STUDIES ON A NEW LONG-ACTING INSULIN: ZINC METHYL-ALBUMIN INSULIN

By

Ole Skensved

Insulin is freely soluble at the pH of the blood and is therefore absorbed within a few hours. In order to obtain a retarded absorption and consequently a prolongation of the therapeutic effect over a period of up to 24 hours, the insulin must be slowly liberated at pH 7.

Among the different methods of producing such a «depot» preparation it is worth mentioning those of Hagedorn et al. (1936) and Krarup (1935). In these methods insulin negatively charged at pH 7 is combined with protamine extracted from fish sperm which is positively charged at the same pH. The protamine is not antigenic.

Among other basic proteins, histones and globins (Mac Bryde & Reiss, 1944, Bailey & Marble, 1942) have been used, but the results have not been good enough to give them much therapeutic significance.

Scott & Fischer (1935) found that the depot effect of protamine insulin could be further prolonged by the addition of zinc. Ever since, this compound has, along with protamine insulin, been widely used in the management of diabetes.

The latest development is the discovery of insulin-zinc suspensions in an amorphous and/or crystalline state in acetate buffer without zinc precipitating ions and without the addition of proteins (Hallas-Møller et al., 1954).

It seems strange that for 20 years only the naturally occurring alkaline proteins have been used to obtain the depot action. The fact is, that they have been believed to be the only available media that were not at the same time antigenic, but this is not so.

Human albumin is eo ipso a non-antigenic, well-defined substance. Its reaction

1. Zinc Methyl-Albumin Insulin was kindly supplied by Roskilde Medical Company Ltd., Roskilde, Denmark.
is acid, but a methylation process blocks the acid groups, so that the methyl albumin behaves as a basic protein. Accordingly, it can become bound to the acid insulin, thus forming an insulin preparation, slightly soluble at pH 7.

The use of human albumin is attractive, and the incidence of allergic reactions to this preparation might be expected to be reduced compared with previous retardation media.

**ZINC METHYL-ALBUMIN INSULIN**

*Components of Zinc Methyl-Albumin Insulin (ZMAI)*

The insulin component of this preparation is obtained from the pancreas of slaughtered animals and the albumin component from human serum purified at the Statens Seruminstitut, Copenhagen, Denmark.

One litre of ZMAI, 40,000 international units, contains:

1.74 gm. insulin, 1.74 gm. methyl albumin, 16 gm. glycerol, 1.5 gm. tricresol, 0.6 gm. phenol, 80 mg. zinc.

Its pH ranges from 6.8 to 7.3.

The preparation is made from re-crystallized insulin containing not less than 24,000 I. U. per gm.

*Test for Antigenicity of Methyl-Albumin*

To this end the author used the method advocated e. g. by Nathan & Kallos (1932): 0.01 ml. of the test substance is injected intracutaneously. One week later, the intracutaneous injection of the same dose is repeated. If the substance is antigenic, a cutaneous manifestation of sensitization will appear within 24 hours of the second injection.

Forty-nine adults were tested by this method with a diluted solution of methylated human albumin with a nitrogen content corresponding to that of guinea-pig serum.

According to Nathan & Kallos the method should definitely show whether or not the injected substance is antigenic.

*None* of the 49 subjects exhibited skin reactions after the second injection.

**EXPERIMENTAL TEST METHODS**

Whenever a new insulin preparation is released, it has to be assessed in relation to existing preparations, not only clinically, but also experimentally. For this purpose the author tested two methods, viz: Gerritzen (1952, 1953) and Hallas-Møller (1945).

(1) *Gerritzen's method:*

Gerritzen showed, that blood sugar (Bₙ) remains at a constant level, about 85–90 mg. per cent. for 24 hours in healthy subjects during special standard condition.
Gerritzen thus injected 20 I. U. insulin of different types into these subjects while they were under the same standard conditions.

He then found a decrease in Bs, in each case with a characteristic rate of fall and in the time required for the Bs to return to the initial value.

By these experiments on a limited test material Gerritzen was able to demonstrate qualitatively the duration of action of the different insulins used. He also found a close parallelism between these results and the properties of the insulins already known and in clinical use.

Thus it seemed of value to investigate ZMAI under the above mentioned standard conditions in order to see, how the action on Bs compared with previous insulins in use.

**Test: Gerritzen I**

**Name:** K. I.

**Age:** 38

**Meals:** 50g potatoes + 30cc water

Blood sugar curve during Gerritzen test I (control test) showing marked increases at the time of meals for the other patients in the same room.

**Author’s tests by the Gerritzen method**

4 non-diabetic subjects were tested. However, in the control tests it proved impossible to obtain a constant blood sugar level throughout the 24 hours in every case. A typical specimen is shown in Fig. 1. A marked increase (up to 180 mg. per cent) occurred at the time of the usual meals. In one experiment in which a subject was isolated while the others having their meals, the increases were eliminated, but the blood sugar fluctuated between 122 and 60 mg. per cent irrespective of meals or other known factors.

