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PRELIMINARY COMMUNICATIONS

Controlled Extension of Oral Antidiabetic Therapy on Former Insulin Dependent Diabetics by Means of the Combined i.v. Glibenclamide-Glucose-Response-Test

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Summary. A new sulphonylurea response test is described for predicting the results of long-term treatment with a recently developed sulphonylurea compound, glibenclamide, particularly in insulin-dependent tolbutamide-non-responsive elderly diabetics. The test is based on the observation that the insulin-stimulating capacity of glucose and the determination of the insulin increases are strikingly potentiated following glibenclamide plus glucose i.v. (25 γ plus 0.33 g/kg body weight) in serum samples where insulin binding antibodies have been removed. 11 out of 40 diabetics demonstrating between 60 and 90 min following injection, a mean increase of insulin of more than 500 per cent above the initial values, correlated satisfactorily with successful long-term oral treatment with glibenclamide. A positive glibenclamide-glucose-response test contrasted with primary failure of glibenclamide therapy in only one patient suffering from haemochromatosis. Oral treatment with glibenclamide may have certain advantages over insulin therapy, especially in elderly diabetics suffering from visual impairment, who are unable to inject themselves with insulin.

Extension contrôlée de la thérapeutique antidiabétique orale à des diabétiques précédemment insulino-dépendants à l'aide du test de réponse au glibenclamide-glucose I.V.: communication préliminaire

Résumé. Les auteurs dé crivent un nouveau test de réponse au sulfonylurée destiné à prévoir les résultats d'un traitement à long terme avec la Glibenclamide, composé sulfonylurée récent, particulièrement intéressant pour les diabétiques âgés, insulino-dépendants et ne réagissant pas à la tolbutamide. Le test est basé sur l'observation que la capacité du glucose à stimuler l'insuline et la détermination de l'augmentation de l'insuline sont fortement potentialisées après l'injection intra-veineuse de Glibenclamide plus glucose (25 γ plus 0.33 g/kg poids corporel). Les anticorps fixant l'insuline ont été extraits préalablement des échantillons de sérum. 11 diabétiques sur 40 montrant en moyenne plus de 500% d'accroissement d'insuline au-dessus des valeurs initiales, entre 60 et 90 mn. après l'injection, étaient en corrélation satisfaisante avec l'efficacité d'un traitement oral à long terme à la Glibenclamide. Le test positif de réponse à la Glibenclamide plus glucose a contrasté avec l'échec initial de la thérapeutique à la Glibenclamide chez un seul malade souffrant d'hémochromatose. Le traitement oral à la Glibenclamide peut avoir certains avantages sur la thérapeutique insulinique, particulièrement chez les diabétiques âgés souffrant de troubles visuels et ne pouvant s'injecter eux-mêmes l'insuline.

Zusammenfassung. Ein neuer Sulfonylharnstoffvorhersagetest wird beschrieben. Er soll ermöglichen, das Ergebnis einer Langzeit-Therapie mit einem in letzter Zeit entwickelten Sulfonylharnstoffabkömmling (Glibenclamid) besonders bei insulinpflichtigen, auf Tolbutamid nicht mehr ansprechbaren älteren Diabetiker vorauszusagen. Der Test beruht auf der Beobachtung, daß die insulinstimulierende Kapazität der Glucose und die Stärke des Insulinanstieges beträchtlich potenziert werden können, wenn Glibenclamid und Glucose zusammen gegeben werden (25 γ Glibenclamid plus 0.33 g/kg Körpergewicht Glucose). Das Insulin wurde bestimmt nach entsprechender Entfernung der insulinbindenden Antikörper. Von 40 Diabetikern zeigten 11 mehr als einen 500 prozentigen Insulinanstieg über den Ausgangswert zwischen der 60. und 90. Minute nach der Injektion. In diesen Fällen war der positive Test gut mit einer erfolgreichen Langzeittherapie korreliert. Nur bei einem Patienten mit Haemochromatose war trotz positivem Ausfall des Testes ein Versagen der Langzeit-Glibenclamidbehandlung zu beobachten. Die orale Therapie mit Glibenclamid hat gewisse Vorteile gegenüber der Insulintherapie, besonders bei älteren Patienten, die ein schlechtes Sehvermögen haben und daher unfähig sind, sich selbst Insulin zu spritzen.

