BIOMEDICAL CHROMATOGRAPHY, VOL. 11, 36–41 (1997)

Simultaneous Determination of Carbamazepine and Carbamazepine 10,11-Epoxide by Using Microcolumn HPLC: Study of Pharmacokinetics of Carbamazepine in a Volunteer

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A highly-sensitive microcolumn HPLC method for the simultaneous determination of carbamazepine and carbamazepine-10,11-epoxide in human serum and saliva is described. The method was successfully employed for the study of pharmacokinetics of carbamazepine in humans. After oral administration of 100 and 200 mg of carbamazepine to a volunteer, multiple peaks were observed on the kinetic curves. They were symbathic in the serum and saliva. This indicated the presence of multiple peaks which characterize both free and protein-bound fractions of the drug.

The existence of multiple peaks on the kinetic curves implies that the kinetic of carbamazepine cannot be described with the one-compartment linear model. Nevertheless, each peak was treated within the range of a one-compartment linear model of absorption and the results obtained were compared with published data.

For the elucidation of the nature of multiple peaks the graphical differentiation of ascending and descending branches of all peaks were carried out. On this basis the dependence of the absorption and elimination rates on time was constrained. The analysis of experimental data resulted in the following conclusions: (a) the presence of multiple peaks on the kinetic curves is induced by the interrupted character of carbamazepine absorption that is caused by the very poor solubility of carbamazepine; (b) the elimination of the drug from blood serum occurs in two phases. Binding of carbamazepine with tissues takes place in the first phase, and biotransformation and excretion occurs in the second phase. It is possible that the presence of multiple peaks on the kinetic curves is partially caused also by redistribution of the drug from the comparatively easily accessible to the less accessible tissues. This requires further investigation.

Biomed. Chromatogr. **11**, 36–41 (1997)
No. of Figures: 6. No. of Tables: 2. No. of Refs: 11.

INTRODUCTION

Carbamazepine (CBZ), the widely prescribed antiepileptic drug, is successfully used in the treatment of psychomotor and generalized tonic–clonic seizures, and also grand mal and complex partial seizures. In humans, carbamazepine is partially metabolized to carbamazepine-10,11 epoxide (ECBZ), which possesses an anti-convulsive effect, analogous to the parent drug. Therefore, simultaneous determination of their levels in serum or saliva is most important for the correction of doses of patients receiving CBZ, as well as for the study of the pharmacokinetics of CBZ and its metabolite (Herkes and Eadie, 1990). Simultaneous determination of CBZ and ECBZ can be simply and precisely realized by using reversed-phase HPLC, which precedes either liquid–liquid extraction of these compounds from serum and saliva or non-extractive methods of sample preparation (MacKichan, 1980; Elyas *et al.*, 1982; Kumps, 1984; Herkes *et al.*, 1989). A certain amount of difficulty is created by the poor absorbance of ECBZ in the UV-range, which has been avoided either by switching the detection wavelength during analysis (Mihaly *et al.*, 1977), or by choosing the most suitable wavelength for both compounds (Elyas *et al.*, 1982). If it is also taken into account that after a single dose the concentration of ECBZ in biological media is very low, then the use of microcolumn HPLC becomes expedient, owing to the small dilution of samples which provides high sensitivity of analysis, thereby reducing the sample volume needed for analysis.

The pharmacokinetics of CBZ at the present time are considered within the range of a one-compartment linear model (Faigle and Feldmann, 1982; Cereghino, 1982). Apart from the data about dependence of absorption and elimination constants from the doses (Eadie and Tyrer, 1983), the exact value of bioavailability of CBZ has not been clarified, but apparently is considerably less than 100%. In calculations, the total bioavailability of CBZ was used and this resulted in increased values of both partition volume and clearance (Eadie and Tyrer, 1983). Therefore the kinetic mechanism of conversion of CBZ cannot be considered finally established.

In this communication, the pharmacokinetics and metabolism of CBZ were investigated by using microcolumn HPLC after a single dosing of CBZ by a volunteer.

EXPERIMENTAL

Reagents. Carbamazepine and carbamazepine 10,11-epoxide were obtained from Geigy Pharmaceuticals (Horsham, UK). Halodiph

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CCC 0269–3879/97/010036–06

Received 9 May 1996
Accepted 2 July 1996

was kindly supplied by the Laboratory of Organic Synthesis of Tomsk Polytechnic Institute (Tomsk, Russia).

