One observer appeared to be biased against and/or the other towards finding abnormalities in the cotton-workers. The same observer bias occurred in taking histories of symptoms.

The importance of observer error in epidemiological studies and possible methods of reducing it are briefly discussed.

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CLINICAL EXPERIENCE OF THE INSULIN ZINC SUSPENSIONS*

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In introducing the insulin zinc suspensions, Hallas-Møller *et al.* (1952) claimed that they enabled the majority of diabetics requiring insulin to be controlled with a single daily injection. By mixing in different proportions the relatively quick-acting amorphous and the longer-acting crystalline forms, insulins with a wide range of prolonged action could be obtained. These mixtures were consistent in their effect, unlike, for example, those of protamine zinc (P.Z.I.) and soluble insulin (S.I.). The Danish workers found that a mixture of seven parts crystalline with three parts amorphous was suitable for most patients; this was placed on the market as "lente insulin" and is known in this country as insulin zinc suspension (I.Z.S.). The amorphous and crystalline forms are also available separately as insulin zinc suspension (amorphous)—I.Z.S.(A)—and insulin zinc suspension (crystalline)—I.Z.S.(C). It was claimed, too, that the absence of any foreign protein and the use of thrice-recrystallized insulin in the preparation of the insulin zinc suspensions reduced the incidence of sensitivity reactions. Preliminary studies in this country (Lawrence and Oakley, 1953; Oakley, 1953; Murray and Wilson, 1953; Nabarro and Stowers, 1953a, 1953b) confirmed these

findings, and the new insulins were made generally available in Great Britain in October, 1953. We have now used them in the routine management of 240 diabetic patients in the clinics at University College Hospital and in the Dundee area, and this report is a critical review of the results we have obtained.

Results

Patients already on insulin were selected for trial of the new preparations only if their previous regime was proving unsatisfactory. They therefore do not represent a cross-section of all diabetics requiring insulin, but rather some of the more difficult ones. A series of 205 patients already taking insulin have been tried on the insulin zinc suspensions, and the indications for transfer and the results obtained are shown in Table I. The term "poor control—hyper-

TABLE I.—Indications for Transfer (205 Cases)

	No.	Trial Discontinued	Satisfactory Result
Multiple injections ..	120	5	98 (81%)
Poor control—hyperglycaemia ..	93	4	70 (76%)
" hypoglycaemia ..	47	—	39 (83%)
Allergy " " " " " " ..	9	1	6

The sum of the number of indications exceeds the total number of patients because in a proportion multiple indications were present.

glycaemia" is used for patients with diabetic symptoms (thirst, polyuria, or unintentional loss of weight) or in whom the blood sugar was found to be over 250 mg. per 100 ml. on an ordinary day. Of the patients transferred because their previous regime involved multiple injections, 81% were satisfactorily controlled with a single injection of the new preparations. About 80% of those who had hyperglycaemia, diabetic symptoms, or repeated hypoglycaemic attacks were improved. Of those in whom the results of transfer have so far proved unsatisfactory, the trial was discontinued in 10 for reasons that are given in a subsequent paragraph. In the remainder further adjustments of the proportions of I.Z.S.(A.) and I.Z.S.(C.), as well as alterations of the diet, are being tried to improve the control of the diabetes.

The alterations in dose that were required when the patients were transferred from different insulin regimes to the insulin zinc suspensions are shown in Table II. Patients who had been taking two or three doses of S.I. usually

TABLE II.—Alterations of Dose in Transfer to I.Z.S.

	No.	Mean Previous Dose	Mean Alteration	% Change
2 or 3 doses S.I. ..	19	47 (20–116)	+1.7 (–24 to +26)	+3.6
2 doses G.I. ..	11	53 (44–88)	+12 (–12 to +24)	+23
1 dose G.I. ..	19	40.5 (20–124)	+5.7 (–16 to +36)	+14
1 " P.Z.I. ..	36	32.2 (10–60)	+12.4 (0 to +40)	+38.5
P.Z.I.+S.I. a.m.	82	53.9 (10–104)	+5.9 (–28 to +58)	+10.9
P.Z.I.+S.I. " and S.I. p.m.	21	78.5 (36–128)	+10.2 (–16 to +78)	+12.9
Miscellaneous ..	7			

needed little if any change in dosage, whereas those previously on a single dose of P.Z.I. required on an average 40% more. The difference between these two figures is highly significant, being five times the standard error. Patients previously using globin insulin (G.I.) or P.Z.I. plus S.I. required usually a slight increase in dose, but the differences do not attain the level of statistical significance. The alterations of insulin requirements that follow transfer from P.Z.I. or S.I. to the insulin zinc suspensions can to some extent be explained by the pharmacological properties of these insulins. P.Z.I., if given in a dose large enough to control postprandial hyperglycaemia in the day, is virtually certain to produce hypoglycaemia in the night or early morning. Therefore patients on P.Z.I. alone usually have to take a

