since they are marketed as preformulated dosing solutions, the same variations in K_a with dosing volume might be expected. Part of the variations noted earlier may be due to the effects of the different recommended dosing schedules and different dosing solution volumes on the volumes administered. The question of variations in aminoglycoside absorption rates is an interesting phenomenon that warrants further investigation.

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Theophylline Absorption and Disposition in Rabbits: Oral, Intravenous, and Concentration-Dependent Kinetic Studies

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Abstract □ Theophylline pharmacokinetics following oral and intravenous administration were studied, and the absolute bioavailability of five commercially available products was determined using the rabbit as an *in vivo* model. Concentration-dependent clearance studies were performed by multiple constant-rate infusion and multiple bolus dose administration of aminophylline. Theophylline pharmacokinetics following the oral adminstration of these products obeyed the one-compartment open model adequately. However, the data obtained following rapid intravenous aminophylline administration in the rabbit fit either the one-compartment model (half-life = 2.8 hr and the volume of distribution = 0.586 liter/kg) or the two-compartment model (β-phase half-life = 4.4 hr and $V_{d(\beta)}$ = 0.708 liter/kg). There were no significant product-to-product differences in the time to peak (t_{max}), the rate constant of absorption (k_a), or the percent of dose absorbed at 1 hr (F_1); however, differences in the absolute bioavailability (F), dose-normalized peak serum concentration ($C_{max(n)}$), and percent of dose absorbed at 6 hr (F_6) were significant. There was no evidence of concentration-dependent clearance for theophylline in the rabbit in the serum concentration range studied, but the results of the multiple constant-rate infusion study suggest that total clearance decreases at higher serum theophylline concentrations.

Keyphrases □ Theophylline—pharmacokinetics, oral and intravenous administration, concentration-dependent clearance □ Pharmacokinetics—theophylline, oral and intravenous administration, concentration-dependent clearance □ Bioavailability—theophylline, oral administration, aminophylline, intravenous administration

Several pharmacokinetic studies have investigated theophylline bioavailability from various dosage forms (1–5). Two studies (3, 5) determined the absolute theophylline bioavailability from oral dosage forms. However, additional research is needed to examine the intrasubject variability in theophylline elimination kinetics (6).

The present work concerned theophylline pharmacokinetics in the rabbit following intravenous and oral administration to determine the absolute bioavailability of theophylline from five commercially available dosage forms using the rabbit as an *in vivo* model and to examine the possibility of concentration-dependent clearance of this drug. Although no reports suggested dose-dependent kinetics for theophylline in the rabbit, it may be that at sufficiently high dosages the relationship between the dose and the serum concentration–time integral becomes nonlinear. Such a finding would preclude the use of traditional approaches for quantitating absolute or relative bioavailability.

EXPERIMENTAL

Materials and Methods—All dosage forms studied (A[1], B[2], C[3], D[4], and E[5]) were purchased commercially. Anhydrous theophylline[6] was used as supplied. Aminophylline[7] injection, 250 mg (25 mg/ml), was used for intravenous administration.

Animals—Male New Zealand White rabbits, 2.4–3.9 kg, were maintained on commercial rabbit food[8] and tap water and were fasted overnight prior to each oral experiment. Water was allowed *ad libitum* during fasting and throughout the experiment. Each animal received one oral dosage form of theophylline, followed 24 hr later by an intravenous dose of aminophylline.

Oral Administration of Drugs—The animal was restrained, and its mouth was opened by inserting hemostatic forceps from the side into the oral cavity immediately behind the rabbit's incisors. A small animal capsule administration device, with the tablet or capsule in its slotted end, was placed over the rabbit's tongue and advanced ~4–5 cm into the pharynx. The capsule or tablet then was released by pushing the plunger rapidly and completely. This administration was followed by ~10 ml of water. After the device and forceps were removed, the rabbit's mouth and nostrils were held closed until swallowing occurred.

Rapid Intravenous Administration of Aminophylline—A dose of 24.33

[1] Tablets (aminophylline, 200 mg), lot 776-991, Searle, Chicago, Ill.
[2] Capsules (theophylline, 200 mg), lot 1W60531, Cooper, Wayne, N.J.
[3] Tablets (theophylline, 125 mg), lot 68308, Riker Laboratories, St. Paul, Minn.
[4] S. R. Capsules (theophylline, 2 gr), lot 6090204, Fleming, Fenton, Mo.
[5] S. R. Capsules (theophylline, 1 gr), lot 6090204, Fleming, Fenton, Mo.
[6] Anhydrous theophylline, Nutritional Biochemicals, Cleveland, Ohio.
[7] Aminophylline Injection, Searle, Chicago, Ill.
[8] Purina Laboratory Rabbit Chow, Ralston-Purina, St. Louis, Mo.

