for phase separation within the lipid phase (Phillips et al., 1970; Shimschick & McConnel, 1973) UDP-glucose inhibited the activity of UDP-glucuronyltransferase. There was no inhibition at any temperature above 16°C. Similarly, at temperatures below that for phase separation within the lipid portion of the membrane, UDP-glucuronyltransferase was insensitive to activation by UDP-N-acetylglucosamine. Interactions between UDP-glucuronyltransferase and the lipid portion of untreated lipid microsomal preparations are hence not essential for catalytic activity, but they are critical for physiological function. This is true not only for perturbations which increase the fluidity of membrane lipids, but holds as well for decreases in the fluidity of membrane lipids.

Since activation of UDP-glucuronyltransferase by phospholipases and detergents is not unique to this enzyme, the significance of the activation of other enzymes like UDP-glucuronyltransferase may lie also in control of enzyme functions other than the catalytic rate constant.

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The Role of the Microsomal Membrane in Control of Uridine Diphosphate Glucuronyltransferase Activity in vitro and in vivo

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Liver microsomal UDP-glucuronyltransferase is one of many membrane-bound enzymes whose activity is influenced by membrane structure. Evidence for this has been obtained by treating microsomal preparations with detergents and other surface-active agents, phospholipases and proteases, all of which perturb the structure of the microsomal membrane and profoundly affect rates of glucuronidation in vitro (Leuders & Kuff, 1967; Winsnes, 1969; Graham & Wood, 1969, 1972, 1973; Attwood et al., 1971; Mulder, 1970; Mowat & Arias, 1970; Vessey & Zakim, 1971, 1972a,b; Hänninen & Puukka, 1971; Puukka & Hänninen, 1972).

Studies with phospholipase A

Brief treatment of rat liver microsomal preparations with phospholipase A in the presence of Ca2+ increases their UDP-glucuronyltransferase activity (acceptor p-nitrophenol) severalfold (Graham & Wood, 1973). The products of phospholipase A action, namely lysophosphatidies and fatty acids originating from the 2-ester position of microsomal phospholipids, are surface-active compounds capable of altering membrane structure and hence of affecting UDP-glucuronyltransferase activity. The effect of phospholipase A can be simulated by adding to native rat liver microsomal fractions lysophosphatidylcholine, linoleic acid or arachidonic acid, or equimolar mixtures of

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Fig. 1. Effects of phospholipase A hydrolysis products on rat liver microsomal UDP-glucuronyltransferase activity

Microsomal suspensions were treated with various concentrations of palmitic acid (□), stearic acid (■), linoleic acid (▲), arachidonic acid (▲), lysophosphatidylcholine (○), and an equimolar mixture of linoleic acid and lysophosphatidylcholine (▼). Enzyme activity is expressed relative to that of an untreated microsomal suspension. For comparison is included the effect of phospholipase A (●).

These, in amounts approximately equal to those liberated by phospholipase A (Fig. 1). These results are similar to those of Hänninen & Puukka (1971). However, unlike linoleic acid and arachidonic acid (the major unsaturated fatty acids of rat liver microsomal phospholipids) palmitic acid and stearic acid, the major saturated species, did not affect UDP-glucuronyltransferase activity.

Thus the effect of phospholipase A appears to be due to the products of phospholipid hydrolysis rather than a result of any direct effect hydrolysis might have on membrane structure. This conclusion is supported by the finding that addition of serum albumin to phospholipase A-treated microsomal preparations abolished the phospholipase-induced activation. Presumably this is due to binding of lysophosphatides and fatty acids and their removal from the microsomal membrane (Duttera et al., 1968; Fiehn & Hassebach, 1970; Meissner & Fleischer, 1972). Micellar dispersions of phosphatidylcholine, phosphatidyethanolamine, phosphatidylinositol and sphingomyelin abolish activation similarly. This non-specific effect probably is due also to binding of lysophosphatides and/or fatty acids. Neither albumin nor the phospholipids affected the UDP-glucuronyltransferase activity of untreated microsomal preparations.

Studies with protein-deficient rats

The question whether the effects of membrane perturbants on UDP-glucuronyltransferase activity in vitro have any physiological significance has been debated somewhat
inconclusively (see, e.g., Winsnes, 1972). We recently investigated one situation where such observations in vitro do seem to have physiological relevance. Microsomal fractions from protein-deficient rats have enhanced UDP-glucuronyltransferase activities with p-nitrophenol or o-aminophenol as acceptors (Wood & Woodock, 1970; Woodcock & Wood, 1971). Preliminary isolated-liver-perfusion experiments (B. G. Woodcock, unpublished work) suggest that these findings reflect an increased capacity of the liver from protein-deficient rats to synthesize glucuronides. Protein-deficient rats also show an enhanced rate of biliary excretion of bilirubin, presumably as the glucuronide (Eakins & Slater, 1973).

