Morihara, M., Aoyagi, N., Kaniwa, N., Kojima, S. and Ogata, H.*: **Assessment of gastric acidity of Japanese subjects over the last 15 years**
Biol.Pharm.Bull., **24**, 209-320 (2001).

The gastric acidity of young to elderly Japanese subjects from 1989 to 1999 was assessed and compared with that obtained in 1984, using GA-Test capsules containing acid-dissolving granules of riboflavin. The percentage of achlorhydric subjects increased with the age as observed before, which, however, tended to decrease in all age categories year by year. The percentage of achlorhydric subjects of 50 years in 1995-1999 was about 40%, which was lower than that (60%) in 1984. However, such a chronological change was not observed when the percentage of achlorhydric subjects was determined according to the birth year, indicating that it is related with the birth year of subjects. The percentage of achlorhydric subjects is correlated with that of the infection of Helicobacter pylori. Considering the high percentage of achlorhydric elderly that still exists, bioavailability and bioequivalence studies should be performed by taking into consideration the effects of gastric acidity on the in vivo performance of drug products.
Keywords: gastric acidity, achlorhydric elderly, Helicobacter pylori
*明治薬科大学

Izutsu, K., Kojima, S.:
Freeze-concentration separates proteins and polymer excipients into different amorphous phases
Pharm. Res., **17**, 1316-1322 (2000).

The miscibility of proteins and polymer excipients in frozen solutions and freeze-dried solids was studied as protein formulation models. Thermal profiles of frozen solutions and freeze-dried solids containing various proteins (lysozyme, ovalbumin, BSA), nonionic polymers (Ficoll, PVP), and salts were analyzed by DSC. The polymer miscibility in frozen solutions was determined from the glass transition temperature of maximally freeze-concentrated solute (Tg'). All the protein and polymer combinations (5% w/w, each) were miscible in frozen solutions and presented single Tg's that rose with increases in the protein ratio. Various salts concentration-dependently lowered the single Tg's of the proteins and Ficoll combinations maintaining the mixed amorphous phase. In contrast, some salts induced the separation of the proteins and PVP combinations into protein-rich and PVP-rich phases among ice crystals. Freeze-concentration separates some combinations of proteins and nonionic polymers into different amorphous phases in a frozen solution.
Keywords: Freeze-drying, Phase separation, Formulation

伊豆津健一, 小嶋茂雄:
凍結濃縮による生体高分子の相分離と共存物質の影響
低温生物工学会誌 **46**, 91-94 (2000)

Miscibility of polymers in aqueous frozen solutions was studied through thermal analysis. Freeze-concentration separated some polymer combinations (e.g., PVP and dextran) in single-phase initial solutions into different amorphous phases among ice crystal. Other polymer combinations (e.g., PVP and ovalbumin) were concentrated into mixture amorphous phase. Addition of salts altered the polymer miscibility by covering the electrostatic interactions and hydration state of polymers. Possible mechanisms and effects of the phase separation were discussed.
キーワード：相分離、凍結濃縮、製剤

Yoshioka, S., Aso, Y. and Kojima, S.: **Temperature Dependence of Bimolecular Reactions Associated with Molecular Mobility in Lyophilized Formulations**
Pharm. Res., **17**, 925-929 (2000)

The temperature dependence of acetyl transfer between aspirin and sulfadiazine, a bimolecular reaction, in lyophilized formulations at temperatures near the glass transition temperature (Tg) and NMR relaxation-based critical mobility temperature (Tmc) was studied to further understand the effect of molecular mobility on chemical degradation rates in solid pharmaceutical formulations. The temperature dependence of the hydrolysis rates of aspirin and cephalothin was also studied. The diffusion barrier of water molecules in lyophilized formulations appears to be smaller than the activational barrier of the hydrolysis of aspirin and cephalothin based on the results of this study that the temperature dependence of the hydrolysis rate is almost linear regardless of Tmc and Tg. The diffusion barrier of aspirin and sulfadiazine molecules appears to be comparable to the activational barrier of the acetyl transfer reaction between these compounds, resulting in nonlinear temperature dependence.
Key words: acetyl transfer, lyophilized formulation, molecular mobility.

吉岡澄江、阿曽幸男、小嶋茂雄:**タンパク質凍結乾燥製剤の安定性に及ぼす分子運動性の影響に関する検討**
低温生物工学会誌, **46**, 8-11 (2000)

種々の高分子添加剤を含有するタンパク質凍結乾燥製剤の分子運動性をパルス1H-NMRおよび高分解能13C-NMRを用いて測定し、製剤中のタンパク質の安定性との関連を検討した。温度がプロトンのスピン-スピン緩和から測定した分子運動性の限界温度(Tmc)以上になると高分子の運動性が急激に高まり、それに連動してタンパク質の分子運動性も高まることが明らかになった。Tmcは添加剤の種類および水分含量に大きく依存することが分かった。さらに、温度がTmc以上に上昇すると製剤中のタンパク質分子の凝集が顕著になり、安定性が著しく低下することが明らかになった。
Key words: protein stability, lyophilized formulation, molecular mobility.

坂本知昭, 花尻瑠理, 石橋無味雄, 小嶋茂雄:**日本薬局方の参照吸収スペクトルに関する研究 その2 参照紫外可視吸収スペクトルに関する研究 (I)**
医薬品研究, **31**, 871-882 (2000)

第十四改正日本薬局方(JP14)では, 医薬品各条の紫外可視吸収スペクトルによる確認試験, 参照スペクトルあるいは標準品を測定して得られた吸収スペクトルのパターンを, 試料を測定して得られた吸収スペクトルのパターンとの比較により行う方法が全面的に導入される. 著者らは, JP14に収

載予定の医薬品のうち，250品目（第一部249品目，第二部1品目）について，紫外可視吸収スペクトルを測定し，参照スペクトル法と従来法を比較した．紫外可視吸収スペクトルは，各医薬品に固有のパターンを示すため，参照スペクトル法を用いれば，吸収の極大波長を用いた従来の方法より高い精度で確認試験が行える．しかしながら，本研究において，酷似したスペクトルパターンを示す医薬品が存在することが判明し，ほぼ同一のスペクトルパターンを有する医薬品も存在することが分かった．一般的に，医薬品の確認試験では数種類の試験を行った結果を総合して判定するため，類似した紫外可視吸収スペクトルを与える医薬品が存在しても直ちに重大な問題とはなることはないが，個々の確認試験の精度を向上させるとの観点からは参照スペクトルと試料のスペクトルパターンの比較を行うのみばかりではなく，吸収の極大波長，そして必要に応じて，吸光係数（ε）や局方規定濃度で測定したときの吸光度などを参考情報として付記しておくことが望ましいと思われる．

Keywords: Ultraviolet and visible absorption, The Japanese Pharmacopoeia, Reference spectrum

Saisho, K., Tanaka, E.* and Nakahara, Y. : **Hair Analysis for Pharmaceutical Drugs. I. Effective Extraction and Determination of Phenobarbital, Phenytoin and their major metabolites in Rat and Human hair.**

Biol.Pharm.Bull., **24**, 59-64 (2001)

In order to establish an analytical method for the determination of phenobarbital (PB), phenytoin (PPH) and their hydroxylated metabolites in hair, animal model experiments were performed. Five male dark-agouti pigmented rats, aged 5 weeks, were intraperitoneally and orally administered PB or PPH independently at 25 mg/kg once a day for 5 successive days. The growing back hair was collected 15 days after the first administration. Four typical extraction methods were evaluated using the rat hair samples containing PB or PPH. Methanol-acetone-NH_4OH (10:10:1) was the best extraction method from all aspects. The analytes in the extract were methylated in acetonitrile with 20% tetramethylammonium hydroxide and methyliodide at 70℃ for 10 min. After purification with Bond Elut Certify, the methylated products were analyzed by GC-MS. From rat hair, PB, p-hydroxy PB, PPH and p-hydroxy PPH were detected at average concentrations of 26.9, trace, 4.2 and 0.4 ng/mg with an ip injection, and at 30.9, trace, 4.0 and 0.4 ng/mg with oral administration, respectively. This method was applied to the head hair of two patients who orally took toxic amounts of PB and two volunteers who orally took 100 mg of PPH daily for 5 days. The hair concentrations of PB in the two patients were 16.2 and 14.7 ng/mg, and those of PPH in the two volunteers were 3.3 and 0.1 ng/mg.

Keywords: hair analysis, phenytoin, phenobarbital

*筑波大学社会医学系

Kaddoumi, A.*, Nakashima, M.*, Wada, M.*, Kuroda, N.*, Nakahara, Y., Nakashima, K.* : **HPLC of (±) fenfuramine and phentermine in plasma after derivatization with dansyl chloride.**

J.Liq.Chrom. & Rel. Technol., **24**, 57-67 (2001)

A high-performance liquid chromatographic method is described for the simultaneous determination of the dansyl derivatives of (±)-fenfluramine (Fen) and phentermine (Phen) in addtion to four other sympathomimetic amines, norephedrine(NE), ephedrine (E), 2-phenylethylamine (PEA) and 4-bromo-2,5-dimethoxyphenethylamine (2-CB). The separation was performed on a reversed phase C18 column with fluorescence detection. The dansylation conditions were examined and the optimum derivatization was obtained at pH 9.0 with 4.7 mM dansyl chloride. A preliminary study for the simultaneous determination of the dansyl derivatives in spiked human plasma was showed good linearities from 0.005 to 2 μM with detection limits ranging from 16 to 255 fmol on the column. Extraction recovery of the compounds was greater than 90%. The application of the method was studied in rat plasma following an intraperitoneal administration of Fen and Phen.

Keywords: HPLC, sympathomimetic amines, dansyl chloride

*長崎大薬学部

早川堯夫，内田恵理子，黒澤努*1，白倉良太*2：**トランスジェニック動物由来細胞の品質・安全性確保に関する基礎的研究**

医薬品研究，**31**, 791-817 (2000)

This study is aimed to provide points to consider on the assurance of quality and safety of cellular products derived from transgenic animals for human therapy. Points were described based on the characteristics of the cellular products derived from transgenic animals and on scientific principles of guidelines of biotechnology products for human therapy. The most careful consideration must be given to prevent the transmission of infectious diseases from cellular products derived from transgenic animals to the xenograft recipients, and to minimize potential risks to public health. Consideration required for the production and maintenance, stability and safety of transgenic animals, quality and safety of cellular products, and the system for the surveillance of xenograft recipients, archiving health records and specimens of recipients and donor animals to prevent infections after clinical trials, are also described.

Keywords:Transgenic animal, Cellular therapy, Xenotransplantation

*1 大阪大学医学部付属動物実験施設
*2 大阪大学医学部付属バイオメディカル教育研究センター

早川堯夫，真弓忠範*1，黒澤 努*2，豊島 聰*3，山口照英，川西 徹：**トランスジェニック動物由来医薬品の品質・安全性確保に関する基礎的検討**

医薬品研究，**32**, 223-246 (2001)

Recently the pharmaceuticals, which were produced using transgenic animals, have been developed, and will be submitted for registration in nearly future in Japan as well as in USA and EU. This study has been, therefore, undertaken to establish the technical requirement for registration. The pharmaceuticals should be evaluated from the following standpoints: 1) Transgene construct and its characterization; 2) Creation and characterization of the transgenic founder animal; 3) Establishment of a reliable and continuous source of transgenic founder animals; 4) Generation and selection of the production animals; 5) Maintenance of

transgenic animals; 6) Recovery of products from transgenic animals; 7) Purification of products; 8) Characterization of products; 9) Process evaluation and in-process test; 10) Specification of products, 11) stability of products, 12) Preclinical safety evaluation and clinical evaluation.

Keywords: transgenic animal, quality evaluation, clone animal

*1 大阪大学大学院薬学研究科
*2 大阪大学医学部
*3 星薬科大学薬学部

Kawakami, N.*, Kita, K*., Hayakawa, T., Yamaguchi, T., and Fujimoto, S.*: **Phorbor myristate acetate induces NADPH oxidase activity of cytochalasin B-primed neutrophils through the protein kinase C-independent pathway**

Biol. Pharm. Bull, **23**, 1100-1104 (2000)

We examined the effect of cytochalasin B (CB) or granulocyte colony stimulating factor (GCSF) on superoxide radical (O_2^-) production of neutrophils by phorbor myristate acetate (PMA)-stimulation. It was observed that O_2^- generation of intact and GCSF-treated neutrophils by PMA-stimulation showed a lag during the early stage, and was largely inhibited by 1-(5-isoquinoline-sulfonyl)-3-methyl-piperazine (200 microM) or GF109203X (GFX) (0.2 microM), but not by ethanol (1%) and wortmannin (100 nM). In contrast, O_2^- generation of CB-pretreated neutrophils by PMA-stimulation did not show a lag, but was less than that of intact cells, and was only minimally depressed by the above inhibitors, but was markedly depressed by the simultaneous addition of GFX and ethanol or GFX and wortmannin. Although translocation of p47phox and p67phox to the membrane fraction by PMA-stimulation of intact and GCSF-treated neutrophils occurred in parallel with O_2^- production, that of CB-treated neutrophils by PMA-stimulation was not always proportional to O_2^- production. These findings suggest that pretreatment of neutrophils with CB dramatically alters the PMA response of the cells; that is, the protein kinase C-dependent pathway is largely depressed, and a phospholipase D-dependent one for NADPH oxidase activation appears in CB-treated cells.

Keywords : NADPH oxidase, protein kinase C, neutrophils

*京都薬科大学

Shimohama, S.*1, Tanino, H.*2, Kawakami, N.*2, Okamura, N.*3, Kodama, H.*3, Yamaguchi, T., Hayakawa, T., Nunomura, A.*4, Chiba, S.*4, Perry, G.*5, Smith, M.A.*5, and Fujimoto, S.*2 : **Activation of NADPH oxidase in Alzheimer's disease brains**

Biochem. Biophys. Res. Commun., **273**, 5-9 (2000)

The present study is the first to show that superoxide (O_2^-) forming NADPH oxidase is activated in Alzheimer's disease (AD) brains by demonstrating the marked translocation of the cytosolic factors p47-phox and p67-phox to the membrane. In conjunction with a recent *in vitro* study showing that amyloid beta activates O_2^- forming NADPH oxidase in microglia, where these phox proteins are localized in this study, the present results suggest that, in AD, NADPH oxidase is activated in microglia, resulting in the formation of reactive oxygen species which can be toxic to neighboring neurons in AD.

