Condensing Effect of Cholesterol: A Physical Model

JOEL L. ZATZ

Abstract □ A physical model is proposed to account for the "condensing effect" of cholesterol in mixed monolayers. If the other component of the monolayer has its polar groups in contact, a cholesterol molecule may interact sufficiently with the hydrocarbon tails to orient them vertically, thus creating free space for itself. In cholesterol-rich films, the polar groups of the phospholipid do not contribute to the area occupied. Both effects lead to a reduction in the observed mean molecular area.

Keyphrases □ Cholesterol—condensing effect in mixed monolayers, physical model, equations, π–A plots □ Membranes—physical model proposed for condensing effect of cholesterol in mixed monolayers, equations, π–A plots □ Films, monolayer—physical model proposed for condensing effect of cholesterol □ Monomolecular films—physical model proposed for condensing effect of cholesterol

Monomolecular films provide a useful model for studying the properties of biological membranes. Of particular interest are mixed monolayers containing cholesterol and a phospholipid (1–8). In most cases, the behavior of these mixed systems has been analyzed by plotting the mean area per molecule as a function of monolayer composition at constant surface pressure. If the molecular area occupied by each component in the mixed film is the same as in a monolayer of the pure material, the mean area plot is linear. This type of behavior is often observed in mixed monolayers containing cholesterol, but, more frequently, the mean area plot exhibits negative deviations from linearity. The apparent area occupied by the phospholipid in the mixed monolayer is less than that in a pure monolayer of the same material, so a "condensing effect" has been ascribed to cholesterol.

There is general agreement that cholesterol will cause condensation of another lipid only if that substance exhibits an expanded phase in its π–A (area) isotherm. But some practical problems cloud interpretation of the published work. Working with ostensibly the same "pure" materials, investigators have reported π–A isotherms and mean molecular area plots that disagree with other published reports. Furthermore, the experimental error involved in determination of the mean molecular area may be of the order of ± 2Å^2 (5, 8). Perhaps some "breaks" in published mean molecular area plots are due to impurities and experimental error rather than specific geometrical arrangements at particular mole ratios.

Some workers have attributed the condensation in mixed films largely to van der Waals' interactions between the hydrocarbon chains of the components (2, 6). On the other hand, Shah and Schulman (4) proposed that certain lipids form cavities due to steric effects (unsaturated fatty chains) or thermal motion (saturated fatty chains) which are capable of including cholesterol molecules. Neither theory is quantitative or capable of predicting what type of behavior will be encountered in untested systems.

In this paper, a quantitative model (the heads-or-tails model) is proposed to explain the effect of cholesterol in mixed monolayers.

THEORY

From its force–area curve, the cholesterol molecule at an air–water interface may be visualized as a rigid structure, oriented so that its single polar group is in contact with the subphase. At nearly all values of surface pressure, these molecules are close packed; the nonpolar portions are in contact and determine the area occupied by each molecule. Long-chain lipids (in a monolayer of the pure material) may be in "tail contact," in which case the molecules are close packed and vertically oriented. In this state, the area per molecule corresponds very nearly to that of the hydrocarbon portion of the molecule. This description applies to substances that form condensed monolayers (9). Some materials that exhibit π–A curves characterized as "expanded" may also be in tail contact at higher values of surface pressure, *e.g.*, oleic acid. Although the π–A curve of oleic acid is of the expanded type, it is generally agreed that the area per molecule at high surface pressures is that of the hydrocarbon portion of the molecule, since further reduction in molecular area is prevented by the "kink" in the hydrocarbon chain due to the *cis*-double bond (10).

It is also possible, in the case of a coherent film, that the hydrocarbon tails are not closely packed. The molecules may be in "head contact." The effective area occupied by each molecule is then determined by the size of the polar group and associated water molecules (11).

Some substances, as shown by their π–A curves, are in a state of tail contact at nearly all values of surface pressure, *e.g.*, cholesterol and dipalmitoyl phosphatidyl ethanolamine at 22° (2). Other lipids such as L-α-dipalmitoyl lecithin at 25° are in tail contact at high values of π but in head contact at lower surface pressures where the film is liquid (12). When the hydrated polar heads fix the surface area, the hydrocarbon tails are free to assume a random configuration (13). If a molecule of cholesterol is added, there may be sufficient intermolecular interaction (due to van der Waals' forces) to cause the hydrocarbon tails to orient vertically, thereby allowing the cholesterol molecule to fit into some free space in the surface.

