Comparative bioavailability of three brands of ampicillin

C. MacLeod, M.D., H. Rabin, M.D., F.R.C.P.[C], J. Ruedy, M.D., C.M., F.R.C.P.[C], M. Caron, B.SC.PHARM., D. Zarowny, M.SC., M.D. and R. O. Davies, M.D., PH.D., *Montreal*

Summary: The relative biological availability (bioavailability) of three brands of ampicillin trihydrate capsules marketed in Canada was studied by means of a crossover experimental design and was evaluated by analysis of variance. After administration of a single oral dose (500 mg.) to volunteer subjects, the mean peak ampicillin serum concentration and the area under the time ampicillin serum concentration curve (AUC) were higher for one of the products than for the other two. A second study utilizing the same experimental design revealed no differences in these parameters when different production lots of the more available product were compared. Product bioavailability was not related to the *in vitro* dissolution time of the capsules. The experimental design and methods of statistical analysis are discussed.

Résumé: ***Comparaison de la disponibilité biologique de trois marques d'ampicilline***

On a évalué, au cours d'une étude croisée, la disponibilité biologique de trois marques de gélules de trihydrate d'ampicilline commercialisées au Canada. Les résultats ont été évalués par analyse de variance. Consécutivement à l'administration d'une seule dose orale de 500 mg à des volontaires, les pics moyens de la concentration sérique d'ampicilline ainsi que l'aire sous la courbe temps/concentration sérique d'ampicilline (ASC) ont été plus élevés après l'administration d'un produit que des deux autres. On a procédé à une deuxième étude selon le même schéma expérimental; or, on n'a constaté aucune différence dans ces paramètres lors de la comparaison des différents lots du produit dont le taux de disponibilité biologique est le plus élevé, celle-ci n'étant pas reliée à la vitesse de dissolution des gélules *in vitro*. Le protocole expérimental et les méthodes d'analyse statistique sont passés en revue.

From the Division of Clinical Pharmacology, Montreal General Hospital and the Department of Clinical Pharmacology, Ayerst Laboratories.

Reprint requests to: Dr. D. P. Zarowny, Assistant Director, Dept. of Clinical Pharmacology, Ayerst Laboratories, P.O. Box 6115, Montreal, Quebec.

Evaluation of the equivalency of products containing the same drug is of concern to the government, the pharmaceutical industry, the medical profession and the consumer. While chemical equivalence is an integral part of official standards, no official compendium specification for biological equivalence has been established. Determination of biological availability (bioavailability), broadly defined as the efficiency of drug absorption and distribution in relationship to drug elimination, is a recognized method for investigating equivalence. This technique most often involves the comparison of blood and/or urine concentrations following either single or repeat doses of the products which are being compared.

Schneller[1] and Wagner[2] have drawn attention to the number of drugs now considered to be subject to problems of bioavailability. On the other hand, at least one author suggests that inequivalence of bioavailability between formulations is "probably of very minor significance"[3] and cites studies in Canada with phenylbutazone,[4] sulfadiazine,[5] sulfisoxazole,[6] acetaminophen,[7] nitrofurantoin[8] and hydrochlorothiazide[9] to support this opinion. Recently, inequivalent bioavailability has been reported following oral administration of single doses of different formulations of oxytetracycline,[10] warfarin,[11] digoxin[12] and tetracycline.[13] While a crossover design was used in all of these investigations, the number of subjects studied and the statistical methods of analysis differed. The design employed in the following study for the comparison of the bioavailability of different products is a practical method and is evaluated by suitably powerful statistical analysis.

The object of the study was to compare the bioavailability of three ampicillin products presently marketed in Canada using a crossover experimental design. When the comparison revealed differences in availability between the products, a second study was designed to compare different production lots of one of the products.

Methods and materials

Products and brands studied: During study I, three brands of 250 mg. ampicillin trihydrate capsules, each from a different manufacturer, were compared: Products A1, B and C as listed in Table I. In the second study, product A1 and two additional production lots from the same manufacturer were compared: Products A2 and A3. Products A1, A2 and A3 were chosen from

nine lots manufactured in the first half of 1971 with the specification that they should be representative of the complete range of dissolution times of the nine lots. Products B and C were obtained by purchase from a retail outlet located in a large metropolitan area and to our knowledge were manufacturered during the same time interval as products A1, A2 and A3.

