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SOLUBLE PROTAMINE INSULIN

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6 Claims. (Cl. 167—75)

This invention relates to water soluble protamine insulin and the process of producing the same.

By this invention protamine insulin can be produced in a very rapid and convenient manner without requiring expensive apparatus and without as much difficulty as is experienced in producing insoluble protamine insulin that is now on the market. The protamine insulin of this invention possesses the prolonged hypoglycemic effect that has been observed with insoluble protamine insulin and at the same time it has all the advantages due to its being soluble in water.

It is well known that the administration of insulin causes a very rapid lowering of blood sugar with subsequent rapid return to the initial level. From clinical and animal experimentation it has been found that the effect of insulin on blood sugar does not usually last more than 6 or 7 hours, so that many diabetic patients need to be given as many as four injections of insulin daily. Investigators have therefore been working diligently toward the preparation of an insulin that would exert a prolonged hypoglycemic effect in the system, thus reducing the number of necessary daily injections. Emulsions of insulin with oil have been tried and have been found to produce a somewhat more prolonged hypoglycemic effect than regular insulin. However, since such preparations were not homogeneous they were not satisfactory to the clinicians and the diabetic patients.

It has also been known for some time that so-called protamine insulin could be produced by precipitating insulin from a water solution by the addition of protamine and that the precipitated insulin-protamine complex, when administered to patients, acted more slowly on the blood sugar of the system and maintained a low blood sugar level over a longer period of time than the regular insulin does. Some of the disadvantages of such precipitated protamine-insulin preparations or complexes are:

(*a*) They are not stable over a long period of time, and therefore an expiration date must be placed on each package of them.

(*b*) Such preparations do not remain homogeneous upon standing at room temperature, and quite often a sticky material is formed on the sides of the containers.

(*c*) It has ben observed that nodules are often found at the site of injection of such protamine-insulin preparations.

(*d*) The action of the precipitated protamine-insulin complex is slow, so that a considerable post-prandial rise in the blood sugar takes place after its administration necessitating a simultaneous injection of regular insulin to compensate for this deficiency.

(*e*) In view of the fact that such a precipitated material has a hydrogen ion concentration suitable for bacterial growth, the utmost precaution is necessary to insure complete sterility, for unless the final preparation is perfectly clear, it is extremely difficult to know, by mere inspection of the vial, whether or not the material has become contaminated by bacteria.

In the preparation of the precipitated protamine-insulin complex of the prior art, 0.75 to 1.25 milligrams of protamine were used per 100 units of insulin dissolved in water and about 1 cc. of a 2 per cent solution of di-sodium hydrogen phosphate was added to raise the hydrogen ion concentration of the mixture of insulin and protamine to about pH 7.0. It was found that such addition of protamine to insulin, and the subsequent injection as a clear solution did not produce a more prolonged action on the blood sugar than that of regular insulin but that the product had to be precipitated in order for it to produce a prolonged hypoglycemic effect. This precipitated product is now on the market.

By the present invention I use a method that enables me to prepare a clear solution of insulin-protamine complex or preparation that is almost as rapid initially in its action as regular insulin and yet as prolonged in its effect as the precipitated insulin-protamine.

In my investigations of the effect of protamine on insulin with reference to the prolongation of the action of the latter, I have found that by using 2.5 to 5.0 milligrams of protamine to 100 units of water solution of insulin, and by heating the insulin and protamine and allowing the mixture to stand for several days, a complex or preparation is formed that has an initial rapid hypoglycemic effect, and yet the duration of hypoglycemia is greatly prolonged. Such a preparation has been carefully studied on animals and human beings and has been found to possess the following distinct advantages over other protamine-insulin preparations now on the market:

(*a*) This new preparation of a clear insulin-protamine solution is permanently stable and therefore does not require an expiration date to be placed on the packages thereof.

(*b*) Its initial action is rapid so that this clear insulin-protamine begins to take effect at once upon administration, thus eliminating the neces-

sity for a simultaneous injection of regular insulin.

(c) The prolongation of the action of the clear insulin-protamine can be so regulated or controlled by the size of the dose that when it is injected into a diabetic patient, one dose a day is sufficient to control the blood sugar of the diabetic, thus preventing the cumulative effect which as a rule disturbs the equilibrium of insulin intake by the system.

(d) The simplicity of preparation and filling into vials or containers avoids the necessity of careful adjustment of the hydrogen ion concentration.

(e) The danger of bacterial contamination is considerably reduced. My preparation is sterilized by one filtration, whereas with the prior product, that is, a precipitated or cloudy insulin-protamine, one must sterilize insulin or insulin and protamine and introduce them into a sterile vial and then into this vial a sterile solution of phosphate buffer must be added for the adjustment of the hydrogen ion concentration.

(f) The subcutaneous administration of this clear solution of insulin-protamine does not cause the formation of nodules at the site of injection.

The following are given as specific examples to illustrate the invention, but it is to be understood that the invention is not restricted to the specific details or the exact temperatures, materials, or proportions thereof that are mentioned:

Example I

To a known concentration of insulin 5.0 milligrams of a highly purified protamine for every 100 units of insulin are added. The hydrogen ion concentration need not be carefully adjusted, other than to maintain an acidity slightly below that of the precipitation of insulin, for example, pH 3.0 to 3.5. The mixture is then carefully warmed in a water bath below the temperature at which the insulin protein would be denatured. I have found that temperatures of about 50–55° C. are suitable for this and that about 2 or 3 hours are need at such temperatures. The volume is adjusted by adding an isotonic medium such as water and glycerine, for example, to produce the desired concentration of units of insulin per 1 cc. The material is next rendered sterile by any of the usual methods.

Example II

To a known concentration of insulin, preferably a solution of crystalline insulin, approximately 5 milligrams of pure protamine to every 100 units of insulin are added, followed by a small quantity of an acid phosphate, such as sodium or potassium di-hydrogen phosphate, and about 0.2 milligram of zinc. The purpose of the latter is to prevent deterioration of the potency of the final product. The mixture is then heated to about 50 to 55° C. for 2 or 3 hours, then kept in an incubator at about 50° C. for approximately 24 to 48 hours, and is then brought to the desired concentration and sterilized.

I claim:

1. The process of producing a protamine-insulin that is entirely water soluble which comprises combining protamine and insulin in the proportions of at least 2.5 mg. of protamine to 100 units of insulin in an aqueous medium whose pH is below 3.5 at a temperature below about 55° C.

2. The process of producing a protamine-insulin that is entirely water soluble which comprises combining protamine and insulin in the proportions of at least 2.5 mg. of protamine to 100 units of insulin in an aqueous medium whose pH is below 3.5, and heating to about 50 to 55° C.

3. The process of producing a protamine-insulin that is entirely water soluble which comprises combining protamine and insulin in the proportions of at least 2.5 mg. of protamine to 100 units of insulin in an aqueous medium whose pH is below 3.5, and heating to about 50 to 55° C. for about 2 or 3 hours.

4. The process of producing a protamine-insulin that is entirely water soluble which comprises combining protamine and insulin in the proportions of at least 2.5 mg. of protamine to 100 units of insulin in an aqueous medium whose pH is below 3.5 in the presence of about 0.2 mg. of zinc per 100 units of insulin at a temperature below about 55° C.

5. A clear stable solution containing insulin to which has been added from 2.5 mg. to 5 mg. of protamine for each 100 units of the insulin, said solution having an acidity of about pH 3.0.

6. A clear stable solution containing insulin to which has been added zinc and from 2.5 mg. to 5 mg. of protamine for each 100 units of the insulin, said solution having an acidity of about pH 3.0.

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