DRUG METABOLISM— Principles and Significance in Drug Therapy

by

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The metabolism of a drug in the body is the process by which the drug is converted to highly ionic water-soluble compounds to facilitate excretion from the body. The process is chemical in nature and can be regarded as consisting of two reactions viz: Phase 1 and Phase 2 reactions.

Drug **Phase 1** Modified Drug **Phase 2** Conjugated Drug ——> ——>

The phase 1 reactions involve the formation of more polar compounds than the original drug and result from such processes as oxidation, reduction and hydrolysis.

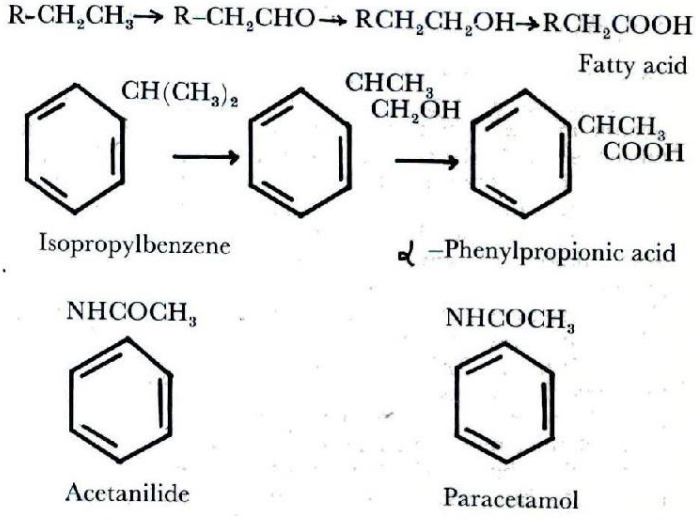
1. Oxidation in metabolism:

Oxidation of foreign substances in the body is by far the commonest and readily the most important route in the metabolism of most drugs. It occurs mainly in the liver in the presence of hepatic microsomal enzymes as catalysts. The enzymes were first isolated as mixed function oxidases which require molecular oxygen and cofactors like reduced Nicotine adenine diphosphopyridine nucleotide phosphate (NADPH), flavoproteins and iron proteins. These oxidases are believed to be responsible for the metabolic oxidation of several drugs of varying structures. Recently, however, various workers have shown that some oxidative enzyme systems specific for certain groups of drugs do exist in the body. The isolation of the amine-N-oxidase as the enzyme system responsible for the oxidation of basic drugs at the N-centre by Das and Ziegler (1970) serves as an example.

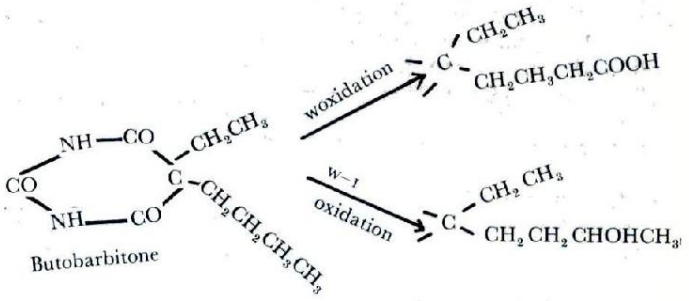
Metabolic oxidation may be considered under the following headings:

a) C-Oxidation

A reaction which may involve free radical attack on the C-atom, occurs generally in the presence of microsomal cytochrome P-450 system. There is evidence that this reaction involves a binding of the drug to cytochrome P-450. The course of events in this metabolic conversion has been worked out. The C-atom being oxidised may be in an aliphatic system, in an aliphatic side chain on an cromatic ring or in an aromatic ring. While in an aliphatic system the oxidation may proceed right down to the fatty acid derivative in an aromatic ring this reaction is less favoured and when it does occur it yields the phenolic derivative for which reason the reaction is called aromatic hydroxylation.