The author, therefore, abandoned the Gerritzen method and tried instead the method used and described by
as follows: »Under standard conditions and at suitable intervals a diabetic is subjected to 24 hours test. In these 24 hours tests one uses successively the insulin preparations which it is desired to compare, so that they are characterized by a 24 hours blood sugar curve as well as excreted quantities of sugar in fractionally collected urine.«

On the day prior to the test (= »pre-days«) the subject is given only regular insulin at suitable intervals, the last dose not later than midnight.

This arrangement is designed to have the following advantages: (1) that the patient can be adjusted to a suitable level at 8 a.m. on the test day, (2) that the effect of the long-acting insulin given previously during the pre-day is eliminated before the commencement of the test day, (3) that during the pre-day it can be determined whether the patient was out of control (in which case the test is of no value), and (4) that the last dose of regular insulin has entirely ceased acting by 8 a.m. on the test day – so that at this juncture the patient is not exposed to the action of »exogenous« insulin.

According to Hallas-Møller this method classifies the insulin preparations better than ordinary clinical testing. In the latter an average Bs curve (and one for the urinary sugar excretion too) may be plotted for a number of days, but the frequently marked fluctuations around this mean may make it difficult to tell whether the curve is representative of the effect of the preparation in the individual patient.

By this method called Bed – Standard – Diet – Method (B. St.) Hallas-Møller (1945, 1954), obtained extremely illustrative curves (and constant upon repetition) representing the different insulin preparations used in relatively small series. His curves are in good agreement with the clinical experience in large series treated with the tested insulin preparations.

Author's tests by the Hallas-Møller method

In the present study, the author used Hallas-Møller's method with 2 modifications:

(1) The patients had the diet to which they were previously adjusted, in the same quantity and of the same nature, on pre-days as well as on test days. This should maintain standard conditions, and should give a reasonable possibility of comparing the results obtained before and possibly after the test (with a constant dosage of insulin), taking into account the lack of physical activity on the days of the test.

(2) The test period was prolonged up to 27 hours in order to include a possible effect lasting for more than 24 hours. The patients had their usual breakfast at 8 a.m., 24 hours after the commencement of the test day.

On the days of the testing the Bs was determined 14 times at suitable intervals. The urinary excretion of sugar was measured in 5 portions.

It would be most reasonable to expect that the Bs curve should be higher particularly during that part of the test day on which the patient was usually ambulatory and performing quite considerable physical activity in the form of exercise (on unchanged diet), or perhaps of the same, but at any rate not at a lower level.

The series comprises 7 patients. B. St. has been performed both with adjustment on previously injected insulin and with adjustment on ZMAI. In the present case the latter is of greatest interest.

The control on the pre-day was on the whole satisfactory.

In 2 cases, and to some extent in one more case, there was a parallelism between B. St. and the 5 days' clinical trial, in some cases with the expected effect of physical activity, whereas 3 cases did not show the expected parallelism. One case cannot be definitely assessed. They differ as follows: (a) A higher level in the morning: 2 cases, (b) a lower level in the middle of the 24 hours: 1 + perhaps 1 more case, (c) a higher
level after midnight: \(3 + \) perhaps \(1\) more case. Of these only \((a)\) can be ascribed to the absence of physical activity on the test day whereas \((b)\) (the fall in middle of the 24-hours) and \((c)\) (the nocturnal increase) cannot be attributed to physical inactivity.

The findings \((b)\) and \((c)\) will be called »distortion«.

Hence in 3 out of 6 cases there is an exaggerated ~ shaped Bs curve in B. St. compared with a 5-days' clinical testing. Since clinical testing of ZMAI appears to have shown that the preparation is satisfactory in clinical practise, and since in the 3 above-mentioned cases it gave better results in clinical practise than in B. St. it seems justified to assume that the B. St. method is not reliable in assessing the ZMAI preparation. On the other hand, it is probably suitably for testing insulins with a less prolonged type of action such as e.g. insulin of the NPH 50 type.

The »distortion« in the latter part of the experimental 24 hours with a higher Bs level also affects the assessment of the duration of the effect of ZMAI, which on the whole proved to be 3 hours short of 24 hours in the B. St. test. This does not correspond to clinical results in general, which seem to indicate that it is approximately 24 hours.

In trying to ascertain why the B. St. method failed in essential respects to assess the effect of ZMAI, it seems reasonable to point out that the cases showing »distortion« were rather severe. The other cases had considerably less insulin. Moreover the first case mentioned had ZMAI before B. St. for on an average \(1\frac{1}{2}\) day while in the latter this averaged \(4\frac{1}{2}\) days.

The latter finding is in agreement with the fact that during the adjustment to ZMAI, some days were required before the Bs curves were stabilized, presumably because of the marked depot effect of the preparation, cf. Wilder (1937).

Hence, a possible explanation of the inaccuracy of the method in testing ZMAI is that the pre-day administration of regular insulin decisively breaks the ZMAI control obtained by using the preparation for some consecutive days.