Capsule content and dissolution studies: The ampicillin content of individual capsules was determined by use of the microbiological assay method specified by the United States Food and Drug Administration[14] modified by replacing petri dishes with large agar plates containing 64 wells. The mean of individual determinations of the content of six capsules is expressed as ampicillin (anhydrous).

Dissolution times were determined for the products according to the general method described in the United States Pharmacopeia XVIII.[15] The time for 25%, 50%, 75% and 90% of the ampicillin content of a single capsule to dissolve in 900 ml. of 0.1 N HCl was measured. Each capsule was placed in a wire basket which rotated at 100 r.p.m. in a glass tank containing the acid solution. Aliquots of the solution were passed through a circuit containing a Hitachi Perkin Elmer model 139 spectrophotometer attached to a Sargent model SRL recorder and returned to the tank. The optical density of the solution was measured at 256 mμ. The dissolution time for each product is expressed as the mean of the results of five determinations.

Ampicillin serum concentration method: Serum samples were assayed in triplicate for ampicillin content independently by two laboratories using the method of Bennett *et al*:[16] one laboratory in the Clinical Pharmacology Division, Montreal General Hospital (Laboratory 1) and one at the Department of Microbiology, Ayerst Laboratories (Laboratory 2). The samples were identified to laboratory personnel by subject number and sampling time without reference to the identity of the products that had been administered. Standards were prepared with ampicillin trihydrate using fresh pooled human serum. The microorganism for all assays was *B.subtilis* ATCC 6633.

Human bioavailability method: This investigation was conducted in the Division of Clinical Pharmacology of the Montreal General Hospital. Study I was carried out in 18 healthy men between 20 and 40 years of age, weighing between 60 and 90 kg. Prior to the study a complete medical assessment was obtained which included a history, a physical examination, and routine biochemical and hematological tests and urinalysis. All volunteers denied a history of allergy to penicillin. Informed written consent was obtained. The subjects were divided into three equal groups by random assignment. The order of product administration is presented in Table II. Each subject received a single oral dose of 500 mg. of one of the three ampicillin preparations given as two 250 mg. capsules on each investigational day. One week elapsed between test days. The dose was administered to each subject with 250 ml. of water at approximately 8:30 a.m. All subjects fasted from midnight prior to and until two hours after drug administration. Blood samples were obtained by venipuncture before drug administration (time 0) and at one-half, one hour, two hours, three hours, four hours, and six hours following drug administration.

Blood was allowed to clot for one hour at room temperature, then was centrifuged and the serum drawn off using sterile Pasteur pipettes. The serum obtained was divided equally into two samples which were assayed independently by the two laboratories. All serum specimens for Laboratory 1 were refrigerated at 4°C. until the end of each experimental day at which time the assay was carried out. The serum specimens from the first study for Laboratory 2 were frozen overnight for assay the following day. During Study II, Laboratory 2 carried out the assays at the end of each experimental day without freezing the specimens.

Study II was carried out on 12 of the subjects from Study I and six other men. Additional blood samples were obtained at one and one-half hours and five hours

Table I
Ampicillin content* of 250 mg. ampicillin capsules from three manufacturers

Product code	Brand name	Lot no.	Ampicillin content mg./capsule	Percent of labelled dose
A-1	Penbritin	2054PD	251.3 ± 3.13	100.5
A-2	Penbritin	2717PG	240.3 ± 4.33	96.1
A-3	Penbritin	2422PF	247.5 ± 3.74	99.0
B	Ampen	800 PA	244.5 ± 2.93	97.8
C	Novo-ampicillin	104207	238.6 ± 4.84	95.4

*Measured as ampicillin trihydrate and expressed as ampicillin (anhydrous). Each value is the mean ± standard error of six determinations. All products manufactured during the first half of calendar year 1971.

