Loss of Sensitivity in Distinguishing Real Differences in Dissolution Rates Due to Increasing Intensity of Agitation

By W. E. HAMLIN, E. NELSON†, B. E. BALLARD†, and J. G. WAGNER

Two polymorphic forms of methylprednisolone, designated I and II, were compressed into constant surface pellets and their dissolution rates determined *in vitro* by four different methods and *in vivo* following implantation in rats. There was a highly significant difference between the rates of dissolution of forms I and II *in vivo*. There were also highly significant differences between the rates of dissolution of forms I and II in the two *in vitro* tests where the intensity of agitation was of a relatively low order of magnitude. There were no significant differences in the rates of dissolution of forms I and II in the two *in vitro* tests which produce the greatest velocity of dissolution medium across the surface of the pellet. One of the *in vitro* methods which gave no significant difference in rates for forms I and II employed the machine of Souder and Ellenbogen, rotating at 40 r.p.m. This machine is now being used in interlaboratory tests of timed release and prolonged action oral products in an attempt to "develop a test procedure which may be generally useful as a routine control procedure." The implications of our results in relation to such testing procedures are discussed.

IN PERFORMING dissolution rate experiments several investigators (1–6) have used relatively high intensities of agitation, and Nelson (7) has described a method in which free convection, diffusion coefficient, and concentration were stated to be the rate determining factors. Several authors (4, 5, 8) have reviewed the literature concerning the effect of agitation speed on the velocity of heterogeneous reactions and the kinetics of dissolution; some of these results are discussed in the Appendix. We have studied the *in vitro* dissolution of many substances by several methods and have attempted in some cases to correlate the results with *in vivo* measurements of absorption rate, either after implantation of the substances or following their oral administration. With respect to relative intensity of agitation (rate of stirring or velocity of the dissolution medium across the surface of the dissolving substance), we have been interested in determining: (*a*) what *in vitro* method correlates best with *in vivo* results; (*b*) whether differences between substances or lots of the same substance or product can be differentiated with respect to dissolution rate at different intensities of agitation; and (*c*) whether the error in the measured dissolution rate is independent of or dependent upon the relative intensity of agitation. Results of such investigations are important, not only from a theoretical viewpoint but also from a very practical viewpoint. Recently the Pharmaceutical Contact Section of the Pharmaceutical Manufacturer's Association (9) has been carrying out interlaboratory tests on timed release and prolonged action oral products. These investigations are intended to "develop a test procedure which may be generally useful as a routine control method." One of us (J. G. Wagner) wrote a memorandum (10) to the Tablet Subcommittee of the Contact Section outlining some of the pitfalls in the current tests under consideration. One of the pitfalls mentioned was that the intensity of agitation resulting from use of the machine described by Souder and Ellenbogen (11), and used in these interlaboratory tests, may be too great. On the basis of theory it was hypothesized that there may be examples where products would give a significant difference in results *in vivo*, yet by such a method the difference would not be measurable *in vitro*. We now have some experimental data obtained *in vivo* and by several *in vitro* methods to support this viewpoint.

EXPERIMENTAL

In order to test the above hypothesis we chose to use constant surface pellets of two polymorphic forms of methylprednisolone, hereafter termed forms I and II. Form II has a greater water solubility than form I, hence one could deduce that form II would have a greater initial dissolution rate in water than form I on the basis of dissolution rate theory (8). We chose to use pellets compressed at high pressures since with methylprednisolone I and II we observed these remained intact during dissolution and the surface areas during the experiments were accurately known. By operating in the region where the concentration of methylpredni-

Received August 7, 1961, from the Pharmacy Research Section, Product Research and Development Unit, The Upjohn Co., Kalamazoo, Mich.

Accepted for publication August 21, 1961.

† School of Pharmacy, University of California Medical Center, San Francisco 22.

Presented to the Scientific Section, A.PH.A., Las Vegas meeting, March 1962.