Keywords : Alzheimer's disease, NADPH oxidase, superoxide

*1 京都大学医学部
*2 京都薬科大学
*3 広島大学医学部
*4 旭川医科大学
*5 Case Western Reserve University

Okabe,J.*, Eguchi, A.*, Msago, A.*, Hakayawa, T., and Nakanishi, M.* : **TRF1 is a critical trans-acting factor required for de novo telomere formation in human cells**

Hum. Mol. Genet., **9**, 2639-2650 (2000)

The duplex telomere repeat (TTAGGG)n is an essential cis-acting element of the mammalian telomere, and an exogenous telomere repeat can induce chromosome breakage and *de novo* telomere formation at the site of a break (telomere seeding). Telomere seeding requires the telomere repeat (TTAGGG)n more stringently than does an *in vitro* telomerase assay, suggesting that it reflects the activity of a critical *trans*-acting element of the functional telomere, in addition to telomerase. Furthermore, telomere seeding is induced at a frequency fluctuating widely among human cell lines, suggesting variation in the activity of this hypothetical factor among cells. In this study, we investigated the cellular factor(s) required for telomere formation using the frequency of telomere seeding as an index and identified TRF1, one of the telomere repeat binding proteins, as an essential trans-acting factor. The exogenous telomere repeat induces telomere formation at a frequency determined by the availability of TRF1, even in telomerase-negative cells. Our study shows clearly that TRF1 has a novel physiological significance distinct from its role as a regulator of telomere length in the endogenous chromosome. The possible role of TRF1 in cell aging and immortalization is discussed.

Keywords: telomere, TRF1, chromosome

*大阪大学微生物病研究所

Kawasaki, N., Ohta, M., Hyuga, S., Hyuga, M. and Hayakawa, T. : **Application of liquid chromatography/mass spectrometry and liquid chromatography with tandem mass spectrometry to the analysis of the site-specific carbohydrate heterogeneity in erythropoietin**

Anal. Biochem., **285**, 82-91 (2000)

High-performance liquid chromatography with electrospray ionization mass spectrometry (LC/MS) and liquid chromatogtaphy with tandem mass spectrometry (LC/MS/MS) were applied to the analysis of the site-specific carbohydrate heterogeneity in erythropoietin (EPO) used as a model of the sialylated glycoprotein. *N*-linked oligosaccharides were released from recombinant human EPO expressed in Chinese hamster ovary cells enzymatically and reduced with $NaBH_4$. Many different sialylated oligosaccharides of EPO were separated and characterized by LC/MS equipped with a graphitized carbon column (GCC). Glycosyaltion sites and the preliminary glycosylation pattern at each glycosylation site were determined by LC/MS of endoproteinase Glu-C digested EPO. The detailed site-specific carbohydrate heterogeneity caused by the differences in the molecular weight, branch, linkage, and sequence were elucidated by GCC-LC/MS of the *N*-linked oligosaccharides released from the isolated gly-

copeptides. Structural details of the isomers were analyzed by LC/MS/MS, and it was indicated that di- and trisialylated tetraantennary oligosaccharides are attached to Asn24, 38, and 83, whereas their isomers, di- and trisialylated triantennary oligosaccharides containing N-acetyllactosamines, are combined with Asn24. Our method is useful for determining of glycosylation sites, the site-specific carbohydrate heterogeneity of glycoproteins, and carbohydrate structure.
Key words: ESI-LC/MS, erythropoietin, carbohydrates

Ohta, M., Kawasaki, N., Hyuga, S., Hyuga, M., and Hayakawa, T. : **Selective glycopeptide mapping of erythropoietin by on-line high-performance liquid chromatography/electrospray ionization mass spectrometry**
J. Chromatogr. A., **910**, 1-11 (2001)

Selective glycopeptide mapping of recombinant human erythropoietin (rhEPO) used as a model glycoprotein was successfully carried out by on-line high-performance liquid chromatography/electrospray ionization mass spectrometry (ESI-LC/MS) using a Vydac C18 column eluted in acetonitrile-1mM ammonium acetate, pH 6.8. RhEPO expressed in a Chinese hamster ovary clone was exhaustively digested into four glycopeptides and nine peptides with endoproteinase Glu-C. Both glycopeptides and peptides were eluted with trifluoroacetic acid as the eluent, whereas only glycopeptides were eluted selectively with ammonium acetate in the following order: *N*38, *N*24, *O*126, and *N*83. Furthermore, many glycoforms included in each glycopeptide were found to be separated by differences in the numbers of sialic acid and *N*-acetyllactosaminyl repeats. Twenty, 16 and 22 different *N*-linked oligosaccharides were determined at Asn24, 38, and 83, respectively, and two different *O*-linked oligosaccharides were observed at Ser126. Our method is simple, rapid, and useful for determining the carbohydrate structures at each glycosylation site and for elucidating the site-specific carbohydrate heterogeneity.
Key words: glycopeptide mapping, ESI-LC/MS, erythropoietin

Oda, Y.*[1], Nakayama, K.*[1], Abdul-Rahman, B.*[2], Kinoshita, M.*[1], Hashimoto, O., Kawasaki, N., Hayakawa, T., Kakehi, K.*[1], Tomiya, N.*[2], and Lee, Y. C.*[2] : **Crocus sativus lectin recognizes (Man)$_3$GlcNAc in the N-glycan core structure**
J. Biol. Chem., **275**, 26772-26779 (2000)

Crocus sativus lectin (CSL) is one of the truly mannose-specific plant lectins that has a unique binding specificity that sets it apart from others. We studied sugar-binding specificity of CSL in detail by a solution phase method (fluorescence polarization) and three solid phase methods (flow injection, surface plasmon resonance, and microtiter plate), using a number of different glycopeptides and oligosaccharides. CSL binds the branched mannotriose structure in the *N*-glycan core. Substitution of the terminal Man in the Manalpha(1-3)Man branch with GlcNAc drastically decreases binding affinity much more than masking of the terminal Man in the Manalpha(1-6)Man branch. Most interestingly, the beta-Man-linked GlcNAc in *N*-glycan core structure contributes greatly to the binding. The effect of this GlcNAc is so strong that it can substantially offset the negative effect of substitution on the nonreducing terminal Man residues. On the other hand, the GlcNAc that is usually attached to Asn in *N*-glycans and the L-Fuc linked at the 6-position of the GlcNAc are irrelevant to the binding. A bisecting GlcNAc neither contributes to nor interferes with the binding. This unique binding specificity of CSL offers many possibilities of its use in analytical and preparative applications.
Keywords : *crocus sativus* lectin, (Man)$_3$GlcNAc
*[1] 近畿大学薬学部
*[2] Department of Biology, Johns Hopkins University

Hashimoto, O., Kawasaki, N., Tsutida, K.*[1], Shimasaki, S.*[2], Hayakawa, T., and Sugino, H*[1] : **Difference between follistatin isoforms in the inhibition of activinsignalling: Activin neutralizing activity of follistatin isoforms is dependent on their affinity for activin**
Cell. Signal., **12**, 565-571 (2000)

We demonstrate the difference between the follistatin isoforms (FS-288 and FS-315), two activin-binding proteins, in the neutralizing activity for activin signalling. Transcriptional reporter assay using 3TP-Lux, an activin-responsive reporter construct, showed that the inhibitory effect of FS-288 on activin-induced transcriptional response is more potent than that of FS-315. The potency was not influenced by the presence of heparan sulfates, by which FS, in particular FS-288, associates with cell surfaces at a high affinity. Furthermore, FS-288 inhibited the binding of activin to its type II receptor more markedly than did FS-315, as evidenced by surface plasmon resonance and affinity cross-linking experiments. Moreover, the Kd of FS-288 and FS-315 for activin A was estimated to be 46.5+/-0.37 pM and 432+/-26 pM, respectively, by surface plasmon resonance experiments. These results indicate that the different potency between the two FS isoforms in the inhibition of activin activities depends on their affinity for activin A.
Keywords: Follistatin, FS-288, FS-315
*[1] 徳島大学分子酵素学研究センター
*[2] School of Medicine, University of California

Kawai, H., Kotake, Y.*, and Ohta, S.* : **Inhibition of Dopamine Receptors by Endogenous Amines: Binding to Striatal Receptors and Pharmacological Effects on Locomotor Activity**
Bioorg. Med. Chem. Lett., **10**, 1669-1671 (2000)

Endogenous amine 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ) derivatives are synthesized, and their activity for dopaminergic systems are evaluated *in vitro* and *in vivo* by receptor binding assay and pharmacological tests. It is proposed that 1BnTIQ derivatives can act as endogenous dopaminergic antagonists.
Keywords: endogenous amine, dopamine receptor, locomotor activity
*広島大学医学部

Kawai, H., Kotake, Y.,*, and Ohta, S.*: **Dopamine transporter and catechol-O-methyltransferase activities are required for the toxicity of 1-(3',4'-Dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline**
Chem. Res. Toxicol., **13**, 1294-1301 (2000)

1-(3',4'-Dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (3',4'DHBnTIQ, 1) is an endogenous parkinsonism-inducing substance. It is taken up into dopaminergic neurons via the dopamine transporter, inhibits mitochondrial respiration and induces parkinsonism in mice. We synthesized four derivatives (aromatized, *N*-methylated, *N*-methyl-aromatized, and *O*-methylated, 2 - 5) and studied the cellular uptake and cytotoxicity of 1 - 5, as well as the metabolism of 1. All except the *O*-methyl derivative (5) were specifically taken up by the dopamine transporter, but 1 was taken up most efficiently. Relative to 1, oxidation reduced V_{max}, *N*-methylation markedly increased *K*m , and *O*-methylation eliminated the uptake activity. Cytotoxicity of 1 - 5 was examined in a mesencephalic cell primary culture. Compound 1 reduced cell viability by nearly 80% at 100 μM, but the other compounds had little or no effect on cell viability. *In vivo* and *in vitro* studies revealed that 1 was *O*-methylated by soluble catechol-*O*-methyltransferase (COMT). Aromatization and *N*-methylation of 1 were not observed. We found that dopamine transporter inhibitors and a COMT inhibitor each blocked the cytotoxicity of 1, indicating that uptake and *O*-methylation are both necessary for neurotoxicity. Thus, we consider that 1 is taken up into dopaminergic neurons via the dopamine transporter and then converted by COMT to 5, which has cytotoxic and parkinsonism-inducing activities.

Keywords: metabolism, dopamine transporter, parkinsonism

*広島大学医学部

Niimi, S., Takizawa, M.*, Sugimura, Y.*, Seki, T.*, Ariga, T.*, Kobayashi, T. and Hayakawa, T. : **Effect of lactacystin on the herbimycin-A-dependent decrease in glucocorticoid receptors in primary cultured rat hepatocytes**

Recent Res. Devel. Steroid Biochem. Mol. Biol., **1**, 53-62 (2000)

We previously showed that herbimycin A causes a decrease in glucocorticoid receptor (GR) level in primary cultured rat hepatocytes and suggested that the decrease was due to degradation of GR protein. In this paper, we examined the nature of the protease involved in the degradation by using a specific protease inhibitor. The proteasome-specific inhibitor lactacystin prevented the herbimycin-A-induced decrease in GR protein and binding capacity. Lactacystin also prevented the decrease in GR level during culture in the absence of herbimycin A. These results suggest that proteasome may be involved in herbimycin-A-dependent and independent decrease in GR protein.

Keywords: glucocorticoid receptor, proteasome, degradation

*日本大学生物資源科学部

Mizuguchi, H., Kay, M. A.*, and Hayakawa, T. : **In vitro ligation-based cloning of foreign DNAs into the E3 as well as E1 deletion region for generation of recombinant adenovirus vector**

BioTechniques, **30**, 1112-1116 (2001)

We develop a simple system that allows for the insertion of foreign genes into the E3 deletion region as well as into the E1 deletion region of adenoviral vector. By using this system, we constructed, as a model, a self-contained Ad vector carrying a tetracycline-regulatable gene expression system. The tet-responsive transcriptional activator gene was inserted into the E3 deletion region, while the luciferase gene with a tetracycline-regulatable promoter was inserted into the E1 deletion region. Tetracycline-regulatable luciferase expression was observed in the cells transduced with such a vector. This single vector system, combined with tetracycline-regulatable gene expression system, should greatly facilitate the application of the adenovirus vector for gene therapy and gene transfer experiment.

Keywords: adenovirus vector, gene therapy

*Stanford University

Mizuguchi, H., Koizumi, N., Hosono, T., Utoguchi, U.*[1], Watanabe, Y.*[1], Kay, M. A.*[2], and Hayakawa, T. : **A simplified system for constructing recombinant adenoviral vectors containing heterologous peptides in the HI loop of their fiber knob**

Gene Ther., **8**, 730-735 (2001)

The use of recombinant adenovirus (Ad) vectors containing genetically modified capsid proteins is an attractive strategy for achieving targeted gene transfer. The HI loop of the fiber knob is a promising candidate location for the incorporation of foreign ligands for achieving this goal. However, the method of constructing an Ad vector containing a foreign ligand in the HI loop of the fiber knob has proven difficult. In this study, we developed a simple system to construct fiber-modified vectors. To do this, a vector plasmid containing a complete E1/E3-deleted Ad type 5 genome and a unique *Csp*45I and/or *Cla*I site between positions 32679 and 32680 of the Ad genome (residues threonine-546 and proline-547 of the fiber protein) was constructed. Oligonucleotides corresponding to the Arg-Gly-Asp (RGD) or Asn-Gly-Arg (NGR)-containing peptide motif (as a model) and containing a *Csp*45I and/or *Cla*I recognition site, were ligated into the *Csp*45I and/or *Cla*I digested plasmid. The simplicity of this method allows not only for easy construction of fiber-mutant Ad vectors, but also for screening of the peptides that target the vector to the desired cells and tissues.

Keywords: adenovirus vector, gene therapy, targeting

*1 昭和薬科大学
*2 Stanford University

Okada, N.*[1], Tsukada, Y.*[2], Nakagawa, S.*[2], Mizuguchi, H., Mori, K.*[1], Saito, T.*[1], Fujita, T.*[1], Yamamoto, A.*[1], Hayakawa, T., and Mayumi, T.*[2] : **Efficient gene delivery into dendritic cells by fiber-mutant adenovirus vectors**

Biochem. Biophys. Res. Commun., **282**, 173-179 (2001)

Recent studies have demonstrated the usefulness of dendritic cells (DCs) genetically modified by adenovirus vectors (Ad) to immunotherapy, while sufficient gene transduction into DCs is required for high doses of Ad. The RT-PCR analysis revealed that the relative resistance of DCs to Ad-mediated gene transfer is due to the absence of Coxsackie-adenovirus receptor expression, and that DCs expressed adequate alpha(v)-integrins. Therefore, we investigated whether fiber-mutant Ad containing the Arg-Gly-Asp (RGD) sequence in the fiber knob can efficiently transduce and express high levels of the LacZ gene into DCs. The gene delivery by fiber-mutant Ad was more efficient than that by conventional

Ad in both murine DC lines and normal human DCs (NHDC). Furthermore, NHDC transduced with fiber-mutant Ad and conventional Ad at 8000-vector particles/cell resulted in a 70-fold difference in beta-galactosidase activity. We propose that alpha(v)-integrin-targeted Ad is a very powerful tool with which to implement DC-based vaccination strategies.

