insulin preparation for injection as claimed in any one of claims 1 to 4, which comprises suspended insulin crystals in an aqueous medium which contains at least one of the metals specified.

6. A process for producing an insulin preparation for injection as claimed in any one of claims 1 to 4, which comprises suspending insulin crystals in an aqueous medium not containing the metals specified or containing the metals in an insufficient amount, the insulin crystals themselves having such a metal content that the desired metal content of the final preparation is obtained.

7. A process as claimed in either claim 5 or 6, in which the composition of the aqueous medium is such that the suspended insulin crystals have a content of the metals specified of at least 0.25 milliequivalent per gram when the aqueous medium has a pH value of approximately 7.

8. A process as claimed in claim 7 wherein the suspended insulin crystals have a content of the metals specified of between 0.25 and 0.83 milliequivalent per gram when the aqueous medium has a pH value of approximately 7.

9. A process as claimed in any one of claims

1.6% sodium chloride
0.2% methyl-*p*-hydroxybenzoate
1/60 molar sodium acetate

and is given a pH value of 5.5 by addition of HCl. To this suspension are added 50 millilitres of a zinc solution of the same acidity and containing 0.016% zinc. A zinc solution of an acidity other than that of the suspension medium may also be employed, in which case a regulation of the pH value of the final suspension may have to be done afterwards, if desired. By changing the pH value of the suspension medium to 7, the suspended insulin crystals will contain about 2.2% zinc, provided the crystals originally contained about 0.4% zinc.

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In the above Example the metal in question is added to the suspension medium through the crystals, that is during the suspension. This may also occur by suspending crystals with a normal metal content while simultaneously adding the metal in question.

The Examples given above are only to be regarded as illustrating embodiments of the invention, the main characteristics of which consist in the provision of suspensions of insulin crystals, which exhibit a content of the metals zinc, cobalt, nickel, cadmium, copper, manganese and/or iron which is larger than the content mentioned in the preamble of the specification.

According to the invention it is preferred to give the insulin crystal suspension a total content of at least one of the metals referred to of from $13 \times A \times 10^{-3}$ to $127 \times A \times 10^{-3}$ milli-equivalents per litre, 'A' indicating the number of international units of insulin per millilitre of the suspension. Within this metal range it is considered possible to obtain the degree of protraction which is desired for practical clinical purposes.

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It should finally be noted that the insulin preparations according to the invention may be put on the market in two ampoules of which one contains the suspension medium and the other the insulin crystals, which have beforehand been given an increased content of the mentioned metals, if desired.

WHAT WE CLAIM IS:—

1. An injectable insulin preparation having a protracted action comprising a liquid medium having insulin crystals suspended therein, the preparation containing at least $13 \times A \times 10^{-3}$ milliequivalents per litre of at least one of the metals zinc, cobalt, nickel, cadmium and copper, wherein "A" is the number of international units of insulin per millilitre of the preparation.

2. An insulin preparation for injection as claimed in claim 1 in which the liquid medium is an aqueous medium and in which the insulin crystals have a content of the metals specified of at least 0.25 milliequivalent per gram when the pH of the aqueous medium is, or is adjusted to, approximately 7.

3. An insulin preparation for injection as claimed in claim 1 or 2, having a total content of at least one of the metals specified of from $13 \times A \times 10^{-3}$ to $127 \times A \times 10^{-3}$ milliequivalents per litre, wherein "A" is the number of international units of insulin per millilitre of the preparation.

4. An insulin preparation for injection as claimed in any one of claims 1 to 3 having also amorphous insulin suspended in the liquid medium.

5. A process for producing an insulin preparation for injection as claimed in any one of claims 1 to 4, which comprises suspended insulin crystals in an aqueous medium which contains at least one of the metals specified.

6. A process for producing an insulin preparation for injection as claimed in any one of claims 1 to 4, which comprises suspending insulin crystals in an aqueous medium not containing the metals specified or containing the metals in an insufficient amount, the insulin crystals themselves having such a metal content that the desired metal content of the final preparation is obtained.

7. A process as claimed in either claim 5 or 6, in which the composition of the aqueous medium is such that the suspended insulin crystals have a content of the metals specified of at least 0.25 milliequivalent per gram when the aqueous medium has a pH value of approximately 7.

8. A process as claimed in claim 7 wherein the suspended insulin crystals have a content of the metals specified of between 0.25 and 0.83 milliequivalent per gram when the aqueous medium has a pH value of approximately 7.

9. A process as claimed in any one of claims

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5 to 8, in which the aqueous medium is given a pH value of approximately 7.

10. A process as claimed in any one of claims 5 to 8, in which the aqueous medium is given an acid reaction.

11. A process as claimed in claim 9 or claim 10, in which the adjustment of the pH of the aqueous medium is effected with a buffer substance or a mixture of buffer substances.

12. A process as claimed in claim 11, in which a buffer substance is used the anion of which does not bind the metals specified at or below pH 7.

13. A process as claimed in any one of claims 5 to 12, in which the aqueous medium is given a content of amorphously precipitated insulin.

14. A process as claimed in any one of claims 5 to 13, in which an isotonic aqueous suspension medium is used.

15. A process as claimed in any one of claims 5 to 14, in which a preserving agent is added to the aqueous medium.

16. A process as claimed in any one of claims 5 to 15, in which crystallization of the insulin is effected in the aqueous suspension medium.

17. A process as claimed in any one of claims 5 to 16, in which the mother liquid of the insulin crystals admixed with any missing substances, such as isotonics, zinc or similar metal and/or pH-correcting substances, is used as suspension medium.

18. A process for producing insulin preparations substantially as described with reference to any one of the specific examples hereinbefore set forth.

19. Insulin crystal suspensions when produced by a process as claimed in any one of the preceding claims 5 to 18.

Dated this 3rd day of April, 1951,
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