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Table I—Results of the Multiple-Rate Intravenous Infusion Experiments for the Concentration-Dependent Clearance Study

Experiment	Rabbit Weight, kg	Infusion Time, hr	Infusion Rate, mg/hr	$\bar{C}_{ss}$, mg/liter	Cl_{TB}, liter/hr/kg
P IF	4.13	0–24	3.60	14.4	0.060
P IF	4.13	24–48	7.20	28.7	0.061
P IF	4.13	48–66	10.80	56.3	0.046
Q IF	3.21	0–24	2.59	7.5	0.108
Q IF	3.21	24–48	5.18	16.9	0.095
Q IF	3.21	48–72	7.77	41.7	0.058

Table II—Pharmacokinetic Parameters for Theophylline following Intravenous Administration in the Rabbit Using the One-Compartment Open Model

Experiment	k_d, hr^{-1}	$t_{1/2}$, hr	V_d, liter/kg	Cl_{TB}, liter/kg/hr
A1 IV	0.322	2.2	0.666	0.214
A2 IV	0.303	2.3	0.544	0.165
A3 IV[a]	0.083	8.4	0.770	0.064
B3 IV	0.218	3.2	0.420	0.091
D1 IV	0.190	3.6	0.575	0.109
D2 IV	0.201	3.4	0.620	0.125
D3 IV	0.403	1.7	0.628	0.253
E3 IV	0.210	3.3	0.650	0.137
Mean	0.264	2.8	0.586	0.156
SD	0.080	0.73	0.085	0.059

[a] Values not included in the calculation of the mean and standard deviation.

mg of aminophylline injection USP/kg (25 mg/ml), equivalent to 15 mg of theophylline/kg, was administered through a catheter into the marginal ear vein of the rabbit over 5 min.

Concentration-Dependent Kinetic Studies—*Multiple-Dose Rapid Intravenous Administration*—After the cannulation of the marginal ear vein, a dose equivalent to 15 mg of theophylline/kg was administered intravenously into the catheter. A second dose, equivalent to 22.5 mg of theophylline/kg, was administered 24 hr after the first injection; a third dose, equivalent to 30 mg of theophylline/kg, was injected 24 hr after the second dose.

Multiple-Rate Infusion—The infusion of aminophylline was carried out using a variable-speed, syringe-type infusion pump[9] at an initial rate for the first 24 hr, at twice this rate for the second 24 hr, and at triple this rate for the rest of the experiment. The infusion rates and periods are listed in Table I.

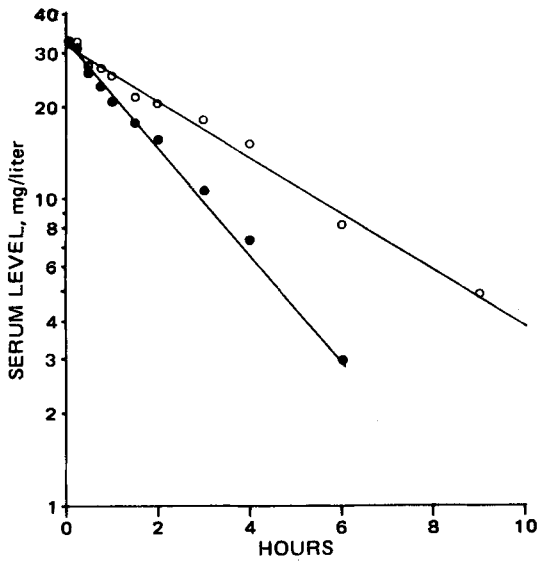

Figure 1—*Serum theophylline concentration–time data fitted to the one-compartment model. Key: ●, Experiment D3 IV; and ○, Experiment E3 IV.*

[9] Model 975, Harvard Apparatus Co., Millis, Mass.

