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THE MECHANISM OF GLUCURONIDE FORMATION

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Abstract—The mechanism of formation of simple glucuronides in animal tissues is reviewed. So far glucuronide synthesis has been found to occur in liver and to a lesser extent in kidney of a number of animal species. Of the mammals studied cat liver slices formed very little glucuronide. More recently glucuronide formation has been discovered to occur in slices of gastrointestinal mucosa supplied with glucose. It would appear that a necessary requisite for the formation of glucuronides is the presence of glycogen or feeding of animals with carbohydrates which points to the essentiality of carbohydrates in the mechanism of synthesis. Starting with glucose the possible steps in glucuronide metabolism are given.

THIS short review will be restricted to the mechanism of formation of simple glucuronides within the animal body. It will deal with the elucidation of this mechanism and with the various tissues in which it is found. No attempt will be made to present a complete picture of glucuronide metabolism and its significance, which would be impossible in this compass.

STRUCTURE OF GLUCURONIDES

Glucuronic acid may be regarded as a D-glucose molecule in which the alcoholic group at carbon atom (6) has been oxidized to that of a carboxylic acid. The reducing properties at carbon atom (1) still remain, however, and the molecule is therefore still able to form glycosides, termed in this case glucuronides or, more accurately, glucosiduronic acids. These glucuronides are of two main kinds: the "ether" type in which carbon atom (1) of glucuronic acid is joined to an hydroxyl group of the aglycone by a relatively stable glycosidic bond, and the "ester" type, in which this carbon atom is linked with a carboxyl group of the aglycone through the much less stable acylal bond. The aglycones can be aliphatic, alicyclic, aromatic or heterocyclic. Linkage may also in a few cases be through a nitrogen or sulphur atom, with an amine or a mercapto compound, respectively; the former is extremely unstable,[1] and the latter has not yet been much investigated.[2] (For comprehensive treatment of the chemistry and occurrence of glucuronides, see Teague[3].)

HISTORICAL

The first glucuronide was unwittingly isolated in 1855 by Schmid[4]; Jaffe[5] and Schmiedeberg and Meyer[6] were the earliest to identify glucuronic acid in such a conjugate. At this time, hypotheses on glucuronide formation necessarily lacked experimental backing. Schmiedeberg and Meyer,[6] for example, suggested that glucose was normally oxidized through glucuronic acid in the course of its complete bodily

oxidation to CO_2 and water; any aglycone that happened to be available was captured by this glucuronic acid and a glucuronide thereby formed. It was difficult to see how carbon atom (6) of simple glucose could be oxidized without the more reactive carbon atom (1) undergoing the same fate, and other workers[7, 8] postulated that glucose itself first combined with the aglycone and that the resulting glucoside, its carbon atom (1) masked, was then oxidized to the glucuronide. When evidence became available, this was shown to be unlikely, for it seemed that glucosides were rapidly hydrolysed in the body, no more glucuronide appearing after their administration than if the free aglycone had been given, and in both cases formation also of the aglycone sulphate was observed.[9] (For an excellent historical review, see Williams[10].)

EVIDENCE FOR MECHANISM

One pathway of glucuronide synthesis is now known, which savours a little of each of these early hypotheses. Its elucidation will now be described.

The first clues were obtained with work on whole animals. It was soon apparent that liver was a major site, and that this organ formed glucuronides more efficiently if it contained glycogen, or if the animals were fed carbohydrate.[11, 12] Though argument raged hot over possible precursors of glucuronic acid, it was obvious that, beyond illustrating such indubitable connexions between glucuronide formation and carbohydrate availability, results with intact animals were inadequate.

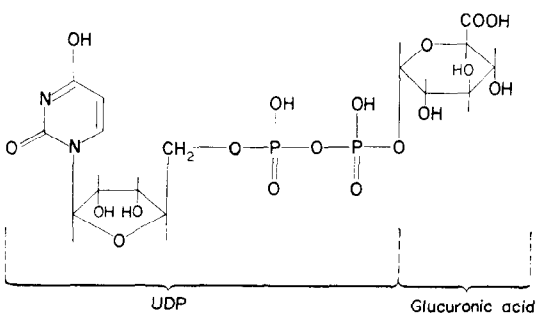

FIG. 1. Uridine diphosphate glucuronic acid.

