

The Metabolism of Drugs and Toxic Substances

D. V. PARKE

Professor of Biochemistry, University of Surrey, Guildford GU2 5XH

Summary of a lecture given at a meeting of the Lancaster and District Chemical Society at Ulverston on 30 December 1976.

The past fifty years have seen a marked development in the use of selectively toxic chemicals. Most commonly, the interest has been in chemicals that will kill or impair a particular species, such as insects, moulds or micro-organisms and leave man unharmed. In addition the chemical industry prepares about 10,000 new chemicals each year and it becomes necessary to assess the risk associated with them.

The study of the metabolism of various chemicals tends now to be called Xenobiotics. The German organic chemists of a century ago often tested their new discoveries on dogs, and they discovered most of the fundamentals of the science. Until about 30 years ago their belief that the body simply detoxicated the chemicals that it did not want remained in vogue, but now we realise that some chemicals can be made more toxic, some pro-drugs can be converted by the body into active drugs and most carcinogenic chemicals are activated in the body after gaining access to it in a non-carcinogenic form.

We know that the same enzymes that metabolise foreign chemicals also metabolise endogenous chemicals, and just recently it has been suggested that perhaps many of the remaining diseases may be due to derangements of the same enzymes which are involved in ordinary metabolism.

The metabolism of chemicals is studied usually for four reasons. Firstly, in connection with the safety and efficacy of new drugs and food additives. Secondly, as clinical pharmacology, i.e. where chemicals go when they enter the body or the environment, and how much is needed to produce the desired effect. Thirdly, for forensic purposes (5 per cent. of all hospital admissions are patients suffering from the adverse effects of chemicals, usually self-administered drugs). Fourthly, to assist in the design of new drugs, pesticides, etc. (This is an area where the Japanese are very active.)

A good example of the sort of transformations one encounters is found in the antidepressant drug imipramine. Imipramine itself has an iminodibenzyl structure with a side-chain attached to the nitrogen atom and terminating in a dimethylamine group. The first phase of metabolism is to remove one of its methyl groups, producing desmethylinipramine, which has the actual antidepressant activity. The next phase of metabolism is mostly hydroxylation of one of the benzene rings to give 2-hydroxy-desmethylinipramine, which is inactive. The last stage is glucuronide conjugation, in which glucuronic acid

reacts with the phenolic grouping to give an inactive conjugate that is readily excreted. Other examples of deactivation mechanisms include the methylation of adrenaline and the reaction of salicylic acid with glycine to form salicyluric acid. Sometimes there are chemical transformations. The Americans call them Bio-transformations but in Britain they are called Metabolic Transformations.

In general, the mammalian tissues perform oxidations, while micro-organisms perform reductions or hydrolyses, but we have micro-organisms in our gut that metabolise chemicals taken by mouth or that reach the gut through being excreted into the bile. Some mammalian enzymes also perform hydrolyses.

Microsomal enzymes may be mentioned. The microsomes are the fragments of the endoplasmic reticulum found in all living cells. The endoplasmic reticulum is concerned with the synthesis of protein, but it contains much phospholipid, and because so many chemicals are lipophilic they arrive at the endoplasmic reticulum, in which many of the enzymes which metabolise foreign chemicals are found.

Many microsomal drug-metabolising enzymes perform oxidations and oxygenations. Thus phenobarbitone is changed to p-hydroxyphenobarbitone and as a result loses its sedative action. The tranquilliser meprobamate has a methyl group converted to CH_2OH and loses its tranquillising action. The antihistamine chlorcyclizine has an N-methyl converted to N-oxide, and mescaline has its amine group oxidised to aldehyde and then to carboxylic acid, and the activities disappear.

In a microscopic section of a liver cell one may see the nucleus and various other bodies, among which the endoplasmic reticulum is quite conspicuous because of a series of dots along it which are the polyribosomes, essential to protein synthesis. When the polyribosomes are attached to the endoplasmic reticulum, it is thought that the cell is making protein for export, mostly glycoproteins, many of which are concerned with the immune response, but when the ribosomes leave the endoplasmic reticulum they synthesise protein required for the cell's own growth.

