Development of *In Vitro* Dissolution Tests Which Correlate Quantitatively with Dissolution Rate-Limited Drug Absorption in Man

By GERHARD LEVY*, JACK R. LEONARDS†, and JOSEPHINE A. PROCKNAL*

The development of a single *in vitro* dissolution rate test is described, which correlates quantitatively with the gastrointestinal absorption, in man, of a test drug (aspirin) from three markedly different types of dosage forms. The *in vitro* conditions that yield such multiple correlations may be expected to be relatively similar to dissolution conditions found *in vivo*. It is suggested that the inclusion of an increasing number of variables (different drugs and different types of dosage forms) in future efforts to develop *in vitro–in vivo* correlations will permit further refinement of the *in vitro* test. This could eventually lead to a relatively generalized test procedure suitable for development and control purposes, and for inclusion in official compendia as a test of physiologic availability.

THE DEVELOPMENT of *in vitro* dissolution rate tests capable of reflecting the absorption rate, in man, of drugs contained in various dosage forms is probably the most important task in biopharmaceutics today (1). The recently achieved total quantitative correlation between the gastrointestinal absorption and *in vitro* dissolution of aspirin administered in compressed tablets (1, 2) is an encouraging indication of the possibility that more broadly applicable *in vitro* methods may be developed. Efforts in this direction often yield additional dividends because pharmaceutical dosage forms can be used as biopharmaceutical probes to establish the role of various physiologic and physicochemical factors in drug absorption (3–5).

Efforts toward the development of more generalized *in vitro* dissolution rate tests can proceed along two logical pathways: the development of a single test which will correlate with *in vivo* absorption of several different drugs administered in one type of dosage form, or the development of a single *in vitro* procedure which will correlate with *in vivo* absorption of one drug given in different types of oral dosage forms. Both of these approaches are used in investigations now in progress (6); the present report deals with a study which resulted in the development of a single *in vitro* dissolution rate test for three different types of dosage forms of one drug (aspirin). These dosage forms differ markedly in drug absorption rate as well as in the principal mechanism involved in the release of drug to the dissolution medium. Consequently, modifications in composition of the medium and in agitation intensity have different effects on the drug release rate from each of these dosage forms. The *in vitro* conditions under which drug release rate from *all* of the dosage forms studied show the same type of correlation with the respective absorption rate may therefore be expected to be relatively similar to conditions found *in vivo*. It is anticipated, however, that future investigations with even more variables will lead to further refinement of the *in vitro* methodology.

In the course of the present study it has been possible also (*a*) to determine (apparently for the first time) the gastrointestinal absorption kinetics, in man, of aspirin given in solution and (*b*) to assess the relationship between drug absorption rate and intersubject variability of absorption.

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EXPERIMENTAL

Clinical Study.—The test panel consisted of 12 healthy subjects, 9 males and 3 females [average age 23 years (range: 19 to 26 years) average weight 74 Kg. (range: 55–98 Kg.)]. Four different dosage forms of aspirin (650 mg.) were administered to each subject in random order, usually 1 week apart. The drug was given with 200 ml. water in the morning on an empty stomach. Tablets were swallowed whole. No food was permitted for 2 hr. Blood samples were taken 10, 20, 30, 45, 60, and 120 min. after drug administration. The concentration of total salicylate in the plasma was determined by the method of Brodie *et al.* (7) after heating the plasma with 2 *N* HCl at 100° for 10 min.

Dosage Forms.—(*a*) Rapidly disintegrating tablets, each containing 650 mg. aspirin as microencapsulated particles prepared by the National Cash Register microencapsulation process; (*b*) rapidly disintegrating "plain" aspirin tablets, each containing 325 mg. aspirin; (*c*) rapidly disintegrating tablets, each containing 325 mg. aspirin and alkaline additives (aluminum glycinate and magnesium carbonate); and (*d*) aspirin in solution as the sodium and calcium salts (5) containing 650 mg. of aspirin equivalent in 200 ml. of water.

Calculation of Absorption Rates.—The per cent

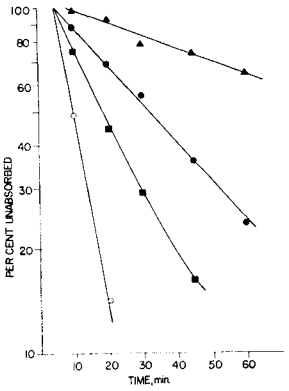

Fig. 1.—Absorption of 0.65 Gm. of aspirin from solution (○), from tablets containing alkaline additives (■), plain tablets (●), and microencapsulated particles (▲). Average of 12 subjects.

