Surface Area Term in Myhill–Piper Equation

Keyphrases ☐ Antacids, dissolution rate—surface area term, Myhill–Piper equation ☐ Surface area—effect on dissolution rate, antacids, Myhill-Piper equation ☐ Dissolution, antacids—surface area term, Myhill–Piper equation

Sir:

A paper by Myhill and Piper (1) is frequently quoted in antacid work. In their treatment, using their nomenclature, $r(t)$ = amount of antacid present in the stomach at time t, κ = emptying rate constant of excess antacid, s = rate per unit time at which antacid is neutralized by acid secreted by the stomach, and τ = "starting index" or time after administration of antacid when exponential emptying rate commences.

This leads to the differential equation:

$$dr(t)/dt = -\kappa r(t) - s \qquad \text{(Eq. 1)}$$

which can readily be integrated. This equation, although useful as an initial equation, does not hold in general, since it assumes a constant dissolution rate, s, of the antacid independent of time. The overall dissolution rate would be a function of surface area, which changes with time, and would be related to $[r(t)]^{2/3}$. It furthermore depends on pH (which also changes with time), and Notari and Sokoloski (2) showed that a cube root law ensues when proper treatment is applied. Denoting the pH (or time) dependence of s, $\phi(t)$, changes Eq. 1 to:

$$dr(t)/dt = -\kappa r(t) - q\phi(t)[r(t)]^{2/3} \qquad \text{(Eq. 2)}$$

which is sufficiently complicated to prohibit solution in closed form.

Of course, $\phi(t)$ and $r(t)$ may change at different rates with time. If the stomach emptying time is long, then pH [and $\phi(t)$] may change fairly rapidly with small amounts of dissolved antacid [*i.e.*, in this situation, $r(t)$ does not change much], so that under these circumstances $\phi(t)$ would change much more rapidly than $r(t)$. In such a case, the circumstances are simplified sufficiently to allow Eq. 2 to be solved; it now can be put in the form:

$$dr(t)/dt = -\kappa r(t) - s(t) \qquad \text{(Eq. 3)}$$

where $s(t) = q\phi(t)[r(t)]^{2/3} \sim \text{constant} \cdot \phi(t)$. When the dissolving medium has constant volume, then [as may be implied from the work of Notari and Sokoloski (2)] the volume V of hydrochloric acid consumed at time t can be expressed by:

$$V_\infty - V = V_\infty e^{-\omega t} \qquad \text{(Eq. 4)}$$

where infinity denotes final state and where ω is a function of the original surface area. Therefore, with the assumption made:

$$s(t) = V_\infty \omega e^{-\omega t} = \alpha e^{-\omega t} \qquad \text{(Eq. 5)}$$

and Eq. 3 now takes the form:

$$dr(t)/dt + \kappa r(t) + \alpha e^{-\omega t} = 0 \qquad \text{(Eq. 6)}$$

The solution to Eq. 6 is obtained *via* the integration factor $e^{(\kappa t+\gamma)}$ and is:

$$r(t) = -\frac{\alpha}{\kappa - \omega} e^{-\omega t} + Ce^{-\kappa t} \qquad \text{(Eq. 7)}$$

where C would depend on initial conditions (*e.g.*, on τ).

The point to be stressed is that the surface area is related to $[r(t)]^{2/3}$ and that s in Eq. 1 is a function of this (and of pH); therefore, *surface area* must be a part of the resulting equations.

(1) J. Myhill and D. W. Piper, *Gut*, **5**, 581(1964).

(2) R. E. Notari and T. D. Sokoloski, *J. Pharm. Sci.*, **54**, 1500 (1965).

