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# The *In Vivo* Significance of *In Vitro* Test Procedures for the Evaluation of Drug Products*

M. Pernarowski**

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The last decade of this century is now the accepted birth date of that sub-discipline of pharmacy that is now called 'biopharmceutics'. Wagner[1] defines biopharmaceutics 'as the study of the influence of formulation on the therapeutic activity of a drug product.' More specifically, he states that biopharmaceutics encompasses the study of the relationship between the nature and intensity of the biological effects observed in animals or man and the following factors:

1. The nature of the form of the drug (ester, salt, complex, etc.).
2. The physical state, particle size, and surface area.
3. Presence or absence of adjuvants with the drug.
4. The type of dosage form in which the drug is administered.
5. The pharmaceutical process(es) used to make the dosage form.

The philosophy inherent in this definition has revolutionized our thinking with respect to product development, quality control, and to the practice of pharmacy itself. Although the emphasis herein will be on quality control, the interrelationship between this and the other areas of pharmacy will be evident.

The principles of quality control dictate that a wide variety of techniques be used to evaluate the quality of a dosage form. Since quality must be built into a dosage form, the pharmaceutical scientist begins the process at the research stage, continues it during the production stage, and ends it by applying the tests and procedures established by pharmacopeial commissions. These stages are usually separate and distinct and, because of this, product quality has become synonymous with compliance with pharmacopeial specifications.

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** Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada.

This, in essence, is the basis of the generic equivalency hypothesis which states that dosage forms containing the same quantity of active ingredient but manufacured by different companies are biologically and therapeutically equivalent. This hypothesis is, in some instances, true but, as our knowledge of the interrelationship between formulation and/or process variations and biological or therapeutic activity increases, this concept becomes untenable.

Pharmacopeial standards are usually minimal. Standards will differ from pharmacopeia to pharmacopeia and the specifications for a monographed drug product will depend on the characteristics of the drug itself. However, for certain basic types of dosage forms(e. g., tablets and capsules), it is possible to outline the general requirements which are an inherent part of all monographs. These requirements are based on:

1. an identification of the active ingredient(s) in the dosage form.

2. an identification and a quantitative assessment of degradation products, if such are known.

3. a quantitative assay for the amount of active ingredient in the dosage form. Since analytical procedures are usually based on the assay of composite samples(e.g., 20 tablets), the values obtained are, in effect, average values and do not give any indication of the amount of drug in each and every dosage form.

4. a weight variation test. This test is an attempt to control variations in drug content between tablets. It involves the weighing of individual tablets and assumes that there is a uniform distribution of active ingredient throughout the mass of the tablet granulation and, hence, a direct relationship between tablet weight and tablet potency. Many investigators[2,3] have now shown that tablets containing varying quantities of active ingredient will still comply with this specification.

In an attempt to prevent such tablets from reaching the market place, the *United States Pharmacopeia* and the *National Formulary*[4,5] have described a content uniformity test for those dosage forms which contain small quantities of active ingredient and large amounts of adjuvants(e.g., Prednisone Tablets). The test involves the analysis of individual tablets and, in this way, attempts to control intertablet variation. Unfortunately, this test is not applicable to all tablets.

5. a tablet disintegration test. Although all tablet disintegration tests measure only the time required for a tablet to break up into small particles, it is often implied that tablet disintegration *per se* has biological significance. Studies have been carried out which indicate that there is an apparent correlation between tablet disintegration time and *in vivo* availability[6,7] but, at the same time, other researchers[8,9] have shown that tablets which disintegrate in the time limits specified by pharmacopeias are not clinically or biologically efficacious.

These five general tests are, therefore, poor indicators of the way in which a dosage form will perform in the body. This, in noway, implies that pharmacopeias are not good

sources of information on product control. They do provide the necessary standards and, if these are carefully applied, the probability of sub-standard products reaching the market place are minimal. Unfortunately, not all manufacturers choose to follow these specifications and, more important, do not add to them on the basis of knowledge which is now present in the pharmaceutical literature.

Pharmacopeias are not static. They do change. One of the more dramatic changes during the past few years has been in the area of dissolution testing. The latest editions of the *United States Pharmacopeia* and the *National Formulary* now list procedures for determining the dissolution characteristics of a limited number of products (a diagram of the U.S.P. dissolution apparatus is shown in Fig. 1).