Chromatographic procedure. Chromatographic analysis was carried out by using a microcolumn high-performance liquid chromatograph Milichrom (Nauchpribor, Oriol, Russia) with a UV detector, which permitted fixation of the entire spectrum of absorption within the range 190–360 nm. Detection of analytes was realized at a wavelength of 210 nm. The separation of CBZ and ECBZ and the internal standard Halodiph (HD) was carried out on a stainless steel column (62 × 2 mm i.d.) packed with the sorbent Separaon-C_{18} (Lachema, Brho, Czechoslovakia) of particle size 5 μm.

The mobile phase composition was 0.05 M Na_2HPO_4 with acetonitrile at a ratio of 40:60 (v/v). The pH of the eluent was adjusted with phosphoric acid to 4.0. The flow-rate of the mobile phase was 50 μL/min.

Sample preparation. Samples of saliva (0.5 mL) or serum (0.2 mL) were added to a 10 mL glass centrifuge test-tube. The samples were treated with 0.2 mL of internal standard (solution of HD in chloroform at the concentration of 0.01 mg/mL). Sodium hydroxide (100 μL, 4.0 M) was then added and vortexed briefly. The analytes were extracted with 3 mL of chloroform. The extraction mixture was agitated by hand for 1 min and centrifuged for 10 min. The organic layer was filtered, transferred into a clean test-tube and evaporated to dryness at room temperature with a gentle current of air. The residue was dissolved in 40 μL of mobile phase and 15–20 μL was injected for HPLC analysis.

RESULTS AND DISCUSSION

For the simultaneous determination of CBZ and ECBZ the detection wavelength of 210 nm was chosen for two reasons: (a) it is close to the maximum absorption of ECBZ (206 nm in the mobile phase) and detection of trace quantities is possible; (b) at this wavelength, the absorption of CBZ is weak (since the maximum absorption of CBZ in the eluent was at 228 nm) and therefore the peak-height is comparable with that of ECBZ (concentation of ECBZ in humans makes up to 10–25% of the total concentration of CBZ). Owing to this, two internal standards have been used (MacKichan, 1980). In Fig. 1, a typical chromatogram from the extracted saliva of the volunteer 12 h after a single oral dose of CBZ (200 mg) is illustrated. The concentration of CBZ and ECBZ was 0.05 and 1.2 μg/mL, respectively. Interferences from other antiepileptic drugs of acidic nature (phenobarbital, primidon, phenytoin etc.) were avoided by carrying out the extraction step in the alkaline medium. The basic recovery and precision characteristics of the proposed method are briefly summarized in Table 1. The dynamics of CBZ concentrations in the saliva and serum of a normal

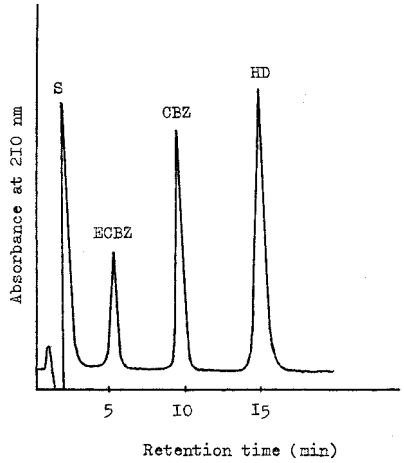

Figure 1. Chromatogram of serum extract of the volunteer 12 h after oral administration of 200 mg of CBZ. ECBZ, 0.05 μg/mL; CBZ, 1.2 μg/mL; HD, internal standard; S, solvent peak.

female volunteer of 55 kg after single oral doses of 100 and 200 mg were studied. The interval between doses of 100 and 200 mg of CBZ was 10 days. In the case of 100 mg, concentrations of CBZ and ECBZ were determined in both saliva and blood serum (Fig. 2), while after a dose of 200 mg CBZ, the concentrations of CBZ and ECBZ were determined in saliva only (Fig. 3).

As Fig. 2 shows, after oral administration of 100 mg of CBZ, more than one peak was observed on the kinetic curves. Symbathicity of between the kinetic curves of CBZ in saliva and in serum (Fig. 2) indicated that the presence of multiple peaks characterizes not only the free, but also the protein-bound fraction of the drug. ECBZ appeared in serum at once after the second peak, but somewhat later in saliva. The concentration of ECBZ in both serum and saliva was equal to approximately 10% of the respective values of CBZ concentration.

