BIOTRANSFORMATION OF XENOBIOTICS AND HORMONES

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Keywords: foreign compounds, xenobiotics, bioactivation, detoxification, oxidation, cytochrome P-450, hydrolysis, conjugation, glucuronides, glutathione

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Summary

The xenobiotics are usually lipophilic. Therefore they are easily absorbed from the food and drinks as well as from air through the mucous membranes of the gastrointestinal and respiratory tracts, respectively. They can also penetrate the skin. They are poorly excreted, because the cells in the walls of the urinary and gastrointestinal tracts have lipid rich membranes. The excretion of xenobiotics requires thus metabolic modification. Usually several metabolic pathways are used. In the first phase functional groups are generated by oxidation and then an endogenous hydrophilic residue like glucuronic acid is added to the functional group. At the same time the biological activity of the xenobiotic is lost or greatly modified, and it is detoxified. During metabolism often also more active metabolites are formed, and they can be reactive and attach also to the DNA. This may lead to mutation and formation of malignant cells. During the oxidation also reactive forms of oxygen are generated, and this can again be harmful. Many endogenous compounds like hormones (e.g. steroid hormones) are inactivated in the same pathways as are the xenobiotics. This explains many of the interactions of the xenobiotics in the body. Drugs used in therapy of diseases are also metabolized along the same routes, which again explain some of the interactions. The enzymes of biotransformation are often readily induced by xenobiotics. This helps to get rid of

them, but at the same time the interference with the hormone metabolism may become harmful and the responses to drug therapy unexpected. The plants have also their own metabolic pathways to inactivate the xenobiotics, but they must store the metabolites in vacuoles due to lacking excretory mechanisms (aquatic plants can of course release the metabolites into water).

1. Introduction

All organisms are exposed through air, food, drinks and skin contacts to chemicals which are either harmful or unnecessary. Such chemicals are called xenobiotics, because they are foreign - i.e. not necessary - to the organism studied. Most of those compounds are lipophilic, and the organism can get rid of them only through metabolism. This biotransformation is a chain of reactions, which increase the water solubility. Xenobiotics can be either natural chemicals which e.g. the plants make to defend themselves against the herbivores or man made for various purposes like drugs to treat diseases, pesticides to protect plants and animals from their diseases and vermin losses, food additives, which e.g. prolong the life span of the product or provide some needed property to help the processing. Some food additives are, however, physiological compounds like vitamin C (L-ascorbic acid). Some mimic compounds, which occur widely in nature like benzoic acid derivatives. Many so called environmental chemicals are man made wastes, which are released by industrial processes in air or effluents in wastewaters.

The pollution of air by a great variety of chemicals is a serious problem in many countries especially in megacities and even in smaller cities, if the climatic stagnation occurs. Energy production and traffic are serious sources of pollution of air (see *Biomonitoring of Environmental Pollution*).

The metabolic modification in biotransformation usually takes place in two main steps in sequence with a loss of the possible biological activity (detoxification). Many enzymes often contribute to the metabolism of every single xenobiotic. As many enzymes contribute to the metabolism of a xenobiotic, one usually finds a wide spectrum of metabolites even from simple organic molecules.

In the so called First Phase functional groups are introduced by oxidation (see Figure 1) or reduction or by unmasking them by hydrolysis with the aid of enzymes having rather low specificity. These functional groups may increase the biological activity of the xenobiotic and increase the reactivity and thus the toxicity (bioactivation). Thus e.g. benzo(a)pyrene, common component of the e.g. tobacco smoke, is rather harmless itself, but the epoxides generated in the oxidation process can react with the DNA and other compounds to initiate the cancer formation.

After the functional groups are available in the xenobiotic a normal metabolite of the intermediary metabolism is enzymatically attached in the co called Second Phase reactions (see Figure 1). Because the products are now much modified and usually soluble in water, they have lost all or most of their toxic character. Therefore this modification of xenobiotics is called detoxication or better detoxification.

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The enzymes catalyzing detoxification occur in the common portals of entry of xenobiotics. The activities are high in the gastrointestinal mucous membrane a well as in the liver. These enzymes reduce the biological activity of xenobiotics and eliminate them partly already before they reach portal blood or before the blood passes the liver. This phenomenon is called the First Pass Elimination.

