Journal of
Environmental and Applied Bioresearch
Published online May 27, 2015 (http://www.scienceresearchlibrary.com)

ISSN: 2319 8745
Vol. 3, No. 2, pp. 40-48

Review Article **Open Access**

APPLICATION OF BIOCHEMICAL SCIENCE IN AGRICULTURE WITH SPECIAL EMPHASIS ON ENVIRONMENT

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Received: April 24, 2015 / Accepted : May 04, 2015

Abstract

Biochemistry is an experimental science that defines the role of various biomolecules and their reactions in maintaining the cell vitality. The understanding of biochemistry has major impacts on medicine, agriculture, nutrition, ecology, and many other facets of life. Biochemical researches can uncover the cause and the subsequent cures of diseases; improve crop productivity, optimize our nutrition; and provide solutions for environmental pollutions. All in all, biochemistry provides an understanding of life processes down to the molecular level.

The knowledge in biochemistry has been applied for thousands of years in human history. Our ancestors made use of cow pox as a vaccine to get immunity against small pox as well as biochemical reactions occurring in yeast to ferment juices and to bake bread. Animal clinical biochemistry at large is a very efficient tool to contribute to the diagnosis of disaeses. It can also be used in toxicology and for environment monitoring. Information thus provided lead to postulate the existence of a chemical defense system. In toxicology, clinical biochemistry can be used to search for diverse toxic effects in laboratory animal species through experiments about the evaluation of the toxicity of xenobiotics such as pesticides, human or veterinary drugs. As environment is concerned, animal clinical biochemistry can be used to investigate the possible effects of endocrine modulators in various species, which is currently a major environmental concern. It can also help evaluate the effects of pollution in fish. All the results obtained with clinical biochemistry lead to an evidence : in all animals, there is a system of chemical defense based on a large number of mechanisms, which allow to fight efficiently against this type of aggression. This system of defense has many similarities with the immune system : immaturity at birth, inducibility, specificity, overflow by toxic effects when doses are too high. Moreover, the actions of this system allow to better understand the concept of hormesis, elaborated in the latest years, which shows that in many cases low doses of toxicants can have beneficial effects on vital processes.

The knowledge in biochemistry has b

Key words: Biochemistry; Biomolecules; Clinical biochemistry; Environmental monitoring; Environmental pollution; Xenobiotics.

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Introduction

There are several relation which show that biochemical sciences is truly an integral part of environmental studies.

Experimental Toxicology

Toxicological experiments on laboratory animals, dogs or monkeys are necessary to determine the dangers of new chemical substances such as pesticides, veterinary drugs and human drugs.

Numerous specifics (OECD, EU, EPA) have been established to check such compounds for acute toxicity, medium toxicity, long term toxicity, reproductive toxicity, carcinogenicity and immunotoxicity. Each of these protocols involves animal clinical chemistry through the determination of numerous parameters in blood and urine samples. This is now possible because of the efficient automated systems at our disposal which enable large numbers of results, including enzymes and hormones, to be obtained from very small samples. Certain mistakes should be mentioned concerning the choice of parameters and their interpretation. It is not uncommon for example to see dossiers which include a determination of GGT activity on rat sera , even though this enzyme is known not to be of value in this species. Although all these experiments include a control group, which might suggest that their interpretation would be straightforward, parameter variations may often occur which are very difficult to judge. We need then to respect the basic rule of toxicology, which is the dose-response effect. Doseresponse assessment is the process of characterising the relationship between the dose of product and the incidence/degree of an adverse effect as variation in blood biochemical parameters. Factors to consider in the dose assessment are the intensity or frequency of the response with increasing dose, the shape and slope of the dose-response curve and the methods used for extrapolation of data from surrogate or sentinel species to ecological endpoint or to humans. Thus the sole and unique observation of a parameter variation, is not sufficient to permit a conclusion.

Numerous cases are very difficult to interprete because their biological significance is not well understood. This is the case when a decrease in the activities of certain enzymes such as phosphatases, transaminases, etc. is observed. It is nevertheless clear that in appropriate use of clinical biochemistry in experimental toxicology, is of more specific value compared with the observed variation in histopathological results. In fact in toxicology, to assess the danger of a particular compound, we need to assemble all the information available (clinical results, pathology, biochemistry, histopathology, pharmacokinetics, etc....) so as to try to obtain a clear picture of the situation as a whole.