Figure 3 shows the relationship of CBZ concentration in saliva with time after a 200 mg dose. As compared with 100 mg of CBZ the number of peaks has increased abruptly and reached five. The presence of multiple peaks on the kinetic curves of CBZ (as well as the above-mentioned dose dependent character of K_{el} and K_{abs}) indicated that description of CBZ kinetic within the range of a one-compartment linear model is impossible. However, it was decided to treat each peak individually within the range of a one-compartment linear model with absorption and to compare the obtained results with data in the literature. According to this model, the concentration of CBZ is described by the biexponential equation (Soloviov *et al.*, 1980)

$$C(t) = B(e^{-K_{el}t} - e^{-K_{abs}t}), \quad (1)$$

where

Table 1. Analytical recoveries and extraction precision, within-day and day-to-day precision of the present method

Compounds	Concentration (ng/mL)	Absolute recovery (%, $n=5$) $\bar{X}$	CV (%)	Within-day precision data ($n=8$) $\bar{X}$	SD	CV (%)	Day-to-day precision data ($n=8$) $\bar{X}$	SD	CV (%)
Carbamazepine	50	95	13.4	51.2	3.18	6.2	52.5	4.30	8.2
	500	88	11.3	498.3	25.19	5.1	495.0	29.40	5.9
Carbamazepine 10,11-epoxide	50	93	15.0	51.8	4.76	9.2	52.6	5.24	10.0
	500	92	11.5	486.8	28.60	5.8	480.4	30.02	6.2

$\bar{X}$, Mean; SD, standard deviation; CV, coefficient of variation.

$$B=\frac{D\times K_{abs}}{V_d(K_{abs}-K_{el})} \quad (2)$$

and D is the dose of drug in mg and V_d is the apparent volume of distribution.

$T_{1/2\,el}$ and $T_{1/2\,abs}$ are calculated by the equations

$$T_{1/2\,el}=\frac{0.693}{K_{el}} \quad (3)$$

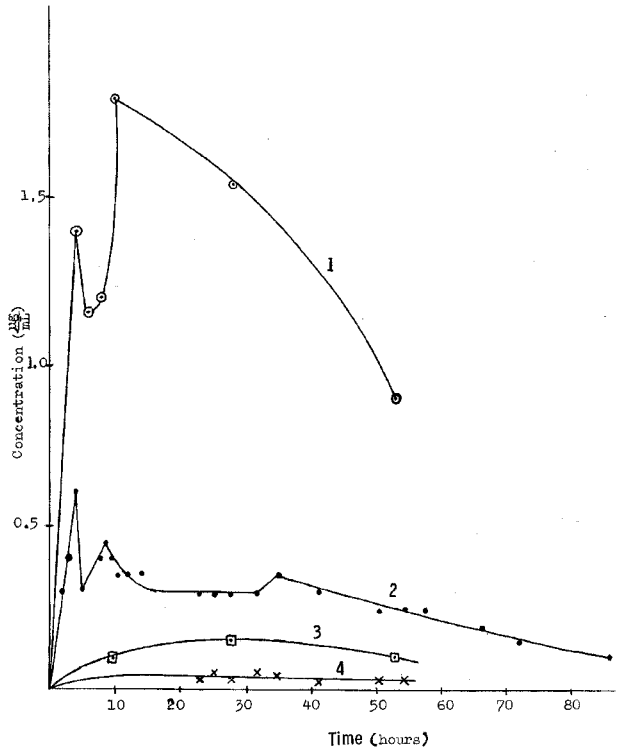

Figure 2. Concentration–time curves in the volunteer after oral administration of 100 mg CBZ. 1 (⊙) concentration of CBZ in serum, 2 (●) concentration of CBZ in saliva, 3 (⊡) concentration of ECBZ in serum, 4 (×) concentration of ECBZ in saliva.