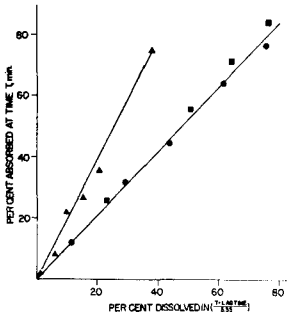

Fig. 2.—Plot of per cent of dose of aspirin absorbed at time T after drug administration *vs.* per cent dissolved *in vitro* at time (T − lag time)/5.35. Dissolution conditions: 0.1 N HCl, 37°, 60 r.p.m. Key: see Fig. 1.

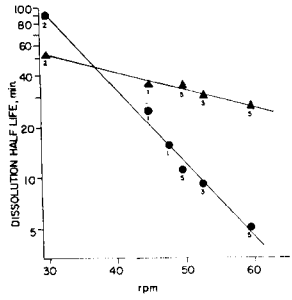

Fig. 3.—Effect of agitation intensity on dissolution rate of aspirin in plain tablets (●) and microencapsulated particles (▲). Numbers under the symbols indicate the number of tablets used for each determination.

of the dose absorbed at various times after drug administration was calculated by the procedure of Wagner and Nelson (8). Considerations regarding the application of this method to the salicylates, despite the complexity of the salicylate elimination process (9), have been discussed elsewhere (1, 2, 4).

Dissolution Rate Determinations.—*In vitro* dissolution rates were determined by the method of Levy and Hayes (10) with recently described modifications (2), using the precision stirring apparatus of Levy and Tanski (11). Stirring was in clockwise direction, and stirring rates were varied as described under *Results and Discussion.*

RESULTS AND DISCUSSION

The time course of drug absorption from the four dosage forms studied is shown in Fig. 1. Aspirin absorption from solution, plain tablets, and microencapsulated particles proceeded by apparent first-order kinetics. A 30-min. data point for solution (not shown because it is below the abscissa) also falls on the line shown in the figure. Only the line fitted to the data from aspirin in tablets with alkaline additives shows a slight but definite curvature. In each instance, a definite absorption lag time was apparent. This phenomenon has already been reported and discussed in previous communications (2, 12). Absorption from the four dosage forms occurred at widely different rates; the shortest absorption half-life (5 min.) was observed when aspirin was given in solution, while the longest absorption half-life (80 min.) was found with microencapsulated particles.[1] The observed half-life for gastrointestinal absorption of aspirin from solution may be compared to the half-life of *gastric* absorption of aspirin in man which is about 30 min. (16, 17). This difference demonstrates the importance of the small intestine for the rapid absorption even of drugs which are readily absorbed from the stomach (18). Determination of the gastrointestinal absorption kinetics of aspirin *from solution*, accomplished apparently for the first time in man, was made possible by use of a strict and intensive blood sampling schedule. The quality of the solution data is indicated by the constancy of A_∞/V values:[2] they were 51.8 mg./L. at 45 min. as well as at 60 and 120 min. Similar good agreement in A_∞/V values was obtained between dosage forms: 51.8, 52.4, 53.4 mg./L. for solution, plain tablets, and tablets containing alkaline additives, respectively. No A_∞/V value could be obtained for the microencapsulated form because drug absorption was not completed at the time when the last blood sample was taken. The data shown in Fig. 1 are based on average plasma salicylate concentrations; similar calculations based on individual plasma values showed that no distortions of kinetic characteristics and rate constants were caused by use of averaged values.[3]

Correlation of *in vivo* absorption and *in vitro* dissolution data was carried out by essentially the same technique used in previous studies (1, 2): the per cent of the dose absorbed at each time T was plotted against the per cent dissolved at (T − lag time)/intensity factor. The intensity factor is the ratio of rate constant for dissolution rate: constant for absorption (or, if the processes are not apparent first order, the ratio of time for 50% absorption : time for 50% dissolution). This type of a plot will yield regression lines of slope unity and zero intercept, regardless of the kinetics involved, if perfect correlation between absorption and dissolution data has been achieved. Figure 2 is such a plot, based on the *in vivo* data shown in Fig. 1 and on dissolution data obtained at a stirring rate of 60 r.p.m. using 0.1 N HCl as the dissolution medium. The intensity factor of 5.35 was based on the ratio of rate constants for dissolution : absorption of the plain aspirin tablets. It can be seen that the experimental data for both plain tablets and tablets with alkaline additives fit a common regression line

[1] Readers interested in assessing the potential utility of this dosage form as a prolonged-release preparation may wish to examine the data shown in Fig. 1 against the background of the principles and criteria presented in *References 13–15.*

[2] A_∞ is the total amount of drug absorbed eventually, and V is the apparent volume of distribution (8).

