chelation with endogenous zinc in a bound or free state, it could then be assumed that the metal requirements of fetuses on Days 8 and 9 of gestation are considerably higher or more important than the demands for the metal after Day 9.

Complex II, in a dose of 25 mg/kg, failed to elicit significant skeletal and soft tissue anomalies when injected on Days 8–11, suggesting that any lower dose would be unlikely to cause teratogenesis and toxicity in fetuses and maternal animals, respectively. A dose of 50 mg/kg of the complex did produce significant incidences of skeletal and soft tissue defects when given on Day 8 or 9. When the complex was administered in this dose on Day 10, increased toxicity to both gravid mice and fetuses overshadowed its teratogenic actions. Yet, when the complex was given on Day 11, skeletal instead of soft tissue anomalies appeared in significant incidences. Furthermore, although the zinc is bound with 1,10-phenanthroline when injected as a complex, sufficient amounts of the metal apparently are available to elicit teratogenic effects in a manner comparable to that achieved by free zinc since ripple ribs occurred only when large doses of the complex were given and in an incidence considerably lower than that induced by the intermediate dose (20.5 mg/kg) of the zinc salt on Days 11 and 9.

CONCLUSION

On the basis of these results utilizing lower species, it is recommended that similar studies be carried out in higher mammals to determine more precisely the relevancy of deprivation and excessive challenge of zinc as etiologic factors in the development of human teratogenesis.

REFERENCES

(1) J. Raulin, *Ann. Sci. Nat. Bot. Biol. Veg.*, 11, 93 (1869).

(2) R. E. Lutz, *J. Ind. Hyg.*, 8, 177 (1926).

(3) B. J. Poiesz, N. Battula, and L. A. Loeb, *Biochem. Biophys. Res. Commun.*, **56**, 959 (1974).

(4) M. C. Scrutton, C. W. Wu, and D. A. Goldthwait, *Proc. Natl. Acad. Sci. USA*, **68**, 2497 (1971).

(5) J. P. Slater, A. S. Mildvan, and L. A. Loeb, *Biochem. Biophys. Res. Commun.*, **44**, 37 (1971).

(6) C. F. Springate, A. S. Mildvan, R. Abramson, J. L. Engle, and L. A. Loeb, *J. Biol. Chem.*, **248**, 5987 (1973).

(7) B. L. Vallee, in "Advances in Protein Chemistry," M. L. Anson, V. Bailey, and J. T. Edsall, Eds., Academic, New York, N.Y., 1955, p. 317.

(8) A. S. Prasad, A. Miale, Jr., Z. Farid, H. H. Sandstead, and A. R. Schulert, *J. Lab. Clin. Med.*, **61**, 537 (1963).

(9) H. H. Sandstead, A. S. Prasad, A. R. Schulert, Z. Farid, A. Miale, Jr., S. Bassily, and W. J. Darby, *Am. J. Clin. Nutr.*, **20**, 422 (1967).

(10) L. S. Hurley and H. Swenerton, *Proc. Soc. Exp. Biol. Med.*, **123**, 692 (1966).

(11) H. Swenerton and L. S. Hurley, *Science*, **173**, 62 (1971).

(12) V. H. Ferm and S. J. Carpenter, *Nature*, **216**, 1123 (1967).

(13) R. E. Staples and V. L. Schnell, *Stain Technol.*, **39**, 61 (1964).

(14) J. G. Wilson, in "Teratology Principles and Techniques," J. G. Wilson and J. Warkany, Eds., University of Chicago Press, Chicago, Ill., 1965, p. 267.

(15) G. G. Simpson, A. Roe, and R. C. Lewontin, "Quantitative Zoology," Harcourt, Brace, New York, N.Y., 1960, pp. 186–195.

ACKNOWLEDGMENTS AND ADDRESSES

Received November 29, 1976, from the *Department of Pharmacology, School of Pharmacy, Temple University, Philadelphia, PA 19140.*

Accepted for publication March 11, 1977.

