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IN VITRO AND IN VIVO AVAILABILITY OF SPIRONOLACTONE FROM VARIOUS ORAL PREPARATIONS

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SUMMARY

The dissolution rates in vitro and the bioavailability in humans were determined for 6 preparations containing 25 mg spironolactone and 5 preparations containing 100 mg spironolactone. Linear relationships were obtained by pairwise correlation of in vitro parameters with in vivo parameters. The following parameters were used.

In vitro parameters of dissolution:

1. The area under the dissolution-time-curve up to 1 h
2. The fraction of active ingredient dissolved within 20 min.
3. The slope of the dissolution-time-curve at 50 % dissolution
4. The dissolution rate constant
5. The time up to 50 % dissolution of the substance
6. The maximum slope of the dissolution-time-curve

In vivo parameters of bioavailability:

1. The time of maximum plasmaconcentration
2. The area under the plasmaconcentration-time-curve up to 1 h and 2 h after application
3. The quantities of active ingredient excreted in the urine up to 2 h after application

The highest correlation coefficient was found between the areas beneath the dissolution-time-curve and the plasmaconcentration-time-curve up to 1 h each.

No significant correlations were found between the within 1 h dissolved substance and maximum plasmaconcentration, the area under the plasmaconcentration-time-curve up to 4 h and 24 h and quantities of active ingredient excreted in the urine up to 4 h after application.

INTRODUCTION

A prerequisite for the efficacy of a medicament is its adequate bioavailability. This can be determined by measurement of the concentration of the active ingredient in the plasma (1). Investigations on bioavailability require extensive analyses and experimental procedures. In the development of new formulations and in the monitoring of the production of medicinal products simple test methods are easier to apply. A simple test that is adequate for these purposes is the determination of the dissolution rate of the active ingredient from the drug form in vitro (2). However, before conclusions with respect to bioavailability can be drawn from an in vitro experiment it should be checked by an in vivo experiment. A particularly large part of the variability of in vitro experiments is based on different apparaturs used.

The selection of different parameters for the dissolution rate in vitro and for the bioavailability may give different conclusions. In the present paper an investigation into the correlation between the dissolution rate measured under defined in vitro conditions and the bioavailability measured as the plasma level and the excretion in the urine is reported, using spironolactone as an example. Those parameters of the in vitro and in vivo experiments will be selected that are suitable for prediction of bioavailability.

MATERIALS AND METHODS

Preparations: The investigation was carried out on a total of 11 oral preparations. Table 1 gives the 6 formulations containing 25 mg spironolactone, 3 of which were commercially available preparations. Table 2 gives the 5 formulations containing 100 mg spironolactone, 4 of which were commercial preparations. In the following, the preparations will only be identified by the numbers given in the tables.

Dissolution rate (3): Three experiments were carried out for each preparation containing 25 mg active ingredient, and 2 experiments for each preparation containing 100 mg active substance. In each case 1 tablet or 1 sugar-coated tablet containing 25 mg spironolactone was dissolved in 2 litre 0.1 N HCl at 37°C. Preparations containing 100 mg spironolactone were dissolved in 4 litres 0.1 N HCl at 37°C. The solutions were stirred in a round-bottomed flask with a paddle stirrer mounted 4 cm above the base of the flask and rotated at a speed of 120 rev/min. The paddle was in the form of a segment of a circle with

TABLE 1

Preparations investigated containing 25 mg spironolactone

Preparation No.	Preparation name
1	Aldactone^{R} A 25 mg tablet , Marupi-Searle/Japan, Batch GU 482 GU
2	Aldactone^{R} 25 mg tablet , Searle/Australia, Batch BO 16009
3	Aldactone^{R} 25 mg sugar-coated tablet , Boehringer/GFR*, Batch 713723
4	Film tablet 25 mg, Schering/GFR, Batch 102
5	Film tablet N 25 mg, Schering/GFR, Batch 101
6	Sugar-coated tablet 25 mg Schering/GFR, Batch 101