The authors greatly appreciate the samples of the polymorphic forms of methylprednisolone supplied by Dr. John W. Shell, The Upjohn Co.

solone in the dissolution medium was very small compared with the concentration of a saturated solution, the mathematics was greatly simplified. In the *in vitro* tests such low concentrations were readily determined by means of ultraviolet spectrophotometry using the Cary recording spectrophotometer. See Fig. 1. In the implantation experiments in rats, rate of loss of weight was used as a criterion of dissolution and absorption rate. However, for constant surface pellets we have correlated rate of loss of weight with rate of appearance in the dissolution medium. Most of the details of the *in vivo* and *in vitro* experiments are given below. An adequate description of the pellet holder and the Wruble machine (12) will be made at a later date.

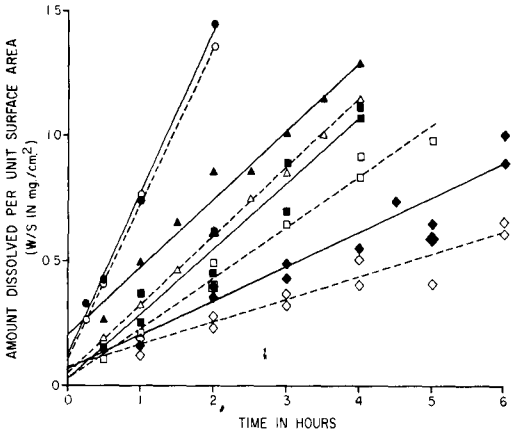

Fig. 1.—Pseudo zero-order plots for dissolution of methylprednisolone polymorphs I and II from constant surface pellets in water at 37°. ●—Polymorph II and ○—polymorph I, pellet holder method, 40 r.p.m. in the Souder and Ellenbogen machine. ▲—Polymorph II and △—polymorph I, pellet holder method, 12 r.p.m. in the Wruble machine. ■—Polymorph II and □—polymorph I, pellet holder method, 6 r.p.m. in the Wruble machine. ◆—Polymorph II and ◇—polymorph I, hanging pellet method.

Pellet Implants in Rats.—Pellets of pure methylprednisolone I and methylprednisolone II, 0.25 inch in diameter, were compressed at high pressure. Two disks of one polymorph were implanted in each rat. After different intervals of time the pellets were removed and weighed. Knowing the loss in weight, the surface area, and the time of exposure, the rate of weight loss, equivalent to the rate of absorption with opposite sign, was calculated. Thirty separate determinations involving three series of 10 different rats were used with methylprednisolone I, and 15 separate determinations involving three series of five different rats were used with methylprednisolone II. The overall average rates obtained are shown in Table I. The complete data have been published by Ballard and Nelson (13).

Hanging Pellet Method.—Pellets of pure methylprednisolone I and methylprednisolone II, 0.50 inch in diameter, were compressed at high pressure. The rates of dissolution in deionized water at 37° were determined by the method of Nelson (7), with the exception that instead of determining weight loss, the fluid surrounding the pellet was assayed for the steroid by ultraviolet spectrophotometry.

Pellet Holder in Wruble Machine.—Pellets of pure methylprednisolone I and methylprednisolone II, 0.375 inch in diameter, were compressed at high pressure. The rates of dissolution in deionized water at 37° were determined as follows. The pellets were held in a polyethylene holder at the center of oval 4-fl. oz. bottles containing 120 ml. of deionized water. The bottles were attached to the wheel of the machine described by Wruble (12). After rotation for different intervals of time, the fluid surrounding the pellet was assayed for steroid by ultraviolet spectrophotometry. The dimensions of the pellet initially and after exposure were determined and the average surface area during exposure to the water calculated. The weight of methylprednisolone in solution, which had been released by the pellet, was divided by the average surface area during exposure. The latter values were plotted against time of exposure to the water. The points were fitted by the method of "least squares." The intercepts and slopes (rates) of the lines are shown in Table I. Experiments were made with the wheel rotating at 6 r.p.m. and at 12 r.p.m.

Pellet Holder Method in the Machine of Souder and Ellenbogen (11).—The experiments were performed similarly as described above for the Wruble machine, except the machine described by Souder and Ellenbogen (11) was used in place of the Wruble machine. The bottles were rotated at 40 r.p.m. during exposure in a constant temperature bath held at 37°.

