Second Edition Revised & Enlarged

DETOXICATION MECHANISMS

The Metabolism and Detoxication of Drugs Toxic Substances and Other Organic Compounds

R. TECWYN WILLIAMS

Ph.D. (Wales) D.Sc. (Birmingham)

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LONDON
CHAPMAN & HALL LTD.
37 ESSEX STREET, W.C.2
1959

Preface to the Second Edition

THE FIRST EDITION of this book appeared twelve years ago in 1947 and since that time there has been a rapid increase in our knowledge of the fate of foreign organic compounds in the animal body. When the book was first written in 1945, the available information on this subject, which may well be called the biochemistry of foreign organic compounds (or xenobiochemistry; *xenos*, Greek for strange or foreign), was fragmentary to say the least, and little, if anything, was known about the enzymology of foreign compounds. The increase in knowledge in this field has been so great that the book has had to be completely rewritten and its size enlarged two and a half times. The rapid development of this field was inevitable as soon as its practical applications were realized. Since the last war there has been a considerably increased use of synthetic organic chemicals in all spheres of human activity and these chemicals include drugs, insecticides and various other pesticides, food additives including colours, artificial flavours and antioxidants, industrial chemicals such as solvents, dry cleaning agents, and so on. The study of the fate of these compounds not only helps in explaining toxicity and drug action and the consequent prevention of toxicity and improvement of drugs, but it also contributes to the general fund of biochemical theory. The manuscript of this book was completed approximately in the first half of 1957 and by that time something was known about the metabolism of most of the major groups of synthetic organic compounds; furthermore, important advances had been made in understanding the enzymic mechanisms responsible for the metabolism of foreign compounds. Although much remains to be done, the subject is growing up. The study of the biochemistry of foreign compounds offers an apparently unlimited field of research when one considers the number of organic compounds now in existence and the number of different species of animals and plants in which each of these compounds could be studied. It is inevitable that in time this subject will become a subject of undergraduate study as it already has in some American universities, and it is already being applied with great effect in many fields, particularly those of industrial toxicology and drug metabolism.

The short title *Detoxication Mechanisms* has been retained because the book had become well-known under this title. The sub-title is a truer indication of the content of the book.

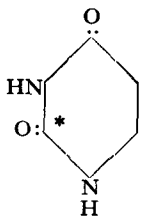
It is with great pleasure that I acknowledge the valuable help that I have received from many friends and colleagues. These include Drs G. King, D. V. Parke, D. Robinson and J. N. Smith, members of my staff at St Mary's Hospital Medical School, who helped at some or all of the stages of the book, Drs W. V. Thorpe, H. G. Bray and Sybil James of the University of Birmingham, who read the galley proofs with great care and made most valuable suggestions, and Professor Leslie Young of St Thomas's Hospital Medical School who read the chapters on aromatic hydrocarbons. For assistance in preparing the indexes, I have to thank my wife and two sons who stuck to a boring job most admirably. In the preparation of the manuscripts and the checking of references, I had the able assistance of Miss Helen Wingrave, my former secretary, and Miss E. Lewis, my present secretary. At the Library of St Mary's Hospital Medical School, the Librarian, Miss W. M. Gallagher, and her staff were most helpful and courteous in getting the journals and papers necessary for writing this book. I thank also Mr E. W. Hamilton of Messrs Chapman & Hall, Ltd., for his help and advice in getting the book through the press.

R. T. W.

Contents

IMPORTANT NOTE

Isotopic atoms are indicated in the formulae by an asterisk *, for example:



CHAPTER ONE

Introductory and Historical

ORGANIC COMPOUNDS which are normally considered foreign to the body are now employed by man on a vast scale and they enter into almost every phase of civilized existence. Thus synthetic organic compounds are used as drugs for sickness, pesticides of various kinds (herbicides, insecticides etc.) for agricultural and health purposes, colouring matters, emulsifiers and stabilizing agents for food and drink, dyes for clothes, cleansing agents for all manner of purposes, beauty preparations, spermicides for population control, explosives and poison gases for military use and so on. It is therefore important to know what happens to these compounds when they get into the body, since it is essential that the body can get rid of them without being damaged in the process or, if they cause damage, that their use should be avoided and a search be made for a less harmful substance. The present book is therefore an account of the chemical aspects of the fate of organic compounds other than those normally considered under fat, carbohydrate, protein, vitamin, steroid and mineral metabolism.