Key words: Oral antidiabetic agents, sulphonylureas, insulin response tests, Glibenclamide, prediction of long-term treatment, formerly insulin-dependent maturity onset diabetics.

Introduction

After sulphonylurea therapy had been used for a short time, oral tolbutamide-response tests for predicting results of long-term treatment were suggested in 1957 by Pfeiffer *et al.* [15] and Camerini-Davalos *et al.* [1]. Blood sugar decreases of more than 30% of the initial values were taken as indicating favourable responses to long-term tolbutamide treatment. Later, i.v. tolbutamide tests were suggested for differentiating normal subjects from latent or chemical diabetes [11,

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18] or for recognizing hypoglycaemia due to insulinoma [4, 6, 14].

After the criteria for oral antidiabetic therapy were firmly established there was no further need for prediction tests. However, the new drug glibenclamide was tried not only in patients with secondary response failures to tolbutamide or other oral drugs [16, 17], but also in an increasing percentage of rather severe elderly diabetics with clear-cut insulin-dependence [9].

Experimental Concept and Technical Approach

Again, the question of selection criteria arose, i.e. whether or not prediction of the outcome of long-term therapy with glibenclamide was possible. Combined intravenous administration of glibenclamide plus glucose was preferred to glibenclamide injection alone to provide maximum stimulation of the releasable pancreatic insulin reserves. As published elsewhere [5, 13, 16], isolated Langerhans' islets and the isolated perfused rat pancreas as well as normal human beings responded to the combined action of glibenclamide-glucose, even under repeated administration, with higher insulin releases than to the combined tolbutamide-glucose injection or to glucose, tolbutamide or glibenclamide alone. In addition, those patients with secondary failures of tolbutamide therapy responded to glibenclamide plus glucose with more vigorous insulin secretions than to glibenclamide alone [12, 16].

Unfortunately, in the more severe cases treated with insulin for extended periods of time evaluation of blood glucose levels after stimulation with glibenclamide plus glucose became somewhat difficult. This applied particularly to those patients who, according to the established rules, were not regarded as suitable cases for oral antidiabetic therapy. The low glucose disappearance rates (K-values) were not markedly influenced by either glibenclamide or glibenclamide plus glucose. This suggested the necessity of measuring and comparing the concentrations of immunologically measurable insulin (IMI) concentrations in serum before and after injection of glibenclamide plus glucose.

However, it had to be considered that extended periods of treatment with exogenous insulin (at least 40 U of long acting insulin/day, sometimes more than 100 U/day) had induced some kind of immunological insulin resistance in the majority of those cases where insulin-binding capacities in serum were increased.

Hence, treatment of serum prior to insulin measurement was carried out according to Heding [7]: the antibodies were separated from the antigen by serum acidification followed by alcohol precipitation in alkali solution. If some exogenous insulin was still contributing to the IMI-values measured, neglecting it seemed justified. After all it was only the increase, particularly the individual increase, in endogenous hormone which accounted for the increment following stimulation.

Patients and Methods

Forty 43—75 year old insulin-dependent subjects with diabetic conditions of up to 21 years duration were submitted to identical response tests. They were then transferred from insulin to glibenclamide in doses of 5—15 mg, remaining in hospital under constant observation. Severe retinopathy with impairment of visual function made the change from insulin to tablets advisable in a number of these diabetics. 29 of the 40 patients did not respond to long-term glibenclamide therapy and had to be changed back to insulin after two or three days. In this connection "excellent" means that the blood sugar amounted to less than 160 mg% and a glucose excretion of less than 2 g during the 24 h period; "good" means blood sugar values lower than 180 mg% and a glucose excretion of less than 7 g during the 24 h period; whereas the term "failure" marks rises in blood sugar values above 200 mg% and a glucose excretion exceeding 10 g during the 24 h period.