Acetylcholinesterase

A good example of the use of clinical biochemistry consists of the assessment of the toxicity and effects of inhibitors of this enzyme, such as the organophosphorus esters (OP). These man-made esters mimic the normal biological ester substrate (such as acetylcholine : ACH) and interfere specifically with the ester-hydrolysing enzyme fraction for which they form a "surrogate substrate". Most of the targets of organophosphates are therefore proteins with this ester-hydrolysing or analogous capacity. The principal targets are acetylcholinesterase (AChE), and neuropathy target esterase (NTP). Inhibition of the former is mainly implicated in the acute effects, and the latter in induced polyneuropathy, where the nature of the subsequent damage closely resembles Wallerian degeneration and the lesion is highly selective. An attempt can be made to estimate these effects by the determination of activity of AchE or NTP, but interpretation of the results is not as easy as it might seem. This interpretation was reviewed at a recent Joint Meeting Pesticides Residues (JMPR). Part of the document in reproduced below:

"The JMPR considers the inhibition of brain acetylcholinesterase activity and clinical signs to be the primary endpoints of concern in toxicological studies on compounds that inhibit acetylcholinesterases. Inhibition of erythrocyte acetylcholinesterase activity is also considered to be an adverse effect, insofar as it is used as a surrogate for brain and peripheral nerve acetylcholinesterase inhibition, when data on the brain enzyme are not available. Toxicological significance of cholinesterase inhibition Butyrylcholinesterase The JMPR has consistently considered that the inhibition of plasma and brain butyrylcholinesterase is not a toxicologically significant effect for the purpose of establishing the acceptable daily intake (ADI). The reason is that there is no evidence that butyrylcholinesterase inhibition has any adverse effect. It can be used as an indicator of absorption of the inhibitor, and, as such, it is still a useful tool for monitoring occupational exposure. Data on statistically significant inhibition of butyrylcholinesterase activity should therefore always be included. Brain and erythrocyte acetylcholinesterase Regulatory agencies have traditionally used various thresholds, such as inhibition, 20 % inhibition, or any statistically significant inhibition, in defining biologically significant depression of enzyme activity. The Meeting considered that statistically significant inhibition by 20 % or more represents a clear toxicological effect and any decision to dismiss such findings should be justified. The Meeting also agreed that statistically significant inhibition of less than 20 % or statistically insignificant inhibition above 20 % indicate that a more detailed analysis of the data should be undertaken. The toxicological significance of these findings should be determined on a case-by-case basis. Considerations affecting such determinations include, amongst others, the shape or slope of the dose-response curve, assay

variability, and correlation with clinical signs." In summary, this example provides a good demonstration of the value of clinical biochemistry in assessing the danger of various compounds.

Control of the environment

Endocrine Disruptors

An environmental endocrine disrupter is defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behaviour. A series of field and laboratory investigations with marine invertebrates has suggested that tributyltin compounds, which are used as antifouling paints on ships, can have significant hormonal effects on some snail species at sublethal exposure concentrations. Controlled dose-response studies, have shown that these compounds can induce irreversible induction of male sex characteristics on females (imposex), which can lead to sterility and reduced reproductive performance. The possibility that other molluscs (e.g., oysters) could also be sensitive to tributyltin compounds is of considerable ecological concerns, as is the fact that such compounds accumulate in the food chain and might thus affect fish, wildlife and humans. A wide variety of compounds and environmental settings have been associated with potential reproductive and developmental anomalies in fish. Hermaphroditic fish, for example, have been observed in rivers downstream from sewage treatment plants, and masculinization, altered sexual development, and decreased fertility have been noted in certain fish species in proximity to the discharge of ipulp and paper plant effluent. The extent to which these observations are associated with significant changes in population dynamics are however, unclear from such studies. The primary causes of these perturbations are also generally unclear and could include synthetic chemicals as well as naturally occurring plant-derived compounds. However, correlative data, supported in some cases by controlled laboratory studies, suggest that alkyl phenol ethoxylates and their degradation products, chlorinated dibenzodioxins and difurans, and polychlorinated biphenyls (PCBs), among other compounds, could be contributing causative agents.

