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Pharmacokinetics and Pharmacodynamics as well as Metabolism Following Orally and Intravenously Administered C^{14}-Glipizide, a New Antidiabetic

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Summary. Peak serum concentrations are reached after about 2 h following oral administration of 5 mg radioactive glipizide, and in the case of intravenous administration of 1 mg radioactive substance they are reached immediately following the injection. The half-life amounts to 36 min (compartment 1) and 3.6 h (compartment 2) resp. The distribution volume of the substance is 11,100 ml. — 5 metabolites were detected in the urine; 2 of them remained unidentified; the same metabolites could partly be detected in the serum. — As regards the insulin levels, two peaks can be seen following oral as well as following intravenous administration; the first peak is reached after absorption or after the injection as the case may be, the second one is reached at midday postprandially. — Of the orally administered substance, the greatest share of total activity eliminated in the urine is observed during the first 24 h, 65.2% of the administered activity are renally eliminated during 48 h. The corresponding value for 120 h being 65.4% after oral and 64.8% after intravenous administration. — These data verify complete absorption following oral administration.

Key words: ^{14}C-labelled glipizide, pharmacokinetics, pharmacodynamics, metabolism, protein-binding, insulin concentration, glucose determination.

Before a drug is introduced into clinical use, basic data on its pharmacokinetics in man should be available. Since there is a tendency to minimize the quantity of substance administered, it is often difficult to test distribution of the drug with chemical methods because of insufficient sensitivity of assay methods.

To an increasing degree, therefore, test procedures are adopted using radioactive-labelled preparations which permit most accurate demonstration of smallest quantities of a substance without affecting other tests performed simultaneously.

After the effectiveness of the substance had been proven in preliminary experiments on animals and in clinical trials, it was reasonable that glipizide (glibenese®)[1] — a new oral antidiabetic — should be checked for its fate in the organism by means of nuclear-medical methods, and at the same time the changes of the insulin concentration in the serum be tested [1, 2, 3, 4, 6, 12, 14, 17].

Material and Methods

We used ^{14}C-labelled glipizide which was supplied by Pfizer, Karlsruhe. The compound designed for oral administration had a specific activity of 69 μCi/mg (variation coefficient ± 0.4 μCi/mg); tablets of 5 mg = 345 μCi were given. Dry frozen substance with specific activity 23 μCi/mg was dispensed in ampoules for intravenous use. 1 mg was administered after having dissolved the compound in 5ml aqua bidest.

5 healthy male test persons ranging in age from 22 to 30 years received the tablets, while the solution was given to 3 healthy male subjects 29 to 49 years of age. Hypersensitivity reactions to sulfonylureas, diseases of the liver, kidney or metabolism were not present in any of the subjects; during the study they received no other drugs.

On the evening before the study the subjects were given a normal meal. On the study day while fasting they received the compound at 6^{30} a.m., from 7^{30} a.m. they received 2 Bahlsen-Leibniz-biscuits and water ad libitum every hour, from the 4th hour they received a normal diet. During the first hour after I.V. administration the test subjects were resting, subsequently they pursued their normal work as craftsmen or attendants.

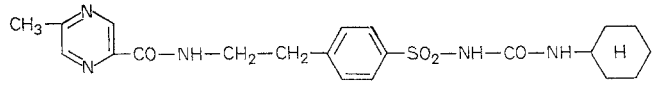

Fig. 1. Structural formula glipizide

Blood samples were taken on the study day prior to the I.V. injection, immediately after and 10, 30 min, 1, 2, 4, 6 and 8 h later. Furthermore, blood was taken 24, 48, 72 and 96 h after the injection.

1 Pfizer GmbH Karlsruhe.

The test persons receiving the preparation by the oral route had blood taken prior to as well as 30, 60, 90 min, 2, 4, 6, 8, 24, 48, 72 and 96 h after the dose was administered.

The test persons were asked to empty their bladder prior to the administration of the preparation. This urine sample served as a control sample. The other collection periods were from 0—4, 4—8, 8—24, 24—48, 48—72, 72—96 and 96—120 h after the administration of the dose.

Determination of Glipizide-C^{14} and Its Metabolites in Serum

In 3 parallel determinations 0.1 ml serum was pipetted into plastic volumetric flasks and mixed with 0.5 ml H_2O and 1.0 ml digestin[1]. After the addition of 10 ml scintillation solution (200 g naphthalene, 10 g PPO[2], 0.30 g POPOP[3], dioxane ad 2000 ml) and pre-cooling the samples, measurement was carried out in an automatic liquid scintillation counter (Picker-Liquimat).

The channels-ratio-method served for determining the recovery and quenching: varying amounts of serum and urine were mixed with a defined C^{14}-standard and measured in 2 channels with separate discriminator adjustment. Subsequently, 1—5 ml methanol was added to each sample for further quenching, the measurements being made in the same way. The quotient of the counting rates of the two channels is related to the efficiency of the measurement. After correction of the individual values, the concentrations expressed as µg/ml can be calculated from the specific activity of the glipizide used; this procedure does not allow for metabolism. The statistical error of counting and the pipetting error were established by repeated measurements of the 3 parallel samples. The mean error of the mean value was

10^{-6} mg/ml approx. 20%
10^{-4} mg/ml approx. 3%.

The O-value of the measuring arrangement was approx. 40 ipm (impulses per minute); this counting rate would correspond to a concentration of 0.002 µg/ml.

For determination of the metabolites in the serum only the 4 h and the 8 h sera of 5 test persons were used, since the radioactivity was too slight in the sera obtained later than this.

In order to isolate the radioactivity, the samples of serum were mixed with a nine-fold volume of acetone and then centrifuged. Then the acetone phase was concentrated to about 2 ml, mixed with 5.0 ml buffer of pH 4.5 and the aqueous phase was extracted five times with 20 ml dichloromethane. The solvent was dried (no loss of activity) and distilled off. The residue was absorbed in 0.5 ml chloroform and 0.5 ml ethanol and chromatographed several times on silica gel plates (H_F 254 Merck) with chloroform/methanol/acetic acid: 95/5/1. After scanning, areas corresponding to the peaks were scraped out and their radioactivity measured in the Liquid-Scintillation-Counter (Messrs. Packard, Tricarb. Model 2420; measuring solution: 10 ml Instagel and 6 ml Toluol and 0.5 ml ethanol and 0.5 ml water).