In the past the metabolic changes of foreign organic compounds have been referred to as 'detoxication mechanisms', but since there are many instances in which a foreign compound is converted in the body to a more toxic substance, this term cannot be used in the strict sense to cover all the reactions of foreign compounds, although formerly it had often been used for any reaction which apparently did not fit into the usual schemes of normal intermediary metabolism. The detoxication of foreign compounds no doubt occurs *in vivo*, for a large number of compounds are converted into less toxic and harmless products in the body, and are, thereby, eliminated. The term, detoxication, will therefore be used in the text only in those instances where it actually occurs.

THE BIOCHEMICAL REACTIONS OF FOREIGN ORGANIC COMPOUNDS

The majority of foreign organic compounds undergo definite

chemical changes in the animal body resulting in the excretion, usually by the kidney, of specific metabolites. There are, however, some compounds which are not metabolized in the body and are excreted unchanged; we may refer to these as biochemically inert compounds although they may be pharmacologically active. Excretion by other channels such as the expired air, the bile and the faeces, the saliva and the skin may also occur. The type of change which occurs depends primarily upon the structure of the compound, but other factors such as species, route of administration and diet may also be involved.

These changes, which are sometimes referred to in the American literature as 'bio-transformations', can be divided into four main types, namely *oxidations*, *reductions*, *hydrolyses* and *syntheses*. The oxidations, reductions and hydrolyses are many and varied, but in general, compounds of similar structure are oxidized, reduced or hydrolyzed in a qualitatively similar manner. The synthetic reactions, or conjugation processes as they are often called, appear to be relatively few in number. They are mainly reactions involving carbohydrates and amino acids, and seem to be the following:

(*A*) *Reactions involving carbohydrate* (*glycoside formation*)

(1) Glucuronic acid conjugation (glucuronide or glucosiduronic acid formation).
(2) Glucoside formation (in insects and plants).
(3) Riboside formation (see p. 6, 133).

(*B*) *Reactions involving amino-acids directly*

(4) Glycine conjugation or hippuric acid synthesis.
(5) Cysteine conjugation or mercapturic acid synthesis.
(6) Ornithine conjugation or ornithuric acid synthesis (lysine conjugation has been noted in chicken kidney slices).
(7) Glutamine conjugation.
(The conjugation of xanthurenic acid with serine has recently been observed (see p. 645).)

(*C*) *Reactions involving sulphur* (*from amino-acids indirectly*)

(8) Sulphate conjugation or ethereal sulphate synthesis.
(9) Thiocyanate detoxication mechanism.
(10) Sulphide detoxication of metals in insects.

(*D*) *Reactions involving alkylation and acylation*

(11) Methylation.
(12) Acetylation.

Whether or not a given compound will undergo any of the above syntheses will depend upon its possessing particular chemical groups or 'centres for conjugation'; these are discussed later. If the compound does not carry such a group, it may acquire one by oxidation or reduction or some other process; in fact, the acquisition of a centre for conjugation is a very common reaction of foreign organic compounds. Thus phenol possesses a hydroxyl group which can combine or conjugate in the body with glucuronic acid or sulphate; benzene, however, contains no centre for conjugation, but acquires one by oxidation in the body, since it is oxidized to phenol which is then excreted in a conjugated form.

Regarding species differences, it has been found that some synthetic processes are confined to particular classes of animals or even to species. Thus the glutamine conjugation has been found only in man and the chimpanzee (see p. 374), whereas the ornithine conjugation occurs, as far as is known, only in birds and reptiles. Some of the synthetic reactions, however, seem to be of general distribution among species. Thus the glucuronic acid and sulphate conjugations have been found in most species examined (glucuronic acid conjugation does not occur in insects, and there is now evidence to suggest that it may not occur extensively in cats).

A further point to be noted about the synthetic mechanisms is that part of the molecule synthesized is provided by the organism. This moiety may be referred to as the conjugating agent. When the dose of the foreign compound is not excessive, the conjugating agent may be provided from waste material or from the tissues, without undue strain upon the resources of the animal. With excessive doses, however, the conjugating agent may be utilized at the expense of material required for the well-being of the organism. This is well illustrated by the case of bromobenzene which, if fed to growing animals in excess of a certain dose, causes cessation of growth because the animal is unable to provide sufficient cysteine for both growth and the conjugation of the excessive amounts of bromobenzene.

OXIDATION

Oxidation is one of the most general reactions of foreign compounds in the body. It includes such reactions as the oxidation of alcohols and aldehydes to acids, the hydroxylation of ring systems, oxidation of alkyl groups and chains to alcohols and acids, oxidative deamination of amines, oxidative dealkylation, oxidation of sulphur compounds to sulphoxides and sulphones, the oxidative splitting of rings, de-

halogenation, and a variety of other processes. In some instances oxidation of the foreign compound may proceed as far as carbon dioxide which is usually eliminated in the expired air. The mechanism of these reactions, however, is unknown except in a few cases. Details of oxidation processes are given in the body of the text.