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Received July 25, 1972.
Accepted for publication August 30, 1972.
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Effect of Microsomal Activation on Interaction between Isophosphamide and DNA

Keyphrases ☐ Isophosphamide interaction with DNA—effect of microsomal activation ☐ DNA interaction with isophosphamide—effect of microsomal activation ☐ Microsomal activation—effect on isophosphamide interaction with DNA

Sir:

We previously showed that the activation to alkylating materials of isophosphamide [3-(2-chloroethyl)-2-(2-chloroethyl)aminotetrahydro-1,3,2-oxazaphosphorine-2-oxide, I][1], an antineoplastic analog of cyclophosphamide, is mediated through the NADPH-dependent liver microsomal oxidase system (1) and is increased by pretreatment of animals with phenobarbital (2). We have now investigated the effect of this activation on DNA synthesis and on the reaction between I and DNA *in vitro* in order to clarify the effect of this activation on antitumor activity.

[1] NSC-109724; Ifosfamide, Mead Johnson.

Table I—Effect of Microsomal Activation of Isophosphamide on Thymidine Incorporation into DNA[a]

Microsomes	Isophosphamide	d.p.m. × 10^{-2}/mcg. DNA
+	−	173 ± 6
−	+	155 ± 20
+	+	82 ± 10

[a] Microsomal suspension (1 ml. = 500 mg. liver) was incubated as described under methods with and without isophosphamide (5.6 m*M*) with an incubation mixture consisting of $MgCl_2$ (100 m*M*), 80 μl.; Na_2HPO_4-KH_2PO_4 buffer (0.4 *M*, pH 7.4), 75 μl.; glucose-6-phosphate (200 m*M*), 85 μl.; NADP (20 m*M*, pH 7.0), 38 μl.; and glucose-6-phosphate dehydrogenase, 20 μl. (2 enzyme units).

After addition of 1 ml. of a rat liver microsomal fraction (≡ 500 mg. wet weight of liver), L-1210 cells (2.7×10^6 cells/3 ml. of Fischer's medium for leukemic cells of mice), cultured by conventional techniques in spinner flasks (3), were incubated for 1 hr. by shaking in air at 37°. The added microsomal fraction, prepared as previously described (4), also contained an NADPH-regenerating system (5) and I (5.6 m*M*, final concentration). This incubate totaled 4 ml. Thymidine-(methyl-^{3}H) (3 μc., 1 c./mmole)[2] was then added and incubation continued for a further 2 hr. The reaction was terminated by the addition of ice-cold trichloroacetic acid (20%, 4 ml.), and DNA was isolated (6) and estimated colorimetrically (7) and by liquid scintillation counting. The results (Table I) indicate that microsomal activation of I leads to inhibition of the incorporation of thymidine into DNA.

To determine the consequences of this inhibition of DNA synthesis on the DNA molecule, we treated calf thymus DNA with I alone and with I after microsomal activation. The microsomal system was retained within a dialysis sac, and calf thymus DNA was dissolved in the dialysis buffer. The DNA was isolated according to Hirt (6), and 0.4 ml. of an extensively dialyzed solution was layered on an alkaline 5–20% sucrose gradient (8)

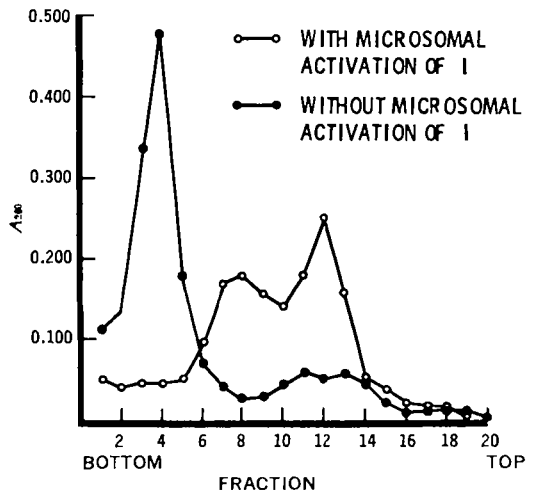

Figure 1—*Effect of microsomal activation of I on calf thymus DNA, showing sucrose zone sedimentation of calf thymus DNA as described in the text. The results of a representative experiment are shown; the pattern was identical in repeat experiments.*

[2] Schwarz/Mann, Orangeburg, N. Y.