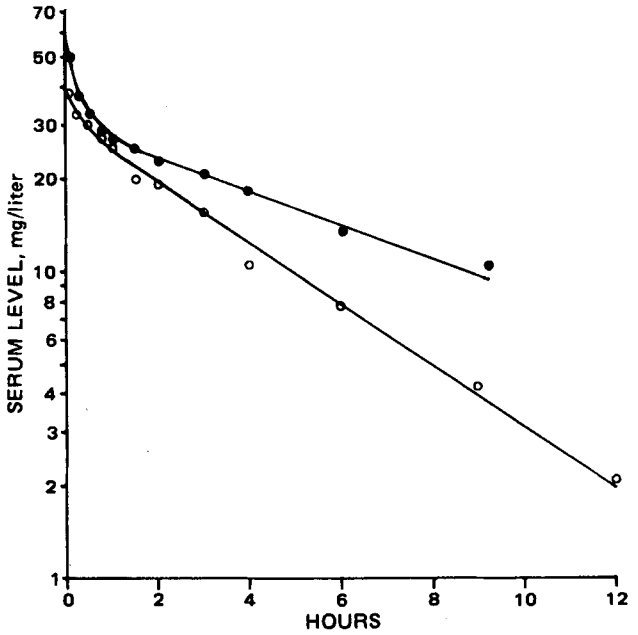

Figure 2—*Serum theophylline concentration–time data fitted to the two-compartment model. Key: ●, Experiment B4 IV; and ○, Experiment E1 IV.*

Analysis of Theophylline in Serum—A modified version of the Schack and Waxler method (7) was used for theophylline analysis in serum. Serum, 0.5 ml, was extracted with chloroform containing 5% (v/v) isopropyl alcohol after the addition of 0.2 ml of 0.1 *N* HCl. The chloroform layer then was back-extracted with 0.1 *N* NaOH, and the absorbance of this solution was read at 272 and 310 nm. The corrected absorbance of the sample was calculated as:

$$A_{sample} = (A_{272} - A_{310})_{sample} - (A_{272} - A_{310})_{blank} \quad \text{(Eq. 1)}$$

where A is the absorbance and the subscript indicates the wavelength at which it was determined.

RESULTS

Each experiment in this study is designated by a letter–number code. The letter indicates the product administered in the oral study, and the number identifies the animal used in the intravenous or oral study.

Rapid Intravenous Administration—The data obtained from the intravenous administration experiments were fitted to either a one- or two-compartment open model using the computer program KINA (8). The parameters obtained from the data that fitted the one-compartment model appear in Table II; those obtained using the two-compartment model are listed in Table III. Typical fits of the data can be seen in Figs. 1 and 2.

Oral Administration—The serum concentration–time data were

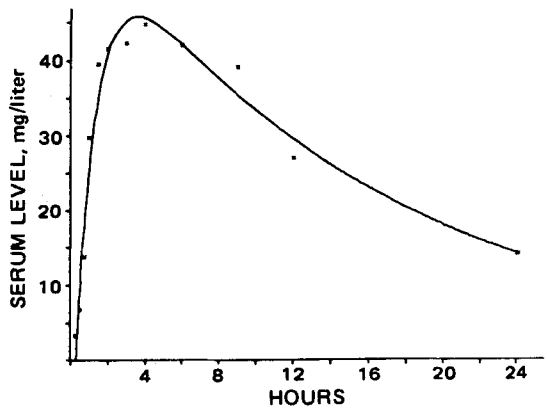

Figure 3—*Computer-generated plot of serum theophylline concentration–time data of Experiment A1 PO fitted to the one-compartment model with first-order absorption.*

Table III—Pharmacokinetic Parameters for Theophylline following Intravenous Administration in the Rabbit Using the Two-Comparment Open Model

Experiment	α, hr^{-1}	β, hr^{-1}	$t_{1/2}$, hr	k_1, hr^{-1}	k_2, hr^{-1}	$V_{d(\beta)}$, liter/kg	V_c, liter/kg	Cl_{TB}, liter/kg/hr
A4 IV	7.18	0.109	6.4	3.10	3.99	0.616	0.343	0.066
B2 IV[a]	2.83	0.038	18.2	1.24	1.56	0.771	0.425	0.029
B4 IV	4.52	0.127	5.5	2.02	2.38	0.665	0.351	0.084
C1 IV	5.84	0.154	4.5	2.12	3.62	0.616	0.383	0.094
C3 IV	1.97	0.255	2.7	0.77	0.90	0.856	0.392	0.218
C4 IV	5.14	0.121	5.7	2.10	2.95	0.664	0.383	0.084
E1 IV	2.11	0.228	3.0	0.43	1.60	0.681	0.517	0.155
E2 IV	4.01	0.258	2.7	1.66	2.14	0.855	0.453	0.220
Mean	4.39	0.179	4.4	1.74	2.51	0.708	0.403	0.132
SD	1.90	0.066	1.6	0.90	1.09	0.104	0.061	0.066

[a] Values not included in the calculation of the mean and standard deviation.