The authors thank Peter Lemke and Ray Brooke for technical assistance.

* Present address: Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, NY 14203.

[x] To whom inquiries should be directed.

Determination of Time Course of Tablet Disintegration I: Numerical Method

K. G. NELSON [x] and L. Y. WANG

Abstract □ A method is described for determining the time course of tablet disintegration. It involves a numerical analysis of the experimental dissolution profile of a tablet and the dissolution characteristics of the primary drug particles in the tablet. The disintegration profile is determined for an acetaminophen tablet to demonstrate the application of the method. Tablet dissolution is simulated with the disintegration–dissolution model, and the interrelationship between the two fundamental processes is studied theoretically by varying the parameters describing the two processes.

Keyphrases □ Disintegration—tablets, time course determined using numerical analysis of dissolution profile □ Dissolution profile—numerical analysis used to determine time course of tablet disintegration □ Tablets—time course of disintegration determined using numerical analysis of dissolution profile

The release of an active ingredient from a tablet involves two distinct processes: disintegration of the tablet and dissolution of the active ingredient. Although both processes commence when the tablet encounters an aqueous environment, the bulk of the active ingredient cannot dissolve until disintegration has occurred. The two processes are thus sequential and occur simultaneously until the tablet has disintegrated completely.

Studies on the temporal aspect of disintegration have largely involved only one time point, the disintegration time. The disintegration time is a subjective measure involving the time it takes the tablet to disintegrate and pass through a screen of arbitrary size in a standard apparatus (1). Attempts at determining the time course of disintegration have been reported (2, 3), but generally research in this area has involved the study of parameters that affect disintegration such as disintegrant type, compression force, and binders, and the results have been monitored by the official disintegration test (4).

Dissolution processes for powders and nondisintegrating tablets have been studied rather extensively. Several reports discuss dissolution theory and provide mathematical models (5–8) which serve as a means to evaluate dissolution rate data and dissolution test systems. A dissolution theory for disintegrating tablets, however, has not been developed, although equations with adjustable parameters have described the process (9, 10).

This paper describes a method for processing dissolution rate data to determine a quantitative description of the disintegration process as a function of time. The method was applied to experimental dissolution rate data from a tablet having a rather idealized formulation to demon-

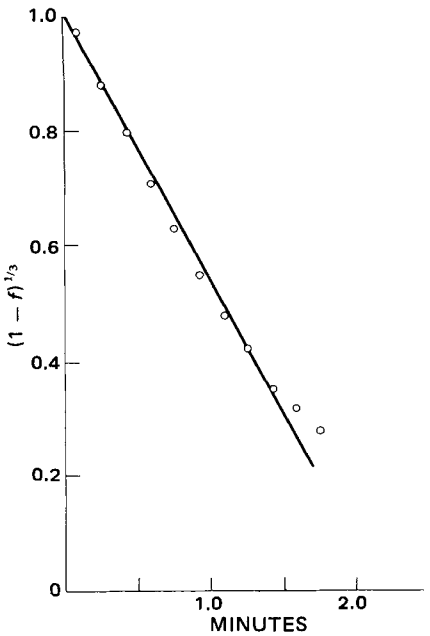

Figure 1—*Powder dissolution data plotted according to the cube-root equation.*

strate the principles of the method. This disintegration–dissolution analysis should provide the framework for a quantitative model for tablet dissolution.

THEORETICAL

Disintegration may be considered to be the process whereby the individual solid drug particles are released from the tablet to the aqueous medium so that they may undergo dissolution. A quantitative description of this process would involve the amount or fraction of the drug in a tablet that has disintegrated as a function of time. Once an individual drug particle is exposed to the aqueous environment, a mathematical expression exists to describe its time course of dissolution, *e.g.*, the Hixson–Crowell equation (5).

The dissolution profile that results from a dissolution test on a tablet in a dissolution apparatus is a composite of the disintegration and dissolution functions. The principle of the method outlined later is the application of a numerical analysis technique to the dissolution profile along with the known dissolution rate function to generate the disintegration function.

