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COMMENTARY

CONTROL OF UDP-GLUCURONYLTRANSFERASE ACTIVITY

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UDP-glucuronyltransferase (GT) as the enzyme, or enzyme family*, responsible for glucuronylating a very wide range of compounds has been assured of continued pharmacological interest since its discovery some 20 years ago [2]. The reasons are clear. It remains the only credible agent for adding glucuronic acid to an aglycone; β-glucuronidase, long coached for the role, still waits expectantly in the wings, and transglucuronylation from one aglycone to another appears [3] to need GT itself acting, as it does with certain substrates [4], reversibly. Moreover, glucuronidation, as the commonest form of conjugation, is probably the most important truly detoxicatory process for, unlike hydroxylation, it does not produce transient toxic intermediates but attaches a strongly polar molecule to the aglycone, thereby increasing solubility and muffling biological activity. Its substrates include endogenous compounds such as thyroid and steroid hormones, catechol amines and bilirubin, as well as a great range of xenobiotics and their metabolites, a selection of which is duly quoted in the introductory paragraphs of most papers dealing with the subject.

The enzyme has, however, proved notoriously difficult to investigate satisfactorily. Its enormous literature confirms its apparently inexhaustible capacity for furnishing episodes of anecdotal natural history—species, strain, sex, tissue, dietary, developmental and substrate specificities—and its equal power of luring kineticists, empirical or dogmatic, into often self-destructive complexities.

The cause of this unsatisfactoriness is now evident. The fault does not lie solely with the investigators. GT is very difficult to study. It is a prime example of a molecule now resisting progress in many fields—the membrane-bound microsomal enzyme. It can be activated and induced to a remarkable degree and its biological properties depend intimately on its readily-modified membrane environment.

GT has therefore become of great current interest to enzymologists as well as to biochemical pharmacologists, and we may expect considerable progress in our understanding of it over the next few years. Recently, however, much has become known, and this commentary will deal with selected aspects of that controversial subject, the control of its activity.

The subject is controversial not only from its intrinsic difficulty, but also from the firm opinions occasionally propagated. As endeavours to confirm, or refute, such opinions have given us most of our present valuable information, we imply no criticism. But a Commentary is by nature selective, personal and speculative, and by no means a fully-documented review. Much excellent work will not be described or even mentioned here. Therefore, for full discussion and references the reader is recommended to those detailed accounts of glucuronidation which already exist [2, 5, 6] or will shortly appear [1]. Brief reviews of specialised aspects will be referred to separately.

We intend to discuss three aspects of GT control. The first and second concern activation and induction, the fine and coarse adjustments respectively of GT activity; the third concerns the coupling of GT with associated enzyme systems.

ACTIVATION

The properties of a bound enzyme are modified by the nature of its binding matrix. This is evident with artificially-immobilised enzymes [7, 8], their pH optima and affinity for various substrates can differ considerably from those of the same enzymes when soluble, and may change with the differing constraint afforded by different matrices. The nature of the matrix can determine local ionisation of substrates or even their physical access. It can determine the conformation of the bound enzyme and hence its catalytic properties. It can exert both these forms of constraint together.

GT is bound to the microsomal membrane and its 'solubilisation' has never yet satisfied all of Razin's [9] criteria. It is presumably equally firmly bound to the endoplasmic reticulum *in vivo*. This brings us our first problem. We can study control of glucuronidation *in vivo*, in perfused organs, in cultures, in cell suspensions, or in tissue slices. But we can only study control of GT itself in homogenates or in microsomes. How similar is GT in microsomes to GT in the endoplasmic reticulum of the cell?

Microsomal GT can usefully be studied in two forms, fresh or fully activated. The difference in activity between them, when measured with high levels of added UDP-glucuronic acid, can rise to 40-fold [10] depending upon source and substrate, but is usually less. Both preparations suffer drawbacks in practice. Fresh microsomal GT ('native' GT) begins to be activated as soon as prepared, even at 0°, so speed is essential: and how far has activation proceeded already, with the time and the ions (or lack

* We cannot discuss here the heterogeneity of GT. The only presently acceptable criteria for heterogeneity are separative, and by these it is provisionally established for a few substrates. (see Ref. 1).

of ions) employed in microsomal preparation? Then again, how 'fully' can one activate GT? For activation may peak and fall into inactivation progressively.