Table II
Order of administration of the products on the investigational days

Study I				Study II			
Group and subject numbers	Investigational day 1	2	3	Group and subject numbers	Investigational day 1	2	3
Group I 4, 5, 10, 13, 14, 17	A-1	B	C	Group I 1, 4, 6, 21, 22, 23	A-3	A-2	A-1
Group II 1, 3, 11, 12, 15, 16	B	C	A-1	Group II 2, 8, 13, 14, 19, 24	A-2	A-1	A-3
Group III 2, 6, 7, 8, 9, 18	C	A-1	B	Group III 3, 7, 10, 11 18, 20	A-1	A-3	A-2

Table III
Dissolution time in minutes of 250 mg. ampicillin capsules from three manufacturers

Product	Dissolution time* T_{25}	T_{50}	T_{90}
A-1	3.2 ± .56	4.1 ± .42	6.4 ± .95
A-2	3.3 ± .88	4.3 ± .74	6.4 ± 1.08
A-3	3.1 ± .26	4.5 ± .36	7.1 ± .70
B	6.4 ± .54	8.9 ± .74	13.1 ± 1.80
C	2.5 ± .29	3.2 ± .30	5.2 ± .66

*Time in minutes for 25%, 50%, 90% of the ampicillin content of a capsule to dissolve. Each value is the mean ± standard deviation of five determinations.

after drug administration. In all other respects the experimental protocol for Study II was identical to that of Study I.

Analysis of results: Area under the time ampicillin serum concentration curve (AUC) from time 0 to six hours was calculated for each subject by the trapezoid rule. The serum concentration at each sampling time, the peak serum concentration independent of sampling time, and AUC were analyzed by an analysis of variance technique for crossover experiments.[17] This technique permitted the total experimental variation to be fractioned into one component that was due to intersubject (between) variation and another due to intrasubject (within) variation. Interaction between groups of subjects (grouped by virtue of the order of product administration) and days may be examined as a subcomponent of the intersubject variation leaving a residual representing intersubject experimental error. Subcomponents of the intrasubject variation may be identified as variation between products, variation between experimental days, interaction between products and days, leaving a residual representing intrasubject experimental error. It was therefore possible to test for differences between pairs of drugs relative to the within subject experimental error. Since the comparison of all possible drug pairs is nonorthogonal, a level of significance of P = 0.01 was chosen to test for differences between product pairs and P = 0.20 for tests of interaction. In addition, whenever significant differences between pairs of products were found, the difference was tested *a posteriori* by the Scheffé method[18] in order to preclude drawing conclusions in the presence of a type I error. The standard error about the mean peak serum concentration and AUC was calculated by use of the formula

$$S_{\bar{x}} = \frac{S}{\sqrt{n}}$$

where S is the square root of the mean square of the error within subjects taken from the analysis of variance and n is the number of observations on which each of the means is based.[19]

Table IV
Mean ± standard error of the area under the time ampicillin serum concentration curve (AUC) from Study I*

Product	Mean AUC & % of A-1
A-1	11.71 ± 0.44† (100)
B	9.14 ± 0.44 (78)
C	8.40 ± 0.44 (72)

*Statistical differences presented in Table V.
†Refer to methods and materials section for calculation of the standard error.

Table V
Analysis of variance of the areas under the time ampicillin serum concentration curve for Study I

Sources of variation	Degrees of freedom	Mean squares	F ratio	"P" value
Between subjects	17	9.7006	2.7515	<0.01
Interaction between products and days	2	0.2463	0.0224	>0.20
Error	15	10.9612		
Within subjects	36	6.6725	1.8930	<0.05
Products	2	54.5620	15.4760	<0.001
A-1 vs B	1	59.6782	16.9275	<0.001
A-1 vs C	1	99.0476	28.0946	<0.001
B vs C	1	4.9600	1.4068	>0.20
Days	2	9.6159	2.7275	<0.10
Interaction between products and days	2	3.0448	0.8640	>0.20
Error	30	3.5255		
TOTAL	53			

Results

The experimental methods were followed as described with two exceptions. One volunteer could not attend the second day of the first study. He completed the third day as scheduled and returned one week later to complete the trial of the drug he would have received on the second day. One subject became dizzy during the venipuncture for the one-hour blood sample on the second day of Study II. His blood pressure was 70/40 mm. Hg. He was considered to have suffered vasovagal syncope and was allowed to rest and break the fast. He recovered rapidly and completed the study without further difficulty.