Perhaps the most fully documented example of putative ecological effects caused by a disruption of endocrine function has been reported for alligators in Lake Apopka, Florida. A series of detailed field and laboratory investigations indicated that it is very likely that a mixture of dicofol, dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylne (DDE) associated with a pesticide spill in 1980 was responsible for a variety of developmental effects resulting in the demasculinization of male alligators and "super-feminisation" of females. The effects of the spill were also reportedto include detrimental effects on hatching success and population levels. Although numerous controlled laboratory studies have demonstrated that a variety of compounds can elicit hormonally mediated effects on reproduction and development in rodents, the establishment of a credible cause-and-effect relationship in wild mammalian population has not been reported in the scientific literature to date. These discrepancy may be explained by the fact that the species in which problems have been identified are all egglaying aquatic species. In this case, the eggs are directly in contact with the toxic agents in the water. This is not the case for mammals. What is the place of animal clinical biochemistry in this area ? Today it is well known that, in a general way, hormones act by binding to specific receptors. The hormonal receptors bound to their ligand act as transcription factors for gene expression in the target tissue. Simple in vitro screening assays based on binding to a receptor are not in themselves sufficient for measuring hormone activity. The binding of ligand to its specific receptor must be correlated with a physiologic response. For such screening assays to be accepted as indicative of hormonal alteration, they must be thoroughly validated in a number of qualified, independent laboratories. Such validation requires the correlation of receptor binding with a physiologic endpoint, for example, the induction of progesterone receptors, increase in uterine peroxidase or an increase in vitellogenin in the case of the oestrogen receptors. These effects could be explored by using biochemistry techniques even though such techniques are not animal clinical biochemistry sensu stricto.

Role of biochemistry in the prevention of Aquatic pollution

The above-cited publication gives a good illustration of the use of blood biochemical analysis to explore the effects of chemical contaminants in water It states that one of the most important potential uses of blood chemistry data, from a toxicological perspective, is to compare animals collected from different locations (i.e., reference vs. contaminated sites), sample the same location over time or compare a contaminated site before and after remediation. For field evaluations, virtually any species that will provide more than a millilitre of blood can be used. However, the

investigator must ensure that the fish collected at different times or from different locations are of the same age and sex. There are many examples in the literature demonstrating significantly different values based on age, sex, and season of the year the fish were collected. Therefore, any investigator considering blood chemistry as a diagnostic tool should establish annual cycles of all the parameters of interest for both sexes in the species/strain to be used.

Measurement of glucose and cortisol can be used to evaluate general stress, electrolytes can be used to assess osmoregulatory ability, lipids and proteins can reflect the general nutritional state of the animal, while the measurement of certain serum enzymes may indicate organ or tissue damage. In situations where there are unexplained fluctuations in year class strength, or an obvious impairment of growth, the measurement of growth-promoting and reproductive hormones may identify the cause. Antibodies to the steroid reproductive and thyroid hormones are widely available whereas antibodies to growth hormone, insulin-like growth factor, gonadotropin-releasing hormone, and gonadotropins appear to be highly species specific, and their availability is limited. As both the steroid and the peptide reproductive hormones are available for salmonids, laboratory exposure to different classes of chemicals may reveal the most sensitive link in the hypothalamic-hypophysial-gonadal axis and provide a single assay for screening chemicals in the future. Other biochemical markers Other biochemical markers, such as cytochrome P 450 and metallothionein, can be used to assess the toxic effect. Cytochrome P 450 Monooxygenase and organic compounds : cytochrome P 450 exists in fish and many aquatic organisms. Its induction may indicate the exposure of fish to polynuclear aromatic hydrocarbon (PAH), polychlorinated biphenyls (PCBs) and other toxic compounds. However this induction is not always a sign of toxicity. Metallothionein and metals the widespread distribution of metallothioneins (MT) in aquatic animals is firmly established, having been reported in at least 80 species of fish and invertebrates. These proteins can be induced by various metals. The toxicological significance of MT induction has been supported by numerous studies conducted in aquatic organisms. The notion that metal-binding by MT is prospective of cellular function is supported by these studies, although the specific intracellular interactions remain to be clarified. From a practical point of view, the premise that MTs can be used for routine assessment of metal-exposed aquatic animals represents a still-to-be realised promise.

