Relationship of *in Vitro* Release to Urinary Recovery in Man of a Sustained-Release Preparation of S^{35} Prochlorperazine

By EARL ROSEN

A sustained-release preparation of S^{35}-labeled prochlorperazine prepared with different *in vitro* release patterns as determined by the rotating bottle method was administered to human subjects. Urinary recovery of S^{35} was determined by liquid scintillation counting. Previously published data on sustained-release preparations of phenylpropanolamine and trimeprazine, treated in the same fashion as the prochlorperazine data, are included. For the specific dosage form of each drug, a direct rank relationship was found to exist between *in vitro* data and the respective *in vivo* data.

EARLY IN the development of sustained-release products it was recognized that a laboratory test method could not be expected to duplicate the *in vivo* release of a product, but should provide an artificial means of assuring batch-to-batch reproducibility of a product proved to be clinically effective on the basis of controlled studies in humans (1, 2). Though numerous sustained-release products have been developed in the past, little objective human data have been reported which correlates differences in *in vitro* release patterns with *in vivo* response. Or, to put it another way, few data have been published to directly correlate the *in vitro* release pattern with clinically satisfactory material of a specific formulation.

This study was undertaken to establish that, for various dosage regimens of sustained-release formulations of S^{35}-labeled prochlorperazine, a relationship exists between *in vitro* release by the Souder and Ellenbogen technique (2) and human *in vivo* response. In addition, the experiment was designed to provide data to verify that the *in vitro* release limits used for *in vitro* control of lot-to-lot reproducibility are realistic.

The procedure utilized in this study is one previously described (3) for preparing radiochemically labeled formulations of pelleted sustained-release medication. The basic approach involves the preparation of "fast," "normal," and "slow" releasing S^{35}-labeled prochlorperazine formulations and the measurement of the radioactivity appearing in the urine after administration to human subjects.

It was recognized that this plan involved certain assumptions, namely, that the human body metabolizes prochlorperazine from any of the three formulations in the same manner, and, secondly, that the observed radioactivity measures only the phenothiazine moiety. In order to validate the treatment of experimental data and to support the conclusions, previously published data by Heimlich, *et al.* (4, 5), were subjected to a method of analysis permitting direct comparison of *in vitro* and *in vivo* results.

EXPERIMENTAL

***Preparation* of Dosage Forms.**—Chemical and radiochemical purity of S^{35} prochlorperazine dimaleate were established by spectrophotometric analysis, infrared analysis, chloride determination, half-life determination, and chromatographic analysis. The labeled compound was synthesized with a specific activity requiring no dilution with unlabeled chemical prior to formulation.

The general procedure used for preparation of sustained-release pellets was the same as previously reported by Rosen and Swintosky (3). The various sustained-release groups thus prepared were combined in varying proportions into three different pellet mixtures each of which had a different *in vitro* release pattern. Each mixture was formulated as capsules containing 15 mg. of prochlorperazine as the dimaleate salt, with an activity of 3 μc./mg. at the time of administration.

The *in vitro* release patterns of prochlorperazine were determined using the apparatus and general method reported by Souder and Ellenbogen (2). The percentage of drug released at specified intervals was obtained by difference after spectro-

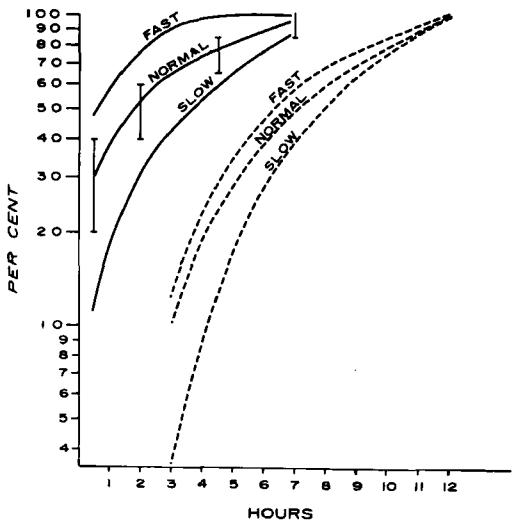

Fig. 1.—Comparison of *in vitro* release and *in vivo* measurements. Solid lines ——— represent per cent *in vitro* release of "slow," "normal," and "fast" pellet type S^{35} prochlorperazine sustained-release formulations. Bars indicate control limits for a specific pellet-type product. Broken line------ represents per cent average cumulative 12-hr. urinary recovery from adult human subjects after administration of "slow," "normal," and "fast" formulations.

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photometrically determining the amount of drug in the undisintegrated residual pellets at each test interval. The *in vitro* release patterns of the three S^{35}-labeled prochlorperazine formulations are given in Fig. 1.