Table II—Effect of Microsomal Activation of Isophosphamide-^{14}C on Alkylation of Calf Thymus DNA *In Vitro*[a]

Pretreatment	d.p.m./mg. DNA	Moles Isophosphamide/ Mole DNA[b]
Saline	620	2.9
Phenobarbital	1074	5.1
Incubation without microsomes	170	—

[a] Isolation and incubation of microsomes as described in Table I. [b] Molecular weight of calf thymus DNA is assumed to be one-half million.

and centrifuged for 10 hr., at 35,000 r.p.m., in a centrifuge[3]. Four-drop fractions were collected, and each was diluted with water (1 ml.). The A_{260} was determined on each diluted fraction. Representative fractions under each peak were scanned from 220 to 320 nm., and all indicated nucleic acid without contamination of protein. The results (Fig. 1) show that the liver microsomal metabolites of I cause the production of low molecular weight DNA.

To determine if this inhibition and chain scission are associated with the binding of metabolites of I to DNA, the microsomal fraction was prepared from the livers of rats which had received phenobarbital intraperitoneally (55 mg./kg. body weight daily × 3) and from rats which had received only saline pretreatment. Isophosphamide-^{14}C (11.5 μmoles) (0.19 μc./μmole) and calf thymus DNA (3 mg.) were incubated with a microsomal fraction (≡250 mg. wet liver weight) and an NADPH regenerating system, as described previously, in a final volume of 3 ml. for 40 min. at 37°. Ice-cold ethanol was used to terminate the reaction, and DNA was extracted by the method of Hirt (6). After extensive dialysis, the Hirt supernate was without radioactivity. The Hirt pellet, suspended in 10 ml. of 1 *M* NaCl, was dialyzed for 3 days at room temperature against 2 × 12 l. of standard saline citrate buffer. The dialyzed Hirt pellet was clarified by centrifugation at 10,000 r.p.m. for 15 min., diluted with water (10 ml.), and lyophilized. The precipitates were dissolved in 10 m*M* NaOH, and aliquots were assayed for radioactivity. It was found (Table II) that microsomal activation promotes the incorporation of radioactivity into DNA, and this incorporation is increased when the animals are pretreated with phenobarbital, in line with the enhancement of microsomal metabolism of I previously shown with phenobarbital pretreatment (2).

Isophosphamide resembles cyclophosphamide in having essentially no cytotoxic activity *in vitro* but in being an effective antitumor agent *in vivo* (9). The present findings that its liver microsomal activation leads to products that inhibit incorporation of thymidine into L-1210 cell DNA and that causes alkylation and fragmentation of calf thymus DNA are therefore of considerable interest in relation to the antitumor activity of I *in vivo* but not *in vitro*.

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(2) L. M. Allen and P. J. Creaven, *Proc. Int. Congr. Pharmacol., 5th*, in press.

[3] Beckman model L, using a SW50.1 rotor.

(3) G. J. Dixon, E. A. Dulmadge, and F. M. Schabel, Jr., *Cancer Chemother. Rep.*, **50**, 247(1966).
(4) P. J. Creaven, D. V. Parke, and R. T. Williams, *Biochem. J.*, **96**, 390(1965).
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Received June 29, 1972.
Accepted for publication September 1, 1972.
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BOOKS

REVIEWS

Organic Reactions in Steroid Chemistry, Volumes I and II. Edited by JOHN FRIED and JOHN A. EDWARDS. Van Nostrand Reinhold Co., 450 W. 33rd St., New York, NY 10001, 1972. 15 × 23 cm. Price: Vol. I (xv + 510 pp.) $26.00; Vol. II (xii + 466 pp.) $24.00; for the 2-volume set $45.00.