In the following discussion, it is assumed that: *(a)* the two components of the mixed monolayer are miscible at the surface, *(b)* there are no specific polar interactions between the two components, and *(c)* hydrocarbon chains are vertically oriented when the two components are in contact.

The mean molecular area, A_m, in a mixed monolayer at constant surface pressure is given by:

$$A_m = \sum A_i X_i \qquad \text{(Eq. 1)}$$

where A_i is the effective area per molecule of the ith component, and X_i is the mole fraction of the ith component in the mixture. Furthermore, let A_i^0 represent the area per molecule in a monolayer containing only the ith component under the same surface pressure as that of the mixed monolayer. For a two-component system, one may write:

$$A_m = A_1 X_1 + A_2 X_2 \qquad \text{(Eq. 2)}$$

in which the subscripts identify the components. Let 1 represent cholesterol and 2 a phospholipid. The dependence of the mean molecular area on monolayer composition will be a function of the properties of the pure phospholipid monolayer, as described in the following four cases.

Case 1—The phospholipid molecules are in tail contact.

At the surface pressure studied, the molecules in a monolayer of pure phospholipid are in tail contact. In a mixed monolayer where the mole fraction of cholesterol equals or exceeds 0.5, each phospholipid molecule occupies the same area as it did in the single-component monolayer (Fig. 1*a*). This is also true when the mole fraction of cholesterol is less than 0.5 (Fig. 1*b*). Therefore, regardless of the composition of the mixed film, $A_1 = A_1^0$ and $A_2 = A_2^0$. Equation 2 becomes:

$$A_m = A_1^0 X_1 + A_2^0 X_2 \qquad \text{(Eq. 3)}$$

In other words, the mean molecular area plot will be linear and no "condensation" will be evident (Fig. 2, curve 1).

Case 2—Phospholipid molecules are in head contact, but there is insufficient space for a cholesterol molecule to fit between the chains.

In the monolayer of pure phospholipid, the molecules are in head contact at the surface pressure of interest. If cholesterol is added, the two species are put into chain contact with each other (Fig. 1*c*). Since the area occupied by the hydrocarbon portion of the phospholipid is less than that occupied by the polar groups, there is an apparent decrease in the total area needed to accommodate the cholesterol. Each molecule of the phospholipid in contact with cholesterol occupies an effective area equal to the area of its hydrocarbon tail, A_{HC}. In cholesterol-rich mixtures, the molecules are always in chain contact as in Fig. 1*a*. The polar groups of the phospholipid do not contribute to the area, so that the effective area of each phospholipid molecule is again A_{HC}. Regardless of composition, $A_1 = A_1^0$. Therefore, Eq. 2 becomes for $X_1 \geq 0.5$:

$$A_m = A_1^0 X_1 + A_{HC} X_2 \qquad \text{(Eq. 4)}$$

and for $X_1 \leq 0.5$:

$$A_m = A_1^0 X_1 + A_{HC} X_1 + A_2^0(X_2 - X_1) = (A_1^0 + A_{HC})X_1 + A_2^0(X_2 - X_1) \qquad \text{(Eq. 5)}$$

The mean molecular area plot for this case is illustrated by Fig. 2, curve 2.

Case 3—The phospholipid molecules are in head contact, and there is just enough space for a cholesterol molecule to fit between the chains.

Now, added cholesterol molecules fit between the chains of the molecules of the other component without requiring additional area (Fig. 1*d*). Effectively, then, the cholesterol molecules appear to require no space as long as the mole fraction of cholesterol does not exceed 0.5. In cholesterol-rich films, the arrangement is once again as shown by Fig. 1*a*. Therefore, for $X_1 \geq 0.5$, Eq. 4 holds. For $X_1 \leq 0.5$:

$$A_m = A_2^0 X_2 \qquad \text{(Eq. 6)}$$

The mean molecular area plot appropriate for this case is illustrated by Fig. 2, curve 3.

Case 4—The phospholipid molecules are in head contact, and there is more than enough space for one cholesterol molecule to fit between the chains but not enough space for two.

As long as the mole fraction of cholesterol does not exceed 0.5, the cholesterol molecules appear to occupy no additional space (Fig. 1*e*). Addition of cholesterol above a mole fraction of 0.5 puts those molecules in chain contact, and the monolayer contains a mixture of molecules in chain contact and head contact (Fig. 1*f*) until the mole fraction of cholesterol reaches 0.667, at which point all of the molecules are in chain contact, as in Fig. 1*a*.