Estimation of Film Thickness.—Under the conditions of our *in vitro* experiments we can substitute D/h for k in Eq. 3 shown in the Appendix, where D is the diffusion coefficient of methylprednisolone in water and h is the diffusion layer or film thickness. Higuchi, Rowe, and Hiestand (14) have estimated D for methylprednisolone in water at 25° to be 4.7×10^{-6} cm.^{2} sec.$^{-1}$ which is equivalent to 1.8×10^{-2} cm.^{2} hr.$^{-1}$ at 37°. Estimations of D/h and h in our experiments, using the latter value, are shown in Table II.

DISCUSSION OF RESULTS

Effect of Intensity of Agitation.—The data in Table I and Fig. 2 indicate that when the intensity of agitation, or velocity of dissolution medium across the dissolving solid, was of a low order of magnitude, then a significant difference in dissolution rates was observed for methylprednisolone forms I and II. When the speed of rotation of the wheel in the Wruble machine was increased from 6 to 12 r.p.m., the rates of dissolution of the two polymorphs became equal within the errors of measurement. Rotation at 40 r.p.m. in the machine described by Souder and Ellenbogen (11) also led to no observable significant difference in rates of dissolution. When implanted in rats the pellets of the two polymorphs lost weight at significantly different rates. These data then indicate that *in vitro* tests in which there is a relatively low intensity of agitation, or only natural convection, correlate best with the *in vivo* implantation data.

TABLE I.—RESULTS OF DISSOLUTION RATE EXPERIMENTS PERFORMED WITH CONSTANT SURFACE PELLETS OF METHYLPREDNISOLONE POLYMORPHS I AND II BY AN *In Vivo* AND SEVERAL *In Vitro* METHODS

Test[a]	Methyl-prednisolone Polymorph	95% Confidence Interval of the Intercept, mg./cm.²	95% Confidence Interval of the Rate, mg./cm.²/hr.	Percentage Difference in Rates[b]	Significance of Difference Between Rates by *t*-test
Pellet implants in rats (13)	I	... ...	0.0179 ± 0.0022	51.1	Sig. 1% level
	II	... ...	0.0302 ± 0.0034		
Hanging pellet method (7)	I	0.077 ± 0.081	0.091 ± 0.021	41.7	Sig. 1% level
	II	0.066 ± 0.119	0.139 ± 0.030		
Pellet holder method in Wruble machine (12), 6 r.p.m.	I	0.029 ± 0.083	0.203 ± 0.027	26.5	Sig. 2% level
	II	0.026 ± 0.123	0.265 ± 0.047		
Pellet holder method in Wruble machine (12), 12 r.p.m.	I	0.048 ± 0.028	0.276 ± 0.011	−0.4	No sig. diff.
	II	0.203 ± 0.115	0.275 ± 0.045		
Pellet holder method in machine of Souder and Ellenbogen (11), 40 r.p.m.	I	0.100 ± 0.039	0.630 ± 0.034	4.0	No sig. diff.
	II	0.118 ± 0.180	0.656 ± 0.156		

[a] Intensity of agitation increasing in descending order. [b] Percentage difference in rates = $\frac{\text{rate for methylprednisolone II} - \text{rate for methylprednisolone I}}{\text{average of rates of methylprednisolone I and II}} \times 100$.

TABLE II.—ESTIMATIONS OF FILM THICKNESSES (h)

In Vitro Method	Methylprednisolone I		Methylprednisolone II	
	D/h,[a] cm. hr.$^{-1}$	h,[b] cm.	D/h,[a] cm. hr.$^{-1}$	h,[b] cm.
Hanging pellet method (7)	0.165	0.11	0.208	0.087
Pellet holder method, Wruble machine (12), 6 r.p.m.	1.73	0.010	1.88	0.0096
Pellet holder method, Wruble machine (12), 12 r.p.m.	2.35	0.0077	1.95	0.0092
Pellet holder method, machine of Souder and Ellenbogen (11), 40 r.p.m.	5.37	0.0034	4.65	0.0039

[a] Obtained by dividing the rate of dissolution by the solubility of methylprednisolone I or II. The latter values are 0.1174 and 0.141 mg. ml.$^{-1}$ at 37°, respectively. [b] Obtained by dividing D, namely 1.8×10^{-2} cm.² hr.$^{-1}$, by the suitable value of D/h.