This work consists of up-to-date critical reviews of the mechanism and scope of a number of types of transformations which have proven to be particularly useful in steroid chemistry. Most authors are experts in the areas which they discuss and, in consequence, they are able to offer many practical suggestions. Selected experimental procedures are given for all recommended reactions. While other authorities might occasionally question the procedures recommended by a given author, those presented are typically procedures which have proven to be particularly reliable.

Volume I begins with a discussion by Dryden of metal–ammonia reductions and reductive alkylations. The excellence of this chapter is marred only by the lack of any mention of related reactions in higher amines or in trimesitylborane. Following authoritative reviews of ketone reductions by hydride reagents, diborane, and iridium complexes by Wheeler and Wheeler and of hydrogenation by Augustine, Tökés and Throop present a definitive account of techniques which have been used for selectively deuterating steroids. Rasmusson and Arth then give a particularly lucid account of selective oxidation of hydroxyl groups in polyfunctional steroids. A long (109 pages), but selective, chapter by Beard provides a good overview of methods for introducing double bonds. This chapter includes a discussion of the preparation of haloketones which is generally quite useful but which does slight several methods and which fails to make clear that 21-halo-20-ketones readily rearrange to 17α-halo-20-ketones. Both of these deficiencies are rectified in a chapter by Oliveto in Volume II (but without cross-referencing or adequate indexing). A chapter by Gardi and Ercoli gives a thorough coverage of a number of methods for selectively protecting carbonyl and hydroxyl groups but fails to even mention the use of *tert*-butyl ethers and silyl ethers for the protection of hydroxyl groups or of the tetramethyl bismethylenedioxy group for the protection of the dihydroxyacetone side chain. The first volume concludes with an excellent review by Josef Fried and N. A. Abraham of methods for introducing fluorine into steroids.

Volume II begins with a chapter by Matthews and Hassner on the synthesis of oxiranes, aziridines, and episulfides. This is followed by a review by Laurent and Wiechert of methods for selectively introducing alkyl and methylene units into steroids. The Wittig reaction is considered in the following chapter, by Oliveto, which focuses on methods for interconverting androstanes and pregnanes and on procedures for oxygenating the side chain of pregnanes. Heusler and Kalvoda contributed a chapter on functionalization of angular methyl groups and on the conversion of C-19 functionalized steroids to 19-norsteroids. The photochemistry of chromophores containing the carbonyl group is reviewed by Schaffner. Ring expansion and simultaneous ring contraction–ring expansion reactions are reviewed by Boswell, and ring contraction reactions are covered by Scribner. Although the material presented by Oliveto, Boswell, and Scribner is excellent, it is to be regretted that none of them included a discussion of the sequences which have been developed for converting D-homosteroids to 20-ketopregnanes.

The utility of these books, particularly for the nonsteroid chemist, could have been improved by a more thorough indexing. Despite this lack, all steroid chemists and any others who may be concerned with analogous reactions (provided that they are put off neither by the title nor by the cost) will want these volumes at their desks.

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Civilization & Science in Conflict or Collaboration? A Ciba Foundation Symposium. American Elsevier, 52 Vanderbilt Ave., New York, NY 10017, 1972. 227 pp. 16 × 24 cm.

The symposium was held in late June 1971, and was attended by scientists, politicians, economists, and historians from several nations. They attempted to review the origins of today's conflict and reexamine the traditional lack of value orientation in science. The antiscience movement is considered along with such problems as scientific involvement in politics and the effects of social conflicts and recent cutbacks in funds on the creative work of scientists.

Staff Review ■

Analytical Emission Spectroscopy, Vol. I, Part II. Edited by E. L. GROVE. Marcel Dekker, Inc., 95 Madison Ave., New York, NY 10016, 1972. ix + 570 pp. 15 × 23 cm. Price $35.75.

This book contains four sections on excitation of spectra, flame spectrometry, qualitative and semiquantitative analysis, and quantitative analysis. Each chapter covers a particular area and coordinates the earlier basic developments with the more recent work in the field.

Staff Review ■