$$T_{1/2\,abs}=\frac{0.693}{K_{abs}} \quad (4)$$

At the single dose of 200 mg of CBZ, K_{el} for each peak was determined from the relationship $\ln C_{saliva}-t$ (Fig. 5). For the first peak K_{abs} and B were also calculated by means of consequent logarithmization (Soloviov *et al.*, 1980). The value of B as determined from the relationship $\ln C_{saliva}-t$ characterizes the free fraction of drug. In our case

$$B_{saliva}=1.99\ \mu g/mL$$

Multiplication of B_{saliva} by four gave a B_{serum} of approximately 7.97 [mg]g/mL, which was used in the calculations by the expression

$$V_d=\frac{D\times K_{abs}}{B(K_{abs}-K_{el})} \quad (5)$$

$$\dot{V}_d=\frac{V_d}{M} \quad (6)$$

where $\dot{V}_d$ is the specific volume of distribution and where M is body weight

$$Cl=K_{el}\times V_d \quad (7)$$

$$\dot{C}i=K_{el}\times \dot{V}_d \quad (8)$$

where Cl is the clearance and $\dot{C}l$ is the specific clearance.

The results in Table 2 show that the determined value of K_{el} was two or three times greater than the values of the elimination constants of CBZ mentioned in the literature (as well as the above-mentioned dose dependent character of the constants of elimination K_{el} and absorbtion K_{abs}). It was only in the case of the last (fifth) peak that our value of K_{el} coincided with the one generally accepted. Such divergence is surely caused by the fact that description of the kinetics of CBZ for the given volunteer using a one-compartment model is impossible. It was only at the end of experiment (fifth peak) that the elimination obeyed the law of first degree.

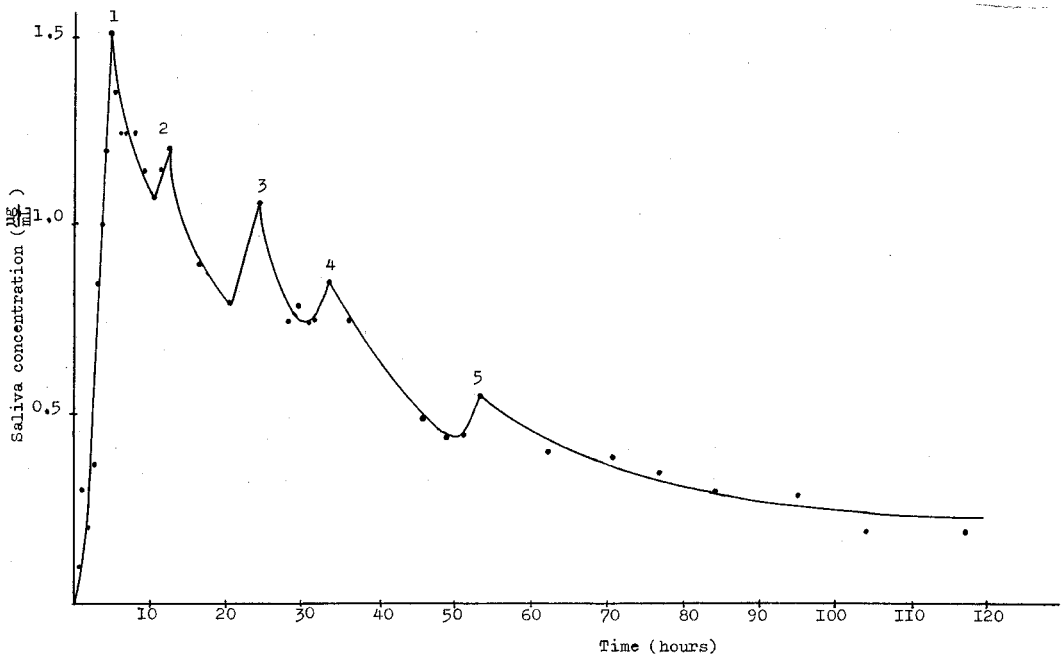

Figure 3. Saliva concentration–time curve in the volunteer after the oral administration of 200 mg CBZ. 1, 2, 3, 4 and 5 mean peak numbers.

It is reported in the literature that considerable variability is remarkable for K_{abs} of CBZ $T_{1/2\,abs} = 2 \div 7$ h (Eadie and Tyrer, 1980). This is related to poor solubility and hence to incomplete bioavailability of CBZ. The value of $T_{1/2\,abs}$ determined for the first peak was equal to 4.1 h, coinciding entirely with existing data. The above-mentioned refers to $\dot{V}_d$ also, which was equal to 0.8 L/kg according to our data (Table 2). Our value of specific clearance was somewhat increased, and is equal to 0.05 L/h·kg. It should be noted once again, that existing literature values of $\dot{V}_d$ and $\dot{Cl}$ were also increased, since at their determination the total bioavailability of CBZ was wrongly assumed.

