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STABILITY AND BIOAVAILABILITY OF FIVE BRANDS OF AMPICILLIN SUSPENSIONS FOLLOWING RECONSTITUTION

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ABSTRACT

The stability of the active ingredient of four generically equivalent brands of ampicillin for oral suspension were studied at three controlled conditions 5,25 and 40°C. All ampicillin suspensions tested, except one brand, did not meet the official compendial stability requirements when stored at the recommended conditions. However the different brands studied were not completely equivalent with respect to stability. In vitro release of ampicillin from 5 brands of ampicillin suspension was

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studied by using dialysis method. Slight differences in dissolution profiles among the studied brands were observed. The bioavailability of 5 brands of ampicillin suspensions was examined in 5 subjects using cross-over experimental design. Based upon the urinary excretion method, the extent and rate of ampicillin bioavailability were determined. Statistically significant differences were found between the brands examined. An insignificant intersubject variation was found between the volunteers participated in the present study.

INTRODUCTION

Recently, considerable attention has been directed towards providing data which would permit a more rational basis for the selection of quality products at reasonable cost among so-called generically equivalent brands of drug dosage forms (1). However, most of the data have centered around the parameter of bioavailability as an indicator of equivalency. For oral liquid dosage forms which require reconstitution prior to dispensing, comparative chemical stability profiles following reconstitution might serve as an even better indicator of brand equivalency or lack of it, since bioavailability problems for liquid dosage forms generally, and solution dosage forms especially, tend to be less prevalent than for tablets and capsules (2). However some reports indicated unsatisfactory bioavailability of drugs from

suspensions (3-8). These findings suggest the possibility of drug adjuvant interactions in suspension formulations that could affect drug dissolution and absorption.

Consequently, the present study was undertaken to determine the dissolution and bioavailability of ampicillin, as a model drug, from different suspension brands. The study also aimed to investigate to what extent the stability-time profiles of the active ingrediant vary among these brands after reconstitution.

Ampicillin was categorized as a drug with "moderate risk potential" for bioavailability failures (9). Furthermore, the statment by the Drug Bioequivalence Panel of the Office of Technology Assessment (10) included ampicillin in the list of 24 drugs that exhibit differences in bioavailability between chemically equivalent products. Various workers have investigated the bioavailability of ampicillin from capsules produced by different manufacturers (11-14). However the results of these studies were conflicting.

Ampicillin is susceptible to B-lactam ring hydrolysis. Hau and Poole (15) investigated the Kinetics of degradation of ampicillin in several buffer systems and at several temperatures. Larsen and Pilbrant (16) studied the stability of ampicillin in aqueous suspension and found that an aqueous suspension of ampicillin

trihydrate (67 mg/ml) was stable for 18 months at + 5°. Jaffe et al. (17) studied the stability of five generically equivalent brands of ampicillin for oral suspension after reconstitution. The results showed that all the ampicillin products tested were stable when stored at the conditions recommended by the manufacturers.

EXPERIMENTAL

Materials

Citric acid, anhydrous disodium hydrogen phosphate and copper sulphate used were of analytical grade. Pure anhydrous ampicillin B.P., supplied by Alexandria Company for Pharmaceuticals and Chemical Industries, Alexandria, Egypt. Five brands of ampicillin suspensions were chosen, 2 locally made (D and E); 2 brand locally made under licence from an international firm (B and C) and one brand was imported (A).

Stability studies

Three samples of each of the commerical products were studied in their original containers at three different temperatures for 10 days. These temperatures were: (1) refrigeration (5±1°C); (2) room temperature (25±2°C) and (3) elevated temperature (40±2°C) which were maintained by means of an incubator. Each sample was assayed to determine the concentration of active ingredient present immediately following reconstitution. Additional

assays for each temperature condition were performed at specific time (day) intervals to permit observations at certain significant times, particularly in reference to the manufacturers recommendations for storage and expiration data.

Method of analysis

The amount of ampicillin in the studied samples was determined chemically, according to Angelucci and Baldiere (18). Three standard concentrations of ampicillin (20,30 and 40 ug/ml) were assayed side by side with the samples to overcome the effect of any fluctuation in the condition of the assay.