Determination of the Insulin Concentration and the Glucose in the Serum

0.1 ml serum was mixed with 0.1 ml I^{125}-insulin solution and 1.0 ml of the suspension of a sephadex-anti-insulin-complex (Deutsche Pharmacia). After twice repeated mixing at 30 min intervals and subsequent incubation for 18 h at room temperature, the samples were again mixed and then centrifuged and washed 3 times with 0.5 ml 0.9% NaCL-solution. The deposit was measured by means of a spectrometer counter after the supernatant had been decanted (Picker-Liquimat). The insulin concentration expressed as µU/ml was calculated by comparing the activity rates with the counting rates of standards and basal values which had been measured simultaneously.

After deproteinisation of 0.1 ml whole blood with 1.0 ml uranyl acetate solution (3.2 g uranyl acetate, 18.0 g Na-chlorate ad 2000 ml aqua bidest) and centrifugation, 0.1 ml of the supernatant was pipetted into 2.5 ml of prepared "Glucose-Reagent" (Messrs. Boehringer) followed by incubation at 37°C for 25 minutes; subsequently it was measured in an Eppendorf-Photometer at 578 mu in 1-ml cuvettes. 0.1 ml standard glucose with 91 mg/ml was pipetted, in each case with the same pipette, into 2.5 ml "Glucose-Reagent". A third glucose-reagent sample of 2.5 ml without additions served as basal value. For measurement purposes, the basal value was compared with the standard and with the sample.

Determination of Glipizide and Its Metabolites in the Urine

The total amount of the particular urinary portions was measured at an accuracy of 1 ml up to 1 l and amounts above 1 l at an accuracy of at least 5 ml. After sufficient shaking of the collecting plastic flasks, 2 ml urine were mixed with 8 ml aqua bidest, and to 2 ml of this dilution 10 ml scintillation solution were added (3 parallel samples in each case). Measurement was carried out under the same conditions as with the blood samples. After correction of the counting rates the C^{14}-amounts of the urinary samples can be calculated as % of the dosage administered. They were not converted into amount of substance

1 Digestin (Merck): 1 M N-ethyl-N-Dodecyl-N, N-dimethylamoniumhydroxide in methanol ($C_{16}H_{37}NO$)
2 PPO: Diphenyloxazol ($C_{15}H_{11}NO$)
3 POPOP: 2.2-p-(phenylen-bis (5-bis-phenylenoxazol) ($C_{24}H_{16}N_2O_2$)

since it had to be assumed that metabolism had taken place. The mean error of the measurement was approx. 1% up to the 48 h fraction, subsequently it rose to 20%.

For purposes of investigation, the 0—4, 4—8, 8—24 and possibly the 24—48 h urine was used, for the samples of urine obtained later than this contained insufficient activity.

10, 50 and 100 ml respectively of urine were buffered at pH 4 and extracted four times with the five-fold volume of dichloromethane. The organic solvent was dried (no loss of activity) and distilled off the residue absorbed in 0.5 ml chloroform and 0.5 ml ethanol and chromatographed several times (in some cases up to eight times) on silica gel plates (HF_{254}Merck) with chloroform/methanol/acetic acid: 95/5/1. Following scanning (Messrs. Berthold), areas corresponding to the peaks were scraped out and their radioactivity was measured in the Liquid-Scintillation-Counter (Messrs. Packard, Tricarb. Model 2420; measuring solution: 10 ml Insta-Gel (Messrs. Packard) + 6 ml Toluol + 0.5 ml ethanol + 05 ml. water).

Determining the Protein-binding of Glipizide

In order to test the protein-binding, the plasma samples of the test persons were used. The determination was performed in Scholtan dialysis chambers with Na-phosphate buffer as dialysing liquid (Na-phosphate buffer 1/15 with pH 7.2; dialysis 3 hours at 37° C).

Table 1. *Glipizide concentration in the serum (in ng/ml)*
1a) Oral application of 5 mg

Time min/h	Test pers. No. / Weight (kg) / Age (years)	1 / 75 / 26	4 / 76 / 22	5 / 63 / 22	6 / 52 / 30	9 / 75 / 25	Mean Value $\bar{x}$	Variance ± 1 s
30 min		257.76	15.51	73.29	73.26	60.20	96.00	92.80
60 min		585.40	223.92	297.19	275.38	335.46	343.47	146.70
90 min		—	—	482.10	281.83	388.22	384.05	100.20
2 h		513.19	453.06	344.13	611.90	304.26	445.30	125.05
3 h		348.61	507.62	—	—	—	428.11	112.44
4 h		276.50	347.97	285.58	522.54	126.13	311.74	143.29
6 h		166.92	254.30	136.34	314.39	—	217.98	81.41
8 h		125.52	190.60	85.06	173.89	94.36	133.89	46.99
24 h		9.26	29.78	6.51	18.39	0.57	12.90	11.40
48 h		2.10	3.95	0.65	1.68	0.19	1.71	1.82
72 h		0.98	1.53	0.10	0.21	0.09	0.58	0.64
96 h		0.87	1.14	0.20	0.00	0.00	0.44	0.42

1b) Intravenous application of 1 mg

Time min/h	Test pers. No. / Weight (kg) / Age (years)	2 / 76 / 49	3 / 78 / 39	7 / 70 / 29	Mean Value $\bar{x}$
immediately		285.52	309.49	308.06	301.02
10 min		143.31	138.16	178.09	153.19
30 min		102.23	100.79	146.59	116.54
60 min		88.84	75.67	119.10	94.54
2 h		54.73	53.81	86.02	64.85
4 h		22.22	34.63	51.75	36.20
6 h		19.32	25.24	34.79	26.45
8 h		10.39	19.83	21.65	17.29
24 h		4.72	4.97	0.90	3.53
48 h		—	4.45	0.60	2.53
72 h		2.36	1.55	0.30	1.40
96 h		—	1.29	0.00	0.64

Results

The glipizide concentrations in the serum after *oral administration* are shown in Table 1 and Fig. 2a. From Table 1a it is evident that the maximum concentration with a mean value of 0.45 µg/ml was reached at 2 h. The standard deviation was 0.13 µg/ml i.e. 29%. The individual distribution of the peak values covered the period from 1 to 3 h. The limit of detection was reached after 48 h. The relatively high concentration in test person 1 which was recorded as early as 30 min after administration was particularly striking. In Fig. 1a these findings are plotted showing not only the curve of the mean values with standard deviation but also the individual values.