For $X_1 \geq 0.667$, Eq. 4 applies. For $X_1 \leq 0.5$, Eq. 6 applies. For $0.667 \geq X_1 \geq 0.5$:

$$A_m = 2A_1^0(X_1 - X_2) + A_{HC}(X_1 - X_2) + A_2^0(2X_2 - X_1) = (2A_1^0 + A_{HC})(X_1 - X_2) + A_2^0(2X_2 - X_1) \qquad \text{(Eq. 7)}$$

The mean molecular area plot appropriate to this case is shown in Fig. 2, curve 4.

Cases 1-4 cover the situations of practical interest encountered in mixed monolayers containing two components. All theoretical curves exhibit a "break" at a mole fraction of 0.5. One consequence of the model is that, in cholesterol-rich mixed films, the mean molecular area plots for a given system at various surface pressures ought to be practically superimposable. This effect (apparent in Fig. 2) is due to the incompressibility of the cholesterol monolayer and the nearly constant area of the vertical hydrocarbon tail of the phospholipid.

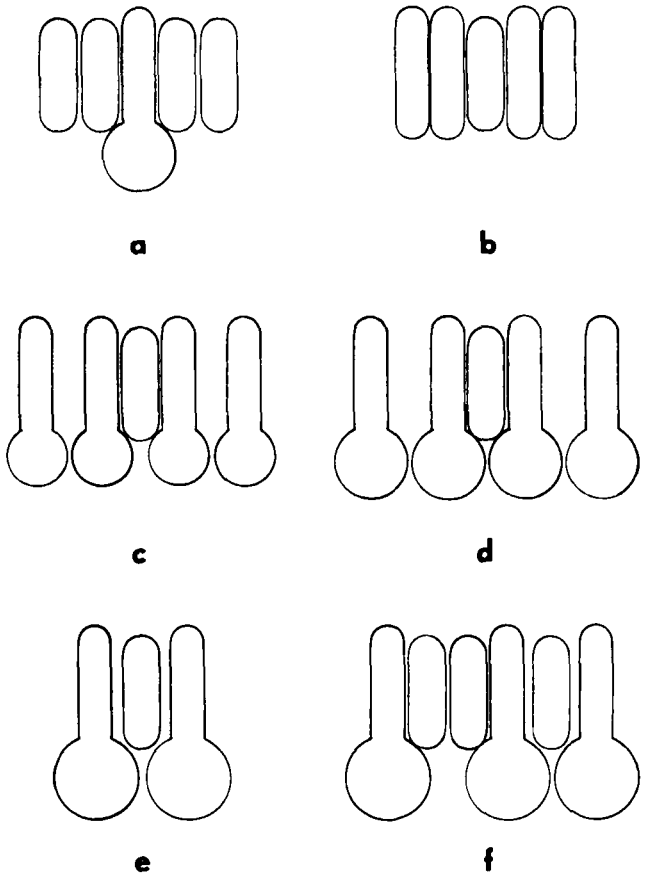

Figure 1—*Representation of molecules in mixed monolayers. The short rod is cholesterol. The other molecule is phospholipid, whose exact shape depends on its state in the surface. (See text for details.)*

DISCUSSION

In applying the heads-or-tails model, the values for X_1, X_2, A_1^0, and A_2^0 are provided by experiment. The cross-sectional area of the hydrocarbon tail of the phospholipid, A_{HC}, may be estimated from molecular models and appropriate surface balance experiments. The value of A_2^0 of a lipid at a particular surface pressure may vary a great deal with changes in environmental conditions, *e.g.*, temperature. Less variation is anticipated in A_{HC}.

If, at a particular surface pressure A_2^0 equals A_{HC}, Case 1 of the theoretical section applies. If the difference between A_2^0 and A_{HC} is significant but less than the value of A_1^0, Case 2 applies. The equations for Case 3 are used when the difference between A_2^0 and A_{HC} equals A_1^0. Case 4 applies when the difference between A_2^0 and A_{HC} exceeds A_1^0.