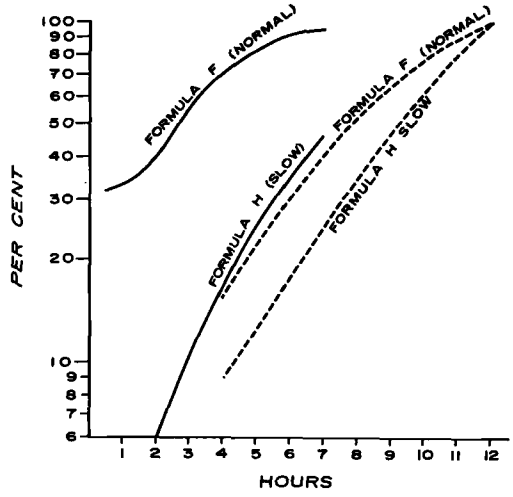

Fig. 2.—Comparison of *in vitro* release and *in vivo* measurements. Solids lines ——— represent per cent *in vitro* release of Formula "F" ("normal") and Formula "H" ("slow") pellet-type trimeprazine sustained-release formulations (4). Broken lines - - - - - - represent per cent average cumulative 12-hr. urinary recovery from adult human subjects after administration of Formula "F" ("normal") and Formula "H" ("slow") (4).

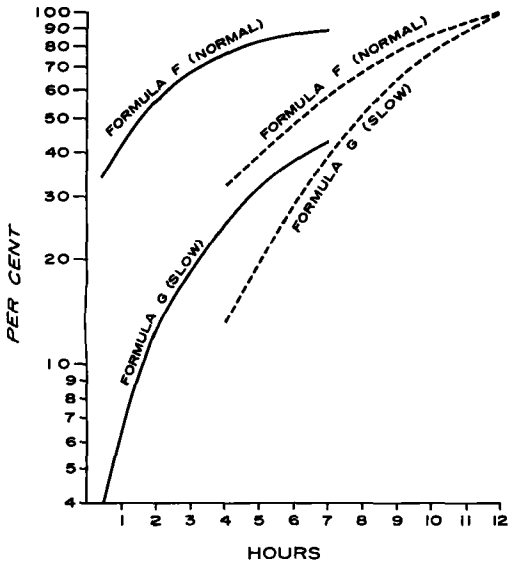

Fig. 3.—Comparison of *in vitro* release and *in vivo* measurements. Solid lines ——— represent per cent *in vitro* release of Formula "F" ("normal") and Formula "G" ("slow") pellet-type phenylpropanolamine sustained-release formulations (5). Broken lines - - - - - - represent per cent average cumulative 12-hr. urinary recovery from adult human subjects after administration of Formula "F" ("normal") and Formula "G" ("slow") (5).

Analysis of Specimens.—Urine samples were counted in a liquid scintillation counting solution adapted from one devised by Kinard (6). This solution consists of dioxane, 400 ml.; naphthalene, 80 Gm.; PPO,[1] 5 Gm.; and POPOP,[2] 50 mg. A sample of urine (0.4 ml. or less) is added to 10 ml. of liquid phosphor contained in a sample vial and, after mixing, this solution is counted in a model 314 Tri-Carb[3] liquid scintillation spectrometer. An internal standard is then added, the sample is again counted and, after proper corrections are made for quench, the counting rate of the urine sample is obtained.

Decay corrections were employed to permit a direct comparison of all experimental results.

Human Studies.—A total of 16 healthy adult subjects were given one or more of the sustained-release formulations. Of these, seven received a second formulation after a lapse of 2 weeks. Seven individuals received the "fast" formulation, nine the "normal" formulation, and seven the "slow" formulation, for a total of 23 observations. All subjects were given the drug in the morning, and urine samples were collected in 3-hr. increments over a 12-hr. period.

RESULTS AND DISCUSSION

In order to provide a basis for direct comparisons of the experimental *in vitro* and *in vivo* data, the per cent of the cumulative 12-hr. S^{35} to be excreted (Fig. 1) was plotted. The *in vitro* and *in vivo* data of Heimlich, *et al.*, on "normal" and "slow" formulations of trimeprazine (4) and "normal" and "slow" formulations of phenylpropanolamine (5) are treated in the same manner in Fig. 3.

Inspection of Figs. 1, 2, and 3 shows a direct rank relationship of the *in vivo* and *in vitro* data, indicating that "slow" or "fast" *in vitro* release patterns correlate with respective *in vivo* measurements. The data also show that formulations outside of the *in vitro* control limits can be differentiated *in vivo*.

For the formulations studied, differences in release are more demonstrable by the *in vitro* test than by *in vivo* response. This feature of the rotating bottle technique provides a measure of assurance that in the cited instances the test is sufficiently sensitive to discriminate between materials which might and those which might not produce a satisfactory clinical effect.

In vitro control specifications of 20–40% at 0.5 hour, 40–60% at 2 hours, 65–85% at 4.5 hours, and not less than 85% at 7 hours are rational and meaningful for control of this particular sustained-release prochlorperazine formulation.