After intravenous administration of 1 mg it can be seen from Table 1b that the peak value of 0.30 µg/ml was present immediately after the injection, and 10 min later it was already reduced to half of its value. Here, too, the concentration decreased to concentrations within the limit of detection 48 h after the injection. The variance was not established because of the small number of test persons.

In Fig. 2b the individual values and the curve of the mean values are shown. It is apparent that there was a relatively small degree of individual fluctuations. The graphical analysis for establishment

of the half life is represented in Fig. 2c. It is evident that after a first fast component with a half life of 36 min a second component with a half life of 3.6 h can be distinguished.

compound can be calculated. It is 11,100 ml. When making allowance for the mean body weight of the 3 test persons of 75 kg we obtain a value of 11,250 ml corresponding to 15% of the body weight. This

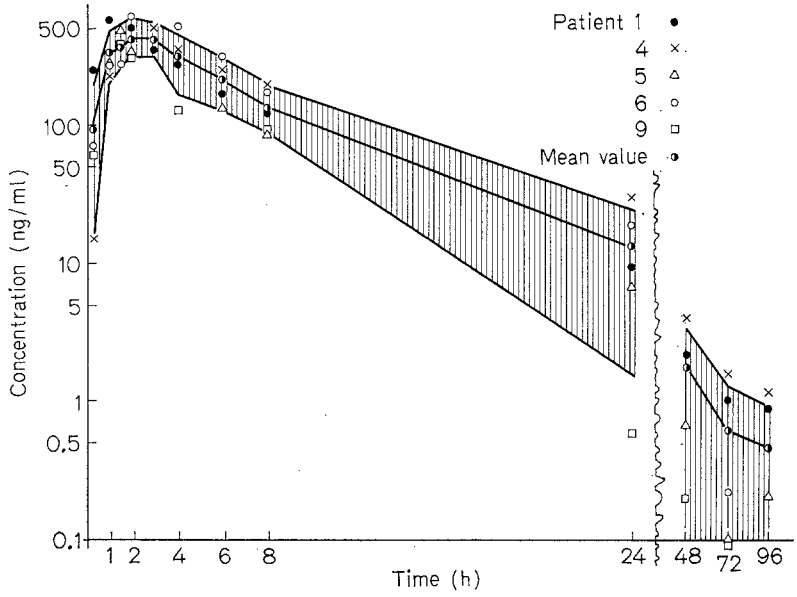

Fig. 2a. Glipizide concentration in the serum after a single oral dose of 5 mg. (Individual and mean values, standard deviations; expressed as μg/ml.)

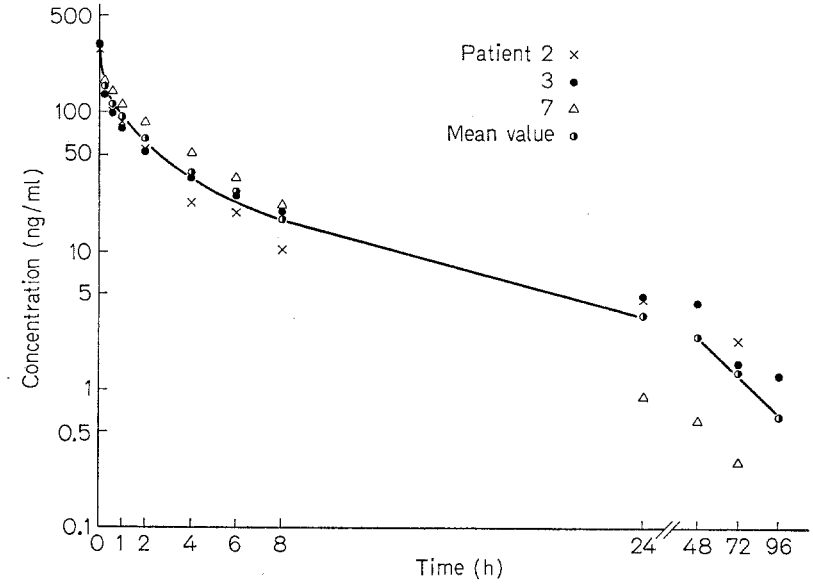

Fig. 2b. Glipizide concentration in the serum after single intravenous dose of 1 mg. (Individual and mean values)

Since the 24 h value is above the extrapolated curve (half life 2), there might be even a third component; the activity rates 24 h after the injection are, however, relatively small, so we have neglected this value in view of the small number of test persons.

From the theoretical concentration at time t_0 and the amount given, the distribution volume of the distribution volume corresponds to the easily interchangeable extracellular water.

If one compares after 5 mg oral application the radioactivity per ml serum with the radioactivity of the extract, converted to 1 ml serum, it will be evident that the radioactivity cannot be completely extracted.