Dissolution dialysis method

Five ml of suspension plus 5 ml of 0.1N HCl were transferred into a dialysis bag (15 cm in length) made from spectrapor membrane tubing (dry cylinder diameter 16 mm, dry thickness 0.0008 inch, Fisher Scientific, PA, U.S.A). The dialysis bag was attached to the shaft of the USP dissolution apparatus rotating at 50 rpm. The contents were dialyzed against 900 ml of 0.1N HCl maintained at 37°C and at specific time intervals samples from the outside solutions were automatically withdrawn, properly diluted and analyzed chemically for drug contents.

Protocol for urine collection

Five healthy normal adult volunteers, four femals, and one male, participated in the present study. Their average age was 30.6 years (range 20-42 years) and their average weight was 68.8 kg (range 62-85 kg). The subjects had no previous history of allergic reaction to penicillins. All subjects were instructed not to take drugs (other than the required doses of ampicillin) one week prior and during each study. The volunteers fasted over night before each treatment and no food or liquid was taken until 4 hr after dosing. On the morning of a treatment, and at zero time the bladder was emptied and sample of blank urine was kept. Each subject ingested 10 ml of the suspension equivalent to 500 mg of ampicillin with 250 ml of water on an empty stomach. The urine was collected quantitatively after 1,2,3,4,6,8 and 12 hours. After each urine collection, the volunteers drank 200 ml water to effect diuresis and to keep sufficient urinary output. Aliquots of the collected urine samples were immediately deep frozen until assayed within 24 hr. The collected urine samples were analyzed chemically according to the procedure proposed by Angelucci and Baldieri (18).

RESULTS AND DISCUSSION

Stability study

The mean concentration of ampicillin in each of three reconstitued suspensions is shown as a function of

time for each of the storage condition temperatures (5, 25 and 40°C) in figure 1. The linearity shown in the figure 1 ($r > 0.9$) indicates a constant degradation rate-independent of concentration-indicative of a pseudo zero order degradation process. Zero order rate constants were calculated and are shown in Table 1. The rate constants indicated that brand E was the most stable at the three temperatures. At 5 and 25°C brand C was the least stable one. Figure 1 indicates that diferences in stability occured among the brands when stored at the same temperature.

Analysis immediately following reconstitution revealed that all studied ampicillin oral suspensions did not meet the USP potency requirement of not less than 90% and not more than 120% of label claim. The main percent of label claim of brands B,C,D and E were 140,129,135 and 87% respectively.

On all labels of the four brands of ampicillin suspension, which were employed in this study, the manufacturer recommends that the product, after reconstitution, is stable for one week when kept at room temperature and two weeks when refrigerated.

In the present study, none of the products maintained the USP required potency (225-300 mg/5 ml) for more than two days at the elevated temperature 40°C (Fig. 1).