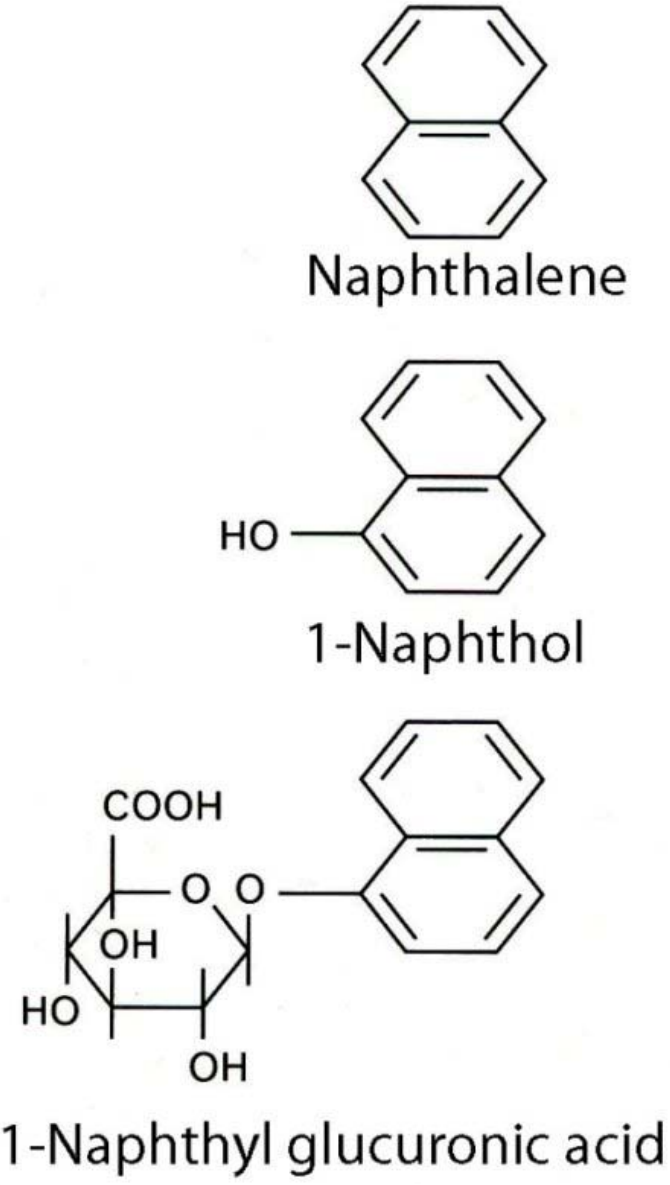

Figure 1. The two phases in the biotransformation of lipophilic naphthalene. In the first phase the oxidation by cytochrome P-450 introduces a hydroxyl group and in the second phase uridine diphosphate glucuronosyltransferase adds a glucuronic acid residue, which makes the product water soluble which is necessary for excretion.

The same enzyme systems, which are metabolizing the xenobiotics, are also contributing to the metabolism of endogenous substances (endobiotics) like steroids and several other hormones. Some of these enzymes catalyze steps in the synthesis and some in the catabolism of the hormones. Therefore one can expect significant interaction in pollutant and hormone metabolism. Rachel Carson's book "Silent

Spring", which was translated into many languages draw the attention of great public on the environmental problems caused by the chemicals spread for various purposes in nature.

2. Absorption of Xenobiotics

The absorption of non-charged xenobiotics takes place usually passively along the concentration gradient. The majority of xenobiotics are lipophilic organic compounds. Lipophilic nature helps their penetration trough the skin and mucous membranes of the animals.

If the xenobiotic is a weak acid or base, the absorption is favoured in human stomach in the first case and in the gut in the second case. The hydrochloric acid as a strong acid inhibits the dissociation of weak organic acids in the stomach and alkaline environment in the gut promotes their dissociation there. The ingested chemicals are spread by the mixing peristaltic movements of the gastrointestinal tract over a very large area, in humans over some 250 square meters. Therefore the absorption is quite extensive if not practically complete. The same is true also in case of skin and respiratory mucous membrane in man. The animals are cleaning their fur by licking, which means that the xenobiotics find their way to the gastrointestinal tract.

In plants the leaves have lipophilic fax layer on their surface of their leaves. That collects the pollutants and helps their penetration into the tissues.