All ampicillin products conformed to British Pharma-

Table VI
Levels of significance from the analysis of variance of the mean ampicillin serum concentration at each sampling time for Study I

Sources of variation	"P" values at sampling hours					
	½	1	2	3	4	6
Between subjects	n.s.*	<0.001	<0.001	<0.001	<0.001	<0.001
Interaction between products and days	n.s.	n.s.	n.s.	<0.20	<0.20	<0.10
Within subjects	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Products	n.s.	<0.01	<0.05	<0.001	=0.05	n.s.
A-1 vs B	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.
A-1 vs C	n.s.	<0.01	<0.01	<0.001	n.s.	n.s.
B vs C	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Days	n.s.	n.s.	n.s.	n.s.	<0.05	<0.001
Interaction between products and days	n.s.	<0.20	<0.20	n.s.	n.s.	n.s.

* n.s. = not significant at P >0.20 for tests of interaction and P >0.01 for product pair comparisons

Table VII

Mean ± standard error of the peak ampicillin serum concentration measured in both laboratories in Study I*

	Mean peak ampicillin serum concentration (μg./ml.)	
Product	Laboratory 1	Laboratory 2
A-1	4.21 ± 0.19†	4.04 ± 0.24
B	3.13 ± 0.19	3.38 ± 0.24
C	2.87 ± 0.19	2.95 ± 0.24

*Statistical differences presented in Table VIII.
†Refer to methods and materials section for calculation of the standard error.

Table VIII

Levels of significance from the analysis of variance of the mean peak ampicillin serum concentration measured in both laboratories in Study I

Sources of variation	Laboratory 1	Laboratory 2
Between subjects	<0.01	<0.01
Interaction	>0.20	>0.20
Within subjects	<0.10	<0.05
Products	<0.001	<0.05
A-1 vs B	<0.001	<0.10
A-1 vs C	<0.001	<0.01
B vs C	>0.20	>0.20
Days	<0.20	<0.01
Interaction	>0.20	>0.20

copoeia specifications of 100 ±7.5% of labelled content (Table I). Dissolution time varied slightly, with 90% dissolution of product C in the shortest time (5.2 minutes) while product B required the longest (13.1 minutes) (Table III).

The results of the assays of ampicillin serum concentration in Studies I and II from both laboratories were similar. The coefficients of correlation between laboratories for each assay day ranged from 0.89 to 0.96. A small but consistent variation occurred between laboratories which could be explained in part by differences between standard curves. For clarity, the assay results from Laboratory 1 (Division of Clinical Pharmacology, Montreal General Hospital) are presented. The results from Laboratory 2 were evaluated independently and the conclusions were identical to those in this report with the exception of one of the comparisons of mean peak serum concentration as discussed in subsequent sections.

Study I: After administration of product A1, higher mean serum concentrations of ampicillin at all sampling times were observed in comparison with products B and C (Fig. 1). Product A1 was common to both studies and was used as the comparison product for relative bioavailability. Products B and C achieved only 78% and 72% respectively of the bioavailability of A1 as calculated from the area under the time ampicillin serum concentration curve (Table IV).

The results of the analysis of variance (Table V) show that the difference in AUC between product A1 compared with products B and C was statistically significant at P <0.001. The "P" values for the comparison of serum concentrations at each sampling hour are shown in Table VI. Products B and C did not differ from one

Table IX

Comparison of the differences between products that were measured by two laboratories in Study I

	Difference in peak ampicillin serum concentrations†	Critical value for the Scheffé test* P = 0.05	P = 0.01	Difference in area under the time ampicillin serum concentration curves†	Critical value for the Scheffé test* P = 0.05	P = 0.01
Laboratory 1						
A-1 vs B	19.84			46.35		
A-1 vs C	24.12	12.73	16.22	59.71	29.02	36.98
B vs C	4.67			13.36		
Laboratory 2						
A-1 vs B	11.75			42.53		
A-1 vs C	19.75	15.84	20.18	60.00	31.82	40.55
B vs C	8.00			17.46		

†Peak concentration and AUC values are the differences between the totals of the individual measurements from each of the 18 subjects following each product.
*The critical value by Scheffé's method is the smallest difference that must be measured in order to avoid a probability of type I error larger than 5% and 1% respectively.
The underlined difference values are significant.