On the basis of the present findings, therefore, it seems justifiable to conclude that in assessing ZMAI the B. St. method does not afford more information about the preparation than ordinary clinical testing, despite the uncertainty attached to the latter.

This leaves only the possibility of assessing the preparation by the usual clinical testing in diabetics.

**Clinical Testing of ZMAI**

**Material:**

During a certain period all diabetics admitted to the department and in whom insulin was indicated, were adjusted to the preparation to be tested. This gave a total of 22 patients most of whom were admitted for diabetes which had been out of control – with or without insulin.

It will be seen from Table 1 and 2 that the material is fairly representative with the exception unfortunately, that it does not include children.

**Method:**

An attempt was made to control the patients, who had previously been receiving insulin, on their usual preparation in the course of \(\frac{1}{2} - 2\) weeks. If they remained out of control, as happened in many cases, an attempt was made to adjust them to other insulins. The Bs was determined at 8 a.m. (fasting),
Table 1.
The material.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Sex</th>
<th>Duration of diabetes (years)</th>
<th>Duration of insulin therapy (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20: 3</td>
<td></td>
<td>&lt; 5: 7</td>
<td>5: 6</td>
</tr>
<tr>
<td>21-40: 2</td>
<td>♂</td>
<td>5-10: 6</td>
<td>5-10: 5</td>
</tr>
<tr>
<td>41-60: 9</td>
<td>7</td>
<td>&gt; 10: 6</td>
<td>&gt; 10: 4</td>
</tr>
<tr>
<td>&gt; 60: 8</td>
<td>♀</td>
<td>newly</td>
<td>started in course of present study: 7</td>
</tr>
<tr>
<td>min.: 17</td>
<td></td>
<td>total: 22</td>
<td>max.: 16 years</td>
</tr>
<tr>
<td>max.: 75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>nearly 51 years</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.
Insulin doses.

<table>
<thead>
<tr>
<th>Previous dose of insulin (best obtainable)</th>
<th>ZMAI (in brackets fresh cases)</th>
<th>Decrease in units on ZMAI</th>
<th>Increase in units on ZMAI</th>
<th>Unchanged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once daily</td>
<td></td>
<td>Once daily</td>
<td>Twice daily</td>
<td></td>
</tr>
<tr>
<td>Units (No. of patients)</td>
<td>(No. of patients)</td>
<td>From 36 to 4 units in 8 cases, average 17.5 units</td>
<td>4 units in 3 cases, average 4 units</td>
<td>4</td>
</tr>
<tr>
<td>12-20</td>
<td>2</td>
<td>2 (+2)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>21-40</td>
<td>1</td>
<td>3 (+4)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>41-60</td>
<td>4</td>
<td>7 (+1)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

average: 38 units in the 24 hours

11 a. m., and 4 p. m. and only in exceptional cases at other times of the day.

The patients were then adjusted to ZMAI – in 21 cases 1 daily injection was administered at 8 a.m. – with an initial dose corresponding in units to the preparations given up to date.

When the patients had been stabilized on the new preparation and the dose seemed to have been fixed, usually at the end of 5–8 days, the following features were studied on a constant dosage of insulin for 5 consecutive days:
Determination of the Bs at 8 and 11 a.m., at 4, 6, and 10 p.m., and at 2 and 5 p.m. as well as measurement of the urinary sugar excretion in 4 portions: from 8–11 a.m., from 11 a.m. to 4 p.m., from 4–10 p.m., and from 10 p.m. to 8 a.m. This will be called the 5-day clinical test.

Only in cases in which for private reasons the patients had to restrict their stay in hospital as far as possible, the period before starting ZMAI was shortened. In the case of 3 patients this was only slightly less than 5 days with determinations of Bs at 8 a.m., 11 a.m., and 4 p.m. and of the 24-hour urinary excretion of sugar (Us. D.).

During the 5-day test all the patients were ambulatory and, so far as their condition permitted, had ample exercise throughout the day.

Diet:

Practically all the patients had a full diet less sugar, caloric value 1500–3500 with a carbohydrate content of from about 100 to a little over 300 gm. daily (the latter being rare). The ratio of the relative carbohydrate content of the meals was as a rule as follows: 8 a.m.: 1. 11.30 a.m.: 3–5. 3 p.m.: 1/2–1. 5.30 p.m.: 3–4. 8–9 p.m. Only in 1 case, in which the control was poor, was an endeavour made to obtain improvement by weighed amounts of bread: about 100 gm. and potatoes: 100–150 gm. daily. A few overweight individuals had a 1200-calorie diet without sugar. In a few cases, moreover, the relative size of the individual meals and their carbohydrate content were changed according to the schedule given by Duncan (1952).

The patients were instructed to take, as far as possible, the same amount and kind of food every day. The results would no doubt have been more «perfect», if they had been given an accurately and weighed diet as done by e.g. Hallas-Møller (1953) and Duncan (1952). However, the author made a point of testing the preparation under conditions as close as possible to the «home pattern».

