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Environmental Chemicals, Enzyme Function and Human Disease

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Chairman's introduction

T.A. CONNORS

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This is an opportune time for the discussion of environmental chemicals and their interaction with drug-metabolizing enzymes. Research in this area has, over the past few years, led us to revise our opinion of the safety of many chemicals to which human beings are exposed through the general environment (at work, for example, and in food additives, medicines and pollutants). We once thought these chemicals were innocuous because people could be exposed to them for long periods without developing any obvious symptoms of toxicity. However, extensive research, some of which we shall discuss at this symposium, has shown that many of these environmental agents may be associated, in the long term, with the development of cancer or other serious diseases. One of our symposium members, Dr Higginson, believes that we have reasonable evidence that most human cancers are related to the environment; we now know some of the hazards of the chemicals in our environment, and their interaction with drug-metabolizing enzymes.

A key discovery in this field was that chemically inert substances causing cancer in animals could be converted, by drug-metabolizing enzymes, into reactive metabolites (Miller & Miller 1974). This explained many observations that were not previously understood. About 40 years ago Sir Alexander Haddow commented that it was strange for the chemically reactive nitrogen-mustard, mechlorethamine, to share so many biological properties, including cancer induction, with what he called the 'inert' benzo[*a*]pyrene; all was explained once it was demonstrated that such 'inert' chemicals could be metabolized, *in vivo,* to electrophilic reactants. This discovery demonstrated a simple molecular mechanism whereby structurally dissimilar chemicals could initiate cancer via a common pathway. The reactive species produced could bind covalently with DNA to form promutagenic bases, and this constituted the initiating step in the process of cancer induction. A further conse-

quence of the discovery was that the somatic mutation theory of cancer was strengthened; this led to the development of simple tests on the interaction of a chemical (or its metabolite) with DNA, by which the carcinogenic potential of the chemical could be predicted. At present many agents are *suspected* of being carcinogenic to humans but we do not yet have enough evidence to determine whether they are hazardous to humans under *normal* conditions of exposure.

However, I believe that we can already classify some chemicals as human carcinogens. The alkylating agents and related cytotoxic agents used in the treatment of cancer are reactive species and they bind extensively to DNA. These must be considered hazardous, and there is evidence that some members of the class have caused cancer in humans. The 5-nitrofurans are used to treat human infections, but they probably act only after reduction of the nitro group and generation of electrophilic reactants. Since mammalian enzymes can also reduce the nitro group, alkylation of DNA and initiation of cancer remains a possibility when the 5-nitrofurans are used therapeutically.

However, with most chemicals that are suspected of being carcinogenic, reactive species may be formed only by a minor pathway and under abnormal conditions. In these cases, reactive metabolites may have been detected, in the first place, only by highly sensitive analytical techniques or *in vitro* tests. Evidence for carcinogenicity in animals may have come only from bioassays in which large and sometimes toxic doses of the chemicals were administered for long periods. As more and more chemicals are suspected of being carcinogens, there will be increasing pressure on us to define more precisely the relationship between these tests and the risks to humans. Recently I searched for published reports of medicines that have been both used in the last 15 years and tested for carcinogenicity. Of 84 substances tested, 66 had produced evidence of carcinogenicity in animals. I hope that as a result of the discussions at this symposium we may gain a better understanding of the dangers to humans when these chemicals are used under normal conditions.

We shall be discussing how drug-metabolizing enzymes are influenced by a variety of endogenous and exogenous factors in both *in vitro* systems and controlled animal experiments. We shall then consider how these results apply to humans and we should identify the areas where more research is needed. I hope also that we may be able to formulate guidelines about the use of substances that are now suspected to be human carcinogens. What, for instance, is the relevance of animal experiments in which only high doses are carcinogenic? Also, how do we begin to extrapolate from animals to man in our attempts to quantify and predict risks of carcinogenicity? I hope these topics will lead to some fruitful discussion in the next three days.