*GFR = German Federal Republic

TABLE 2

Preparations investigated containing 100 mg spironolactone

Preparation No.	Preparation name
1	Aldactone^{R} 100 mg sugar-coated tablet , Searle/France, Batch 1013C4
2	Aldactone^{R} 100 mg sugar-coated tablet , Lepetit/Italy, Batch 06001036
3	Aldactone^{R} 100 mg tablet , Searle/Australia, Batch B 114222
4	Osyrol^{R} 100 mg sugar-coated tablet , Hoechst/GFR*, Batch E 014
5	Sugar-coated tablet 100 mg, Schering GFR, Batch 101

*GFR = German Federal Republic

a chord length of 8 cm segment width of 2 cm. The increasing concentration of the dissolved active ingredient was determined continuously over 65 min by measurement of the extinction difference with a two wavelength photometer at the wavelengths 250 and 275 nm in a 1 cm flow cell. Filtration of the solution was unnecessary as by measuring the extinction difference, interference by the suspended particles is eliminated. The values were recorded "on line" with an IBM 1800 computer and the following parameters were calculated as criteria.

The fraction of active ingredient dissolved within 20 min (C_{20})

The fraction of active ingredient dissolved within 60 min (C_{60})

The dissolution half-life of the substance (t_{50})

The slope of the dissolution-time-curve at 50 % dissolution (St_{50})

The maximum slope of the dissolution-time-curve (St_{max})

The dissolution rate constant (K) from the equation up to 1 h:

$$c_i/c_{max} = K \cdot t_i/(+ K \cdot t_i)$$

The dissolution integral (A) from the equation up to 1 h:

$$A = \sum_{i=0}^{n-1} \frac{(c_i + c_{i+1})\,(t_{i+1} - t_i)}{2 \cdot t_n \cdot c_{max}}$$

c_i is the concentration at the time t_i, c_{max} is the concentration on complete dissolution of the active ingredient, and i takes the values 0, 1,2.....n.

Bioavailability: In the in vivo experiments the preparations were administered to the proband at 8.00 a.m.

after a 10 h fasting period together with a standard breakfast so that uniform absorption could be expected. Between each administration there was a period of one week. In total 22 subjects of both sexes aged 21 - 36 years participated in several multiple change over trials. Eight preparations were tested in 8 subjects, and 3 of the 25 mg formulations in 6 subjects each. Organic diseases were excluded by clinical and biochemical examinations according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie (4) (German Society for Clinical Chemistry). Blood samples were taken at o.5, 1, 2, 3, 4, 5, 6, 10, and 24 h after oral application of the preparations, and urine was collected in 0 - 2 and 2 - 4 h portions. The blood was heparinised, centrifuged and the plasma and urine were frozen at -20°C until analysis. The spironolactone concentrations in the plasma and the quantity excreted in the urine were determined by means of the 6 derivative, aldadiene (canrenone). Aldadiene is regarded as the biologically active metabolite of spironolactone (5). The determination was carried out fluorimetrically on the basis of the method described by Sadée, Dagcioglu and Riegelman (6). By this method aldadiene and aldadienic acid were determind simultaneously in the plasma and in the urine both of these and the glucuronide. The following parameters for bioavailability were selected.

The maximum concentration in the plasma (P_{max})
The time of maximum concentration (t_{max})
The area under the concentration-time-curve in the plasma up to 1, 2, 4, and 24 h after application (F_1), (F_2), (F_4), (F_{24})
The quantities of active ingredient excreted in the urine up to 2 and 4 hours after application (U_2), (U_4)

Correlation: In order to compare the results of in vitro and in vivo investigations, correlation coefficients (r) were calculated according to the equation (7)

$$r = \frac{Sxy}{Sxx \cdot Syy}$$

where Sxy = Covariance and Sxx, Syy = variances of the parameters to be compared.

