THE INSULIN ZINC SUSPENSIONS

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The insulin zinc suspensions are a group of long-acting insulins recently developed by Hallas-Møller and his colleagues (1952b) at the Novo Laboratories in Copenhagen. They contain insulin precipitated with zinc and resuspended in an acetate buffer. The duration of the hypoglycaemic action depends chiefly on the size and form of the insulin particles. Two basic suspensions were prepared. One, with amorphous insulin particles, had a hypoglycaemic action lasting up to 16 hours, and was called "semilente" (insulin zinc suspension—amorphous). The second contained crystalline insulin, had an action lasting up to 30 hours or more, and was called "ultralente" (insulin zinc suspension—crystalline). Mixtures of the two suspensions remained stable and had the same effect as separate injections. By varying the proportions in the mixture it was possible to obtain insulins with a wide range of duration of action. Clinical trials in Denmark showed that most diabetic patients could be controlled with a single daily injection of a mixture containing 3 parts of the amorphous to 7 parts of the crystalline suspension (Hallas-Møller *et al.*, 1952a). This mixture was called "lente insulin" (insulin zinc suspension), and it is to be noted that the ratio of the two suspensions in this standard mixture was decided after trials in Denmark, using diets somewhat different from those customary in Great Britain. The descriptive features of these three preparations are tabulated below.

British Name	Danish Name	Duration in Hours	Strengths Available (Units/ml)
Insulin zinc suspension	Lente	app. 24	40 and 80
Insulin zinc suspension (crystalline)	Ultralente	30+	40
Insulin zinc suspension (amorphous)	Semilente	12-16	40

Through the courtesy of the Novo Laboratories we have been studying the actions of these new insulins in the Diabetic Clinic at University College Hospital since October, 1952. The introduction of three new insulin preparations will complicate the management of patients with diabetes, and we have been investigating the possibility of shortening the action of I.Z.S. and I.Z.S. (crystalline) by the addition of soluble insulin instead of I.Z.S. (amorphous), thus making it unnecessary to release the latter as a separate preparation.

Clinical Trials

For the initial studies of the insulin zinc suspensions patients were admitted to hospital. Most of them were selected because they required large doses of insulin and morning and evening injections. They were given a strictly controlled diet with a distribution of carbohydrate suitable for the I.Z.S. (lente) (see Fig. 1). When the insulin dose had been adjusted, blood-sugar levels were studied over a 24-hour period. Capillary blood samples were collected at 8.15 a.m., 10 a.m., 12 noon, 2.15 p.m., 6 p.m., and 11 p.m., and the sugar content was estimated in duplicate by the Nelson modification of Somogyi's method (Nelson, 1944). Urine was collected in six-hour fractions and the glucose content measured by Benedict's method. The insulin was

injected 45 minutes before breakfast. In many of the patients the blood-sugar levels were studied on a number of occasions with different doses and combinations of I.Z.S., I.Z.S. (amorphous), and I.Z.S. (crystalline). At least one clear day was allowed between a change of insulin and investigation of the blood sugars. When repeated studies were made on a single patient the distribution of dietary carbohydrate was kept constant to simplify comparison of the results obtained, although the distribution was primarily devised for use with I.Z.S. and was not really ideal for some of the combinations.

Of the 22 patients studied, 19* were found to need more than 48 units a day of I.Z.S. The three milder cases and 9 of the 19 more severe ones were adequately controlled with I.Z.S. alone. This insulin proved unsatisfactory in

FIG. 1.—Diurnal variations in the blood sugar in patients with severe diabetes poorly controlled on I.Z.S. alone. Mean of six patients. Dose range, 48 to 108 units (mean 72). On the left I.Z.S. alone. On the right the same patients on combinations of I.Z.S. and I.Z.S. (crystalline) in the ratio 1:1 (two patients) and 2:1, 3:1, 4:1, and 5:1 (one patient each). Glucose equivalent of the diet = g. carbohydrate + 58% g. protein + 10% g. fat.

10 patients, all needing more than 48 units a day. One had a very variable insulin requirement, and in another the insulin action in the early part of the day was inadequate. The defect in the remaining eight was an evening and early morning hyperglycaemia. In six it was found that this could be prevented by the use of a combination of I.Z.S. and I.Z.S. (crystalline) in ratios varying from 5:1 to 1:1. These results are shown in Fig. 1. In the two other cases combinations of I.Z.S. and I.Z.S. (crystalline) were not used owing to the high doses needed and the consequent large volume of injection (over 3 ml.) using 40 units insulin per ml.