Reference

Miller EC, Miller JA 1974 Biochemical mechanisms of chemical carcinogenesis. In: Busch H (ed) The molecular biology of cancer. Academic Press, New York, p 377-402

The role of the drug-metabolizing enzymes

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Abstract Drug-metabolizing enzymes function in the biotransformation of both endogenous and exogenous lipophilic compounds. Phylogenetic studies indicate that the drug-metabolizing enzymes were a late evolutionary development. Stimuli for the evolution of these enzymes were probably movement to a terrestrial environment, a diet of higher plants and an increasing tissue specialization, with a consequential need for the formation and inactivation of hormones, bile salts etc. Most drug-metabolizing enzymes exist in multiple forms. Some are concerned solely with the metabolism of a very limited range of endogenous lipids; others such as 'phenobarbitone-type cytochrome *P*-450' seem to be concerned mainly with the metabolism of exogenous compounds. In mammals the liver and intestine have a major role in the biotransformation of exogenous compounds, whereas in other tissues the primary function of the drug-metabolizing enzymes appears to be the metabolism of endogenous lipids such as steroids, lipid-soluble vitamins and fatty acids.

The metabolism of exogenous lipophilic compounds (xenobiotics or foreign compounds) by higher animals occurs predominantly in the liver and intestine but is also brought about by other tissues. This metabolism is normally a two-stage process. In the first phase (phase 1) an electrophilic substituent group such as $-OH$, $-NH_2$, $-COOH$ or $-SH$ is inserted or revealed through the action of oxidation, reduction or hydrolytic enzymes. In the second phase (phase 2) this substituent group is conjugated, typically with a carbohydrate, amino acid or inorganic acid. The overall effect of this metabolism is to convert lipophilic compounds, which tend to pass readily into cells and bind to cellular components, into more lipophobic products which can be excreted actively or passively by cells. The enzymes carrying out these two phases of metabolism are commonly referred to as the *drug-metabolizing enzymes*. The name 'drug-metabolizing enzymes' is a misnomer for the enzymes which, in addition to transforming drugs, metabolize a very wide range of synthetic and

naturally occurring xenobiotics. Many endogenous lipophilic compounds are also metabolized to more or less bioactive products by the single or combined action of oxidative, reductive, hydrolytic or conjugative enzymes. The enzymes that metabolize endogenous lipophilic compounds appear in many cases to be similar or identical to those concerned with exogenous compounds. Therefore the true function of the drug-metabolizing enzymes may be either to facilitate the clearance from the body of exogenous lipophilic compounds which it is unable to prevent from being absorbed, or to control the synthesis and degradation of bioactive endogenous lipophilic compounds such as sterols, steroids, prostaglandins, thyroxine, bilirubin and similar substances. The true function(s) of the drug-metabolizing enzymes may be derived from consideration of their distribution and activities under different circumstances.

EVOLUTIONARY DEVELOPMENT OF DRUG-METABOLIZING ENZYMES IN ANIMALS

Let us consider first the evolution of the enzymes that metabolize *exogenous* lipophilic compounds. In the simpler organisms, many xenobiotics are degraded by hydrolytic, reductive or oxidative enzymes to less complex structures which can be used wholly or partially in intermediary pathways of metabolism. In higher organisms this degradative ability is largely lost and as a consequence some means of clearing non-degradable exogenous lipophilic compounds from the body is more necessary. This is reflected, by and large, in a parallelism between the complexity of an animal and the range and total activity of its drug-metabolizing enzymes. The conjugating reactions appear to have developed considerably earlier than the forms of cytochrome *P*-450 with a broad substrate specificity (see Fig. 1) in spite of the fact that non-*P*-450 cytochromes are found in all animals. The *Platyhelminthes* (flatworms) are the simplest animals in which drug-metabolizing enzyme activity has been detected. As we ascend the phylogenetic tree, an increasing range and activity of conjugating enzymes is observed (Smith 1977). Interestingly, on the arthropoda branch, glucose is the main form of carbohydrate conjugate whereas on the vertebrate branch glucuronic acid is preferred. Oxidative activity of the cytochrome *P*-450-type is very low in crustaceans but is much higher in other arthropods, such as insects and arachnids. With the exception of glucuronic acid conjugation, insects appear to show all the major drug-metabolizing reactions observed in mammals. Reptiles, amphibia, fish, birds and mammals have qualitatively similar pathways of drug metabolism. However, considerable quantitative differences are apparent, the usual order

