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COMMENTARY

HOW DOES THE MICROSOMAL MEMBRANE REGULATE UDP-GLUCURONOSYLTRANSFERASES?

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The amounts and functional states (activity/molecule enzyme) of UDP-glucuronosyltransferases modulate the toxicity of a broad array of xenobiotics by directly inactivating pharmacologically active compounds, by facilitating their excretion, and by removing many xenobiotics from oxidative pathways that produce proximate toxins. It is widely acknowledged, therefore, that the UDP-glucuronosyltransferases are important for the maintenance of health.

UDP-glucuronosyltransferases are integral components of membranes [1–3], and interactions with the matrix of membranes appear to have important regulatory functions [4–9]. The enzymes are most abundant in the microsomal fraction of cells [1]. And, since this organelle has been the primary focus of research on UDP-glucuronosyltransferases, our comments are limited to their properties in microsomes.

An interesting characteristic of all UDP-glucuronosyltransferases studied to date is that activities are less in untreated microsomes as compared with microsomes treated with a variety of mechanical (grinding in sand, sonic energy, high hydrostatic pressure) or chemical (detergents, staphylococcal α-toxin) perturbants [4–12], which indicates that some factor in microsomes constrains activities. Identification of this factor is a challenging problem with practical importance. The presence of constraint on the activities of glucuronidating enzymes, which appears to occur in perfused liver as well as in microsomes [13], raises the possibility that the efficiency of glucuronidation reactions could be enhanced in intact animals by increasing the activity/molecule of UDP-glucuronosyltransferases. Devising strategies with this goal in mind depends, obviously, on defining the basis for constraint. There is considerable controversy in this area in that two basically different ideas—the compartmentation [10, 14–18] and conformation [7, 19, 20] hypotheses have been proposed. Sufficient data are available at present for deciding which of these models accounts for the properties of the enzyme. Our purpose in this commentary is to review the relevant data and to show that the conformation hypothesis not the compartmentation hypothesis is the mechanism for regulating UDP-glucuronosyltransferase in microsomes.

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Compartmentation and conformation hypotheses for regulating UDP-glucuronosyltransferases

The compartmentation hypothesis is based on the following assumptions [10, 14, 18]. (i) UDP-glucuronosyltransferases are oriented with their active sites within the lumen of the microsome. As a result, the microsomal membrane prevents free passage of UDP-glucuronic acid to the active site of the enzyme. (ii) To account for glucuronidation in untreated microsomes, it is proposed that the microsomal barrier is short-circuited by a UDP-glucuronic acid-transport protein that (iii) is regulated by UDP-N-Ac-glc [15], which is a known positive, allosteric effector of UDP-glucuronosyltransferase [9, 21]. (iv) The rate of facilitated transport of UDP-glucuronic acid into the lumen of microsomes, not the inherent properties of UDP-glucuronosyltransferase, limits the activity of UDP-glucuronosyltransferase in untreated microsomes. (v) Treatments that remove constraint on the activity of UDP-glucuronosyltransferase destroy the vesicular structure of microsomes (detergents), or create holes in the membrane (α-toxin) thereby allowing free passage of UDP-glucuronic acid to the active site of the enzyme.

The conformation hypothesis is far more general than the compartmentation hypothesis and assumes only that UDP-glucuronosyltransferases can exist in different conformational states that have variable kinetic properties, According to this proposal, treatments that activate UDP-glucuronosyltransferase change the relative stabilities of these states by altering the hydrophobic region of the microsomal membrane. Thus, the lipid environment is considered to be an important determinant of the functional state of the enzyme [7, 19, 20–24].

The compartmentation hypothesis is depicted easily in a simple drawing. It is not possible to represent the conformation hypothesis in a seemingly precise picture because to do so requires detailed understanding of the three-dimensional structure and molecular dynamics of the enzyme. No informed statements can be made in these contexts. Nevertheless, the conformation hypothesis is a simple scheme for regulating UDP-glucuronosyltransferase, and it is an idea that has been validated as the basis for regulating a large number of proteins. By contrast, there is no established precedent for regulation of a microsomal enzyme by the mechanism of compartmentation. This mechanism has been offered as the basis for regulation of microsomal

UDPase [25, 26] and glucose-6-Pase [27, 28]. But, as for UDP-glucuronosyltransferase, there is no direct evidence to support this idea, as it applies to glucose-6-Pase. There is, however, a reasonable experimental basis for rejecting it [29–33].

It is difficult to obtain direct physical proof for either the compartmentation or conformation hypotheses. For example, the orientation of the active site of UDP-glucuronosyltransferase has not been established by direct experiment. And, experience with other membrane-bound enzymes [34–37] demonstrates that this will be exceedingly difficult to achieve in a structure as complex as the microsome. It can be argued too that there is no certain relationship between the orientation of UDP-glucuronosyltransferase reconstituted into pure lipid bilayers and the orientation of enzyme *in situ* in microsomes. Moreover, one cannot disprove directly that a putative transport protein for UDP-glucuronic acid does not exist. So solving the problem of how UDP-glucuronosyltransferase is regulated depends on interpretations of data from indirect experiments that are designed to test the applicability of the assumptions that are the basis for each hypothesis summarized above. Thus, if a given hypothesis is not compatible with the observed properties of the enzyme, it cannot be a suitable model for regulation, whether or not it accounts for the limited issue of release of constraint. It is especially important to realize that a given experimental result cannot be interpreted without considering how this interpretation fits with all other data relating to the problem, i.e. no data can be ignored. Much of the literature on UDP-glucuronosyltransferase suffers from this deficiency.

Use of progress curves for production of glucuronides as a test for the compartmentation hypothesis

UDP-glucuronic acid enters the lumen of microsomes [38, 39,*]. To compare the properties of UDP-glucuronosyltransferase predicted by the compartmentation hypothesis with actual properties, we assume that UDP-glucuronic acid interacts with the active site of UDP-glucuronosyltransferase within the lumen of the microsome.