Re-adjustment from Previous Preparation to ZMAI

As a rule, this was accomplished without major difficulties. Occasionally, however, the patients showed increased 24-hour urinary sugar excretion and a higher Bs at 8 and 11 a.m. and a lower at 4–6 p.m. during the first few days, but this was in most cases stabilized within 4–6 days.

Results with ZMAI

In order to get as wide a range of evaluations as possible, the author selected the following features from those which influence the character of diabetic control:

1. Mean blood sugar for the 24 hours determined in the case of ZMAI, by calculating the mean of 7 daily determinations for 5 consecutive days (m. Bs. D5).
(2) Mean of daily maximum and minimum of blood sugar (determined as the
mean of 5-day values, e. g. at 8, 11 a. m. etc.). The distance between the
maximum and minimum m. Bs. is called \( \Delta \) Bs.

(3) Mean urinary excretion for 5 days (m. Us. D5).

(4) Persistent insulin reactions – or possibly insulin shock.

(5) The extent to which the mean blood sugar curve for 5 days is representa-
tive. The fact is that extremely varying values from day to day and from
hour to hour may easily give a »perfect« m. Bs. curve, though without any
relevance to the clinical use of the preparation.

This was attempted by calculating \( S_y \) – the standard error – uncertainty on the
mean value for each of the 7 Bs values obtained in the 24 hours, for the 5
consecutive days, on the basis of the formula \( S_y = \sqrt{\frac{\sum (x - \bar{x})^2}{n \times (n - 1)}} \). Thus, it is
impossible to arrive at a final value for the 24-hour period, and this quantity
cannot be fitted into Table 3, but the \( S_y \) values are given on the curves to
supplement the evaluation. (It must be mentioned that if only a small number
of determinations is made, \( S_y \) will be relatively high and vice versa).

To illustrate the quantities representing \( S_y \), two m. Bs. curves are set out,
giving the individual Bs at the different hours as well as the \( S_y \) values, Figs.
2 and 3.

On the basis of (1), (2), (3), (4), and an estimate of (5) the classification is
set out in Table 3.

In Table 4 each patient is characterized exclusively by the poorest result
obtained with regard to items 1 to 5 in Table 3, e. g.: a case with findings in
the groups (1), (2), (3): excellent and in group (4): bad, would be classified
as: bad.

The 15 patients who had been controlled with one or perhaps more insulin
preparations before being started on ZMAI are, with a few exceptions, only

**Table 3.**

Classification of results on ZMAI and on other insulins.

<table>
<thead>
<tr>
<th>m. Bs. D.</th>
<th>Max. m. Bs.</th>
<th>Min. m. Bs.</th>
<th>( \Delta ) Bs.</th>
<th>Persistent, minor insulin reactions</th>
<th>Sy</th>
<th>m. Us. D.</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;150</td>
<td>200</td>
<td>100</td>
<td>&lt;100</td>
<td>no</td>
<td>Acc.</td>
<td>&lt; 5 gm.</td>
<td>excellent</td>
</tr>
<tr>
<td>&lt;175</td>
<td>230</td>
<td>80</td>
<td>&lt;150</td>
<td>no</td>
<td>to</td>
<td>&lt; 15 gm.</td>
<td>good</td>
</tr>
<tr>
<td>&lt;200</td>
<td>260</td>
<td>70</td>
<td>&lt;190</td>
<td>no</td>
<td>esti-mate</td>
<td>&lt; 25 gm.</td>
<td>fair</td>
</tr>
<tr>
<td>&lt;210</td>
<td>300</td>
<td>70</td>
<td>&lt;230</td>
<td>yes</td>
<td></td>
<td>&lt; 50 gm.</td>
<td>not sufficient</td>
</tr>
<tr>
<td>&gt;210</td>
<td>&gt;300</td>
<td>60</td>
<td>&gt;230</td>
<td>yes</td>
<td></td>
<td>&gt; 50 gm.</td>
<td>poor</td>
</tr>
</tbody>
</table>

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Case XIII
Name: o  Age: 54
Dose: 24 i.u. ZMAI

Fig. 2.
Mean blood sugar curve for 5 days. The single blood sugar determinations are marked as dots. The S\(\bar{y}\) values are small, i.e.: representative mean curve.

Case VI
Name: o  S. Age: 39
Dose: 24 i.u. NL + 24 i.u. SL 8 a.m.

Fig. 3.
Mean blood curve for 5 days with big S\(\bar{y}\) values, i.e.: not representative mean curve.

representated by m. Bs. determined at 8 a.m., 11 a.m., and 4 p.m. as well as m. Us. D. When the results on the former preparations are compared with those on ZMAI, the corresponding values are given for the latter. As regards the 9 patients who had previously received another insulin and who are classified as excellent, good, and fair (vide infra) the findings were as follows: In no case was the control on ZMAI poorer than on a previous preparation; in 3
Table 4.
Results on ZMAI.

