[*Journal of Health Science*, 45(5), 282-288 (1999)] [Lab. of Hygienics]

Rapid Analysis of Pesticides Causing Acute Poisoning in Patients by High-performance Liquid Chromatography with Column-switching Technique.

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We investigated a more rapid method of screening and quantitative analysis of 10 important fat-soluble pesticides employing column-switching HPLC-DAD together with a direct injection method of biological samples. The detection limits of the 10 pesticides were less than 10 ng per injection, except for isoxathion in artificial gastric juice and urine. This method was applied to three actual cases of acute poisoning, and we were able to provide important information to doctors on the basis of the rapid method of screening and quantitative analysis of pesticides in biological samples. The direct injection method in this paper could shorten analyzing time by 1－1.5h compared to the method that necessitating solvent extraction prior to HPLC analysis.

[*Biol. Pharm. Bull.*, **22**, 741-744 (1999)] [Lab. of Biochemistry]

Inhibition of Human Aldehyde Reductase by Drugs for Testing the Function of Liver and Kidney.

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Drugs for testing the function of liver and kidney (sulfobromophthalein, phenolsulfonphthalein, indigo carmine and indocyanine green) and other organic anions (rose bengal and haematin) were found to potently inhibit human liver aldehyde reductase that is involved in the detoxification of 3-deoxyglucosone and methylglyoxal, reactive intermediates, during the formation of advanced glycation end products. The results suggest that these potent inhibitors bind weakly to the free enzyme and tightly to the enzyme-NADP binary complex.

[*Pharmacogenetics*, **9**, 763-771 (1999)] [Lab. of Biochemistry]

Characterization of a Novel Variant (S145C/L311V) of 3α-Hydroxysteroid/Dihydrodiol Dehydrogenase in Human Liver.

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Human liver 3α-hydroxysteroid/dihydrodiol dehydrogenase (DD) is involved in the metabolism of steroid hormones and polycyclic aromatic hydrocarbons, and is also responsible for the reduction of ketone-containing drugs. To account for the interindividual difference in the activity, we isolated and characterized clones for the human liver enzymes. The sequence of the cDNA clone coding for the variant differed from that coding for the wild-type DD by two nucleotides (substitutions of C with G at positions 434 and 931) which caused two amino acid replacements, Ser^{145} to Cys (S145C) and Leu^{311} to Val (L311V). The introduction of S145C/L311V double mutations resulted in three- to five-fold decreased activities for xenobiotic and steroidal substrates, whereas no significant change was observed by an introduction of the S145C mutation alone. The results substantiate the existence of polymorphic forms for human liver DD, and also suggest the importance of the residue at position 311 for substrate binding to the enzyme.

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Cloning and Sequencing of the cDNA Species for Mammalian Dimeric Dihydrodiol Dehydrogenases.

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Cynomolgus and Japanese monkey kidneys, dog and pig livers and rabbit lens contain dimeric dihydrodiol dehydrogenase (DD, EC 1.3.1.20) associated with high carbonyl reductase (CR) activity. Here we have isolated cDNA species for the dimeric enzymes by reverse transcriptase-PCR from human intestine in addition to the above five animal tissues. In contrast with previous reports that other types of DD, CR and enzymes with either activity belong to the aldo-keto reductase family or the short-chain dehydrogenase/reductase family, dimeric DD showed no sequence similarity with the members of the two protein families. The dimeric enzyme aligned with low degrees of identity (14-25%) with several prokaryotic proteins, in which 47 residues are strictly or highly conserved. Thus dimeric DD has a primary structure distinct from the previously known mammalian enzymes and is suggested to constitute a novel protein family with the prokaryotic proteins.