The environmental pollutants are often enriched to livings organisms in several folds, because they can be considered behave as lipid droplets in their surroundings. All cell organelles have lipid backbone, therefore lipophilic xenobiotics are concentrated to the vital structures.

The food chains promote the enrichment of persistent xenobiotics several folds. The herbivores get them from the plants and the predators pick the pollutants from the herbivores in their food chain, when eating them (see *Biomonitoring of Environmental Pollution*).

Because the routes of excretion like the gastrointestinal and urinary tracts are lined by single cell layers i.e. lipid films the reabsorption is efficient. Only after a loss of their lipophilicity the chemicals leave the organisms. Volatile compounds can, however, evaporate through the skin and leave the body via lungs during expiration.

The terrestrial plants do not have similar excretory mechanisms as animals. Their strategy is therefore different, and the metabolites are stored as secondary metabolites e.g. in vacuoles.

3. Detoxication and Bioactivation

3.1. Oxidation of Xenobiotics

3.1.1. Cytochrome P-450

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The oxidation is a very common step in the metabolism of xenobiotics. It is catalyzed in most cases by a superfamily enzyme system called cytochrome P-450. At present this superfamily is known to consist of about 20 enzyme families. The genes and thus also the amino acid composition of these enzymes are related. They have probably originated all from the same gene after duplication and further modifications. The name comes from the color i.e. spectral properties of the reduced enzyme when bound to carbon monoxide. This is due to the prosthetic group, heme.

Cytochrome P-450 (see Figure 2) catalyzes monooxygenation in which one oxygen atom is incorporated into the substrate. The other atom is reduced to water with the aid of reducing equivalents derived from glucose via pentose phosphate pathway and NADPH and NADPH-cytochrome P-450 reductase. The cytochrome P-450 produces also various intermediates of the oxygen metabolism, which are quite reactive.