*The results in this paper formed the basis of a communication given at the Annual Scientific Meeting of the British Diabetic Association on July 16, 1954.

smaller dose of it than of an insulin which acts mainly in the day and less at night, like I.Z.S. S.I., on the other hand, has a rapid hypoglycaemic action, which is apt to bring into effect counter-regulatory mechanisms for raising the blood sugar, and, to control this, extra S.I. may need to be given at the next injection. With some patients on two or three doses of S.I. this leads to an increase in the apparent insulin requirement. The moderate increase of dose in the case of patients previously taking G.I. or P.Z.I. + S.I. may reflect in part the closer attention accorded by the physician in an attempt to obtain optimal control with a new preparation. FitzGerald *et al.* (1954) found that four out of eight patients transferred from two doses of S.I. to the same total dose of I.Z.S. suffered a deterioration in control, and although this experience has proved exceptional in our series, it illustrates the variability in individual response in the same circumstances. It is such variations that require adequate numbers of cases to be studied before reliable conclusions can be drawn.

In deciding the initial dose to be given when transferring a patient to the new insulins the previous regime must be taken into consideration. If the patient was on P.Z.I. alone we now suggest an increase of 20% for the initial dose of I.Z.S., with patients on G.I. or P.Z.I. + S.I. an increase of 10%, and for those who had been taking two or three doses of S.I. the same total dose as before, assuming that the patient had been having an optimal dose of the previous insulin.

The present insulin dose of patients transferred to the insulin zinc suspensions is shown in the Chart. Most cases fell into the group requiring 30 to 70 units a day, but a

Chart showing the final dose of I.Z.S. in 230 diabetics.

number were well controlled on a single dose of over 100 units, and one patient takes 160 units. We have previously pointed out (Nabarro and Stowers, 1953b), that patients needing large doses may require more I.Z.S.(C.) than in the standard 3:7 mixture (I.Z.S.). On the latter these patients, despite the exclusion of a bed-time snack, tended to get early morning hyperglycaemia, and any attempt to increase the insulin dose resulted in the development of hypoglycaemia during the day. In this series we have so far found that about 14% of the patients required the addition of extra I.Z.S.(C.) to the standard I.Z.S. mixture, whereas only 2.5% needed extra I.Z.S.(A.). It therefore appears that, although 3:7 is the ratio most commonly required, a significant proportion of diabetics need a modification of the standard mixture. Results from other British clinics (Oakley, 1953; Venning, 1954) have differed to some degree regarding the proportions of patients needing more or less crystalline zinc suspension than is present in the I.Z.S. (lente) mixture. Such differences are probably based mainly on different distribution of the diet and a different timing of the blood

and urine tests. Where mixtures other than the standard I.Z.S. have been indicated, we provide them already mixed in sterile phials, or the more capable patients prepare them themselves. Since the 80 units per ml. strength of I.Z.S.(A.) and I.Z.S.(C.) has become available, it has been possible to provide special mixtures for patients with high insulin requirements.

It is our practice when transferring patients to the new insulins to review the distribution of the dietary carbohydrate. I.Z.S. acts most strongly between about 12 noon and 6 p.m., and therefore the carbohydrate content of the lunch and tea meals should be considerable. The usual distribution used for these patients is shown in Table III. A few tended to get hypoglycaemic before lunch, and in these the carbohydrate content of the mid-morning snack was increased. Dietary adjustments have proved a valuable help in improving control in patients taking the insulin zinc suspensions. The patients were advised to take their insulin

TABLE III.—Distribution of Dietary Carbohydrate Equivalents (Calculated from the Grammes of Carbohydrate Plus 58% g. Protein + 10% g. Fat.)

Breakfast	..	..	17%	Tea	..	..	25%
Mid-morning	..	..	5%	Supper	..	..	21%
Lunch	..	..	32%				

three-quarters to one hour before breakfast when possible, in order to prevent the postprandial hyperglycaemia which occurs if the interval is shorter.