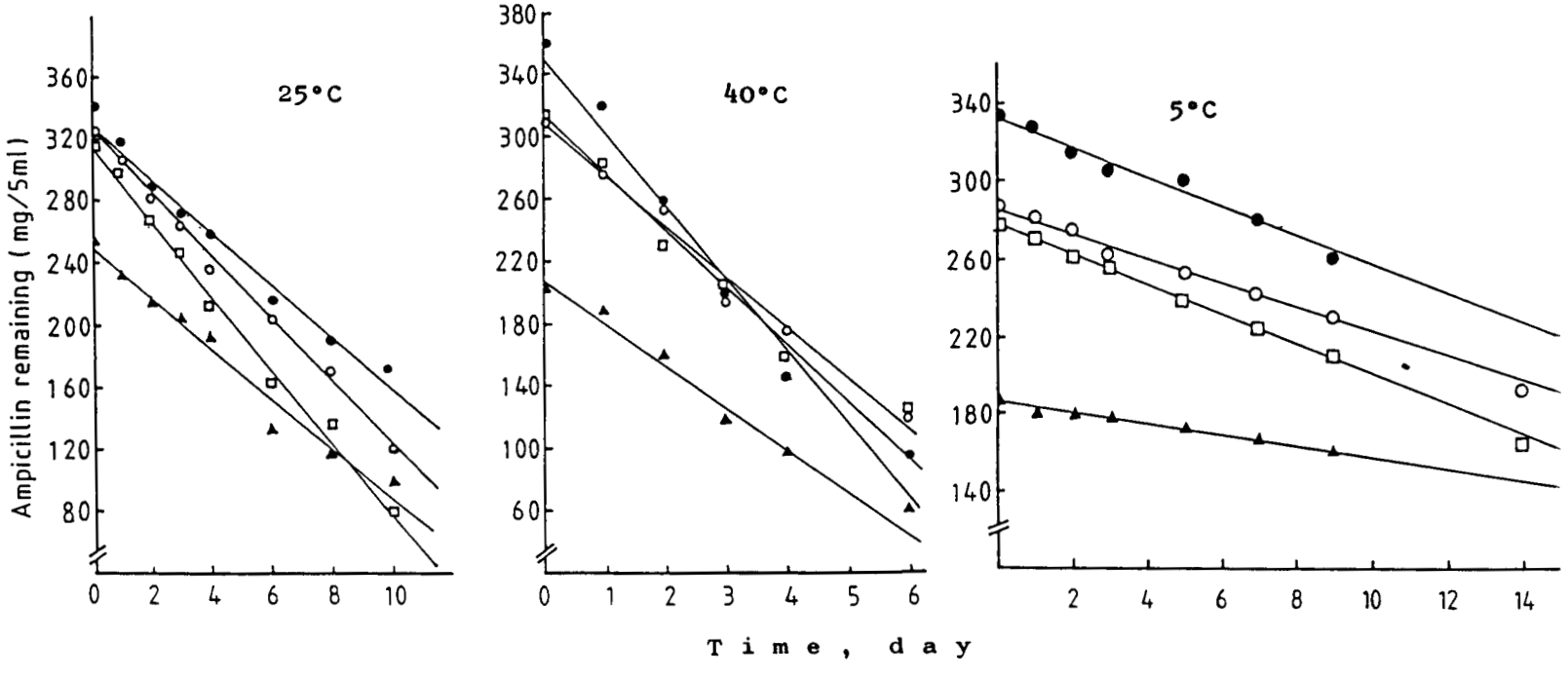

FIGURE 1

Degradation of commercial ampicillin oral suspensions stored at refrigerated temperature (5°C), room temperature (25°C) and elevated temperature (40°C). Brand B (●), brand C (○), brand D (□), and brand E (▲).

TABLE 1

Calculated rate constants K, (mg/5ml)/day for ampicillin oral suspensions at various storage temperatures.

Brand	Temperature		
	5°	25°	40°
B	7.757	16.617	47.27
C	8.088	23.81	33.328
D	6.578	20.72	32.54
E	2.815	15.9	25.57

At room temperature 25°C, all brands showed decomposition of more than 10%, the maximum limit allowed by USP, after storage for 7 days. Brands B,C,D and E maintained the USP required potency (225-300 mg/5 ml for 6,4,5 and 2 days respectively on storage at 25°C (Fig. 1). The trihydrate form of ampicillin (brand B) exhibited greater stability at room temperature than anhydrous forms (brands C,D and E). This is probably due to the higher solubility of the anhydrous form of ampicillin (3.9×10^{-2} mole/L at 20°C) than the trihydrate form(1.7×10^{-2} mole/L at 20°C) (16). The solubility of the anhydrous

form decreases with increasing the temperature while that of the trihydrate increases (19). This may be responsible for the higher degradation rate constant of brand B (trihydrate) at 40°C (Table 1).

When refrigerated brands B,C and D maintained the required potency for 14,7 and 9 days respectively(Fig. 1). It is worthy to note that brand B maintained the USP potency (225-300 mg/5 ml) when stored at 5° and 25°C for 14 and 7 days respectively while brand E did not maintain this potency although its decomposition rate constants at these two temperatures are lower (2.8,15.9 mg/5 ml.day^{-1}) than that of brand B (7.8,16.6 mg/5 ml.day^{-1}). This may be attributed to the higher intiatial ampicillin potency of brand B 350 mg/5 ml than that of brand E (216 mg/5ml).