[3] It is believed that kinetic analysis of averaged data, *provided that examination of individual data shows that averaging does not cause distortions*, is preferable because the results thus obtained are based on fewer manipulations (*i.e.*, curve fittings).

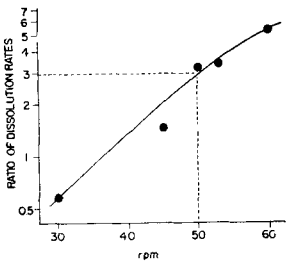

Fig. 4.—Ratio of dissolution rates, plain tablets: microencapsulated particles, as a function of agitation intensity. Broken line indicates the ratio found *in vivo* and the corresponding *in vitro* agitation intensity.

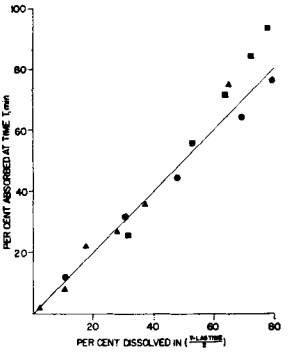

Fig. 5.—Plot of per cent of dose of aspirin absorbed at time T after drug administration *vs.* per cent dissolved *in vitro* at time $(T - \text{lag time})/2$. Dissolution conditions: 0.1 *N* HCl, 37°, 50 r.p.m. Key: see Fig. 1.

of slope 1.05; however, the data for microencapsulated particles yield a separate regression line of markedly different slope. According to Fig. 2, *in vitro* dissolution from the microencapsulated particles is too rapid, relative to the other dosage forms, when compared with the results of the *in vivo* study.

In view of the results depicted in Fig. 2, it was considered advisable to examine the relationship between stirring rate and dissolution rate of aspirin from the microencapsulated particles and plain tablets. It was found that dissolution from plain tablets was very much more sensitive to stirring rate than was dissolution from microencapsulated particles (Fig. 3). The relative insensitivity of the latter to stirring rate is consistent with the mechanism of drug release from this dosage form—namely, inward diffusion of water and outward diffusion of drug solution through the microcapsule (*Reference 19* and microscopic observations in this laboratory). Figure 4 shows the relationship between the ratio of dissolution rates, plain tablets: microencapsulated particles, as a function of stirring rate. From this graph it was found that the ratio of the *in vivo* absorption rates of aspirin from these two dosage forms is equal to the ratio of their *in vitro* dissolution rates at a stirring rate of 50 r.p.m. only. A plot of *in vivo versus in vitro* data (Fig. 5), using an intensity factor of 2 (calculated as described in the preceding paragraph), shows very good correlation between drug absorption and *in vitro* dissolution for all three dosage forms.

The successful quantitative correlation of drug absorption from three different dosage forms with the results of an *in vitro* dissolution rate test is a most encouraging indication that the development of more generalized dissolution rate tests may be feasible. The results are particularly significant

TABLE I.—COEFFICIENT OF VARIATION OF PLASMA SALICYLATE LEVELS AS A FUNCTION OF DOSAGE FORM AND TIME AFTER ADMINISTRATION OF 0.65 GM. OF ASPIRIN TO 12 SUBJECTS

Time, min.	Coefficient of Variation			
	Soln.	Tbs. with Alkal. Additives	Plain Tbs.	Micro-encaps. Particles
10	50	89	95	207[a]
20	27	68	64	98
30	31	51	56	88
45	29	45	35	63
60	27	39	29	54
120	33	30	25	36

[a] Not meaningful since less than 2% of dose absorbed.