Table 2. *Insulin concentration in the serum after application of glipizide (in μU/ml)*
2a) Oral application of 5 mg

Time min/h	Test pers. No.	1	4	5	6	9	Mean value $\bar{x}$	Variance $\pm$ 1 s
	Weight (kg)	75	76	63	52	75		
	Age (years)	26	26	22	30	25		
prior to		5.6	10	7	10	10	8.5	2.1
30 min		5.2	17	17	11	16	13.2	5.1
60 min		18.5	40	24	24	36	28.5	9.1
90 min		—	—	34	16	27	25.7	9.1
2 h		5.0	37	14	32	18	21.2	13.1
3 h		11.0	17	—	—	—	14.0	4.2
4 h		6.0	12	11	5	12	9.2	3.4
6 h		90.0	65	39	8	51	50.6	30.5
8 h		52.0	33	45	11	20	32.2	17.0
24 h		8.0	10	8	5	13	8.8	3.5
48 h		4.5	12	8	5	4.5	6.8	3.3
72 h		9.0	13	5	7.5	7	8.3	4.8
96 h		11.0	14	5	2	5	7.4	5.0

2b) Intravenous application of 1 mg

Time min/h	Test pers. No.	2	3	4	Mean Value $\bar{x}$
	Weight (kg)	76	78	70	
	Age (years)	49	39	29	
prior to		2	4	20	8.7
immediately		0	9	18	9.0
10 min		16	37	65	39.3
30 min		4	13	35	17.3
60 min		3.5	3.5	16	7.7
2 h		2	13	18	11.0
4 h		2	6	12	6.7
6 h		19	23	31	24.3
8 h		8.5	5	11	8.2
24 h		2	3.5	8.5	4.7
48 h		2.5	6	10.5	6.3
72 h		4	6	6	5.3
96 h		3	10	12	8.3

The evaluation of the thin layer chromatogram showed that about 2/3 of the extracted radioactivity is to be ascribed to glipizide. A further very small peak seems to indicate that either the 4-trans-hydroxycyclohexyl derivative or the 3-cis-hydroxycyclohexyl derivative or both are present. It is not possible to make a more detailed statement on account of the slight radioactivity and the poor separation of these two substances.

In view of the small amount of radioactivity following intravenous injection of 1 mg glipizide, determination of the metabolites in the serum was not performed.

In Table 2a the *insulin concentrations* in μU/ml serum measured in the serum after oral administration are compiled. A first peak with a mean value of 28.5 μU/ml was reached at 60 min, the variance was 9 μU/ml, i.e. 32%. A second peak of 50.6 μU/ml

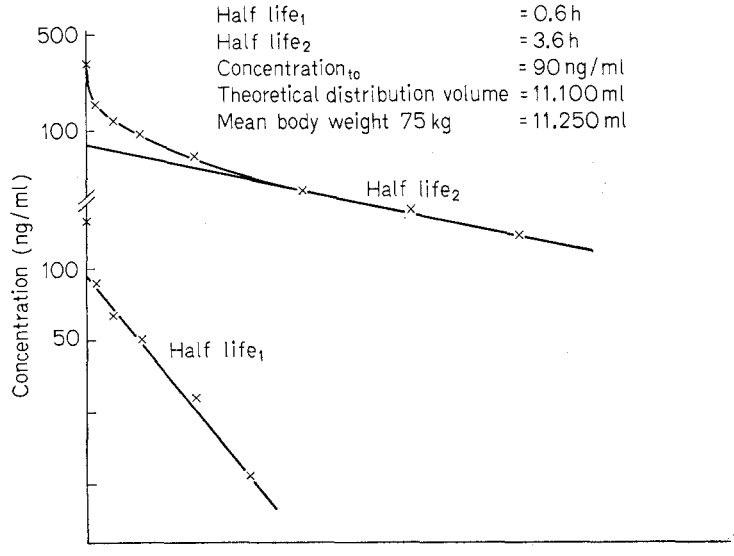

Fig. 2c. Graphic analysis of the serum elimination of glipizide after an intravenous dose of 1 mg. (Curves of mean values)

(variance 30.5 μU/ml) was observed at 6 h. This new increase was probably due to the meal given after 4 h. The first peak occurred between 1 and 2 h and the second between 6 and 8 h in the individual test persons.

was noted after 10 min and the second with a mean value of 24.3 μU/ml 6 h after the injection. It is interesting to note that the 6 h peak is only half as high as after oral administration of the preparation. This phenomenon may be due to the glipizide concen-

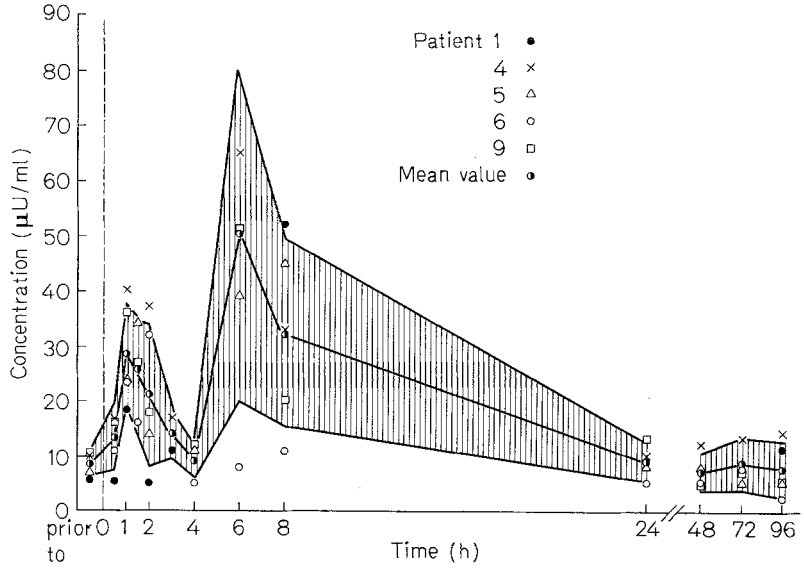

Fig. 3a. Insulin concentration in the serum after single oral dose of 5 mg glipizide (Individual and mean values, standard deviations; expressed as μU/ml)