REDUCTION

Reduction is usually less common than oxidation, but for several types of foreign compound, it is a general reaction. Thus ketones are frequently reduced in the body to secondary alcohols. Reductions which have been observed in the body are the following:

(*a*) Conversion of some aldehydes to alcohols, $R.CHO \longrightarrow R.CH_2OH$
(*b*) Reduction of ketones to secondary alcohols, $R.R'.CO \longrightarrow R.R'.CHOH$
(*c*) Double bonds may be saturated, $R.CH:CH.R' \longrightarrow R.CH_2CH_2R'$
(*d*) Nitro groups are often reduced to hydroxylamines and amines,
$$R.NO_2 \longrightarrow R.NO \longrightarrow R.NHOH \longrightarrow R.NH_2$$
(*e*) Azo groups are converted to hydrazo compounds and amines,
$$R.N:N.R' \longrightarrow R.NH.NH.R' \longrightarrow R.NH_2 + R'.NH_2$$
(*f*) Hydroxamic acids may be reduced to amides,
$$R.CONHOH \longrightarrow R.CONH_2$$
(*g*) Pentavalent arsenic is often reduced to trivalent arsenic,
$$R.AsO(OH)_2 \longrightarrow R.AsO$$
(*h*) Disulphides may be reduced to sulphydryl derivatives,
$$R.S.S.R' \longrightarrow R.SH + R'.SH$$

The above list is not intended to be exhaustive. Frequently reduction, like oxidation, is a process which may produce a centre for conjugation, as in the case of chloral, which is reduced to trichloroethanol *in vivo*. Trichloroethanol possesses a hydroxyl group which can conjugate with glucuronic acid, and, in fact, the main metabolite of chloral is trichloroethyl glucuronide:

$$CCl_3CHO \longrightarrow CCl_3CH_2OH \longrightarrow CCl_3CH_2O.C_6H_9O_6$$

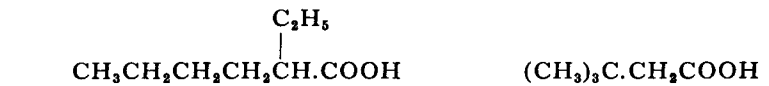
THE SYNTHETIC MECHANISMS

(1) **Glucuronide** *or* **Glucosiduronic acid formation** (see also p. 284). This appears to be one of the most common of the synthetic processes. Compounds which usually form glucuronides are those which possess hydroxyl, carboxyl, amino and sulphydryl groups or can form them in the body by oxidation, reduction or some other process.

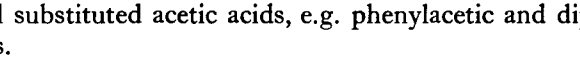
(*a*) Hydroxyl groups in almost any type of compound are potentially capable of conjugating with glucuronic acid in the body. They include those of primary, secondary and tertiary alcohols and of

phenols. Hydroxyl groups of carbohydrates and of hydroxylamines (R.NHOH) have not, so far, been shown to form glucuronides.

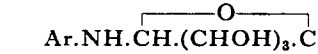
(*b*) Carboxyl groups are also potentially capable of conjugating with glucuronic acid. Conjugation has been found to occur with aromatic acids, Ar.COOH, in which the aromatic ring may be carbocyclic or heterocyclic, aliphatic acids in which β-oxidation is hindered in some way, such as by substitution on the α- or β-carbon atom, e.g. α-ethylhexoic acid and *tert*-butylacetic acid,



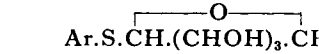
and phenyl substituted acetic acids, e.g. phenylacetic and diphenylacetic acids.



(*c*) Both aliphatic and aromatic amino groups are also potentially capable of combining with glucuronic acid. The conjugation has been observed with the aromatic amino group ($Ar.NH_2$) as in the case of diaminodiphenylsulphone. The compounds formed are presumably *N*-glycosides.

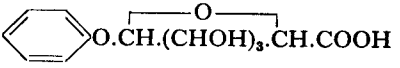


(*d*) The sulphydryl group (SH) as the analogue of the OH group should also be a group capable of conjugating with glucuronic acid *in vivo*. Such a conjugation has been observed with thiophenol (C_6H_5SH) and 2-mercaptobenzothiazine, which form the corresponding thioglucosiduronic acids.