Concept of chemo-defence and hormesis

All living organisms possess a number of evolutionary derived systems which enable them to cope with the chemicals existing in their environment. Biotransformations Biotransformation processes exist in the liver, kidney and other tissues of humans and other species that are capable of transforming the structures of xenobiotics, thereby decreasing the toxicity of the compounds and facilitating their elimination.

Classically there are two phases in this PROCESS:

– Phase I: This is a structural biotransformation involving P 450 cytochrome monooxygenases constituted by a superfamily of more than 400 hemothiolates-enzymes. These enzymes permit the introduction of hydroxyl groups into the structure of lipophilic compounds thereby increasing their polarity and facilitating excretion.

– Phase II or conjugation phase: The biotransformation products and others, if their structure is suitable, are conjugated with polar agents of conjugation, which increase their urinary excretion. This is the case for example of glucuronic acid, sulphates, etc....All these enzymes are inducible.

Metallothionins

In the precedent chapter, we briefly discussed the presence of these sulfur proteins in aquatic organisms. But this is also true of mammals and birds. These molecules are able to complex heavy metals and decrease their toxicity (Hg, Cd, etc....). These proteins are inducible, as are cytochromes P 450. Other systems DNA repair systems, rapid exfoliation of the cells subjected to exposure such as cells the mouth, oesophagus, stomach and gut, are very useful.

Chemo-defence system versus immunodefence system

The analogies between these two systems are summarized in the table I. It is clear that during evolution living organisms have developed biochemical processes to react against the aggressions of the environment.

Hormesis Concept

Hormesis can be defined as the stimulatory effects caused by low levels of toxic agents. This situation has been described for radiations but also for some chemical agents. Assessment of the biological effects and risk of low level exposure is very important. The induction of detoxifying enzymes and other proteins could explain this particular effect. However until now this new concept, which supports the fact that toxic agents that are detrimental to

human and animal health above certain threshold levels may induce positive effects at a dose that is significantly lower than the classical "No effect level", has not been wholly accepted by part of the scientific community or by the regulatory agency. In conclusion, it is clear that clinical biochemistry provides an excellent tool in many areas. In toxicology and environment control it could help scientists to develop new approaches, new strategies and support the concepts of chemo-defence and hormesis.

Role of Biochemistry in Agriculture in Phytoremediation

Over the past few decades, heavy metal contamination of aquatic system has attracted the attention of several investigators both in the developed and developing countries of the world. Many industrial and agricultural processes have contributed to the contamination of fresh water systems thereby causing adverse effects on aquatic biota and human health. The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the environment make these toxicants deleterious to the aquatic environment and consequently to humans who depend on aquatic products as sources of food. Heavy metals can accumulate in the tissues of aquatic animals and as such tissue concentrations of heavy metals can be of public health concern to both animals and humans.

Organic pollutants are absorbed by plants both from the air, and from the soil after being dissolved in water and taken up together with nutritional components. The absorption of pollutants through roots and leaves differs: to get into the root, a substance must penetrate only through unsuberificated (i.e. not containing the reinforcing compound suberin) cell walls free of cuticle; but to get into the inner part of the leaf, a substance must penetrate either through the stomata or through the cuticle of the epidermis. The absorption of dissolved compounds by the plant is a controllable process. The rhizodermis of young roots absorbs foreign compounds by osmosis. This absorption depends greatly on such external factors as temperature and pH of the nutritional ambient and the soil. Other factors that significantly influence the penetration of toxicants into the plant are their molecular weights and their lipophilicities (hydrophobicities), principally the latter determining movement of organic pollutants across the plant cell membrane. After passage across the membrane, xenobiotics are distributed throughout the entire plant. What then is the further fate of the pollutant molecule? In order to carry out the oxidative degradation of toxicants, the plant cell, in addition to some ultrastructural reorganization, undergoes other intracellular changes including the formation of conjugates and their deposition in vacuoles. These processes imply the expenditure of energy, supplied as reducing equivalents from NADPH generating systems (photosynthesis, pentose cycle) in mitochondria, from freely available molecular oxygen, and from free radicals produced by the peroxidation of lipids. Within our present state of knowledge the following aspects of the fate of toxicant conjugates within plant cells remain unclear: (i) their stabilities and half lives; (ii) the activities of toxicants after conjugate dissociation; (iii) the extents of oxidative degradation— in the context of conjugation—of different xenobiotics; and (iv) the physiological consequences for the plant itself of the conjugation of organic pollutants. Changes in cell metabolism occur due to conjugate formation, especially where concentrations of toxicants are high. The excessive expenditure of cell energy associated with the creation of conjugates impairs cellular function. Vitally important endogenous metabolites are sequestered during the process of conjugate formation. On entering the cellular food chain, conjugates dissociate and may consequently release toxic components. The conjugation process can, therefore be considered as an intermediate stage resulting in the incomplete transformation of the toxic parts of xenobiotics by their temporary conjugation to endogenous metabolites.