Considerable advances came with the advent of techniques for isolated tissues. Lipschitz and Bueding[13] and Storey,[14] using slices from these organs, showed that glucuronide synthesis occurred in liver and, to less extent, in kidney. Various aglycones were employed, and for them all the process appeared strictly aerobic, most probably involving endergonic phosphorylative reactions.

About the same time evidence from work with isotopes was becoming available, and although this was to some extent conflicting it did make clear that the carbon chain of administered glucose came down unbroken to the glucuronic acid of excreted glucuronide.[15–17] Glucurone or glucuronate was not a direct source.[18]

To pursue the question further, broken cell preparations were employed.[19] The rapid and reasonably specific method of Levvy and Storey[20] was modified to study formation of *o*-aminophenyl glucuronide in liver homogenates, and it was soon clear that an unknown factor was involved. Synthesis did not occur unless a boiled liver extract

was added, and no known compound, even the likely glucuronic acid-1-phosphates, could reproduce this effect. The unknown factor was isolated, and on analysis uridine, stable phosphate, labile phosphate and glucuronic acid were found in equivalent proportions;[21] the parent "uridine diphosphate glucuronic acid" (UDPglucuronic acid) was analagous to the recently discovered UDPglucose.[22] The nucleotide was in effect an "active form" of glucuronic acid and transferred this molecule to the aglycone under the influence of an enzyme (preferably termed "UDPtransglucuronylase": see Dixon and Webb[23]). The transfer was anaerobic and seemed to represent the final stage in that formation of glucuronides observed in liver slices.

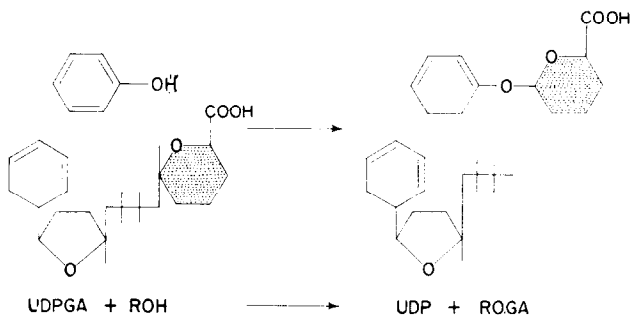

FIG. 2. Schematic outline of glucuronyl transfer from UDPglucuronic acid to an acceptor substrate

The preceding stages presumably formed UDPglucuronic acid itself, and were soon clarified. Strominger *et al.*[24] showed that UDPglucuronic acid was immediately derived from the oxidation of UDPglucose, diphosphopyridine nucleotide (DPN) and oxygen was required together with the enzyme UDPglucose dehydrogenase. UDP-glucose can be made by the action of a pyrophosphorylase on uridine triphosphate (UTP) and α-glucose-1-phosphate; UTP is of course dependent on the supply of adenosine triphosphate (ATP). The constituent reactions may therefore be outlined as follows:

$$\text{Glycogen} \xrightarrow{H_3PO_4} \alpha\text{-glucose-1-phosphate} \quad (1)$$

$$\alpha\text{-Glucose-1-phosphate} + \text{UTP} \rightarrow \text{UDPglucose} + \text{PP}^{25} \quad (2)$$

$$\text{UDPglucose} \xrightarrow{\text{DPN}, O_2} \text{UDPglucuronic acid}^{24} \quad (3)$$

$$\text{UDPglucuronic acid} + \text{R.OH} \rightarrow \text{R.O.glucuronic acid} + \text{UDP}^{19, 21, 26-28} \quad (4)$$

Production of glucuronides by this pathway touches on both the early suggestions quoted; a line of glucose oxidation is followed, but carbon atom (1) is masked by UDP. Observations that carbohydrate, oxygen and energy are required, that the glucose chain comes down unbroken and that free glucuronic acid plays no part are all now explicable by this scheme.