<table>
<thead>
<tr>
<th>excellent</th>
<th>good</th>
<th>fair</th>
<th>not sufficient</th>
<th>poor</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6</td>
<td>4*</td>
<td>5**</td>
<td>2***</td>
<td>total 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>total 7</td>
</tr>
</tbody>
</table>

*) of these 4 patients: 2 had not been tested on another insulin.
2 had been »not sufficient« on another insulin.

**) of these 2 patients: 2 had not been tested on another insulin.
1 had proved not sufficient on the best of other insulins.
2 were classified as poor on another insulin.

*** of these 2 patients: 1 was classified as not sufficient on the best of other insulins.
1 was classified as poor on a number of other insulins.

it was equally good on ZMAI once daily, as on a previous preparation twice daily, in 1 case it was equally good on ZMAI once daily as on a previous preparation once daily, in 2 cases it was far better and in 2 a little better on

Case XIX
Name: T. T. Age: 59
Dose: 48 i.u. NL 8 a.m. + 16 i.u. NL 6 p.m.

Fig. 4.
Result on ZMAI classified as excellent.

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ZMAI once daily, than on a previous preparation twice daily, lastly in 1 case it was better on ZMAI once daily than on a previous preparation once daily.

The results on ZMAI were distributed as shown in Table 4.

Excellent results are exemplified in Fig. 4 (Case XIX), Fig. 5 (Case I), and Fig. 2 (Case XXII), good results in Fig. 6 (Case XXI), fair results in Fig. 7 (Case XIII).

Below a few details will be given regarding cases classified as (1) not sufficient (not. suff.) and (2) poor.
Case XIII

Name: K. Age: 68.

Dose: 24 i.u. ZMAI 8 a.m.

<table>
<thead>
<tr>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>22</th>
<th>24</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[Graph showing blood sugar levels]

Urine sugar

Fig. 7.
Result on ZMAI classified as fair.

Re (1): not. suff.
Case V. I.G., ♂, aged 57 with diabetes of 4 years' duration treated with insulin for 4 years. Psychic instability, previous difficulties of control. Admitted with rather severe exacerbation. On insulin Retard, 40 units in the morning, the m. Bs. D. was 276 mg. per cent and on 52 units in the morning 231 mg. per cent with hypoglycaemia in the evening. On insulin Novo lente, 44 units in the morning: Hypoglycaemia every day from 6–8 p.m. On ZMAI, 52 units in the morning: Less hypoglycaemia in the early hours, but definite hypoglycaemia from 6–8 p.m. This left the only possibility of 2 daily injections (ZMAI 24 units at 8 a.m. and 16 units at 6 p.m.). This resulted in some improvement. For lack of time, this patient could not be tested on other preparations twice daily. For most m. Bs. values the S₁ was rather high, corresponding to the patient's instability - diabetic as well as psychic.

This is the only case requiring ZMAI twice daily.

To sum up. a patient who was difficult to control, who had a high △ Bs. as well as a high m. Bs. D. and a tendency to hypoglycaemia on the depot preparations (Retard, Lente, and ZMAI) once daily, showed improvement on ZMAI twice daily.

Case IX: Fig. 8. M. H., ♂, aged 22, with diabetes of 12 years' standing, treated with insulin for 12 years. On 6 occasions admitted to hospital for control purposes which had always proved difficult. Previously, she had been receiving insulin Retard, up to 60 units in the morning + 60 units in the evening. Recently a number of hypoglycaemic reactions on this dosage, also on admission. The dose of Retard was quickly reduced. ZMAI once daily, at the outset 72 units decreasing to 56. At 6 p.m. some insulin reaction, so the dosage was reduced despite a rather high fasting Bs. Int. al. she had several episodes of counter-regulation. cf. Jersild (1953). It was considered advisable to divide the dosage into two daily injections but the patient objected violently. Despite warnings, her food intake was extremely irregular.

To sum up. prolonged diabetes, overdosed on 60 + 60 units of insulin Retard. Discharged on 56 units of ZMAI with a high △ Bs.

Case XII: E. M., ♂, aged 63, with diabetes of 9 years' duration, not previously treated with insulin. The patient was admitted with ureteral calculus, infected hydro-
nephrosis, and a flare-up of the diabetes. Transferred to another department with hyperpyrexia. The calculus was voided without operation, but the temperature did not return to normal until 2 days prior to discharge. Only the m. Bs. values from 8 a.m., 11 a.m., and 4 p.m. are available: 195 – 254 – 164 mg. per cent. m. Bs. D. 204 mg. per cent and m. Us. D.: 22 gm. Dose: 24 units of ZMAI in the morning. The control will presumably became more satisfactory, when the infection has entirely subsided.

To sum up, a patient who had not previously been treated with insulin. Exacerbation owing to infected hydronephrosis which had not entirely subsided, when the patient was controlled on 24 units of ZMAI daily.