Functionalization

Often nonpolar toxicants do not contain the functional groups necessary for a conjugation reaction. In order to increase the reaction potential of these nonpolar compounds, incorporation of the necessary functional groups into the xenobiotic molecule is required. This process of functionalization increases the polarity of the whole molecule and is performed enzymatically by oxidation, reduction or hydrolysis. The resulting hydrophilic products are much more readily utilized by plant cells. Functionalization requires a high activation energy and is the most severely rate-limiting reaction in the entire multistep process of intracellular toxicant transformation.

Hydroxylation is one of the most widely encounte- red functionalization reactions in plants and is effected by insertion of an oxygen molecule into a C-H bond of the xenobiotic molecule.

The reaction requires the participation of active oxygen. In plants, hydroxylation is catalyzed by metalloenzymes: cytochrome P450-containing monooxygenases, peroxidases and polyphenoloxidases. The monooxygenase system is localized in the endoplasmic membrane and is a highly organized multienzyme complex. For activation of molecular oxygen this system exploits NADH (produced mainly in mitochondria) and NADPH (produced mainly in chloroplasts) reduced equivalents. The individual components of the monooxygenase system—NADPH-cytochrome P450-reductase, cytochrome b5 and cytochrome P450— possess sufficient redox potential in order to apply molecular oxygen to the oxidation of organic compounds. The terminal electron acceptor, cytochrome P450, is a hemoprotein. The iron ion situated in the active site of this molecule binds molecular oxygen and the xenobiotic molecule activates oxygen by electrons received from the redox chain, and one of its atoms is then incorporated into the substrate molecule.

The enzymes of oxidation

After functionalization of the xenobiotic in plants, during which its primary structure remains intact, extensive oxidative degradation takes place. This has been confirmed by numerous studies showing the inclusion of the transformed intermediates in the Krebs cycle, other cell processes, and the excretion of $14CO_2$ following exposure to 14C-labelled xenobiotics. Xenobiotic conjugates accumulated in vacuoles are highly likely to undergo extensive oxidation after their release. Hence, functionalization prepares a xenobiotic either for immediate further oxidation, or for conjugation, whereby oxidation is deferred until the eventual release of the compound. A classical example of extensive oxidation of an organic pollutant in plants is shown: the full degradation of an aromatic carbon skeleton down to carbon dioxide. Muconic acid is the first aliphatic compound formed as a result of aromatic ring splitting, which is further oxidized to fumaric acid and then participates in the cell metabolism as a standard endogenous substrate.

It is apparent that the importance and universality of plants in bioremediation lies in their ability to take up from the air, water and soil all types of organic pollutants and thereafter to oxidatively degrade them down to carbon dioxide. As the result of such transformation, all the carbon atoms of the toxicant acquire the ability of insertion in the pathways of biogenic migration, i.e. participation in the characteristic cycle of carbon circulation. This is the ultimate basis of the phytoremediational process. All the important pathways of xenobiotic oxidation (mediated by iron and copper containing enzymes) operate in plant cells consecutively or simultaneously. The organization and functioning of the cytochrome P450-containing monooxygenase systems in procaryotic and eucaryotic organisms are distinguished by specialized features. Procaryotes contain soluble forms of this enzymatic system. In eucaryotes, the quinary structure of the hemoproteins is established by their incorporation into the endoplasmic membrane. The classic example is liver cytochrome P450, which is readily incorporated into the membrane structure. The individual components of the monooxygenase system are positioned along the entire membrane. In this configuration they are in close contact with the lipid matrix, which at the same time appears to have a barrier function; therefore oxidative hydroxylation in microsomes is preceded by penetration of the xenobiotic through the membrane lipid layer. Formation of a catalytically active complex between cytochrome P450 and the xenobiotic determines its movement from the aqueous to the phospholipid phase.