Nevertheless the two states of fresh and fully activated microsomal GT can be approximated to. Which state most resembles the enzyme *in vivo*? Or, to put the question differently, how 'latent' is GT in the cell?

Two approaches have been employed. One works back from glucuronidation rates in intact organ or cells, and compares them with the respective GT activities displayed by the two microsomal preparations [11, 12]. The other, bolder and more relevant to our subject here, compares the kinetic efficiency and susceptibility to control of the two preparations *in vitro*. Both approaches assume that UDP-glucuronic acid (UDPGA), UDP-*N*-acetylglucosamine, UDP and similar accessory molecules exist around GT in the cell at concentrations similar to the concentrations measured in extracts of whole liver. They assume no compartmentation or other restriction of access to GT. We shall return to the validity of this assumption later. We will first outline the findings of the kinetic approach, which in one case have been assembled into a satisfyingly coherent theory. As its authors admit in a forthcoming short review [13] this theory applies in its details so far only to one species, guineapig, one tissue, liver, one substrate, *p*-nitrophenol and two activators, UDP-*N*-acetylglucosamine and phospholipase A. Its more general application to GT activities *in vitro* remains unproved, though suggestive. Even if it turns out to be totally incorrect, which seems unlikely, it has provided for the GT field a necessary focus of constructive, idea-oriented research. Though several earlier, and some contemporary, workers have demonstrated similar evidence or advanced similar views, the following is the only detailed hypothesis advanced (for summaries see Refs. 14, 13) and is therefore outlined here.

GT fully activated by membrane perturbation would be kinetically inefficient *in vivo*. Activation by phospholipase A decreases K_{UDPGA} but increases affinity of the UDPGA active site for the UDP moiety of UDPGA and for UDP itself; it also abolishes specificity of the UDPGA active site, allowing competition from other UDP sugars. Only *in vitro* at artificially high levels of added UDPGA, can high activity be obtained from the activated enzyme. GT in untreated microsomes, however, accepts UDPGA specifically. But as this 'native' GT also is strongly inhibited by the small UDP levels occurring (overall) in liver, it too might appear catalytically inefficient *in vivo*. However, at physiological temperatures and concentrations of UDP-*N*-acetylglucosamine and divalent metal ions [15], UDP-*N*-acetylglucosamine acts like an allosteric effector of untreated GT, enhancing binding of UDPGA and decreasing binding of UDP. By decreasing binding of UDP, it also inhibits the reverse reaction of GT. UDP-*N*-acetylglucosamine is thus a suitable physiological modifier of GT if the enzyme occurs latent *in vivo*: it activates the forward reaction, inhibits the reverse reaction, limits end-product inhibition and preserves donor substrate specificity [14, 13]. This concept may appear rather too neat and tidy. Have we stumbled on the only endogenous modifier? Are there no others? Product glucuronides can inhibit GT *in vitro*, but in untreated microsomes with physiological concentrations of UDPGA, these glucuronides may also activate GT by increasing its affinity for UDPGA [16]; a useful property if glucuronides accumulate during biliary stasis. Doubtless room could be found for other, undiscovered, endogenous modifiers, positive and negative, within this model.

The general conclusion that GT is controlled *in vivo* over a small range of activity by endogenous modifiers acting on an essentially 'inactivated' enzyme has also been reached by workers who compare rate of overall glucuronidation in intact organisms, tissues or cells, with specific GT activity in microsomal preparations [11, 12], though not by all [17, 18].

It should now be clear that the validity of this conclusion depends largely on free availability to GT *in vivo* and *in vitro* of nucleotides, ions and other agents at the concentrations measured in whole liver, especially when kinetic interpretations become complex. Resistance by some workers to the idea of compartmentation of GT in the endoplasmic reticulum or microsome is therefore understandable. The various stages of GT activation brought about by successive surfactant or enzymic procedures are considered by them not to reflect peeling off of barrier membranes or modification of permeases, but to result from conformational changes in an always largely-exposed GT enzyme, following changes in its supporting (but not enclosing) microsomal membrane.