It is also evident that no selective "chemical defence" is operating (as was once supposed) with conjugation only of ingested toxic compounds, for enzymic transference of glucuronic acid from UDPglucuronic acid occurs for all aglycones (except sugars) so far examined: for ether and ester types, aromatic or aliphatic, "foreign" or

"physiological",[28] and for aromatic amines;[1] thyroxine,[27] various steroids[27, 28] and bilirubin[29] are examples of particular physiological aglycones handled in this way. Whether the same enzyme transfers glucuronic acid to all these substrates is so far uncertain, but in each case it is located in the microsomes, is unstable, requires Mg ions and has a fairly broad pH optimum around pH 7·6.

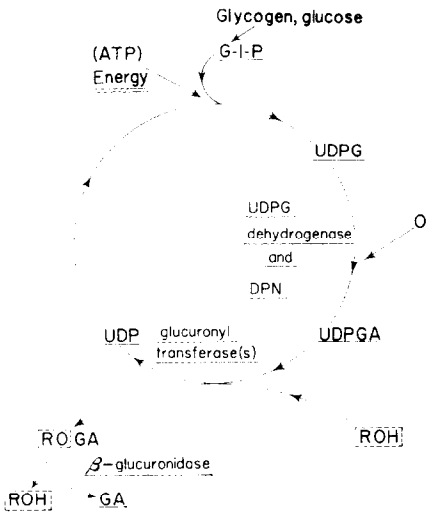

FIG. 3. Simplified scheme of glucuronide formation. G-1-P, glucose-1-phosphate; UDP, uridine diphosphate; G, glucose; GA, glucuronic acid; ROH, acceptor substrate. UDPtransglucuronylase is here termed, less satisfactorily, "glucuronyl transferase".

OCCURRENCE OF THIS HEPATIC MECHANISM

The glucuronyl transfer described above occurs in liver of man, pig, sheep, dog, rabbit, guinea pig, rat, mouse, pigeon, hen and frog,[30, 31] a sex difference being reported in the rat.[52] The system may be present to a very limited extent in brown trout liver, being there extremely thermolabile,[32] but is apparently absent from other fish and from tadpoles.[33] Among mammals, the cat is an exception. Hartiala[34] pointed out that cat liver slices form very little glucuronide, and this has been confirmed for the intact animal by others;[35, 36] the reason is not lack of UDPglucuronic acid, but of UDPtransglucuronylase.[30]

Conjugation with glucuronic acid is also low in foetal and neonatal liver,[37, 38] where it increases with growth. Since bilirubin is excreted largely as a glucuronide[39–41] and excess unconjugated bilirubin is typical of non-haemolytic non-obstructive "physiological" jaundice of the new-born, foetal conjugating mechanisms are of interest. Work with livers of infant mice and foetal guinea pigs [30, 42] demonstrated the low conjugation to be due, not to hydrolysis of formed glucuronide by the large amount of β-glucuronidase present in infant tissues or to an inhibitory factor in foetal liver, but to true low levels of both UDPglucuronic acid and UDPtransglucuronylase; these were virtually absent in new-born mice, in foetal guinea pig up to 5 weeks and in the 3–4 month human foetus. The guinea pig does not reach adult levels in these substances until several days after birth. This has been shown for several aglycones, including bilirubin.[43, 44] It might therefore be that "physiological" jaundice of the new-born is due to survival of foetal characteristics into the neonatal state.

Several other jaundiced conditions, in both rat and man, are accompanied by reduced conjugation of bilirubin and other aglycones with glucuronic acid; in these cases only the UDPtransglucuronylase may be deficient.[45–49]

Bilirubin can be conjugated also with sulphate[50] and one might expect this pathway to be followed in such animals as the cat whose glucuronide-synthesizing capacity is poor. Yet bilirubin glucuronide has been found in cat bile[51] and Lathe and Walker[43] report formation of this compound by cat liver preparations; this may be the first indication that more than one "UDPtransglucuronylase" exists in the microsomes, though evidence is by no means conclusive.