Keywords: adenovirus vector, gene therapy, dendritic cells

*1 京都薬科大学
*2 大阪大学大学院薬学研究科

Nakanishi, M.*1, Akuta, T.*1, Nagoshi, E.*1, Eguchi, A.*1, Mizuguchi, H., and Senda, T.*2: **Nuclear Targeting of DNA**
Eur. J. Pharamaceu. Sci., **13**, 17-24 (2001)

The nuclear membrane is a tight barrier for cytoplasmic proteins, but nuclear proteins have the intrinsic ability to overcome this barrier by an active signal-mediated process. Specific cytoplasmic carrier proteins have the responsibility to escort these proteins into the nucleus through the nuclear pore. The nuclear membrane is also a tight barrier for exogenous DNA delivered by synthetic vehicles, while many of the karyophilic viruses have a mechanism to actively deliver their genome through the nuclear pore. Virus DNA and RNA cannot move into the nucleus by themselves and require the viral structural proteins for efficient nuclear transport. In this article, we review the recent progress in understanding the mechanism of the nuclear transport of proteins and the virus genome, and discuss the possibility of developing synthetic gene-delivery systems based on these outcomes.

Keywords: adenovirus vector, gene therapy, dendritic cells

*1 大阪大学微生物病研究所
*2 名古屋大学医学部

Nagayama, Y.*1, Nishihara, E.*1, Namba, H.*1, Yokoi, H.*1, Hasegawa, M.*2, Mizuguchi, H., Hayakawa, T., Hamada, H.*3, Yamashita, S.*1, and Niwa, M.*1: **Targeting the replication of adenovirus to p53-defective thyroid carcinoma with a p53-regulated cre/loxP system**
Cancer Gene Ther., **8**, 36-44 (2001)

In this article, we evaluated the feasibility of the restricted replication-competent adenoviruses for treatment of anaplastic thyroid carcinomas (ATCs), which are very aggressive and difficult to treat. Because ATCs very often harbor p53 mutations, we used wt-p53 as a regulatory factor to restrict virus replication and cytopathic effect to p53-mutated cells. The recently reported "gene inactivation strategy" using p53-regulated Cre/loxP system was employed; this system consists of two recombinant adenoviruses. One has an expression unit of the synthetic p53 - responsive promoter and the Cre recombinase gene (Axyp53RECre), and another contains two expression units; the first consists of E1A gene flanked by a pair of loxP sites downstream of the constitutive CAG promoter and the second E1B19K gene under the control of the CMV promoter (AdCALE1AL). In vitro data demonstrate that although infection of AdCALE1AL alone led to E1A expression, viral replication and cytolysis in all the thyroid cells examined irrespective of their p53 status, the double infection did so in FRO cells (p53-null ATC) but not in FRO cells stably expressing wt-p53 and normal thyroid cells with wt-p53. These data indicate that our double infection method may have a potential for treatment of ATC and probably also other p53-defective cancer cells.

Keywords: adenovirus vector, gene therapy, p53

*1 長崎大学医学部
*2 ディナベック研究所
*3 札幌医科大学

Imazu, S.*1, Nakagawa, S.*1, Nakanishi, T.*1, Mizuguchi, H., Uemura, H.*2, Yamada, O.*2, and Mayumi, T.*1: **A novel nonviral vector based on vesicular stomatitis virus**
J. Control. Release, **68**, 187-194 (2000)

Here we report a simple and efficient method for nonviral gene transfer using liposomes which have envelope protein of vesicular stomatitis virus (VSV) on their surface (VSV-liposomes). We prepared VSV-liposome by fusing simple liposomes with VSV particles. To evaluate whether these particles can efficiently introduce their internal contents into the cytoplasm of mammalian cells, we examined the delivery of fragment A of diphtheria toxin (DTA) by VSV-liposomes into the cytoplasm of FL cells. We found that VSV-liposomes encapsulating DTA were highly cytotoxic to the cells, while empty VSV-liposomes and plain liposomes encapsulating DTA were not, suggesting that VSV-liposomes delivered DTA into cytoplasm. Consistent with this, the cells cultured with plasmid DNA entrapped in VSV-liposomes and coding for firefly luciferase showed significant luciferase expression, whereas cells culture with plasmid DNA in plain liposomes and plasmid DNA-cationic liposomes complex did not. Thus, VSV-liposomes function as a simple and efficient nonviral vector for the delivery of DNA.

Keywords: vesicular stomatitis virus, gene therapy

*1 大阪大学大学院薬学研究科
*2 扶桑薬品

Kondoh, M.*1, Matsuyama, T.*1, Suzuki, R.*1, Mizuguchi, H., Nakanishi, T.*1, Nakagawa, S.*1, Tsutsumi, Y.*1, Nakanishi, M.*2, Sato, M.*3, and Mayumi, T.*1: **Growth inhibition of human leukemia HL-60 cells by antisense phosphodiester oligonucleotide encapsulated into fusogenic liposome**
Biol. Pharm. Bull., **23**, 1011-1013 (2000)

We report here the antisense effect of phosphodiester oligodeoxynucleotide (D-ODN) using fusogenic liposomes (FL) as its carrier. Using antisense (AS) D-ODN 15-mer complementary to the c-myc proto-oncogene mRNA, including the translation initiation codon site, we analyzed the growth of HL-60 cells by [^{3}H]-thymidine uptake. AS-ODNs encapsulated in FL inhibited the growth by about 70% that of the control HL-60 cells at 2.48 microM. In contrast, sense and scramble D-ODNs encapsulated in FL showed no effect of the growth of HL-60 cells at the same concentration. Even at 50 microM, free form D-ODNs did not show any effect. These results suggest that FL is potentially a useful delivery vehicle for oligonucleotide-based therapeutics, and that D-ODN may be a likely candidate for oligodeoxynucleotides when an efficient delivery system is used.

Keywords: vesicular stomatitis virus, gene therapy

*1 大阪大学大学院薬学研究科
*2 大阪大学微生物病研究所

*3 徳島文理大学薬学部

Eguchi, A.*1, Kondoh, T.*2, Kosaka, H.*3, Suzuki, T.*3, Momota, H.*4, Masago, A. *1, Yoshida, T. *5, Taira, H. *6, Ishii-Watabe, A., Okabe, J. *1, Hu, J. *1, Miura, N. *7, Ueda, S. *1, Suzuki, Y. *3, Taki, T*4., Hayakawa, T., and Nakanishi, M. *1: **Identification and characterization of cell lines with a defect in a post-adsorption stage of Sendai virus-mediated membrane fusion**

J. Biol. Chem. **275**, 17549-17555 (2000)

In the early stage of infection, Sendai virus delivers its genome into the cytoplasm by fusing the viral envelope with the cell membrane. Although the adsorption of virus particles to cell surface receptors has been characterized in detail, the ensuing complex process that leads to the fusion between the lipid bilayers remains mostly obscure. In the present study, we identified and characterized cell lines with a defect in the Sendai virus-mediated membrane fusion, using fusion-mediated delivery of fragment A of diphtheria toxin as an index. These cells, persistently infected with the temperature-sensitive variant Sendai virus, had primary viral receptors indistinguishable in number and affinity from those of parental susceptible cells. However, they proved to be thoroughly defective in the Sendai virus-mediated membrane fusion. We also found that viral HN protein expressed in the defective cells was responsible for the interference with membrane fusion. These results suggested the presence of a previously uncharacterized, HN-dependent intermediate stage in the Sendai virus-mediated membrane fusion.

Keywords: Sendai virus, fusion, HN protein

*1 大阪大学微生物病研究所
*2 University College London
*3 静岡大学薬学部
*4 大塚製薬
*5 広島大学医学部
*6 岩手大学農学部
*7 浜松医科大学

Kawahara, N., Nozawa M., Flores, D., Bonilla P.,*1 Sekita, S. Motoyoshi Satake : **Sesterterpenoid from *Gentianella alborosea***

Phytochemistry, **53** (8) 881-884 (2000).

Gentianella alborosea and *G. nitida* (Gentianaceae), commonly known as "Hercampuri" or "Hircampure" are biennial medicinal plants growing in the Andes Region. The aqueous extracts of the whole plants have been used in traditional Peruvian folk medicine as a remedy for hepatitis, as a cholagogue and in treatment of. During our on going research on the plants above mentioned, we have isolated a novel sesterterpenoid, named alborosin, from the $CHCl_3$ extract of *G. alborosea*. The paper describes the structure elucidation of the above compound, occurring in the plant together with xanthones and phenolic compounds.

Keywords : Hercampuri, *Gentianella alborosea*, alborosin

*1Fucultad de Farmacia, Universidad Nacional Mayor de San Marcos

Kuroyanagi, M.,*1 Kazumasa Sugiyama,*2 Kanazawa M.,*3 Kawahara N. : **Novel A-*Seco*-Rearranged Lanostane Triterpenoids from *Abies sachalinensis***

Chem. Pharm. Bull., **48** (12), 1917-1920 (2000).

From the needles of *Abies sachalinensis*, novel rearranged lanostane type triterpenes, 1-4, were isolated along with a known triterpene (5). The structures of the new compounds, 1-4, were elucidated to be 3,4-*seco*-8-(14→13*R*)*abeo*-17,13-*friedo*-9 β -lanosta-4(28),7,14(30),22*Z*,24-pentaen-26,23-olide-3-oic acid, methyl 3,4-*seco*-8-(14→13*R*)*abeo*-17,13-*friedo*-9β-lanosta-4(28),7,14(30),22*Z*,24-penten-26,23-olide-3-oate, 3,4-*seco*-8(14 13R)*abeo*-17,13-*friedo*-9 β -lanosta-4(28),7,14,22*Z*,24-pentaene-26,23-olide-3-oic acid and methyl 3,4-*seco*-8(14→13*R*)*abeo*-17,13-*friedo*-9β -lanosta-4(28),7,14,22*Z*,24-pentaene-26,23-olide-3-oate, respectively, by means of spectral experiments, especially two dimensional NMR spectroscopy, such as ^{1}H-detected multiple quantum coherence (HMQC), ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) and ^{1}H-^{1}H-correlation spectroscopy (COSY) experiments. These new compounds have novel structures containing A-*seco*, rearranged spiro structure and a γ -lactone conjugated with a diene. Some of these compounds showed potent antibacterial activity against gram positive bacteria.

Keywords: *Abies sachalinensis*, Pinaceae, rearranged lanostane

*1 広島県立大学生物資源学部
*2 静岡県立大学薬学部
*3 拓豊産業

Yun Y. S., Sugimoto, N., Sekita S., Maitani T., Yamada T., Satake M.: **Two New Alkaloids from Cigarette Smoke Condensate**

Chem. Pharm. Bull., **48**, 1990-1991 (2000).

The structural elucidation of minor constituents in mainstream smoke of cigarette was carried out. After mainstream smoke was collected on the grass filters by inhalation apparatus "Hamburg II", the condensate was extracted by MeOH. Seven known alkaloids and two new alkaloids named Cigatin A (**1**) and B (**2**) were isolated. Their structures were determined, on the basis of spectral data and chemical method

Keywords : Cigarette, Cigatin A, Cigatin B

Tsuchiya, T.: **A useful marker for evaluating tissue-engineered products: Gap-junctional communication for assessment of the tumor-promoting action and disruption of cell differentiation in tissue-engineered products**

J. Biomater. Sci. Polymer Edn., **11 (9)**, 947-959 (2000)

An in vitro system for evaluating the safety of tissue-engineered products is a convenient because of its rapidity and low cost. On the basis of recent studies, intercellular channels called gap-junctions are considered to play an important role on the tumor-promotion stage during the tumorigenesis induced by polyurethanes. Further, we also demonstrate the significance of the intercellular communication during neuronal cell differentiation. From these results, we propose a survey of the function of the gap-junctional communication as a probable useful marker for evaluating the safety of tissue-engineered products.

Keywords: tissue-engineered products, cell differentiation, tumor-promotion

角出泰造, 土屋利江:**代謝協同試験によるソフトコンタクトレンズ用化学消毒剤の評価**
生体材料, **19**, 93-97 (2001)

For the evaluation of long-term safety of contact lens care solution used repeatedly for human eyes, inhibitory effects of chemical disinfectants on the gap-junctional intercellular communication (GJIC) were studied with the metabolic cooperation (MC) assay. Three types of contact lens care solutions were investigated using a colony formation test and MC assay, its ingredients, poloxamine and polyhexamethylenebiguanide (PHMB), were investigated as well. Poloxamine showed an inhibitory effect on the GJIC, whereas PHMB had no effect. The MC assay was concluded to be suitable for evaluating the inhibitory activity of GJIC at non-cytotoxic concentrations. To support this MC assay, further studies are needed to evaluate the long-term safety of contact lens care solutions.

Keywords: metabolic cooperation assay, colony formation test, polyhexamethylenebiguanide

Kondo, S.*, Haishima, Y., Ishida, K.*, Isshiki, Y.*, Hisatsune, K.* : **The O-polysaccharide of lipopolysaccharide isolated from Vibrio fluvialis O19 is identical to that of Vibrio bioserogroup 1875 Variant**
Microbiol. Immunol., **44**, 941-944 (2000)

Vibrio bioserogroup 1875 Variant株と同一の抗原性 (01コレラ菌イナバ因子C, 1875株共通抗原因子DおよびE) を持つ新規分離株 **Vibrio fluvialis** O19 (Kobe 株) のLPS O-抗原多糖部分のエピトープ構造解析を行った. その結果, Kobe株のO-抗原特異鎖はN-3-hydroxypropinyl-D-perosamine (4-amino-4,6-dideoxy-D-mannnopyoranose) のα(1→2)結合ホモポリマーにより構成されており, 1875 Variant株LPS O-抗原特異鎖と同一の構造を持つことが明らかになった.

Keywords: LPS, Vibrio fluvialis, Vibrio bioserogroup 1875
*城西大学

Haishima, Y., Hayashi, Y., Yagami, T., Nakamura, A.: **Elution of bisphenol-A from hemodialyzers cosisting of polycarbonate and polysulfone resins**
J. Biomed. Mater. Res. (Appl. Biomater.), **58**, 209-215 (2001)

ポリカーボネートおよびポリスルホン製血液透析器からのビスフェノールA (BPA) の溶出状況を検討するため, 原材料ペレット中のBPA総含量試験, ウシ血清を含めた各種溶媒を使用したハウジングケースおよび血液透析器からの溶出試験を行った. BPAの溶出は全ての回収実験において認められたが, その溶出量は最大でも2090 ng/modul (ウシ血清使用時) であり, 溶出したBPAによる人体への影響は殆どないものと判断された. また, 血液透析器の実際の使用条件に最も近似した回収条件と思われるウシ血清循環と同等のBPA溶出を与える簡易疑似溶媒組成を検討した結果, 17.2% エタノールが使用できることが明らかになった.