Case XVII: Fig. 9. K. P., aged 60, with diabetes of 14 years' duration, treated with insulin for 7 years. Previously admitted 6 times for control which had always proved difficult. This time, admitted in precoma and with fresh coronary occlusion, incipient uraemia, nephrosclerosis and gouty arthritis. At the outset, greatly increasing insulin requirement. The 5-day clinical test was performed 6 weeks after admission. Considering the severity of the associated diseases and the size of the dosage: 80 units of ZMAI in the morning, the results must be called satisfactory. A few months later, this patient was re-admitted for another reason, and at that time the result was even better, but is not included in the present analysis.

To sum up, a patient admitted in precoma with coronary occlusion and renal damage. Discharged on 80 units of ZMAI once daily.

Case XVIII: O. L., aged 47, with recently discovered diabetes which had not previously been treated with insulin. Signs of precocious arteriosclerosis. Chest radiography: Hilar adenitis. The patient discharged himself just before the 5-day clinical test was to be instituted. m. Bs. (4 days) at 8 a.m., 11 a.m., and 4 p.m.: 139 – 269 – 146 mg. per cent. Sj 10, 13, 25, m. Bs. D. 185 mg. per cent. Urinary sugar excretion averaged from 8 a.m. to 6 p.m.: 16.8 gm., from 6 p.m. to 8 a.m.: 1.1 gm. m. Us. D. 17.9 gm. Too little effect of the preparations from 10 a.m. to 1 p.m., satisfactory effect during the remaining 21 hours. Dose 48 units of ZMAI in the morning.

To sum up, recently discovered diabetes in a patient who discharged himself before satisfactory control had been achieved. 48 units once daily on discharge.
Re (2) (2 cases classified as poor).

Case III (Fig. 10). I. S., Q., aged 17, with diabetes which had been treated with insulin for 5 years. Hospitalized 4 times for control. Insulin Retard twice daily: not satisfactory. Insulin Novo lente 44 units + ultra lente 8 units in the morning: m. Bs. D7: 189 mg. per cent, m. Us. D7: 46 gm. On three occasions severe insulin reactions occurred. The m. Bs. curve was not so bad (Δ Bs.: 136 mg. per cent), but is probably not representative as also shown by the S̄Y values.

ZMAI, 44 units in the morning: m. Bs. D5: 208 mg. per cent, m. Us. D5: 65 gm., insulin reactions 2 or 3 times daily, and higher Δ Bs.: 212 mg. per cent. S̄Y values high, approximately as stated above. Insulin Novo lente, 44 units in the morning showed: m. Bs. D3: 188 mg. per cent. Δ Bs.: 123 mg. per cent, m. Us. D3: 27 gm., no insulin reaction. S̄Y values very high (but the number of days is small, cf. formula).

The reason why, despite the above-mentioned short-comings in control, the patient was discharged on NL, 44 units, classified as not sufficient (ZMAI: poor), was that (1) this did not give rise to insulin reactions and that (2) the m. Us. D. fell.

To sum up, severe juvenile diabetes, difficult to control with different insulin combinations, also with ZMAI. Discharged on Novo lente, 44 units, which gave somewhat better results than ZMAI.

Case VIII: H. P., Q., aged 61, with diabetes which had been treated with insulin for 14–15 years. Fifteen (1) times previously the patient had been hospitalized for control purposes (as well as for treatment of rheumatoid arthritis). The insulin requirement was constantly fluctuating, there being sometimes hypoglycaemia and sometimes threatened precoma on the same dosage, e.g. m. Us. D.: 7 to 120 gm. on the same dose of the same insulin at 3 days' interval. Always fairly poor control, usually on insulin Retard alone, which had been the best product obtainable.

The following trials were made: (1) Insulin Retard in various doses. (2) Insulin Novo lente, 68 units in the morning (best dose): m. Bs. at 8 a.m., 11 a.m., and 4 p.m.: 102 – 297 – 218 mg. per cent. m. Bs. D4: 206 mg. per cent., m. Us. D4: 21.6 gm. S̄Y: 25, 18, and 15, periodically severe hypoglycaemia, down to 42 mg. per cent. Classification
(if anything) poor. (3) Insuline Novo semilente, 60 units in the morning (best dose): m. Bs. at 8 a.m., 11 a.m., and 4 p.m.: 314 – 397 – 107 mg. per cent., m. Bs. D4: 267 mg. per cent, m. Us. D4: 45 gm, Sy: 41, 23, and 8, persistent hypoglycaemia from 6 to 8 p.m. Classification: poor. (4) Insulin Novo lente, 40 units + Insulin Leo 20 units, both in the morning: m. Bs. at 8 a.m., 11 a.m., and 4 p.m.: 189 – 270 – 202 mg. per cent, m. Bs. D9: 220 mg. per cent, m. Us. D9: 40 gm., Sy: 33, 30, and 30, periodically hypoglycaemia. Classification: poor. (5) ZMAI 52–56 units in the morning: m. Bs. (7 values): 290 – 410 – 297 – 240 – 206 – 193 – 286 mg. per cent, m. Bs. D6 (3 values at 8 a.m., 11 a.m., and 4 p.m.): 331 mg. per cent. All 7 values: 274 mg. per cent, m. Us. D6: 84.2 gm., Sy: 13, 13, 19, 26, 31, 46, and 26. No hypoglycaemia. Classification: poor.