of enzyme activities being amphibia = fish < birds < mammals. In addition, mammals are considerably more versatile than fish and birds in the range of substrates metabolized effectively by individual phase I reactions. This difference is most marked in the case of cytochrome *P*-450-dependent reactions. Such differences might be explained by the development of additional isoenzymes in mammals; e.g. in mammalian liver, phenobarbitone is able to induce a form of cytochrome *P*-450 that has a particularly broad substrate specificity. This form of cytochrome *P*-450 is not induced in fish, in early mammalian fetuses or in de-differentiated hepatocytes in culture (Elcombe & Lech 1979, Guenther & Mannering 1977, Bridges & Fry 1978), whereas

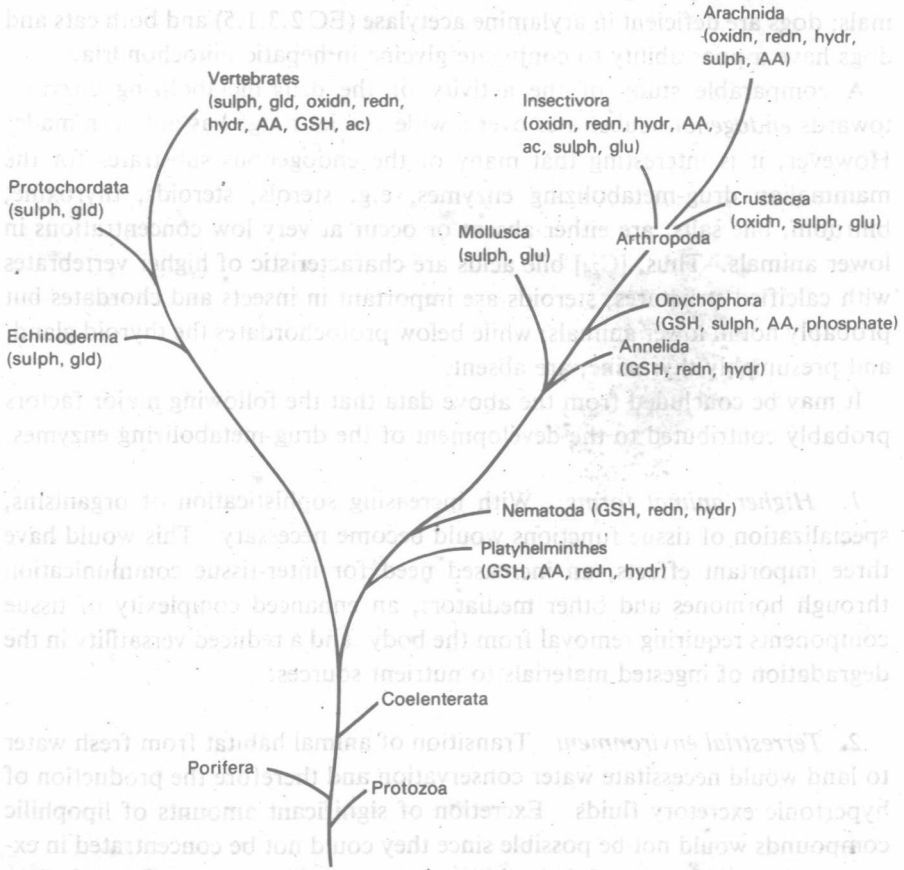

FIG. 1. Phylogenetic tree showing the occurrence of drug-metabolizing enzymes. (oxidn = oxidation, redn = reduction, hydr = hydrolysis, AA = amino acid conjugation, GSH = glutathione conjugation, ac = acetylation, sulph = sulphate conjugation, glu = glucose conjugation and gld = glucuronic acid conjugation).