The carbonyl groups when present on drugs are usually converted to fatty acid but when they are of low molecular weights aldehydes, primary and secondary alcohols are oxidised to carbon dioxide and water. Barbiturates belong to a class of drugs in which the major route of metabolism is C-oxidation. The duration of action of barbiturates depends on their rate of inactivation by metabolism and rate of excretion of the unchanged drugs from the body.



b) N-Oxidation

Is the oxidation occurring at the N-centre in a N-containing compound. There are many basic drugs which possess primary, secondary and tertiary amino groups which are known to undergo metabolic conversion both **in-vitro** and **in-vivo** to yield the primary hydroxylamine, secondary hydroxylamine and N-oxide derivatives respectively. N-Oxidation of drugs takes place mainly in the liver but minor N-oxidising activity occurs in the lung, the kidney and the corpora lutea. Various workers (Booth & Boyland, 1964; Bickel, 1971; Ziegler **et al,** 1971, Beckett **et al,** 1971) have postulated mechanisms by which they believe the N-oxidation occurs.

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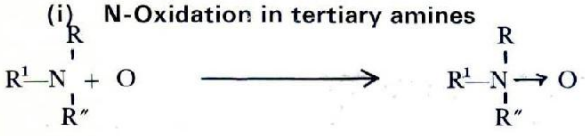
(i) N-Oxidation in tertiary amines



3° amine 3° amine N-oxide

There are four types of tertiary N-environments available for N-oxide formation (Jenner, 1971). The reaction occurs in the microsomal fraction of the liver in the presence of NADPH and molecular oxygen. Investigations have revealed that this route is not blocked by compounds such as carbon monoxide and SKF525—A (*B*-diethyl-aminoethyldiphenylpropylacetate) which are well known inhibitors of microsomal enzyme system responsible for C-oxidation. The enzyme system responsible for N-oxidation is the mixed function amine oxidase which probably contains a flavoprotein (Ziegler **et al,** 1971; Gorrod, 1973). The view held in the past was that where there were other routes of metabolism, the N-oxidation route was a minor route. Several workers have since demonstrated both in **in-vitro** and **in-vivo** studies that the N-oxidation route is not only an important route but may sometimes be the major route in the metabolism of several drugs like Chlorpromazine and other phenothiazines, Morphine, Codeine, Nicotine, Methadone, Orphenadrine and Dimethylamphetamine.

(ii) N-Oxidation in secondary amines

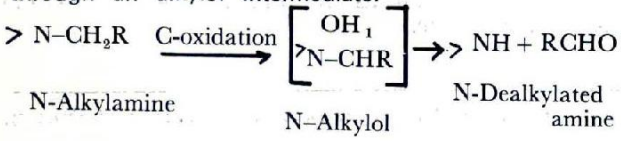
yields the hydroxylamine derivatives and involves the same enzyme system as for the tertiary amine. The various N-environments identified in tertiary amines also exist in the secondary amine drugs. One group of secondary amine drugs which are being studied in details by the Beckett group is the N-arylalkylamines to which several classes of basic drugs belong. A general metabolic pathway has been proposed for the oxidation on and around the N-centre in the N-arylalkylamines (Ogunbona, 1973). This is demonstrated in the **in-vitro** metabolic studies on fenfluramine (Ponderax), an anti-obesity drug (Beckett **et al,** 1973).

N-Oxidation of primary amines

gives the hydroxylamine derivatives. The enzyme system catalysing this reaction has been shown to be different from that catalysing the N-oxidation of tertiary and secondary amines. It was a difficult task to detect the presence of hydroxylamines of some arylkylamines because of their inherent instability (Beckett and Al-Sarraj, 1972). It was much easier to demonstrate **in-vivo** in several animals the presence of the hydroxylamine derivatives of aromatic amino compounds like 2-acetylaminofluorine (a known carcinogen), Naphthylamine and p-aminopropiophenone.

c) Dealkylation:

This can be the removal of the alkyl group from a nitrogen atom (N-dealkylation), a sulphur atom (S-dealkylation) or an oxygen atom (O-dealkylation). It is an example of a C-oxidation reaction which passes through an alkylol intermediate.



N-Alkylamine N-Alkylol N-Dealkylated amine

d) Deamination

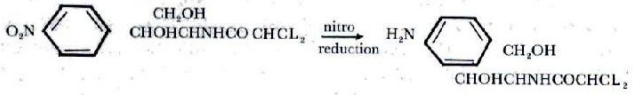
is also a metabolic C-oxidation occurring at the position to the nitrogen like the N-dealkylation. The enzyme system is the same and an alkylol intermediate is also implicated.