Results on ZMAI classified as poor, on Novo lente as not sufficient.
Thus, on insulin Retard the control was not much better or much worse than on the other preparations, but there was no hypoglycaemia, and the patient felt best on this preparation.

There is little doubt that any preparation, administered in a fairly constant dosage, must fail to control the diabetes in this case.

To sum up, a patient who was extremely difficult to cope with and who had previously been admitted 15 times. Numerous insulins and combinations of insulins had been tried with almost equally poor results. Discharged on insulin Retard, 52 units, as the patient felt best on this preparation.

Lastly, it is worth mentioning:

Case VI: I. S., ♂, aged 39, with diabetes treated with insulin for 15 years. Admitted 6 times plus another 6 times to a diabetic hospital, each time for control purposes. As was to be expected this proved extremely difficult. On present admission: (1) Insulin Retard 20 units in the morning and 24 units in the evening: hyperglycaemia and glucosuria. (2) Insulin Novo lente 44–48 units in the morning: m. Bs. D.: 268 mg. per cent, m. Us. D.: 39 gm. (3) Insulin Novo lente 28 units + Insulin Leo 16 units, both in the morning: m. Bs. D.: 219 mg. per cent, m. Us. D.: 31 gm. (4) Insulin Retard 48–52 units + Insulin Leo (regular) 12–8 units, both in the morning: m. Bs. D.: 200 mg. per cent, m. Us. D.: 22 gm. (5) ZMAI 52 units in the morning (5-day clinical test): m. Bs. D5 determined at 8 a.m., 11 a.m., and 4 p.m., as in the above-mentioned cases: 187 mg. per cent, m. Us. D5: 20.7 gm. Determined at all 7 values: m. Bs. D5: 142 mg. per cent. Δ Bs. 159 mg. per cent. When assessed on the basis of 3 as well as 7 values, the result was classified as fair on ZMAI. The Ŝy values for (4) and (5) were fairly good and identical – for all the others far worse.

Subsequently, we have learned from a diabetic hospital, where the patient was admitted because she was out of control, that she is now being maintained on a depot preparation + regular insulin in the morning and a depot preparation in the evening as well as on a weighed diet. Considering the numerous previous admissions and attempts at control, it is not surprising that this patient could not be maintained on ZMAI once daily.

The two cases classified as poor were the only ones of a total of 22 who had to be discharged on a preparation other the ZMAI. A third patient who was subsequently so discharged, case VI, is mentioned immediately above.

The remainder of the series, i.e. 19 patients, are still being controlled on ZMAI, more than 2 years after the end of the present trial.

Duration of Effect of ZMAI

On the basis of all 22 mean blood sugar curves, the duration of the effect of ZMAI may be estimated as an average of 21–24 hours, the minimum being about 18 hours and the maximum presumably in many cases more than 24 hours.

Maximum Effect:

Usually occurred between 4 and 12 p.m., but in a few cases later, i.e. between 2 and 4 a.m. or perhaps maintained until this time.
Initial Effect:
In 18 instances the m. Bs. at 11 a.m. was higher than at 8 a.m., while in 2 cases it was a little lower (at relatively high fasting values) and in 2 cases practically the same at both times (both being relatively low).
In one case the m. Bs. at 4 p.m. was 18 mg. per cent higher than at 11 a.m., and in 2 cases it was the same at 11 a.m. and 4 p.m.
Hence as a general rule it may be said that breakfast causes a (usually moderate) increase in Bs., an increase which has been completely or partially eliminated by 4 p.m.
Considering the relatively marked depot effect of ZMAI it could not be expected that breakfast would fail to produce some effect on the blood sugar values.
The B. St. curves on 6 patients for ZMAI (not reproduced) in which the Bs. was determined 14 times throughout the day, in all 6 cases showed an increase after breakfast, in only 2 (out of 6) cases an increase after lunch, in only 1 case an increase after the meal in the middle of the afternoon, and lastly in 2 cases a moderate and in another 2, a negligible increase after supper.
Accordingly, the effect of meals upon the Bs. is slight after 11 a.m.—1 p.m.

Insulin Reactions
Actual insulin shock, i.e. loss of consciousness, did not occur in the clinical ZMAI material, but in one case, on another insulin.
In Case III (Fig. 10) on the ZMAI 5-day test, an insulin reaction occurred once or twice daily (and also twice daily on other preparations). This also happened in Case V while on ZMAI once daily (and in the same case on another 2 preparations) but not on 2 daily injections. Case VI had a very mild insulin reaction on one occasion (more distinct on another 4 preparations). Lastly, Case VIII had insulin reactions on other preparations, but not on ZMAI, on which the Bs. level was higher than on other preparations.
The tendency to these reactions in this series might therefore be said to be the same or perhaps less than with other preparations of the same nature.