In order to demonstrate once more the enormous differences between the amounts of insulin released in normal and diabetic subjects following stimulation, as well as the time differences between peak increments in blood insulin in normals and diabetics following tolbutamide and glibenclamide administration, twelve normal individuals were included. Each of the subjects was tested on five consecutive days, which were sometimes interrupted by an interval of two days during which exogenous insulin was given again, with i.v. glucose loads (0.33 g/kg) to establish the basic response in absolute insulin concentrations per ml, the percentage of the individual increases and the time periods at which the maximum rise in insulin was recorded; to i.v. tolbutamide[1] (25 mg/kg) and tolbutamide + glucose (25 mg + 0.33 g/kg) as well as to i.v. glibenclamide (25 µg/kg) and glibenclamide[1] + glucose (25 µg + 0.33 g/kg) loads. The stimulatory substances were slowly injected over a period of 2 min. Venous blood samples for determination of blood glucose and insulin were secured at 5, 10, 20, 30, 40, 50, 60, 90, 120, 150 and 180 min following injection. The fasting patients were examined between 8 and 9 a.m. 36 h after withdrawal of exogenous insulin blood sugar was determined according to Hoffmann [8] by auto-analyzer. The blood was centrifuged at low temperatures and the serum separated and stored in the deep-freeze at −30° C. After pretreatment of serum [7], insulin was determined according to Melani *et al.* [10] by separating free and bound insulin by means of amberlite (CG 400 type II). Glucose disappearance rates were calculated according to Conard [19] in the normal subjects and according to Dost [2, 3] in the diabetics.

1 We are indebted to Farbwerke Hoechst A.G. and Boehringer-Mannheim for ample supply of the insulin stimulating sulphonylureas ready for injection.

Table 1. *Changes in serum insulin induced by glucose and sulphonylureas i.v. in normal subjects*

Age sex	Height (cm)	Body weight (kg)	Max. Insulin release following glucose i.v. a) μU/ml b) % Increase[a] c) Time in min[b]	Max. Insulin release following tolbutamide i.v. a) μU/ml b) % Increase[a] c) Time in min[b]	Max. Insulin release following tolb. + gluc. i.v. a) μU/ml b) % Increase[a] c) Time in min[b]	Max. Insulin release following HB 419 i.v. a) μUml b) % Increase[a] c) Time in min[b]	Max. Insulin release following HB 419 + gluc. i.v. a) μU/ml b) % Increase[a] c) Time in min[b]	K-value following glucose i.v.	K-value following gluc. + tolb. i.v.	K-value following gluc. + HB 419 i.v.
29 years	165	61	55	90	110	40	210			
L.M. ♀			275.0	450.0	550.0	200.0	1050.0			
			5	5	5	20	15	1.76	2.05	2.50
45 years	172	69	65	80	125	35	220			
S.G. ♀			361.1	444.4	694.4	194.4	1222.2			
			5	5	5	15	20	1.40	1.90	2.30
49 years	171	74	75	95	115	55	198			
Q.M. ♂			312.5	395.8	479.1	229.1	825.0			
			5	5	5	30	15	1.96	2.00	3.22
22 years	182	80	69	80	130	48	238			
P.O. ♂			313.6	363.6	590.9	218.1	1081.8			
			5	5	5	15	20	1.86	2.16	2.40
29 years	192	95	45	65	120	42	218			
R.V. ♂			155.1	224.1	413.7	144.8	751.7			
			5	5	5	30	30	1.60	2.20	2.52
52 years	187	92	66	80	95	57	160			
M.T. ♂			212.9	258.0	306.4	167.7	516.1			
			5	5	5	15	15	1,46	1.66	1.98
61 years	172	71	52	75	115	60	236			
K.D. ♀			305.8	441.1	676.4	352.9	1388.2			
			5	5	5	20	30	1.70	1.99	2.17
62 years	162	59	40	65	90	38	217			
O.F. ♀			190.4	309.5	428.5	180.9	1033.3			
			5	5	5	20	15	2.00	2.50	3.12
45 years	172	62	68	68	95	44	215			
M.S. ♀			342.8	485.7	678.5	314.2	1525.7			
			5	5	5	30	20	1.77	1.97	2.16
22 years	191	79	58	70	110	38	220			
S.F. ♂			305.2	368.4	578.9	200.0	1157.8			
			5	5	5	15	15	1.49	1.86	2.02
33 years	174	73	55	70	90	28	222			
E.M. ♂			229.1	291.6	375.0	116.6	925.0			
			5	5	5	20	30	1.77	1.96	1.86
44 years	165	69	70	90	120	38	240			
P.Q. ♂			259.2	33.3	444.4	140	888.8			
			5	5	5	20	20	1.45	1.70	2.11
24 years	165	59	60	80	120	38	212			
E.K. ♂			249.2	34.5	444.4	120	780			
			5	5	5	15	15	1.50	1.90	2.42
32 years	172	70	59	70	120	38	238			
I.L. ♀			312	363	581	218	1090			
			5	5	5	15	20	1.70	2.20	2.92
19 years	165	61	60	100	120	50	220			
E.F. ♂			280	500	560	210	1060			
			5	5	5	15	20	1.90	2.90	3.00