It is apparent from this study that when biochemical measurements of a drug and its metabolites in humans are available in conjunction with objective and subjective clinical studies, there exists a scientific basis for evaluating the effectiveness of sustained-release formulations and for establishment of a realistic *in vitro* procedure to assure lot-to-lot reproducibility of the specific formulation prepared in the specific manner.

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[1] 2,5-Diphenyloxazole.
[2] 1,4-Bis-2[5-phenyloxazolyl]-benzene.
[3] Packard Instrument Company, Inc., LaGrange, Ill.

REFERENCES

(1) Blythe, R. H., *Drug Standards*, 26, 1(1958).
(2) Souder, J. C., and Ellenbogen, W. C., *ibid.*, 26, 77 (1958).
(3) Rosen, E., and Swintosky, J. V., *J. Pharm. Pharmacol.*, 12, 237T(1960).
(4) Heimlich, K. R., MacDonnell, D. R., Polk, A., and Flanagan, T. L., THIS JOURNAL, 50, 213(1961).
(5) Heimlich, K. R., MacDonnell, D. R., Flanagan, T. L., and O'Brien, P. D., *ibid.*, 50, 232(1961).
(6) Kinard, F. E., *Rev. Sci. Instr.*, 28, 293(1957).

X-ray and Crystallographic Applications in Pharmaceutical Research III. Crystal Habit Quantitation

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It is customary practice when describing the crystal habit of a given compound to make use of qualitative terms, such as "needles" or "plates." These terms are useful in describing crystal habit as it relates to some important pharmaceutical characteristics, such as suspension stability and syringeability, but inadequate for the more involved processes in which crystal habit affects tableting ability. In this paper a method is presented for describing the crystal habit of a given compound in quantitative terms which may be used, in some instances, to predict tableting behavior and to serve as specifications for tableting materials.

THE SYMMETRY of a crystal is fixed by the crystal system and class to which it belongs. Its relative dimensions, however, are independent of its symmetry. As a crystal grows from solution, a variety of factors, notably crystallization rate and the presence of impurities, tend to influence the amount of growth on each of the possible faces. Extremes of the possible conditions result in acicular, or needle-shaped crystals as a consequence of unidimensional growth (bidimensional retardation) and tabular, or plate-shaped crystals, as a consequence of bidimensional growth (unidimensional retardation). Terms such as acicular, equant, and tabular describe crystal habit in a qualitative manner.

Crystal habit often exerts a dominant influence on some important pharmaceutical characteristics, such as suspension stability, suspension syringeability, and the behavior of powder mixes during a tablet-compressing process. In the case of suspension syringeability, the influence is mostly mechanical. A suspension of plate-shaped crystals, for instance, may be injected through a small needle with greater ease than one with needle-shaped crystals of the same overall dimensions.

In the case of tableting behavior, however, the influence of the crystal habit of the active ingredient is more involved. The mechanical influence of crystal shape just mentioned is one factor, but there is another, sometimes dominant one, which results from the anisotropy of cohesion and of hardness which is possessed by organic (low symmetry) crystals and, therefore, of most pharmaceutically important compounds. It is significant that this anisotropy bears a fixed relation to the fundamental crystallographic directions. Therefore, as crystal habit varies, the dominant faces may vary in their relation to this anisotropy, and it is the influence of the dominant faces which tends to orient the crystals during a packing or compression process. Thus, major habit variations of an active ingredient can influence greatly the ease or the difficulty of making satisfactory compressed tablets. This is particularly true when the active ingredient makes up a large portion of the total tablet mass.

In order to evaluate tableting behavior as influenced by crystal habit, the habit must be expressed in quantitative terms which reflect some relationship between the dominant faces and the principal crystallographic directions. Qualitative terms describing shape are, in some instances, not sufficient.

Relating the Dominant Faces to the Crystallographic Directions.—An ideal situation exists when the crystals are less than about 0.2 μ in size, for in this range there is measurable "line-broadening" in the X-ray powder diffraction pattern, and the average crystal sizes in each of the crystallographic directions can be measured directly. Thus, needle-shaped crystals elongated along the c axis show sharp (001) reflections, and broadened (hk0) reflections. The actual dimensions can be measured from the width of each of the appropriate peaks at half maximum height. However, most crystalline preparations for pharmaceutical use are out of the X-ray line-broadening range, being larger, usually, than one micron, and this procedure cannot be applied.

For a typical pharmaceutical composition, it has been found that a quantitative description of crystal habit as it affects tableting behavior can be based upon measurements of preferred orientation. After relating habit extremes to tableting behavior by experimentation, optical and X-ray crystallographic studies on representative single crystals allow the designation of the dominant faces by their Miller indexes. An X-ray powder diffraction

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