Table X

Mean ± standard error of peak ampicillin serum concentration and mean ± standard error of area under the time ampicillin serum concentration curve (AUC) from Study II

Product	Mean peak serum concentration μg./ml. and % of A-1	"P" value	Mean AUC & % of A-1	"P" value
A-1	4.56 ± 0.17* (100)	n.s.†	12.90 ± 0.40 (100)	n.s.
A-2	4.25 ± 0.17 (93)	n.s.	13.28 ± 0.40 (103)	n.s.
A-3	4.41 ± 0.17 (97)	n.s.	13.36 ± 0.40 (104)	n.s.

*Refer to methods and materials section for calculation of the standard error.
†n.s. = not significantly different (P >0.20 for all possible product comparisons).

another in serum concentration at each sampling hour or AUC.

In both Study I and Study II statistical analysis of the results from both laboratories led to identical conclusions about the products except for the mean peak ampicillin serum concentration. When the results from both laboratories are compared (Table VII) the peak serum concentration of ampicillin after products A1, B and C differs only slightly. The administration of product A1 was associated with a peak serum concentration of ampicillin (4.21, 4.04 μg./ml.) that was significantly higher than the peak concentration following products B and C according to analysis of the results from Laboratory 1 (Table VIII). However, the difference between products A1 and B was not significant according to analysis of the results from Laboratory 2. The peak concentration after product A1 remained significantly higher than that after product C in spite of the larger experimental variation associated with Laboratory 2 and indicated in Table VII by the larger standard error of the mean.

The critical values for the Scheffé test at 5% and 1% levels of significance (Table IX) indicate that the significant differences demonstrated above were not compromised by a high risk of type I error, i.e. finding a difference between products when no real difference existed.

Study II: Following administration of products A1, A2 and A3, the ampicillin serum concentration (Fig. 2), the peak serum concentration and the AUC (Table X) were similar. Analysis of variance revealed no significant differences between products for these parameters.

In summary, while capsule content was uniform and within British Pharmacopoeia specifications, a difference in bioavailability was observed between some of the products. No relationship was observed between dissolution time and bioavailability in either study.

Factors influencing the experimental design: In both studies there was a large variation in sampling time serum concentration, peak serum concentration and AUC among the 18 volunteers ($P < 0.01$, Tables V, VI, VIII). This was an expected source of variation and was