[a] Expressed as percentage increase above initial levels (= 100%). [b] Of highest peak of serum insulin recorded during test.

Table 2. *Clinical data and changes in serum insulin induced by glucose and sulphonylureas i.v. in 12 insulin-dependent elderly diabetics, non-responding to long-term treatment with glibenclamide*

Age sex	Height (cm)	Body weight (kg)	Duration of diabetes (years)	Insulin treatment (years)	Insulin requirement (morning-night)	Max. insulin release following glucose i.v. a) μU/ml b) % Increase[a] c) Time in min[b]	Max. insulin release following tolbutamide i.v. a) μU/ml b) % Increase[a] c) Time in min[b]	Max. insulin release following tolb. + Gluc. i.v. a) μU/ml b) % Increase[a] c) Time in min[b]	Max. insulin release following HB 419 i.v. a) μU/ml b) % Increase[a] c) Time in min[b]	Max. insulin release following HB 419 + gluc. i.v. μU/ml b) % Increase[a] c) Time in min[b]	a) Result of long term treatment with HB 419 b) Dose c) Time
72 years V.S. ♂	177	82.0	16	6	36—32 U Lente	10.0 142.8 10	12.0 171.4 10	13.0 185.7 15	18.0 257.1 70	16.0 228.5 80	failure 15 mg 7 days
65 years H.L. ♀	165	62.0	12	3	48 U Rapitard	6.0 100.0 10	10.0 166.6 15	9.0 150.0 20	12.0 200.0 70	17.0 283.3 90	failure 15 mg 10 days
47 years K.M. ♀	158	77.0	4	1	32 U Comb. 16 U Lente	14.0 280.0 15	18.0 360.0 20	16.0 320.0 15	10.0 200.0 80	15.0 300.0 80	failure 20 mg 14 days
43 years G.B. ♂	181	79.0	6	2	40—24 U Depot	6.0 60.0 20	10.0 100.0 15	14.0 140.0 10	12.0 120.0 70	11.0 110.0 80	failure 15 mg 4 days
66 years M.E. ♂	175	70.0	19	7	52 U Lente	7.0 87.5 15	12.0 150.0 20	13.0 162.5 15	15.0 187.5 90	14.0 175.0 80	failure 15 mg 5 days
71 years H.B. ♀	162	69.0	8	2	32—16 U Depot	20.0 285.7 20	24.0 342.8 15	28.0 400.0 15	32.0 457.1 70	33.0 471.4 70	failure 15 mg 6 days
62 years G.R. ♂	182	92.0	6	4	48 U Depot	14.0 233.3 15	15.0 250.0 15	17.0 283.3 10	20.0 333.3 70	24.0 400.0 90	failure[a] 20 mg 10 days
70 years R.P. ♀	154	57.0	20	5	32 U Lente	18.0 225.5 20	22.0 275.0 15	29.0 362.5 20	36.0 450.0 90	40.0 500.0 80	failure 15 mg 6 days
59 years G.H. ♀	160	69.0	7	2	48 U Depot	12.0 133.3 15	12.0 133.3 20	21.0 233.3 20	19.0 211.1 80	24.0 266.6 70	failure 15 mg 7 days
49 years H.S. ♂	163	67.5	4	1	38—12 U Comb.	20.0 285.7 10	19.0 271.4 20	18.0 257.1 10	20.0 285.7 70	16.0 228.5 70	failure 15 mg 3 days
72 years J.F. ♂	180	85.5	14	4	36—12 U Depot	15.0 214.2 15	22.0 314.2 15	17.0 242.8 15	19.0 271.4 90	22.0 314.2 90	failure 15 mg 8 days
61 years K.G. ♂	172	71.0	13	10	28—20 U Depot	7.0 87.5 15	15.0 187.5 10	12.0 150.0 20	16.0 200.0 80	18.0 225.0 80	failure 15 mg 10 days