Dissolution procedures attempt to measure the amount of drug released from a dosage form into water or a simulated gastric or intestinal fluid (See Fig. 2). Wagner[10] lists many different approaches to the determination of dissolution characteristics. One of

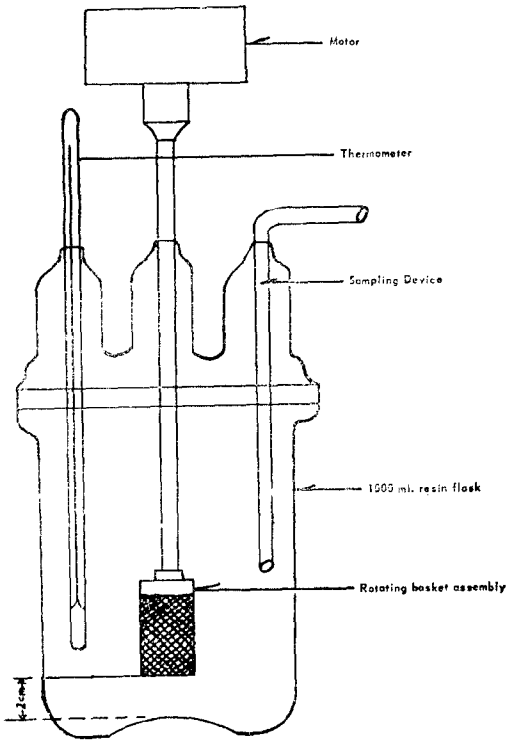


Fig. 1—The U.S.P. dissolution apparatus (Specifications and procedural details are given on p.934 of the U.S.P.).

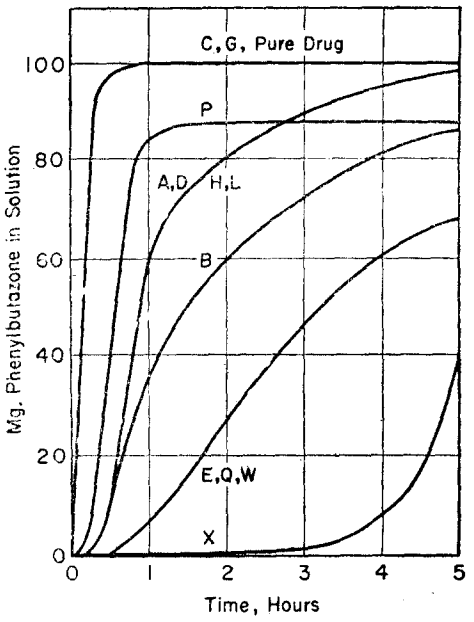


Fig. 2—The dissolution characteristics for pure phenylbutazone and for 12 brands of phenylbutazone tablets (These characteristics were determined by using a modified U.S.P. dissolution apparatus and simulated intestinal fluid U.S.P. as the test medium).

these that developed in this laboratory[11] is shown in Fig. 3. Unlike other dissolution methods, this approach is based on a continuous flow principle and produces a different form of dissolution curve. An example of such acurve is shown in Figure 4.