The existence of multiple peaks on the kinetic curves of CBZ is of particular interest. It should be noted that secondary and tertiary peaks were also observed during a chronic regimen (Morselli and Bossi, 1982). For the elucidation of their nature, graphic differentiation of the ascending and descending branches of each peak was carried out. Determined in this way, the dependences $W_{abs} - t$ and $W_{disapp} - t$ are given in Fig. 4 and Fig. 6 (W_{abs} is the rate of absorbtion; W_{disapp} denotes the total rate of distribution of drug through organs and tissues and the rate of its elimination: $W_{disapp} = W_{distrib} + W_{el}$). As Fig. 4 shows, in the case of the first peak W_{abs} increased with time and reached the maximal value not long before C_{max}. The rates of absorption of other peaks were approximately equal to each other and were constant during absorption. The interval between the start of absorption of adjacent peaks was the same for the first three pairs of peaks and was approximately equal to 10 h. For the last pair of peaks (the fourth and fifth peaks) this interval was equal to 20 h (Fig. 3 and Table 2).

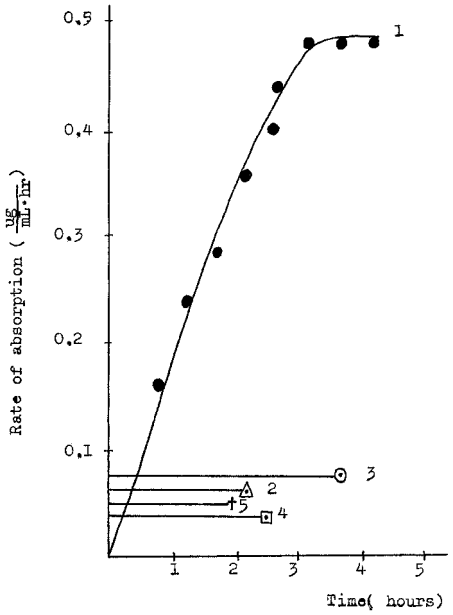

Figure 4. Dependence of the rate of absorption W_{abs} on time after the oral administration to the volunteer of 200 mg of CBZ. 1 (●), 2 (△), 3 (⊙), 4 (⊡) and 5 (+) correspond to the 1, 2, 3, 4 and 5 peaks in Fig. 3.

Increase of W_{abs} during the process (Fig. 4, curve 1) and some S-likeness of kinetic curves (Fig. 1, curves 1, 2 and Fig. 3, the first peak) indicated that absorption of the drug in blood plasma consisted of two consecutive stages clearly distinguished from each other by the rate. Besides, the second stage rate surpassed the first. The apparently kinetic stages making up the absorption of CBZ in blood plasma are both dissolution of CBZ (first stage) and its absorption through the gastric–intestinal highway (second stage). It is probably due to the poor solubility of CBZ in water (Morselli and Bossi, 1982) limited character of this stage results in the observed kinetics: S-like kinetic curves and a rise of W_{abs} during the process.

The appearance of CBZ in saliva and serum following the first peak (ascending branches of peaks on kinetic curves) is explained as follows: after absorption of the first basic portion of dissolved drug a comparatively negligible part of the drug is retained in the gastric–intestinal highway. Since the dissolution of CBZ proceeded with difficulty, some time was required for the accumulation of a sufficient amount of the dissolved drug to make the rate of absorption of