[a] Expressed as percentage increases above initial levels (= 100%). [b] Of highest peak of serum insulin recorded during test.

Table 3. *Clinical data and changes in serum insulin induced by glucose and sulphonylureas i.v. in 12 insulin-dependent diabetics; responding satisfactorily to long-term treatment with glibenclamide*

Age sex	Height (cm)	Body weight (kg)	Duration of diabetes (years)	Insulin treatment (years)	Insulin requirement (morning-night)	Max. insulin release following glucose i.v. a) μU/ml b) % increase[a] c) time in min[b]	Max. insulin release following tolbutamide i.v.	Max. insulin release following tolb. + gluc. i.v.	Max. insulin release following HB 419 i.v.	Max. insulin release following HB 419 + gluc. i.v.	a) Result of long term treatment with HB 419 b) Dose c) time in month
56 years	163	77.0	18	3	40 U	12.5	15.0	25.0	20.0	48.0	excellent
G.L ♀					Rapitard	208.3	250.0	416.6	333.3	800	7.5 mg
						15	15	15	60	80	7 months
70 years	168	65.0	9	2	40—20 U	14.0	22.0	20.0	29.0	42.0	excellent
K.B ♂					Depot	175.0	275.0	250.0	362.5	525.0	7.5 mg
						20	20	15	70	60	8 months
68 years	158	56.0	15	1	36—24 U	6.0	14.0	22.0	26.0	36.5	excellent
M.M. ♀					Rapitard	85.7	200.0	314.2	371.4	521.4	10 mg
						15	20	10	80	70	7 months
49 years	174	80.0	17	12	60 U	8.0	17.0	15.0	32.0	46.0	good
K.A. ♂					Zink-	88.8	188.8	166.6	355.5	511.1	15 mg
					Protamine	15	15	20	60	80	9 months
61 years	164	95.0	21	10	32—16 U	18.0	35.0	30.0	40.0	52.0	good
B.S. ♀					Depot	180.0	350.0	300.0	400.0	520.0	7.5 mg
					long acting	10	15	15	80	70	5 months
68 years	159	61.2	8	2	56 U	10.0	17.0	35.0	25.0	45.0	good
S.S. ♀					Depot	181.1	309.0	636.3	454.5	818.1	15 mg
						15	10	15	70	60	7 months
65 years	175	70.0	10	1	40—16 U	7.0	12.0	7.5	26.0	56.0	excellent
J.B. ♂					Depot	116.6	200.0	125.5	433.3	933.3	12.5 mg
						15	15	10	60	70	6 months
71 years	162	68.0	13	7	50—30 U	9.5	14.0	12.5	22.5	42.5	good
K.M. ♀					Depot	118.7	175.0	156.2	281.2	531.2	15 mg
						20	20	20	70	70	6 months
68 years	172	74.2	18	4	40 U	12.0	28.0	28.0	32.0	48.5	excellent
E.W. ♂					Depot	171.4	414.8	400.0	457.1	692.8	5 mg
						20	15	10	80	70	6 months
69 years	168	65.0	5	5	40—20 U	18.0	20.0	19.0	36.0	56.5	failure[c]
S.J. ♂					Depot	225.0	250.0	237.5	450.0	706.2	15 mg
						15	20	10	70	80	10 days
49 years	157	57.9	8	3	112 U	6.0	22.0	19.0	22.0	38.0	excellent
R.A ♀					Ultralente	66.6	244.4	211.1	244.4	422.2	7.5 mg
					+ 1 g Tolb.	10	15	15	70	70	2 months
43 years	154	57.0	3	2½	40—36 U	16.0	17.0	12.0	33.0	60.5	good
L.L. ♀					Depot	266.6	283.3	200.0	550.0	1008.3	10 mg
						10	15	20	70	80	2 months

