Dissolution Rates under Sink Conditions

PAUL J. NIEBERGALL, EDWIN T. SUGITA, and ROGER L. SCHNAARE

Abstract □ Equations are given for the dissolution of a drug into an aqueous phase overlayered with a lipid phase, in which back transfer from the lipid phase is assumed possible. Consideration of both the presence and absence of back transfer, assuming in each that the amount of drug in the aqueous phase either approaches saturation or remains negligible, results in four equations for following the amount of drug in the lipid phase *versus* time. Three of the four equations are identical in form, predicting that after a brief lag time, a plot of the amount of drug in the lipid phase *versus* time will become linear. However, only in the instance for which no back transfer occurs and for which the buildup of drug in the aqueous phase is negligible (Case IV) is the slope of the line equal to the zero-order dissolution-rate constant, k_0. In the other cases, the slope represents a fraction of k_0, depending upon the relative rates of dissolution, partitioning, and back transfer. Thus, it becomes important to be completely certain that the conditions under which dissolution studies into a "perfect sink" are carried out do actually meet the requirements of Case IV.

Keyphrases □ Dissolution rates under sink conditions—equations presented considering back transfer □ Drug dissolution—equations developed considering back transfer □ Sink conditions, dissolution rates—role of back transfer from lipid phase, equations presented

The concept of dissolution of a solid into an aqueous phase followed by partitioning into a lipid phase, presumably acting as a perfect sink, was investigated (1, 2) and found to be applicable to dissolution-rate and dissolution–partitioning-rate studies. In both of these reports, it was assumed that the lipid phase acted as a perfect sink, and no transfer of the drug from the lipid phase back into the aqueous phase could occur. However, the data of Khalil and Martin (3) for systems containing similar phases to those used previously (1, 2) indicate that back transfer from the lipid phase could easily occur. It was decided, therefore, to investigate all of the possible conditions that could exist when a solid dissolves into an aqueous phase overlayered with an organic phase.

THEORY

The dissolution of a solid into an aqueous phase with subsequent partitioning into an immiscible organic phase may be represented by the following kinetic model:

$$A \xrightarrow{k_1} B \underset{k_3}{\overset{k_2}{\rightleftharpoons}} C \qquad \text{(Eq. 1)}$$

in which A represents the amount of drug in the solid form, B represents the drug dissolved in the aqueous phase, and C represents the amount of drug in the lipid phase; k_1, k_2, and k_3 are first-order rate constants.

The following differential equations can be used to describe the system:

$$-dA/dt = k_1(W_s - B) = k_0 - k_1B \qquad \text{(Eq. 2)}$$

$$dB/dt = k_1(W_s - B) - k_2B + k_3C = k_0 - (k_1 + k_2)B + k_3C \qquad \text{(Eq. 3)}$$

$$dC/dt = k_2B - k_3C \qquad \text{(Eq. 4)}$$

Table I—Definition of the Constants α, β, and γ of Eq. 7 for Cases II, III, and IV: $C = \alpha t - \beta(1 - e^{-\gamma t})$

Case	α	β	γ
II	$\frac{k_0k_2}{(k_2 + k_3)}$	$\frac{k_0k_2}{(k_2 + k_3)^2}$	$k_2 + k_3$
III	$\frac{k_0k_2}{(k_1 + k_2)}$	$\frac{k_0k_2}{(k_1 + k_2)^2}$	$k_1 + k_2$
IV	k_0	k_0/k_2	k_2

in which W_s represents the amount of drug needed to saturate the given volume of the aqueous phase, and k_0 is the zero-order dissolution-rate constant. These equations were integrated under the following four sets of conditions, using Laplace transforms:

Case I: $k_3 \sim k_2$ and $B \sim W_s$ (back transfer occurs)
Case II: $k_3 \sim k_2$ and $B \ll W_s$ (back transfer occurs)
Case III: $k_3 = 0$ and $B \sim W_s$ (no back transfer)
Case IV: $k_3 = 0$ and $B \ll W_s$ (no back transfer)