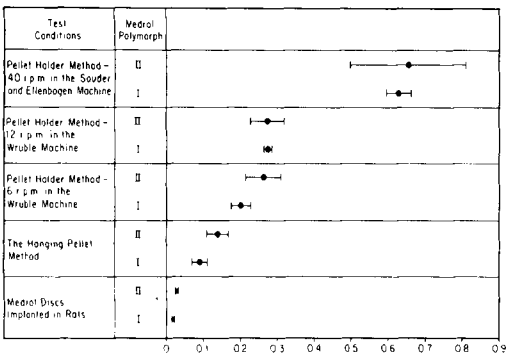

Fig. 2.—Dissolution rates and their 95% confidence intervals for methylprednisolone polymorphs I and II determined under various conditions.

The implications of these results with respect to *in vitro* tests for timed release or prolonged action oral products are important. Before an *in vitro* test is accepted as a routine procedure to be applied to a wide variety of products, one should be sure that the test can differentiate differences between lots of a given product and between different products which do show a difference *in vivo* by their intended route of administration. Otherwise the control test is just a routine operation without any meaning.

Film Thicknesses.—Parrott, Wurster, and Higuchi (2) calculated a diffusion layer or film thickness of 3.28×10^{-3} cm. for benzoic acid dissolving in pure water under their experimental conditions. This is very comparable to the values of 3.4×10^{-3} and 3.9×10^{-3} cm. which we estimated for methylprednisolone I and II, respectively, when the $^3/_8$ inch pellets in our tests were held in the center of a 4-fl. oz. bottle containing 120 ml. of water rotated in the machine described by Souder and Ellenbogen (11). Under the usual conditions in which this machine is used to test timed release or prolonged action products, the effective film thickness would be much less and the intensity of agitation much greater since the tablets or granules are allowed to fall freely through the fluid during the test and are not held rigidly in the center of the bottle as in our tests. Hence, one might expect much greater difficulty in distinguishing between lots of a product by the test used by the Pharmaceutical Contact Section (9) than we experienced in our tests.

In the hanging pellet test we estimated a diffusion layer or film thickness of 0.11 and 0.087 cm. for methylprednisolone I and II, respectively. These film thicknesses are 33 and 22 times, respectively, those estimated when the machine of Souder and Ellenbogen was used. When the Wruble machine, rotating at 6 r.p.m., was used in our tests, the film thicknesses estimated were 1.0×10^{-2} and 9.6×10^{-3} cm. for methylprednisolone I and II, respectively. These are about one-tenth the film thicknesses estimated for the hanging pellet tests.

Hence, diminishing the film thickness by increasing intensity of agitation beyond this point led to failure to distinguish between the polymorphs with respect to dissolution rate.

APPENDIX

Some Literature Concerning Kinetics of Dissolution and Effect of Intensity of Agitation.—Hixson and Crowell (8) derived the relationship shown as Eq. 1.

$$dW/dt = kS(C_s - C) \qquad \text{(Eq. 1)}$$

where $-(dW/dt)$ is the rate of change of weight of the dissolving substance or velocity of solution of a dissolving substance and dW/dt is the rate of appearance of the dissolving substance in the dissolution medium, k is a constant with dimensions distance/time, S is the surface area of the dissolving substance, C_s is the concentration of a saturated solution of the dissolving substance in the dissolution medium, and C is the concentration of the dissolving substance in the dissolution medium at time t. The value of the constant k has been shown (8) to be dependent upon the temperature, the structure of the surface of the dissolving substance, the arrangement of the apparatus, and the rate of stirring or agitation (or the velocity of the dissolution medium across the surface of the dissolving substance).