Table IV—Pharmacokinetic Parameters for Oral Theophylline Products

Parameters	Product A	B	C	D	E
Number of experiments	4	4	4	3	3
$t_{1/2}$, hr	13.4[a] (5.1)[b]	15.5 (5.8)	7.3 (2.1)	16.6 (8.5)	15.9 (8.1)
k_a, hr^{-1}	0.447 (0.291)	0.899 (0.638)	0.260 (0.267)	0.174 (0.163)	0.993 (1.32)
AUC $\int_{0(n)}^{\infty}$[c], kg hr/liter	19.6 (4.8)	19.0 (8.6)	15.4 (3.1)	9.2 (8.9)	12.2 (8.3)
$C_{max(n)}$[d], kg/liter	0.763 (0.170)	0.708 (0.285)	0.724 (0.095)	0.205 (0.182)	0.388 (0.120)
t_{max}, hr	6.2 (2.3)	7.1 (7.9)	6.7 (2.6)	12.3 (8.1)	6.1 (4.3)
$f_1 \times 100$, %	40.8 (14.5)	45.7 (31.2)	32.7 (29.3)	28.1 (29.0)	39.5 (30.4)
$f_6 \times 100$, %	90.4 (13.0)	81.4 (38.0)	65.0 (28.1)	52.9 (29.6)	85.7 (8.00)
F, %	68.7 (15.9)	50.2 (22.9)	120 (13.7)	19.1 (10.9)	39.0 (13.9)
F_1, %	27.8 (12.5)	21.1 (17.8)	22.1 (13.5)	6.46 (9.41)	13.3 (5.76)
F_6, %	61.8 (16.6)	39.8 (32.2)	62.1 (14.4)	10.5 (10.7)	33.3 (9.50)

[a] Mean. [b] Standard deviation. [c] Normalized area under the curve (AUC/dose). [d] Normalized peak serum concentration (C_{max}/dose).

fitted to the one-compartment open model with first-order absorption and elimination using the computer program KINA. The parameters obtained are listed in Table IV. A typical fit of the data is shown in Fig. 3.

Determination of Absolute Bioavailability (F)—The absolute bioavailability (F) was calculated according to:

$$F = \frac{k_{d_{or}} \int_0^{\infty} C\,dt_{or} D_{iv}}{k_{d_{iv}} \int_0^{\infty} C\,dt_{iv} D_{or}} \times 100 \qquad \text{(Eq. 2)}$$

where D is the dose, $\int_0^{\infty} C\,dt$ is the area under the serum concentration–time curve, and k_d is the rate constant for elimination. The subscripts or and iv refer to the oral and intravenous routes. The values of F are presented in Table IV.

Determination of Cumulative Percent of Dose Absorbed at 1 (F_1) and 6 (F_6) hr—The cumulative fraction of drug absorbed at 1 (f_1) and 6 (f_6) hr was calculated according to the Wagner–Nelson equation (9):

$$f_t = \frac{C_t + k_d \int_0^{t} C\,dt}{k_d \int_0^{\infty} C\,dt} \qquad \text{(Eq. 3)}$$

where C_t is the serum theophylline concentration at time t.

Using the values of f_1 and f_6, the cumulative percent of dose absorbed at 1 (F_1) and 6 (F_6) hr was calculated as follows:

$$F_1 = f_1 F \qquad \text{(Eq. 4a)}$$

and:

$$F_6 = f_6 F \qquad \text{(Eq. 4b)}$$

The values of F_1 and F_6 are given in Table IV.

Determination of Time to Peak (t_{max}) and Peak Serum Concentration (C_{max})—The time to peak was calculated from:

$$t_{max} = \frac{1}{k_a - k_d} \ln \frac{k_a A}{k_d B} \qquad \text{(Eq. 5)}$$

where k_a is the absorption rate constant, A is the preexponential coefficient for the absorption phase, and B is the preexponential coefficient for the elimination phase. All four parameters (k_a, k_d, A, and B) were calculated by KINA.