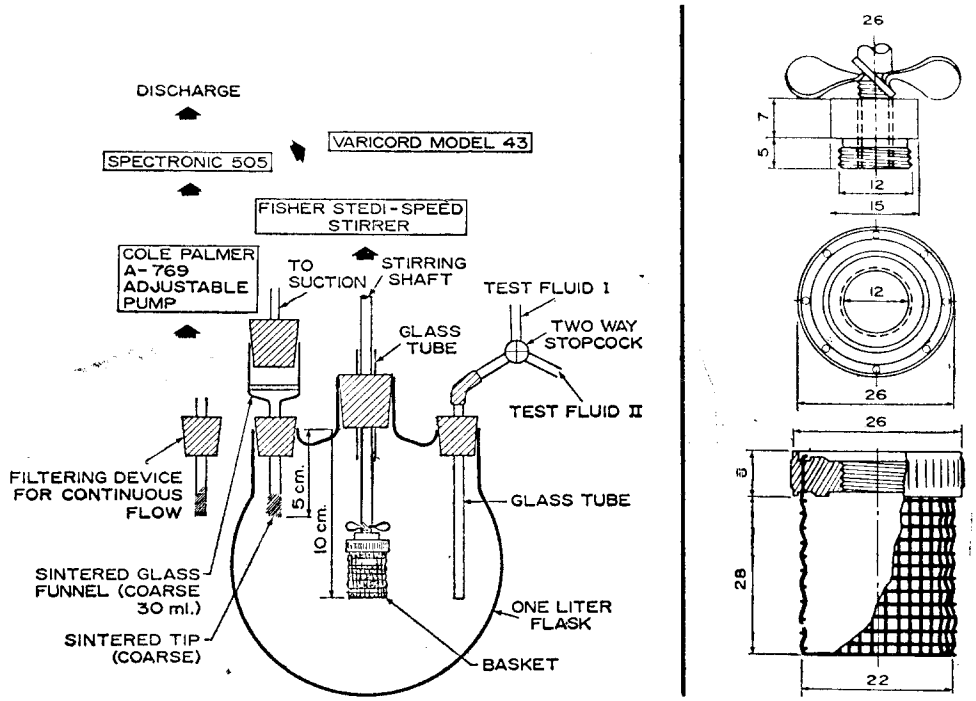


**Fig. 3**—A continuous flow dissolution apparatus. (A tablet is placed in the basket which is attached to the stirrer, and inserted into flask. The basket is rotated at 100 rpm. The flask is filled with simulated gastric fluid U.S.P. and pumped through the apparatus at 60 ml. per minute for 30 minutes. The two way stopcock is then changed to the test fluid II position (simulated intestinal fluid U.S.P.)and the procedure is continued until dissolution is complete. See reference 11 for further details.

The ultimate objective of any dissolution method is to produce a correlation with some *in vivo* parameter, e.g., area under a blood level curve, amount of drug excreted in the urine, etc. The apparatus illustrated in Figure 3 was tested for such correlation by using several brands of phenylbutazone tablets. Each brand (200mg phenylbutazone, i.e., two tablets) was administered to three healthy subjects, blood samples taken over a 30 hour period of time, and the areas under the blood level curve were compared with $T_{50}$% values (i.e., the time required for 50% of the drug in the tablets to go into solution). Results are shown graphically in Fig. 5. It would appear, therefore, that it is possible to compare *in vitro* dissolution values with results obtained *in vivo*.

Unfortunately, many dissolution studies do not produce results similar to those given herein and, more important, make no attempt to correlate *in vitro* data with some *in vivo*

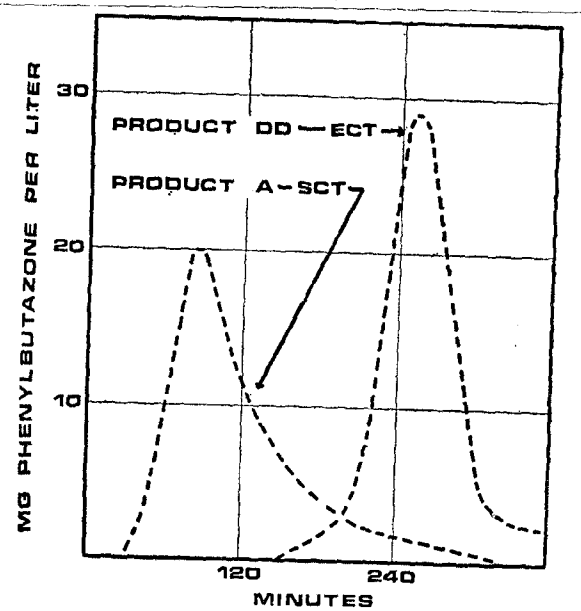


**Fig. 4**—Dissolution profiles for sugar-coated(product A) and enteric-coated(product DD)phenylbutazone tablets. Profiles were obtained by using the continuous flow dissolution apparatus diagrammed in Fig. 3.

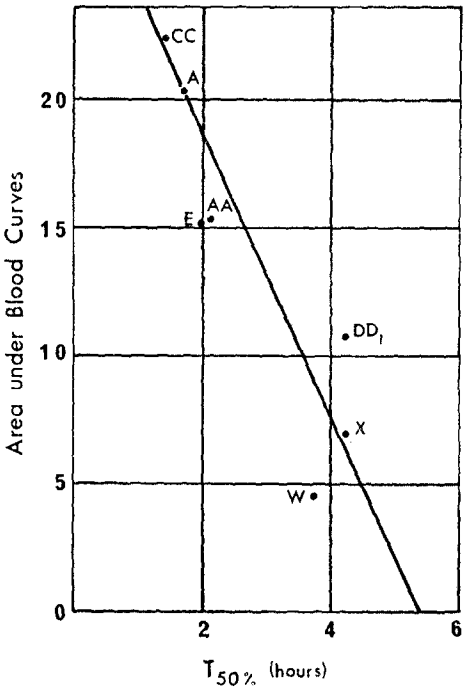


**Fig. 5**—The correlation between area under a blood level curve and $T_{50}\%$ values for several brands of phenylbutazone tablets.

parameter. Furthermore, there is no guarantee that an untested brand of phenylbutazone tablets will follow the pattern indicated in Fig. 5. All of these factors lead to the conclusion that dissolution testing *per se* is not an absolute guarantee of product efficacy. However, it is probably the best quality control tool available at the present time. It not only guarantees uniformity between batches of a particular dosage form but also is an excellent research tool. With respect to the latter, it can be used to check several experimental dosage form and, in this way, determine which of these is the most suitable for further evaluation.