The resulting equation for the appearance of drug in the lipid phase for Case I is:

$$C = \frac{k_0k_2}{k_1k_3} + \frac{k_0k_2}{m_1(m_1 - m_2)}e^{-m_1t} - \frac{k_0k_2}{m_2(m_1 - m_2)}e^{-m_2t} \qquad \text{(Eq. 5)}$$

in which:

$$m_1 = -1/2\left[-(k_1 + k_2 + k_3) + \sqrt{(k_1 + k_2 + k_3)^2 - 4k_1k_3}\right] \qquad \text{(Eq. 6a)}$$

$$m_2 = -1/2\left[-(k_1 + k_2 + k_3) - \sqrt{(k_1 + k_2 + k_3)^2 - 4k_1k_3}\right] \qquad \text{(Eq. 6b)}$$

A plot of C *versus* time would be constantly curving until equilibrium between the aqueous and lipid phases had been attained. The equilibrium amount of drug in the lipid phase at this point, C_∞, would be equal to k_0k_2/k_1k_3 or W_sk_2/k_3. Use of the feathering or backward projection technique on Eq. 5 would then enable m_1 and m_2 to be evaluated. The sum of $m_1 + m_2$ gives the quantity $(k_1 + k_2 + k_3)$, while the product of m_1m_2 gives k_1k_3. However, unless either k_2 or k_3 is known from an independent partitioning study in which the drug in solution is followed kinetically as it transfers between the two phases, the zero-order rate constant, k_0, cannot be evaluated from Eq. 5. The nonlinearity in a plot of C *versus* time for Case I distinguishes it from the other three cases, all of which show linear segments when C is plotted *versus* time.

Cases II, III, and IV are interesting in that they can all be represented by the following general equation:

$$C = \alpha t - \beta(1 - e^{-\gamma t}) \qquad \text{(Eq. 7)}$$

in which α, β, and γ are constants which are defined for each case in Table I. The importance of Eq. 7 is that it illustrates the fact that a plot of C *versus* time for these three cases would be identical in shape, becoming linear when $e^{-\gamma t}$ becomes much less than 1. However, only in Case IV would the slope of the linear portion of the plot be equal to the zero-order dissolution-rate constant, k_0. In the other cases, the slope would represent only a fraction of k_0, depending upon the relative rates of dissolution, partitioning, and back transfer. Furthermore, for Cases II and III, the zero-order dissolution-rate constant cannot be obtained from plots of Eq. 7. Either k_2 or k_3 must be obtained from independent partitioning-rate studies. Since these three cases (Cases II, III, and IV) cannot be distinguished from each other by a plot of C *versus* time, the lipid phase must be chosen with great care to ensure that perfect sink

conditions do actually exist. For this to happen, the partitioning rate constant, k_2, must be appreciably greater than both k_1 and k_3.

REFERENCES

(1) P. J. Niebergall, M. Y. Patil, and E. T. Sugita, *J. Pharm. Sci.*, **56**, 943(1967).

(2) M. Gibaldi and S. J. Feldman, *ibid.*, **56**, 1238(1967).

(3) S. A. Khalil and A. N. Martin, *ibid.*, **56**, 1225(1967).

ACKNOWLEDGMENTS AND ADDRESSES

Received April 26, 1971, from the *Department of Pharmacy, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104*

Accepted for publication July 8, 1971.

Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, San Francisco meeting, March 1971.

Hepatic Injury Caused by *N*-γ-Phenylpropyl-*N*-benzyloxyacetamide: A Light and Electron Microscopic Study

K. KOVACS, B. D. GARG, and J. D. KHANDEKAR*

Abstract □ W-1372 (*N*-γ-phenylpropyl-*N*-benzyloxyacetamide), a hypolipemic and antiatherosclerotic compound, given orally to rats in corn oil induces fatty degeneration of the hepatocytes. Electron microscopically, it was possible to note accumulation of lipid droplets and liposomes, progressive dilatation, disorganization and degranulation of the rough-surfaced endoplasmic reticulum, and injury of the mitochondria as well as distension of the sacs of the Golgi apparatus with disappearance of their vacuolar content. Possibly, a defect in hepatocellular lipoprotein synthesis or release might be responsible for this lipid accumulation.