UDP-glucuronic acid enters the lumen of microsomes slowly [38, 39], but only very small amounts of UDP-glucuronic acid need to enter this compartment to achieve equilibrium with the extralumenal compartment [40,*]. In the absence of aglycon, when there is no utilization of UDP-glucuronic acid, it is possible to equilibrate UDP-glucuronic acid across the microsomal membrane. There should be no constraint in this setting on access of UDP-glucuronic acid to the active site of the UDP-glucuronosyltransferase. So there should be no constraint on the initial rate of enzyme activity. Hence, the initial rate of glucuronidation by untreated microsomes containing UDP-glucuronic acid equilibrated between intra- and extralumenal compartments should equal the steady-state rate for unconstrained enzyme. Figure 1A is a representation of the progress curve (product formed versus time) predicted by the compartmentation model, for the conditions just given. The initial rate of activity in Fig. 1A is shown as 10-fold greater than the steady-state rate of constrained enzyme because this is the typical extent to which activity increases when constraint is released. The initial rate decays quickly, however, as intralumenal UDP-glucuronic acid is consumed. The rate of glucuronidation decays to the steady-state rate for enzyme in untreated microsomes not preloaded with UDP-glucuronic acid. Given an intralumenal volume of about 1 μL/mg protein [40, 41], it is simple to arrange an experiment in which there should be a "burst" of glucuronidation lasting about 1 min at 30° and longer at lower temperatures. Attempts to verify the result predicted (Fig. 1A), however, have always produced instead the behavior depicted in Fig. 1B, which is not compatible with the compartmentation hypothesis. We are unaware too that any laboratory has observed the kinetic pattern predicted by the compartmentation hypothesis (Fig. 1A).

If glucuronidation occurs via reaction (1), when UDP-glucuronic acid is present *only* in the intralumenal space, then the progress curve for glucuronidation will appear as shown in Fig. 1C: a rapid initial rate, equivalent to that for unconstrained enzyme, that decays to zero as UDP-glucuronic acid is consumed.

$$\text{Aglycon} + \text{UDP-glucuronic acid} \leftrightarrow \text{aglycon-glucuronic acid} + \text{UDP} \quad (1)$$

The interpretation of the data from such an experiment will not be confounded by the possibility of end-product inhibition, which must be considered for a reaction within a small, confined space. Glucuronides are poor inhibitors of UDP-glucuronosyltransferase and are easily outcompeted by aglycon [23, 42], which will be present in great excess relative to product. In addition, UDP is not normally found as a product of reaction (1) in untreated microsomes. It is metabolized rapidly to UMP, unless it is trapped [43].

There are no published data to compare with the result predicted in Fig. 1C. Vanstapel and Blanckaert [41] reported that bilirubin was not conjugated with glucuronic acid when UDP-glucuronic acid was present only in the intralumenal compartment of the assay system. Blanckaert and co-workers reported more recently, however, that this reaction occurs*, but no progress curves have been published. On the other hand, there are relevant data for the conjugation of bilirubin with UDP-glucose [41]. This reaction shares with (1) the property of constraint in untreated microsomes that is relieved by treatment with detergent. The time-dependent formation of bilirubin–glucose conjugates, when UDP-glucose was only present intralumenally, did not correspond to Fig. 1C. The initial rate of conjugation, using rat liver microsomes, was less than 10% of the rate of unconstrained enzyme; and rates were nearly linear for as long as they were examined. Moreover, the initial rate of product formation by microsomes preloaded with UDP-glucose was smaller than for microsomes not preloaded with UDP-glucose. One might attempt to rationalize these data with the

* Blanckaert N, Xiao Wei L, Bossuyt X and Vanstapel F, Transmembrane transport of UDP-glucuronic acid into rat liver microsomes. In: *Workshop on Glucuronidation*, Abstr. No. 18, Noordwijkerhout, The Netherlands, 1991.

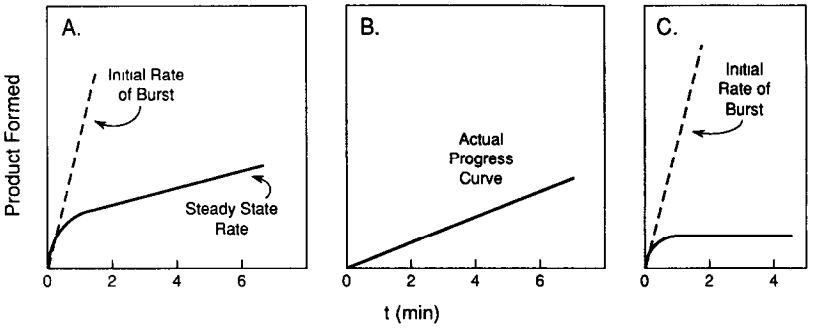

Fig. 1. Predicted and observed progress curves for UDP-glucuronosyltransferase. The time-dependent production of glucuronide in panel A is based on the assumptions of the compartmentation hypothesis for assays in which microsomes were pretreated with UDP-glucuronic acid to allow for equilibration of intra- and extralumenal compartments with UDP-glucuronic acid prior to adding aglycon. Extralumenal UDP-glucuronic acid is not washed off prior to adding aglycon in panel A but is washed off in panel C. The kinetics in panel B are the actual observed kinetics for glucuronidation under the conditions established in panel A.

compartmentation hypothesis by arguing that microsomal preparations contain some vesicles that are leaky to UDP-glucose and/or are inside/out so that assays in untreated microsomes reflect the activity of unconstrained enzyme in "disrupted" vesicles not the activity of constrained enzyme in "intact" vesicles. But, the rate of conjugation was about 5-fold greater when UDP-glucose was present extralumenally versus intralumenally. Hence, the argument invoking leaky vesicles implies that nearly all the untreated microsomes would be leaky, under which condition the enzyme would not appear to be constrained.

Kinetic constants of constrained enzyme give an accurate value of K_{eq} *for glucuronidation of* p-*nitrophenol*

The compartmentation theory predicts that the kinetic properties of UDP-glucuronosyltransferase cannot be measured for enzyme in untreated microsomes. This is so because activity in this setting is limited by the rate at which UDP-glucuronic acid enters the lumen. As a result, kinetic constants derived from assays of UDP-glucuronosyltransferase in untreated microsomes contain rate constants for the uptake process in addition to rate constants for the glucuronidation reaction. It is possible to determine whether the true kinetic constants of UDP-glucuronosyltransferase can be measured in untreated microsomes. This is so because of the relation between K_{eq} for the reaction catalyzed by UDP-glucuronosyltransferase and the kinetic constants of the enzyme, which is given in (2) [23, 44].

$$K_{eq} = (V_f/V_r)\, K_{UDP} K'_{PNPGA}/K_{UDPGA} K'_{PNP} \quad (2)$$

V_f and V_r are activities at V_{max} in forward and reverse directions. The kinetic mechanism that applies to UDP-glucuronosyltransferase and for which (2) is valid (random order, rapid equilibrium), as well as the physical significance of the kinetic constants in (2), are given in Fig. 2.