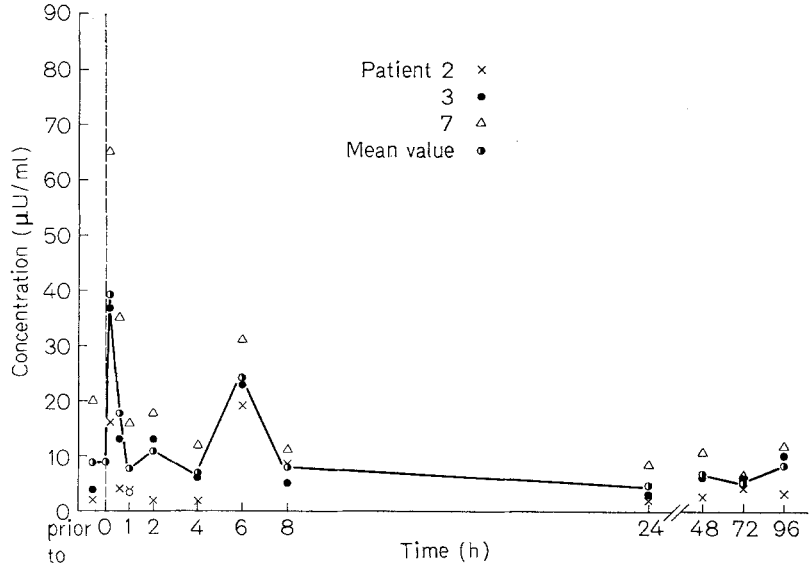

Fig. 3b. Insulin concentration in the serum after single intravenous dose of 1 mg glipizide (Individual and mean values, expressed as μU/ml)

The values are plotted in Fig. 3a showing again the individual values, the curve of the mean values and the dispersion range (1 s).

The findings observed after I.V. administration are compiled in Table 2b. In all test persons, the first insulin peak with a mean value of 39.3 μU/ml

tration which at this time is smaller by one power of ten.

Fig. 3b shows the curve of the mean values including the individual values.

The *blood sugar values* recorded after oral administration are compiled in Table 3a. The fasting

Table 3. *Glucose concentration in the blood after application of glipizide (in mg/ml)*
3a) Oral application of 5 mg

Time min/h	Test pers. No.	1	4	5	6	9	Mean value $\bar{x}$	Variance $\pm$ 1 s
	Weight (kg)	75	76	63	52	75		
	Age (years)	26	26	22	30	25		
prior to		55	55	64	67	67	62	6.2
30 min		55	55	63	65	47	57	7.2
60 min		40	80	58	62	60	60	14.3
90 min		—	—	49	45	65	53	7.5
2 h		40	70	66	71	84	66	16.1
3 h		55	75	—	—	—	65	10.0
4 h		65	45	67	73	85	67	14.8
6 h		64	59	46	51	116	67	28.1
8 h		69	64	69	69	102	75	15.2
24 h		77	71	66	65	74	71	5.1
48 h		87	72	81	78	82	80	5.5
72 h		80	74	75	70	80	76	4.3
96 h		99	85	80	63	75	80	11.8

3b) Intravenous application of 1 mg

Time min/h	Test pers. No.	2	3	7	Mean value $\bar{x}$
	Weight (kg)	76	78	70	
	Age (years)	49	39	29	
prior to		50	60	77	62
immediately		45	60	60	58
10 min		50	60	33	48
30 min		30	30	53	37
60 min		35	50	72	52
2 h		60	75	66	67
4 h		65	85	60	70
6 h		69	79	56	68
8 h		83	79	74	79
24 h		66	82	68	72
48 h		77	77	75	76
72 h		80	84	72	79
96 h		81	85	65	77

value prior to the administration of the sulfonylurea averaged 62 mg/100 ml, 30 min after it fell slightly to 57 mg/100 ml and after a modest increase (60 min) it showed a decrease to 53 mg/100 ml at 90 min. The most pronounced decrease occurred between 30 min and 4 h in the individual test persons. The 30 min and 90 min values had a variance of 7.2 and 7.5 mg/100 ml respectively corresponding to 12.6% and 14%.

The individual values, the curve of the mean values and the simple sigma range are represented in Fig. 4a. When compared with Fig. 3a it becomes apparent that the maximum insulin increase precedes the maximum decrease of the glucose level in the blood.

Table 3b contains the glucose concentrations recorded after the intravenous dose. In 2 test persons the maximum decrease was noted 30 min after in-

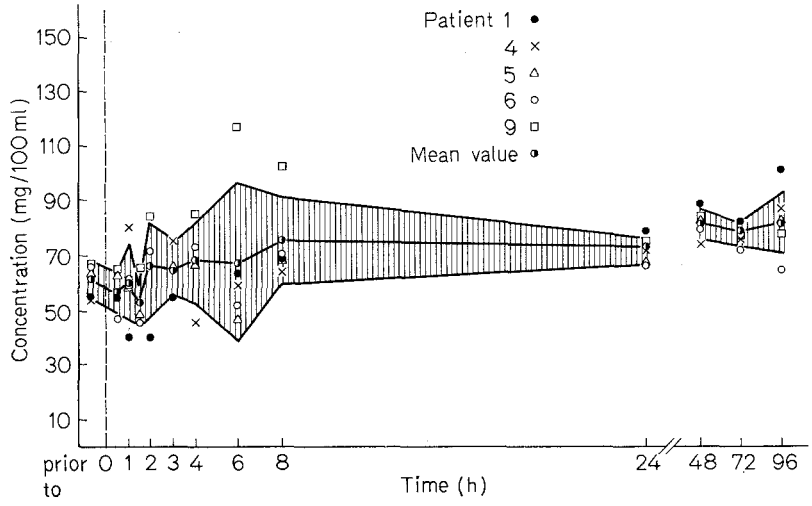

Fig. 4a. Glucose concentration in the blood after single oral dose of 5 mg. glipizide (Individual and mean values, standard deviations; in mg/100 ml)

Table 4. *Glipizide elimination in the urine (expressed as % of the ^{14}C-dose applied)*
4a) Oral application of 5 mg