According to above data it could be concluded that all ampicillin suspension tested, except brand B, did not meet the official compendial stability requirements when stored at the recommended conditions. However, the different studied brands were not completely equivalent with respect to stability. This may be attributed to different stabilizing agent, suspending agents, bufferes and sugars used in the formulation of ampicillin suspension. Also, the present stability study suggest that storage time of ampicillin suspension should be 3 days at room temperature or 7 days under refrigeration.

TABLE 2

Apparent release rate parameters of various brands of ampicillin suspensions.

Brand	K	K_f (min^{-1})	$t_{1/2}$ (min)
B	0.0560	0.0057	121.15
C	0.0767	0.0078	89.42
D	0.0528	0.00533	130.0
E	0.0524	0.0053	131.0

Dissolution dialysis

Table 2 demonstrates the apparent dialytic rate constant (K), apparent first order rate constant (K_f) and half lives ($t_{1/2}$) for the studied brands. The calculations of the dialytic rate constants were based on the equation derived by Davis et al. (20). From Table 2, it is apparent that the brands B,D and E have approximately the same K_f values, while brand C has slightly higher value. The release rate of ampicillin from brand A was negligible. Very low amounts were dialysed when the sample was stirred at high speeds (150 rpm). Dialysis method indicates that ampicillin from reconstituted suspensions

(brand B,C,D and E) will be more available than that from brand A.

Bioavailability of five brands of ampicillin suspensions

The bioavailability of ampicillin from different suspension brands was determined using the urinary excretion method. To compare the relative bioavailability of five brands of ampicillin suspensions tested, five parameters describing the urinary excretion data were evaluated. These were a) The cumulative mg of ampicillin excreted after 8 hours, b) the urinary peak height(mg/hr), c) The time to reach that peak (hr), d) the urinary concentration after 2 hours following oral administration (mg/ml), and e) The elimination and absorption rate constants (hr^{-1}).

The cumulative mg ampicillin excreted following oral administration of 5 brands of ampicillin suspensions to 5 subjects are summarized in Table 3 and Figure 2. The Figure 2 showes significant differences between the studied brands in all subject. According to the amount excreted after 8 hr (Table 3), an order describing all brands may be drawn up as follows:

A > B > D > C > E.

The average mg excreted after 8 hr for all tested brands is 220.5 mg (range 130-283.8mg).The analysis of variance of these data showed significant differences between

TABLE 3

Average pharmacokinetic parameters per brand following oral administration of 5 brands of ampicillin suspension to 5 subjects.

Brand	Cumulative mg excreted after 8 hr	Urinary peak height (mg/hr)	Time of peaking (hr)	Urinary concentration after 2 hr (mg/ml)	K_e^b (hr^{-1})	K_a^c (hr^{-1})
A	283.8 (15.47)	70.9 (4.58)[a]	2.3 (0.2)	0.66 (0.24)	0.333 (0.066)	0.385 (0.047)
B	253.9 (11.51)	64.6 (3.45)	2.3 (0.2)	0.5 (0.13)	0.259 (0.039)	0.69 (0.24)
C	192.6 (16.33)	53.6 (4.44)	2.5 (0.00)	0.25 (0.07)	0.31 (0.049)	0.368 (0.09)
D	242 (24.02)	56.7 (7.72)	2.5 (0.00)	0.62 (0.22)	0.359 (0.073)	0.297 (0.058)
E	130 (10.23)	34.5 (3.38)	2.1 (0.25)	0.14 (0.01)	0.411 (0.094)	0.595 (0.08)

a= Numbers in parentheses represent the standard error of the mean.

b= Elimination rate constant.

c= Absorption rate constant.