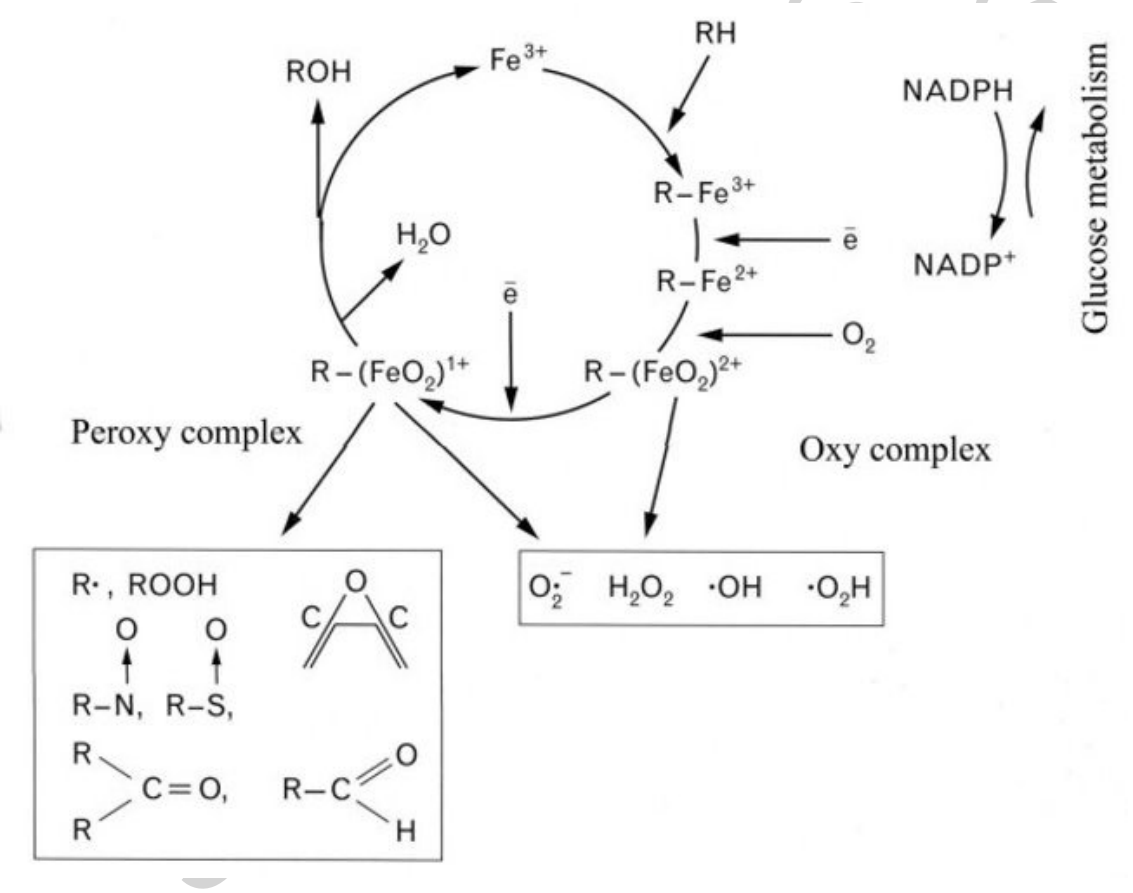

Figure 2. Reaction mechanism of cytochrome P-450 in the oxidation of xenobiotics RH and production of different reactive forms of oxygen.

Cytochrome P-450 can catalyze a hydroxylation of an aliphatic or aromatic carbon, an epoxidation of a double bond, heteroatom (S-, N-, I-) oxygenation, a N-hydroxylation, heteroatom (O-, S-, N-) dealkylation, oxidative group transfer, cleavage of esters and dehydrogenation. It can also catalyze the reduction of azo and nitro compounds and cause reductive dehalogenation. In the synthesis of endogenous compounds cytochrome P-450 enzymes catalyze the cleavage of C-C-bonds in the side chain of cholesterol and

aromatization of androgens to estrogen. This list indicates the unique role of the cytochrome P-450 system in the overall metabolism of pollutants and the xenobiotics.

The protein part of the cytochrome P-450 enzymes determines the substrate specificity i.e. what compounds that particular isoenzyme can oxidize. The substrate specificity of some of the enzymes is rather broad while that of some others is very specific. The cytochromes P-450 are now abbreviated in codes as CYP followed by a number to indicate the family and an letter to specify the subfamily and number, which codes further the enzymatic activity of that particular protein. The gene of such a specified protein may mutate further and in some cases several alleles have been described. Such mutations may have great significance in drug therapy, because the mutation may cause a lowering or loss of the catalytic activity i.e. the individual lacks that metabolic reaction.

CYP1-3 families have significance in the oxidation of pollutants and drugs while other families are catalyzing the metabolism of endogenous substances.

Recently CYP2A6 gene has received considerable interest, because its allele frequencies vary in different populations as is also coumarin metabolism and smoking responses as well as lung cancer susceptibility. It is the key enzyme in the metabolism of nicotine, too.

Those cytochrome P-450 enzymes, which have a role in the metabolism of endogenous compounds, contribute to the synthesis of e.g. steroid hormones. CYP11A catalyzes the oxidation of the side chain of cholesterol in pregnenolone synthesis, aromatase CYP19 catalyzes the conversion of androstendione to estradiol and also the catabolism of many more hormones.

The endogenous products of some cytochrome P-450 forms are powerful regulators of functions. So for instance CYP4A and CYP2C families produce in kidneys hydroxyeicosateraenoic (HETE) and epoxyeicosatrienoic as well as dihydroeicosatrienoic acids. These compounds play central roles in the regulation of renal tubular and vascular functions.

Another example of very necessary reaction catalyzed by a cytochrome P-450 enzyme (25-hydroxyvitamin D-1-alpha-hydroxylase) is the synthesis 1,25(OH)(2)D(3) ie the active form of vitamin D. It is regulating calcium homeodynamics, control of bone differentiation and even modification immune responses. This cytochrome is also expressed in the kidneys.

The reaction products of cytochrome P-450 may be quite reactive. Epoxides have an oxygen bridge (see Figure 2). Epoxides easily react e.g. with macromolecules. Product DNA adducts hamper the genomic functions. Benzo(a)pyrene (see Figure 3) is one such example i.e. this compound as such is not so dangerous, but its epoxide derivatives are. Also other reactive products are produced. Thus e.g. CYP1A2 activates heterocyclic amines to mutagenic products.