Time h	Test pers. No. / Weight (kg) / Age (years)	1 / 75 / 26	4 / 76 / 26	5 / 63 / 22	6 / 52 / 30	9 / 75 / 25	Mean value $\bar{x}$	Variance $\pm$ 1 s
0— 4		29.64	19.87	27.66	17.70	21.83	23.34	4.63
4— 8		20.25	12.25	4.60	22.95	40.75	24.05	12.02
8— 24		18.43	24.84	14.10	16.13	7.67	16.23	17.33
24— 48		1.47	6.50	1.34	3.83	0.58	2.74	1.57
48— 72		0.07	0.47	0.11	0.33	0.05	0.20	0.17
72— 96		0.03	0.06	0.02	0.04	0.03	0.03	0.01
96—120		0.01	0.02	0.01	0.02	0.03	0.02	0.01
0— 8							47.39	
0— 24							63.62	
0— 48							65.19	
0— 72							65.36	
0— 96							65.37	
0—120		69.90	64.01	47.84	61.00	70.94	65.38	

4b) Intravenous application of 1 mg

Time h	Test pers. No. / Weight (kg) / Age (years)	2 / 76 / 49	3 / 78 / 39	7 / 70 / 29	Mean value $\bar{x}$
0— 4		43.81	39.29	35.56	39.55
4— 8		14.57	16.86	5.51	12.31
8— 24		4.74	13.06	14.29	10.69
24— 48		1.35	1.82	2.32	1.83
48— 72		0.13	0.14	0.25	0.17
72— 96		0.11	0.06	0.26	0.14
96—120		0.19	0.11	0.11	0.13
0— 8					51.86
0— 24					62.55
0— 48					64.38
0— 72					64.55
0— 96					64.69
0—120		64.90	71.34	58.30	64.82

jection, in 1 test person 10 min after injection the lowest mean value of 37 mg/100 ml was observed about 30 min after the injection. In Fig. 4b the individual values and the curve of the mean values are plotted. In this case, too, the decrease of glucose level follows the rise of serum insulin.

With regard to glipizide *elimination in the urine after oral administration* Table 4a summarises the amount of activity eliminated during the particular collecting periods. They were not converted into amount of substance because of metabolism. Since test person 5 had not collected quantitatively the 4—8 h fraction, this value was entered though not considered in the calculation of the mean value, the variance and the total excretion. The bottom part of the table gives the cumulative elimination, i.e. the total amounts excreted up to the respective times.

The table reveals that with the first two 4 h frac-

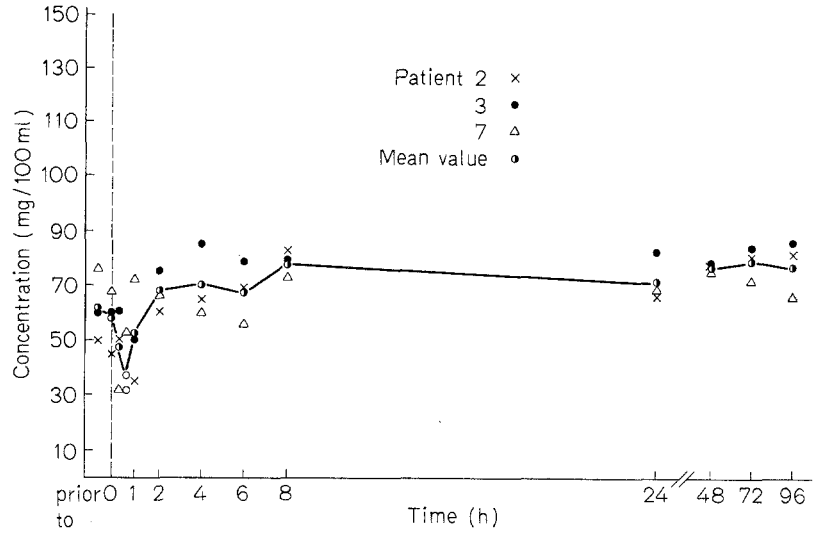

Fig. 4b. Glucose concentration in the blood after single intravenous dose of 1 mg. Glipizide (Individual and mean values; in mg/100 ml)

tions 23% and 24% respectively of the dose were eliminated, during the following 16 h another 16% were excreted in the urine. In the following 24 h approximately 3% of the dose appeared in the urine, after 48 h the excretion was less than 1%. The total amount eliminated within 24 hours averaged 63.6%, within 48 h 65.2% and within 120 h 65.4% of the dose. The individual fluctuations ranged between 61.0% and 70.9%.

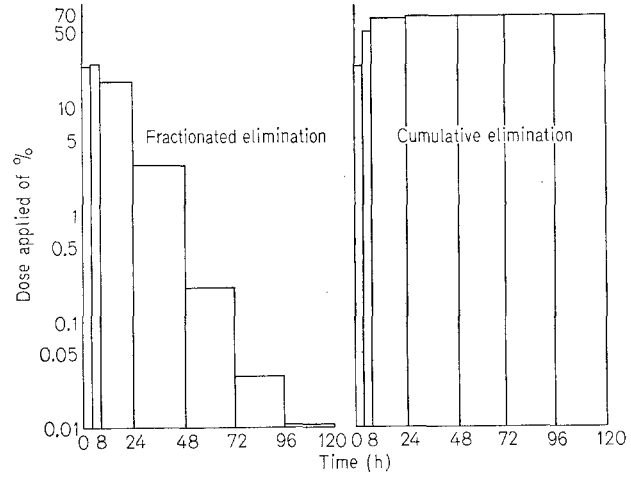

Fig. 5a. Glipizide elimination in the urine after single oral dose of 5 mg. (Mean values; expressed as % of the ^{14}C-dose applied)

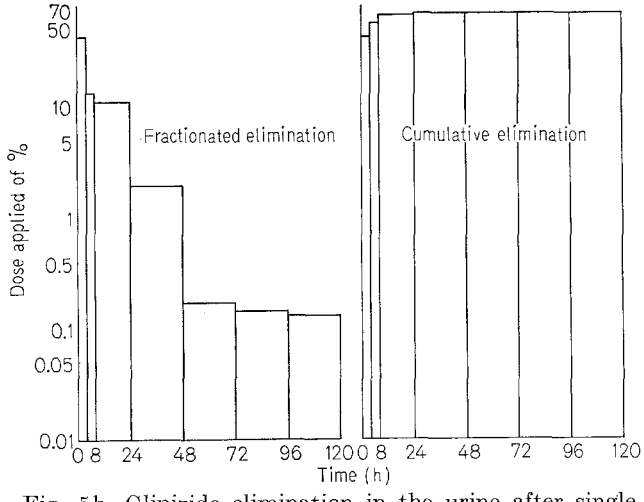

Fig. 5b. Glipizide elimination in the urine after single intravenous dose of 1 mg. (Mean values; expressed as % of the ^{14}C-dose applied)

A graphic representation of the findings is given in Fig. 5a.