A rearrangement of the Hixson–Crowell cube-root equation (5) describes the dissolution of a powder:

$$f = 1 - (1 - Kt)^3 \qquad \text{(Eq. 1)}$$

where f is the fraction dissolved, t is the time, and K is an experimentally determined constant, which depends upon the initial particle size and the solubility of the drug among other things. Of course, f cannot exceed 1, so f is defined equal to 1 when t gets greater than the time for complete dissolution.

If the time course of tablet disintegration is considered in terms of many discrete steps, then the fraction of drug in the tablet that has dissolved after the first small time interval may be obtained by applying Eq. 1:

$$M_1 = w_0[1 - (1 - Kt_1)^3] \qquad \text{(Eq. 2)}$$

where M_1 is the fraction of drug in the tablet that has dissolved at time t_1, and w_0 is the fraction of the drug in the tablet that was disintegrated at the beginning of the time interval, *i.e.*, t_0 (=0). For the second time interval:

$$M_2 = w_0[1 - (1 - Kt_2)^3] + w_1\{1 - [1 - K(t_2 - t_1)]^3\} \qquad \text{(Eq. 3)}$$

where the first term on the right is analogous to Eq. 2 but at t_2, and the second term now accounts for the fraction of drug dissolved during the second time interval that arises from the fraction of drug disintegrated, w_1, at the beginning of the second interval (at t_1). In general:

$$M_n = \sum_{i=0}^{n} w_i\{1 - [1 - K(t_n - t_i)]^3\} \qquad \text{(Eq. 4)}$$

where M_n is the fraction of drug in the tablet that has dissolved after n time intervals, corresponding to time t_n. As before, the term in braces that arises from Eq. 1 should be defined as 1 once it attains that value.

The cumulative fraction of drug disintegrated, W_n, up to time t_n then is:

$$W_n = \sum_{i=0}^{n} w_i \qquad \text{(Eq. 5)}$$

Thus, the disintegration profile can be constructed from Eq. 5.

To determine the w_i values, the experimental dissolution profile of a tablet is divided into many small intervals, *e.g.*, 30, and the fractional amount dissolved is determined at each interval. The intervals are numbered and, considering that M_n is the fraction dissolved at the nth interval, Eq. 4 is written out for each interval. If K is known, then the set of Eq. 4 for all intervals gives a system of linear equations with the same number of unknowns, *i.e.*, the w_i values, which can then be solved.

Once the system is solved for the w_i values, the disintegration profile can be obtained by applying Eq. 5 for each time interval.

EXPERIMENTAL

Tablet Preparation—Tablets (500.0 mg) were prepared from 25.0 mg of acetaminophen[1], 15.0 mg of sodium carboxymethyl starch[2], 75.0 mg of microcrystalline cellulose[3], 382.5 mg of lactose (spray dried)[4], and 2.5 mg of magnesium stearate[5]. The acetaminophen crystals were ground in a mortar and separated into a 70–80-mesh fraction with a U.S. Standard sieve series[6] and a sieve shaker[7]. All other ingredients were passed through a 60-mesh sieve, and the formulation was well mixed. The 500-mg tablets were compressed individually with a laboratory press[8], using a 1.27-cm (0.5-in.) die and flat-faced punches. The compressional force was increased slowly to 1360 kg (3000 lb) and retained for 30 sec before release.

Dissolution Rate—The tablet dissolution profile was determined in water using a rotating-filter–stationary basket dissolution test apparatus[9] (11) at 37° and 400 rpm. The amount of drug dissolved with time was monitored by circulating (flow rate of 71 ml/min) the filtered dissolution fluid through a flowcell in a recording spectrophotometer[10] and returning it to the vessel. After accounting for the holdup volume, a chart of the absorbance of acetaminophen (at 243 nm) *versus* time thus represented the dissolution profile of the tablet, and the fractional amount released at any time could be readily determined by dividing the absorbance at that time by the absorbance after complete dissolution.