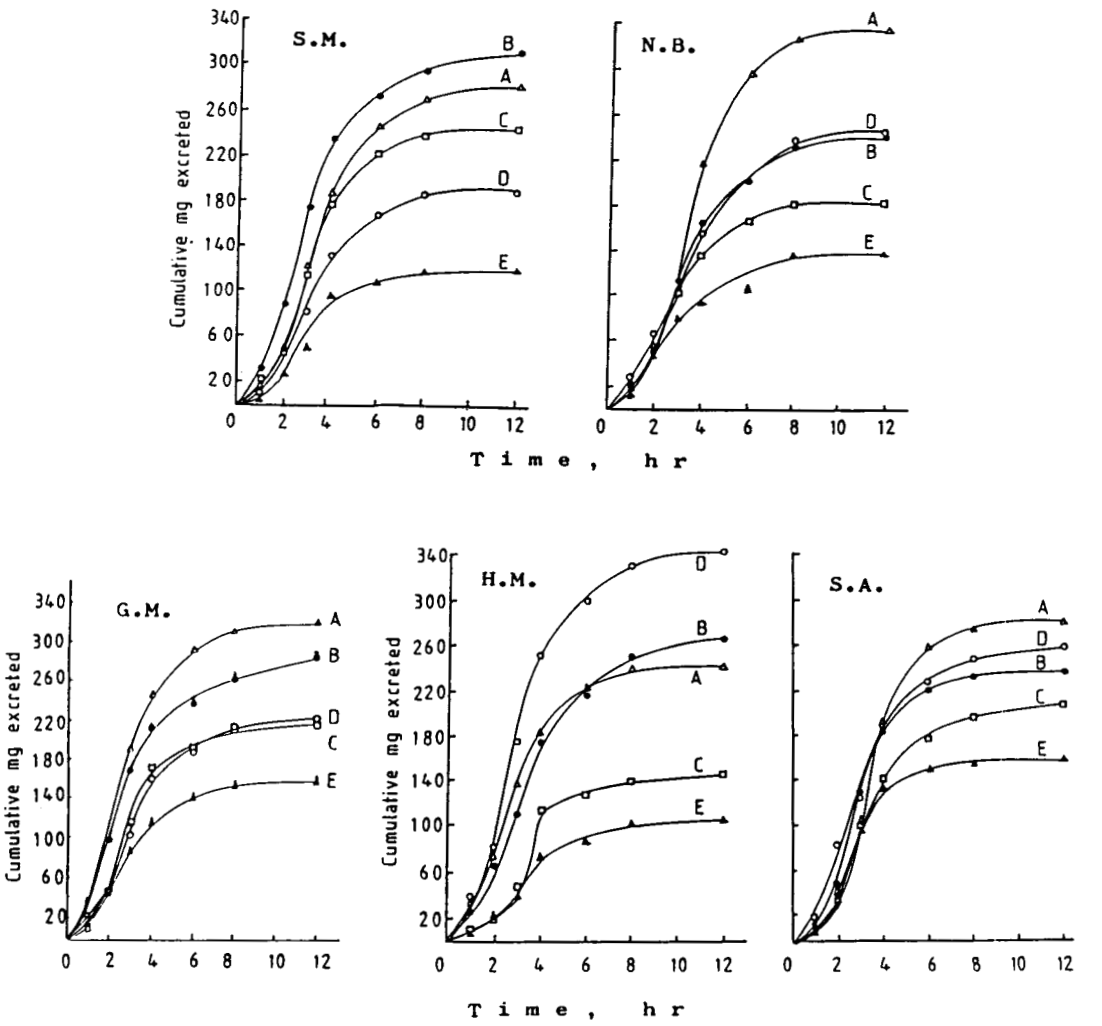

FIGURE 2

Cumulative mg ampicillin excreted following oral administration of 5 brands of ampicillin suspensions to 5 subjects.

brands ($p = 0.05$) and insignificant intersubject variation was observed (Table 4).

The average urinary excretion rate data of the various studied brands are summarized graphically in Fig. 3 and Table 3. It is clear that brand A produced the heighest peak (70.9 mg/hr), while brand E gave the lowest one (34.5 mg/hr). Also, the average value of the urinary peak height for all tested brands was 56.06 mg/hr. From Table 4, it is apparent that significant differences between brands were observed at the 5% level of confidence from the peak height data, while intersubject variation were still insignificant ($p = 0.05$). The average time to achieve the peak urinary concentration for all tested brands was 2.34 hr (Table 3). No statistically significant differences ($p > 0.05$) were observed between brands, while intersubject variation were significant (Table 4).