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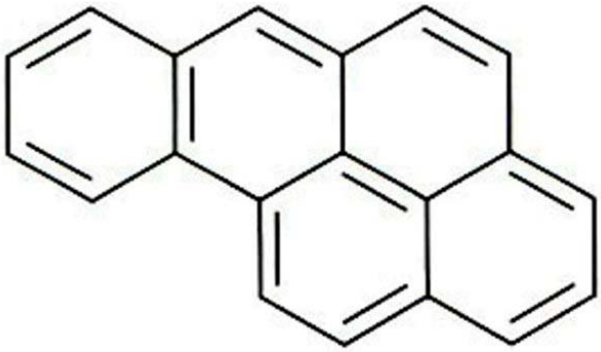

Benzo(a)pyrene

Figure 3. Structure of benzo(a)pyrene, carcinogen of e.g. tobacco smoke.

CYP enzymes split the dioxygen molecule. This opens also a possibility to the formation of oxygen derived radicals, if the catalytic cycle is uncoupled. If this takes place after the first electron, oxygen is released as superoxide and if the catalytic cycle is interrupted after the second electron, oxygen is released as hydrogen peroxide (see Figure 2). These reactive oxygen species (ROS) are also harmful, if not controlled. They can initiate chain reactions, which can ruin the membrane structures of the cells due to the peroxidation of lipids and the carbonylation of proteins. Furthermore the nucleic acids are also sensitive to these radicals. The CYP enzymes occur widely in the tissues. They can be found in the portals through which the xenobiotics enter the body (gastrointestinal mucosa, skin and respiratory tract) and also in the liver through which the blood from the gut passes. Thus the blood returning from the gut to heart has been rather efficiently cleaned. Because many orally used drugs have thus lost a significant fraction of the effective molecules in First Pass Effect.

In the cells the CYP enzymes are located mostly in the endoplasmic reticulum and outer membrane of nuclei. This means that the lipophilic xenobiotics are automatically coming to the immediate neighbourhood of this oxidizing enzyme system. The number of cytochrome P-450 molecules in the endoplasmic reticulum membrane is so high that the enzyme molecules most probably form clusters. Cytochrome P-450 cooperates with NADPH cytochrome P-450 reductase. The cluster structures promote this cooperation and the transfer or reducing equivalents.

The level of CYP enzymes varies a lot depending on the chemical loading of the animals and also in man. There are situations, when the CYP enzymes are the most prominent proteins in the liver. Several chemicals like arylhydrocarbon carcinogens TCDD and dioxins are know to induce the synthesis of one or more CYP enzymes. One of the most widely studied models of induction is CYP1A1. It metabolizes a large number of xenobiotics to cytotoxic and/or mutagenic derivatives. Its induction activates a cascade of the Ah receptor. It starts with the binding of the compound to this receptor, heterodimerization Arnt protein, constitution of a complex with xenobiotic responsive element (XRE) and finally gene activation. The synthesis of new enzyme proteins is regulated so that the messenger RNAs are translated into protein copies. The increased

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reading of the genes and enzyme synthesis, i.e. induction takes place very fast. In few hours time the levels of CYP enzyme activity can increase from very low levels to many folds (see Figure 4). At the same time the production of many other enzymes related to the biotransformation and detoxification is induced. Thus diaphorase and the second phase enzymes like UDPglucuronosyltransferase and glutathione-S-transferase activities are much increased.

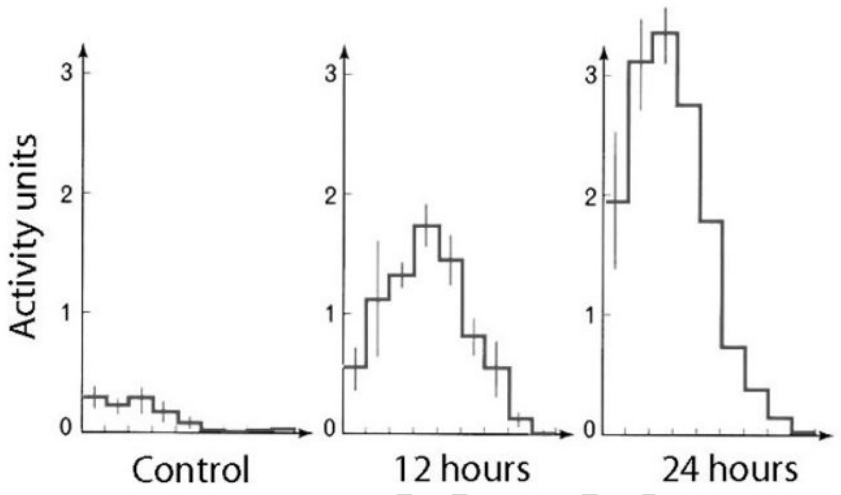

Figure 4. Induction of benzo(a)pyrene hydroxylation (CYP1A1) by 3-methylcholantrene administration in the gastrointestinal mucosa of the rat.