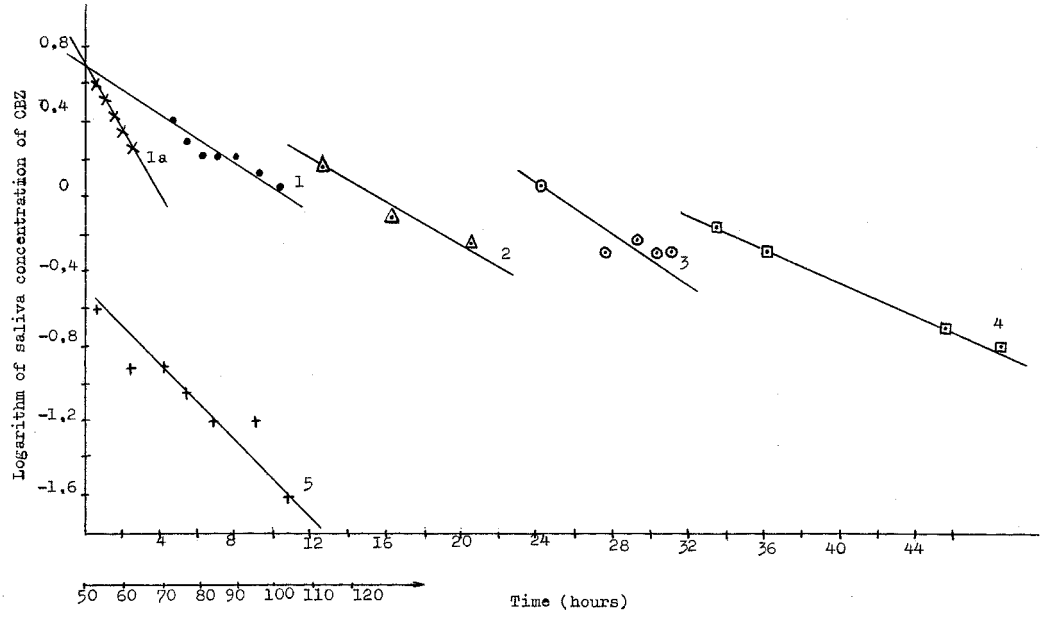

Figure 5. Relationship between logarithm of saliva concentration of CBZ and time after oral administraton of 200 mg CBZ by the volunteer, the straight lines 1 (●), 2 (△), 3 (⊙), 4 (⊡) and 5 (+) correspond to the descending branches of peaks 1, 2, 3, 4 and 5 in Fig. 3; 1a (×) corresponds to the ascending branch of the 1 peak. The upper scale on the abscissal axis corresponds to the straight lines 1, 2, 3, 4, 1a and the lower scale to the straight line 5.

Table 2. Pharmacokinetic parameters of CBZ from saliva recalculated to serum after single oral administration of 200 mg of CBZ to the volunteer

Number of peaks	K_{el}[a]/h	$T_{1/2\,el}$/h	K_{abs}/h	$T_{1/2\,abs}$/h	V_d (L)	$\dot{V}_d$ (L/kg)	CL (L/h)	$\dot{C}L$ (L/h/kg)	Δt_{abs} (h)	Δt between beginning of absorption of neighbouring peaks (h)	W_{abs} (μg/mL/h)	Δt_{disapp} (h)	Δt between beginning of elimination of neighbouring peaks (h)	W_{disapp} (μg/mL/h)
I	0.06	11.6	0.17	4.1	38.8	0.78	2.33	0.047	4.6		0.041	5.75		0.36
				(2–7)[b]		0.8[b]		(0.01–0.02)[b]		10.3	0.48			
II	0.06	11.6							2.3		0.06	7.9		0.12
										10.2			11.8	
III	0.068	10.1							3.7		0.074	6.1		0.106
										10.6			9.3	
IV	0.044	15.6							2.5		0.04	16.5		0.06
										20			19.5	
V	0.021	33.4							2		0.05	64[c]		0.025
		(38)[b]												

[a] K_{el} is determined for each peak separately.
[b] Data from Eadie and Tyrer (1983).
[c] Elimination is not finished.

significant value. During this time a reduction of CBZ concentration was observed on the kinetic curves, which was caused by the redistribution and elimination of the drug. When W_{abs} exceeded W_{disapp} the revival of drug in plasma was observed.

Study of the relationship $W_{disapp} - t$ (Fig. 6) shows that during this process a decrease in W_{disapp} for all peaks takes place. A particularly abrupt decrease in the rate was found for the first peak, but the diminution of rate was less sharply expressed for the other peaks. The largest value of $W_{initial\ disapp}$ was that of the first peak, which gradually decreased with transference to the other peaks. Accordingly, the duration of interval of disappearance Δt_{disapp} increased (Table 2).