If the phospholipid is in a condensed state, its molecules will be in tail contact in the pure monolayer and in the mixed films. The mean molecular area plot should be linear (Case 1 of the theoretical section). In accord with the model, mixed films of cholesterol and dipalmitoyl phosphatidyl ethanolamine, distearoyl phosphatidyl ethanolamine, or distearoyl lecithin yield linear mean area curves (2, 3). Dipalmitoyl lecithin, which is in tail contact only at high surface pressures, exhibits a linear plot only at high pressures (4).

At low surface pressure, dipalmitoyl lecithin molecules are in head contact. By taking the area per hydrocarbon chain to be 20

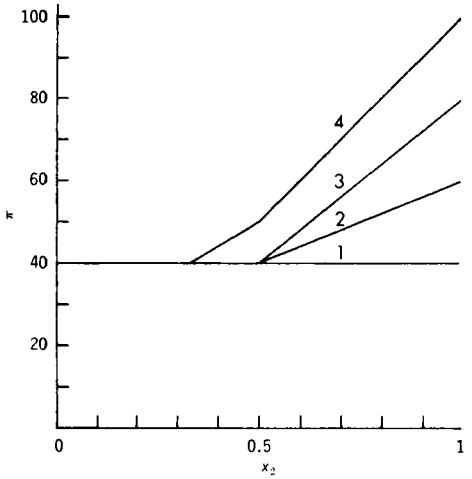

Figure 2—*Mean molecular area plots for hypothetical mixed films. A molecular area of 40 Å²/molecule is assumed for cholesterol in all cases. The hydrocarbon tails of the phospholipid have a cross-sectional area of 40 Å². Numbers correspond to the cases discussed in the text.*

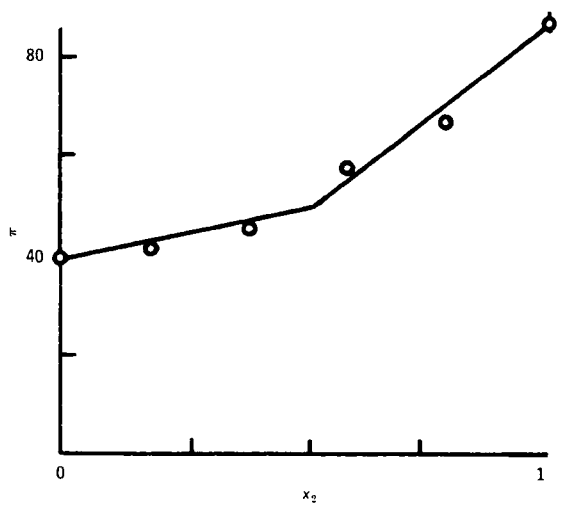

Figure 4—*Mean molecular area plot for cholesterol and* L-*stearoyl-linoleoyl lecithin at 10 dynes/cm. Key:* ○, *experimental data (Ref. 7); and* ——, *theoretical curve.*

Å² (9), A_{HC} is 40 Å², a value that agrees with the close-packed area per molecule obtained from the π–A curve of this phospholipid at 25° (4, 12). At this temperature, under a surface pressure of 5 dynes/cm, mixed monolayers of dipalmitoyl lecithin and cholesterol provide an example of Case 2 behavior. In Fig. 3, the theoretical curve is plotted and compared with the experimental results of Shah and Schulman (4). The agreement is quite good.

Many studies of mixed monolayers have included unsaturated phospholipids. Tinoco and McIntosh (7) published mean molecular area plots for several mixed films, including the combination of cholesterol with L-stearoyl-linoleoyl lecithin at 10 dynes/cm. By assuming an area of 20 Å² for the stearoyl moiety and 40 Å² for the unsaturated chain (14), a value for A_{HC} of 60 Å²/molecule is determined, close to the area per molecule occupied by this lipid under high surface pressure (7). As shown in Fig. 4, there is good agreement between theory and experiment. This is another Case 2 situation.

The mean molecular area plot for mixtures of cholesterol and dioleoyl lecithin at 12 dynes/cm was reported (2). At this surface pressure, A_2^0 exceeds A_{HC} [obtained from the π–A curve for this lecithin (2)] by only about 10 Å². The relatively small deviation observed in the mean area plot is, therefore, in accord with the heads-or-tails model but not with the cavity model of Shah and Schulman (4).