The average urinary concentration after 2 hours from administration of ampicillin suspension, for all tested brands was 0.43 mg/ml (Table 3). Tables 3 and 5 illustrate the data of the calculated elimination rate constants (Kel) per brand and per subject respectively. The mean Kel for all tested brands using five volunteers was 0.334 hr^{-1} (S.E.= 0.025) and the average half-life calculated was 2.12 hr (S.E.= 0.176). The data of the calculated absorption rate constants (Ka) are shown in Tables

TABLE 4

Analysis of variance for the cumulative mg excreted after 8 hrs (C. mg E.), peak height (mg/hr) and the time to reatch the peak (hr).

Source of variance	C.mg.E.			Peak height (mg/hr)			time of peaking, hr		
	df	M.S	Fratio	df	M.S	Fratio	df	M.S	Fratio
Between brands	4	17995.5	11.22[a](S.)	4	958.58	6.81 (S.)	4	0.14	1.56(N.S)
Between subjects	4	177.8	0.111[b](N.S)	4	55.34	0.393(N.S)	4	0.34	3.78(S.)
Error	16	1604.44		16	140.755		16	0.09	

a= Significant at p= 0.05

b= Insignificant at p= 0.05

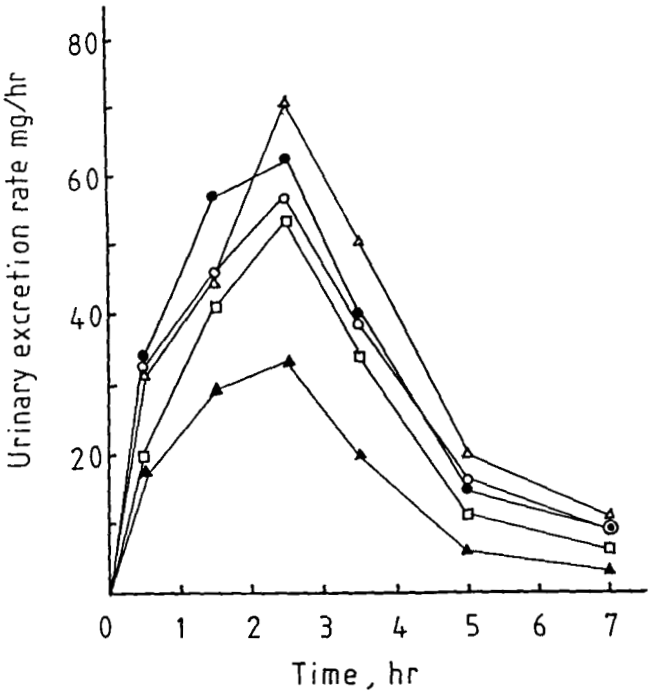

FIGURE 3

Average urinary excretion rates following oral administration of 5 brands of ampicillin suspensions to 5 subjects, brand A (▲), brand B (●), brand C (○), brand D (□) and brand E (▲).

3 and 5. The mean Ka was found to be 0.467 hr^{-1} (S.E.= 0.075) and the average half life calculated was 1.64 hr (S.E.= 0.244). Table 6 reveals that no statistically significant differences (p= 0.05) between brands and between subjects were observed from the urinary concentration after 2 hrs data, elimination rate constant data and absorption rate constants data.

TABLE 5

Average pharmacokinetic parameters per subject following oral administration of 5 brands of ampicillin suspension to 5 subjects.

Subject	Age (years)	Weight (Kg)	Cumulative mg excreted after 8 hr	Urinary peak height (mg/hr)	Time of peaking (hr)	Urinary concentration after 2 hr (mg/ml)	K_e (hr^{-1})	K_a (hr^{-1})
G.M.	20	70	227.6 (27.02)	58.9 (6.83)	1.9 (0.25)	0.560 (0.26)	0.298 (0.038)	0.37 (0.053)
S.M.	25	65	221.0 (32.21)	57.5 (7.79)	2.5 (0.00)	0.43 (0.08)	0.33 (0.076)	0.389 (0.08)
N.B.	34	62	223.1 (31.7)	51.7 (8.65)	2.3 (0.2)	0.16 (0.02)	0.452 (0.089)	0.466 (0.094)
H.M.	32	85	211.3 (40.93)	53.4 (9.64)	2.5 (0.0)	0.65 (0.24)	0.33 (0.066)	0.474 (0.129)
S.A.	42	62	220.1 (20.79)	58.9 (5.42)	2.5 (0.0)	0.37 (0.106)	0.263 (0.025)	0.388 (0.046)