However, some degree of compartmentation, even if embarrassing to more detailed speculations, could still be compatible with the general conclusion. The allosteric sites of the kinetic theory are then regarded as permeases (that for UDPGA being activated by, for example, UDP-*N*-acetylglucosamine); the spectrum of multiple conformations becomes a progression from actively-regulated to partially-disorganised transport complexes. Unfortunately, whilst proponents of compartmentation can show much supporting evidence [19–23], they cannot, from the nature of their proposed mechanism, yet go beyond the more obvious inferences of its role in the control of GT. As this is a Commentary on control of GT, not on its topology, we can do no more here than bring readers' attention to this vigorous, and invigorating, dialogue which will certainly give us further exciting information. To an observer it would seem that, like an artificially-immobilised enzyme, GT could well be constrained both by conformation and by limited access: doubtless at present a tepidly equivocal opinion.

INDUCTION BY XENOBIOTICS

Membrane constraint, by the one mechanism or the other, or by both, being so important for GT activity, we can consider its role in procedures that alter that activity. This must blur the distinction between activation and induction of the enzyme. Activation is increased in catalytic activity of an enzyme molecule; induction is increased in concentration of enzyme molecules. GT could be activated by a change in membrane constraint brought about by synthesis of membrane protein and/or membrane lipid. But if this new material were part of the GT catalytic complex, then the process might be called induction of

GT. Until we know more about what is, and what is not, GT and where its active sites are, we shall have to use the term induction to cover the slow increase of GT activity observed in the living cell; and reserve activation for the effect, not involving protein synthesis, seen *in vitro* (and also in the dying cell, where it strikingly parallels activation *in vitro* [24] and where it would, if activated GT played a physiological role, be an efficient scavenger of glucuronidogenic toxins).

The role of the membrane during induction of GT is evident. It is well known that pretreatment of animals with, for example, phenobarbital alters the composition of the microsomal membrane. Pretreatment of rats with phenobarbital increases liver GT activity towards its substrate, but this increase is obvious only when perturbants are present during the assay [25, 26]; i.e. a large part of the induced activity remains latent in fresh microsomes (ill-termed 'increased latency'). Treatment with trypsin sufficient to remove a small percentage (superficial layer?) of microsomal protein allows activation of GT in normal rats [19]; after phenobarbital administration, a greater percentage of microsomal protein is removed by trypsin before activation occurs [27]. Existing constraint of GT has been strengthened after phenobarbital treatment; this may be because its 'native' conformation has been more securely anchored, a surrounding annulus of superficial protein has been thickened, or because the 'new' GT is sequestered in new 'holes' deeper in the enlarged and modified endoplasmic reticulum.

Various other activation characteristics alter following induction of GT by xenobiotics e.g. [26, 27]; kinetic parameters change e.g. [26, 11], as do relative activities to various substrates [28]. Moreover, the changes induced both in GT and in membrane characteristics differ between inducers such as phenobarbital and methylcholanthrene [28, 29, 12]. These effects, although occurring in an essentially fluid membrane, recall those brought about by changes in the supporting matrix of immobilised enzyme.

Induction of GT requires protein synthesis [28] and may be directly related to it [30], but it is often difficult to determine how far increased concentration of GT catalytic units is responsible for the increases of GT activity observed *in vitro* and of glucuronidation observed *in vivo*.

INDUCTION FROM ZERO LEVELS BY ENDOGENOUS INDUCERS

One good example of an obvious increase in such catalytic units must be the development of GT from zero levels in the early foetus to adult levels at, or after, birth (for reviews see [1, 2, 6, 31]). This presumably reflects hormonal control, i.e. induction by endogenous inducers.

Xenobiotic and endogenous compounds are complementary in the study of enzymic control. Xenobiotic inducers can provoke dramatic responses; once we know that such responses are possible, we search for endogenous inducers, the responses to which are more specific and so usually less obvious. With GT, hormonal 'induction' and 'repression' was noted many years ago [32]; but this concerned sex hormones and affected existing high levels of GT. It could have operated through progressive membrane change. More recently [33] thyroidectomy or hypophysectomy of adult rats has been shown to lower liver GT activity to *o*-aminophenol. However, involvement of membrane environment is suggested by change in activation characteristics of the enzyme [34] and by the remarkable fact that full activity was restored on addition of diethylnitrosamine to the microsomes. This compound had earlier been shown [35] to restore normal 'activated' GT activity towards *o*-aminophenol in Gunn rat liver microsomes, once thought to lack such activity; they possess it, but in a form almost wholly latent for that substrate unless diethylnitrosamine is present. Diethylnitrosamine therefore modifies environmental constraint imposed genetically and/or by hormonal imbalance; it is tempting, despite the displeasure of conformationists, to suggest it facilitates access of *o*-aminophenol.