Keywords : bisphenol-A, hemodialyzer, endocrine disruptor

Haishima, Y., Murai, T., Nakagawa, Y., Hirata, M.*, Yagami, T., Nakamura, A.: **Chemical and biological evaluation of endotoxin contamination on natural rubber latex products**
J. Biomed. Mater. Res., **55**, 424-432 (2001)

ラテックス製品による発熱とエンドトキシン (LPS) 汚染の相関性について検討した. ウサギ発熱活性を指標として市販ラテックス製品のサーベイ試験を行った結果, 数種の製品抽出液が発熱陽性を示した. これらの各抽出液の化学的性状 (エンドトキシン常成分のGC/MS分析) および各種生物学的性状 (菌体成分分析, 発熱動態, ヒト由来単球様細胞株に対する各種サイトカイン産生誘導能, LPS 阻害剤による同誘導能の抑制試験, LPS除去試験) に関して検討した結果, これらのラテックス製品には相当量のLPSが混入しており, 同製品による発熱は混在するLPSに由来していることが明らかになった.

Keywords: pyrogenicity, endotoxin, latex
*岩手医科大

Yagami, T., Kitagawa, K.*[1], Aida, C.*[1], Fujiwara, H.*[1], Futaki, S.*[2]: **Stabilization of a tyrosine *O*-sulfate residue by a cationic functional group: Formation of a conjugate acid-base pair**
J. Peptide Res., **56**, 239-249 (2000)

硫酸化チロシン残基は, 蛋白質間の相互作用に重要な役割を果たすと考えられている. 本研究では, 硫酸化チロシン残基と塩基性官能基との相互作用をMSとHPLCで調べた. 硫酸化チロシン残基が塩基性残基とイオンペアを形成している場合, 気相及び液相中で脱硫酸化反応を起こしにくくなることから, 脱硫酸化の程度を基に相互作用の強さを推測することができる. まず, ペプチド中の塩基性残基数が増すにつれ, 脱硫酸化反応が起こりにくくなることが確かめられた. アルギニン残基はリジン残基よりも強い安定化能を示した. また, 複数のアルギニン残基と硫酸化チロシン残基との分子間相互作用が, MALDI-TOFMSスペクトルから確認された. 以上の結果は, 特にアルギニン残基とイオンペアを形成することによる硫酸化チロシンの安定化が, 蛋白質のフォールディングや蛋白質間の相互作用を推進する力の一つとなっていることを示唆するものであった.

Keywords: tyrosine *O*-sulfate, acid-base pair, protein interaction
*[1] 新潟薬科大学
*[2] 京都大学化学研究所

Yagami, T., Haishima, Y., Nakamura, A., Osuna, H.*, Ikezawa, Z.*: **Digestibility of allergens extracted from natural rubber latex and vegetable foods**
J. Allergy Clin. Immunol., **106**, 752-762 (2000)

天然ゴムラテックスや植物性食品に由来する交叉反応性アレルゲンの幾つかは, 植物の生体防御に関わる蛋白質であることが既に明らかにされている. 一方, 遺伝子組み換え法など用いてこういった抵抗性蛋白質を人為的に発現させる試みが多くなされており, 農作物中のアレルゲン量が増加する可能性が懸念される. 本研究では, 発現させた蛋白質が食物アレルゲンとなるかどうかを評価する手法の一つとして用いられている消化性試験により, 天然ゴムラテックスや植物性食品に含まれるアレルゲン蛋白を実際に検出できるかどうかを調べた. その結果, ラテックスアレルギーあるいは口腔アレルギー症候群 (OAS) と診断された患者のIgE 抗体が認識する蛋白質抗原は, 必ずしも消化耐性を示さないことがわかった. この実験結果を基に, 消化試験は経口感作を成立させるような食物アレルゲンの検出法としては有効であ

るものの，感作抗原との交叉反応性に基づき，主に口腔症状を誘発するような食物アレルゲンの検出法としては有効ではないと結論した．

Keywords: allergen, cross-reactivity, digestibility

*横浜市立大学医学部

Kitagawa,K.*[1], Aida,C.*[1], Fujiwara,H.*[1], Yagami,T., Futaki, S.*[2], Kogire,M.*[3], Ida, J.*[3], Inoue, K.*[3]: **Facile solid-phase synthesis of sulfated tyrosine-containing peptides: Total synthesis of human big gastrin-II and cholecystokinin (CCK)-39**

J. Org. Chem., **66**, 1-10 (2001)

硫酸化チロシンが酸性溶液中で非常に不安定であることから，この修飾残基を含むペプチドの化学合成は極めて困難であると考えられてきた．そこで本研究は，硫酸化チロシン含有ペプチドの一般的な化学合成法を確立することを目的に行われた．まず，アリール硫酸エステルの酸性溶液中における脱硫酸化反応のメカニズムを基に，硫酸化チロシン残基が分解しないような酸脱保護反応の条件を詳細に検討した．その結果，イオン化能の高いトリフルオロ酢酸中で低温という条件では，脱硫酸化反応の速度が極めて小さいことを見いだした．そして，酸に鋭敏なクロロトリチル樹脂を用いる固相ペプチド合成法にこの酸脱保護条件を組合せ，硫酸化ペプチドの一般的で容易な合成法を確立し，ビッグガストリンーIIやCCK-39の化学合成に応用した．

Keywords: tyrosine *O*-sulfate, peptide synthesis, deprotection

*[1] 新潟薬科大学
*[2] 京都大学化学研究所
*[3] 京都大学医学部

Futaki, S.*, Suzuki, T.*, Ohashi, W.*, Yagami, T., Tanaka, S.*, Ueda, K.*, Sugiura, Y.*: **Arginine-rich peptides: An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery**

J. Biol. Chem., **276**, 5836-5840 (2001)

HIV-1のTat蛋白に由来する塩基性ペプチド（48-60）には，細胞膜を通過し核に蓄積する傾向があることが知られている．この研究では，多数のアルギニン残基を持つ様々なペプチドがTat(48-60)と同様な細胞膜透過作用を示すことを，マクロファージRAW264.7細胞の蛍光顕微鏡観察により見いだした．作用が認められたペプチドの例として，各種ウイルス蛋白のRNA結合ドメインや，ロイシンジッパー蛋白のDNA結合セグメント等が挙げられる．アルギニンを多く含むことを除き，これらのペプチドの1次構造や2次構造に共通性は認められなかった．また，細胞膜透過性は4℃においても認められた．さらに，アルギニンを8残基ほど含むペプチドが最も効率的に細胞膜を通過することが，(Arg)n（n=4～16）という合成ペプチドを用いた実験で明らかになった．一連の結果は，アルギニンを多く含むペプチドに，従来のエンドサイトーシス機構とは異なった共通の細胞膜透過機構がある可能性を示唆するものであった．

Keywords: internalization, protein delivery, translocation

*京都大学化学研究所

中島晴信*[1], 松永一朗*[1], 宮野直子*[1], 宮内留美*[2], 羅川日出男*[2], 増田ゆり*[2], 伊佐間和郎, 五十嵐良明, 鹿庭正昭：**抗菌防臭加工剤の安全性評価に関する研究ー大阪府における抗菌製品の市場実態調査(1991年度から1999年度)ー**

大阪府立公衛研所報，38, 21-32 (2000)

抗菌防臭加工剤の安全性評価の一環として，大阪府における抗菌製品の市場実態調査を1991年度から1999年度にかけて行った結果，抗菌製品において抗菌剤名が具体的に表示されたものが少なく，消費者が抗菌剤に関する情報をほとんど得ることができない実態が明らかにされた．

*[1] 大阪府立公衆衛生研究所
*[2] 大阪府健康福祉部環境衛生課

Huh, W.-K.*[1], Masuji, Y.*[2], Tada, J.*[1], Arata, J.*[1], Kaniwa, M.: **Allergic contact dermatitis from a pyridine derivative in polyvinyl chloride leather**

Am. J. Contact Dermatitis, **12(1)**, 35-37 (2001)

椅子の表地として使用されたポリ塩化ビニル製合成皮革（ビニルレザー）によって大腿部にアレルギー性接触皮膚炎を生じた事例において，ビニルレザーの抗菌処理に使用されたピリジン系抗菌剤の2,3,5,6-tetrachloro-4-(methylsulfonyl) pyridineが原因化学物質であったことを明らかにした．

Keywords: household product, allergic contact dermatitis, antimicrobial agent

*[1] 岡山大学医学部皮膚科
*[2] 岡山協立病院皮膚科

五十嵐良明，鹿庭正昭，中村晃忠：**椅子張り地に用いられる人工皮革中の抗菌剤10,10'-oxybis-10*H*-phenoxarsineの分析**

YAKUGAKU ZASSHI, **120**, 795-799 (2000)

最近，抗菌剤10,10'-oxybis-10*H*-phenoxarsine(OBPA)を使用したポリ塩化ビニル(PVC)製の椅子用張り地によってアレルギー性接触皮膚炎を起こした症例が報告されたことから，原因物質を特定するため人工皮革中のOBPAの定量法を開発した．OBPAはメタノールで抽出し，酸化アルミニウムカラムにかけ，ジエチルエーテル：ヘキサンで洗浄後，エタノール：ヘキサンで溶出した．溶出物はメタノールに溶解後，ODSカラムとUV検出器（波長300nm）をつけたHPLCに注入した．OBPAの検量線は0.1～100μg/mlの範囲で直線性が得られ，検出限界及び定量限界はそれぞれ0.07及び0.25μg/gであった．本方法を用いて椅子張り地に用いられる8種のPVCシートを分析した結果，2種からそれぞれ52.7, 84.9μg/gのOBPAを検出したが，皮膚炎を起こしたPVC製品からOBPAは検出されなかった．

Keywords : antimicrobial, poly(vinyl chloride), HPLC

Ikarashi, Y., Kaniwa, M.: **Determination of *p*-phenylenediamine and related antioxidants in rubber boots by high performance liquid chromatography. Development of an analytical method for *N*-(1-methylheptyl)-*N'*-phenyl-*p*-phenylenediamine**

J. Health Sci., **46**, 467-473 (2000)

We developed a method for the determination of PPD derivatives, such as *N*-(1-methylheptyl)-*N'*-phenyl-*p*-phenylenediamine (MHPPD), *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine (IPPD) and *N*-1,3-dimethylbutyl-*N'*-phenyl-*p*-phenylenediamine (DMBPPD). The PPD derivatives were extracted from rubber boots with acetone:chloroform (1:1). The extract was loaded then on to a silica-gel column. MHPPD and DMBPPD were eluted in

the diethylether:hexane (10:90) fraction. IPPD was detected in the diethylether:hexane (20:80) fraction. Each fraction was subjected to HPLC with an ODS column and a UV detector (detection wavelength 290 nm). The mobile phase was methanol:water (85:15). MHPPD was not found in any of the rubber boots, but DMBPPD and IPPD were detected.
Keywords: *p*-phenylenediamine, antioxidant, HPLC

Ikarashi, Y., Tsuchiya, T., Kaniwa, M., Nakamura, A.: **Activation of osteoblast-like MC3T3-E1 cell responses by poly(lactide)**
Biol. Pharm. Bull., **23**, 1470-1476 (2000)

This study examined the osteoblast-like MC3T3-E1 cell responses to poly(DL-lactide) (PDLLA) and poly(L-lactide)(PLLA) with different weight average molecular weight (Mw). The protein, DNA and hydroxyproline (HYP) content and alkaline phosphatase (ALP) activity for cells cultured on the PLLA (Mw. 270000 or 1370000) were almost similar to those on glass. The ALP activity of the cells cultured on low Mw PLLA (Mw. 20000) increased. The addition of low Mw PDLLA (Mw. 5000 or 10000), L-lactide or L-lactic acid into culture increased the protein, DNA and HYP content and ALP activity for cells. The release of L-lactic acid from PLLA and PDLLA into aqueous solution during incubation was little. These results suggested that increased osteoblast differentiation was induced by low Mw PDLLA and PLLA, and these may be used as a effective material in the field of orthopedic and drug delivery systems for the treatment of bone diseases.
Keywords: poly(DL-lactide), poly(L-lactide), osteoblast

Takayama, M.*, Hayashi, Y.: **Prediction of measurement precision based on FUMI theory for quantitative mass spectrometry with electron ionization**
J. Mass Spectrom. Soc. Jpn., **48**, 248-253 (2000)

The precision or relative standard deviation (RSD) of measurements in a sector type mass spectrometer equipped with an electron ionization system is examined experimentally and theoretically. The observed RSD is obtained by the usual replication and the theoretical RSD is predicted from the probabilistic properties of the baseline noise by the uncertainty theory called the FUMI theory (Function of Mutual Information). The peak corresponding to the limit of detection (RSD = 33%) is demonstrated.
Keywords : precision, mass spectrometry, FUMI theory
*東邦大学

Shintani, H : **Pretreatment and chromatographic analysis of phthalate esters and their biochemical behavior in blood products**
Chromatogr., **52**, 721-726 (2000)

医療用具に用いられているポリ塩化ビニルの可塑剤であるフタル酸エステルの内分泌撹乱作用が問題になっている．本論文では血液バッグからのフタル酸ジエステル，フタル酸モノエステルならびにフタル酸が血液中の酵素によって生成する．これらの成分をHPLC法で分離，分析した．またそれらの成分の選択的な固相抽出前処理法を開発した．
Keywords : phthalate esters, blood products, solid phase extraction

Shintani, H : **Determination of the endocrine disrupter bisphenol-A in the blood of uremia patients treated by dialysis**
Chromatogr., **53**, 331-333 (2001)

透析患者に使用されている人工透析器にはポリサルフォン，ポリカーボネート，ポリスチレンなどが使用されている．これらは内分泌撹乱作用を有すると言われているBisphenol-Aならびにスチレンオリゴマーなどを含んでいる．本論文では人工透析器からのBisphenol-A，スチレンオリゴマーならびにシクロスチレンオリゴマーの放射線滅菌ならびに高圧蒸気滅菌での生成とその安全性について議論した．
Keywords : bisphenol-A, endocrine disrupter, artificial dialyzer

Shintani, H, Akers J.E.*: **On the cause of performance variation of biological indicator used for sterility assurance**
PDA J. Pharm. Sci. Technol., **54**, 332-342 (2000).