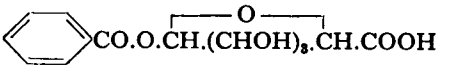


The glucosiduronic acids formed are without exception β-D-glucuronides, but they differ in their stability. Those formed from alcohols or phenols are *O*-glycosides and are commonly known as 'ether glucuronides' and are non-reducing to the usual copper reagents. They can be hydrolysed by acids, but their stability to acid varies greatly. Thus menthyl glucuronide is readily hydrolysed by dilute hydrochloric acid, whereas *o*-aminophenylglucuronide is only hydrolysed with great difficulty with the 10N-acid. They are usually stable to dilute alkali. The glucuronides of carboxylic acids are known as 'ester glucuronides' and are labile to dilute alkali; consequently they usually reduce alkaline copper reagents. A third type

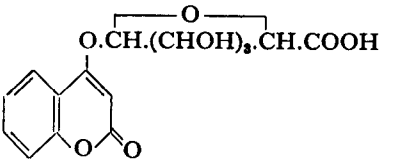
of *O*-glucuronide has been found which is also labile to alkali and therefore reduces alkaline copper reagents. These are the glucuronides which possess an 'enol glycoside' structure (see Ballou, 1954) such as that found in 4-hydroxycoumarin glucuronide. Typical examples of these glucuronides are shown below.



Phenyl glucosiduronic acid
(ether type)



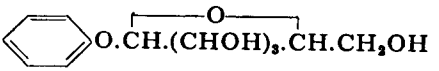
Benzoyl glucosiduronic acid
(ester type)



Coumarin 4-glucosiduronic acid
(enol glucuronide)

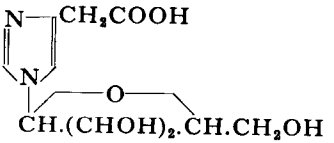
The *N*-glucosiduronic acids are usually labile to alkali and reduce alkaline copper reagents, but very few of these have been isolated and studied. The *S*-glucuronides, so far studied, have been *S*-ether glucuronides, and, as far as is known, are stable to alkalis.

(2) **Glucoside formation.** Glucuronide formation has not been found in insects, which convert phenols into β-glucosides. This has been observed in certain species of Orthoptera, Coleoptera, Lepidoptera and Hemiptera (Smith, 1955).



Phenyl β-glucoside

(3) **Riboside formation.** The conversion of imidazole-4-acetic acid into a ribose derivative has recently been observed in rats, mice, rabbits and to a small extent in cats (see Schayer, 1956). The importance, if any, of this mechanism in the metabolism of other compounds has not yet been investigated.

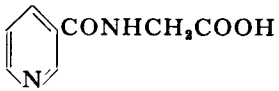


1-Ribosylimidazole-4-acetic acid

(4) **Glycine conjugation—Hippuric acids.** Conjugation with glycine in the animal body is a reaction of the carboxyl group only. It is most commonly observed with aromatic acids (the glycine conjugates of which are sometimes known as hippuric acids), but it is also found with other types of carboxylic acids. The types of acids which are known to undergo glycine conjugation are the following:

(*a*) *Aromatic acids* including benzoic acid, substituted benzoic acids, naphthoic acids, pyridine carboxylic acids, furoic acid and thiophene-α-carboxylic acid.

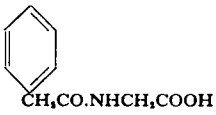
Hippuric acid



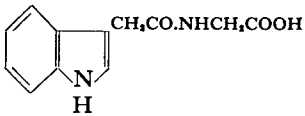
Nicotinuric acid

Pyromucuric acid

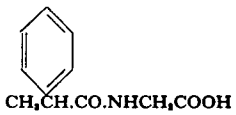
(*b*) *Substituted acetic acids* including arylacetic acids such as phenyl-, substituted phenyl-, indolyl- and naphthyl-acetic acids; alkylaryl-acetic acids such as hydratropic acid (methylphenylacetic acid); and possibly trialkylacetic acids such as trimethylacetic acid.



Phenaceturic acid

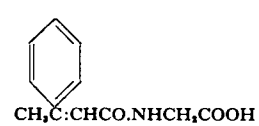


Indolylaceturic acid



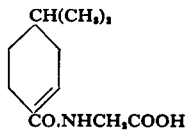
Hydratropoyl glycine

(*c*) *β-Substituted acrylic acids*, i.e. acids containing an α:β-double bond. Those known to conjugate with glycine are cinnamic acid, β-methylcinnamic acid, furylacrylic acid and phellandric acid (4-*iso*propyl-Δ^1-*cyclo*hexenecarboxylic acid).