For each preparation the means were used for the in vitro experiment, and individual values for each subject for the in vivo results. The use of the mean of the in vitro data is necessary as it only characterises the totality of the galenical formulation, and not the individual units for the bioavailability. Therefore, individual values cannot be compared in pairs. Linear relationships are assumed to exist if the correlation coefficient found is significantly different from zero (7). Tests were carried out with an error risk of 1 %, related to the single test - not multiple.

RESULTS

Dissolution rate: Fig. 1 shows the mean dissolution curve calculated from the three dissolution experiments for each preparation.

The preparations containing 25 mg active ingredient can be divided into a group of rapidly dissolving preparations, consisting of preparations 1, 4, 5 and 6 and a group of more slowly dissolving preparations consisting of preparations 2 and 3. This subdivision was confirmed by the parameters for the dissolution rate in Table 3.

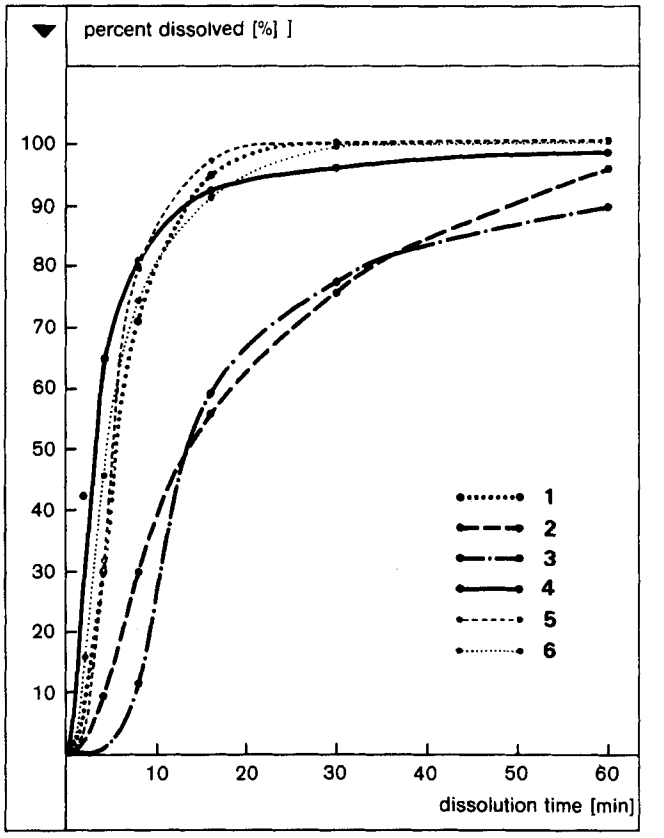

FIGURE 1

Quantity of spironolactone dissolved from various 25 mg preparations in relation to time (in vitro)

The preparations containing 100 mg active ingredient (Fig. 2) can be divided into a group of rapidly dissolving preparations consisting of preparations 3 and 5, and a group of more slowly dissolving preparations consisting of preparations 1, 2 and 4. Preparation 2 also differs from the other preparations by the very slow disintegration. The subdivision of the preparations by means of the

TABLE 3

Parameters for the dissolution rate of spironolactone in preparations containing 25 mg active ingredient

Preparation No.	C_{20} mg/l	C_{60} mg/l	t_{50} min	St_{50} %/min	St_{max} %/min	K_1 min^{-1}	A_1 1
1	12.5	13.0	5.1	12.4	12.4	0.415	0.784
2	8.4	12.3	13.3	2.7	6.8	0.079	0.477
3	8.1	11.4	13.4	5.1	6.9	0.069	0.416
4	12.1	12.5	3.2	17.3	17.3	0.468	0.815
5	12.6	12.5	5.0	19.2	21.8	0,358	0.805
6	12.0	12.5	4.0	14.9	19.7	0.391	0.800