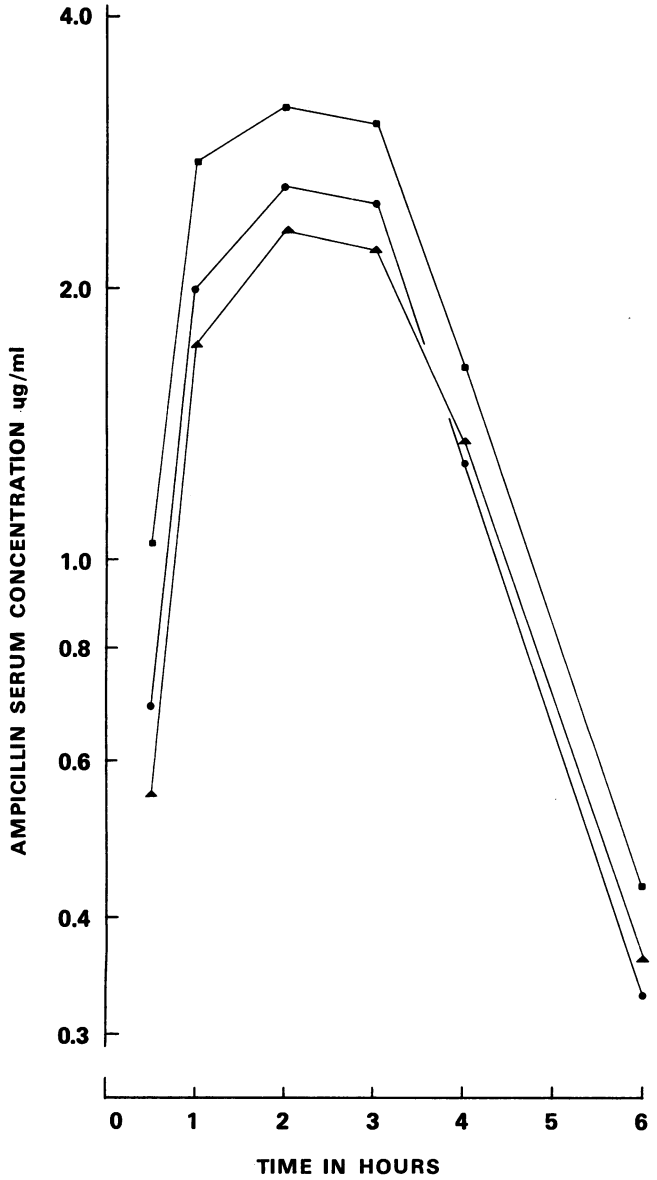

FIG. 1—Mean serum concentrations of ampicillin in 18 subjects after 500 mg. p.o. in Study I.

■ Product A-1 ● Product B ▲ Product C

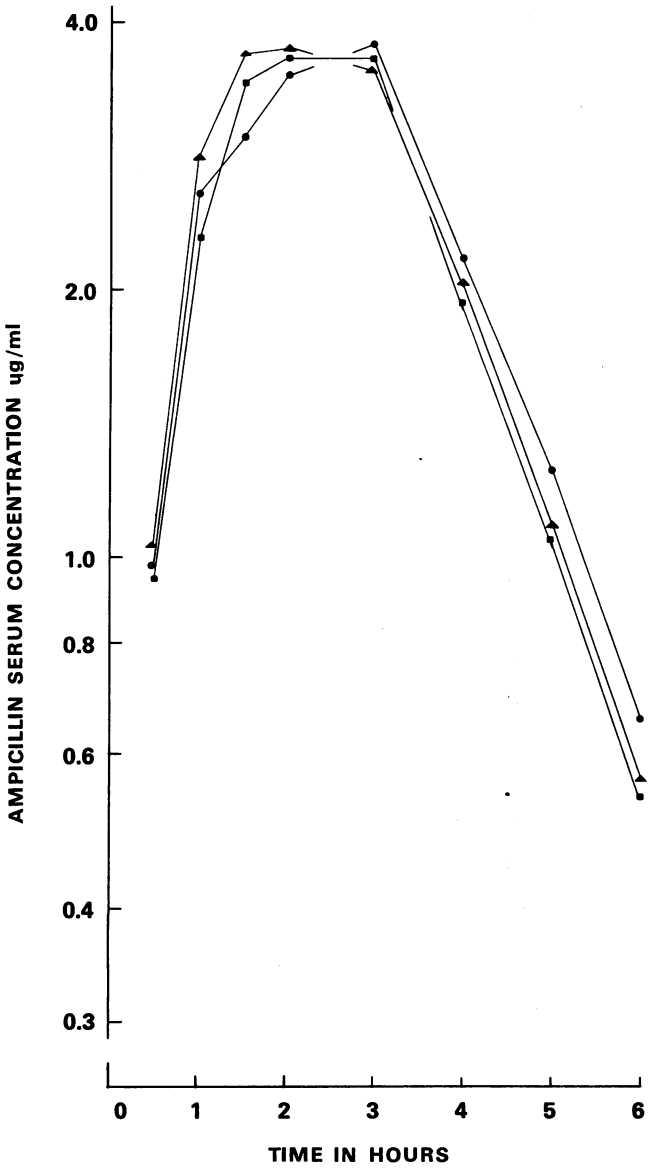

FIG. 2—Mean serum concentrations of ampicillin in 18 subjects after 500 mg. p.o. in Study II.

■ Product A-1 ● Product A-2 ▲ Product A-3

the reason for the choice of a crossover design experiment utilizing six subjects per drug per day. The analysis of variance permitted separation of product-to-product differences from person-to-person variation.