Biochemical Markers of Aquatic Environment Contamination – Cytochrome P450

The fish, as a bioindicator species, plays an increasingly important role in the monitoring of water pollution because it responds with great sensitivity to changes in the aquatic environment. The sudden death of fish indicate heavy pollution; the effects of exposure to sublethal levels of pollutants can be measured in terms of biochemical, physiological or histological responses of the fish organism (Mondon at al. 2001). Changes in age and species distribution in a stock fish population are general indicators of water pollution, but there are also responses specific to a single pollutant or a group of contaminants (Svobodová 1997). Biochemical markers are biochemical responses induced in the presence of a specific group of contaminants that have the same mechanism of toxic activity.

Biochemical markers

Entering to an organism, xenobiotics bind to specific cellular structures called receptors that are localised on the cell surface or inside the cell either in its cytoplasm or on cell organelles. The binding of a xenobiotic with its receptor may induce cellular processes that have toxic or other adverse effects on the cell. In macroorganisms, these processes subsequently affect organs, the organism itself or even the whole population involved. Biochemical markers are measurable responses to the exposure of an organism to xenobiotics. They usually respond to the

mechanism of toxic activity and not to the presence of a specific xenobiotic and, therefore, may react to a group of either similar or very heterogeneous xenobiotics. Biochemical markers detect the type of toxicity; in some of them, the magnitude of their response correlates with the level of pollution. Biochemical markers have been used in research in toxicology, ecotoxicology and pharmacology. Most of these studies have utilised in vitro assays (White et al. 1997b; Fent et al. 1998; Rogiers and Vercruysse 1998) whereas in vivo tests have been employed in research concerned with aquatic environments (Machala 1995; White et al. 1997b; Anzenbacherová and Anzenbacher 1999; Schlenk and Di Giulio 2002). The majority of reports on biochemical markers deal with pharmacological or toxicological aspects of new drugs introduced to the market. The great advantage of biochemical markers is providing evidence of the state of pollution in a comprehensive way based on the synergistic and antagonistic effects of all contaminants involved. In fish, however, the physiological values of many parameters may vary greatly in relation to the species of fish, their age and sex as well as seasons of the year. It is, therefore, very important to seek and make use of indicators independent of such physiological fluctuation (Svobodová 1997; Schlenk and Di Giulio 2002; Jorgensen et al. 2002). One of the most intensively studied biomarkers, in both laboratory and field conditions, is cytochrome P450. The determination of cytochrome P450 levels as a response of the organism to the presence of pollutants in the aquatic environment has been reported by many studies from all over the world (Payne et al. 1987; Curtis et al. 1993; Aas et al. 2001; Mondon et al. 2001; Ba rd et al. 2002; Jewett et al. 2002; Ruus et al. 2002; Moore et al. 2003) including those on river pollution in the Czech Republic (Machala et al. 1997; Machala et al. 2000).