β-Methylcinnamoyl-glycine

Furfuracryluric acid



Phellanduric acid

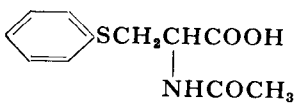
(*d*) Certain naturally occurring *steroid acids* such as cholic and deoxycholic acids.

As far as is known ordinary fatty acids do not conjugate with glycine in the whole animal, but may do so under certain conditions

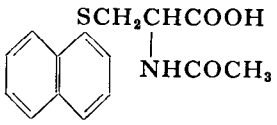
in tissue extracts (see p. 352). Acids other than carboxylic acids, e.g. sulphonic acids, have not been shown to conjugate with glycine.

(5) **Cysteine conjugation and Mercapturic acid synthesis.** The mercapturic acid synthesis involves the addition of an L-acetylcysteyl residue to an aromatic ring or rarely to a benzyl residue. The types of compounds undergoing this process are few in number and all belong to the aromatic series. They include the following.

(*a*) *Aromatic hydrocarbons* such as benzene, naphthalene and anthracene. Mercapturic acid formation takes place with replacement of nuclear hydrogen by the acetylcysteyl residue, i.e. acetylcysteyl-dehydrogenation.

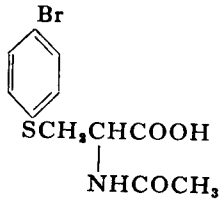


Phenylmercapturic acid

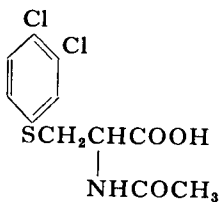


α-Naphthylmercapturic acid

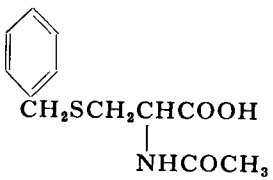
(*b*) *Halogenated aromatic hydrocarbons* such as bromobenzene, *o*- and *m*-dichlorobenzene but not *p*-dichlorobenzene, and benzyl chloride. Except in the case of benzyl chloride, the mercapturic acid is formed by acetylcysteyl-dehydrogenation.



p-Bromophenylmercapturic acid (from bromobenzene)



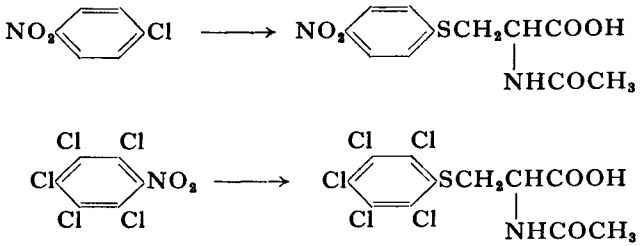
3:4-Dichlorophenylmercapturic acid (from *o*-dichlorobenzene)



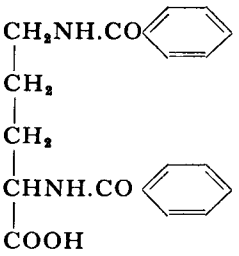
Benzylmercapturic acid (from benzyl chloride)

In the case of benzyl chloride, the chlorine is replaced by the acetylcysteyl residue to form benzylmercapturic acid, i.e. by acetylcysteyl-dechlorination.

(*c*) *Halogenated Nitrobenzenes.* These compounds form mercapturic acids either by replacement of halogen or by replacement of the nitro group (i.e. acetylcysteyl-denitration). Replacement of halogen occurs, for example, with 4-chloronitrobenzene, and, of the nitro group, with pentachloronitrobenzene.



(6) **Ornithine conjugation or Ornithuric acid synthesis.** The synthesis of ornithuric acids is a reaction which has been found, so far, only in birds and reptiles, and appears to be the equivalent of hippuric acid synthesis in mammals. It occurs with aromatic acids such as benzoic, phenylacetic, pyromucic, and the pyridine carboxylic acids. The products of the conjugation of these acids with ornithine are called ornithuric acids (since they were first discovered in birds) which are *N*:*N*′-diaroyl derivatives of ornithine. Ornithuric acid itself is *N*-2:5-dibenzoylornithine (see below).



Ornithuric acid

Recently it has been shown that in birds and reptiles the ornithine conjugates are usually accompanied by smaller amounts of the corresponding glycine conjugates.

(7) **The Glutamine conjugation.** Whereas most mammals excrete phenylacetic acid mainly combined with glycine (e.g. the dog and the rabbit) and hens excrete it combined with ornithine, man and the chimpanzee excrete it in combination with glutamine as phenylacetylglutamine. As much as 95% of moderate doses of the acid is excreted in man combined with glutamine, the rest forming a glucuronide.