because the three dosage forms differ so markedly with respect to the mechanism governing drug release (19, 20) and the rate of drug absorption. The importance of stirring rate, stressed previously (20–22), is shown dramatically in Fig. 3: at 30 r.p.m. drug dissolves more slowly from the more rapidly absorbed plain tablets than from the much more slowly absorbed microencapsulated particles! Similar reversals have been observed with other drugs as a function of the composition of the dissolution medium (23).[4] These observations suggest that the apparent failure of some dissolution tests to reflect the results of *in vivo* studies (occasionally reported to us in private communications) may be due to improper test conditions. The profound effect which even minor changes in test conditions can have is illustrated in Figs. 2 and 5, where a change of only 20% in stirring rate made the difference between successful correlation and failure. Changes in composition of the dissolution medium had similar effects, as will be shown in a subsequent report. The *in vitro* conditions which will yield good correlation with *in vivo* absorption of drugs in dosage forms with widely different rates and mechanisms of drug release are likely to be quite similar to the dissolution conditions encountered *in vivo*. Thus, the development of *in vivo–in vitro* correlations with diverse dosage forms and/or different drugs is also a method for obtaining more information about those physicochemical and physiologic conditions in the gastrointestinal tract which affect the dissolution of drugs.

APPENDIX

In the course of the study described above it was possible to examine the effect of absorption rate on the intersubject variability of drug absorption, as reflected by the variability of drug concentration levels in the plasma. The coefficients of variation of plasma salicylate levels as a function of time after oral administration of 0.65 Gm. of aspirin in four different dosage forms are listed in Table I. It may be noted that the coefficient of variation at any one time tends to be higher with the more slowly absorbed dosage forms. However, when the coefficient of variation was plotted as a function of per cent of the dose absorbed (Fig. 6), it becomes evident that there is no relationship between drug

[4] The good correlations observed in the present study with 0.1 *N* HCl as the dissolution medium suggest that dissolution occurred mainly in the stomach and/or that the relative effects of changes in gastrointestinal pH on *in vivo* dissolution rate were similar with each of the dosage forms studied.

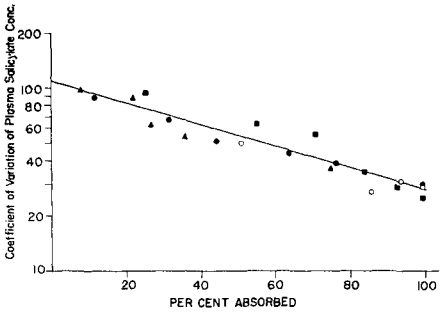

Fig. 6.—Coefficient of variation of plasma salicylate concentrations as a function of per cent of dose absorbed. Key: see Fig. 1.

absorption rate and variability of drug absorption. Rather, it is found that, under the conditions of the present study, the coefficient of variation of plasma salicylate concentrations decreased as the per cent of the dose absorbed increased.[5] The limiting value of the coefficient at 100% absorption reflects mainly the variation in plasma salicylate levels due to intersubject differences in relative and absolute apparent volumes of distribution. The coefficient at times prior to the completion of drug absorption reflects intersubject differences both in absorption rates and in volumes of distribution. When absorption is slow and elimination is rapid, the coefficient of variation is affected also by individual differences in drug elimination rate constants. After drug absorption is completed, intersubject variability of drug concentrations in the plasma may be expected to increase with time, due to intersubject differences in elimination kinetics.

[5] The same type of relationship is suggested by the data in Fig 3 of a recent paper by Lieberman *et al.* (24).

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Method for Determining Dissolution Rates of Multiparticulate Systems

By ARTHUR H. GOLDBERG*, MILO GIBALDI, JOSEPH L. KANIG, and JAI SHANKER

A method and apparatus are described for the measurement of dissolution rates of multiparticulate systems. Dissolution studies were conducted with benzoic acid and salicylamide in order to determine the applicability of the method. Replication between experiments was well within accepted limits. Furthermore, the results obtained demonstrated reasonable agreement with the extended Hixson-Crowell equation.

THE ABSORPTION of a large number of drugs is rate-limited by the dissolution process. Consequently, studies of dissolution rates can be useful in evaluating the prospective absorption rate and physiologic availability of these drugs. A number of methods for studying dissolution rates have been proposed. Levy (1, 2) has described a method involving the use of rotating nondisintegrating disks. Milosovich (3) employed a tablet mounted in a die which was subjected to solvent agitation. The use of disks or tablets of pure drug with either method permits the determination of intrinsic dissolution rates as a function of temperature and agitation.

Levy (4) has noted that a knowledge of intrinsic dissolution rate is not necessarily sufficient to determine the dissolution rate of a drug in particulate form. Frequently, certain complications arise that necessitate experimental measurement

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* Present address: College of Pharmacy, University of Michigan, Ann Arbor.