生物指標メーカーならびに同一メーカーでロットが異なることに拠る抵抗値（D値）の差の原因について調べた．
Keywords : biological indicator, D value, variation
*Akers Kennedy & Associations

Sasaki, K.*[1], Shintani, H., Itoh, J.*[2]: **Effect of calcium in assay medium on D value of *Bacillus stearothermophilus* ATCC 7953 spores**
Appl. Environm. Microbiol., **66**, 5509-5513 (2000)

SCD培地のロット間ならびに／あるいはメーカー間の差により滅菌保証を達成するためのD値が異なることが報告されている．違いの現象については既に報告があるが，培地組成のどの成分が生物指標（BI）のD値の差に起因しているのか明らかにされていない．著者らはSCD液体培地（SCDB）ならびにSCD寒天固形培地（SCDA）の組成を個々に検討した．その結果D値の差を生じさせるのはSCD培地組成中のカルシウム（Ca）イオン量であることを同定した．SCDAでのD値はSCDBより顕著に高く，それはSCD培地中のCa量の差によることを究明した．
Keywords: sterility assurance, calcium ion, culture medium
*1 エーザイ（株）美里工場
*2 ベクトンデケンスン（株）福島工場

Kim, J.-G.*[1], Lee, Y.-W.*[2], Kim, P.-G.*[3], Roh, W.-S.*[4], Shintani, H.: **Reduction of aflatoxins by Korean soybean paste and its effect on cytotoxicity and reproductive toxicity-Part 1. Inhibition of growth and aflatoxin production of Aspergillus parasiticus by Korean soybean paste (Doen-jang) and identification of the active component**
J. Food Production, **63**, 1295-1298 (2000)

The inhibitory effect of methanol extract of Korean soybean paste on the mold growth and aflatoxin production of a toxigenic strain of *Aspergillus parasiticus* ATCC 15517 was studied using different concentrations of the extract in yeast-extract sucrose broth.
Keywords: soybean paste, aflatoxin, *Aspergillus parasiticus*
*1 Department of Public Health. Keimyung University. Taegu
*2 Graduate School of Public Health, Seoul National University
*3 Department of Environmental Health, Yongin University
*4 Korea Health Industry Development Institute

安藤正典:**インド・バングラディシュにおける地下水ヒ素汚染と健康影響**
公衆衛生研究, **49**(3), 266-274 (2000)
平成4年に水道水質基準の改正が行われたが, ヒ素は地下水の利用や温泉排水などの混入によって水質基準を超える可能性の高い原水が多く存在していることが明らかになってきた. 更に最近, US. EPAではヒ素のリスクアセスメントにおける暴露評価の結果から高いリスクがあることが明らかで基準値の見直しを検討中である. 著者は国際協力事業の一つとして, インドやバングラディシュの西ベンガル周辺地域における地下水ヒ素汚染に伴う健康影響の改善のためヒ素の自然環境での挙動, 健康影響及び暴露量を考察すると共に, ヒ素の地下水汚染の現状と課題の概要についてインド国西ベンガル州の状況について述べた.
Keywords : arsenic, India, contamination of ground water

安藤正典, 徳永裕司:**化粧品の将来に向けての産官学の共同研究の展望**
フレグランスジャーナル, **29**(1), 37-40 (2001)
化粧品の規制緩和が2001年4月を目指して, 秒読み体制に入り, 化粧品の承認許可制度に大きな変革の波が押し寄せている. 従来は, 化粧品の新規原料に関して, その安全性の面から, 化粧品の使用の承認・許可を行ってきたが, 化粧品の規制緩和以降は, 製造, 品質確保, 安全性確保などに関し企業の自己責任を前提に, 消費者への化粧品の安全性と有効性の情報の提供を充実させることが大きな狙いいとなっている. 本編では21世紀を展望した化粧品の在り方あるいは化粧品に関する産官学の共同研究の在り方を展望する.
Keywords : cosmetics, efficacy,effectiveness

安藤正典, 五十嵐良明, 鎌田栄一:**ホルムアルデヒドの暴露評価と健康影響**
アレルギーの臨床, **21**(2), 113-118 (2001)
生活環境には数え切れないほどの化学物質や微生物その他の生物が存在し, その発生源も極めて多様な挙動を示している. これらの化学物質や生物は, 食品, 水などを介して経口的に, また居住空間や労働環境の空気あるいは大気を通して経気道的に, 更に化粧品等の故意の接触や家庭用品や建築資材などの居住環境を通して経皮的にヒトを絶えず暴露している.
特に, 室内空気中に多く存在する化学物質は濃度も高く, しかも飲食に伴う食品や水などのように間欠的摂取による暴露ではなく1日の大部分を継続して暴露している. このことから, 室内空気中化学物質が健康に影響を及ぼす可能性について懸念されることは当然であり, その結果としてシックハウス症候群や化学物質過敏症として知られる疾病の発症原因として取り扱われている. 特に, ホルムアルデヒドはその代表的化学物質で、目や粘膜などに対する刺激性と共に, アレルギーとの関連性が指摘されている. 本稿では, こうしたホルムアルデヒドによる発症要因や健康影響を検討すると共に, アレルギーとの関連性について紹介した.
Keywords : hormaldehyde, risk assessment, carcinogenicis

安藤正典:**水質管理の課題とその対応**
資源環境対策, **37**(3), 242-246 (2001)
2000年末, 生活環境審議会 (厚生省の諮問機関) は, ①水道事業の一部を第三者に委託することを可能にすること, ②水道による専用水道の規制対象を居住人口 (100人以上) から, それと同等の給水の能力がある水道も加えること, ③未規制の小規模受水槽 (10m³以下) 水道の管理の充実, ④水道事業における情報提供の制度的位置づけ, などの水道法一部改正について答申した. これを受けて厚生労働省は水道法の改正案を提出する予定である.
このような新たな施策を推進することは, きわめて好ましいことではあるが, このことはひるがえると旧来の水道における水質管理の在り方に大きな課題を突きつけられたことに他ならない. 本稿では, 厚生労働省が新たに施行するに至った水質管理について水道事業体の観点からの問題点を考えてみる.
Keywords : control of drinking water quality,water sources, drinking water treatment

安藤正典:**室内化学物質の毒性と対策**
建築雑誌, **116**(4), 75 (2001)
室内空気中に存在する化学物質によって, シックハウス症候群や化学物質過敏症などの疾病が発生する可能性が高いことが社会的に大きな問題となっている. これらの疾病は, 慢性的な微量・長期又は多量・短気暴露によって誘発され, それ以降, 微量で発症すると考えられている. しかしながら, 室内空気に関連する疾病であるシックハウス症候群は, それと関連すると思われる揮発性有機化合物の特定あるいはそれらの毒性が動物実験によって証明されているわけではなく, まして, 暴露量と健康影響の量的関連性が示されているわけでもない. 本稿では, 室内化学物質の有害性の指摘と, その有害性を回避する対策としてのガイドライン値設定の骨組みを紹介した.
Keywords : indoor air,guideline,sick house sindrom

熊谷一清[*1], 坊垣和明[*2], 池田耕一[*3], 堀 雅宏[*4], 松村年郎, 飯倉一雄[*5], 吉澤 晋[*6]:**実大実験住宅における内装材の室内化学物質濃度に及ぼす影響に関する研究**
日本建築学会計画系論文集, 第542号, 77-83, (2001)
実大実験住宅において, 各施行段階ごとの気中濃度を測定し, 内装材の室内汚染に及ぼす影響を検討した. 更に, 使用する材料の放散量, 使用する建材・工法の放散量に及ぼす影響, 材料の組み合わせに違い, 外的条件 (温湿度) の影響, 放散量低減対策の効果など, 総合的に検討し, 内装材の室内汚染に及ぼす影響を明らかにした.
Keywords : voc, building meterial, full sized experiment
[*1] 東京大学工学部
[*2] 建築研究所
[*3] 国立公衆衛生院
[*4] 横浜国立大学教育人間科学部
[*5] 東北文化学園大学科学技術学部
[*6] 愛知淑徳大学建築学部

徳永裕司, 鄭 然孫, 内野 正, 安藤正典:**ビスフェノールAのIn Vitro経皮吸収に関する研究**
日本化粧品技術者会誌, **34**, 255-260 (2000)
化粧品に含まれると想定される内分泌かく乱化学物質のビスフェノールA (BA) の経皮級的な検討を行った. モルモットの腹部の剥離皮膚をFranz型拡散セルに装着した. donor

側に10mMドデシル硫酸ナトリウム溶液,10mM塩化ベンザルコニウム(BK)溶液あるいは0.5%ポリオキシエチレン(10)オレイルエーテル(POE.OE)溶液を加え,32℃で2時間放置した.donor側の界面活性剤を除き,0.05%BA溶液を加え,32℃で14～24時間後にreceptor側に透過してくるBAをHPLC法で測定した.また,同様に0.05%BAを含む各種界面活性剤を用いて32℃で14～24時間後にreceptor側に透過してくるBAをHPLC法で測定した.HPLC条件は,Unisil Q C_{18} (4.6mm i.d. × 150mm)カラム及び水/アセトニトリル混液(3:2)を用い,蛍光検出器(励起波長:280nm,蛍光波長:305nm)にて測定を行った.界面活性剤のBK及びPOE.OEで2時間処理された剥離皮膚でのBAのfluxは1.6及び1.2倍と増加した.また,BAを界面活性剤BKあるいはPOE.OEと共存させた場合,fluxが0.1倍及び0.22倍と著しく低下した.

Keywords : bisphenol A, skin permeation, Franz type diffusion cell

Samanta,G.,Chowdhury, UK.*, Mandal, BK.*, Chakraborti, D.*, Chandra S,N., Tokunaga, H., Ando, M.: **High performance liquid chromatography inductivel coupled plasma mass spectrometry for speciation of arsenic compounds in urine**

Microchem.J., **65**, 113-127(2000)

A high performance liquid chromatography inductivel coupled plasma mass spectrometry(HPLC-ICP-MS) system for speciation of arsenic, arsenate, monomethyl arsonic acid, dimethylarsenic acid and arsenobetaine in a single run in urine samples has been developed. Detection limits for the five arsenic species in urine samples are between 0.01 and 0.04 μg/l. To validate the method, SRM2670 containing both normal and elevated levels of arsenic have been analyzed for arsenic species. The method has been applied to determine the arsenic species in urine samples of two groups of people from two arsenic-affected villages of two districts, out of the nine affected districts of West Bengal, India. From their urine speciation, the nature of exposure of indivisuals to arsenic compound could be predicted. It is conclude that, even though these groups are using safe water, they cannot avoid, from time to time, arsenic contamination as any water souces of the surrounding areas are arsenic contaminated.

Keywords : arsenic species in urine samples, HPLC-ICP-MS

*Jadavpur University

内野 正, 徳永裕司, 安藤正典:**高速液体クロマトグラフによる化粧水, 乳液, クリーム中の塩酸プロカイン及び塩酸ジブカインの定量**

粧技誌, **34**, 261-266 (2000)

塩酸プロカイン及び塩酸ジブカインは局所麻酔剤として広く用いられているが,昭和61年の厚生省通知(昭和61年3月12日薬審2第100号)により化粧品への配合禁止成分となっている.我々は配合禁止成分の有無を効率よく確認し,化粧品の安全性を確保するために塩酸プロカイン及び塩酸ジブカインの高速液体クロマトグラフィーによる定量法を確立し,化粧品への応用について検討した.化粧水や乳液及びクリーム中の塩酸プロカイン又は塩酸ジブカインを水又はメタノールで抽出し,ODSカラム(Shiseido CAPCELL PACK C_{18}, 4.6 × 250mm),移動相として50mMリン酸塩緩衝液(pH 5)/アセトニトリル混液(37:3)又は(65:35),検出器として紫外吸光光度計(測定波長296nm又は228nm)を用い,高速液体クロマトグラフィーで分析した.この方法を用いることにより,化粧水や乳液及びクリーム中の塩酸プロカイン又は塩酸ジブカインを原料の影響もなく定量することが出来ることを明らかにした.

Keywords:procaine hydrochloride, dibucaine hydrochloride, HPLC

Hanioka, N., Tanaka-Kagawa, T., Chung, Y.S., Nishimura, T., Jinno,H., Ando M.: **Changes in hepatic cytochrome P450 enzymes by biodegradation products of 4-*tert*-octylphenol polyethoxylate in rats.**

Bull. Environ. Contam. Toxicol., **64**, 804-810 (2000)

The effects of some biodegradation products of 4-*tert*-octyl phenolpolyethoxylate (OPEO), namely 4-*tert*-octylphenol (OP), 4-*tert*-octylphenol diethoxylate (OP2EO) and 4-*tert*-octylphenol monocarboxylate (OP1EC), on hepatic cytochrome P450 enzymes in rats were studied. Among the cytochrome P450-dependent monooxygenase activities, testosterone 2a-hydroxylase activity,was significantly decreased by OP,OP2EO and OP1EC. CYP3A2-dependent monooxygenase, testosterone 6b-hydroxylase activity was also decreased by 50% by OP. Furthermore, immunoblotting showed that OP significantly decreased by 49 and 43%, the CYP2C11/6 and 3A2/1 protein level, respectively. By contrast, CYP1A1/2-, 2A1/2-, 2B1-, 2D1-, 2E1- and 4A1/2/3-dependent monooxygenase activities were not affected by any OPEO biodegradation product. These results suggest that OPEO biodegradation products change themale-specific cytochrome P450 isoform(s) in rat liver (OP>OP1EC=OP2EO), and that these changes may relate to the toxicity of OPEO and its biodegradation products.

Keywords: Cytochrome P450; 4-*tert*-Octylphenol

Hanioka, N., Tatarazako, N.*, Jinno, H., Arizono, K.*, Ando, M.: **Determination of cytochrome P450 1A activities in mammalian liver microsomes by high-performance liquid chromatography with fluorescence detection.**

J. Chromatogr. B., **744**, 399-406 (2000)

A sensitive method for the determination of CYP1A activities such as ethoxyresorufin *O*-deethylase (EROD) and methoxy resorufin *O*-demethylase (MROD) in liver microsomes from human, monkey, rat and mouse by HPLC with fluorescence detection is reported. The newly developed method was found to be more sensitive than previous methods. The detection limit for resorufin was 0.80 pmol/assay. Intra-day and inter-day precisions (RSD) were less than 6% for both enzyme activities. With this improved sensitivity, the kinetics of EROD and MROD activities in mammalian liver microsomes could be determined more precisely. EROD activities in human and monkey liver microsomes, and MROD activities from all animal species exhibited a monophasic kinetic pattern, whereas the pattern of EROD activitiesin rat and mouse liver microsomes was biphasic. Therefore, this method is applicable to *in vivo* and *in vitro* studies on the interaction of xenobiotic chemicals with CYP1A isoforms in mammals.

Keywords: Cytochrome P450; EROD; MROD

*Prefectural University of Kumamoto

Hanioka, N., Jinno, H., Tanaka-Kagawa, T., Nishimura, T., Ando,M:**Determination of UDP-glucuronosyltransferase UGT1A6 activity in human and rat liver microsomes by HPLC with UV detection.**

J. Pharm. Biomed. Anal., **25**, 65-75 (2001)

A simple and sensitive method for the determination of UDP-glucuronosyltransferase UGT1A6 activity using 4-methyl umbelliferone (4-MU) and 4-nitrophenol (4-NP) as substrates in human and rat liver microsomes by HPLC with UV detection is reported. The method was validated for the determination of 4-methylumbelliferyl b-D-glucuronide (4-MUG) and 4-nitrophenyl b-D-glucuronide (4-NPG). The method was found to be more sensitive than previous methods using a spectrophotometer, a spectrofluorimeter and HPLC. The detection limit for 4-MUG and 4-NPG was 14 and 23 nM, respectively. The intra- and inter-day reproducibility (RSD) of UGT1A6 enzyme assay in liver microsomes wasless than 6%. With this improved sensitivity, the kinetics of UGT activities toward 4-MU and 4-NP in human and rat liver microsomes could be determined more precisely. Therefore, this method is applicable to *in vivo* and *in vitro* studies on the interaction of xenobiotic chemicals with UGT1A6 isoform in mammals using small amounts of biological samples.