Obviously, the kinetic constants in (2) will differ for constrained and unconstrained states of UDP-glucuronosyltransferase whether the compartmentation or conformation hypothesis is correct.

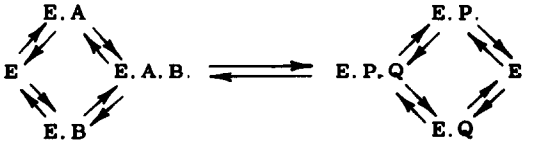

Fig. 2. Rapid equilibrium, random order kinetic mechanism, A and B are, respectively, UDP-glucuronic acid and aglycon. P and Q are glucuronide and UDP. Since either substrate, or product in the reverse direction, can bind to enzyme as the first or second to bind, there are two binding constants for each substrate. K_A and K_B are for binding of A and B to free enzyme. K'_A is for binding of A to enzyme already saturated with B; K'_B has the same meaning for substrate B. Similarly there are K_p and K'_p terms, etc. for binding of P and Q.

According to the conformation hypothesis, K_{eq} calculated from (2) will be the same for constrained and unconstrained states of UDP-glucuronosyltransferase because K_{eq} is not altered by an enzyme. But the compartmentation hypothesis predicts that K_{eq} (for reaction (1)) cannot be calculated from (2) for constrained enzyme because the true kinetic constants of UDP-glucuronosyltransferase cannot be measured for enzyme in untreated microsomes. Thus, comparison of values of K_{eq} calculated from (2) using data for constrained and unconstrained enzyme is a simple experimental test of the compartmentation hypothesis. The relevant data (Table 1) indicate that the predictions of the compartmentation hypothesis are not valid. The data in Table 1 give the result predicted by the conformation hypothesis.

Comparison of rates of glucuronidation and rates of entry of UDP-glucuronic acid into the lumen of the microsome

The compartmentation hypothesis predicts that the maximum rate of glucuronidation (of any aglycon) in untreated microsomes cannot exceed the maximum rate of entry of UDP-glucuronic acid into the lumen of the microsome. The only direct comparison of rates of entry of UDP-glucuronic acid into microsomes with rates of glucuronidation shows

Table 1. K_{eq} for glucuronidation calculated from (2) (see text) for constrained and unconstrained states of UDP-glucuronosyltransferase

Source of microsomes	Treatment of microsomes	K_{eq}
Guinea pig	None	10.4
	PLA	6.7
	Triton	10.3
Wistar rat	None	7.2

Constraint was released by treating microsomes with Triton X-100 or phospholipase A_2 (PLA). Data are from Ref. 23.

that the rate of the former is less than 1% of the rate of conjugation of bilirubin, which is conjugated relatively slowly as compared with phenolic aglycons [38]. Comparisons of rates of entry of UDP-glucuronic acid into the lumen of microsomes from the data in Refs. 38 and 39 with rates of glucuronidation in the literature [23, 38, 45–47] confirm this result. It is clear then that little of the glucuronidation measured in untreated microsomes can occur intralumenally. One could try to explain this result with the argument used above, i.e. the activity of UDP-glucuronosyltransferase within the lumen of microsomes represents a small amount of the total glucuronidating activity in untreated microsome because most enzyme is in vesicles that have been disrupted/made leaky, etc. during preparation. There are no data to support this premise, but two experiments exclude it. First, as indicated already, there will be little or no constraint on enzyme activity in untreated microsomes if the fraction of leaky vesicles is large enough to account for the discrepancy between rates of entry of UDP-glucuronic acid into the lumen and steady-state rates of glucuronidation. Second, assume that the discrepancy in these rates exists because untreated microsomes are leaky to UDP-glucuronic acid. It follows that UDP-glucuronosyltransferase assayed in untreated microsomes has the properties of the unconstrained state. Therefore, release of constraint on enzyme within the lumen of intact microsomes can have no other effect except to increase specific activity by a small extent. It is well-known, however, that release of constraint on the activity of UDP-glucuronosyltransferase in untreated microsomes is associated with profound changes in the properties of the enzyme, in addition to a change in specific activity [8, 12, 21, 23, 48, 49]. Hence, the failure of the compartmentation hypothesis to predict the true behavior of UDP-glucuronosyltransferase cannot be explained away.

Site of production of UDP during glucuronidation

Finch *et al.* [43] used ^{31}P NMR and a transmicrosomal gradient of pH to distinguish between nucleotides on the inside and the outside of rabbit liver microsomes. Their experiments showed that any UDP formed in (1) was hydrolyzed rapidly to UMP. The pH gradient across the microsome would split the ^{31}P signal, however; so signals from intra- and extralumenal UMP would be distinguishable. The data showed that all the UMP produced was present on the outside of the microsome. More importantly, all the UDP produced in (1) could be trapped by adding pyruvate kinase and phosphoenol-pyruvate to microsomes. Thus, if (1) occurs within the lumen, then all UDP must be transported instantly and quantitatively from the intralumenal to the extralumenal compartments, which is a process that cannot be accounted for by a protein-mediated pumping mechanism.

Glucuronidation in the presence of intralumenal but no extralumenal UDP-glucuronic acid

Demonstration of this event [40,*] does not establish that product was formed via reaction (1). Glucuronic acid [40] (or glucose [42]) could be transferred from UDP-glucuronic acid (or UDP-glucose) to microsomal components during the time that UDP-glucuronic acid (or UDP-glucose) is present on the outside of the microsome, i.e. when the lumen was being "loaded" with UDP-sugars. Sugars attached to water-insoluble or membrane-bound components in the loading phase of the experiment would not be washed off with extralumenal nucleotide sugars and hence could transfer subsequently to exogenous aglycon. The latter reaction need not be enzyme-catalyzed; it occurs spontaneously [50]. Proper control experiments are not available; so the exact interpretation of some experiments [40, *] is uncertain. A related issue is that treating microsomes with detergent, after loading of the lumen with nucleotide-sugar and washing of extralumenal substrate, prevented conjugation of bilirubin with UDP-glucose. This result is interpreted as follows: detergent-induced rupture of microsomes containing intralumenal but no extralumenal UDP-glucuronic acid allows intralumenal substrate to be diluted in the entire assay system thereby decreasing the rate of glucuronidation. The interpretation is taken as evidence for the compartmentation hypothesis. The experiment cannot be interpreted fairly, however, if one ignores the general failure of the compartmentation hypothesis to account for the properties of UDP-glucuronosyltransferase. An explanation of this "dilution" experiment must be sought outside the context of the compartmentation hypothesis. For example, detergent may have inhibited whatever process actually accounted for synthesis of conjugates.

