[*Cancer Res.*, **57**, 246-252 (1997)] [Lab. of Biochemistry]

Chemoprevention of 4-Nitroquinoline 1-Oxide-induced Oral Carcinogenesis in Rats by Flavonoids Diosmin and Hesperidin, Each Alone and in Combination.

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The modifying effects of the two flavonoids diosmin and hesperidin given during the initiation and postinitiation phases of oral carcinogenesis initiated with 4-nitroquinoline 1-oxide (4-NQO) were investigated in male F344 rats. The compounds were tested alone and in combination. Dietary administration of these compounds significantly decreased the expression of cell proliferation biomarkers (5-bromodeoxyuridine-labeing index and AgNORs) of the nonlesional tongue squamous epithelium, and caused a significant reduction in the frequency of the tongue carcinoma. These findings suggest that diosmin and hesperidin supplementation is effective in inhibiting the development of oral carcinogenesis initiated with 4-NQO.

[*Biochem. J.*, **322**, 89-93 (1997)] [Lab. of Biochemistry]

Involvement of Two Basic Residues (Lys-270 and Arg-276) of Human Liver 3α-Hydroxysteroid Dehydrogenase in NADP(H) Binding and Actvation by Sulphobromophthalein: Site-directed Mutagenesis and Kinetic Analysis.

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A human liver 3 α -hydroxysteroid dehydrogenase isoenzyme, a member of the aldo-keto reductase family, shows a marked preference for NADP(H) over NAD(H), and is activated by sulphobromophthalein. The mutated enzymes, K270M and R276M, showed increases in the *Km* for $NADP^+$ of 22- and 290-fold respectively, and attenuated activation by sulphobormophthalein. The results suggest that the two basic residues in the 3 α -hydroxysteroid dehydrogenase isoenzyme play crucial roles in binding both the negatively charged 2'-phosphate group of $NADP^+$ and the sulphonic groups of sulphobromophthalein.

[*YAKUGAKU ZASSHI*, **117**, 167-177 (1997)] [Lab. of Biochemsitry]

A Sensitive Fluorometric Assay for Dihydrodiol Dehydrogenase.

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Dihydrodiol dehydrogenase (DD) oxidizes naphthalene dihydrodiol to 1,2-dihydronaphthalene, which is immediately autoxidized to 1,2-naphthoquinone. Here we established a fluorometric assay for the enzyme, which is based on the conversion of 1,2-naphthoquinone to fluorescent compounds by reacting with ethylenediamine. The formed fluorescent compounds were synthetically identified as 6-(2-aminoethylamino)benzo[*f*]quinoxaline and 2,6- or 3,6-bis(2-aminoethylamino)benzo[*f*]quinoxaline, which showed the same fluorescence at 550 nm at an excitation wavelength of 420 nm. The method provides a 9000-fold increase in sensitivity over a currently available assay which measures the change in the absorbance of a cofactor, NADPH. Since this method allowed many samples to be assayed simultaneously, we applied it to the analysis of multiple forms of DD, separated by an anion-exchange chromatography, from six human liver specimens.

[*Carcinogenesis*, **18**, 761-769 (1997)] [Lab. of Biochemsitry]

Modulation of *N*-Methyl-*N*-amylnitrosamine-Induced Rat Oesphageal Tumourigenesis by Dietary Feeding of Diosmin and Hesperidine, Both Alone and in Combination.

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The modifying effects of diosmin and hesperidin during the initiation and postinitiation phases of oesophageal carcinogenesis initiated with N-methyl-N-amylnitrosamine (MNAN) were investigated in male Wister rats. Feeding of these compounds significantly decreased the expression of cell proliferation biomarkers (BrdU-labelling index, AgNORs and polyamine levels). A number of oesophageal neoplasms developed in rats treated with MNAN alone, and feeding of both compounds caused a significant reduction in the multiplicites of oesophagel carcinoma and papiloma. These findings suggest that diosmin and hesperidin supplementation is effective in inhibiting the development of oesophageal cancer induced by MNAN.