Similarly, no conclusions can yet be drawn on the induction of liver glucuronide synthesis, but in the interesting work already quoted[52] sex difference was reversible with hormone treatment and increase of UDPtransglucuronylase activity (in common with that of other microsomal enzymes) was demonstrated in new-born rats after administration of benzpyrene.

OCCURRENCE OF THIS MECHANISM EXTRAHEPATICALLY

Kidney has long been known as a site of glucuronide formation. This organ contains UDPglucuronic acid, can form it by a DPN- and oxygen-dependent enzymic oxidation of UDPglucose, and, in its cortex "microsomes", possesses an enzyme able to transfer glucuronic acid from the nucleotide to "ether" and "ester" acceptors in a manner not yet distinguishable from that of liver UDPtransglucuronylase.[53] Kidney not only resembles liver in one major pathway of its considerable glucuronide synthesis, but also in the gradual development of this pathway through foetal and neonatal life.[42]

Another important extrahepatic site of glucuronide formation has recently been discovered. Conjugation of glucuronic acid with various aglycones including steroids has been demonstrated in slices of gastrointestinal mucosa supplied with glucose.[34,54–57] This tissue, similarly, contains UDPglucuronic acid and a DPN-dependent oxidative enzyme system forming it from UDPglucose, and can transfer the nucleotide's glucuronic acid to various acceptors with the aid of an enzyme found in the particulate fraction of a water homogenate.[53] The enzyme is difficult to characterize, but resembles the UDPtransglucuronylase(s) of liver and kidney. However, in gastrointestinal tract, particularly the stomach, synthesis of glucuronides can occur by this pathway even in the early (5 week) guinea pig foetus, and is then already at, or even above, adult level.[42, 53] This level is, on wet or dry weight basis, only some 10–20 per cent that of adult liver, but must be significant in the otherwise poorly-equipped foetus. Further discussion is not relevant to this brief survey, but it should be noted that foetal tissues contain glucuronide[58] and that placenta appears to form negligible amounts.[38, 42]

Results from other sites, such as connective tissue, are not sufficiently advanced to warrant inclusion here.

OCCURRENCE OF OTHER MECHANISMS

Though the method outlined above is an important one it need not be unique. So far, there seem two possible "alternative pathways". The first is really another means

of forming UDPglucuronic acid and involves pyrophosphorolysis of UTP in the presence of glucuronic acid 1-phosphate:

$$\text{Glucuronic acid-1-P} + \text{UTP} \rightarrow \text{UDPglucuronic acid} + \text{PP}$$

This is reported in mung beans,[59] and if applicable to animals could explain the results[60] wherein ATP, UTP and glucuronate or glucurone appear (to some extent) to replace UDPglucuronic acid.

The second concerns the synthetic, or "transferase", capacity of β-glucuronidase which, it is now clear, is a completely different enzyme from UDPtransglucuronylase. Fishman and Green[61] have succeeded in demonstrating that, in β-glucuronidase preparations under special conditions of substrate concentration, transference of glucuronic acid from a glucuronide to an accepting aglycone can occur. To what extent, if any, this takes place *in vivo* is not yet clear.

This short review has necessarily confined itself to the mechanism of synthesis of simple glucuronides in animals, and many interesting digressions could not be entertained. It is well, however, to include a final note on characterization of glucuronides formed. The amounts of conjugate available in enzyme experiments do not usually permit classical analysis. It is therefore desirable to employ several substrates, the various methods of estimation or identification of whose conjugates cover not only the aglycone but also the uronic acid; this is very necessary when impure UDPglucuronic acid is being used. Hydrolysis by β-glucuronidase should be checked by its specific inhibition by saccharolactone,[62] with due regard for the substrates attacked by the enzyme.[63] Bilirubin is a peculiarly difficult aglycone and though, for example, formation of "direct" van den Bergh-reacting pigment from "indirect" has been observed in gastric mucosal preparations,[31] this by itself reflects solubility in water and not necessarily conjugate formation, still less that of a glucuronide.

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