Is the uptake of UDP-glucuronic acid by microsomes facilitated by a protein?

If UDP-glucuronic acid within the lumen of microsomes is not important for glucuronidation via reaction (1), one might ask why UDP-glucuronic acid enters the lumen. And since the process of entry is temperature-dependent, saturable, and susceptible to inhibition by other nucleotide-sugars [38, 39], properties usually attributed to a protein-based mechanism of transport, one also might ask why a transport protein is elaborated to catalyze a reaction with no apparent utility.

* Blanckaert N, Xiao Wei L, Bossuyt X and Vanstapel F, Transmembrane transport of UDP-glucuronic acid into rat liver microsomes. In: *Workshop on Glucuronidation*, Abstr. No. 18, Noordwijkerhout, The Netherlands, 1991.

There is no evidence to support the idea that UDP-glucuronic acid is transported to the lumen of microsomes by a protein-mediated mechanism. Polar molecules diffuse across lipid bilayers, albeit slowly. Moreover, lipid bilayers, which are not bulk phases, can display properties often attributed to protein-based mechanisms [51–54]. For example, the spontaneous diffusion of UDP-glucuronic acid across a lipid bilayer will depend on spontaneous formation of a hole (thermal fluctuations in packing of polymethylene chains) large enough to accommodate hydrated UDP-glucuronic acid [55–57]. Relatively large energies are needed to create such holes because of the cooperative interactions between polymethylene chains, the negative entropy for forming a void, and the absence of compensating enthalpic effects due to interactions between UDP-glucuronic acid and the polymethylene chains lining the hole. Therefore, hole-formation in a bilayer will have a large energy of activation and be temperature dependent. In addition, a bilayer of finite dimension can contain only a finite number of holes. So the spontaneous rate of passage of UDP-glucuronic acid to the inside of the microsome via permeation of the lipid bilayer region is expected to become saturated as the concentration of UDP-glucuronic acid increases and to show competition between compounds that permeate the membrane via holes that also accommodate UDP-glucuronic acid. Observations that the rate of entry of UDP-glucuronic acid into the lumen of microsomes is temperature dependent, that the rate reaches a limiting value, and/or that there is competition hence are not *prima facie* evidence for a facilitated event in a membrane system.

There are no data for the rate of transmembrane transport of UDP-glucuronic acid in lipid vesicles or for the non-specific effects of protein on this rate. On the other hand, it is known that the rate of entry of UDP-glucuronic acid into the lumen of microsomes is extremely slow (about 0.2 nmol/min/mg protein at V_{max}) [38]. The $T_{\frac{1}{2}}$ for uptake is measured in days. By contrast, passive diffusion of Na^+ across bilayers of dimyristoylPC (liquid crystal phase) has a $T_{\frac{1}{2}}$ of about 1 hr [58]. In addition, limited thermodynamic data for transport of UDP-glucose into the lumen of microsomes are not compatible with a protein-mediated event. Thus, the energy of activation for this process (86 kcal/mol) [41] was reported to be the same at high and low concentrations of substrate. This result is expected for a spontaneous, diffusive process, but it is not compatible with a protein-mediated process. The reason is that the slope of ln v versus $1/K^\circ$, when v is less than V_{max}, contains terms for binding and catalytic steps whereas the slope of ln V_{max} (high concentration of substrate) versus $1/K^\circ$ gives only the energy of activation for the catalytic step. The data are far from convincing, therefore, in demonstrating that entry of UDP-glucose and UDP-glucuronic acid into the lumen of microsomes is facilitated by a protein. In fact, when scrutinized carefully, the available data suggest that uptake is simply diffusive.

A puzzling feature of the transport of UDP-glucose into microsomes, however, is stimulation of efflux by extralumenal UDP-sugars [41]. This phenomenon cannot be explained by the chemical properties of bilayers. Possibly, however, UDP-sugars enter microsomes in part via unrelated transport systems that have less than absolute specificity for ligands. Most important, trans stimulation cannot be taken as support for compartmental regulation of UDP-glucuronosyltransferase when the basic tenets of the compartmentation hypothesis cannot be validated.

Effect of proteases on UDP-glucuronosyltransferase in microsomes

Treatment of microsomes with trypsin activates UDP-glucuronosyltransferase to a small extent [59–61], but addition of proteases to detergent-treated microsomes inactivates this enzyme [16, 60] and leads to degradation products detected by immunoblotting [16]. These results have been interpreted to mean that trypsin has access to sites of proteolysis in disrupted microsomes that are not accessible in untreated microsomes and that this is so because almost all the UDP-glucuronosyltransferase is within the lumen of untreated microsomes. The former part of the interpretation is undoubtedly true, but either the compartmentation theory or the conformational theory accounts for the observations. Thus, it is well known that the conformation of a protein determines accessibility of sites for proteolytic cleavage. Indeed, this is the basis for a classical method for determining whether a given protein exists in different conformational states. Therefore, the experiments with proteases do not distinguish between one or the other theory of constraint, and hence are not useful in resolving the argument.

Proposed transmembrane organization of UDP-glucuronosyltransferase in microsomes

Hydropathy plots based on sequences of UDP-glucuronosyltransferase deduced from cDNAs have been interpreted to mean that the enzyme contains only one transmembrane sequence [62–64]. All the mass of UDP-glucuronosyltransferase except for this sequence is depicted as within the microsomal lumen [62–64]. As shown by the data reviewed above, there is no basis for believing that the active site of the enzyme has this orientation. There is also no firm basis for the conclusions about how much of the protein is embedded within the membrane. For, if a membrane protein contains one highly hydrophobic sequence, it is not possible to establish an exact discriminator for which of the other hydrophobic sequences will span the membrane [65]. Studies of the seven membrane spanning peptides of bacteriorhodopsin show, for example, that not all of these are stable transmembrane peptides when isolated from the intact molecule [66]. Moreover, the proposed topologies for UDP-glucuronosyltransferase are not based on attempts to rationalize the known properties of the enzyme with a reasonable formulation of how it might interact with a lipid bilayer. Proposals about topology have to be compatible with the data indicating the sensitivity of the properties of UDP-glucuronosyltransferase to the composition of the polymethylene chains of bilayers.