Allergic Reactions
None of the patients exhibited allergic reactions to ZMAI, but one (Case VI) to Insulin Novo lente. As mentioned above, this patient has later been adjusted to other preparations in another hospital, where cutaneous tests had shown allergy not only as previously to Novo lente, but now also to ZMAI. No allergy had been demonstrated to insulin Retard-{zinc allergy?}.
That methylated human albumin was not antigenic to man was shown by the intracutaneous technique described by Nathan & Kallos (1932).
SUMMARY AND CONCLUSIONS

The preparation tested in the present study is Zinc Methyl-Albumin Insulin (ZMAI), an insulin bound to zinc and non-antigenic human albumin rendered alkaline by methylation. It was expected to give similar results with regard to a depot effect as other insulin preparations whose depot effect is obtained by binding to another alkaline protein, and in some cases also to zinc.

(1) Experimental

The author first tried to test the insulin preparations by Gerritzen's method. Since this method proved unsatisfactory in that the blood sugar level did not remain constant in a control group, it was abandoned.

The Hallas-Moller method for testing insulin preparations on diabetics—called the Bed-Standard-Diet-Method (B. St.) was then tried on diabetics.

This method was definitely not suitable for testing ZMAI, as the 24-hour blood sugar curves during this test failed to parallel those from a clinical trial.

(2) Clinical Testing

The series comprises 22 patients, 7 men and 15 women, ranging in age from 17 to 75 years, averaging about 51 years. The duration of diabetes ranged from 1 month to 16 years, treatment with insulin in 15 cases lasting from a few weeks to 16 years.

The patients had the same full diet less sugar from day to day. A rather free dietary regimen was maintained, because when at home the patients usually eat approximately as they please. Therefore, the results obtained ought also to be valid, after discharge from hospital.

The procedure was as follows: First an attempt was made to control the diabetes as well as possible on the usual insulin preparation. If this failed, other preparations were tried. The patients were then adjusted to ZMAI, and when the findings were as constant as possible, they had the same dose of ZMAI as well as approximately the same diet both as regards quantity and quality for 5 consecutive days. They were also encouraged to take the same exercise each day. The blood sugar was determined 7 times and the urinary sugar excretion in 4 portions in the 24 hours.

The dose of ZMAI was from 12 to 80 units, averaging 38 units—in 21 cases given once daily, in the morning, and in one case twice daily, morning and night.

Among the 15 patients who had previously been receiving insulin the following findings were made on re-adjustment to ZMAI: 8 exhibited a reduction in insulin requirement of from 36 to 4 units, averaging 17.5 units. In 3 cases the requirement remained unchanged, and in 4 cases it increased by 4 units per patient. The results obtained with ZMAI were classified, according to
principles described in the text. into excellent, good, fair, not sufficient, and poor. The distribution was as follows: excellent 5 cases, good 6, fair 4 – a total of 15. Furthermore, 5 were not sufficient and 2 poor. Out of the 5 cases classified as not sufficient, 2 did not have other preparations, while 2 were worse and 1 remained not sufficient on other preparations (but slightly better than on ZMAI). Of the 2 patients classified as poor on ZMAI one was also poor on other preparations (but slightly better) and the other one was not sufficient on another preparation. Out of the 15 patients who had previously received another insulin 9 were classified as excellent, good, or fair on ZMAI. In none of them was the control poorer on ZMAI. In 4 (including 3 who had previously been receiving 2 daily injections as compared 1 with ZMAI) it was equally good, in 2 equally good to better, and in 3 better. Of the latter, 2 were far better. Both had previously been receiving 2 daily injections as compared with one now. The duration of the effect averaged 21-24 hours, in a few cases about 18 hours, but in others presumably over 24 hours. The initial effect was on the whole of rather slow onset. Thus, in 18 cases the Bs. was (usually somewhat) higher at 11 than at 8 a.m., whereas in 19 instances it was lower at 4 p.m. than at 11 a.m. In most cases, the maximum effect was found between 4 p.m. and midnight, in a few cases somewhat later. Actual insulin shock did not occur during the 5-day clinical testing of ZMAI, but slight insulin reactions occurred in 3 cases which had also exhibited such reactions on other preparations. No allergic reactions were observed to ZMAI in the present series, whereas one patient manifested allergy to another preparation. This patient has, however, subsequently shown cutaneous reactions to ZMAI in another hospital (zinc allergy?).

Out of 22 patients tested with ZMAI 20 were discharged from the hospital on this preparation. One has abandoned it subsequently. The remaining 19 patients are still being controlled on ZMAI more than 2 years after the end of the present trial.

On the basis of the present findings it seems justified to conclude that ZMAI is in advance of other preparations with regard to the manner in which its depot effect is achieved, i.e. by using human albumin. Moreover, its clinical effect in controlling diabetes might be said to be equally as good as that of other long-acting insulins, and in this series often better, and rarely poorer. It is also worth mentioning that its clinical use does not appear to give rise to severe untoward reactions.

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