The 1920 Method to Make Insulin

The method of preparation is as follows.

First a summary of the preparation of the extracts as used in the first clinical cases:

To a small volume of 95%, ethyl alcohol freshly minced mammal's pancreas was added in equal amount. The mixture was then allowed to sit ofr some hours with occasional stirring and shaking. Then it was strained through a clean cheese cloth and the liquid part at once was filtered. The filtrate was then mixed (treated) with two volumes of 95 %, ethyl alcohol. By this the major part of the protein was removed, but the active important part remained in alcoholic solution. After allowing a few hours for the protein precipitation to be effected, the mixture was again filtered.

The filtrate concentrated to small bulk by distillation in vacuo at a low temperature (18° to 30°C, 64°F to 86°F).

The lipoid substances were then removed by twice extracting it with Sulphuric ether in a separating funnel, then the watery solution returned to the vacuum, where it was further concentrated until it was of a pasty consistency.

We added 80% ethyl alcohol, and the mixture was centrifuged again. After centrifuging, four distinct layers were manifested in the tube:

The uppermost was perfectly clear and consisted of alcohol holding all the active principle in solution. Below this layer, in order, were a flocculent layer of protein, and a second clear watery layer saturated with salt, and then a lowermost layer consisting of crystals of salt.

The alcohol layer was then removed by means of a pipette and then was at once delivered into several volumes of 95%, alcohol, or better, of absolute alcohol.

We found that after this final treatment with alcohol of high grade, it caused the precipitation of the active principle along with adherent substances.

Some hours after this final precipitation, the precipitate was caught on a Buchner Funnel, dissolved in distilled water and then concentrated to the desired degree by use of the vacuum still. Finally, it was then passed through a Berkfeld filter, then sterility tests made and then the final product delivered to the clinic.

A more detailed summary:

The pig or cow pancreas is finely minced in a large grinder. The minced material parts is then treated with 5 c.c. of concentrated Sulphuric Acid, which is appropriately diluted, per pound of pancreas glands.

The mixture is then stirred for about 3 to 4 hours and then 95% alcohol is added until the concentration of alcohol is 60% to 70%.

Now two extractions of the glands are done. The solid gland material is partially removed by centrifuging the mixture, and the solution must be further clarified by filtering through paper.

Next the filtrate is practically neutralized with Sodium Hydroxide.

The clear filtrate is concentrated in vacuo to about 1/15 of its original volume.

The concentrate is then heated to about 50 degrees Centigrade, which results in the separation of lipoid and the other materials, are removed by filtration.

Ammonium sulphate (37 grams. Per 100 c.c.) is then added to the concentrate. A protein material which contain all the Insulin floats to the top of the liquid.

The precipitate is then skimmed off and dissolved in hot acid alcohol. And when the precipitate has completely dissolved, add 10 volumes of warm alcohol.

The solution is then neutralized with sodium hydroxide (NaOH) and cooled to room temperature, and kept in a refrigerator at 5 C (41 F) for two days.

At the end of two days, the dark colored supernatant alcohol is decanted off. The alcohol contains practically no potency.

The precipitate is then dried in vacuo to remove all trace of the alcohol.

Next it is dissolved in acid water, in which it is readily soluble.

The solution is then made alkaline with sodium hydroxide (NaOH) to PH 7.3 to 7.5. At this alkalinity a dark colored precipitate will settle out, and then it is immediately centrifuged off.

This precipitate is then washed once or twice with alkaline water of PH 9.0, and the washings are then added to the main liquid.

It is **extremely** important that this process is carried out very quickly because Insulin is destroyed in alkaline solution. The acidity is adjusted to PH 5.0 and a white precipitate readily settles out.

Tricresol is then added to a concentration of 0.3% in order to assist in the isoelectric precipitation and to act as a preservative.

After standing for about one week in an ice chest, the supernatant liquid is then decanted off and the resultant liquid is removed by centrifuging. The precipitate is then dissolved in a small quantity of acid water.

Now a second isoelectric precipitation is carried out by adjusting the acidity to a PH of approximately 5.0.

Next after standing for over a night the resultant precipitate is then removed by centrifuging.

The precipitate, which will contain the active principle in a comparatively pure form, is then dissolved in acid water and the hydrogen ion concentration adjusted to PH 2.5.

The material is then carefully tested to determine the potency, then it is diluted to the desired strength of 10, 20, 40 or 80 units per c.c.

Tricresol is then added to get and secure a concentration of 0.1 percent.

Sufficient sodium chloride is then added to make the solution isotonic.

Finally, pass the solution which contains the Insulin through a Mandler filter.

After passing the solution through the filter, the Insulin is retested carefully to determine its potency.

There is practically no loss in berkefeld filtering.

The tested Insulin can then be poured into sterile glass vials with aseptic precautions.

The sterility of the final Insulin product must be thoroughly tested by approved methods.

A last note on insulin thermo stability

Some researcher state that commercial Insulin is generally more stable and hearty than the label suggests (i.e. 30 days).

Doctors Without Borders commissioned a study to examine the loss of potency. Though heat will denature insulin it has to be pretty intense for long periods of time.

Sunlight and shaking will also destroy potency. However in most cases a person can get months of potency even if it is kept outside of a fridge.

However, what will quickly destroy the insulin though is going the other way - freezing. So be careful not to store directly on ice packs.