Keywords: UDP-Glucuronosyltransferase; 4-MU; 4-NP

豊田正武，酒井　洋[*1]，小林ゆかり[*1]，小松雅美[*1]，星野庸二[*2]，堀江正一[*2]，佐伯政信[*3]，長谷川康行[*3]，辻　元宏[*4]，小嶋美穂子[*4]，豊村敬郎[*5]，熊野眞佐代[*5]，谷村顕雄[*6]：**日本人の食事経由のトリブチルスズ、ジブチルスズ、トリフェニルスズ及びジフェニルスズ化合物の摂取量**

食品衛生学雑誌，**41**, 280-286 (2000)

４種有機スズ化合物の人体暴露量を推定することを目的にトータルダイエットスタディーによる14食品群中の濃度を測定した．1998年度の１日摂取量はTBTCが平均1.73（範囲0～4.70）μg／人，DBTCが平均0.45（範囲0～1.72）μg／人，TPTCが平均0.09（範囲0～0.25）μg／人，DPTCが0μg／人であった．TBTC及びTPTCはほとんど10群（魚介類）由来であり，本群の１日摂取量の経年変化を７年間にわたり調べた．1998年度にTBTCは1991年度の1/2に低下し，TPTCは1/44（約２%）に低下した，TBTCの１日摂取量は我が国のTBTOの暫定ADIの２%に相当した．TPTCの平均１日摂取量は，JMPRのADIの0.3%相当であった．

Key words: organotin compounds, tributyltin, triphenyltin, total diet study, daily intake

[*1] 新潟県保健環境科学研究所
[*2] 埼玉県衛生研究所
[*3] 千葉県衛生研究所
[*4] 滋賀県立衛生環境センター
[*5] 長崎県衛生公害研究所
[*6] 昭和女子大学

佐々木久美子，辰濃　隆[*1]，中村宗知[*2]，今澤　剛[*3]，大島辰之[*4]，近藤安昭[*5]，田形　肇[*6]，千葉　実[*7]，松田りえ子，豊田正武：**食品衛生法告示エスプロカルブ等５農薬試験法の評価**

食品衛生学雑誌，**41**, 219-223 (2000)

エスプロカルブ等５農薬の告示試験法評価のために共同実験を行った．６分析機関で５農薬を添加した玄米等８作物を分析したときの各農薬回収率の平均値は87.2～102.2%，併行精度及び室間再現精度の相対標準偏差はそれぞれ1.1～9.6%，7.9～17.3%と良好な結果が得られた．８作物試験液のガスクロマトグラムには妨害ピークは検出されなかった．平均的な検出限界値は0.001～0.005 μg/gであった．

Keywords: method-performance study, notified analytical method, esprocarb

[*1] 日本食品衛生協会
[*2] 日本食品分析センター
[*3] 東京顕微鏡院
[*4] 日本油料検定協会
[*5] 日本食品衛生協会食品衛生研究所
[*6] 日本冷凍食品検査協会
[*7] 日本穀物検定協会

佐々木久美子，辰濃　隆[*1]，中村宗知[*2]，穴沢　昭[*3]，今澤　剛[*4]，宇都宮　領[*5]，後藤修宏[*6]，田形　肇[*7]，寺澤真二[*8]，松田りえ子，豊田正武：**食品衛生法告示臭素試験法の評価**

食品衛生学雑誌，**41**, 224-227 (2000)

臭素の告示試験法評価のために共同実験を行った．６機関で臭素を添加した玄米等４作物を分析したとき，平均回収率は88.0～89.9%であった．併行精度及び室間再現精度の相対標準偏差はそれぞれ1.7～5.2%，10.4～12.7%であった．４農作物の臭素試験液のクロマトグラムには妨害ピークは検出されなかった．検出限界値は操作ブランクの変動に依存し，0.2 μg/g以下であった．

Keywords: method-performance study, notified analytical method, bromine

[*1] 日本食品衛生協会
[*2] 日本食品分析センター
[*3] 東京都予防医学協会
[*4] 東京顕微鏡院
[*5] 食品環境検査協会
[*6] 日本海事検定協会
[*7] 日本冷凍食品検査協会
[*8] 日本油料検定協会

佐々木久美子，辰濃　隆[*1]，中村宗知[*2]，穴沢　昭[*3]，宇都宮　領[*4]，近藤安昭[*5]，千葉　実[*6]，三浦嘉巳[*7]，松田りえ子，豊田正武：**食品衛生法告示ダミノジッド試験法の評価**

食品衛生学雑誌，**41**, 228-232 (2000)

ダミノジッド(DZ)告示試験法の評価のために共同実験を行った．６機関でDZを添加した玄米等５作物を分析したとき，平均回収率は69.2～84.4%であった．併行精度及び室間再現精度の相対標準偏差はそれぞれ4.1～5.5%，7.4～11.8%であった．５農作物のDZ試験液のクロマトグラムには妨害ピークは検出されなかった．検出限界値はGC-NPDの状態に依存し，0.1 μg/g以下であった．

Keywords: method-performance study, notified analytical method, daminozide

[*1] 日本食品衛生協会
[*2] 日本食品分析センター
[*3] 東京都予防医学協会

*4 食品環境検査協会
*5 日本食品衛生協会食品衛生研究所
*6 日本穀物検定協会
*7 千葉県薬剤師会検査センター

根本 了, 佐々木久美子, 衛藤修一*1, 斎藤 勲*2, 酒井洋*3, 高橋哲夫*4, 外海泰秀, 永山敏廣*5, 堀伸二郎*6, 前川吉明*7, 豊田正武 : **GC/MS(SIM)による農作物中110農薬の一斉分析法**

食品衛生学雑誌, **41**, 233-241 (2000)

農作物からアセトニトリル抽出後, GPC及びカートリッジカラムによるクリーンアップを行い, GC/MS(SIM)測定する110農薬の一斉分析法を作成した. 6農作物に4又は5分析機関で添加回収試験を行った結果, 各農薬の回収率データ中70～120%の良好な結果を示したデータが全体の70%以上を占めた農薬は77種であった. 各農薬の検出限界値は0.0004～0.08 μg/g以下であった.

Keywords: agricultural product, pesticide residue, multiresidue analysis

*1 北九州市環境科学研究所
*2 愛知県衛生研究所
*3 新潟県保健環境科学研究所
*4 北海道立衛生研究所
*5 東京都立衛生研究所
*6 大阪府立公衆衛生研究所
*7 日本食品分析センター

根本 了, 高附 巧, 佐々木久美子, 豊田正武 : **市販魚中のノニルフェノールの分析**

食品衛生学雑誌, **41**, 377-380 (2000)

6店舗で購入した市販魚のノニルフェノール (NP) 汚染を調査した結果, 35検体中24検体から9～800ng/gのNPが検出された (検出限界 8 ng/g). 購入した店舗間で魚のNP汚染に差が認められたことから, 包装材のn-ヘプタンによる溶出試験を行った. その結果, NPが検出された魚の包装に使用されていたラップフィルムから70～931ng/cm^{2}のNPが溶出したが, NP不検出の魚の包装ラップフィルムからはほとんど溶出しなかった. 耐衝撃性ポリスチレン製トレイからもNPが溶出したが, トレイと魚汚染レベルとの関連性は低かった. これらの結果から魚のNP汚染には包装材, 特にラップフィルムからの移行の関与が強く示唆された.

Keywords: fish, nonylphenol, wrapping film

今中雅章*1, 佐々木久美子, 根本 了, 植田英一*2, 村上恵美子*2, 宮田大典*2, 外海泰秀 : **GC/MSによる各種食品中のビスフェノールAの分析**

食品衛生学雑誌, **42**, 71-78 (2001)

各種食品中のビスフェノールAをアセトンで抽出, クリーンアップ後, ヘプタフルオロ酪酸で誘導体化し, GC/MS(SIM)で定量する汎用性のある分析法を確立し, 加工食品, 生鮮食品など95検体の実態調査を行った. その結果, 缶詰, 生鮮魚介類, 肉類, コンビニ弁当からそれぞれ痕跡量～602ng/g, 痕跡量～6ng/g, 痕跡量～2ng/g及び痕跡量のビスフェノールAが検出された. 一方, 乳製品, 野菜, 果実, 精白米からは検出されなかった.

Keywords: bisphenol A, GC/MS, fresh food

*1 岡山県環境保健センター
*2 北九州市環境科学研究所

高附 巧, 根本 了, 堤 智昭, 佐々木久美子, 豊田正武: **HPLCによる農産物中のAcibenzolar-S-methyl及び分解物の分析法**

食品衛生学雑誌, **41**, 381-386 (2000)

HPLCによる農産物中のアシベンゾラル-S-メチル(BTH)及び分解物のbenzo[1,2,3]thiodiazole-7-carboxylic acid(BTC)の分析法を確立した. 試料からリン酸緩衝液(pH8.0)とアセトンで抽出後, BTHは塩基性溶液(pH7.5～8.5)からエーテル－ヘキサン(1:1)で再抽出し, BTCはリン酸酸性溶液(<pH3)から同溶媒で再抽出した. BTHは脱脂後, フロリジルで精製した. BTCは, 直接フロリジルで精製した. UV検出器付きHPLC及びLC/MSで測定を行った.

米, 麦及びトウモロコシにBTH又はBTCを0.1 μg/g添加したときの回収率は, 平均76.8～86.7%, 標準偏差は0.3～2.7%であった. 検出限界はUV検出器付きHPLCで0.003 μg/g(BTH)及び0.008 μg/g(BTC)であった.

Keywords: pesticide residue, HPLC, acibenzolar -S- methyl

豊田正武, 堤 智昭, 柳 俊彦*1, 河野洋一*1, 内部博泰*1, 堀 就英*2, 飯田隆雄*2: **野菜中ダイオキシン類測定における振とう抽出法と還流抽出法の比較**

食品衛生学雑誌, **41**, 316-320 (2000)

野菜試料における, アセトン・ヘキサン振とう抽出法とトルエン還流抽出法のダイオキシン類抽出効率の比較を行った. 抽出試験を3回行った結果, ほうれん草では, 振とう抽出でPCDDs, PCDFs及びCo-PCBsが平均0.48, 0.80及び7.7pg/g検出され, 還流抽出では同様の順に0.43, 0.72及び7.3pg/g検出された. また, ちんげん菜では, 振とう抽出でPCDDs, PCDFs及びCo-PCBsが平均0.67, 0.50及び2.6pg/g検出され, 還流抽出では同様の順に0.81, 0.64及び2.6pg/g検出された. 両抽出法の間で, 抽出量には有意な差はなく, 両者の野菜中ダイオキシン類の抽出効率は同様であることが判明した.

Keywords: shaking extraction, reflux extraction, dioxin

*1 (財)日本食品分析センター
*2 福岡県保健環境研究所

松田りえ子, 佐々木久美子, 酒井洋*1, 青柳由美子*1, 佐伯政信*2, 長谷川康行*2, 日高利夫*3, 石井敬子*3, 望月恵美子*4, 山本敬男*4, 宮部正樹*5, 田村征男*5, 堀伸二郎*6, 池辺克彦*6, 辻元宏*7, 小嶋美穂子*7, 佐伯清子*8, 松岡幸恵*8, 西岡千鶴*9, 藤田久雄*9, 城間博正*10, 大城善昇*10, 豊田正武 : **食品からのアルミニウムの一日摂取量の推定**

食品衛生学雑誌, **42**, 18-23 (2001)

1996年から1998年に, トータルダイエット試料中のアルミニウム濃度を測定しアルミニウムの一日摂取量を推定した. 10カ所の機関でトータルダイエット試料の調製及びアルミニウム濃度の測定を行った. アルミニウムの一日摂取量は平均3.5mgであり, 範囲は1.8mgから8.4mgであった. 分析結果の正当性は, 認証標準試料の分析により保証された.

Keywords: aluminum, daily intake, total diet sample

*1 新潟県保健環境科学研究所
*2 千葉県衛生研究所

*3横浜市衛生研究所
*4山梨県衛生公害研究所
*5名古屋市衛生研究所
*6大阪府立公衆衛生研究所
*7滋賀県立衛生環境センター
*8山口県環境保健研究センター
*9香川県衛生研究所
*10沖縄県衛生環境研究所

Kondo, K., Kurihara, M., Fukuhara, K. : **Mechanism of antioxidant effect of catechins**

Methods in Enzymology, **335**, 203-217 (2001)

The antioxidant mechanisms of EC, EGC, ECG, and EGCG were studied using LC/MS, spectrophotometry, chemiluminescence and electrochemical analyses, and semiempirical MO calculations. These results showed the antioxidant effect of catechins and their mechanisms were complicated. EGC has a lower oxidation potential (159 mV) than EC (307 mV) and, therefore, can scavenge peroxyl radicals more quickly (kinh/kp =232) than EC (kinh/kp =41). This indicates that the pyrogallol structure in the B ring play an important role in the rapid scavenging ability. EGCG having the gallate group at the C-3 position show much more rapid scavenging ability (kinh/kp =628) than EC and EGC, suggesting that as the number of phenolic OHs in catechins increases, the more rapidly they can scavenge peroxyl radicals. However, EGC and EGCG with the pyrogallol structure have a negative aspect. They may generate superoxide and the antioxidant effect of them does not last for a long time. Catechins have both aspects as antioxidants and prooxidants as do other flavonoids. Catechin with the lowest oxidation potential does not exert the strongest antioxidant effect. Also, the effect may change based on the experimental conditions such as the solvent system or radical species being used as an initiator.

Keywords : catechin, oxidation potential, bond dissociation enthalpy

近藤一成，栗原正明，福原 潔，鈴木 隆，宮田直樹，豊田正武：**カテキンの抗酸化作用におけるC-2位プロトンの重要性**

磁気共鳴と医学，**12**, 91-94 (2001)

(-)-Epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (-)-epicatechin gallate and proanthocyanidins exerted a strong antioxidant effect against peroxyl radicals. EGC has a more rapid scavenging effect on peroxyl radicals (kinh/kp = 628 for EGCG, 232 for EGC) than EC (kinh/kp = 41). The antioxidant effect of EC, whose oxidation potential was 304 mV was the same or greater than that of quercetin, which has lower oxidation potential (168 mV). EC, procyanidin B-2 (catechin dimer), and proanthocyanidin extracted from grape seeds (catechin polymer) showed the first oxidation potentials in the following order, EC (304 mV) < procyanidin B-2 (450 mV) < proanthocyanidin (795 mV). These three compounds inhibited peroxyl radicals effectively in the order, suggesting that the first oxidation potential do not reflect the antioxidant effect. The compounds generated from each catechin and a radical initiator 2,2' -azobis(2-aminopropane) dihydrochloride (AAPH) have been investigated using LC/MS. The calculated C-H bond dossociation enthalpies (BDEs) for catechins and catechin dimers at the C-2 position were expectedly low (65-70 kcal/mol) compared with O-H BDEs at phenolic sites (71-75 kcal/mol). Procyanidin B-2 converted into procyanidin A-2 by radical reaction, indicating that the strong effect of catechins on radical scavenging is based on the presence of the C-2 hydrogen in addition to the o-dihydroxyl structure.