RCH_2NHR^1 **deamination** $RCHO + R^1NH_2$

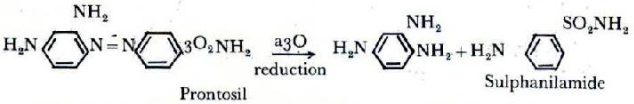
Amine drug Carbonyl compound

(e) S-Oxidation (Sulphoxidation) occurs in sulphur -containing drugs for example the phenothiazines. The reaction which produces the sulphoxide derivative of the drug is catalysed by a NADPH-linked system which is of a different nature from the systems catalysing N–and C-oxidations (Gorrod *et all* 1974).

(II) Reduction in metabolism: Reduction occurs less commonly than oxidation and takes place in the presence of hepatic enzyme systems which require as the hydrogen donor reduced triphosphopyridine nucleotide (TPNH), reduced flavine adenine dinucleotide (FADH) NADPH among others. There can be reduction of a nitro group, for example in chloramphenicol, to the amino group:



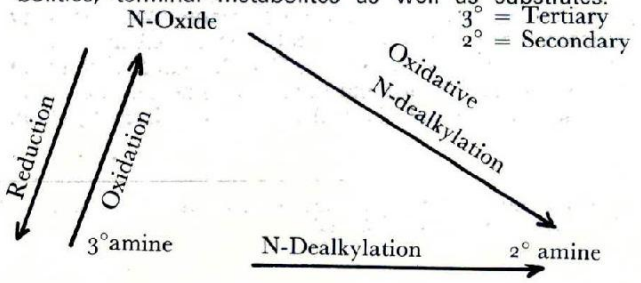
Azo reduction is known to occur *in-vivo*. For example, the antibacterial activity of sulphonamides was first detected from the observation of the *in-vivo* reduction of prontosil to sulphanilamide.



Prontosil Sulphanilamide

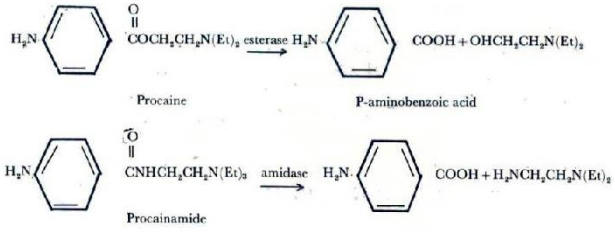
Ketones are reducible metabolically to their corresponding alcohols.

N–Oxides are also reducible in the body, to the corresponding tertiary amines hence the postulation of the role of N-oxides in the Bickel triangle as intermediate metabolities, terminal metabolites as well as substrates.



(III) Hydrolysis in metabolism: Hydrolytic cleavage of drugs possessing either ester or amide groups occurs readily in the plasma and liver in the presence of appropriate hydrolase. Non-microsomal enzymes and enzymes of the intestinal microflora participate actively in this hydrolytic reaction which also covers the hydrolytic ring scission observed in the metabolic deactivation of barbiturates.

In the hydrolysis of esters and amides, amidases have been shown to be much slower than esterases in their action.



Procaine is used as a local anaesthetic while procainamide is not. The latter is employed in the treatment and control of disturbances of the cardiac rythm. The resistance to metabolism makes it possible for procainamide to reach the heart in sufficient concentration to effect its action. To employ procaine for the same purpose it is administered intravenously thus reducing the rate of inactivation since a good proportion will bypass the liver in the first circulatory pass.

Phase 2 reactions are synthetic reactions which involve the formation of conjugates with (usually) glucuronic, sulphuric, acetic or (rarely) phosphoric acids (Gorrod and Beckett, 1969). In addition many carboxylic acids are conjugated with aminoacids such as glycine, ornithine sorine and taurine. The reactions can occur directly with the uchanged drugs or with the products of phase 1 reactions.