Table 4b shows the fractionated C^{14}-elimination in the urine after intravenous administration. The excretion averaged 39.6% within the first 4 h, 12.3% during the following 4 h and 10.7% of the dose within another 16 h. During the following 24 h the renal elimination was about 1.8%, during the following days below 1%.

Thus, 51.9% appeared in the urine within the first 8 h, 62.6% within the first 24 h and 64.4% in the first 48 h. Within a period of 5 days 64.8% could be detected, the individual values ranged between 58.3% and 71.3%.

A comparison with Table 4a reveals that the elimination rates after oral administration do not differ much from those after intravenous administration (65.4% as opposed to 64.8%). This phenomenon suggests that the preparation is practically completely absorbed after oral administration.

A graphic representation of the results will be found in Fig. 5b.

The measurement of the urinary samples prior to and after extraction revealed that about 9% of the radioactivity was not extracted in the urine. A 24 h treatment of the urine with glucuronidase/sulphatase at 37° did not change this value.

The evaluation of the chromatograms reveals that apart from unchanged glipizide two metabolites above all are eliminated, namely the 4-trans-hydroxycyclohexyl derivative and the 3-cis-hydroxycyclohexyl derivative. In addition, small amounts of 3 other metabolites were found in all test persons; one of these proved to be an acid derivative of glipizide, whereas the other two were not identified. In 2 test persons, another metabolite which is already known could be traced, namely a small quantity of N-(2-acetylamino-ethyl-phenyl-sulfonyl)-N'-cyclohexyl urea (DCDA). A point worthy of note is the fast elimination of the 4-trans- and 3-cis metabolites. The small quantity of these metabolites in the serum 4—8 h after the administration corresponds to these findings.

Table 5 indicates the radioactivity of the eliminated *glipizide and its metabolites* as mean values of 4 test persons, as well as its percentage of the amount of

Table 5. *Detection of glipizide and its metabolites respectively in the urine (thin layer chromatography) after oral application of 5 mg ^{14}C-glipizide (345 μCi). Mean values of 4 test persons. Values in μCi and % of the dose applied*

Hours after application	Glipizide		unidentified metabol. RF 0.77		DCDA		3-cis-metabol.		4-trans-metabol.		unidentified metabol. RF 0.11	
	μCi	%	μCi	%	μCi	%	μCi	%	μCi	%	μCi	%
0— 4	10.4	3.02	1.2	0.35	1.1	0.29	13.9	4.04	36.0	10.40	1.1	0.29
4— 8	10.7	3.11	0.9	0.26	0.9	0.26	8.9	2.58	22.5	6.52	0.9	0.26
8—24	9.2	2.66	0.5	0.15	0.85	0.25	4.3	1.25	10.9	3.17	0.77	0.22
0—24	30.3	8.79	2.6	0.76	2.85	0.80	27.1	7.87	69.4	20.9	2.77	0.77

glipizide administered, for the collection periods 0—4, 4—8 and 8—24 h. The sum of the quantities of glipizide and its metabolites detected in the chromatogram shows a difference relative to the total radioactivity measured in the urine. This difference is to be explained by losses during processing.

After both oral and intravenous administration the insulin level showed 2 peaks, the first after 1 h, the second after 6 h. In sequence, the insulin maxima were followed by blood sugar minima. These findings conform with observations made by other authors, who observed, also 60 min p.a., an increase of the

Table 6. *Protein-binding of glipizide in human serum after oral administration (Na-phosphate buffer 1/15 with pH 7.2; dialysis 3 h at 37° C)*

Test person Type of application Dose	Time after application	Concentration (ng/ml)	% bonded	% free	Remarks
1	60 min	585.0	98.16	1.83	
oral	2 h	513.0	98.18	1.81	
345 μCi/5 mg	6 h	166.0	95.62	4.37	
	24 h	9.0	92.50	7.50	small counted yield
	72 h	0.9	—	—	counted yield too small
4	60 min	224.0	99.06	0.93	
oral	2 h	453.0	98.41	1.58	
345 μCi/5 mg	6 h	254.0	95.61	4.38	
	24 h	30.0	95.65	4.34	
	72 h	1.5	—	—	no yield from figures
5	60 min	297.0	98.01	1.98	
oral	2 h	344.0	97.69	2.30	
345 μCi/5 mg	6 h	136.0	96.80	3.20	
	24 h	6.5	98.18	1.81	
	72 h	0.1	—	—	no yield from figures
6	60 min	275.0	98.59	1.40	
oral	2 h	612.0	98.45	1.54	
345 μCi/5 mg	6 h	314.0	97.45	2.54	
	24 h	18.0	95.88	4.11	
	72 h	0.2	—	—	no yield from figures
9	60 min	335.0	98.4	1.5	
oral	2 h	304.0	96.5	3.5	
345 μCi/5 mg	6 h	94.0	96.6	3.4	
	24 h	0.5	—	—	no yield from figures

On account of the very low activity in connection with the intravenous administration of 1 mg of the substance, determination of the metabolites in the urine was not done.

The protein-binding of glipizide fluctuates after oral and intravenous administration between 92% and 99% depending on the concentration of glipizide in the serum (Tables 6 and 7).