Thirteen of the 22 patients have subsequently been maintained as out-patients on I.Z.S. or a combination of I.Z.S. with I.Z.S. (crystalline); in addition a further eight have been given the new insulins as out-patients. In one of these the trial was unsuccessful because at the dose level needed with I.Z.S. alone (120 units a day, 80 units per ml.) the patient had nocturnal thirst and polyuria, and it was considered impracticable to use the appropriate combination of I.Z.S. with I.Z.S. (crystalline), as the latter is at present available only in the strength of 40 units per ml. In 15 patients observed over an adequate period as out-patients, the dose of the new insulins can be compared with that previously required. Of the remaining five, one had not needed insulin before and the other four have not been followed for long enough. The dose is almost identi-

cal (± 4 units) in five; in eight the dose of the new insulins is greater (mean +19 units, or 139% of the previous dose); in two the dose is smaller (10 and 24 units less).

A few hypoglycaemic attacks following the insulin zinc suspensions have occurred in hospital. In each case there was good warning and a gradual onset. This experience has been confirmed by out-patients who have had similar attacks.

There have been no local reactions to the injections of the insulin zinc suspensions. I.Z.S. was given to three patients who were having local reactions to soluble or globin insulin; there were no reactions with the new preparation.

Mixing of Insulin Zinc Suspension with Soluble Insulin

The insulin zinc suspensions are preparations at body pH and are designed to cover a range of duration of action from about 12 to 30 or more hours, but, since soluble insulin will always be needed, the question arises whether the action of I.Z.S. could not be shortened by mixing it with soluble insulin, thus dispensing with the need for I.Z.S. (amorphous). There is no phosphate in British soluble insulin, so the danger of precipitating the zinc in I.Z.S. as zinc phosphate does not exist. Soluble insulin, however, is kept at the acid pH of 3 to 3.5 to obtain optimal stability, and the pH would therefore be variable on mixing I.Z.S. and soluble insulin in different preparations. This factor of pH was shown by Hallas-Møller *et al.* (1952b) to control the form and size of insulin zinc particles and thus their prolongation of effect. Insulin exists as a precipitate in the presence of zinc (0.5 to 2 mg. per 1,000 units) in the pH range of roughly 4.5 to 8, and with a pH of between 5 and 6 the precipitate is in the crystalline long-acting form. If insulin zinc crystals are suspended in buffers between a pH of 4.5 and of 8 they remain crystalline, but when amorphous insulin zinc particles are buffered between a pH of 5 and of 6 they slowly become crystalline, and, furthermore, insulin precipitated from added soluble insulin will also be crystalline. Thus the addition of soluble insulin at this pH, so far from shortening the duration of action, will actually prolong it by increasing the proportion of the crystalline particles. This can be seen from the curves shown in Fig. 2. It can also be seen in this figure that the pH of a mixture of a soluble insulin and I.Z.S. cannot be

reliably predicted from the proportions of the two insulins mixed. This is presumably because there is a variable amount of free hydrochloric acid in soluble insulin. If successful mixing of soluble and I.Z.S. is to be achieved, the I.Z.S. must be buffered effectively outside the range at which crystalline precipitation occurs. We have been able to do this with 0.1 M acetate buffer at pH 4.8 added in equal volume to 80 units I.Z.S. per ml. (see Fig. 3).

FIG. 3.—Action of buffered I.Z.S. and mixtures of buffered I.Z.S. and soluble insulin. Top: Comparison of the effects of plain and buffered I.Z.S. Middle: Comparison of the mixtures of soluble with plain and buffered I.Z.S. In the former (final pH 5.19) the prolonged hypoglycaemic action suggests that additional I.Z.S. (crystalline) has been formed. Below: Comparison of mixtures of soluble and buffered I.Z.S. and I.Z.S. (amorphous) and buffered I.Z.S., showing that the addition of soluble insulin has the same effect as the addition of I.Z.S. (amorphous).

The final concentration of zinc in the mixture is important. Hallas-Møller *et al.* (1952b) have shown that the minimum effective concentration is about 0.5 mg. per 1,000 units, and the preparations of I.Z.S. to be put on the market contain 2 mg. per 1,000 units. To lower the zinc concentration below the critical level at least three volumes of soluble insulin would have to be added to one of the suspension, and this would be most unlikely to be required in practice. In mixtures of soluble insulin with I.Z.S. buffered at pH 4.8 the soluble insulin is at once precipitated, and at this pH the precipitate is in the amorphous form. Further study of this buffered I.Z.S. is required, but it seems likely that with its introduction it should be possible to dispense with the I.Z.S. (amorphous) as a separate preparation.