Although the equations presented were derived with the intent of describing the π–A characteristics of mixed monolayers of cholesterol and phospholipids, they may be applied to other mixed systems. The derived relationships should hold if one surface-active material forms, like cholesterol, a condensed monolayer in which the molecules are in tail contact and if the other has a long hydrocarbon chain and is in head contact on the surface. These conditions appear to be satisfied in mixed monolayers of cetyl alcohol and sodium cetyl sulfate at room temperature (15). At this temperature, cetyl alcohol forms condensed monolayers with an average area per molecule of about 20 Å². This value is approximately the cross-sectional area of a hydrocarbon chain (9) and suggests that the molecules are in tail contact. Therefore, cetyl alcohol corresponds to Component 1 of the theoretical section. Sodium cetyl sulfate, although somewhat soluble in water, was prevented from dissolving by the inclusion of 5% sodium chloride in the subphase (15). Sodium cetyl sulfate has a long, saturated hydrocarbon chain and forms expanded monolayers. It is likely that the molecules are in head contact, so sodium cetyl sulfate corresponds to Component 2 of the theoretical section. A mean molecular area plot at 10 dynes/cm permits a test of the equations describing Case 4 behavior. At this surface pressure, A_1^0 is 20 Å²/molecule, A_2^0 is 54 Å²/molecule, and A_{HC} is taken to be 20 Å². The experimental data (15) are plotted in Fig. 5. The solid line in Fig. 5 was calculated from Eqs. 4, 6, and 7 and is in reasonably good agreement with the experimental points.

The heads-or-tails model is based on the assumption that the hydrocarbon chains of the phospholipid are vertically oriented in mixed films with cholesterol. At very high temperatures or in the case of relatively short hydrocarbon chains, the kinetic energy of the phospholipid tails may be sufficient to preclude vertical orientation. In such cases, the heads-or-tails model is not expected to describe the system. However, the phospholipids found in biological membranes have sufficiently long hydrocarbon chains to satisfy the assumptions made in the model (16). Hopefully, appli-

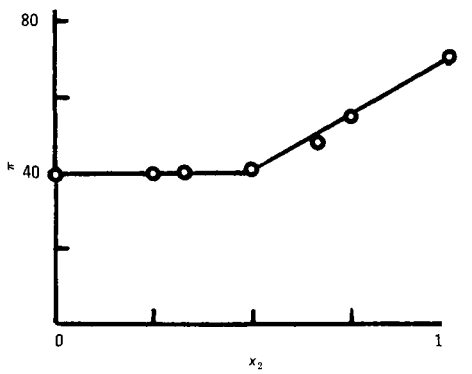

Figure 3—*Mean molecular area plot for cholesterol and dipalmitoyl lecithin at 5 dynes/cm. Key:* ○, *experimental data (Ref. 4); and* ——, *theoretical curve.*

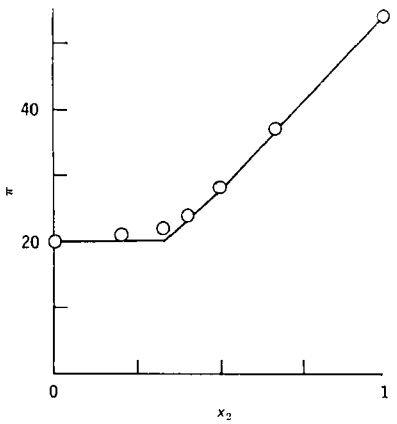

Figure 5—*Mean molecular area plot for cetyl alcohol and sodium cetyl sulfate at 10 dynes/cm. Key:* ○, *experimental data (Ref. 15); and* ——, *theoretical curve.*

cation of this framework will help remove some of the mystery surrounding the role of cholesterol in biological membranes.

REFERENCES

(1) L. De Bernard, *Bull. Soc. Chim. Biol.*, **40**, 161(1958).

(2) R. A. Demel, L. L. M. Van Deenen, and B. A. Pethica, *Biochim. Biophys. Acta*, **135**, 11(1967).

(3) M. M. Standish and B. A. Pethica, *ibid.*, **144**, 659(1967).

(4) D. O. Shah and J. H. Schulman, *J. Lipid Res.*, **8**, 215(1967).

(5) D. A. Cadenhead and M. C. Phillips, *Advan. Chem. Ser.*, **84**, 131(1968).

(6) P. Joos and R. A. Demel, *Biochim. Biophys. Acta*, **183**, 447(1969).

(7) J. Tinoco and D. J. McIntosh, *Chem. Phys. Lipids*, **4**, 72(1970).