(a) Glucuronide formation is the major pathway for conjugation and the enzyme system responsible can be found in the liver, the gastro-intestinal tract and the skin.

$$R\text{–}OH + UDPGA \xrightarrow[\text{transglucuronylase}]{UDP} R\text{–}O\text{–}GA + UDP$$

UDPGA = Uriridine diphosphoglucuronic acid
UDP = Uridine diphosphate

The type of glucuronide formed depends on the nature of the compounds affected. For example, alcohols and phenols form ether-O-glucuronide; carboxylic acids, ester-O-glucuronide; aliphatic and aromatic amines, N-glucuronides and thiols, S-glucuronides.

The reaction is reversed by the enzyme, *B*-glucuronidase. All glucuronides are usually acid labile. Glucuronide formation is partly a way of metabolising glucose and is quite an important reaction for the transport of steroids in the body. The steroids are rendered water-soluble by glucuronide formation and the glucuronides are readily broken down at the site of action by *B*-glucuronidase.

(b) Sulphate formation Sites of sulphate formation are located in the liver, kidney and g.i.t. and the compounds affected are phenols, alcohols and aromatic amines. The reaction can be reversed by hydrolysis or sulphatase.

$$R\text{—}OH + PAPS \xrightarrow{\textbf{sulphotransferase}} R\text{-}O\text{-}SO_3\,H + PAP$$

PAPS = 3^1—Phosphoadenylphosphosulphate

PAP = 3^1—Phosphoadenylphosphate

(c) Methylation of phenols, amines and thiols occurs in the liver, pancreas and salivary gland in the presence of S–adenosyl-methionine (SAM).

$$R\text{–}HN_2 + SAM \xrightarrow{\textbf{N-Methyltransferase}} RNHMe$$

The reaction can be reversed by demethylation in the presence of appropriate demethylase.

(d) Acetylation affects both alipatic and aromatic amines and is effected by enzymes located in the liver and kidney. Reversal of the reaction is by hydrolysis and deacetylases.

$$R\text{–}NH_2 \text{ Acetyl CoA} \xrightarrow[\text{transferase}]{\textbf{Acetyl}} RNHCOCH_3$$

(e) Conjugation with aminoacids takes place converting aromatic carboxylic acids to the aminoacid conjugates with coenzyme A as the catalyst. Hydrolysis effects reversal.

$$R\text{–}COOH + NH_2CH_2COOH \longrightarrow RCONHCH_2COOH$$

glycine — hippuric acid derivative

(f) Mercapturic acid formation which occurs in the liver and kidney affect aromatic hydrocarbons and halogen compounds. These conjugates are acid-labile.

$$R\text{–Drug} \longrightarrow R\text{–}S\text{–}CH_2CH(COOH)NHCOCH_3$$

Mercapturic acid conjugate

Factors influencing the metabolism of drugs

As a process which invloves a number of chemical pathways drug metabolism will be undoubtedly subjected to certain factors like species variation, age, sex and presence of other drugs.

(i) Species: Both qualitative and quantitative differences have been observed in the metabolism of drugs by various species. This is most probaly due to the species variation in the concentration and activity of the enzyme systems responsible for the metabolic pathway of drugs. For example, dogs do not acetylate aromatic amines like sulphonamides while man does. Rabbits have been shown to excrete an acid-labile precursor of banzylmethylketone when given amphetamine while rats do not excrete this metabolite. Several other examples which can be quoted only go to show that extreme care should be taken in extrapolating metabolic data obtained

in laboratory animals to Man as the search continues for a "man - mimetic" animal which will mimic man both in the desired pharmacological response, production of side effects and metabolism of drugs.

(ii) Sex: Sex variation in metabolism has not been well manifested in experimental animals except in rats in which the females metabolize drugs to a lesser extent than the males. In a demonstration of the effect of smoking on oxidative metabolism in man it was reported that non-smoking women excrete more nicotine unchanged than do non-smoking men. Women who smoke were reported to metabolize nicotine more than do non-smokers (Beckett, 1971).

(iii) Age: Newborn animals as well as the aged usually have very low activity of microsomal enzymes required for the metabolism of several drugs. This observation serves to highlight the hazards of indiscriminate administration of drugs to infants and young children.