Discussion

The introduction of three new insulins will complicate the management of patients with diabetes mellitus and should be done only if there is good evidence that they are an improvement on the preparations at present in use. P.Z.I. alone is suitable for the control of mild diabetes, but in the more severe cases soluble insulin is also necessary in the morning and sometimes in the evening as well. The varying excess of protamine in P.Z.I. makes it unwise to mix soluble insulin and P.Z.I. in the syringe or to inject them at the same site; for efficient and consistent control separate injections are required. A single dose of globin insulin given one hour before breakfast is effective in many patients needing less than 48 units a day, but in more severe diabetes early morning hyperglycaemia necessitates a second injection in the evening. Both P.Z.I. and globin insulins contain foreign protein, and sensitization reactions at the site of injection are not uncommon. Isophane (N.P.H.)

FIG. 2.—Comparison of blood-sugar curves obtained in a patient on a constant diet using varying proportions of I.Z.S. and soluble insulin, given by separate injection (on right) and premixed in phial for 24 hours (on left). Curves obtained with soluble insulin alone and I.Z.S. alone are shown at the top. The pH of the mixtures is recorded.

insulin has recently been made available in this country. It does not contain any excess of protamine and can therefore be satisfactorily mixed with soluble insulin. There is some evidence that the duration of action of isophane insulin is too short for a proportion of severe diabetics: it contains a foreign protein and may give rise to allergic reactions. The most outstanding defect of the long-acting insulins at present available is that most diabetics who need more than 48 units a day require morning and evening injections.

The originators of the insulin zinc suspensions claim that a single daily injection is enough for the great majority of diabetics requiring insulin. The standard mixture I.Z.S. (lente) is reported to be suitable for 85 to 90% of cases (Hallas-Møller *et al.*, 1952a); in the remainder a combination with I.Z.S. (amorphous) or I.Z.S. (crystalline) is necessary. These claims were confirmed by Lawrence and Oakley (1953), who obtained good results with I.Z.S. in 11 patients, 7 of whom needed more than 48 units a day. We have made detailed studies of the action of the insulin zinc suspensions in 22 diabetic patients in hospital. Our findings suggest that a single daily injection of the new insulins will be satisfactory in over 90% of diabetics, but in 40% of the more severe cases I.Z.S. (lente) used alone had too short an action, and a combination of I.Z.S. with I.Z.S. (crystalline) was required. The composition of the standard I.Z.S. mixture (lente insulin) was decided on the results of trials in Denmark using diets in which slightly more than half of the day's allowance of carbohydrate was taken before 2 p.m. In a recent memorandum published by the Novo Laboratories it is recommended that the ratio of carbohydrate taken at breakfast and lunch to that taken later in the day should be 120 : 80 or 3 : 2. In the diets in use at the diabetic clinic at this hospital the ratio is 1 : 1. It seems possible that although the standard I.Z.S. mixture with three parts I.Z.S. (amorphous) to seven parts I.Z.S. (crystalline) is optimal for the Danish dietary habits, a ratio of 2 : 8 would be more suitable in this country.

The duration of action of the standard I.Z.S. mixture can be lengthened by combining it with I.Z.S. (crystalline) in varying proportions. We have supplied our patients on such mixtures with phials of the premixed insulin so that the technical difficulties of mixing in the syringe are avoided. Use of these combinations in severe cases is limited by the strength of I.Z.S. (crystalline) available. The patients who might benefit most from the insulin zinc suspensions are those at present requiring large doses of insulin and two injections a day. However, unless the I.Z.S. (crystalline) is made available in the strength of 80 units per ml., many of them will be unable to take advantage of the new preparations.

Our studies have confirmed the value of the new insulins, but have indicated minor modifications that might simplify their introduction. In this country a standard mixture with two parts of amorphous to eight of crystalline may prove more suitable than the present lente mixture, and mean that fewer patients will require combinations of two types. If the standard mixture can be suitably buffered it may be possible to shorten its action, when this is necessary, by the addition of soluble insulin, thus dispensing with the need for I.Z.S. (amorphous). In only one of our patients has it so far proved desirable to shorten the action of I.Z.S. The I.Z.S. (crystalline) should be made available in a strength of 80 units per ml.