(8) D. Ghosh, R. L. Lyman, and J. Tinoco, *ibid.*, **7**, 173(1971).

(9) G. L. Gaines, Jr., "Insoluble Monolayers at Liquid–Gas Interfaces," Interscience, New York, N.Y., 1966, p. 162.

(10) *Ibid.*, p. 233.

(11) F. M. Fowkes, *J. Phys. Chem.*, **65**, 355(1961).

(12) F. Villalonga, *Biochim. Biophys. Acta*, **163**, 290(1968).

(13) I. Langmuir, *J. Chem. Phys.*, **1**, 756(1933).

(14) N. K. Adam and J. W. W. Dyer, *Proc. Roy. Soc. London, Ser. A*, **106**, 694(1924).

(15) F. M. Fowkes, *J. Phys. Chem.*, **67**, 1982(1963).

(16) L. L. M. Van Deenan, in "Progress in the Chemistry of Fats and Other Lipids," vol. 8, part 1, R. T. Holman, Ed., Pergamon Press, Oxford, England, 1965.

ACKNOWLEDGMENTS AND ADDRESSES

Received May 31, 1973, from the *College of Pharmacy, Rutgers—The State University, New Brunswick, NJ 08903*.

Accepted for publication January 22, 1974.

Supported in part by a Merck Grant for Faculty Development.

Bioavailability Determination of Two Crystal Forms of Sulfameter in Humans from Urinary Excretion Data

NAWAL KHALAFALLAH, SAID A. KHALIL[x], and MAMDOUH A. MOUSTAFA

Abstract □ Urinary excretion data were used to determine the bioavailability of crystal Forms II and III of sulfameter in humans. Agreement was observed between the ratio of absorption parameters of the two forms determined in the present study and those previously obtained from blood level data. Although the urine data revealed a significant difference in the rate of absorption of the two forms, no significant difference was observed in the extent of absorption of both forms as indicated by the 72-hr urinary excretion data. Urinary excretion rates during the absorption phase, without further mathematical treatment, were statistically shown to be adequate means for comparing the bioavailability of sulfameter crystal forms. The use of urinary excretion data of sulfameter as an alternative to the use of blood level data is discussed.

Keyphrases □ Sulfameter—bioavailability of two crystal forms, human urinary excretion data □ Sulfonamides—bioavailability of two crystal forms of sulfameter, human urinary excretion data □ Bioavailability—two crystal forms of sulfameter, human urinary excretion data □ Polymorphism—bioavailability of two crystal forms of sulfameter, human urinary excretion data

The polymorphism of sulfameter (1), as well as the GI absorption of its crystal Forms II and III (2), was recently reported. The *in vitro* dissolution behavior of the two crystal forms was shown to be reflected in their bioavailability as determined from blood level data (2). The use of urinary excretion data, however, was thought of as a simpler and perhaps more accurate (3) alternative to the use of blood level data in bioavailability determination. Reduction of costs and the elimination of venipunctures are also obvious advantages. Urinary excretion data have been successfully used to evaluate the bioavailability of various drugs including aspirin (4), riboflavin (5), chloramphenicol (6), tetracycline products (7), and sulfamethizole (8).

Several mathematical treatments have been developed for bioavailability determination using urinary excretion data. The direct proportionality between excretion rate and blood level of free unchanged drug, measured at the mean time of the urine collection period (4, 9), is a common prerequisite to such treatments. Nelson (10) developed an equation for determining the amount of drug absorbed at a certain time. Later, Wagner and Nelson (9), using a one-compartment open model, simplified the Nelson equation for calculating the percentage of drug absorbed from urinary excretion data. A two-compartment open model for data treatment was proposed by Loo and Riegelman (11), who introduced terms describing the tissue distribution phase into the Wagner–Nelson equation. Recently, Perrier and Gibaldi (12) pointed out the possibility of overestimating the absorption rate constant, using either of the previous treatments, in cases of drugs with incomplete availability.

The mechanism of drug excretion may inflict certain complications on the linear relationship between excretion rate and blood level of free unchanged drug and, consequently, upset the fundamental assumption on which the application of urine data is based. A previous report (13) showed that the above-mentioned relationship could be affected by active tubular secretion, passive reabsorption, and protein binding. It was also shown (13) that when the extent of drug protein binding is constant and the passive reabsorption is not affected by urine pH, the urinary