Evidence that UDP-glucuronosyltransferase can exist in different conformational states

The fact that the compartmentation hypothesis

cannot account for the properties of UDP-glucuronosyltransferase in untreated microsomes does not prove that the conformation hypothesis is the correct explanation for regulation of this enzyme. There is direct evidence, however, to support the assumptions of the latter hypothesis. UDP-glucuronosyltransferase appears to exist in different functional states that are related to changes in conformation; and the relative stabilities of these states, as well as rates of interchange between them, depend on interactions between the enzyme and the membrane environment. Studies of pure delipidated UDP-glucuronosyltransferase show that the functional state of the enzyme is highly dependent on the lipid environment into which enzyme is reconstituted [48, 49, 67–74]. Both the polar and non-polar regions of lipids influence function [73], but for a constant polar group, variations of the polymethylene chains modulate V_{max} over a 700-fold range, affinity of enzyme for UDP-glucuronic acid and aglycon, and catalytic specificity [68, 69], repsonsiveness to activation by UDP-N-Ac-glc [71] and the pattern of kinetics (non-Michaelis–Menten or Michaelis–Menten) [69], reaction mechanism [68, 72], and thermotropic properties [49, 74]. Insufficient data are available as yet to allow one to state with certainty how lipids modulate the enzyme. Nevertheless, the effects of lipids on the thermotropic properties of pure UDP-glucuronosyltransferase demonstrate that different polymethylene chain environments have selective effects on the stabilities of conformational isomers of the enzyme [49, 74]. Therefore, the fundamental tenets of the conformation hypothesis are well-supported by direct experiment.

There also are data to support the idea that changes in the conformation of UDP-glucuronosyltransferase account for the specific alterations in the properties of UDP-glucuronosyltransferase, other than increase in specific activity, that accompany relief of constraint. These changes include response to UDP-N-Ac-glc, thermal properties, and a switch in the pattern of kinetics. For example, constrained UDP-glucuronosyltransferase is activated by UDP-N-Ac-glc, but unconstrained enzyme is inhibited by UDP-N-Ac-glc [8, 21]. According to the compartmentation hypothesis, UDP-N-Ac-glc does not interact with UDP-glucuronosyltransferase but with the putative microsomal transport protein for UDP-glucuronic acid [15]. The conformation hypothesis proposes that UDP-N-Ac-glc interacts directly with the constrained state of UDP-glucuronosyltransferase, leading to activation, and with the unconstrained state to cause inhibition [21]. In fact, the effect of UDP-N-Ac-glc on the properties of pure UDP-glucuronosyltransferase can be modulated by the physical state of the lipid matrix in which enzyme is embedded [71].

UDP-glucuronosyltransferase in untreated microsomes displays non-Michaelis–Menten kinetics that bend concave downward [8, 21]. A minor but interesting point is that this pattern is exactly opposite to that expected for rate limitation by access of UDP-glucuronic acid to the active site [21, 23]. A more important point is that the kinetics become Michaelis–Menten when constraint on UDP-glucuronosyltransferase is removed. This switch of kinetic pattern can be reconstituted with pure enzyme, as the physical state of the lipid environment is modified [68–71].

The activity of UDP-glucuronosyltransferase in untreated microsomes increases with temperature to the highest temperatures tested (about 50°) [48]. But, the temperature-dependence of activity of unconstrained enzyme shows a maximum at about 37° [48]. The maximum occurs because the unconstrained enzyme undergoes a rapid, reversible change in state to an inactive form [49]. This is also a property of pure enzyme, for which the temperature of the reversible transition active state ↔ inactive state is modulated by the lipid matrix [49, 74]. The temperature for onset of this reaction can be varied from about 20° to 44°. The detailed thermodynamic properties of the reaction show too that the thermal stability of the active form does not depend on the bulk phase properties of the lipid matrix but on microscopic interactions between protein and lipid [49].

Dietary studies support the conformation hypothesis

Diet has been used as a pharmacologic tool to alter the properties of UDP-glucuronosyltransferase. Castuma and Brenner [75] treated guinea pigs with a rat-free diet for up to 3 weeks. This led to a decrease in linoleic and arachidonic acids and an increase in oleic and palmitoleic acids. This change in microsomal fatty acid composition was associated with a loss of enzyme activity, decreased membrane fluidity and progressive alteration in the apparent negative cooperativity of UDP-glucuronosyltransferase. These authors concluded that changes in the physicochemical properties of the lipid bilayer led to a change in the conformation and properties of UDP-glucuronosyltransferase. In a subsequent study [76], these same investigators showed that a cholesterol-enriched diet resulted in an increase in microsomal cholesterol and a change in the kinetic properties of UDP-glucuronosyltransferase: V_{max} increased while K_m decreased. These findings were reproduced by incorporation of cholesterol *in vitro* into guinea pig microsomes [77]. In a related study, Hietanen *et al.* [78] showed that a cholesterol-rich diet led to a change in sensitivity to stimulation of UDP-glucuronosyltransferase activity by trypsin treatment. The conformation model predicts that a change in the microenvironment of UDP-glucuronosyltransferase that causes a change in enzyme activity should result in altered sensitivity to proteases.

What biologic purpose is served by constraint on the activity of UDP-glucuronosyltransferase in untreated microsomes?