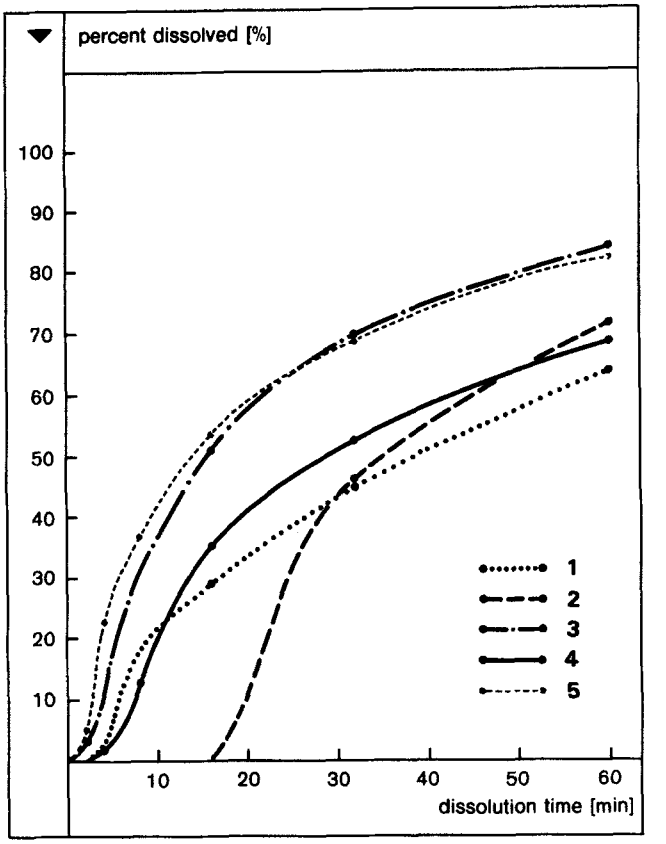

FIGURE 2

Quantity of spironolactone dissolved from various 100 mg preparations in relation to time (in vitro)

dissolution curves is confirmed by the parameters for the dissolution rate in Tab. 4.

Bioavailability: Fig. 3 shows the mean concentration curves for spironolactone in the plasma for the individual 25 mg preparations calculated from the 6 or 8 subjects. The preparations can be subdivided into three groups. A high plasma level is achieved

TABLE 4

Parameters for the dissolution rate of spironolactone in preparations containing 100 mg active ingredient

Preparation No.	C_{20} mg/l	C_{60} mg/l	t_{50} min	St_{50} %/min	St_{max} %/min	K_1 min^{-1}	A_1 1
1	9.7	16.7	35.0	0.7	8.7	0.029	0.277
2	5.2	18.3	32.1	1.0	3.4	0.015	0.117
3	14.8	21.2	14.7	2.0	4.3	0.064	0.416
4	10.9	17.6	28.6	1.1	3.3	0.031	0.279
5	15.0	20.5	13.5	1.8	8.1	0.074	0.460