The dissolution profile for the pure acetaminophen powder (70–80 mesh) was obtained by introducing the powder directly into the vessel with all of the other conditions the same as for the tablets.

RESULTS AND DISCUSSION

The value for the dissolution rate constant, K, was determined by monitoring the dissolution of the 70–80-mesh acetaminophen powder in the dissolution test apparatus. In accordance with Eq. 1, the data were plotted as $(1 - f)^{1/3}$ *versus* time (Fig. 1) and K was obtained directly from the slope. The linearity of this plot was excellent ($r^2 = 0.997$) over the experimental range of f from 0 to 0.96. The least-squares slope in this range yielded $K = 0.465$. The positive curvature at times corresponding to the last 4% of the powder dissolution probably resulted because the powder was not strictly monodisperse.

The experimental dissolution profile for the acetaminophen tablet, as obtained from the spectrophotometer recording, was divided into 36 equal time intervals of 10 sec each for the numerical analysis. The points in Fig. 2 show the dissolution profile thus obtained and represent the required data, along with the value for K, to generate the set of 36 linear equations from Eq. 4. A digital computer[11] was programmed to solve this

[1] Rhodia, Inc., New York, N.Y.
[2] Primojel, Edward Mandell Co., Carmel, N.Y.
[3] Avicel PH-101, Avicel Department, FMC Corp., Philadelphia, Pa.
[4] Fast Flo Lactose, Foremost Foods Co., Foremost-McKesson, Inc., San Francisco, Calif.
[5] Fisher Scientific Co., Fair Lawn, N.J.
[6] W. S. Tyler Co., Cleveland, Ohio.
[7] Ro-Tap shaker, W. S. Tyler Co., Cleveland, Ohio.
[8] Model C, Fred S. Carver, Menomonee Falls, Wis.
[9] Coffman Industries, Kansas City, Kans.
[10] Cary 14, Cary Instruments, Monrovia, Calif.
[11] CDC 3300, Health Computer Sciences, University of Minnesota.

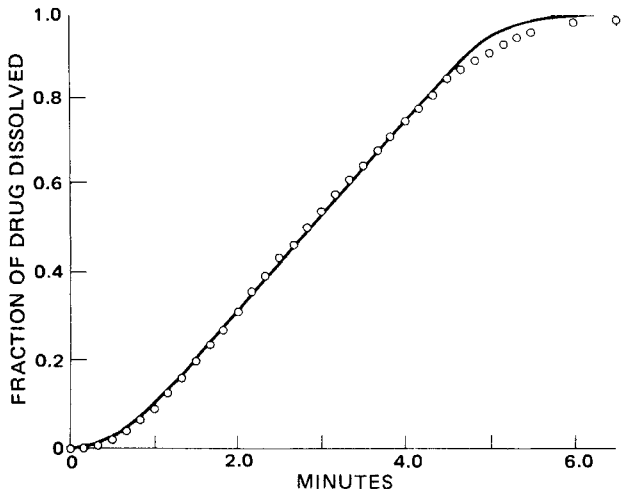

Figure 2—*Tablet dissolution profile (points) and model-simulated curve.*

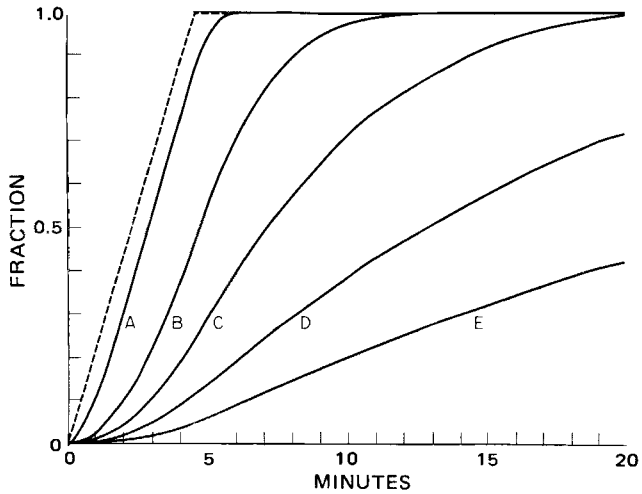

Figure 4—*Disintegration profile (dashed line) and simulated dissolution curves (solid lines) for* $k_0 = 0.219$. *Key: A,* K = *0.465; B,* K = *0.1; C,* K = *0.0465; D,* K = *0.02; and E,* K = *0.01.*