Beta-naphthofavone is another widely studied model compound in the induction studies of xenobiotic metabolism. It is also binding to Ah receptors, but in addition it works via activating the antioxidant responsive element (ARE), which is orchestrating the broader defense as its name suggests to cope with the oxidant stress. Up to now much less has been known about the molecular mechanisms of phenobarbital type of inducers. More than three decades since the induction by phenobarbital - formerly widely used drug - was first reported, a nuclear receptor that mediates the induction has been identified. In response to phenobarbital exposure, a specific protein is translocated from cytoplasm into nucleus, where it forms a heterodimer with a retinoid receptor and activates the phenobarbital response element leading to the concerted induction of numerous genes and those of cytochrome P-450 included. The response is much broader than that of the activation of Ah receptor chain.

Cytochrome P-450 is contributing to the oxidation of fatty acids. CYP4A enzymes catalyze omega and omega-1 oxidation of fatty acids and their derivatives, including prostaglandins. Alcohol is causing a lot of health problems. Some of them are related to the function and induction of alcohol inducible form of cytochrome P-450 (CYP2E1). It is also known as microsomal ethanol oxidizing system. This enzyme produces toxic metabolites, oxygen radicals, and causes lipid peroxidation. CYP2E1 plays a role in the metabolism, endogenous compounds including fatty acids and ketone bodies. The CYP2E1 expression is complex and involves transcriptional, translational and post-translational mechanisms. Hormones like insulin, glucagon, growth hormone and leptin and growth factors including epidermal growth factor and hepatocyte growth factor contribute to the regulation. Because the substrate specificity of CYP enzymes can be

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rather low, and there are several isoenzymes active in the same time the same cells, there are usually several oxidized products released from one xenobiotic. In the organism the xenobiotic is carried by blood to all tissues and their isoenzyme spectra and enzyme levels are most probably different. Thus the product outcome can vary quite a lot in different tissues.

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Biographical Sketch

Dr Osmo Otto Päiviö Hänninen, DMS, Ph..D., Professor of Physiology, Chairman of the Department, University of Kuopio, Finland. Born 1939, Lahti, Finland. He studied at the University of Helsinki and the University of Turku, Finland, where he received his Master of Sciences (Biochemistry) in 1962, Licentiate of Medicine (MD) in 1964, Doctor of Medical Sciences (DMS) in 1966, and passed his dissertation in biochemistry for his Ph.D. in 1968. He has also studied genetics. He has been a specialist in sports medicine since 1986. He served as the Research Assistant of Professor K. Hartiala, 1962–4; Assistant of Physiology, 1964–5; Laborator of Physiology, 1966–7; Docent of Physiology, from 1967, and Associate Professor of Biochemistry, 1969–71, at the University of Turku; Acting Professor in the Planning Office, 1971–2; and from 1972, Professor of Physiology and Chairman of the Department of Physiology, University of Kuopio; Vice-President of the University of Kuopio, 1972–9; and President, University of Kuopio, 1981–4. Furthermore, he served as Visiting Professor of Physiology at Shanghai Medical University, China, 1991–2, and at Sun Yat Sen Medical University, Guangzhou, China, 1998–9; as Foreign Member of the Russian Academy of Natural Sciences, from 1994; and as Secretary General, International Council for Laboratory Animal Science, 1988–95. He was the President of Societas Physiologica Finlandiae, 1990–9, and has been President of the International Society for Pathophysiology and a Member of the Executive Committee since 1994, and the Treasurer of the International Union of Biological Sciences since 1997.

His special interests in research are:

- Biotransformation and adaptation to chemical loading, biomonitoring of toxicants, and comparative biochemical toxicology.
- Muscle metabolism and function.
- Ergonomics.

He has contributed 266 papers in refereed journals and seventy-two in proceedings, and written fifty-five reviews, and thirty books or book chapters. He serves on the editorial board of four international journals and is at present the European Journal Editor of *Pathophysiology*.

Of his post-graduate students (thirty-two in biotransformation, twenty-seven in muscle metabolism and physiology, and five others), twelve serve as professors in China, Finland, Greece, Sweden, and the United States.

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