Indications for the Use of the Insulin Zinc Suspensions

Diabetics at present well controlled on a single daily injection of P.Z.I. or globin insulin are unlikely to benefit in any way from transfer to the new insulin zinc suspensions, unless their present insulin causes allergy. We consider that the indications for trial of the new insulins are: (1) poor control on present insulin; (2) patients at present needing morning and evening injections; (3) patients requiring P.Z.I. and soluble insulin in the morning by separate injection; and (4) patients who show allergic reactions to other types of insulin.

There are also certain contraindications. "Brittle" diabetics whose insulin requirement varies from day to day or during the day will usually be better controlled with morning and evening injections. Patients needing more than 80 units a day are likely to be disappointed with the insulin zinc suspensions at present available, because if they need a combination of I.Z.S. and I.Z.S. (crystalline) they will have to use it in the strength of 40 units per ml.

We have had little experience in the use of insulin zinc suspensions in patients being given insulin for the first time. With P.Z.I. and globin insulin severe local reactions may occur between three and nine months after the injections are started. It seems likely that these will be avoided by the use of the insulin zinc suspensions, and in many new diabetics treatment may begin with the new preparations.

Transfer to the Insulin Zinc Suspensions

It will often be found necessary to adjust both the diet and the insulin dosage when the patients are transferred to the new preparations. The carbohydrate in the diet should be distributed so that the greater part is taken at lunch and tea to cover the period of maximum insulin action. The amount of carbohydrate needed at breakfast-time has varied considerably. We have usually restricted it initially, but in some cases it has had to be increased later on account of morning hypoglycaemia. In all cases we have started with I.Z.S. and the previous total insulin dose. The patients are advised to make the injection 45 to 60 minutes before breakfast, and the importance of shaking the bottle well before withdrawing the insulin is stressed.

Patients are asked to test their urine four times a day during the period of adjustment (before breakfast, lunch-time, and suppertime, and at bedtime). Results of all urine tests are recorded and brought to the clinic on the weekly visits, which are considered desirable during this period. The patients are requested to adjust their own insulin dose if this appears necessary between visits. If the urine contains 2% or more sugar for 48 hours they are advised to increase the dose by 10%. If they experience any hypoglycaemic reactions a comparable reduction is authorized. Reactions may occur at any time of the day, but seem to be most common in the late morning and during the afternoon. They usually indicate the necessity for adjustment of the diet.

In a number of our patients it has become clear after the first few weeks that, although good control can be achieved with I.Z.S. alone during the day, glycosuria is present at bedtime and in the early morning. This suggests that the action of the I.Z.S. should be prolonged by the addition of I.Z.S. (crystalline), and we try first a 1 : 1 mixture, in which the ratio of I.Z.S. (amorphous) to I.Z.S. (crystalline) is 1.5 : 8.5. Some patients can be provided with sterile rubber-capped bottles and allowed to prepare their own mixtures; others have to have this done for them, and special arrangements are required for this in a busy clinic.

Summary

The action of the insulin zinc suspensions has been studied in 22 diabetic patients in hospital. Repeated blood-sugar estimations were performed over a 24-hour period. Insulin zinc suspension (lente) proved unsatisfactory in 10 out of 19 patients requiring more than 48 units a day. In most of these patients the duration of action was too short, and better control was achieved with a mixture of insulin zinc suspension and insulin zinc suspension (crystalline). For this purpose it is recommended that insulin zinc suspension (crystalline) should be made available in a strength of 80 units per ml.

Studies were undertaken to ascertain whether the action of insulin zinc suspension could be shortened by mixing it with soluble insulin, thus dispensing with

the need for insulin zinc suspension (amorphous). It is suggested that this would be possible if the properties of the acetate buffer in insulin zinc suspension were altered, but that further studies are required.

The indications for the use of the insulin zinc suspensions are described, and a plan is outlined for changing patients to the new preparations.

We wish to thank the Novo Laboratories in Copenhagen and Evans Medical Supplies Limited, their distributors in this country, for very generous supplies of these insulins for use in this trial. We would also like to thank Miss A. Moxham, who performed most of the blood-sugar estimations, and the dietitians, sisters, and nurses for the great help they have given us.

REFERENCES

- Hallas-Møller, K., Jersild, M., Petersen, K., and Schlichtkrull, J. (1952a). *J. Amer. med. Ass.*, **150**, 1667.
 ———, Petersen, K., and Schlichtkrull, J. (1952b). *Science*, **116**, 394.
 Lawrence, R. D., and Oakley, W. (1953). *British Medical Journal*, **1**, 242.
 Nelson, N. (1944). *J. biol. Chem.*, **153**, 375.