Given the complex system for regulation of UDP-glucuronosyltransferase one needs to ask why the enzyme is constrained in its native state. There is no certain answer to this question; but there are data to suggest that the constrained versus unconstrained form of enzyme will be more active under conditions that appear to exist in intact liver [22]. For example, UDP-N-Ac-glc not only enhances the avidity of enzyme for UDP-glucuronic acid [9, 21], but it also modulates inhibition by UDP [79], which is

Table 2. Modulation of end-product inhibition by UDP-N-Ac-glc for constrained and unconstrained states of UDP-glucuronosyltransferase

State of enzyme	Addition to assay	Activity (nmol product formed·min^{-1}·mg^{-1})
Constrained	None	1.89
	UDP	1.21
	UDP-N-Ac-glc	12.7
	UDP + UDP-N-Ac-glc	10.1
Unconstrained	None	12.6
	UDP	6.7
	UDP-N-Ac-glc	11.2
	UDP + UDP-N-Ac-glc	5.0

Constraint was released by treating microsomes at 2.2 kbar for 90 min [11]. Assays were at 37° using *p*-nitrophenol as aglycone. The concentration of UDP-glucuronic acid was 2.0 mM. Concentrations of UDP and UDP-N-Ac-glc, when added, were 1.0 and 4.0 mM, respectively.

potentiated by loss of constraint [22]. These effects of UDP-N-Ac-glc are not present in unconstrained UDP-glucuronosyltransferase. The functional importance of the differences in response of constrained and unconstrained states of the enzyme to UDP and UDP-N-Ac-glc is shown in Table 2. Note that the activity of constrained enzyme is 2-fold greater in the presence of UDP and UDP-N-Ac-glc as compared with unconstrained enzyme even though the latter is nearly 10-fold more active than the former in the absence of UDP and UDP-N-Ac-glc. In addition to changes in the response of UDP-glucuronosyltransferase to UDP-N-Ac-glc, the constrained but not the unconstrained state of the enzyme has high selectivity for binding of nucleotide sugars at the UDP-glucuronic acid site; relief of constraint is associated with inhibition of enzyme by several naturally abundant nucleotide sugars [22].

The presence of UDP-glucuronosyltransferase in a state with k_{cat} that is less than the maximal potential value has biologic significance. Relief of this constraint is associated with the loss of regulatory properties that are important for efficient function under conditions presumed to occur *in vivo*. The challenge to increasing the activity/molecule of UDP-glucuronosyltransferase *in vivo* is thus not a simple matter of developing a strategy for relief of constraint on k_{cat}. This has to be done without loss of positive allosteric response to UDP-N-Ac-glc and loss of selectivity for binding at the UDP-glucuronic acid site. Preliminary work from our laboratory (unpublished data) indicates that this is not an unrealistic goal. Therefore, it is reasonable to expect that better understanding of the details of the regulation of UDP-glucuronosyltransferase will lead to practical advances in our ability to enhance the rate of metabolism of xenobiotics via glucuronidation.

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REFERENCES

1. Dutton GJ and Storey IDE, Uridine compounds in glucuronic acid metabolism. I. The formation of glucuronides in liver suspensions. *Biochem J* **57**: 275–283, 1954.
2. Amar-Costesec, A, Beaufay H, Wibo M, Thines-Sempoux D, Feytmans E, Robbi M and Berthet J, Analytical study of microsomes and isolated subcellular membranes from rat liver. II. Preparation and composition of the microsomal fraction. *J Cell Biol* **61**: 201–212, 1974.
3. Dutton GJ, *Glucuronidation of Drugs and Other Compounds*. CRC Press, Boca Raton, FL, 1980.
4. Lueders KK and Kuff EL, Spontaneous and detergent activation of a glucuronyltransferase *in vitro*. *Arch Biochem Biophys* **120**: 198–203, 1967.
5. Isselbacher KJ, Chrabas MF and Quinn RC, The solubilization and partial purification of a glucuronyltransferase from rabbit liver microsomes. *J Biol Chem* **237**: 3033–3036, 1962.
6. Graham AB and Wood GC, The phospholipid dependence of UDP-glucuronyltransferase. *Biochem Biophys Res Commun* **37**: 567–575, 1969.
7. Winsnes A, Studies of the activation *in vitro* of glucuronyltransferase. *Biochim Biophys Acta* **191**: 279–291, 1969.
8. Vessey DA and Zakim D, Regulation of microsomal enzymes by phospholipids. II. Activation of hepatic uridine diphosphate-glucuronyltransferase. *J Biol Chem* **246**: 4649–4656, 1971.
9. Winsnes A, Kinetic properties of different forms of hepatic UDP-glucuronyltransferase. *Biochim Biophys Acta* **284**: 394–405, 1972.
10. Berry C, Stellon A and Hallinan T, Guinea pig liver microsomal UDP-glucuronyltransferase: Compartmented or phospholipid-constrained. *Biochim Biophys Acta* **403**: 335–344, 1975.
11. Dannenberg AJ, Kavecansky J, Scarlata S and Zakim D, Organization of microsomal UDP-glucuronosyltransferase. Activation by treatment at high pressure. *Biochemistry* **29**: 5961–5967, 1990.
12. Dannenberg AJ, Wong T and Zakim D, Effect of brief treatment at alkaline pH on the properties of UDP-glucuronosyltransferase. *Arch Biochem Biophys* **277**: 312–317, 1990.
13. Bock KW and White INH, UDP-glucuronyltransferase in perfused rat liver and in microsomes: Influence of phenobarbital and 3-methylcholanthrene. *Eur J Biochem* **46**: 451–459, 1974.
14. Berry C and Hallinan T, Summary of a novel three-component regulatory model for uridine diphosphate glucuronyltransferase. *Biochem Soc Trans* **4**: 650–652, 1976.
15. Bingham P, Berry C and Hallinan T, Compartmentation of uridine diphosphate glucuronosyltransferase: Effect of uridine diphosphate *N*-acetylglucosamine on the