The phospholipid composition of the microsomal membrane from protein-deficient rats differs slightly from that of preparations from normal animals (Graham et al., 1974). The most striking difference is in their content of lysophosphatides, which in protein-deficient animals comprised 9–14% of the total phospholipids, but in animals fed on a normal diet only 0–2.7%. The increased lysophosphatide content was not due to an endogenous phospholipase A acting after death but, like the enhanced UDP-glucuronyltransferase activity, probably reflects an abnormality occurring in vivo. The amounts of lysophosphatides in microsomal preparations from protein-deficient animals are sufficient to have caused their elevated UDP-glucuronyltransferase activities as judged from results with phospholipase A and lysophosphatidylycholine. Lysophosphatides may therefore contribute to physiological control of UDP-glucuronyltransferase. A similar role for lysophosphatides has recently been suggested in relation to another rat liver microsomal enzyme, UDP-galactose–glycoprotein galactosyltransferase (Mookerjea & Yung, 1974).

In view of the evident importance of membrane structure in relation to enzyme activity it is pertinent to consider the ways in which its influence might be exerted.

**Possible effects of membrane structure on enzyme activity**

(a) *Effects of localized high enzyme concentration.* Diffusion of substrates to the microsomal membrane and through the ‘fixed layer’ surrounding it may be rate-limiting under some circumstances. Such effects are probably overcome in vitro by efficient stirring, but may well be significant in vivo.

(b) *Limitation of access of substrates to enzyme active sites.* If the enzyme is located in the microsomal membrane so that a permeability barrier is interposed between substrates and active site, partition of substrates between the aqueous and membrane phases, permeability of the microsomal membrane and the nature of substrate-transport mechanisms may all affect the overall rate of the enzyme reaction. The size and surface charge of the microsomal vesicles may be important. Lysophosphatides, unsaturated fatty acids, phospholipase A and detergents increase the permeability of microsomal membranes (Fiehn & Hasselbach, 1970; Kreibich et al., 1973) and as a result might affect the apparent activity of rat liver UDP-glucuronyltransferase.

(c) *Effects on the microenvironment of the enzyme.* Depending on the location of the enzyme in the membrane its interaction with substrates may occur in an environment which is different (e.g. in pH) from that outside the microsomal vesicles (Katchalski et al., 1971).

(d) *Effects on the ‘intrinsic activity’ of the enzyme.* A membrane-bound enzyme may, as a result of interaction with other membrane components, have a different conformation (and hence different catalytic activity) from that which it would possess in free solution (Laidler & Bunting, 1973). Further, involvement of specific membrane components in the enzyme-catalysed reaction can lead to absolute membrane-dependence.

These membrane modulation mechanisms may not be equally important with all microsomal enzymes. Even with the same enzyme, UDP-glucuronyltransferase, they may not be equally important in microsomal preparations from different species or isolated by different methods. Assessment of their relative significance must ultimately depend on isolation of a soluble form of UDP-glucuronyltransferase. Meanwhile qualitative information can be gained by comparing the behaviour of microsomal preparations
from different sources which appear to differ in the responses of their UDP-glucuronyltransferase activity to membrane perturbants.

**Comparison of rat and guinea-pig microsomal preparations**

Mild treatment of rat liver microsomal preparations with Triton X-100, deoxycholate or digitonin (Leuders & Kuff, 1967; Heirwegh & Meuwissen, 1968; Winsnes, 1969; Hänninen & Puukka, 1970; Mulder, 1970; Vessey & Zakim, 1971; Graham & Wood, 1973), trypsin digestion (Hänninen & Puukka, 1970) or peroxidation of microsomal phospholipids (Hogberg et al., 1973) activate UDP-glucuronyltransferase in much the same way as do phospholipase A, lysophosphatides and unsaturated fatty acids. In contrast, similar treatments of guinea-pig microsomal fractions (prepared in 0.154 M-KCl) cause very little if any activation. Indeed, some of them (phospholipase A, deoxycholate, trypsin and peroxidation, but not Triton X-100, lysophosphatides or fatty acids) gradually inactivate the enzyme (Graham & Wood, 1969; Wood & Graham, 1972; D. N. Bentley & G. C. Wood, unpublished work). Inactivation by phospholipase A cannot be reversed by serum albumin and is not due to the products of phospholipid hydrolysis (cf. the effect of phospholipase A on rat liver microsomal fractions). However, it can be reversed by adding phosphatidylcholine micelles, a specific effect not shown by other microsomal phospholipids. The responses of microsomal UDP-glucuronyltransferase from the two species to membrane perturbants are thus radically different.