Discussion

The examination of the distribution sample of C^{14}-glipizide in 5 test persons with healthy metabolism revealed, after oral administration of 5 mg, a maximum concentration of 0.45 μg/ml serum 2 h p.a.; 22 h later the serum level was 0.013 μg/ml. The corresponding investigations in 3 test persons following intravenous injection of 1 mg radioactive substance showed a serum level of 0.116 μg/ml 10 min p.i. and 0.01 μg/ml 8 h p.i.

insulin both in healthy persons [13] and in diabetics [5, 7, 9]. The second peak is influenced by the intake of food 4 h after the start of the trial. Marigo *et al.* [13] observed the same findings. The speedy rise of the insulin level and the short duration is reminiscent of the tolbutamide type of the insulin release; however, the second postprandial insulin release corresponds with the behaviour of the sulfonylureas of the so-called 2nd generation which for instance includes glibenclamide. The insulin rise which is, compared to glibenclamide, steep and of short duration, might indicate that insulin is released mainly postprandially which would reduce the danger of hypoglycemia. Renal excretion was relatively quick. The major part of the total activity of the orally applied substance which is excreted in the urine appears within the first 24 h, on an average 63.6%, within 120 h 65.4%. After intravenous administration 64.8% was renally excreted within 120 h. Thus the above data reveal a quantitatively identical excretion of the substance

Table 7. *Protein-binding of glipizide in human serum after I.V. administration (Na-Phosphate buffer 1/15 with pH 7.2; dialysis 3 h at 37° C)*

Test person Type of application Dose	Time after application	Concentration (ng/ml)	% bonded	% free	Remarks
2	10 min	143.0	98.04	1.95	
i.v.	60 min	88.0	92.30	7.69	
21.8 μCi/1 mg	2 h	54.0	96.81	3.18	
	6 h	19.0	92.30	7.69	
	24 h	4.0	—	—	no yield from figures
	72 h	2.3	—	—	no yield from figures
3	10 min	138.0	99.09	0.90	
i.v.	60 min	75.0	89.28	10.71	
22.07 μCi/1 mg	2 h	54.0	93.75	6.25	
	6 h	25.0	93.10	6.89	
	24 h	5.0	—	—	no yield from figures
	72 h	2.0	—	—	no yield from figures
7	10 min	178.0	99.4	0.56	
i.v.	60 min	119.0	86.5	13.5	
22.42 μCi/1 mg	2 h	86.0	99.5	0.5	no yield from figures
	6 h	34.0			
	24 h	0.9	—	—	no yield from figures

in the urine after oral and intravenous administration and suggest almost complete absorption after oral administration. Similar values were measured in the dog [8]. The same applies to glibenclamide where Rupp *et al.* [16] observed a practically complete intestinal absorption, too.

The protein-binding in serum ranged between 92 and 99% for the measured glipizide concentrations. According to investigations made by Hajdu [10] and also by Heptner *et al.* [11], more than 90% of glibenclamide, too, are bound to plasma proteins. The corresponding data for glibornurid vary between 94 and 95% [15].

References

1. Ambrogi, V., Bloch, K., Daturi, S., Griggi, P., Logemann, W., Parenti, M.A., Rabini, T. Tommasini, R.: Arzneimittel-Forsch. **21**, 200—204 (1971)
2. Ambrogi, V., Bloch, K., Cozzi, P., Daturi, S., Logemann, W., Parenti, M.A., Tommasini, R.: Arzneimittel-Forsch. **21**, 204—208 (1971)
3. Ambrogi, V., Bloch, K., Daturi, S., Griggi, P., Logemann, W., Mandelli, V., Parenti, M.A., Rabini, T., Usardi, M.M., Tommasini, R.: Arzneimittel-Forsch. **21**, 208—215 (1971)
4. Ambrogi, V., Bloch, K., Daturi, S., Logemann, W., Parenti, M.A., Tommasini, R.: Arzneimittel-Forsch. **22**, 542—544 (1972)
5. D'Onofrio, F., Pempinello, R., Romis, L.: Arzneimittel-Forsch. **22**, 1879—1881 (1972)
6. Emanueli, A., Molari, E., Colombo Pirola, L., Caputo, G.: Arzneimittel-Forsch. **22**, 1881—1885 (1972)
7. Fedele, D., Tiengo, A., Riva, F., Muggeo, M., Emanueli, A., Crepaldi, G.: Arzneimittel-Forsch. **22**, 1885—1888 (1972)
8. Goldaniga, G.C., Maraone, C., Pianezzola, E., Valzelli, G., Ambrogi, V.: Arzneimittel-Forsch. **23**, 242—247 (1973)
9. Greco, V.A., Fedeli, G., Ghirlanda, G., Lucente, M., Accietto, F., Fenici, R.: Minerva med. **62**, 2829—2839 (1971)
10. Hajdu, P., Spingler, H., Kohler, K.F., Schmidt, F.H.: Tegernsee-Conference, Rottach-Egern, Januar 1969, p. 32
11. Heptner, W.: Tegernsee-Conference, Rottach-Egern, Januar 1969, p. 38
12. Lentini, S., Bossini, A., Colombo Pirola, L.: Arzneimittel-Forsch. **22**, 1169—1173 (1972)
13. Marigo, S., del Nevo, G., Bini, P.P., Sacchetti, G.: Arzneimittel-Forsch. **21**, 215—220 (1971)
14. Pedrazzi, F., Pisani, Ceretti, A., Losi, S., Bommartini, F., Artini, D., Emanueli, A.: Arzneimittel-Forsch. **21**, 220—225 (1971)
15. Rentsch, G.: Arzneimittel-Forsch. **22**, 2209—2212 (1972)
16. Rupp, W., Christ, O., Fülberth, W.: Arzneimittel-Forsch. **22**, 471—473 (1972)
17. Vailati, G., Pagani, G., Montini, M., Caputo, G., Sacchetti, G.: Arzneimittel-Forsch. **22**, 1888—1889 (1972)

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