When C is always negligible in comparison to C_s, Nelson (15) showed that Eq. 1 reduces to

$$dW/dt = kSC_s \qquad \text{(Eq. 2)}$$

Integrating Eq. 2, with S considered a constant, yields

$$W = kSC_st \qquad \text{(Eq. 3)}$$

Polli (4) reviewed the literature concerning the effect of agitation speed on the velocity of heterogeneous reactions. Investigators have usually reported the following relationship

$$K = aV^b \qquad \text{(Eq. 4)}$$

where K is a constant similar to k in Eq. 1, V is the velocity of agitation, and a and b are constants. In accordance with the Nernst-Brunner theory, the value of the exponent b has been found to be 1, or approximately 1, when the reaction taking place was diffusion controlled. On the other hand, for reactions controlled by the rate of the interfacial reaction, values for b were found to approach zero. After studying the dissolution of inorganic salts, Davion (5) reported he obtained a value of 0.5 for the constant b. Hence, in terms of the symbols used above Davion found

$$k = a\sqrt{V} \qquad \text{(Eq. 5)}$$

Van Krevelen and Krekels (16) found a similar relationship for the rate of dissolution of solid substances when they are contacted with a liquid current in the form of a granular bed. Hence a plot of k *vs.* V would pass through the origin and be parabolic with the slope of the line approaching zero, but always positive as V increases.

Linton and Sherwood (6) and Davion (5) applied the methods of investigation applicable to heat transfer to the kinetics of dissolution. The former authors measured the rate of solution of slightly soluble fused salts from the inner walls of cast tubes through which water was forced both in streamline and turbulent flow at velocities from 0.5 to 500 cm./sec. Data were also obtained on the rate of solution of cast plates, cylinders, and spheres of the same materials with water velocities of 5 to 90 cm./sec. For streamline flow in tubes their data checked reasonably well with the Leveque equation, which is

$$\frac{C - C_0}{C_s - C_0} = 5.5\left(\frac{R}{D_L\rho L}\right)^{-2/3} \qquad \text{(Eq. 6)}$$

where C is concentration of exit stream in Gm./ml., C_0 is the concentration of inlet stream, C_s is concentration at saturation, R is the flow rate of the fluid in Gm./sec., D_L is molecular diffusivity in cm.^{2}/sec., ρ is the density in Gm./ml., and L is the length of the tube in cm. Their results supported the assumption of parabolic flow, molecular diffusion, and saturation at the solid-liquid interface.

REFERENCES

(1) Levy, G., and Hayes, B. A., *New Engl. J. Med.*, **262**, 1053(1960).
(2) Parrott, E. L., Wurster, D. E., and Higuchi, T., THIS JOURNAL, **44**, 269(1955).
(3) Higuchi, W. I., Parrott, E. L., Wurster, D. E., and Higuchi, T., *ibid.*, **47**, 376(1958).
(4) Polli, G., "Investigations of Drug Release from Solids: Effect of Adsorption and Viscosity on the Dissolution Rate," Dissertation, The University of Wisconsin.
(5) Davion, M., "Étude sur la Vitesse de Dissolution des Sels Cristallisés," Thesis No. 370, Faculty of Sciences, The University of Paris, 1953.
(6) Linton, W. H., Jr., and Sherwood, T. K., *Chem. Eng. Progr.*, **46**, 258(1950).
(7) Nelson, E., THIS JOURNAL, **47**, 297(1958).
(8) Hixson, A. W., and Crowell, J. H., *Ind. Eng. Chem.*, **23**, 923(1931).
(9) Krueger, E. O., Minutes of the 76th meeting of the Pharmaceutical Contact Section of the Pharmaceutical Manufacturers Association, Shoreham Hotel, Washington, D. C., March 21 and 22, 1961.
(10) Wagner, J. G., "Memorandum to the Tablet Subcommittee of the Contact Section of The Pharmaceutical Manufacturers Association," October 20, 1960.
(11) Souder, J. C., and Ellenbogen, W. C., *Drug Standards*, **26**, 77(1958).
(12) Wruble, M. S., *Am. J. Pharm.*, **102**, 318(1930).
(13) Ballard, B. E., and Nelson, E., *J. Pharmacol. Exptl. Therap.*, **135**, 120(1962).
(14) Higuchi, W. I., Rowe, E. L., and Hiestand, E. N., submitted for publication.
(15) Nelson, E., THIS JOURNAL, **46**, 607(1957).
(16) Van Krevelen, D. W., and Krekels, J. T. C., *Rec. trav. chim.*, **69**, 1519(1950).