THE CLASSIFICATION OF VARIOUS INSULINS

BY

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In an annotation in the *British Medical Journal* (1953) in which the Novo insulin preparations were announced, attention was drawn to the confusion that might arise when a larger variety of insulins became available. This problem needs extensive consideration for several reasons.

In some cases the new insulin preparations show no superiority over those already in use, but in others they are a distinct improvement. Most doctors who treat diabetic patients use a special brand of insulin, and thus do not take advantage of the intensive research that is going on in the laboratories of the insulin manufacturers.

To choose the right insulin for a certain diabetic patient is not a simple matter. It requires a thorough study of the particular manifestations of the case and a knowledge of the characteristics of the various insulins existing, to adapt the insulin therapy to the individual need. It would be of great value, therefore, if the physician could be guided in the choice of the most suitable insulin, or, if the patient is already using insulin, in making a change that might be of benefit in the particular case.

Principles Involved in Choosing Type of Insulin

In this study a method is recommended which has been described earlier (Gerritzen, 1952), but it might be useful to recall attention to the principles that led to its adoption. Though insulin is used almost exclusively in diabetics, no reliable information can be had from an investigation of diabetic patients because they vary widely in sensitiveness to it. The results obtained would tell us more about the variation in sensitiveness of the patients than about the insulin itself. The same consideration would apply if, in the standardization of insulin, diabetic instead of healthy rabbits were used. The results would be far more uncertain if diabetic rabbits with different degrees of the metabolic

disorder served as test animals. Evidently the diabetic patient would not be a suitable subject for testing the properties of different insulins. Even the average of several diabetics, if an average could be calculated, would not be better, as the average diabetic does not exist.

Insulin is measured in terms of the decrease of the blood sugar after injection. This means that if the decrease in the blood sugar is representative of the action of insulin, all other factors influencing the blood sugar must be excluded. Fasting and exercise, which tend to depress the blood sugar, must be avoided; in addition, emotions and meals, which cause an increase of blood sugar, should also be excluded. Elimination of exercise is easy in the human, as the test persons can be kept in bed. The requirements to exclude meals and to avoid a fasting state led me to give a constant amount of carbohydrate every hour, a method that is not practicable in test animals, because the emotion of forced feeding would undoubtedly influence the blood sugar. Test animals in a state of fasting would get low blood-sugar values when insulin was injected, and a regulating mechanism, of which adrenaline is the most important component, might come into action, thus making the results of insulin action very uncertain.

For these reasons I choose healthy students as test persons. The results obtained in regard to duration and character of the action of insulin would not, of course, apply to diabetics, but they would tell us something about the qualities of the various insulins. This has become of special importance since Hallas-Møller *et al.* (1952a, 1952b) published their method of testing insulin in diabetics. In a great number of diabetics they classified the reaction to different insulins as types A, B, and C; type B being the ideal one, as it gives a flat blood-sugar curve throughout the day. It is necessary to give the A-type (∞) patient an insulin that has a more prolonged action with less intensity during the first few hours after injection, while patients of type C (∧) require an insulin of shorter and more intense action.

This logical attempt to adjust the insulin to the type of reaction of the patient presupposes a knowledge of the differences that exist between the various insulins. This study is an effort to classify the insulins in regard to the duration and character of the action.

The persons tested were lying down from 11 p.m. the night before the experiment started and during the experiment. From 6 a.m. on the day of the experiment they ate 50 g. of mashed potatoes (about 10 g. of carbohydrate) and drank 30 ml. of water every hour. From 7 a.m. the blood sugar was determined hourly by the Hagedorn-Jensen method, until the end of the experiment, which was considered to be reached when the average blood sugar had returned to its original level at 8 a.m.

By this method I was able to classify 13 different insulins without difficulty into four categories according to the duration of the action. Differences in the intensity of the action, as measured by the depression of the blood-sugar level, are shown in Figs. 1-4. The insulin mixtures are described in my previous study (Gerritzen, 1952), but are not considered here.

In an earlier investigation (not published) I found that the sensitivity of healthy students to insulin does not vary throughout the day. Therefore the fact that the prolonged insulins act throughout the evening and night does not affect the results.

Duration of Action

Category 1. — Duration, eight hours. Only two insulins were tested—regular

FIG. 1.—Category 1. Duration of action, 8 hours. Both insulins act during the same period of time. Regular insulin (Organon) reaches its maximum in one hour. Alt-insulin (Horm) acts less acutely.