Two phases can be identified in all peaks except the third: in the first phase abrupt reduction of W_{disapp} was observed, but in the second phase its reduction was comparatively smooth. Apparently, in the first phase, rapid distribution of CBZ occurred through organs and tissues. As found in the literature and also obtained by us, the value of $\dot{V}_d = 0.8$ L/kg indicated that CBZ rapidly spreads through all the liquid medium of the organism and binds with tissues to a considerable degree. In the second phase (the sphere of comparatively slow decrease of W_{disapp}) elimination of the drug was chiefly realized, by means such as biotransformation and excretion. During the process (with the ordinal number of peaks increased) the share of distribution through tissue decreased and the specific share of elimination increased. This was emphasized by the rising of the smooth decline region of W_{disapp} with an incease in the ordinal number of peaks (Fig. 6). Particularly significant is the share of elimination region for the fifth peak. This circumstance, in addition to the value of $K_{el} = 0.02\ h^{-1}$ determined by us according to the linear model, shows that in case of the last peak, apparently, elimination was chiefly governed by the law of first degree.

Thus, analysis of experimental pharmacokinetics data of CBZ in saliva and serum of a volunteer after administering 100 and 200 mg of the drug resulted in the following conclusions: the presence of multiple peaks on the kinetic curves is caused by the interrupted character of absorption of the drug from the gastric–intestinal highway into blood serum. This characer of absorption is brought about by the very poor solubility of CBZ. After absorption of the next portion of CBZ, a certain time lapse is required for the accumulation of such an amount of dissolved CBZ in the gastric–intestinal highway, during which W_{abs} exceeds the W_{disapp} of the drug. Only then further increase of concentration of CBZ on the kinetic curve would be realized.

The process of disappearance of the drug from serum

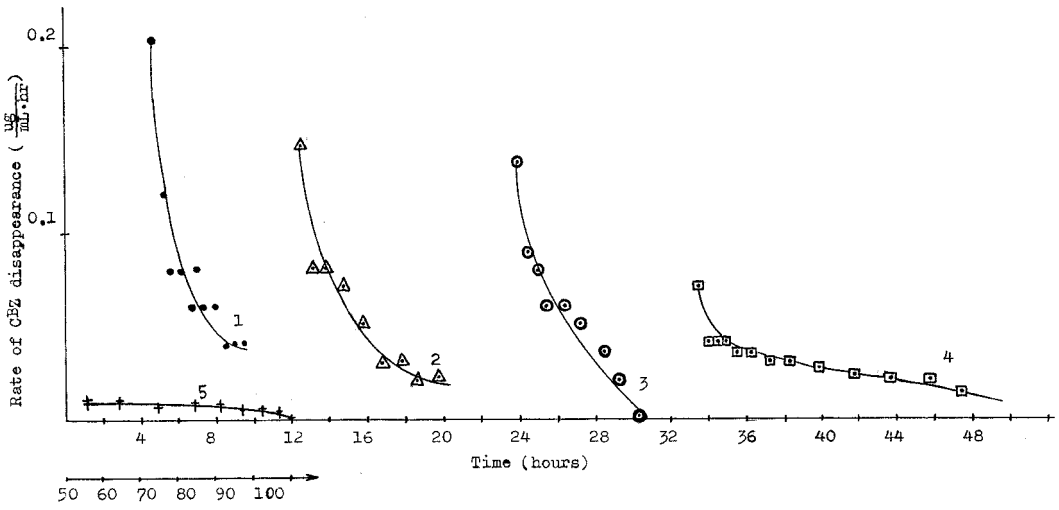

Figure 6. Dependence of the rate of disappearance (W_{disapp}) of CBZ in the saliva vs. time in the volunteer after oral administration of 200 mg of CBZ. 1 (●), 2 (△), 3 (⊙), 4 (⊡) and 5 (+) correspond to the 1, 2, 3, 4 and 5 peaks on Fig. 3. The upper scale on the abscissal axis corresponds to curves 1, 2, 3, 4 and the lower scale to curve 5.

consists of two phases practically separated by time. In the first phase distribution of CBZ occurs through organs and tissues (besides the degree of binding of CBZ with tissues which, presumably, is significant enough), but in the second phase elimination takes place (biotransformation and excretion). In the course of time the specific share of elimination increases. On the last descending branch of the kinetic curve elimination mostly takes place as described by the one-compartment linear model.

It should be noted, in conclusion, that the presence of multiple peaks on the kinetic curves is partially caused also with the redistribution of drug from comparatively the easily attainable to the less accessible tissue. This needs further investigation.

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