apparent V of glucuronidation. *Biochem Soc Trans* **6**: 178–180, 1978.
16. Shepherd SRP, Baird SJ, Hallinan T and Burchell B, An investigation of the transverse topology of bilirubin UDP-glucuronosyltransferase in rat hepatic endoplasmic reticulum. *Biochem J* **259**: 617–620, 1989.
17. Vanstapel F and Blanckaert N, Topology and regulation of bilirubin UDP-glucuronyltransferase in sealed native microsomes from rat liver. *Arch Biochem Biophys* **263**: 216–225, 1988.
18. Tephly TR and Burchell B, UDP-glucuronosyltransferases: A family of detoxifying enzymes. *Trends Pharmacol Sci* **11**: 276–279, 1990.
19. Zakim D and Vessey DA, The effects of lipid–protein interactions on the kinetic parameters of microsomal UDP-glucuronyltransferase. In: *The Enzymes of Biological Membranes* (Ed. Martonosi A), pp. 443–461. Plenum Press, New York, 1976.
20. Zakim D and Vessey DA, The role of the microsomal membrane in modulating the activity of UDP-glucuronyltransferase. In: *Membranes and Transport* (Ed. Martonosi A), Vol. 1, pp. 269–273. Plenum Press, New York, 1982.
21. Vessey DA, Goldenberg J and Zakim D, Kinetic properties of microsomal UDP-glucuronyltransferase. Evidence for cooperative kinetics and activation by UDP-*N*-acetylglucosamine. *Biochim Biophys Acta* **309**: 58–66, 1973.
22. Zakim D, Goldenberg J and Vessey DA, Influence of membrane lipids on the regulatory properties of UDP-glucuronyltransferase. *Eur J Biochem* **38**: 59–63, 1973.
23. Zakim D and Vessey DA, Regulation of microsomal enzymes by phospholipids. IX. Production of uniquely modified forms of microsomal UDP-glucuronyltransferase by treatment with phospholipase A and detergents. *Biochim Biophys Acta* **410**: 61–73, 1975.
24. Vessey DA and Zakim D, Are glucuronidation reactions compartmented? In: *Conjugation Reactions in Drug Biotransformation* (Ed. Aitio A), pp. 247–256. Elsevier/North Holland, Amsterdam, 1978.
25. Brandon E and Fleisher B, Orientation and role of nucleoside diphosphatase and 5′-nucleotidase in Golgi vesicles from rat liver. *Biochemistry* **21**: 4640–4645, 1982.
26. Little JS, Thiers DR and Widnell CC, Latency of inosine-5′-diphosphatase in microsomes isolated from rat liver. *J Biol Chem* **251**: 7821–7825, 1976.
27. Arion WJ, Ballas LM, Lange AJ and Wallin BK, Microsomal membrane permeability and the hepatic glucose-6-phosphatase system. *J Biol Chem* **251**: 4901–4907, 1976.
28. Nilsson OS, Arion WJ, Depierre JW, Dallner G and Ernster L, Evidence for the involvement of a glucose-6-phosphate carrier in microsomal glucose-6-phosphatase activity. *Eur J Biochem* **82**: 627–634, 1978.
29. Zakim D and Edmondson DE, The role of the membrane in the regulation of activity of microsomal glucose-6-phosphatase. *J Biol Chem* **257**: 1145–1148, 1982.
30. Schulze H-U, Nolte B and Kannler R, Evidence for changes in the conformational status of rat liver microsomal glucose-6-phosphate : phosphodydrolase during detergent-dependent membrane modification. Effect of *p*-mercuribenzoate and organomercurial agarose gel on glucose-6-phosphatase of native and detergent-modified microsomes. *J Biol Chem* **261**: 16571–16578, 1986.
31. Schulze H-U, Kannler R and Junker B, Latency studies on rat liver microsomal glucose-6-phosphatase. Correlation of membrane modification and solubilization by Triton X-114 with the enzymatic activity. *Biochim Biophys Acta* **814**: 85–95, 1985.
32. Zakim D and Dannenberg AJ, Thermal stability of microsomal glucose-6-phosphatase. *J Biol Chem* **265**: 201–208, 1990.
33. Berteloot A, Vidal H and van de Werve G, Rapid kinetics of liver microsomal glucose-6-phosphatase. Evidence for tight-coupling between glucose-6-phosphate transport and phosphohydrolase activity. *J Biol Chem* **266**: 5497–5507, 1991.
34. McCrea PD, Engelman DM and Popot J-L, Topography of integral membrane proteins: Hydrophobicity analysis vs. immunolocalization. *Trends Biochem Sci* **13**: 289–290, 1988.
35. Lodish HF, Multi-spanning membrane protein: How accurate are the models? *Trends Biochem Sci* **13**: 332–334, 1988.
36. Paul C and Rosenbusch JP, Folding patterns of porin and bacteriorhodopsin. *EMBO J* **4**: 1593–1597, 1985.
37. Criado M, Hochschwender S, Sarin V, Fox JL and Lindstrom J, Evidence for unpredicted transmembrane domains in acetylcholine receptor subunits. *Proc Natl Acad Sci USA* **82**: 2004–2008, 1985.
38. Nuwayhid N, Glaser JH, Johnson JC, Conrad EH, Hauser SC and Hirschberg CB, Xylosylation and glucuronosylation reactions in rat liver Golgi apparatus and endoplasmic reticulum. *J Biol Chem* **261**: 12936–12941, 1986.
39. Hauser SC, Ziurys JC and Gollan JL, A membrane transporter mediates access of uridine 5′-diphosphoglucuronic acid from cytosol into the endoplasmic reticulum of rat hepatocytes: Implications for glucuronidation reactions. *Biochim Biophys Acta* **967**: 149–157, 1988.
40. Meissner G and Allen R, Evidence for two types of rat liver microsomes with differing permeability to glucose and other small molecules. *J Biol Chem* **256**: 6413–6422, 1981.
41. Vanstapel F and Blanckaert N, Carrier-mediated translocation of uridine diphosphate glucose into the lumen of endoplasmic recitulum-derived vesicles from rat liver. *J Clin Invest* **82**: 1113–1122, 1988.
42. Vessey DA and Zakim D, Regulation of microsomal enzymes by phospholipids. V. Kinetic studies of hepatic uridine diphosphate-glucuronyltransferase. *J Biol Chem* **247**: 3023–3028, 1972.
43. Finch SAE, Slater TF and Steir A, Nucleotide metabolism by microsomal UDP-glucuronyltransferase and nucleotide diphosphatase as determined by ^{31}P nuclear-magnetic-resonance spectroscopy. *Biochem J* **177**: 925–930, 1979.
44. Alberty RA, The effect of enzyme concentration on the apparent equilibrium constant for an enzyme-catalyzed reaction. *J Am Chem Soc* **75**: 1925–1932, 1953.
45. Vessey DA, Goldenberg J and Zakim D, Differentiation of homologous forms of hepatic microsomal UDP-glucuronyltransferase. II. Characterization of the bilirubin conjugating form. *Biochim Biophys Acta* **309**: 75–82, 1973.
46. Vessey DA and Zakim D, Regulation of microsomal enzymes by phospholipids. IV. Species differences in the properties of microsomal UDP-glucuronyltransferase. *Biochim Biophys Acta* **268**: 61–69, 1972.
47. Zakim D, Goldenberg J and Vessey DA, Effects of metals on the properties of hepatic microsomal uridine diphosphate glucuronyltransferase. *Biochemistry* **12**: 4068–4074, 1973.
48. Dannenberg AJ, Rotenberg M and Zakim D, Regulation of UDP-glucuronosyltransferase by lipid–protein interactions. Comparison of the thermotropic properties of pure reconstituted enzyme with microsomal enzyme. *J Biol Chem* **264**: 238–242, 1989.
49. Rotenberg M and Zakim D, Effect of phospholipids on the thermal stability of microsomal UDP-glucuronosyltransferase. *Biochemistry* **28**: 8582–8587, 1989.