We suggested above that induction of foetal GT should provide evidence of its synthesis from zero levels under hormonal influence. But, after the example just quoted, can we assume that GT is truly at zero, or low, levels in foetal liver? Apparently there are no foetal inhibitors of GT present. Yet might not GT exist at high concentration, but latent, awaiting an endogenous inducer (or an accidental xenobiotic comparable with diethylnitrosamine) to rouse it into catalytic activity?

Since the early reports of low liver GT levels perinatally (see [1]), much evidence of occasionally quite high late-foetal or neonatal levels has accumulated. Recent work clearly indicates that substrate and strain differences dominate rates of GT development. At 17 days foetal age, ASH/TO mice possess almost undetectable activity towards bilirubin, 50 per cent of maternal level towards oestriol and 100 per cent towards *o*-aminophenol [36, 37]; 17-day foetal liver from ICR/IAR strain mice possess only 20 per cent adult activity towards *o*-aminophenol*. But in all strains and species tested, foetal liver when taken early enough appears to possess low ($<10\%$ adult) or zero GT activity towards xenobiotic or endogenous substrates, even after activation procedures (including diethylnitrosamine treatment) [1, 2, 6, 36]. One could reasonably argue that procedures activating adult GT might well inactivate foetal GT, especially if membrane characteristics change with age. They do change with age [38, 39]. Period of exposure to sonication, and ratio of perturbant to protein, required for optimal activation are age-dependent; and digitonin concentrations scarcely activating adult GT completely inactivate the foetal enzyme*. However, even with optimal conditions for foetal GT, it has never been reported to be activated above activated adult levels; and in early foetal liver it has remained low or absent, as does the overall glucuronidation rate in intact cells (which would, however, suffer from their low foetal UDPGA synthesis and possibly low secretion). There seems no evidence *in vitro* or *in vivo* for a full adult complement of GT

* J. Leakey, unpublished.

lying wholly latent in early foetal liver; unless we suppose an undetected specific activator for foetal GT, or that we have first activated and then inactivated the enzyme by homogenisation, both somewhat unnecessary hypotheses at present.

Study of induction of mammalian foetal liver GT are complicated by maternal hormonal interference. Chick embryo liver GT is a more convenient model to begin with. It possesses a developmental pattern similar to that of mammals, with a rapid surge from virtually zero to adult levels within 1–3 days after hatching at 21 days (see [31]); maternal influence, if it exists, is restricted to hormonal messages in the yolk; culture of embryo tissues is simple, and other environmental manipulations are readily performed.

Evidence on two aspects of GT control have come from experiments with the chick model (for earlier summaries, see [31]). One may be termed 'spontaneous induction', the other 'induction *in ovo*'. The first is unusual and not yet explained; the second is providing useful information. We shall begin with the first.

When liver or kidney is removed from chick embryo and cultured as organ fragments or as a cell monolayer in a medium of salts, amino acids and vitamins, GT activity to the substrates tested increases rapidly and precociously to adult levels or over [40, 41, 30]. Other microsomal or associated enzymes studied remain unchanged, rise slightly, or fall [30, 31]. Rise of GT is at a constant rate, suggesting initiation by a process completed at beginning of culture, and not by continued increase of a stimulatory condition such as membrane change. The process required protein synthesis. Transcription appears more likely than translation, for the rise was directly inhibited by cycloheximide-pulsing and not unequivocally prevented by actinomycin D [30]. The rise is independent of cell division or tissue architecture and occurs under a wide range of culture conditions which permit either normal morphological maturation or gross cellular degeneration [31]. Activation might be thought the explanation in the latter case, but chick embryo liver, fresh or cultured, possesses negligible latency [42] and cycloheximide or lack of oxygen or of nutrients inhibited the rise. Slices of cultured tissue or sheets of cultured cells also exhibited precociously high levels of overall glucuronidation; so by whatever process GT is stimulated, its stimulation is evident in the intact cell (which possesses adult UDPGA levels: see [38]).