Keywords : catechin, oxidation potential, bond dissociation enthalpy

Terahara, N.*1, Konczak-Islam, I.*2, Nakatani, M.*2, Yamakawa, O.*2, Goda, Y., Honda, T.*3 : **Anthocyanins in callus induced from purple storage root of *Ipomoea batatas* L. Two acylated anthocyanins from purple sweet potato**

Phytochemistry , **54**, 919-922 (2000)

Two anthocyanins were isolated from the highly pigmented callus dereived from the storage root of purple sweet potato (*Ipompea batatas* L.) cultivar 'Ayamurasaki'. One was identified as cyanidin 3-*O*-sophoroside-5-*O*-glucoside, and the other as cyanidin 3-*O*-(2-*O*-(6-*O*-(E)-p-coumaroyl- β -D-glucopyranosyl)- β -D-glucopyranoside)-5- β -D-glucopyranoside, by chemical and spectroscopic anaysis.

Keywords: anthocyanin, *Ipompea batatas*, natural food colorant

*1 College of Horiculture, Minami-Kyushu University
*2 Kyushu National Agricultural Experiment Station
*3 Hoshi University

Matsufuji, H.*, Sakai, S.*, Chino, M.*, Goda, Y., Toyoda, M., Takeda, M.* : **Relationship between cardiac glycoside contents and color of *Corchorus olitorius* seeds**

J. Health, Sci., **46**, 89-93 (2001)

The relationship between the cardiac glycoside contents in *Corchorus olitorius* seeds and the seed color was examined. The seed color was assigned a shade (color value) (L value in UCS system). The dark grayish green seeds showing lower L value, contained more cardiac glycosides than dark grayish yellow seeds showing higher L value. When the total cardiac glycoside contents were plotted against the L values, a positive correlation (r=-0.913) was observed. Also, there was a higher content ratio of strophanthidin glycosides (erysimoside and olitoriside) in the seeds showing lower L value, while there was a lower content ratio of digitoxigenin glycosides (coroloside and glucoevatromonoside) in the seeds showing lower L value.

Keywords: *Corchorus olitorius*, cardiac glycoside, color value

* College of Bioresource Science, Nihon University

Matsuoka, T.*, Kuribara, H.*, Akiyama, H., Miura, H.*, Goda, Y., Kusakabe, Y.*, Isshiki, K.*, Toyoda, M., Hino, A.*: **A Multiplex PCR method of detecting recombinant DNAs from five lines of genetically modified maize**

J. Food Hygienic Soc. Japan, **42**, 24-32 (2001)

Seven lines of genetically modified (GM) maize were authorized in Japan as foods and feeds imported from the USA. We improved a multiplex PCR method described in the previous re-

port in order to distinguish the five lines of GM maize. Genomic DNA was extracted from GM maize by a silica spin column kit, which could reduce experimental time and improve safety in the laboratory and potentially in the environment. We sequenced recombinant DNA (r-DNA) introduced to GM maize, and re-designed new primer pairs to increase the specificity of PCR to distinguish five lines of GM maize by multiplex PCR. A primer pair for the maize intrinsic zein gene (Zel) was also designed to confirm the presence of amplifiable maize DNA. The lengths of PCR products using these six primer pairs were different. The Zel and the r-DNAs from the five lines of GM maize were qualitatively detected in one tube. The specific PCR bands were distinguishable from each other on the basis of the expected length. The r-DNA could be detected from the maize sample containing 0.5 % of each of the five lines of GM maize. The sensitivity would be acceptable to secure the verification of non-GMO materials and to monitor the reliance of the labeling system.

Keywords: genetically modified maize, recombinant DNA, PCR

*National Food Research Institute, Ministry of Agriculture, Forestry and Fisheries

合田幸広, 穐山 浩, 大槻 崇, 藤井明美*, 豊田正武: **多機能カラムとHPLCを利用した食品中のアフラトキシン分析法の応用と改良**

食衛誌, **42**, 56-62 (2001)

アフラトキシン (AF) 通知分析法においても, 健康危害及び環境汚染防止の目的で, 使用溶媒の見直しが要求されている. 我々は既に報告した毒性の高い溶媒を用いない多機能カラムにHPLCを組み合わせたAF分析法の応用範囲を拡大する目的で, 通知で分析が義務付けられたナッツ類及びジャイアントコーンを含め, より多種の試料の添加回収実験を行った. その結果マカデミアナッツ, クルミ, ヘーゼルナッツ, ブラジルナッツ, ジャイアントコーン, コメ, コムギ, ソバで B1, B2, G1, G2 ともに良好な回収率 (85%-106%) を得た. また, 妨害ピークが検出された香辛料 6 種及び紅茶について, 市販のアフィニティーカラムを組み合わせた分析法を検討した. その結果, G2, B2 で一部回収率が低いものの, B1, G1 の回収率は良好 (71-112%) で, これらの試料でも分析可能であることが示された.

Keywords: aflatoxin, multifunctional column, spices

*横浜検疫所 輸入食品・検疫検査センター

Miyahara, M., Ito, H.*1, Saito, A.*2, Nagasawa, T.*2, Kariya, M.*2, Toyoda, M., Saito, Y.: **Detection of meats by HPLC determination for o-tyrosine using novel LASER fluorometric detection with automatic pre-column reaction**

J. Health Science., **46**, 304-309 (2000)

An o-Tyrosine method for detection of irradiation of foods was studied by HPLC using a novel LASAR fluorometric detection system with pre-column reaction. Sample was prepared and purified by eliminating fat and sugars using a mixture of acetone and chloroform, and then the purified protein was hydrolyzed using hydrochloric acid at 110℃ for 24 hours in a vacuum. The sample was reacted with NDB-F reagent by an automatic pipetting system and was introduced into the HPLC system. Irradiated chicken, pork, beef, and tuna were examined by irradiating at 0,1,5,10 kGy. Irradiation of chicken and pork irradiated at or over 10 kGy was successfully detected, but that of beef and tuna were more difficult to detect. After 3 months storage at 20℃, the irradiation was still detectable in chicken irradiated at 10 kGy. Thus this detection procedure can be used to detect irradiation in some chilled meats irradiated at 10 kGy. Non-irradiated o-tyrosine formation and reduction of o-tyrosine by hydroxylation are also discussed.

Keywords: o-tyrosine method, irradiated food detection, NBD-F, HPLC, pre-column derivatization

*1 原子力研究所 高崎研究所
*2 北里大学医療衛生学部

Miyahara, M., Saito, A.*1, Ito, H.*2, Toyoda, M.: **Capability of identification of gamma-irradiated bovine liver by new high sensitivity assay**

Biol. Pharm. Bull., **23**, 1399-1405 (2000)

DNA in food will get damage by gamma radiation. The high sensitive Comet assay was studied using fluorescence- microscopy. Beef lever was irradiated at range of 1 Gy to 8kGy. Single cells were obtained from the irradiated liver, and were analyzed by agarose-gel electrophoresis. The pH of the buffer for electrophoresis was pH13, which is generally utilized for sensitive detection of DNA damage. The pattern formed by DNA was visualized by staining ethidium bromide. The resulted comets were evaluated by the scale we developed and influence scores were calculated based on the Tice method. It is possible to detect irradiation of beef liver at 10Gy. In the other hand, DNA damage will be caused not only by irradiation, but also by the other treatments. Therefore influence of freezing, preservation, irradiating temperature, atmosphere of irradiation, cooking, and homogenizing device were also examined. This new comet assay will be a useful detection procedure of irradiated foods.

Keywords: comet assay, irradiated food detection, DNA damage, beef liver

*1 北里大学医療衛生学部
*2 原子力研究所 高崎研究所

後藤典子*1, 田辺寛子*1, 宮原 誠: **照射鶏肉の炭化水素法およびESR法による検知**

食品照射, **35**, 23-34 (2000)

Chicken meat with bone was irradiated by gamma ray at a-19 ～10℃, and both amount of hydrocarbons formed from fatty acids and intensity of ESR signals in bone fragments were measured. Very good correlation was found between the amount of hydrocarbons and the intensity of ESR signals. The amount of hydrocarbons (Cn-2:1) had 2 carbon atoms less than the original fatty acids and an additional double bond, was almost constant irrespective of the irradiation temperature raised. As the ratio between corresponding fatty acids, the ratio between hydrocarbons (Cn-2:1) is a suitable index in the detection of the irradiation. On the contrary, the ratio of hydrocarbons from same fatty acid, (Cn-2:1) /(Cn-1:0), varied according to the kind of fatty acid and temperature used at the irradiation.

It was found that under irradiation temperature of -19 ～ 10℃, intensity of ESR signals of bone is not affected by the irradiation temperature.

Keywords: irradiated chicken, hydrocarbon method, ESR method, identification

*1 東京都立産業技術研究所

Miyahara, M., Saito, A.*1, Ito, H.*2, Toyoda, M.: **Identification of low level gamma-irradiation of meats by new high sensitivity comet assay**

The 12th International Meeting on Ionization Processing, **http://www.atriplei.com/imrp**, (2001)

DNA in food will sustain damage by gamma radiation. The detection capability of the high sensitivity comet assay was studied using fluorescence-microscopy. Meats were irradiated at a range of 1 Gy to 2kGy. Single cells were obtained from the irradiated meats, then analyzed by agars-gel electrophoresis. The pH of the buffer for electrophoresis was pH13, which is generally utilized for sensitive detection of DNA damage. The pattern formed by DNA was visualized by staining with ethidium bromide. The resulting comets were evaluated with a scale we developed, and influence scores were calculated based on the Tice method. Pork, beef, and chicken that were irradiated at or above 0.5kGy were identified by the method.

Keywords: comet assay, irradiated meats, identification

*1 北里大学医療衛生学部
*2 原子力研究所 高崎研究所

Akiyama, H. ,Sakushima, J., Taniuchi, S.*1, Kanda, T.*2, Yanagida, A.*2, Kojima, T.*1, Teshima, R., Kobayashi, Y.*1, Goda, Y., Toyoda, M.: **Antiallergic effect of apple polyphenols on the allergic model mouse**

Biol. Pharm. Bull., **23**, 1370-1373(2000)

We studied here the antiallergic effect of apple condensed tannins (ACT) administered orally to a type I allergy model transplanted with an IGEL a2 hybridoma secreting anti-2,4,6-trinitrophenyl (TNP) immunoglobulin E (IgE). The oral administration of ACT significantly inhibited the ear swelling responses at 1h after antigen-stimulation with picryl chloride. The response was dose dependent within 0.1 to 10 mg/mouse. The inhibition of the ear swelling response reached the maximal level (90% inhibition) when ACT was administered 2h before the antigen challenge. These findings suggest that ACT has an antiallergic effect on type I allergic symptoms.

Keywords: antiallergic activity,procyanidin,apple

*1 関西医科大学
*2 ニッカウヰスキー㈱

Nagaoka M.H. and Maitani T.: **Differed preferential iron-binding lobe in human transferrin depending on the presence of bicarbonate detected by HPLC/high resolution ICP-MS.**

Biochim. Biophys. Acta, **1523**, 182-188 (2000)

The binding of Fe to human serum transferrin (Tf) was analyzed with an HPLC system equipped with an anion exchange column and directly connected with a HR-ICP-MS for metal detection. Two monoferric-Tfs were assigned based on the results of urea-PAGE and desferrioxamine experiments. When Fe was added as Fe-citrate stepwise to an apo-Tf solution in the presence of bicarbonate, the N-lobe site was the preferential Fe-binding site, while the C-lobe site was preferred in the absence of bicarbonate. In both cases, the Fe-peak areas of the preferential site and Fe_2-Tf increased up to an Fe/Tf molar ratio of 1, and then the peak area of the monoferric-Tf decreased while the peak area of Fe_2-Tf increased. When the Fe/Tf molar ratio was below 1, the amount of Fe bound to the lobe with a weaker affinity was higher in Fe_2-Tf than in the monoferric-Tf in each case. Namely, Fe_2-Tf was the preferential binding state of Fe to human serum Tf. The preference is reasonable for transferring Fe ions effectively to Tf-receptors.

Keywords: Fe, transferrin, HPLC/HR-ICP-MS

Nagaoka M.H. and Maitani T.: **Binding patterns of co-existing aluminium and iron to human serum transferrin studied by HPLC/high resolution ICP-MS**

Analyst, **125**, 1962-1965 (2000)

Serum transferrin (Tf) is an Fe-binding glycoprotein. Al in the blood is bound to Tf. In the present study, the chemical forms of co-existing Al and Fe bound to human serum Tf were studied by on-line combined HPLC/HR-ICP-MS. Samples were subjected to HPLC equipped with an anion-exchange column. The levels of ^{27}Al, ^{56}Fe and ^{32}S, which are interfered by polyatomic ions such as $^{13}C^{14}N^+$, $^{12}C^{15}N^+$ and $^{12}C^{14}N^1H^+$, $^{40}Ar^{16}O^+$ and $^{40}Ca^{16}O^+$, and $^{16}O_2^+$, respectively, in the case of quadrupole ICP-MS, were monitored simultaneously by HR-ICP-MS at resolution $m/\Delta m = 3000$. Al added to apo-Tf as Al-citrate was preferentially bound to the N-lobe site almost selectively. Al in serum from a healthy person without any *in vitro* Al spike was present both as Al_N-Tf and Al_N,Fe_C-Tf. The chemical states were reproduced in apo-Tf solution supplemented with Fe (Fe/Tf = 0.6) and Al (Al/Tf = 1) successively. The cleanup procedures of column were devised, and with the procedures, the detection limit for ^{27}Al was lowered to 0.1 $\mu g\ l^{-1}$ ($3s_B$) at the middle resolution.

Keywords: HPLC/HR-ICP-MS, Al, transferrin

Nagaoka M.H., Maitani T.: **Effects of sialic acid residues of transferrin on the binding with aluminum and iron studied by HPLC/high-resolution ICP-MS.**

Biochim. Biophys. Acta, **1526**, 175-182 (2001)

Carbohydrate-deficient transferrins (Tfs) (CDTs) with fewer sialic acids increased in several diseases. In this study, the affinity of metals (Al and Fe) to Tfs was compared between native- and asialo-Tf by on-line HPLC/HR-ICP-MS, to clarify whether the presence of sialic acids influences the metal binding. Fe added as Fe-citrate in the presence of bicarbonate preferred the N-lobe site and the binding affinity was similar between native- and asialo-Tfs. Al-citrate added also preferred the N-lobe site, while the binding affinity was higher to asialo-Tf than to native-Tf. In Al-oxalate addition, the affinity to the N-lobe site of both Tfs increased further. In the absence of bicarbonate, Al-oxalate showed a preference for the C-lobe site in native-Tf and comparable affinity to both lobes in asialo-Tf. In asialo-Tf, Al_2-Tf was the largest peak. Thus, the lack of sialic acid in glycans and the presence of oxalate enhanced the binding affinity of Al to Tf. Therefore, it was suggested that the binding affinity of Al in patients with CDTs may be enhanced.