(iV) Disease: A damage to any of the two vital organs of metabolism, the liver and kidney, may give rise to unusual toxic effects. Hence, there is need to exercise care in the administration of drugs to patients with history of hepatic or renal damage. Low enzyme activity may accompany liver damage while faulty excretion pattern may result from kidney damage.

(V) Diet: The type of diet a man takes often influences his urinary pH. It has been shown that urinary pH by controlling the rate of drug excretion control the plasma drug levels and may consequently determine the duration of pharmacological responses. This is aptly demonstrated by some workers who administered same dose of amphetamine orally to two groups of subjects. One group (the students) who were on their normal balanced diet had urine with pH 5–6.5. The second group (the laboratory assistants) who were on their normal unbalanced carbohydrate diet (low protein) had urinary pH between 7 and 8.

The students excreted in urine about 25–60% of the dose given as amphetamine while the laboratory assistants all excreted with the same period of time (8hrs) 10% or less of the dose also as amphetamine. This significant difference in amounts and rates of amphetamine excreted can only be accounted for by the difference in urinary pH (due to diet) because when additional protein was included to balance the diet of the laboratory assistants normal urinary pH (5–6.5) was restored and the excretion pattern of amphetamine was similar to that obtained with the students. Withdrawal of the additional protein in their diet caused the urine of the laboratory assistants to become alkaline and a reversal of the excretion pattern to the former situation (Beckett, 1971). The underlining principle illustrated here is that of kidney tubular re-absorption of the basic drug as it goes down the kidney tubules in the prevailing alkaline conditions in the renal system. This is an aspect of drug-diet interaction.

(vi) Drugs: The influence of a drug on the metabolic disposition of another drug is an aspect of drug-drug interaction. Drugs which will alter the pH of urine may like diet bring about an alteration in the rate and degree of excretion and metabolism of a drug. For example, drug like certain diuretics (e.g. chlorothiazide) which render the pH of urine more alkaline than usual extend the biological effects of basic drugs while they reduce the duration of effects of acidic drugs.

The concurrent administration of a compound or another drug which inhibits or blocks the enzymes responsible for the metabolism of a drug will increase the duration of action of the drug in the body. Significant enzyme activity may be induced in animals as well as in man by a wide range of compounds including drugs. However, since in general man receives so many potential inducers from medicines, trace residues in food, drinks, the atmosphere etc. it is only in certain circumstances that effects of particular enzyme-inducing agents may be demonstrated. Barbiturates, for example, have been shown to increase the rate of metabolism of a wide variety of drugs like hydrocortisone, digoxin, diazepam and diphenylhydration. Chronic ethanol administration stimulates its own metabolism and that of meprobamate and pentobarbitone (Misra *et al*, 1971). It seems, therefore, that tolerance to alcohol can be explained partly by enhanced hepatic drug metabolism as well as by nervous system adaptation.

Significance of metabolism in drug therapy

(i) Inactivation of drugs; One obvious consequence of the chemical change due to metabolism is the loss of activity of the administered drug. This becomes prominent in a situation where the metabolite(s) are completely devoid of any activity. One of the main aspects of this deactivation is the rate at which it occurs because this will have a direct bearing on the duration of biological action of the drug in the body. For example, the duration of the hypnotic action of hexobarbitone (a barbiturate of moderate duration of action) is a reflection primarily of rapid metabolism rather than of tissue redistribution.

(ii) Change in activity: In place of a loss of activity there may just be a change from one activity to another completely different activity. It may be a change from little activity to more intense activity (e.g. phenacetin paracetomol) or no activity to activity (i.e. activation) as in prodrugs. A prodrug may be defined as a compound resulting from chemical modification of a biologically active compound which will liberate the active compound *in - vivo* due to enzymatic or chemical attack. Some pharmaceutical reasons for such drug modifications have been outlined by Sinkula (1973) in a review on prodrugs. Examples of prodrugs which after metabolism release the active drugs are found among the ester drugs such as Carbenicillin vidanylsodium, erythromycin estolate, N–2–benzoyloxyethylnorfenfluramine, a new anorectic drug still under investigation.