TABLE 6

Analysis of variance for the urinary concentration after 2 hours (U.C. after 2 hrs), elimination rate constants Kel (hr^{-1}) and absorption rate constants (Ka).

Source of variance	U.C. after 2 hrs (mg/ml)			Elimination rate constants (hr^{-1})			Absorption rate constant (hr^{-1})		
	df	M.S	Fratio	df	M.S	Fratio	df	M.S	Fratio
Between brands	4	0.2616	2.319[a](N.S)	4	0.0162	0.769(N.S)	4	0.1393	1.614(N.S)
Between subjects	4	0.1769	1.568 (N.S)	4	0.0252	1.196(N.S)	4	0.444	0.5145(N.S)
Error	16	0.1128		16	0.0207		16	0.0863	

a= Insignificant at p= 0.05

Comparison between brands

From Tables 4,6 it is apparent that statistical differences between the studied brands were significant only in cumulative mg of ampicillin excreted after 8 hr and urinary peak height. According to the extent and rate of bioavailability, an order describing all the brands, may be drawn up as follows: A > B > D > C > E (Table 3). It is clear that brand A and B have the highest rate and extent of absorption, while brand E shows the lowest bioavailability characteristics. The mean relative bioavailability of brand E/A, based on the cumulative mg excreted after 8 hr and urinary peak height, were found to be 45.8% and 48.6% respectively. The significance of differences between the two brands was tested using the student t-test. The differences resulting from cumulative mg excreted after 8 hr, urinary peak height and the urinary concentration after 2 hr data were statistically significant at $p = 0.01$. The differences resulting from absorption rate constant data was significant at $p = 0.1$ and insignificant at $p = 0.05$.

The observed differences, in the extent and rate of ampicillin bioavailability, between the various brands may be attributed to differences in formulation and manufacturing processes. Previous studies (21,22) indicated that the state of hydration of ampicillin may affect its bioavailability. The suspensions and capsule formula-

tions containing ampicillin anhydrate exhibited superior bioavailability to formulations of the trihydrate (21). Some investigators (23,24) found that capsules containing either form of ampicillin yield essentially identical bioavailability.

In the present study, brand A and B were the only trihydrate forms evaluated. As indicated in table 3 unexpectedly, brand A and B had heigher extent and rate of bioavailability than the other anhydrous forms (brand C,D and E).

Comparison between subjects

Previous studies (25) indicated that the absorption of ampicillin exhibits considerable intersubject variability, with absorption following oral administration ranging from 20 to 70% of the dose. An average of 44% of the dose of ampicillin was excreted after eight hours by the subjects in the present study as calculated from Table 5. This finding agrees with the data of Jusko and Lewis (26) who reported that an average of 32% (ranging from 21 to 46%) of the dose of ampicillin was absorbed from the GI tract by the eight fasting subjects. From Tables 4 and 6, it is apparent that the differences between the subjects in the present study were insignificant. In contrast, a previous study (11) showed significant intersubject variation ranging from 19.2 to 42% for the cumulative percent excreted after 8 hours from oral administration of ampici-

llin capsules. Swahn (27) also found that the absorption of ^{35}S-ampicillin by six healthy subjects varied from 25 to 67%, as calculated from the excretion of radioactivity in urine.

It is well known that differences in age, weight, health conditions and sex between volunteers, contribute significantly to intersubject variations in drug bioavailability (28). No age, weight or sex effect was observed in the present study Table 5.

It could be concluded from the previous results that the different studied brands of ampicillin suspension were not completely equivalent with respect to bioavailability and stability. Therefore evaluation of the various brands of ampicillin suspensions seems to be importance and is highly needed.

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