Nine patients were tried on the new insulins because they were getting sensitization reactions at the site of injection of their previous insulin, and in six there was a considerable improvement. One had been getting local reactions to P.Z.I. and continued to do so with the British I.Z.S.; they ceased, however, when Danish I.Z.S. was used.

The trial of the insulin zinc suspensions was abandoned in 10 cases. In one this was because local sensitization reactions were unimproved and the patient asked to return to his previous insulin. In a second case the insulin requirement proved too variable for any single injection programme. In general we have excluded these "brittle diabetics" from the trials of the insulin zinc suspensions, believing that they are better controlled with two injections of S.I. or G.I. In the remaining eight cases the transfer to I.Z.S. was followed by a steep increase in insulin requirement, and in two ketosis developed. It was not an uncommon experience for the diabetes to escape from control following transfer, if the initial dose of I.Z.S. was the same as that of the previous insulin—especially if the latter was a single injection of P.Z.I. To regain control a large increase of dose was required, sometimes supplemented with S.I. In the cases mentioned above the trial of I.Z.S. was discontinued because the patient, and on occasion the physician as well, became alarmed at the increase of insulin requirement.

The steep increase of dose needed to regain control if the initial dose of I.Z.S. at the time of transfer had been inadequate has also been noted in patients well balanced on I.Z.S. whose stabilization has been upset by an intercurrent infection. When a patient on I.Z.S. becomes unbalanced the insulin dose must be increased more boldly to control the hyperglycaemia and avert ketosis than is the case with patients on S.I. or G.I. Three of our patients have developed ketosis after being satisfactorily balanced on I.Z.S. After treatment with S.I. two of them were re-balanced on I.Z.S. and needed a dose very similar to that taken before the episode of ketosis. This danger of the insulin zinc suspensions has so far not been emphasized. It is important to realize that if thirst and polyuria develop during an intercurrent infection the insulin dose should at once be increased by about 20%, and if the symptoms persist S.I. should be given before the later meals of the day.

In addition to the 205 patients already on insulin 35 new diabetics have been given the insulin zinc suspensions. In the more severe cases which required admission to hospital the symptoms were first controlled with S.I., and in the remainder the insulin zinc suspensions were used from the

start. These new preparations proved very satisfactory for the management of fresh cases of diabetes, and the final doses required are shown in the Chart. One of these patients has had mild local reactions at the site of the injections.

Discussion

Colwell (1954), in a recent editorial discussing the insulin zinc suspensions, suggested that "before new preparations are introduced it should be clearly apparent that they possess decisive advantages over those now available." If the value of the insulin zinc suspensions is to be assessed, the advantages and disadvantages of the insulins previously available must be reviewed briefly and compared with those of the new preparations.

Soluble Insulin.—S.I., with its quick action, is very valuable for emergency use in diabetic ketosis, and for conditions in which the insulin requirement is changing rapidly and is unpredictable—for example, in the presence of an acute infection or after surgery. On the other hand, if it is to be used for the day-to-day routine management of patients, at least two and often three injections are needed.

Protamine Zinc Insulin.—This form is suitable for the treatment of very mild cases, and the exact timing of the injection in relation to breakfast is relatively unimportant. However, its action is slow and the total duration prolonged (up to thirty hours or more). It acts as strongly by night as by day, and, in all but the mildest diabetics, if enough is given to control the daytime blood sugar, hypoglycaemia is apt to occur in the early hours of the morning. For this reason it is often necessary to supplement its action with a dose of S.I. given before breakfast, either mixed with the P.Z.I. or by a separate injection. If the mixing technique is used, inconsistent effects may result from variable degrees of mixing and from the variable excess of protamine present that tends to combine with the S.I. Diabetics requiring doses of 60 units or more may escape from control in the evening unless another dose of S.I. is given before supper. This means that the treatment is somewhat complicated, involving two or three injections daily and two kinds of insulin which have to be varied independently according to the timing of any hypoglycaemic attacks or hyperglycaemic peaks during the twenty-four hours. Regimes with P.Z.I. plus S.I. seldom lead to hypoglycaemic attacks in the late afternoon, a period that is rather difficult for working men with the small tea meal followed by the journey home. Patients taking P.Z.I. and S.I. in the morning and S.I. in the evening are able, when necessary, to vary both the time and the amount of the latter, and this may be very convenient for those who make a practice of dining out. Local reactions at the site of the injection are quite common, and these are usually due to the protamine content of the preparation.