(iii) New drugs: Metabolic studies have led to the discoveries of some drugs. The investigation of the bactericidal properties of azodyes containing a sulphonamide nucleus like prontosil led to the finding that the bactericidal action of the drug was due to a metabolic product, sulphanilamide. This marked the beginning of introduction of sulphonamides as antibacterial agents. It was found that for the antimalarial action of biguanides like proguanil metabolic cyclisation was essential. This stirred up investigations of pyrimidine derivatives for antimalarial activity, a search which yielded an important drug in malarial therapy, pyrimethamine.

A neglect of the significance of metabolism can lead to incalculable error on the part of drug manufacturers in making pronouncements about new drugs. An example of such a situation arose when a drug firm put out a new drug, Cyanoethylamphetamine, with a claim that it is an anorectic drug devoid of the undesirable side effects of ethylamphetamine and amphetamine.

$CH_2CH(Me)NHR$

R
H Amphetamine

$-CH_2CH_3$ **Ethylamphetamine**

$-CH_2CH_2CN$ **Cyanoethylamphetamine**

In the Beckett laboratory it was quickly established that the rate of metabolic N–dealkylation of this new drug to amphetamine was much faster than that of ethylamphetamine. This finding thus negates the claim of the manufacturing firm since the new drug is likely to be more toxic than ethylamphetamine. This led to the immediate classification of the new drug as a dangerous drug under the amphetamines-an action which was a complete contrast to what the innovator wanted.

(iii) Drug interactions: When the effects of diet and concomitant administration of other drugs discussed above are considered there is no doubt that drug-diet and drug-drug interactions can readily occur. The pharmacist should be fully aware of this aspect of drug interactions because providing information on this subject is part of his responsibility to his other colleagues in the health team.

(v) Drug toxicity: The basic objective of the body in metabolism is to rid itself of the presence of the foreign compounds so as to prevent undesirable effects due to the presence of the compounds in the body. It is, thus, rather ironical that some of the steps the body takes to achieve this objective lead to intoxication.

Crystalluria due to sulphonamides can result from the *in-vivo* acetylation of sulphonamides because some acetylated sulphonamides are in fact less soluble than the unchanged drug. This leads to intra-renal deposition of cystals in the kidney tubules or extra-renal deposition of same in the pelvis, urethra and bladder.

But perhaps the most notorious metabolic reaction as far as drug toxicity is concerned is the N-oxidation. Examples of toxicity resulting from N-oxidation can be found in the simplest to the most complex N-containing drugs. Urethane ($NH_2COOC_2H_5$), an antineoplastic agent, is a simple aliphatic amide which is normally metabolised to ethanol, ammonia and carbon dioxide. It has been shown that urethane can also be metabolised in animals to N-hydroxyurethane, its N-hydroxy ester and free radicals, compounds which are believed to be responsible for the induction of cancer by urethane. (Williams & Nery, 1972). A small proportion of a dose of phenacetin is deacedylated (main route being O–dealkylation to paracetamol) to yield p-phenetidine metabolism of which by N-oxidation is implicated in haemoglobin degeneration and cyanosis associated with phenacetin.

The production of coloured free radicals in blood and urine, skin sensitization and/or pigmentation in phenothiazinetreated subjects have been asserted to indicate the biochemical reactions of the phenothiazine nucleus and the N-oxidation products like the nitroxides, the hydroxylamines and the hydroperoxides (Essien, 1974, Beekett *et al,* 1975).

Many aromatic hydroxylamines have been implicated in the induction of tumours and oxidation of haemoglobin. Weisburger and Weisburger (1973), in their excellent review on hydroxylamines and hydroxamic acids, have concluded that N-hydroxylation of aromatic amines and their derivatives may be regarded as a toxification reaction which accounts for many of the pharmacological and pathological, as well as other adverse effects, observed in the studies of these amines. The same may be true of their non-aromatic counterparts (i.e. the aliphatic hydroxylamines) which due to their inherent instability may be reactive entities reacting with essential life-supporting macromolecules like DNA and RNA.

In conclusion, it is pertinent to note that the era is gone when metabolism of a drug is viewed simply as a detoxification process. A critical look is now cast on the results of the metabolic studies of a drug to infer, if any, the instantaneous or cumulative toxic effects of the metabolic products of the drug.

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