The UDP-glucuronyltransferase activity of native rat liver microsomal preparations is very low and activation by membrane perturbants merely raises it to that of guinea-pig preparations (Graham & Wood, 1973). The latter appears to be fully active whereas the activity of native rat preparations is apparently restricted by some 'membrane constraint'. It has been suggested (Mulder, 1970; Winsnes, 1969; Hänninen & Puukka, 1970; Hogberg et al., 1973) that this constraint is due to a membrane barrier restricting access of substrates to enzyme. Vessey & Zakim (1971) have suggested that the phospholipids of the intact membrane constrain the enzyme in a relatively inactive conformation. Present data do not permit us to distinguish between these two mechanisms, if indeed either operates to the exclusion of the other. The observation (Ito & Sato, 1969) that the attack by one of the activators, trypsin, is restricted to proteins on the outer surface of microsomal vesicles suggests that permeability might be rate-limiting.

Whatever the nature of the constraint on UDP-glucuronyltransferase in rat liver microsomal preparations it appears to be almost absent from guinea-pig preparations, at least when they are prepared in KCl. We suggest that the inactivation of guinea-pig enzyme by phospholipase A is due to disruption of membrane structure in the neighbourhood of the UDP-glucuronyltransferase molecule so as to alter its microenvironment or change its conformation to a less active form. Introduction of a reactivator such as phosphatidylcholine places the enzyme in an environment where it can again function actively; although the specificity for reaction is high the new environment is not necessarily identical with that of UDP-glucuronyltransferase in native microsomal fractions.

Inactivation of guinea-pig UDP-glucuronyltransferase by trypsin is also reversed by phosphatidylcholine. Since the attack of trypsin is probably restricted to the outer surface protein of the microsomal vesicles (Ito & Sato, 1969) this indicates that the transferase may be located near the outer surface of the microsomal membrane where it is less likely to be subject to a permeability barrier such as has been postulated for the enzyme in rat liver preparations.

**Solubilization**

Several attempts to solubilize liver microsomal UDP-glucuronyltransferase have used detergents (Mowat & Arias, 1970; Isselbacher et al., 1962; Labow et al., 1971; Bock et al., 1973). In one study (Mowat & Arias, 1970) the product was shown not to satisfy the criteria for solubilization enumerated by Razin (1972), i.e. non-sedimentability at 100000 g in 1 h, inclusion in Sepharose 4B and absence of vesicular particles in electron micrographs. These criteria have not been rigorously applied in other studies, although...
The functional outcome of the hypothalamus mechanism is discussed above.

Investigation of the hypothalamo-cortical modulation mechanisms discussed above.

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Heterogeneity of Hepatic Microsomal Uridine Diphosphate Glucuronyltransferase: A Critical Evaluation

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Hepatic UDP-glucuronyltransferase is located in the endoplasmic reticulum and is, after homogenization of the liver, recovered in the microsomal fraction. It is difficult to determine what differences and artifacts are introduced by this disruption of the cell with regard to UDP-glucuronyltransferase properties.

So far it is generally believed that UDP-glucuronyltransferase is heterogeneous. The idea of heterogeneity originated from results of studies in several animal species lacking glucuronidating activity towards some substrate(s) and from studies on, e.g., development of enzyme activity after birth or tissue distribution of the activity towards different substrates (cf. Dutton, 1971).

Since 1969 many studies have appeared dealing with the consequences of the membrane-bound character of UDP-glucuronyltransferase (Graham & Wood, 1969; Mulder, 1970; Vessey & Zakim, 1971; Winsnes, 1972; Zakim et al., 1973a; Graham et al., 1974). It has become evident that properties of the enzyme are severely influenced by treatment of the microsomal membrane with agents affecting the structure of the membrane. Further, several incongruities in the often rather conflicting results could be attributed to technical differences in assay procedures (Mulder, 1971, 1972; Winsnes, 1971). Thus the 'scenery' of UDP-glucuronyltransferase has changed from that in the sixties and it seems necessary to reconsider the idea of multiplicity in the light of the new findings.

UDP-glucuronyltransferase activity is associated with the enzyme protein, including any factor absolutely required for expression of the activity. Although there is no definite evidence, some phospholipid might be required for activity. The apoenzyme of β-hydroxybutyrate dehydrogenase requires as many as 200 molecules of phosphatidylcholine per molecule of enzyme for full activity (Fleischer et al., 1966); if a similar situation applies to UDP-glucuronyltransferase this may set an upper limit to purification of the enzyme protein.

It seems appropriate to make a sub-division of various kinds of heterogeneity. First there might exist heterogeneity in amino acid sequence, i.e. 'genetic heterogeneity'. Then, quaternary structure ('conformation') of the enzyme protein might be heterogeneous (allosterism in its broadest sense). Because the enzyme conformation depends on interactions with the surrounding membrane, conformational effects seem very prob-