50. March J, *Advanced Organic Chemistry Reactions, Mechanisms and Structure*, 2nd Edn. McGraw-Hill, New York, 1977.
51. Dill KA, Naghizadeh J and Marqusee JA, Chain molecules at high densities at interfaces. *Annu Rev Phys Chem* **39**: 425–461, 1988.
52. Dorsey JG and Dill KA, The molecular mechanism of retention in reversed-phase liquid chromatography. *Chem Rev* **89**: 331–346, 1989.
53. Sentell KB and Dorsey JG, Retention mechanisms in reversed-phase chromatography. Stationary phase bonding density and solute selectivity. *J Chromatog* **461**: 193–207, 1989.
54. De Young LR and Dill KA, Solute partitioning into lipid bilayer membranes. *Biochemistry* **27**: 5281–5289, 1988.
55. Lieb WR and Stein WD, Biological membranes behave as non-porous polymeric sheets with respect to diffusion of non-electrolytes. *Nature* **224**: 240–243, 1969.
56. Trauble H, The movement of molecules across lipid membranes: A molecular theory. *J Membr Biol* **4**: 193–208, 1971.
57. Cruzeiro-Hansson L and Mouritsen OG, Passive ion permeability of lipid membranes modelled via lipid-domain interfacial area. *Biochim Biophys Acta* **944**: 63–72, 1988.
58. Paphadjopoulos D, Nir S and Okhi S, Permeability properties of phospholipid membranes: Effect of cholesterol and temperature. *Biochim Biophys Acta* **266**: 561–583, 1972.
59. Vainio H, Drug hydroxylation and glucuronidation in liver microsomes of phenobarbital-treated rats. *Xenobiotica* **3**: 715–725, 1973.
60. Wilkinson J and Hallinan T, Trypsin-susceptibility of UPD-glucuronyltransferase. *FEBS Lett* **75**: 138–140, 1975.
61. Graham AB, Pechey DT and Wood GC, Responses of microsomal UDP-glucuronyltransferase to trypsin. *Mol Pharmacol* **15**: 375–385, 1979.
62. MacKenzie PI, Rat liver UDP-glucuronosyltransferase. Sequence and expression of a cDNA encoding a phenobarbital-inducible form. *J Biol Chem* **261**: 6119–6125, 1986.
63. Iyanagi T, Haniu M, Sogawa K, Fujii-Kuriyama Y, Watanabe S, Shively JE and Anan KF, Cloning and characterization of cDNA encoding 3-methylcholanthrene inducible rat mRNA for UDP-glucuronosyltransferase. *J Biol Chem* **261**: 15607–15614, 1986.
64. Coffman BL, Tephly TR, Irshaid YM, Green MD, Smith C, Jackson MR, Wooster R and Burchell B, Characterization and primary sequence of a human hepatic microsomal estriol UDP-glucuronosyltransferase. *Arch Biochem Biophys* **281**: 170–175, 1990.
65. Popot J-L and deVitry C, On the microassembly of integral membrane proteins. *Annu Rev Biophys Biophys Chem* **19**: 369–403, 1990.
66. Hunt JF, Bousche O, Meyers KM, Rothschild KJ and Engelman DM, Biophysical studies of the integral membrane protein folding pathway. *Biophys J* **59**: 400a, 1991.
67. Hochman Y, Zakim D and Vessey DA, A kinetic mechanism for modulation of the activity of UDP-glucuronyltransferase by phospholipids. Effects of phosphatidylcholines. *J Biol Chem* **256**: 4783–4788, 1981.
68. Magdalou J, Hochman Y and Zakim D, Factors modulating the catalytic specificity of a pure form of UPD-glucuronyltransferase. *J Biol Chem* **257**: 13264–13269, 1982.
69. Hochman Y, Kelley M and Zakim D, Modulation of the number of ligand binding sites of UDP-glucuronyltransferase by the gel to liquid-crystal phase transition of phosphatidylcholines. *J Biol Chem* **258**: 6509–6516, 1983.
70. Hochman Y and Zakim D, A comparison of the kinetic properties of two different forms of microsomal UDP-glucuronosyltransferase. *J Biol Chem* **258**: 4143–4146, 1983.
71. Hochman Y and Zakim D, Evidence that UDP-glucuronyltransferase in liver microsomes at 37°C is in a gel phase lipid environment. *J Biol Chem* **258**: 11758–11762, 1983.
72. Hochman Y and Zakim D, Studies of the catalytic mechanism of microsomal UDP-glucuronyltransferase. Glucuronidase activity and its stimulation by phospholipids. *J Biol Chem* **259**: 5521–5525, 1984.
73. Zakim D, Cantor M and Eibl HJ, Phospholipids and UDP-glucuronosyltransferase. Structure/function relationships. *J Biol Chem* **263**: 5164–5169, 1988.
74. Rotenberg M and Zakim D, Effects of cholesterol on the function and thermotropic properties of pure UDP-glucuronosyltransferase. *J Biol Chem* **266**: 4159–4161, 1991.
75. Castuma CE and Brenner RR, Effect of fatty acid deficiency on microsomal membrane fluidity and cooperativity of the UDP-glucuronyltransferase. *Biochim Biophys Acta* **729**: 9–16, 1983.
76. Castuma CE and Brenner RR, Effect of dietary cholesterol on microsomal membrane composition, dynamics and kinetic properties of UDP-glucuronosyltransferase. *Biochim Biophys Acta* **855**: 231–242, 1986.
77. Castuma CE and Brenner RR, Cholesterol-dependent modification of microsomal dynamics and UDP-glucuronosyltransferase kinetics. *Biochemistry* **25**: 4733–4738, 1986.
78. Hietanen E, Hanninen O, Laitinen M and Lang M, Dietary cholesterol-induced enhancement of hepatic biotransformation rate in male rats. *Pharmacology* **17**: 163–172, 1978.
79. Zakim D and Vessey DA, Regulation of microsomal UDP-glucuronyltransferase. Mechanism of activation by UDP-*N*-acetylglucosamine. *Biochem Pharmacol* **26**: 129–131, 1977.