set of equations for the w_i values, and the disintegration profile was then generated by repetitive application of Eq. 5. The disintegration profile obtained in this manner is shown as the points in Fig. 3. At 4.25 min, the tablet had disintegrated such that it passed entirely through the wire mesh basket. This time would be analogous to the USP disintegration time (1).

Figure 3 shows that the disintegration profile is quite linear for this formulation. A linear regression of the data up to t = 4.17 min gives:

$$W = 0.007 + 0.219t \qquad \text{(Eq. 6)}$$

with a coefficient of determination of 0.995. Because of the good linearity, the coefficient of t can be considered to be an apparent zero-order disintegration rate constant, k_0. To test the self-consistency of the method, Eq. 6 can be used to calculate the w_i values for Eq. 4, which, together with the value for K, can be used to simulate the tablet dissolution profile. The dissolution profile generated in this way is shown as the curve in Fig. 2. It is apparent that the simulation is quite good, except for some moderate deviation at large times. This result may be expected, however, because in this region the dissolution would be influenced by the disintegration function when disintegration is nearing completion. Hence, the variation at large times results from the deviation of Eq. 6 from the determined disintegration profile, as is apparent in Fig. 3, as well as the experimental problem regarding Eq. 1 at high fractions dissolved.

To explore theoretically the interaction between the disintegration and dissolution processes and the resultant drug release pattern, the values for K and k_0 can be systematically varied. Shown as the dashed line in Fig. 4 is the disintegration profile drawn according to Eq. 6; curve A is the generated dissolution function. Because these functions are parallel for the greater part of the dissolution curve, the drug release from this particular acetaminophen formulation is largely controlled by the disintegration rate. If K is now decreased in value stepwise from 0.465 to 0.1, 0.0465, 0.02, and 0.01, then the four dissolution profiles can be generated from Eq. 4 (curves B, C, D, and E, respectively, in Fig. 4). For the case with a relatively small K (curve E), the calculated time for complete dissolution is about 100 min, which is considerably larger than the disintegration time. Thus, curve E represents the case where drug release is controlled by the dissolution rate.

Curve C in Fig. 4 represents an example of the intermediate case where both disintegration and dissolution influence drug release. Up to the time of complete disintegration, the dissolution curve has positive curvature because additional solid drug is released to the medium at a rate faster than it can dissolve. At the disintegration time, the dissolution curve goes through an inflection point because the drug release subsequently occurs by dissolution of the suspended particles, which is analogous to powder dissolution.

The effect of changing k_0 while holding K constant can also be simulated. In Fig. 5, curves C represent the disintegration and dissolution determined for the tablet as before. Decreasing k_0 by 50% results in curves D, which show a disintegration-controlled release. Increasing k_0 to 0.439 (curves B) demonstrates a case where the disintegration control is shorter; with k_0 = 4.39 (curves A), which corresponds to a disintegration time of 13 sec, the large region of negative curvature on the dissolution profile indicates that the drug release is largely dissolution rate controlled.