Globin Insulin.—Diabetes of mild or moderate intensity may often be treated by a single daily injection of this insulin, and severe "brittle diabetics" may be well controlled by two injections—one given about three-quarters of an hour before breakfast and the second before tea or supper. Severe diabetics cannot be controlled with a single injection because its action is too short and the blood sugar rises in the evening and during the night. Hypoglycaemia tends to occur in the late afternoon and a large tea meal is required. Sensitivity reactions to the globin in this preparation are not uncommon.

Isophane (N.P.H.) Insulin.—This modification of P.Z.I. has only recently been made available in this country, although it is at present the most popular insulin in the U.S.A. It is designed to exert its hypoglycaemic action for twenty-four hours, and, since it contains no excess protamine to combine with the insulin, it can be mixed with S.I. without significantly modifying the rapid action of the latter. Apparently in a proportion of cases its action fails to last for the full twenty-four hours, and early morning hyperglycaemia may be troublesome. Like P.Z.I., it contains a foreign protein and, as would be expected, produces local sensitivity reactions in a number of patients.

The insulin zinc suspensions overcome many of the disadvantages of these preparations. The duration of action of the I.Z.S.(C.) is more than twenty-four hours, and during the day this can be reinforced by the addition of I.Z.S.(A.). The actions of mixtures of these two preparations are consistent and predictable, being those of the two insulins given separately. Most diabetics are well controlled on the 3:7 mixture—I.Z.S.—but a few require a different ratio. The insulin zinc suspensions cannot be mixed with S.I. because the acid in the latter overcomes the weak buffer in the suspensions, and, by lowering the pH, may modify the particle size and lead to unexpected alterations in the duration of action. This is not a serious disadvantage, because I.Z.S.(A.) and S.I. come into action at much the same rate and if it is necessary to accelerate the action of I.Z.S. it can be done by adding extra I.Z.S.(A.). The morning injection of I.Z.S. should probably be given three-quarters of an hour to one hour before breakfast, and this may be a disadvantage in later risers. The maximal hypoglycaemic action of I.Z.S. is in the afternoon; and if hypoglycaemic attacks at that time are to be avoided a good lunch and tea are required; some patients find this difficult to arrange.

The theoretical advantages of the insulin zinc suspensions have been borne out by our finding that 80% of the more difficult diabetics attending our clinics have benefited from being transferred to them. At the same time they have been in general use in this country for only one year, and it is possible that further experience will reveal shortcomings. We are a little apprehensive about the difficulty in regaining control with them once it has been upset by, for example, an intercurrent infection.

Summary

The results of a trial of the insulin zinc suspensions in 240 diabetic patients are described. Thirty-five were new patients and satisfactory stabilization was achieved. The remainder were transferred to the new insulins because their previous regime had disadvantages. Over 75% of these cases benefited from the transfer. Patients previously on a single injection of protamine zinc insulin needed a 40% increase in dose, those on other programmes with long-acting insulins needed smaller increases. Patients having two or three injections of S.I. were balanced on a similar total dose of the insulin zinc suspensions. When patients are being transferred to the new insulins it is necessary to take into consideration the type of insulin the patient had been using when assessing the initial dose of insulin zinc suspension to be given.

In the majority of patients insulin zinc suspension (lente), with the ratio three parts amorphous to seven parts crystalline, proved satisfactory: 14% needed a larger proportion of the crystalline and 2.5% a larger proportion of the amorphous.

The comparative advantages and disadvantages of the current British insulins have been reviewed. It is concluded that the insulin zinc suspensions have definite advantages over the other preparations, and that probably over 90% of all diabetics requiring insulin can be satisfactorily controlled by a single daily injection of suitable proportions of crystalline and amorphous insulin zinc suspensions. Attention is, however, drawn to the difficulty in regaining control with these preparations if the balancing of the diabetes is upset by an intercurrent infection.