Many examples of the inability of standard test procedures to detect sub-standard products are reported in the literature. The following, however, are sufficient to emphasize the importance of biopharmaceutical evaluation prior to marketing.

Varley[12], in a paper on the generic equivalency of tolbutamide tablets, published data which showed that minor pharmaceutical changes produced significant differences in blood levels and the amount of glucose in the blood after administration of the tested dosage forms. Two formulations were tested. Both products contained identical quantities of tolbutamide, were formulated under identical conditions, but one product contained twice the quantity of disintegrant present in the second tablet. This minor change in composition altered the disintegration time from two minutes to 7.6 minutes.

A double-blind, crossover clinical study was arranged in which ten healthy, non-diabetic volunteers received both formulations of the drug. Serum tolbutamide and glucose levels were determined and the results for the two formulations compared. The area under the average serum drug curves over the eight hour period was 3.57 times greater for the product which contained slightly more disintegrant. Similarly, the area under the curve for the average glucose level was 2.09 times higher(less glucose)for Orniase, the first product, than for the U.S.P. equivalent product.

Tolbutamide is an example of a drug which is relatively insoluble in aqueous and acidic media. Many investigators, during the 1960's concluded that if a problem existed, it was related to drug solubility in aqueous media. Tetracycline HCl, however, is very soluble in such media. Simple dosage forms containing highly soluble drugs of this type should not, at least on the basis of statements which have appeared in various papers during the past decade, show significant *in vivo* differences.

Barr and his coworkers [13], in 1969, examined three commercial preparations and, on the basis of plasma concentrations and amounts of free drug excreted in the urine, concluded that—

> In addition to Product A providing greater absorption, there was significantly less variation between subjects for A than B and C. The cumulative amounts of free drug excreted in 72 hours following administration of 250mg. of each drug product in a 9 subject complete cross-over study was 159±26mg for A, 117±40mg for B, and 116±37 mg for C.

MacDonald and his coworkers[14], in the same year, studied Achromycin V and three other brands of tetracycline HCl capsules. Like Barr and his coworkers, they concluded that Achromycin V produced higher blood levels following administration of both single and multiple doses of drug than did any of the other brands tested.

In the same year, Brice and Hammer[15] conducted a series of serum level studies on 16 lots of oxytetracycline capsules, labelled 250mg. potency, and distributed by 13 different suppliers. The cross-over serum level study with 20 subjects was done on all 16 lots, with a single lot of Pfizer oxytetracycline capsules as the control in each case. Two sentences for their paper adequately summarize the results obtained.

Of the 16 lots tested, seven produced blood levels which are generally considered to be below the usually accepted minimum therapeutic level of 0.6 $\mu$g/ml. None of the 16 lots produced over-all levels equal to those obtained from Pfizer oxytetracycline.

Certification of all oxytetracycline capsules except for the Pfizer products was suspended May 1, 1969. On December 6, 1969, the Food and Drug Administration ordered the recall of forty million capsules manufactured by eight companies.

Further examples will add little to the basic concept being set forth in this paper. A summary of the many papers published on this subject can be found in the reference cited at the end of this paper[16].

The ultimate test of product quality is, therefore, its behaviour *in vivo*. This creates new and difficult problems for pharmacopeial officials. What is a suitable *in vivo* standard? How many subjects should be used in an *in vivo* evaluation? What *in vivo* parameter most accurately reflects product quality? Should bioavailability tests be carried out on each lot of a particular drug product?

The Academy of Pharmaceutical Sciences has attempted to answer a number of these questions by publishing guidelines for bioavailability testing[17]. These guidelines are useful in the research stages of product formulation but few manufacturers have the resources which would permit for massive testing on this scale. This implies the need for a suitable and sensitive dissolution method which would detect changes from the researched formulation in subsequent batches.

It is doubtful if any one dissolution method will satisfy the requirements for all manufacturers. The method now official(Figure 1) has been criticized by a numer of researchers and, with this in mind, the compendia have created a Joint U.S.P.-NF Panel on Disintegration and Dissolution which will investigate these criticisms and dissolution methodology in general. The deliberations of this Committee will, hopefully, produce better methods and guideline and, therefore, provide tools for better product control.

The last decade has seen a transition from a chemically-oriented approach to quality control to an approach which is based on biological and/or therapeutic parameters (biopharmaceutics). Not all scientists are convinced that such an approach is necessary. However, after little more than a decade, biopharmaceutics is a well established discipline within the

pharmaceutical sciences and the evidence produced by scientists in this area makes it doubtful if we can ever again accept a simple, chemically-oriented approach to the control of pharmaceuticals.

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