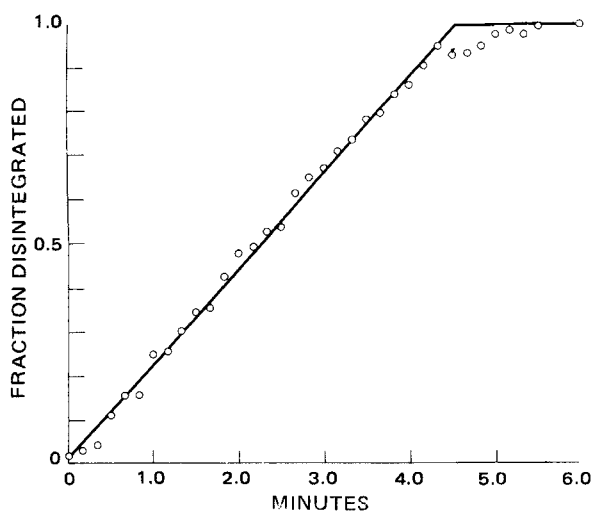

Figure 3—*Disintegration profile determined by numerical analysis of dissolution data. The line represents linear regression up to* t = *4.17 min.*

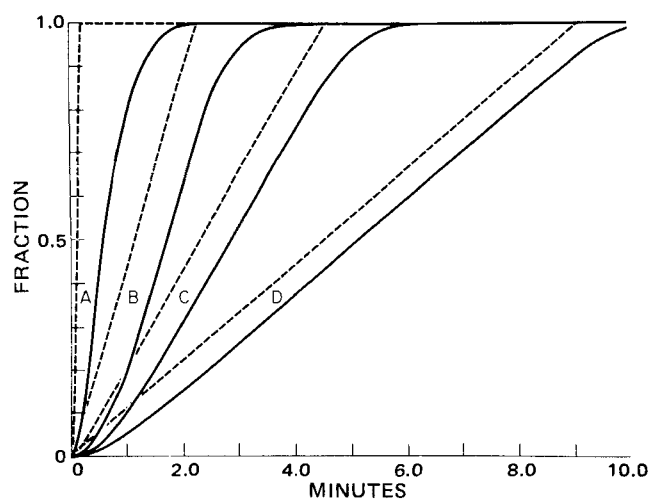

Figure 5—*Simulated disintegration (dashed lines) and associated dissolution (solid lines) profiles for* K = *0.465. Key: A,* k_0 = *4.39; B,* k_0 = *0.439; C,* k_0 = *0.219; and D,* k_0 = *0.109.*

The described concept of the disintegration-dissolution analysis can be rather general, but several restrictions were employed to study a simple system and to demonstrate the use of the method. First, the tablet was prepared by direct compaction, which permits disintegration directly into primary particles. A granulation may introduce a more complex disintegration pattern and may also modify somewhat the primary particles. Second, the Hixson–Crowell equation (5) was employed. Alternative theories for powder dissolution exist, but the resulting mathematical equations do not describe the experimental dissolution process as well as the cube-root law (12).

Third, the drug powder was of a uniform particle size. If a powder with a wide size distribution were used, an additional feature would have to be included to account for the change in size distribution over time for each fraction disintegrated. Fourth, it was assumed that the compression force did not alter the primary particles, *i.e.*, that K for the pure powder represented K for the disintegrated powder. Although compression can fracture particles, the relatively low force used apparently did not have a significant effect because the experimentally observed disintegration time was in close agreement with the time for complete disintegration on the disintegration profile determined by Eqs. 4 and 5.

Finally, because the disintegration profile for the acetaminophen tablet was zero order, the discussion regarding the linearity of the dissolution profile vis-á-vis the disintegration-controlled release and the significance of the inflection points are restricted to this case. As further studies indicate other disintegration profiles, *e.g.*, with negative curvature or sigmoidal, model simulations can be employed to explore these effects.

The described method for determining the time course of disintegration can provide a means to study tablet disintegration under idealized conditions. Work is being continued to extend the model so that regular tablet formulations, *i.e.*, not idealized, can be evaluated.

REFERENCES

(1) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 650.

(2) H. Nogami, J. Hasegawa, and Y. Nakai, *Chem. Pharm. Bull.*, **7**, 331 (1959).

(3) M. H. Rubinstein, J. I. Wells, and C. A. Holland, *J. Pharm. Pharmacol. Suppl.*, **27**, 54 (1975).

(4) W. Lowenthal, *J. Pharm. Sci.*, **61**, 1695 (1972).