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TREATMENT OF DIABETES MELLITUS WITH INSULIN ZINC SUSPENSION

A CLINICAL STUDY BASED ON 479 CASES

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The clinical assessment of the new insulin zinc preparations introduced by Hallas-Møller *et al.* (1952a, 1952b) has necessarily been divided into two stages. The first, in which detailed observations were made on a small number of diabetic patients in hospital under controlled conditions, was necessary to verify their metabolic activity and provide familiarity with their clinical potentialities. Our own initial observations (Lawrence and Oakley, 1953; Oakley, 1953) have been confirmed by those of Drury (1953), Murray and Wilson (1953), Nabarro and Stowers (1953), and Venning (1954), all of whom obtained satisfactory results. From this preliminary work it was evident that in carefully selected and well-treated groups of patients a considerable measure of success could be obtained using insulin zinc suspension ("insulin lente"; I.Z.S.) in a single daily injection.

The second and equally important stage in its evaluation was the study of a large number of diabetics in conditions of everyday life. Variations in diet and activity, exposure to intercurrent disease, and the inevitable spontaneous day-to-day fluctuation in the diabetic state might lead to results at variance with those obtained in the earlier studies.

Present Study

This paper is based on our observations on 479 patients seen in the Diabetic Department of King's College Hospital, treated with I.Z.S. in the period July, 1953, to June, 1954. The length of treatment ranged from three months to one year, the majority having been maintained on I.Z.S. for over six months. When out-patients were changed to the

new insulin, reattendance was demanded during the first six weeks at intervals of a week or a fortnight. Special notes were kept with records of the night and morning urine tests for sugar and ketone bodies, blood sugar, weight, hypoglycaemia, local reactions, the patient's subjective observations, and alterations in diet. Some degree of selection was inevitable, as cases already on insulin tended to be chosen because they were judged likely to do well on I.Z.S., and those with insulin requirements of over 80 units a day were usually excluded. A relatively larger proportion of patients were, for instance, on protamine zinc (P.Z.I.) rather than soluble insulin (S.I.). Almost all new patients seen during this period were tried on a single injection of I.Z.S., those with severe ketosis being first treated with S.I. We must emphasize that at this time it was our policy not to alter the treatment of well-controlled diabetics, and only about 10% of insulin cases attending the clinic were changed to I.Z.S. The frequency of visits also made big demands on the patient's co-operation, and those who could not attend as often as we required were not permitted to change. The total of patients treated at King's College Hospital on I.Z.S., I.Z.S. amorphous ("semilente"), or mixtures of the latter with I.Z.S. crystalline ("ultralente") is now well over 700, and we think that enough clinical experience has been gained to justify a definite decision on their value.

Criteria of Control

There are no clear-cut criteria by which the clinical superiority of any type of insulin can be assessed. We have analysed our results so as to take into account both subjective and objective factors, and have compared these with the degree of control previously achieved using other types of insulin.

Subjective.—By this we mean the opinion of the patient concerning his well-being and freedom from hypoglycaemia and diabetic symptoms, as compared with his condition either before any form of insulin was started in new cases or on his previous regimen. Since the patient's attitude to any new form of therapy is likely to be biased this information is of limited value.

Objective.—To assess *absolute control* we have taken into account (A) the degree of night control as indicated by the early morning urine tests; (B) day control, judged by the blood sugar before the midday meal; and (C) alterations in weight. Night control was regarded as good when on at least 75% of examinations the early morning Benedict's test was blue or green ($\frac{1}{2}\%$; one plus). Day control was accepted as satisfactory if the capillary blood sugar was under 200 mg. per 100 ml. (Folin and Wu). Changes in weight of ± 2 lb. (0.9 kg.) were ignored, and only larger variations were deemed significant. When both night (A) and day control (B) were good, and when weight was static or had increased (C), control was considered excellent and the A B C classification applied. As in many patients a slight loss of weight was desirable, we have grouped those with A B C and A B classifications together and described the control as good. When only one or two of the criteria were satisfied control was assessed as fair, and if none were satisfactory it was assessed as poor and recorded as O.

Comparative control was assessed in the same way by comparing the results achieved with I.Z.S. with those over a similar retrospective period of treatment with other insulins, and classified as better, the same, or worse.

Analysis of Results

In order to facilitate discussion we have divided our cases into the following groups:

Adults:	{ Old insulin cases	..	..	312
	{ New cases	..	..	122
Children:	{ Old cases	..	..	30
	{ New cases	..	..	15
I.Z.S. abandoned, including adults and children				46

Table I shows that 433 of the 479 cases receiving I.Z.S. remained on it at the close of the trial, and that in only 32 was this form of treatment regarded as a failure.