The rise appears to be initiated by removal from the embryonic environment. Culture on the chorioallantoic membrane (CAM) of another egg resulted in little, if any, rise, whereas subsequent transfer of such grafted tissue to a dish resulted in stimulation of GT activity. Conversely the GT activity attained after brief culture failed to increase when grafted on to the CAM [41]. One could invoke poor nutrition of the graft as explanation, but it was considered more likely that the embryonic environment was repressing GT synthesis. Control of GT synthesis by this environment was further suggested from comparison of spontaneous inductions achieved by culture of 11- and 5-day embryo liver. In both cases GT reached a plateau after 8 days' culture, but in the older liver GT did not usually exceed adult values whereas in the younger liver it regularly rose 5–10 times above them [41]. Possibly a control mechanism limiting GT rise to adult levels develops between 5 and 11 days *in ovo*. If so, this control appeared over-ridden by phenobarbital, for if that drug was present in the culture medium GT development was accelerated at both ages, and in the older livers then reached the high levels of the cultured younger livers, whose absolute level was little changed by phenobarbital [30]. However, this age difference is not so clear cut in all chick strains and may affect, not specifically GT control, but age-dependent response to culture conditions.

The results certainly indicated that GT can develop without any obvious endocrine influence and that phenobarbital induces GT directly in the liver [30] (the latter is also suggested by work on adult heptocyte survival: see [43]). They focus attention on the problem of induction *in ovo*. Can GT be induced in that environment at all, or is competence only achieved at the 21st day? Phenobarbital was therefore injected into the egg [44]. It initiated a dose-dependent surge in liver and kidney GT activity from zero levels, increasing with length of exposure. Response was detectable as early as 96 hr of age. Embryo liver thus seems competent from its earliest development to form GT following signals supplied by xenobiotics such as phenobarbital.

What, then is the endogenous inducer of GT at hatching? Or what factors at that time counteract an *in ovo* repression? Changes in oxygen or CO_2 tension did not stimulate or inhibit the normal perinatal pattern [44]. Moreover, GT rise *in ovo* could be dissociated from the physical process of hatching. Thiourea injections disturbed growth relationships and GT rise occurred near the hatching period without initiation of shell-pecking; hatching of 1-day embryos into dishes allowed development up to 8 days, but GT did not increase [44]*.

Hormones were then injected. Various corticosteroids evoked responses from enzymes such as liver phenylalanine hydroxylase, but no conclusive change in GT activity. Glucagon, adrenaline, thyroxine, serotonin and several pituitary hormones or extracts, some of chicken origin, were also injected. Results were inconclusive. Low doses, which may have been inactivated below threshold, had no effect; higher doses killed the embryo. Occasional elevations of GT were not reproducible. Whilst new injection methods were being devised a more physiological approach was adopted, which might uncover an unknown factor.

Hypophysectomy at 38 hr produced embryos typically anencephalic and unable to retract their yolksac, but did not affect GT development *in ovo* or on culture. Pituitary factors therefore appeared unlikely to repress GT *in ovo* or stimulate it on removal to culture. Endocrine or control tissues were then grafted on to the CAM of 12-day embryos. Sexual, adrenal and thyroid glands proved negative, but with pituitary tissue precocious GT development was initiated. Activity towards *o*-aminophenol and *p*-nitrophenol in liver and kidney reached adult levels within 4 days [45]*, i.e. 5 days precociously. Stimulation of anilene hydroxylase activity was also

* G. J. Wishart and G. J. Dutton, unpublished.

noted [45]*. Donors could be adult birds, or embryos down to 15 days, the youngest tested. By weight, the most effective donors were embryos just about to hatch. Presumably a factor was released from the graft into the bloodstream. Its levels were critical and displayed a threshold effect. Two pituitaries from a 6-week chicken invariably killed the embryo; two from an 18 day embryo provoked a response several-fold greater than one gland. Release of factor appeared to initiate GT rise, for its secretion (judge from potency of pituitary tissue) peaked just before the surge of GT.