The peak serum concentration was calculated by substituting the t_{max} value in:

$$C_{max} = -Ae^{-k_a t_{max}} + Be^{-k_d t_{max}} \qquad \text{(Eq. 6)}$$

Values of t_{max} and C_{max} appear in Table IV.

Concentration-Dependent Clearance Studies—*Multiple-Dose Rapid Intravenous Administration*—The pharmacokinetic parameters obtained from these experiments using KINA are listed in Table V.

Multiple-Rate Infusion—The mean steady-state serum concentration ($\bar{C}_{ss}$) at different infusion rates (k_0) and the corresponding total body clearance (Cl_{TB}) obtained from these experiments are presented in Table I.

The total body clearance for theophylline was calculated according to:

$$Cl_{TB} = \frac{k_0}{\bar{C}_{ss}} \qquad \text{(Eq. 7)}$$

DISCUSSION

Rapid Intravenous Administration—For animals whose data fitted the one-compartment model, there appeared to be a fairly rapid elimination of theophylline with a mean half-life of 2.8 hr (Table II). The mean value of the volume of distribution was 0.609 liter/kg, and the mean total body clearance was 0.144 liter/hr/kg. However, for the other eight rabbits whose data fitted the two-compartment model, the mean β-phase half-life, $t_{1/2(\beta)}$, was 4.4 hr. This value is similar to the $t_{1/2(\beta)}$ reported for humans (4.4–6.7 hr) (10, 11). A mean distribution phase rate constant (α) of 4.2 hr^{-1} was found for theophylline in this study in the rabbit (Table III). This value is close to that obtained in humans (5.8 hr^{-1}) (11). The volume of the central compartment was 0.30 liter/kg for humans (11). A slightly higher value (0.41 liter/kg) was found in this study for the rabbit.

Oral Administration—The mean values of the absolute bioavailability (F) for Products A, B, C, D, and E were 68.7, 50.2, 120, 19.1, and 39.7%, respectively. The analysis of variance showed that the difference in F among products was significant ($p < 0.001$). The absolute bioavailability (F) was determined according to Eq. 2, in which it is assumed that the volume of distribution remains constant and the area–dose relationship is linear. Both assumptions appear to be valid since the volume of distribution and the total body clearance did not change as the dose was increased (Table V).

Equation 2 also considers differences occurring in the apparent half-life observed following intravenous and oral administration in the same rabbit. It is assumed that differences in clearances following intravenous

Table V—Results of the Multiple-Dose Rapid Intravenous Administration Experiments for the Concentration-Dependent Kinetic Study

Experiment	Rabbit Weight, kg	Dose, mg/kg	k_d, hr^{-1}	V_d, liter/kg	$AUC \int_0^\infty$, mg hr/liter	Cl_{TB}, liter/hr/kg
K1	3.44	15.0	0.185	0.781	104	0.144
K2	3.44	22.5	0.172	0.750	175	0.129
K3	3.44	30.0	0.187	0.789	203	0.148
L1	3.48	15.0	0.166[a]	0.714[b]	127	0.118
L2	3.48	22.5	0.247	0.549	166	0.136
L3	3.48	30.0	0.258	0.638	182	0.164
M1	2.87	15.0	0.199	0.512	147	0.102
M2	2.87	22.5	0.205	0.504	218	0.103
M3	2.87	30.0	0.200	0.501	300	0.100

[a] β. [b] $V_{d(\beta)}$.

and oral administration in the same animal are reflected by the elimination half-life of the drug and that this half-life is derived from the terminal log-linear portion of the serum concentration–time curve in experiments involving oral theophylline administration.

It was reported (12) that the rabbit presents problems as a model for drug absorption studies since fasting markedly prolongs stomach emptying. It was shown (12) that it is difficult to achieve an empty stomach in the rabbit by withholding food. Other investigators (13) suggested the use of a special solid diet to control gastric emptying rates in the rabbit. More rapid theophylline absorption might have been observed in the present study if the animals had not been fasted prior to oral dosing.