The idea that oxygen is essential to life, not merely for respiration but for the synthesis of oxygenated chemicals, goes back to Lavoisier. The mechanism seems to involve a terminal enzyme called cytochrome P-450. The two reduced nicotinamide derivatives NAD and NADP transfer electrons through the flavoprotein to the terminal oxygenase cytochrome P-450, which is then able to transfer one atom of Oxygen to the chemical substrate and the other to two hydrogens to form water. The P-450 is associated with phospholipid but it has been separated from it and from the flavoproteins experimentally, and after being chromatographed, the components have all been put back together to restore the former activity. Some people think there are several cytochrome P-450's but there is probably only one, but because it can carry out different chemical reactions it is highly versatile; the activities are known as mixed function oxygenases. The most marked of its characteristics is its inhibition by carbon monoxide, when one gets a different spectrum with an absorption maximum at $450\text{ m}\mu$ (hence the name) but there is a related form where the absorption occurs at $448\text{ m}\mu$.

A suspension of liver cells *in vitro* will hydroxylate aniline, or demethylate aminopyrine, but the introduction of only as little as 2 per cent. of carbon monoxide will reduce the conversions almost to half (an observation which provokes speculation about the effect of smoking on enzyme activity in the lungs).

The metabolic pathways of several common drugs are known, and it is known that there are three pathways for the tranquilliser meprobamate. Hexobarbitone (BPC 1959) has at least four metabolic pathways. The widely used drug phenacetin is interesting as an example of enzyme activity simultaneously detoxicating and intoxicating. One end of the molecule can be de-ethylated to give *p*-acetamidophenol which becomes conjugated with glucuronic acid and excreted, while at the other end of the molecule de-acetylation produces *p*-phenetidine which can affect the blood by making methaemoglobin. Meanwhile hydroxylation of the aromatic ring can give 2-hydroxyphenacetin. The route involves the formation of an epoxide which can damage both kidney and liver. The two major metabolic routes for amphetamine are interesting because the preferred route in rabbit metabolism differs from that in man.

Although the enzyme system is sometimes called non-specific, a different end-product is obtained from glutethimide depending on whether one starts with the *d* or the *l* form. It would therefore be more accurate to describe it as highly specific but versatile.

The same enzyme system converts the sulphur in parathion to oxygen, producing the very toxic paroxon. At the same time the molecule is hydrolysed to *p*-nitrophenol and then reduced to *p*-aminophenol.

Malathion is virtually non-toxic to man because it is rapidly converted to the di-acid, which is non-toxic and is quickly excreted. In insects, the sulphur is changed into an oxygen, forming the highly toxic malaoxon. Malathion is added to grain in the storage silo to control weevil attack, and so, every time we eat bread or cereal we eat malathion.

DDT has saved many lives but in the mouse it is carcinogenic. Some people have suggested that this is because it is converted into DDE in the mouse and not in other species. That particular conversion is not the whole reason however, because it has recently been shown that DDE is less of a carcinogen than DDT.

Ethylene glycol became the centre of some controversy about ten years ago because of the possibility of its giving rise to oxalate which produces kidney stone. Millions of pounds worth of plant for drying and freeze drying vegetables was at risk until it was shown that the oxalate pathway was almost unique to cats. In man the route is mainly via glycine.

Many people once believed that banning cyclamate artificial sweetener had been a mistake, because it passes through man practically unchanged. It can, however, be metabolised by gut bacteria to cyclohexylamine which in man will give rise to toxic metabolites. Normally gut bacteria would metabolise only about 0.1 per cent. of cyclamate, but it has recently been shown that the gut bacteria of people who take cyclamates continuously become trained to metabolising it, and by the end of a year they can be metabolising as much as 40 per cent. of it.

Acetamidofluorene was proposed as a new pesticide some 15 years ago

but when tested it was found to be carcinogenic. Later it was realised that the material itself was not carcinogenic but that its metabolites included a carcinogen. This was first believed to be the N-hydroxylated molecule, but later still it was shown that this only became carcinogenic when it was sulphonated. The actual mechanism is now believed to involve the formation of a charged carbonium ion, which is capable of arylating DNA, and this is now believed to be the basis of chemical carcinogenesis. It is further believed that over 90 per cent. of human cancers are due to chemicals in food or in the environment, but that the great majority of those chemicals must be activated in the body before they become carcinogens . . . so the enzymes which metabolise or detoxicate chemicals are involved.