A significant source of variation was found in the ampicillin serum concentration on the three study days. This day-to-day variation occurred in Study I at four and six hours (Table VI) and in Study II in the peak serum concentration ($P < 0.01$) and the two-hour sample ($P < 0.05$). Day-to-day variation occurred repeatedly during Study I in the results from Laboratory 2. These day-to-day variations in both laboratories probably reflected uncontrolled factors in both the clinical and the laboratory portions of the experiment. All experimental conditions were constant until the serum specimens were divided between the two laboratories. At this point in Study I a difference in handling of the specimens and their standard solutions was encountered (freezing overnight prior to assay). It is possible that this deviation from parallel procedure affected Laboratory 2 results in the form of day variation. This more frequent day-to-day variation contributed to the larger total variation seen in the analysis of variance of Laboratory 2 results as compared to that of Laboratory 1 and may have accounted for the only disagreement in results between the laboratories, i.e. the failure to demonstrate a significant difference between peak serum concentration following products A1 and B. This demonstrates the need for assessing day-to-day effects in addition to the other variables in crossover studies.

Analysis of variance did not show any product and day interaction for peak serum concentration and AUC so that evaluation of differences between products in these characteristics could be made in the face of significant day-to-day differences.

The sensitivity of the present experimental design was judged mathematically and it was found that differences in AUC between products of 12 to 15% would have been significant ($P = 0.01$). This was calculated by use of the formula

$$F \text{ ratio} = \frac{d^2}{\text{degrees of freedom within subjects} \times \text{error within}}$$

and solving for d^2 by substituting the F ratio for 1 and 30 degrees of freedom at $P = 0.01$, the degrees of freedom within subjects and the mean square of the error within; "d" becomes the difference in total AUC significant at $P = 0.01$. In Study I a difference in total AUC of 30.98 resulted. This was 14.7% of product A1 AUC for Study I. The calculated value for AUC in Study II was 27.97 or 12% of product A1, AUC. Therefore, between-product differences in total AUC of this magnitude for the respective studies would have been significant at $P = 0.01$.

Discussion

The results of the present study indicate that a crossover design experiment using an adequate number of subjects is a sensitive method for comparing the bioavailability of different formulations. In spite of a small difference (± 7.5%) in capsule content of ampicillin, a significant difference in peak serum concentration and area under the time ampicillin serum concentration curve was found between the products tested. Moreover, the difference was significant at $P < 0.001$ when the products achieved 72% and 78% of the AUC of the reference product. Indeed, significant differences would have been demonstrated had the products achieved as much as 88% of the AUC of the reference product. Studies sponsored by the Food and Drug Directorate (Canada)[4-8] using crossover experimental design have frequently failed to find differences of this magnitude between products "statistically significant". Presumably this is the reason that they have "elected to consider satisfactory a bioavailability of 80% or more of a Reference Standard."[9] However, their studies were designed to compare up to 10 formulations in a single experiment utilizing one subject per drug per day. In this situation the experiment may not be sufficiently powerful to reveal differences between products in the presence of wide differences between individual subjects. Therefore, while providing useful screening data to detect gross difference between products, these reports cannot be considered as rigorous comparisons and may not answer the question of equivalency between any two products. The present design, using six subjects per drug per day, was employed in order to increase the power of the experiment. This was accomplished with an acceptable risk of making a type I error.

The results demonstrate that ampicillin capsules taken from lots produced by two manufacturers were similar to each other but both produced smaller peak serum concentration and AUC than those produced by a third manufacturer. Therefore ampicillin trihydrate may be added to the list of antibiotics for which inequivalency between formulations has been reported. As in other studies[5, 20] absence of correlation between *in vitro* dissolution times and *in vivo* bioavailability assay underlines the inadequacies of dissolution methodology for prediction of product equivalency. In addition, biological inequivalency was demonstrated between products which fell within the narrow range of capsule content allowed by the British Pharmacopoeia standard for chemical equivalence. Therefore these tests for content and dissolution do not assure equivalent product quality or reliability.

Conclusions

1. A carefully designed crossover experiment with appropriate statistical analysis is essential for a comparison of dosage forms in bioavailability investigations. In the studies reported here differences between products of ± 12 to 15% in area under the concentration curve would have been statistically significant.