3-methylcholanthrene, which promotes the synthesis of a form of cytochrome *P*-450 (*P*-448) with a much more limited substrate range, is an effective inducer in all these circumstances. The range of drug-metabolizing enzyme activities (expressed in nmoles·mg protein^{-1}·min^{-1}) among vertebrates for cytochrome *P*-450-dependent reactions, glucuronyl transferase (EC 2.4.1.17) and epoxide hydrolase (EC 3.3.2.3), is greater than 1000-fold. Among both insects and the various classes of vertebrates there is a general tendency for flesh eaters to have lower drug-metabolizing enzyme activities than herbivores (Walker 1980, Brooks 1979). Carnivorous mammals are deficient in a number of important conjugation enzymes. The cat shows a much more limited range of glucuronic acid conjugation reactions than other mammals; dogs are deficient in arylamine acetylase (EC 2.3.1.5) and both cats and dogs have a poor ability to conjugate glycine in hepatic mitochondria.

A comparable study of the activity of the drug-metabolizing enzymes towards *endogenous* substrates over a wide species range has not been made. However, it is interesting that many of the endogenous substrates for the mammalian drug-metabolizing enzymes, e.g. sterols, steroids, thyroxine, bilirubin, bile salts, are either absent or occur at very low concentrations in lower animals. Thus, [C_{24}] bile acids are characteristic of higher vertebrates with calcified structures; steroids are important in insects and chordates but probably not in lower animals; while below protochordates the thyroid gland, and presumably thyroxine, are absent.

It may be concluded from the above data that the following major factors probably contributed to the development of the drug-metabolizing enzymes.

1. Higher animal forms. With increasing sophistication of organisms, specialization of tissue functions would become necessary. This would have three important effects, an increased need for inter-tissue communication through hormones and other mediators, an enhanced complexity of tissue components requiring removal from the body, and a reduced versatility in the degradation of ingested materials to nutrient sources.

2. Terrestrial environment. Transition of animal habitat from fresh water to land would necessitate water conservation and therefore the production of hypertonic excretory fluids. Excretion of significant amounts of lipophilic compounds would not be possible since they could not be concentrated in excretory organs because of their ability to pass readily across cell membranes back into the body. Therefore, such compounds would need to be metabolized to non-lipophilic forms in order to be excreted. Brodie & Maickel (1961) have suggested that fish and amphibia do not need detoxification enzymes

because they can excrete lipophilic substances through their gills and skin respectively. However, Adamson & Sieber (1974) have shown that fish are unable to clear such compounds readily through their gills. Metabolism followed by excretion in the urine and the bile seems to be an important clearance route (Guarino & Anderson 1976), even in aquatic vertebrates.

3. Diet. Carnivorous animals are exposed through their diet to a relatively narrow range of non-nutrient chemicals. In contrast, herbivores, particularly those with catholic tastes in higher plants, ingest an extremely complex range of chemical structures, e.g. flavonoids, alkaloids, quinones, phenols and terpenes. Many of these substances are potentially toxic (Harborne & Simmonds 1964) and require a means of effective inactivation.

4. Environmental chemicals. Within the last two hundred years, all organisms have become exposed to an increasing number of synthetic organic chemicals. Those animal species exposed to high doses of chemicals, and having a short period for the onset of sexual maturity, may have mutated to forms that had elevated activities of certain drug-metabolizing enzymes. In this context, it is interesting to note the wide range of insects that have developed resistance to pesticides, through an improved capacity to metabolize them (Brooks 1979) and also that pesticide-resistant strains of fish are emerging in heavily polluted waters (Wells et al 1973). These rapid evolutionary developments are likely to have increased the protection against acute toxins but probably not that against slowly acting toxins (e.g. carcinogens), many of which are effective towards the end of an animal's reproductive life.

From the data on species distribution currently available it is not possible to resolve which, if any, of the above factors was the primary influence in the development of the drug-metabolizing enzymes in animals.

TISSUE DISTRIBUTION AND SUBSTRATES OF THE DRUG-METABOLIZING ENZYMES IN MAMMALS

All the data from vertebrates that I have discussed so far were obtained from studies of the liver. In all the mammals that have been examined, the liver has proved to be the most active organ in the metabolism of exogenous compounds. The intestinal wall and, to a lesser extent, the lung are relatively versatile drug-metabolizing organs. Many other organs are significantly able to conjugate but appear to have limited oxidative enzymes active against exogenous model compounds. Cytochrome *P*-450-dependent aryl hydrocarbon hydroxylase (*P*-448, EC 1.14.14.1) is present at low concentrations in most