[a] Expressed as percentage increase above initial levels (= 100%). [b] Of highest peak of serum insulin recorded during test.
[c] Patient suffering from haemochromatosis.

Results

As shown in Table 1, glucose as well as tolbutamide acted much faster than glibenclamide in fifteen normal subjects on insulin release, but the effect of glibenclamide plus glucose combined quantitatively exceeded all others.

The clinical and laboratory findings in an arbitrary selection of 12 out of 29 subjects with primary failures

The group of patients successfully treated with glibenclamide for up to 7 and 8 months following the response tests (until November 1971 more than 18 months) did not differ clinically from those who did not respond to long-term treatment. The insulin responses to glucose and tolbutamide or glibenclamide alone were not very different from those seen in non-responsive subjects (Table 3).

In addition, the effect of tolbutamide plus glucose

Table 4. *Average maximum increases in absolute insulin concentrations and in the mean percentage increments above initial values in the three groups following the various types of stimulation of insulin secretion*

	Insulin-dependent elderly diabetics (successfully treated with HB 419) $n = 12$		Normal subjects $n = 15$		Insulin-dependent elderly diabetics (failures of long-term treatment with HB 419) $n = 12$	
	μU/ml (Mean ± SEM)	Mean % increment (Mean ± SEM)	μU/ml (Mean ± SEM)	Mean % increment (Mean ± SEM)	μU/ml (Mean ± SEM)	Mean % increment (Mean ± SEM)
Glucose i.v.	11.4 ± 1.2	156.9 ± 17.8	58.1 ± 3.1	264.2 ± 21.7	12.4 ± 1.5	177.9 ± 24.5
Tolbutamide i.v.	19.5 ± 1.9	261.6 ± 20.1	77.3 ± 2.9	363.7 ± 23.9	15.9 ± 1.4	226.8 ± 25.0
Tolbutamide + glucose i.v.	20.4 ± 2.3	284.5 ± 41.4	109.5 ± 4.0	518.0 ± 37.2	17.2 ± 1.7	240.6 ± 25.2
Glibenclamide (HB 419) i.v.	28.6 ± 1.7	391.0 ± 24.3	43.1 ± 2.6	204.8 ± 19.9	19.0 ± 2.2	264.4 ± 30.1
Glibenclamide + glucose i.v.	47.6 ± 2.1	665.8 ± 54.7	216.1 ± 6.1	1031.3 ± 80.4	20.8 ± 2.4	291.8 ± 33.4