2. In spite of a difference of less than 7.5% in capsule content of ampicillin, the bioavailability as measured by area under the time ampicillin serum concentration curve of the reference product, Penbritin® (100%), was greater than that of Ampen® (78%) and Novo-Ampicillin® (72%). In addition, the mean peak serum concentration of ampicillin was higher following Penbritin® (4.21 μg./ml.) than after Novo-Ampicillin® (2.87 μg./ml.).

3. There was no relationship between peak serum concentration or area under the time ampicillin serum concentration curve and dissolution time.

The authors express their appreciation to the volunteers who took part in this investigation and to the following for their assistance in laboratory determinations: Joyce Flood, Dr. C. Vézina, Dr. H. Baker, Mrs. Anne Sidorowicz and Dr. R. De

Angelis. Dr. Léon Tétreault provided invaluable comment regarding statistical design and analysis.

The raw data from both studies (serum concentrations and statistical analyses) are available as an appendix upon request.

References

1. SCHNELLER GH: Status report on drug bioavailability *Am J Hosp Pharm* 27: 485-488, 1970
2. WAGNER JG: *Biopharmaceutics and Relevant Pharmacokinetics,* First Edition, Hamilton, Illinois, Drug Intelligence Publications, 1971, Chapter 24
3. BOYD EM: The equivalence of drug brands. *Rx Bulletin* 2: 101-120A, 1971
4. VAN PETTEN GR, FENG H, WITHEY RJ, et al: The physiologic availability of solid dosage forms of phenylbutazone. Part I. *In vivo* physiologic availability and pharmacologic considerations. *J Clin Pharmacol* 11: 177-186, 1971
5. VAN PETTEN GR, BECKING GC, WITHEY RJ, et al: Studies on the physiological availability and metabolism of sulfonamides. I. Sulfadiazine *Ibid* pp 27-34
6. *Idem:* Studies on the physiological availability and metabolism of sulfonamides II. Sulfisoxazole. *Ibid* pp 35-41
7. MCGILVERAY IJ, MATTOK GL, FOOKS JR, et al: Acetaminophen II. A comparison of the physiological availabilities of different commercial dosage forms. *Can J Pharm Sci* 6: 38-42, 1971
8. MCGILVERAY IJ, MATTOK GL, HOSSIE RD: A study of bioavailabilities and dissolution rates of commercial tablets of nitrofurantoin. *J Pharm Pharmacol* 23 (suppl): 2465, 1971
9. Drug quality and price, *Rx Bulletin* 2: 75-80, 1971
10. BLAIR DC, BARNES RW, WILDNER EL, et al: Biological availability of oxytetracycline HCl capsules. *JAMA* 215: 251-254, 1971
11. WAGNER JG, WELLING PG, LEE KP, et al: *In vivo* and *In vitro* availability of commercial warfarin tablets. *J Pharm Sci* 60: 666-667, 1971
12. LINDENBAUM J, MELLOW MH, BLACKSTONE MO, et al: Variation in biologic availability of digoxin from four preparations. *N Engl J Med* 285: 1344-1347, 1971
13. BARR MH, GERBRACHT LM, LETCHER K, et al: Assessment of the biologic availability of tetracycline products in man. *Clin Pharmacol Ther* 13: 97-108, 1972
14. Amendment published in *Federal Register* 34: 9333, 1969
15. Dissolution. *U.S. Pharmacopeia XVIII,* p 934
16. BENNETT JV, BRODIE JL, BENNER EJ, et al: Simplified, accurate method for antibiotic assay of clinical specimens. *Appl Microbiol* 14: 170-177, 1966
17. LINDQUIST EF: *Design and Analysis of Experiments in Psychology and Education,* New York, N.Y., Houghton Mifflin Company, 1956, p 273
18. WINER BJ: Chapter 3 in *Statistical Principles in Experimental Design,* New York, N.Y., McGraw-Hill, 1962
19. EDWARDS AL: *Experimental Design in Psychological Research,* 3rd edition, Cambridge, Mass., Holt, Rinehart and Winston, Inc., 1968, p 130
20. WITHEY RJ, FENG H, COOK D, et al: The physiologic availability of solid dosage forms of phenylbutazone. Part II. Correlation of *in vivo* physiologic availability and *in vitro* dissolution parameters. *J Clin Pharmacol* 11: 187-196, 1971