Cytochrome P450

The metabolism of xenobiotics is a two-phase process. The first phase involves reactions giving rise to more polar compounds. This may either result in reducing the effectiveness of the metabolised xenobiotic, which is described as bioelimination, or produce an active metabolite, in terms of both pharmacology and toxicology, which is regarded as bioactivation. The first-phase reactions include oxidation, reduction and hydrolysis and the greatest importance is ascribed to oxidation enzymes involved in the metabolism of the majority of xenobiotics. In second-phase reactions, the metabolites produced in the first phase or, occasionally, also the original substances, are conjugated with products of the endogenous metabolism (glucuronate, glutathione, 3'-phosphoadenosine-5'- phosphosulphate, etc.) to give rise to polar compounds subsequently eliminated from the body. Metabolites produced in the first phase are eliminated only exceptionally. The most important oxidation enzymes of the first phase are P450 cytochromes, formerly "mixed function oxidases" (Lewis 2001). The term "cytochrome" is not very appropriate because it was initially used to designate electron-transporting proteins with a haem prosthetic group and not haem enzymes (Anzenbacherová and Anzenbacher 1999; Lewis 2001). Cytochromes are present, at high levels, in the liver, accounting for 1 to 2% mass of hepatocytes (Lester et al. 1993; Lewis 2001). However, they are also found in the intestine, kidney, lungs, brain, skin, prostate gland, placenta, etc. (Anzenbacherová and Anzenbacher 1999, 2001; Arukwe 2002; Ortiz-Delgado et al. 2002). Cytochrome P450 was described by Klingenberg in 1948 and, since then, this protein has been studied most intensively (Kvasniãková 1995; Anzenbacherová and Anzenbacher 1999; Lewis 2001). As demonstrated, the enzyme is not a single entity but includes a large number of isoforms; up till now over 1000 isoenzymes have been isolated (Stoilov *et al*. 2001; Lewis 2001).

The basic structure of each isoenzyme is a haem skeleton similar to that present in other enzymes, such as cytochrome c oxidase. Cytochrome P450 is classified as a b-type haemoprotein (haem skeleton of this type also has haemoglobin, myoglobin and certain peroxidases) associated with membranes of the endoplasmic reticulum. In eukaryotic cells, it is also bound to mitochondrial membranes and, in bacteria, it is present in the cytosol in a soluble form. Its name is derived from the fact that it was discovered as a pigment that, in a complex with CO, absorbed light at 450 nm. The inactive form of cytochrome P450 has an absorption maximum of 420 nm, which is similar to other haemoproteins.

Assessment of cytochrome P450

The degree of surface water pollution is monitored by means of indicator fish species, i.e., species most common in the region investigated. In the Labe River (Czech Republic), these are bream (Abramis brama L.), perch (Perca fluviatilis L.) and, in the Morava River (Czech Republic), this is chub (Leuciscus cephalus L.) (Svobodová 1997). The toxicological examination includes determination of the amount of mRNA induced or measurement of the catalytic activity of the cytochrome arising in response to xenobiotic exposure. The presence of CYP 1A is associated with the activity of EROD that catalyses the production of 7-

hydroxyresorufin from ethoxyresorufin. This fluorescent product can be detected by several methods, of which fluorescence spectrofluorometry is most common (Machala 1997; Nilsen *et al.* 1998; Chang and Waxman 1998; Machala *et al*. 2000; Schlenk and Di Giulio 2002). Another method evaluates the hydroxylation of benzo[a]pyrene metabolised by aryl hydrocarbon hydroxylase. However, this procedure requires the use of radioisotopes and is currently replaced by other methods. Cytochrome P450 can also be detected by the enzyme-linked immunosorbent assay (ELISA) because antibody against fish CYP 1A has become available (Nilsen *et al.* 1998; Schlenk and Di Giulio 2002). These measurements are accurate only under specific conditions. The catalytic activity of enzymes is very sensitive to thermal denaturation (Schlenk and Di Giulio 2002) and therefore the immediate freezing of samples followed by storage at temperatures lower than -70 °C is a prerequisite. The enzymes are also sensitive to the presence of trace metals or organic metal compounds that may destroy them or interfere with their determination (Schlenk and Di Giulio 2002).

Plant Enzymes involved in Phytoremediation

Another indirect role that plants play in the degradation of petroleum hydrocarbons involves the release of enzymes from roots. These enzymes are capable of transforming organic contaminants by catalyzing chemical reactions in soil. Schnoor et al. (1995) identified plant enzymes as the causative agents in the transformation of contaminants mixed with sediment and soil. Isolated enzyme systems included dehalogenase, nitroreductase, peroxidase, laccase, and nitrilase. These findings suggest that plant enzymes may have significant spatial effects extending beyond the plant itself and temporal effects continuing after the plant has died (Cunningham *et al*., 1996).

Conclusion

Thus it may be concluded here that biochemical reactions are an essential process of environmental reactions and both the things are interrelated to each other in a proper way. Hence there is a hope in finding new ways for the betterment of environment through biochemistry. These are finding which tell us that a lot more can be done on research basis in this regard.

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