Pituitary factor acts on embryo liver tissue *in situ* or on liver fragments on the CAM; it so far has not influenced spontaneous GT rise in liver cultured *in vitro*, either alongside viable factor-producing pituitaries on a raft or below them on a paper strip down which medium (and presumably factor) is passing*. Moreover, GT is not increased in liver of host embryos younger than 11 days when grafted. Response in these 11-day embryos is first seen 3 days later; allowing 1 day for vascularisation of the graft and 12–24 hr for onset of GT induction, this places response to secreted factor at 13 days. The pituitary-thyroid, pituitary-adrenal and pituitary-gondal axes mature in chick embryo at 11, 13–14 and 13–14 days respectively. These findings suggest that pituitary control of GT operates via a secondary endocrine system. The only pituitary area supplying the factor is the cephalic portion [45], which produces TSH, ACTH and FSH in the chicken. The role of these hormones and their secondary agents is now being investigated with new techniques of administration. Involvement of ACTH and corticosteroids appears likely from several lines of evidence [64]. It should be noted, however, that an inducer (such as the pituitary factor) which 'triggers' induction of an enzyme is not necessarily that which subsequently maintains its synthesis (see [46]). Maintainers of induced GT activity may well operate on the membrane environment, as suggested above for thyroxine in rats [33]. The 'trigger' for GT therefore need not be a hormone (such as TSH) active simply through thyroxine release, although this trigger may render GT competent to respond subsequently to, say, thyroxine. We cannot speculate further here; the complex feed-back interrelationship of hormonal effects demand too much space.

To link this induction by pituitary factor *in ovo* with the spontaneous induction on culture is at present difficult. The former is a hastening of natural regulation, the latter a gross disruption of it. If liver from a pituitary-grafted embryo is subsequently cultured, the high 'pituitary-induced' GT level rises and then falls, and then recovers to rise again in the usual 'spontaneous induction' pattern. It is not likely that the same initial inducers are involved; as may be guessed from the different age-dependences of the two processes.

How do these effects in embryo liver relate to control of GT in developing mammalian foetal liver? Two differences between GT control in embryo and foetus must be noted. Firstly, GT has never developed precociously by 'spontaneous induction' in cultured foetal liver; it merely increases at the normal *in utero* rate [31, 36] and if it has already reached adult level *in utero* it decreases. Secondly, early foetal liver GT does not seem competent to respond to phenobarbital in culture or *in utero* [31, 36, 37]. Maternal ASH/TO mice fed phenobarbital possessed high hepatic phenobarbital levels and GT activity double that of control mothers. Their foetuses possessed similar high phenobarbital levels in liver but their GT was no higher than that of control foetuses until 3 days before birth. By one day before birth, GT activity was double that of controls, and it continued higher neonatally. Neonatal induction of GT is familiar (see [2, 6]) but it is surprising to find liver GT, unlike liver microsomal monooxygenases, not responding foetally until near birth. This lack of competence was demonstrable in ASH/TO foetuses with all three substrates employed, *o*-aminophenol, oestriol and bilirubin [36, 37]; GT develops, it will be remembered, for each of them at quite a different rate. It is unlikely that the enzyme is being induced in a latent or easily inactivated form for all three substrates; widely varied preparative procedures have given no evidence of hidden induction. Synthesis *de novo* appears more probable.

Is a pituitary factor responsible for triggering this synthesis of GT in foetal mammals? Hypophysectomy or thyroidectomy of adult rats increased the latency of GT activity towards *o*-aminophenol but left activity towards bilirubin unchanged [33]. However, once synthesis of an enzyme has been triggered, other factors maintain the synthesis and the original trigger may appear to have diminished potency [46]. One could speculate that a pituitary factor was needed to trigger GT activity towards bilirubin and also to give the enzyme competence to respond subsequently to bilirubin as inducer, so that maintenance of activity towards bilirubin henceforth depended largely on bilirubin and (as found—see[33]) not on pituitary or thyroxine. Hypophysectomy of an adult rat is therefore not comparable to the pituitary 'insufficiency' of a foetus, and we are free to consider that a pituitary factor could well trigger the perinatal surge of all GT activities in the foetal liver.