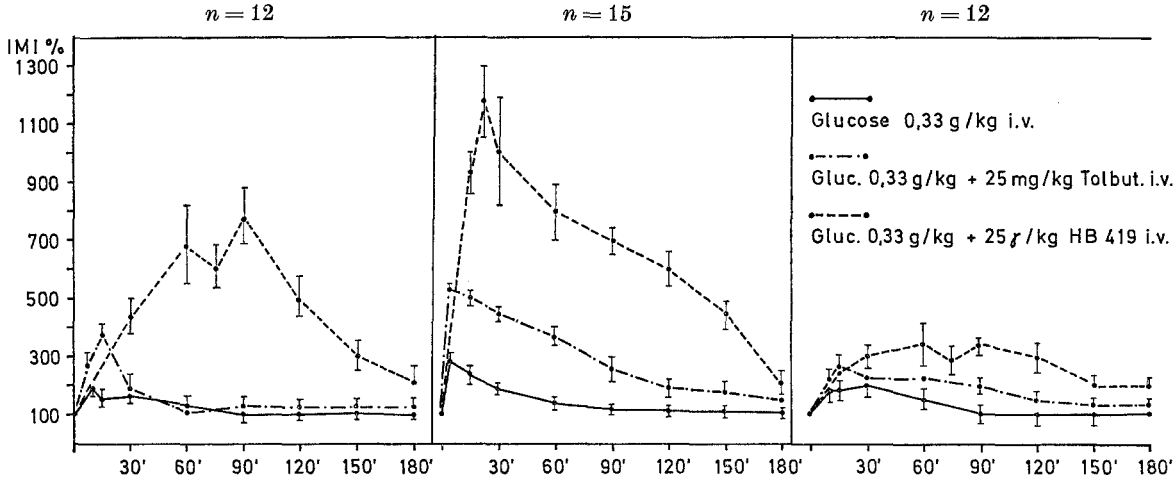

Fig. 1. Insulin responses (expressed as percentage increase above initial levels) to glucose, glucose plus tolbutamide, and to glucose plus glibenclamide i.v., in insulin-dependent elderly diabetics successfully treated with HB 419 afterwards (left part of figure), of normal subjects (middle part of figure), and of insulin-dependent elderly diabetics displaying failure of long-term treatment with HB 419 (right part of figure), respectively (Mean ± SEM)

are demonstrated in Table 2. The responses to glucose and tolbutamide as well as to tolbutamide plus glucose were very modest, and glibenclamide alone also did not effect remarkable rises in plasma insulin. The average increases following glibenclamide plus glucose amounted to only 20.8 ± 2.4 μU/ml, i.e. 291.8 ± 33.43 mean percentage increment above the initial levels. It should be noted that the insulin peaks following tolbutamide and glibenclamide, respectively, occur at different time periods.

was not greater in the responsive as against the non-responsive subjects. Glibenclamide plus glucose, however, induced average insulin increases of 47.6 ± 2.1 μU/ml, i.e. 665.8 ± 54.7 mean percentage increment above initial values. The differences in the percentage increases in the three groups following glucose, glucose plus tolbutamide and glucose plus glibenclamide, respectively, are illustrated in Fig. 1.

A positive reaction to the glibenclamide + glucose load contrasted with a primary response failure ap-

pearing 10 days after initiation of oral treatment in only one case (S.J. Table 3).

The average increases in absolute insulin concentrations and in mean percentage increment (above initial values) for all three groups in response to the five stimulants are summarized in Table 4.

Conclusions

From these results it was inferred:

1. that the "Glibenclamide-Glucose-Response-Test" may be helpful in predicting the outcome of long-term glibenclamide treatment, even in more severe elderly diabetics who were previously considered to be insulin-dependent and non-responsive to sulphonylureas;
2. that the correlation between the positive Glibenclamide-Glucose-Response-Test (i.e. mean percentage insulin increases of more than 500% above initial values) and successful long-term treatment favours the hypothesis that a continuous stimulation of endogenous insulin secretion is the predominant mechanism of action of the new compound following both single and permanent application;
3. that, surprisingly enough, the relation between the absolute amounts of insulin released in response to the various stimuli is between the normal subjects and the diabetics (the positive responders) in the same order of about 5 : 1;
4. that, therefore, the synergistic action or potentiation of glibenclamide and glucose and *vice versa* suggests an action on the receptor site of the β-cell in both the normal and the diabetic subjects, and no particular action of the new drug on the diabetic β-cells alone;
5. that the proportion of elderly diabetics formerly considered to be "purely" or "truly" insulin dependent, i.e. diabetic subjects unable to synthesize even minute amounts of insulin, is much less than was held before;
6. that a practical advantage of glibenclamide over insulin therapy in this type of patient should prove to be the case in subjects suffering from severe retinopathy or other ocular complications inducing impairment of visual function and incapacity to inject oneself.

Further mathematical analysis of the blood glucose changes induced by the various stimulations will show whether or not a simple formula exists which would permit rapid evaluation of the Glibenclamide-Glucose-Response-Test without having to rely upon serum insulin determinations.

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