If one administers phenobarbitone, glutethimide or chlorpromazine repeatedly, the effectiveness of the drug wears off; one has to increase the dose to get the same effect, and this occurs for up to four or five days. The drugs seem to stimulate their own deactivation by the synthesis of the drug-metabolising enzymes. Early work by Kato showed that the administration of phenobarbitone would increase cytochrome P-450 some two and a half times. But having done this, one also finds that if another drug is then administered, its metabolism too has been stimulated. Similarly, at the same time, the metabolism of DDT, parathion, etc., will have been stimulated. This can be a problem in drug administration because doses need readjustment. It is also found that vitamin C excretion is increased. Rats can synthesise vitamin C and the pathway of synthesis runs parallel to the enzyme synthesis pathways, and this is why stimulation of enzyme production also stimulates vitamin C production. This even happens in the guinea-pig, which normally is like man in its inability to synthesise vitamin C. Guinea pigs treated with prednisone were able, after about 50 days, not only to make do with a smaller vitamin C intake than one would have expected in view of the vitamin C demand which normally accompanies drug ingestion, but even to overcome their normal lack of reductase which blocks the final stage in the synthesis of vitamin C, so that they were able to generate that vitamin.

Enzyme-stimulating drugs taken over periods of 15 years or so were found to give rise to folate deficiency, and as the folate went down the enzyme induction was reduced. Experiments with rats showed that large healthy babies resulted when all nutrients were available to the mother and no drugs were given, and a slightly smaller baby was produced when a typical antiepileptic drug dosage was given. On a normal diet, in which there was some doubt whether all nutrients were available, the same pattern showed, but with both groups of babies smaller than in the first group. When a deficient diet was given, undersized babies had been born to the drug-free mother, whereas those of the mother on drugs showed considerable folate deficiency with broken and deformed bones. The parallel in humans has been observed in mothers on anti-epilepsy drugs whose contraceptive pills had failed because of the enzyme induction of the drugs. Many of the children born in these circumstances have had deformities such as hare-lip or cleft palate. So, long-term drug administration can give rise to induction of drug-metabolising enzymes, increased demand for folate, folate deficiency which reduces enzyme induction,

followed by a decrease in rate of drug metabolism, which then causes drug toxicity.

The carcinogenic polycyclic hydrocarbons also induce enzymes, but whereas the drugs induce cytochrome P-450, these induce cytochrome P-448. It is probably the same enzyme system, modified.

The generally-accepted scheme for protein synthesis involves DNA, which undergoes transcription to give messenger RNA followed by translation to give the protein. It has been suggested that in the gene the structural genes are controlled by an operator gene which can be switched off by a repressor from a regulator gene. If the repressor is removed, the operator switches on to make protein. It is believed that harmful environmental chemicals can remove the repressor in the genome that controls the enzymes metabolising these chemicals, so that structural genes then synthesise the enzyme proteins at a rapid rate. Carcinogenic chemicals are believed to de-repress similarly, but additionally to be metabolised to carbonium ion which can alkylate or arylate DNA or RNA.

The self-stimulated enzyme induction occurs with the environmental chemicals as with drugs, but in one case another mechanism seems to be involved. The chemical biphenyl $C_6H_5-C_6H_5$ consists of two benzene rings linked carbon to carbon, and the molecule can be hydroxylated by enzymes at the two ends (the 4 positions) or in the middle (the 2 positions). Biphenyl-4-hydroxylase is induced in the organism in the conventional way, but the biphenyl-2-hydroxylase is capable of increasing immediately and rapidly, though only when the induction is brought about by carcinogens.

This observation has been adapted to make an *in vitro* test-tube test for chemical carcinogens. Endoplasmic reticulum from any animal tissue is incubated for about ten minutes in the presence of NADP and the test chemical. Biphenyl is then added and, after a further ten minutes incubation, the rate of increase of biphenyl-2-hydroxylase is measured. If there is a marked increase, the chemical is likely to be carcinogenic. There is every hope that tests of this kind (e.g. the Ames test) will replace some of the long-term life-span testing of chemicals that has been necessary up to the present time.