It is possible that theophylline absorption in the rabbit is sufficiently slow that the terminal portion of the serum concentration curve represents absorption, especially since gastric emptying is quite slow. This possibility may explain the discrepancy between the apparent half-lives observed following oral and intravenous administrations in the same animal. In this situation, it is incorrect to use the rate constant derived from the terminal portion of the serum concentration–time curve as $k_{d_{or}}$ in Eq. 2. If it can be assumed that the clearances are not changing:

$$F = \frac{\int_0^\infty C\,dt_{or} D_{iv}}{\int_0^\infty C\,dt_{iv} D_{or}} \qquad \text{(Eq. 8)}$$

When F was calculated using Eq. 8, 69% of the values were >100%, suggesting that the assumption of constant clearance is incorrect. Only 19% of the F values calculated using Eq. 2 were >100%, and all of these values occurred with Product C (Table IV).

The existence of a "flip-flop" model (14), which precludes the use of rate constants from the terminal portion of the serum concentration–time curve in Eq. 2, would be supported by a correspondence between the elimination rate constant determined from intravenous data and the rate constant associated with the rising portion of the serum concentration–time curve observed on oral theophylline administration to the same animal. However, these parameters were not similar in this study.

The calculation of the cumulative fraction of drug absorbed at various times using Eq. 3 assumes that theophylline distributes rapidly throughout its volume of distribution and is eliminated by a first-order process. Since the data from some animals receiving intravenous theophylline were fitted by the two-compartment open model, the Loo–Riegelman method (15) may be appropriate for the estimation of the absorption rate constant in these cases. However, this method requires that the model parameters for both modes of administration be similar, which was not the case in the present study. Although intravenous data are not needed in the calculation of the f values using Eq. 3, translation of these parameters into cumulative percent of dose absorbed requires an estimate of F, as indicated in Eq. 4.

The time to peak (t_{max}) was calculated using Eq. 5, which includes the terms A and B. In the traditional equation used for calculating t_{max}, A and B are assumed to be equal. The use of these terms provides better estimates of t_{max} where a lag phase exists.

There was no significant difference in t_{max}, k_a, or F_1 among the products studied; however, product-to-product differences in the dose-normalized C_{max} [$C_{max(n)}$] and F_6 values were significant ($p < 0.005$ and < 0.05, respectively).

Concentration-Dependent Clearance Studies—*Multiple-Dose Rapid Intravenous Administration*—As shown in Table V, changes in k_d, V_d, and total body clearance for Rabbits K and M as the dose was increased were nonexistent or very slight. The fact that there was no change in total body clearance for Rabbits K and M and only a slight increase for Rabbit L suggests that theophylline demonstrates concentration-independent clearance in the rabbit at doses up to 30 mg/kg.

Multiple-Rate Infusion—The results of the multiple constant-rate intravenous infusion studies indicate that the dose–*AUC* relationship in the concentration range utilized here is linear; however, at higher concentrations, this finding may not be true. This phenomenon is shown in Table I, where increasing the infusion rate to three times the initial value resulted in steady-state serum concentrations which were increased 4- and 5.6-fold, respectively. This decrease in the total body clearance at higher infusion rates may be brought about by saturable metabolism or excretion processes, a pharmacodynamic effect of the drug resulting in reduced clearance, or a change in the function of eliminating organs with time. The possibility of time-dependent elimination kinetics may be investigated by performing the experiment in order of descending infusion rates. Although no evidence for capacity-limited elimination of theophylline was observed in another study of four rabbits receiving the drug as an intravenous bolus (16), the dose administered was only 10 mg/kg.

Dose-dependent kinetics of this drug in children were demonstrated by a reduction in clearance at increasing serum theophylline concentrations during infusion at differing rates (17). These results were interpreted in terms of Michaelis–Menten kinetics and suggested the possibility of at least one saturable elimination process among the multiple parallel pathways involved. The evidence for capacity-limited pharmacokinetics of theophylline in humans recently was reviewed (18), and the problems associated with dose-dependent elimination in theophylline bioavailability studies in humans also was addressed (19).

CONCLUSIONS

On the basis of the results of this study, it appears that the variability in the elimination kinetics of theophylline in the rabbit following intravenous administration was relatively small compared to that in humans, as evidenced by the variation in total body clearance and volume of distribution. Moreover, there were significant differences in the absolute bioavailability of the products investigated in the rabbit. Further studies in humans are needed to determine the clinical significance of such differences. In addition, theophylline did not exhibit concentration-dependent kinetics in the rabbit in the serum concentration range studied; however, at higher serum levels, total body clearance decreased substantially.