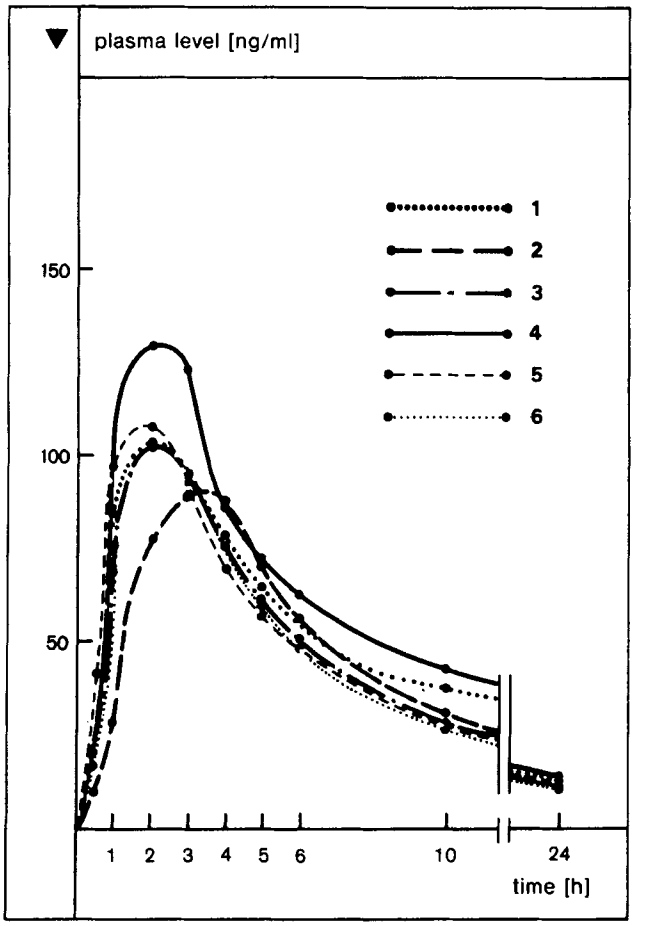

FIGURE 3

Aldadiene concentration in the plasma after the oral administration of 25 mg spironolactone in 6 different preparations; means from 6 or 8 subjects

with preparation 4. A relatively low plasma level is obtained with preparation 2. The preparations 1, 3, 5 and 6 occupy an intermediate position, and cannot be differentiated from each other. This assessment is in agreement with the parameters of bioavailability (Tab. 5).

TABLE 5

Parameters for the bioavailability of spironolactone in the plasma of humans from preparations containing 25 mg active ingredient, determined from 6 or 8 subjects

Preparation No.	P_{max} µg/ml	t_{max} h	F_1 $\frac{ng}{ml} \cdot h$	F_2 $\frac{ng}{ml} \cdot h$	F_4 $\frac{ng}{ml} \cdot h$	F_{24} $\frac{ng}{ml} \cdot h$	U_2 µg	U_4 µg
1	105	2.3	37	125	306	1025	291	765
2	80	3.1	18	76	246	851	202	766
3	105	2.4	32	124	208	884	281	872
4	140	2.3	42	140	379	1149	331	950
5	110	1.8	40	134	307	847	364	823
6	105	2.2	41	133	316	863	334	908

Fig. 4 shows the mean concentration curves for spironolactone in plasma for the individual 100 mg preparation calculated from the 8 subjects. They cannot be subdivided directly by means of the plasma level curves into rapidly and slowly absorbed prepa-

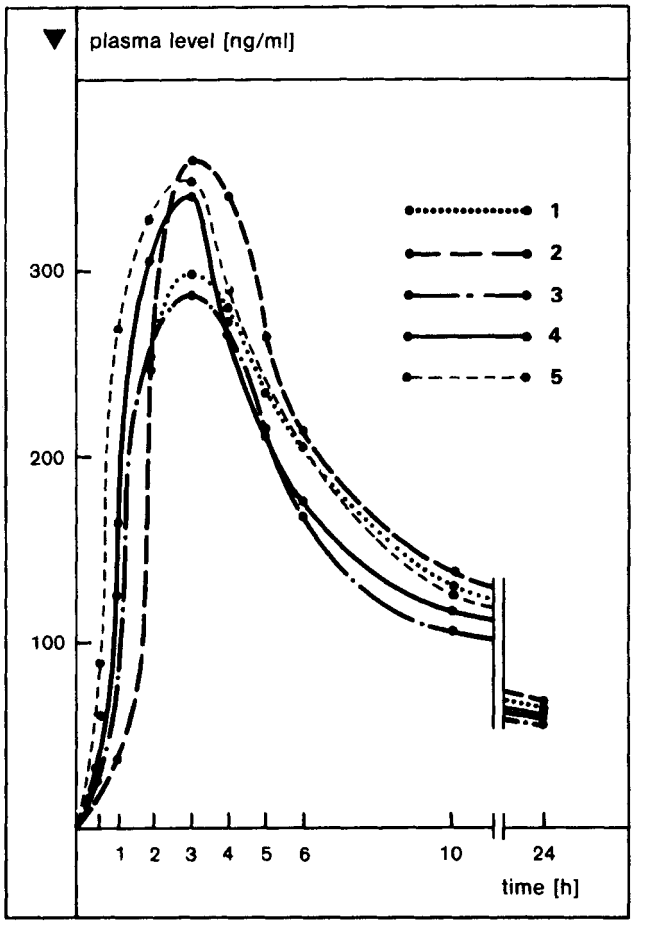

FIGURE 4

Aldadiene concentration in the plasma after the oral adminstration of 100 mg spironolactone in 5 different preparations; means from 8 subjects

rations. With the aid of the parameters (Tab. 6), however, the preparations can be differentiated. The preparations 4 and 5 are rapidly absorbed, whereas preparations 1, 2 and 3 are slowly absorbed.