(5) A. Hixson and J. Crowell, *Ind. Eng. Chem.*, **23**, 923 (1931).

(6) W. I. Higuchi and E. N. Hiestand, *J. Pharm. Sci.*, **52**, 67 (1963).

(7) K. G. Nelson and A. C. Shah, *ibid.*, **64**, 610 (1975).

(8) L. J. Leeson and J. T. Carstensen, "Dissolution Technology," Academy of Pharmaceutical Sciences, American Pharmaceutical Association, Washington, D.C., 1974.

(9) J. G. Wagner, *J. Pharm. Sci.*, **58**, 1253 (1969).

(10) F. Langenbucher, *J. Pharm. Pharmacol.*, **24**, 979 (1972).

(11) A. C. Shah, C. B. Poet, and J. F. Ochs, *J. Pharm Sci.*, **62**, 671 (1973).

(12) P. V. Pedersen and K. F. Brown, *ibid.*, **65**, 1442 (1976).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 22, 1976, from the *College of Pharmacy, University of Minnesota, Minneapolis, MN 55455.*

Accepted for publication March 11, 1977.

Supported by a grant from the Pharmacy Research Unit, The Upjohn Co., Kalamazoo, Mich.

[x] To whom inquiries should be directed.

Bioavailability of Three Isoniazid Formulations

S. SVED [x], I. J. McGILVERAY, and N. BEAUDOIN

Abstract □ The bioavailability of three isoniazid formulations was assessed using a procedure specific for the free drug. Nine human volunteers, all slow acetylators, were each given 4 × 100 mg of isoniazid of three different tablet formulations at weekly intervals; the plasma drug levels were measured at different times during the first 24 hr. No significant differences ($p > 0.05$) were detected among the three products as to relative bioavailability, peak plasma concentrations, C_{max}, and the time of C_{max}, t_{max}. Analysis of variance of the pharmacokinetic parameters obtained according to a one-compartment open model did not demonstrate any significant formulation or time effect but revealed a significant intersubject variation in all parameters involved.

Keyphrases □ Isoniazid—bioavailability of three commercial formulations compared □ Bioavailability—isoniazid, three commercial formulations compared □ Tuberculostatic antibacterials—isoniazid, bioavailability of three commercial formulations compared

Isoniazid has been used since the early 1950's for the treatment of tuberculosis and has been largely responsible for the virtual eradication of this disease in certain parts of the world. It is widely used in preventive therapy in the United States and Canada. Its clinical efficacy and bioavailability assessment are complicated by methodological and metabolic problems. First, the presence of slow and fast acetylator genotypes in the population has been demonstrated (1–5), and the inclusion of both types in a study could lead to increased variability and difficulties in design. Second, isoniazid tends to form relatively stable, probably less active, hydrazones with either physiological (sugars and pyruvate) or excipient (lactose and glucose) aldehydes and ketones (6–9), thus necessitating an assay that can distinguish between the free drug and its hydrazones.

Earlier studies (10) on isoniazid bioavailability did not demonstrate any difference between six commercial preparations available in the United States. The method used, however, could not distinguish between free isoniazid and its hydrazones. This study was initiated to compare the bioavailability of three Canadian isoniazid preparations using a homogeneous group and an assay that measures only free isoniazid in the plasma.

EXPERIMENTAL

Materials—Isoniazid tablets, 100 mg, were supplied[1] from current production lots. Evacuated heparinized blood collection tubes were obtained locally[2]. Extractions were done in glass tubes with polytef-lined screw caps[3]. 2,4-Pentanedione (practical), diethanolamine, ethylene glycol, potassium carbonate, zinc acetate (all reagent grade)[4], methyl

[1] See "Quad Review 4," Health Protection Branch, Health and Welfare Canada, Ottawa, Canada, 1975, p. 44.

[2] Vacutainers, Becton-Dickinson; obtained through Canlab, Ottawa, Canada.

[3] Catalog No. T 1356-1, Canlab, Ottawa, Canada.

[4] Baker; obtained through Canlab, Ottawa, Canada.