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NOTES

Effect of Capsule Size on Permeability of Gelatin–Acacia Microcapsules toward Sodium Chloride

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Abstract □ The effect of capsule size on the permeability of gelatin–acacia microcapsules toward sodium chloride was investigated. Gelatin–acacia microcapsules containing olive oil were prepared by phase separation. The encapsulated olive oil was extracted with acetone and the acetone-loaded microcapsules dispersed in acetone were fractionated by a series of mesh screens. The core material of acetone then was replaced by water. The permeability of each capsule fraction toward sodium chloride was estimated from the change in electrical conductance with time of the mixture of microcapsule suspension and sodium chloride solution. The permeability decreased with decreasing capsule size. Structured water in and around the capsule wall may be the cause of the observed size effect.

Keyphrases □ Gelatin–acacia microcapsules—prepared by phase separation, capsule-size effect on permeability toward sodium chloride □ Permeability—gelatin–acacia microcapsules, evaluated for capsule-size effect □ Capsule size—effect on permeability of gelatin–acacia microcapsules □ Microcapsules, gelatin–acacia—capsule-size effect on permeability toward sodium chloride

Relatively few papers (1–4) have dealt with the permeability characteristics of microcapsules, and many uncertainties remain (5) in spite of their importance in the sustained release of encapsulated drugs and chemicals and the application of microcapsules in enzyme technology and therapy and in the removal of waste products by polymer-coated charcoal. Among the various permeability characteristics of microcapsules, the effect of capsule size seems to be the most ignored; only one paper (6) described the size effect on the permeability of ethylcellulose microcapsules toward electrolytes. This situation prompted the present study to see if capsule size affects the permeability of microcapsules made of polymers other than ethylcellulose. The observed size effect on the permeability of gelatin–acacia microcapsules toward sodium chloride is reported here.

EXPERIMENTAL

Preparation of Microcapsules—The gelatin–acacia microcapsules were prepared as described earlier (7). Gelatin and acacia solutions were made by dissolving separately 5 g each of gelatin[1] (pI 5.0) and acacia[2] in 100 ml of distilled water. These solutions were allowed to hydrate for 10 min at room temperature and then for 30 min at ~47°. Coacervation was induced at 47°.

Fifty milliliters of olive oil was added in small portions to the gelatin solution with gentle stirring. The stirring rate then was increased for 15 sec. The acacia solution then was added in portions with moderate stirring to the newly formed emulsion. With this procedure, a favorable degree of emulsification and spherical mononuclear microcapsules were obtained. The system was adjusted to pH 3.5 by dropwise addition of 10% acetic acid after the addition of the acacia solution.

Coacervation was brought about by adding 170 ml of distilled water prewarmed at 47° in 3-ml portions during 20 min. The temperature was decreased to 5° at a rate of 0.3°/min. This cooling rate was found to be very important in obtaining spherical microcapsules. When the temperature reached 5°, a dilute formaldehyde solution was added to the microcapsule dispersion to make the final aldehyde concentration ~6 m*M*, and the pH was raised to 8.5 by the addition of 10% NaOH. After a 2-hr reaction time, the insolubilized microcapsules were separated by centrifugation in a low field of no higher than $100 \times g$ to avoid possible breakdown of the capsules.

Fractionation of Microcapsules—A wet mass of the microcapsules (~10 g) was transferred into 1000 ml of acetone to extract the encapsulated olive oil. The suspension was stirred vigorously by a magnetic stirrer to prevent capsule aggregation. Ten minutes later, the microcapsules were filtered and transferred into 200 ml of acetone. After this procedure was repeated four times, the acetone-loaded gelatin–acacia microcapsules dispersed in acetone were obtained.

The acetone-loaded microcapsules were fractionated in acetone by a series of mesh screens. Each capsule fraction of a given size was filtered through a coarse filter paper. The separated microcapsules of each fraction were transferred into a large volume of distilled water with vigorous stirring. In this way, acetone was replaced by water. The procedure was not repeated more than twice, because the water-containing gelatin–acacia microcapsules thus obtained were sticky to the surface of the filter paper.

Determination of Microcapsule Size—To determine the size of the microcapsules, a sample of each capsule fraction was placed on a hemocytometer, and the microcapsules were photographed under an optical microscope. The photographed film was projected on a large section of paper, and 500 enlarged capsule images were measured to the nearest 0.8 μm. The scale in the hemocytometer was used for calibration. Finally,

[1] Nippi Co., Tokyo, Japan.
[2] Gum acacia JP, Kokusan Chemicals, Tokyo, Japan.

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