Correlation: In table 7 are shown the correlation coefficients between the parameters for the in vitro and the in vivo results with the 25 mg preparations, as well as the number of significant correlations for each individual parameter. Similar results are shown in table 8 for the 100 mg preparations. The correlation between the in vitro and the in vivo results is demonstrated by the significant correlation coefficients of several parameters, both for the 25 mg preparations and the 100 mg preparations. The frequency of the proven correlations depends on the choice of parameters, which can be seen from tables 9 and 10. For example, most correlations are shown between the dissolution integral for the in vitro experiments and the time of maximum plasma level for the in vivo experiments. No correlations were found between the peak plasma level, the total area under the plasma curve, the quantity excreted in the urine in 4 h and the fraction of active ingredient dissolved within 60 min.

In tables 9 and 10 the parameters are ranked according to the frequency of demonstrated correlations per parameter. In case of equal frequencies, the size of the correlation coefficient was decisive. According to this rank order appropriate parameters can be chosen to describe availability in in vitro and in vivo experiments.

TABLE 6

Parameters for the bioavailability of spironolactone in the plasma of humans from preparations containing 100 mg active ingredient, determined from 8 subjects

Preparation No.	P_{max} µg/ml	t_{max} h	F_1 $\frac{ng}{ml} \cdot h$	F_2 $\frac{ng}{ml} \cdot h$	F_4 $\frac{ng}{ml} \cdot h$	F_{24} $\frac{ng}{ml} \cdot h$	U_2 µg	U_4 µg
1	290	3.0	45.1	234	792	3239	726	2770
2	390	3.5	11	151	806	3437	515	2661
3	280	3.0	67	255	819	3031	755	2709
4	370	3.0	59	299	913	3144	993	3089
5	380	3.0	109	407	1073	3546	1231	3305

TABLE 7

Linear correlation coefficients (r) between the parameters of the dissolution rate in vitro and the bioavailabiliy of spironolactone from 6 preparations containing 25 mg active ingredient. Each preparation was adminstered to 6 or 8 subjects

Parameter in vitro / Parameter in vivo	C_{20}	C_{60}	t_{50}	St_{50}	St_{max}	K_1	A_1	No of significant correlations $p \leq 0.01$
P_{max}	0.111	0.003	-0.156	0.161	0.104	0.169	0.128	0
t_{max}	-0.413**	-0.213	0.416**	-0.502**	-0.457**	-0.394**	-0.394**	6
F_1	0.285	0.160	-0.304	0.314	0.285	0.295	0.281	0
F_2	0.235	0.096	-0.253	0.278	0.240	0.246	0.250	0
F_4	0.209	0.059	-0.253	0.261	0.203	0.258	0.217	0
F_{24}	0.160	0.087	-0.190	0.146	0.052	0.227	0.167	0
U_2	0.306	0.175	-0.315	0.361	0.347	0.295	0.300	0
U_4	-0.085	0.052	-0.153	0.163	0.157	0.139	0.111	0
No of significant correlations $p \leq 0.01$	1	0	1	1	1	1	1	

** Correlation at $p \leq 0.01$

TABLE 8

Linear correlation coefficients (r) between the parameters of the dissolution rate in vitro and the bioavailability of spironolactone from 5 preparations containing 100 mg active ingredient. Each preparation was administered to 8 subjects