Human pituitary increases secretion of TSH 5–10 fold at birth [47]. Although earlier work (see Ref. 1) demonstrated no effect on perinatal liver GT from implanted pituitary or injected hormones, the experiments were limited. GT status is now being investigated‡ in foetuses treated *in utero* with pituitary factors and with hormones, and in foetal liver grafted on to the CAM together with chick or mouse pituitary tissue. It seems likely that the factors controlling endogenous development of GT in both chick and mammalian tissues will be identified in the near future. Membrane changes undoubtedly accompany this induction, but there seems no reason to doubt that increased synthesis of GT catalytic units is involved and that in chicken at least development of this enzyme is fundamentally controlled by the cephalic portion of the anterior pituitary glands.

COUPLING TO ASSOCIATED SYSTEMS

How closely is GT coupled to metabolically-related enzyme systems, such as those determining levels of

* J. Leakey, unpublished.

† G. J. Wishart and G. J. Dulton, unpublished.

‡ J. Leakey, G. J. Wishart and G. J. Dutton, unpublished.

UDPGA, of glucuronide hydrolysis and of hydroxylated aglycones?

GT activity depends on the UDPGA available. But UDPGA levels in the liver cell need not reflect the UDPGA available to GT. As suggested by its turnover rate [48] UDPGA has many fates [2, 5], and intracellular channelling to selected enzyme systems, if necessary by compartmentation, appears likely. Neither do UDPglucose levels directly determine UDPGA formation; feeding with D-glucosamine lowers liver UDPglucose but raises UDPGA [49]. Close linkage in development of UDPglucose dehydrogenase and GT need therefore not be expected, and it is not found [50], the dehydrogenase being much less readily influenced by drugs or culture. Similarly, neither activation [51–53] nor induction [54, 38]* of GT is accompanied by parallel changes in UDPGA pyrophophatase of β-glucuronidase. The last enzyme is usually studied as whole cellular activity at low pH; relationship to GT would be best examined at higher pH in the common microsomal fraction.

Coupling of GT to microsomal monooxygenases has been attractively sketched [55, 56] but never demonstrated. These two systems can appear in different tissues, develop at different times [57], be induced separately [58, 59] and respond differently to membrane perturbation [56]. The monooxygenase is much more sensitive to perturbation than GT, and so is thought to be nearer the membrane surface [20, 56]; certainly procedures which activate GT inactivate the monooxygenase. One could speculate that perhaps GT appears activated in these instances because the monooxygenase system is destroyed; that coupling may indeed occur between some GT and monooxygenase, as between monooxygenase and some epoxide hydrase (see [60]). Such logically-extended coupling would further assist detoxicatory removal of cytotoxic lipophilic compounds by taking structural advantage of the endoplasmic reticulum membrane. Membrane integrity would be needed to ensure funnelling of hydroxylated metabolites towards GT; it would 'choke' GT and limit its accessibility for added hydroxylated substrates *in vitro*, much as added UDPGA is suggested to be less available to fresh microsomal GT than is UDPGA generated intracisternally [3]. Induction by phenobarbital or methylcholanthrene would preferentially induce the coupled GT; hence its high observed degree of latency. (Methylcholanthrene induces the coupled epoxide hydrase, phenobarbital the free: Ref. 60). Activation would structurally uncouple the two neighbouring complexes by destroying the monooxygenase and all GT would then be free to accept the substrate added *in vitro*. This idea is quite compatible with the separation of GT from cytochrome P-450 on chromatographic purification after perturbation (e.g. [61]), but less so with its separation during centrifugation of apparently native microsomes [62]. Also, a considerable degree of compartmentation must be assumed *in vivo*, and channelling of hydroxylated products, when at low levels, towards sulphate conjugation [63] must be taken into account.

To sum up, it is clear that study of the control of GT is intimately concerned with study of its membrane environment. Existing proposals for any one type of constraint may have to be modified, as they have been for membrane-bound systems in the mitochondria. Study of mitochondrial constraint is easier. The microsome is an artefactual organelle, heterogeneous, of inconveniently small luminal volume, and derived from a cellular structure resistant to isolation, analysis and even to satisfactory description. We cannot wait until we understand the function of the microsomal membrane before deducing control of GT. Understanding of the membrane function will only come from continuing experiment, and argument, concerning the control of refractory enzymes such as GT, both latent and activated.

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* J. Fyffe and G. J. Dutton, unpublished.

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