Keyphrases □ *N*-γ-Phenylpropyl-*N*-benzyloxyacetamide—hepatic injury, detection by light and electron microscopic study □ Hepatic lipid accumulation—induced by W-1372, detected by electron microscopy □ Electron microscopy—detection of liver damage induced by *N*-γ-phenylpropyl-*N*-benzyloxyacetamide

N-γ-Phenylpropyl-*N*-benzyloxyacetamide (W-1372) lowers the blood level of cholesterol, phospholipids, and triglycerides (1, 2). In addition, it reduces the extent of aorta atherosclerosis in squirrel monkeys and rabbits fed on a cholesterol and fat-rich diet (1–3). The light and electron microscopic changes found in the liver of rats treated with this hypolipemic and antiatherosclerotic compound are described here.

EXPERIMENTAL

Materials and Methods—Twenty-four female ARS/Sprague-Dawley rats[1], with a mean initial body weight of 95 g. and maintained *ad libitum* on Purina laboratory chow[2] and tap water, were divided into four equal groups, one of which served as untreated controls. The second group received 1 ml. of corn oil, by stomach tube, twice daily for 3 days. The animals of Groups 3 and 4 were given 5 or 10 mg. of W-1372 (Wallace), respectively, in 1 ml. of corn oil twice daily *per os*, also for 3 days. All treated rats were killed without anesthesia, by destruction of the medulla oblongata, 16 hr. after the last gavage. The untreated animals were sacrificed at the same time.

For light microscopic examination, fresh liver tissue was fixed in alcohol formol or neutral formaldehyde and embedded in paraffin. Sections (4–8 μ thick) were cut and stained with hematoxylin–phloxine or by the Periodic Acid Schiff (PAS) technique. Frozen sections were also cut and stained with Oil red O or Sudan black B.

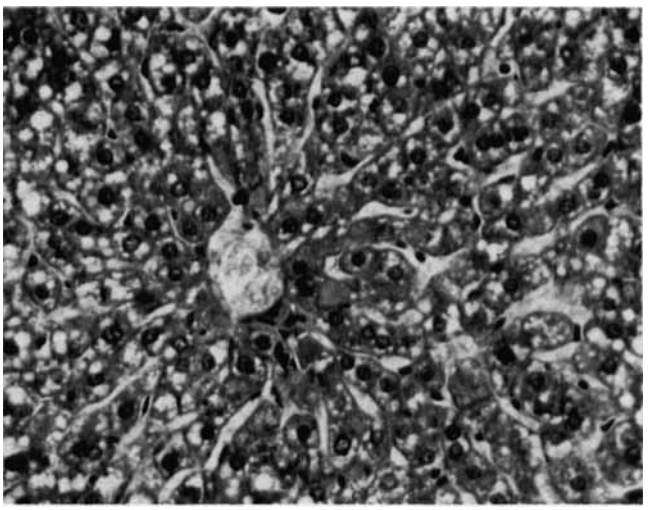

Figure 1—*Liver of a rat treated with 10 mg. of W-1372 for 3 days. Numerous lipid droplets are seen in the cytoplasm; hematoxylin–phloxine.* (×300)

Material for electron microscopic examination was obtained from three rats per group by excising a small portion of tissue from the left lateral lobe of the liver and placing it in Millonig's osmium fixative where it was minced into tiny cubes and kept for 1 hr. at 4°. The specimens were then dehydrated in graded ethanol and embedded in Epon resin. Sections (0.5 μ thick) were cut on a microtome[3], stained with toluidine blue, and examined under a light microscope. Ultrathin sections (approximately 50 nm.) were cut from selected midzonal areas, stained with uranyl acetate and Reynolds' lead citrate, and examined under an electron microscope[4].

RESULTS

The livers of rats treated with 10 mg. of W-1372 were enlarged, smooth-surfaced with somewhat rounded edges, pale brownish-yellow, and more fragile than those of the controls. A mild yellow discoloration was also noticeable after treatment with 5 mg. of W-1372, but other gross changes were not evident. No alterations were observed in the livers of animals that received corn oil alone.

[1] Madison, Wis.
[2] Ralston Purina Co. of Canada.
[3] Porter-Blum MT-2.
[4] Carl Zeiss EM 9A.