Parameter in vitro / Parameter in vivo	C_{20}	C_{60}	t_{50}	St_{50}	St_{max}	K_1	A_1	No of significant correlations $p \leq 0.01$
P_{max}	-0.063	0.070	-0.052	-0.058	-0.071	0.002	-0.053	0
t_{max}	-0.230	-0.034	0.133	-0.218	-0.036	-0.164	-0.214	0
F_1	0.532**	0.335	-0.464**	0.503**	0.274	0.529**	0.546**	5
F_2	0.443**	0.246	-0.375	0.414**	0.219	0.430**	0.452**	4
F_4	0.205	0.145	-0.221	0.185	0.109	0.233	0.220	0
F_{24}	-0.055	0.004	-0.009	-0.074	0.099	0.011	-0.026	0
U_2	0.411**	0.179	-0.332	0.375	0.213	0.390	0.421**	2
U_4	0.184	0.062	-0.153	0.160	0.111	0.181	0.193	0
No of significant correlations $p \leq 0.01$	3	0	1	2	0	2	3	

**Correlation at $p \leq 0.01$

TABLE 9

Rank of the parameters of the dissolution rate in vitro according to the number of demonstrated correlations

Rank	Parameter	Number of significant correlation coefficients $p \leq 0.01$
1	A_1	4
2	C_{20}	4
3	St_{50}	3
4	K_1	3
5	t_{50}	2
6	St_{max}	1
7	C_{60}	0

TABLE 10

Rank of the parameters of bioavailability according to the number of demonstrated correlations

Rank	Parameter	Number of significant correlation coefficients $p \leq 0.01$
1	t_{max}	6
2	F_1	5
3	F_2	4
4	U_2	2
5	$F_4/P_{max}/F_{24}/U_4$	0

DISCUSSION

The observed significant correlation coefficients of ca. 0.5 are low due to the well-known individual scatter (8) from subject to subject. Therefore only a tendency for dependence of the bioavailability on the dissolution rate in vitro can be shown. The correlation at only 1 % error risk on the other hand is obvious, so that the procedure used for the speed of dissolution in vitro can be used as a test method for the development and for the monitoring of the production of solid dosage forms. We must qualify this, however, by saying that the correlation for formulations with other active ingredients is likely, but has not been confirmed.

The various parameters of the bioavailability reflect different aspects of bioavailability. Thus, for example, the area under the plasma level curve up to 24 h is a measure of the completeness of absorption. It does not show any correlation with the in vitro results. The areas under the plasma level curves at the shorter periods of 1 and 2 h and the excretion in the urine in the first 2 h are measures of the speed of absorption. These parameters are related to the dissolution rate in vitro.

The time of maximum plasma level also tends rather to reflect the speed of absorption. It proved, however, not to be a reliable parameter as no correlation could be established by it with the 100 mg preparations. Other authors have carried out in vivo and in vitro experiments with spironolactone (9) and came to similar conclusions. Using three preparations with different in vitro results the same plasma peaks and the same quantity of 6 derivative - alda-

diene - excreted in the urine up to 4, 6 and 24 h after administration were obtained in vivo. The time of maximum plasma level and the quantity excreted in the urine 2 h after administration were different but in agreement with the results described here.

The various parameters of the dissolution rate also reflect different aspects of the dissolution process. The dissolution integral and the constants for dissolution speed give global information on the overall dissolution process. Correlations are demonstrated with these parameters.

The other parameters used only give information on a particular instant in the dissolution process. Therefore the correlation demonstrated with them cannot be generalized. For example, no correlation is demonstrated with the quantity dissolved within 60 min, whereas the quantity dissolved within 20 min was correlated with 4 in vivo parameters. Prior knowledge of a preparation should help the investigators to choose suitable parameters. For example, in an experiment with slowly dissolving preparations parameters referring to 60 min may be more favourable than 20 min values. In part this modification also applies to the half-life and to the slope at half-life. In comparison with the global parameters they have the disadvantage that they do not reflect the entire course of the dissolution, but the advantage that they characterize the mid-point of the dissolution curve. In the present example with spironolactone they proved, with certain reservation, to be suitable parameters for investigations of bioavailability.

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