Keywords: carbohydrate-deficient Tfs, HR-ICP-MS, Al

宇野喜貴*1, 大本俊郎*1, 後藤康慶*1, 浅井以和夫*1, 中村幹雄*1, 米谷民雄：**GPC/ICP-AES法によるカラギナン分子量測定方法**
日本食品化学学会誌, **8**, 33-43 (2001)
カラギナンは分子中に多数の硫黄を有する天然増粘安定剤である．その平均分子量を求めるために，GPCカラムを装着したHPLC法と硫黄検出のICP発光分光法を直結した，GPC/ICP-AES法を応用した．注入λタイプ精製カラギナンからの高分子由来硫黄の回収率は，97-108%と良好であった．測定した平均分子量は，GPC/RI法による分子量と同程度であったが，若干の差異が認められた．その原因としては，カラギナンの分子量による硫黄含有率の違いと，ICP-AES側の要因が考えられた．
Keywords: carrageenan, GPC/ICP-AES, average molecular weight
*1 三栄源エフ・エフ・アイ㈱

宇野喜貴*1, 大本俊郎*1, 後藤康慶*1, 浅井以和夫*1, 中村幹雄*1, 米谷民雄：**GPC/ICP-AES法による食品中のカラギナン分析方法（第 1 法）**
日本食品化学学会誌, **8**, 48-56 (2001)
カラギナンは硫酸多糖の構造を有する天然増粘安定剤である．GPC/ICP-AES法により測定した高分子由来の硫黄量から食品中のカラギナンを分析できるかを，12種の市販デザートゼリーを対象に検討した．κタイプ精製カラギナン0.3%を含む調製ゼリーからの，カラギナンの回収率は103%であった．GPC/ICP-AES法では簡単な前処理のみで分析できる利点があるが，カラギナンの硫酸基の規格が15-40%であるため分析値に約2.7倍の差が生じ，また，ファーセレランなどの硫酸多糖が同時に使用されている場合にも誤差が生じるが，添加物が判明している食品製造ラインでは，品質管理等において有効な方法と考えられた．
Keywords: carrageenan, GPC/ICP-AES, dessert jelly
*1 三栄源エフ・エフ・アイ㈱

Akiyama, T., Yamada, T. and Maitani, T.: **Analyses of enzymatically glucosylated flavonoids by capillary electrophoresis**
J. Chromatogr. A, **895**, 279-283 (2000)
HPCE with a UV detector was applied for the analyses of enzymatically glucosylated flavonoids, which are used as natural food additives in Japan. Four items, which have flavonol or flavanone as aglycone, were analyzed. Each of these items is a mixture of glycosides with various lengths of maltooligosaccharide chain. On capillary zone electrophoresis with untreated fused-silica capillary at alkaline pH, glycosides with longer sugar chain migrated more rapidly. Flavonol glycosides with 1 - 13 glucose units were distinguished with the borate buffer (pH 10.0). Flavanone glycosides needed higher pHs for good separation than flavonol glycosides.
Keywords: capillary zone electrophoresis, flavonoid, malto-oligosaccharide

Akiyama, T., Yamada, M., Yamada, T. and Maitani, T.: **Naringin glycosides α-glucosylated on ring B found in the natural food additive, enzymatically modified naringin**
Biosci. Biotechnol. Biochem., **64**, 2246-2249 (2000)
Enzymatically modified naringin is a natural food additive, which is prepared with cyclodextrin glucanotransferase. Its constituents were structurally analyzed. Four constituents were isolated from the glucoamylase-treated sample. An NMR analysis revealed that two of them were novel compounds having 4'-*O*-α-glucosyl moieties on ring B of the naringenin aglycone. Both the aglycone and the glucose moiety in naringin are shown to be simultaneously glucosylated.
Key words: enzymatically modified naringin, cyclodextrin glucanotransferase, nuclear magnetic resonance

Liu, H.-M., Akiyama, T., Sugimoto, N. and Maitani, T.: **Isolation and identification of main constituents in an enzymatically hydrolysed licorice extract sweetener**
Food Additives and Contaminants, **18**, 281-284 (2001)
Enzymatically hydrolyzed licorice extract (EHLE) is also used as a sweetener in Japan. Three oleanane-type monoglycosides along with glycyrrhizin (**1**) and 3-*O*-[β -D-glucuronopyranosyl-(1→2)- β -D-glucuronopyranosyl]-18 β -liquiritic acid (**2**) were isolated from EHLE. The structures of the three compounds have been determined to be 3-*O*- β -D-glucuronopyranosyl-24-hydroxy -18 β -glycyrrhetinic acid (**3**), 3-*O*- β -D-glucuronopyranosyl-18 β -glycyrrhetinic acid (**4**) and 3-*O*- β -D-glucuronopyranosyl-18 β -liquiritic acid (**5**) based on MS and NMR. **4** was the monoglycosylated derivative of glycyrrhizin (**1**). Compounds **3** and **5** are the monoglycosylated derivatives of the minor constituents in licorice extract. They were first isolated from EHLE, and compound **5** was a new compound.
Keywords: glycyrrhizin, sweetener, enzymatically hydrolyzed licorice

Liu, H.-M., Sugimoto, N., Akiyama, T. and Maitani, T.: **Constituents and their sweetness of food additive enzymatically modified licorice extract**
J. Agric. Food Chem., **48**, 6044-6047 (2000)
Enzymatically modified licorice extract (EMLE) is a natural sweetener, which is prepared with cyclodextrin glucanotransferase. The structures of six major constituents isolated from EMLE were determined, and their sweetness was studied. The isolated compounds were glycyrrhizin (**1**), 3-*O*-[β -D-glucuronopyranosyl-(1 → 2)- β -D-glucuronopyranosyl]liquiritic acid (**2**), and their derivatives glucosylated at the C-4 position of the terminal glucuronopyranose with additional one (**3** and **4**, respectively) and two (**5** and **6**, respectively) glucose moieties. Compounds **3** - **6** were new compounds isolated for the first time. Compound **2** was sweeter than compound **1**. Interestingly, compound **3**, which was a monoglucosylated derivative of compound **1**, was sweeter than compound **4**. Compounds **5** and **6**, which have additional two glucose moieties, showed only slight sweetness.
Keywords: Glycyrrhiza, sweetener, enzymatically modified licorice

石川恵子*1, 久保木均*2, 佐藤恭子, 米谷民雄, 布村 伊*3：**4 倍体シシトウ果実の形態とカプサイシノイド含量**
日本食品化学学会誌, **7**, 74-77 (2000)

トウガラシ属における染色体倍加による倍数体を利用した品種改良の可能性を検討するため，シシトウ（*Capsicum annuum* L. cv. 'Shishitoh'）の4倍体を作出し，当代の果実について種子数，果実の大きさ，カプサイシノイド及びその前駆体含量を調べた．その結果，4倍体の果実は2倍体の約4割の種子しか持たず，果実の幅は同じであるが，長さは6割に減少していた．カプサイシノイド及びその前駆体含量は2倍体と同様であり，カプサイシノイドの生合成には促進あるいは抑制されているステップはないことが明らかとなった．

Keywords: Shishitoh, tetraploid, capsaicinoid

*1 千葉大学園芸学部
*2 福島県立白河実業高等学校
*3 日本園芸生産研究所

阿部有希子，武田由比子，石綿 肇，山田 隆：**甘味料アセスルファムカリウムの規格試験法の検討及び純度と含量**

食品衛生学雑誌，**41**, 274-279 (2000)

アセスルファムカリウムの規格作成のための試験法について検討し，試供品に適用した．試験法はJECFA及びFCCの方法を大きく変える必要はなかった．4ロットの試供品について，性状，極大吸収，沈殿反応，フッ化物，紫外線吸収不純物，乾燥減量，及び含量について測定したところ，JECFA及びFCCの規格に適合した．pHを第7版食品添加物公定書に準じて測定したところpH5.75～5.83で，FCC規格（6.5～7.5）より酸性を示したが，脱二酸化炭素水を用いることにより6.71～6.99を示し，FCC規格に適合した．含量は99.6～100.3%であった．

Keywords: acesulfame potassium, specification, purity test

杉田たき子，平山クニ*1，新野竜太*2，石橋 亨*2，山田隆：**ポリ塩化ビニル製玩具中のフタル酸エステル含有量**

食品衛生学雑誌，**42**, 48-55 (2001)

1998年10月に入手した玩具68検体の材質試験を実施したところ，全ての玩具からフタル酸エステル（PAE）類が検出された．検出されたPAEはフタル酸ジイソノニル(DINP)，フタル酸ジ(2-エチルヘキシル)(DEHP)，フタル酸ジブチル，フタル酸ジノニル，フタル酸ジヘプチルの5種類で，その他にアジピン酸ジ(2-エチルヘキシル)が検出された．DINPは48検体から検出され，含有量は15～580mg/g，平均308mg/g，含有量が最も多かったのはおしゃぶり玩具であった．DEHPは20検体から検出され，含有量は2.0～380mg/g，平均162mg/gであり，国産品では15検体，60%から検出された．

Keywords: polyvinyl chloride toy, diisononyl phthalate, di(2-ethylhexyl) phthalate

*1 神奈川県衛生研究所
*2 ㈱東京顕微鏡院

武田由比子，川崎洋子，石綿肇：**スクラロースの食品添加物成分規格のための定量法に関する検討**

日本食品化学学会誌，**7**, 56-59 (2000)

平成11年7月30日，厚生省令第75号で食品添加物として指定され，告示第167号により，規格が定められた．告示に先立ち，純度試験など成分規格設定に関する検討を行った．含量規格に関する定量法は米国食品添加物規格（FCC）および国際規格（CFAS）では標準品を用いたHPLC法であるが，標準品の入手が困難で，また，わが国の指定の食品添加物の含量規格の定量法は標準品不要の方法が一般的である．そこで，スクラロース分子中の塩素を水酸化ナトリウム溶液で遊離し，硝酸銀で滴定する簡便な方法を考察した．試料約1gを精密に量り，100mlとした試料溶液10mlに水酸化ナトリウム溶液（1→10）10mlを加え，30分間反応後，指示電極に銀，参照電極に銀－塩化銀電極を装着した自動滴定装置を用い，0.1mol/l硝酸銀溶液で滴定した．n=6の変動係数は0.4%であった．

Keywords: sucralose, sweetener, potentiometric titration

武田由比子，阿部有希子，石綿 肇，山田隆：**HPLCによる粉末スープ中のポリソルベートの分析法**

食品衛生学雑誌，**42**, 91-95 (2001)

HPLCによる粉末スープ中のポリソルベートの定量法について検討した．試料中の油脂成分をn-ヘキサンで除去後，アセトニトリルでポリソルベートを抽出した．抽出液をボンドエルートシリカゲルカートリッジ（500mg）に負荷し，酢酸エチルで粉末スープ由来の妨害物質を流去した後，アセトニトリル－メタノール(1:2)でポリソルベートを溶出した．溶出したポリソルベートをチオシアン酸コバルトと反応させ，生成した青色の化合物を，GPCカラムを使用したHPLCで測定した．粉末スープでの添加回収率は75%以上であり，定量限界は0.04mg/gであった．市販の粉末スープ16件につき本法で分析したところ，いずれも不検出であった．

Keywords: polysorbate, powdered soup, cobalt tiocyanate

河村葉子，前原玉枝，飯嶋広代，山田隆：**食品用プラスチック製品及び玩具中のノニルフェノール**

食品衛生学雑誌，**41**, 212-218 (2000)

プラスチック製器具・容器包装及び乳幼児用玩具中のノニルフェノール(NP)残存量をGC/MS-SIMにより測定したところ，ポリ塩化ビニル(PVC)製ラップフィルムと手袋において，全検体で10～2,600 μg/gと高濃度の残存がみられ，ポリスチレン(PS)製使い捨てカップでも高頻度であった．その他，ポリカーボネート，ポリプロピレン，ABS樹脂，スチレンブタジエン製品からも検出されたが，ポリエチレン，AS樹脂，ポリ塩化ビニリデン製品では検出されなかった．NPが残存していたPSカップ，PVCラップフィルム及び手袋について溶出試験を行ったところ，*n*-ヘプタンにより極めて高い溶出が認められた．一方，NPを含有する試料から酸化防止剤のトリス(ノニルフェニル)フォスファイトが検出され，これが分解してNPが生成したものと推察された．

Keywords: nonylphenol, polyvinyl chloride, tris (nonylphenyl) phosphite

河村葉子，前原玉枝，鈴木 隆*，山田 隆：**ガスクロマトグラフィー／原子発光検出器(GC/AED)による食品用器具・容器包装及び玩具中の有機スズ化合物の分析**

食品衛生学雑誌，**41**, 246-253 (2000)

ガスクロマトグラフィー／原子発光検出法を用いた9種類の有機スズ化合物の分析法を検討した．選択性が高く，検出限界は標準溶液で1pgと高感度であった．食品用器具・容器包装及び玩具を分析したところ，ポリ塩化ビニル(PVC)製容器では全検体から安定剤のジオクチルスズ(DOT)が検出

され，残存量はほぼ数千μg/gであった．さらにその不純物や分解物のモノ及びトリオクチルスズ(MOT及びTOT)，ジブチルスズ(DBT)などが検出され，ジメチルスズの残存もみられた．また，PVC製手袋からDOT，MOT，TOT，シリコーン加工クッキングシートからDBT，モノ及びトリブチルスズが検出された．PVC製容器のDOT及びDBTは比較的溶出しにくかったが，手袋及びシートからは極めて容易に溶出した．

Keywords: organotin compounds, gas chromatography/atomic emission detection method (GC/AED), polyvinyl chloride

*京都府立大学

河村葉子，左山佳代，山田 隆：**食品用ポリエチレン，ポリプロピレン及びポリスチレン製品へのガンマ線照射の影響－添加剤及びその他の化合物**

食品照射，**35**, 7-14 (2000)

市販ポリエチレン及びポリプロピレン製品に10～50kGyのガンマ線を照射したところ，残存していた添加剤のうち，酸化防止剤はいずれもすみやかに減少した．減少の程度はIrgafos 168の減少が最も顕著であり，Yoshinox 2246RやYoshinox SRはやや照射抵抗性があった．滑剤では，脂肪酸アミド類に減少がみられたが，脂肪族炭化水素には変化はみられなかった．また，可塑剤のDBPは比較的安定であった．酸化防止剤の分解物のうち，1,3-di-*tert*-butylbenzene及び2,6-di-*tert*-butyl-1,4-benzoquinoneは照射分解物と考えられたが，2,4-di-*tert*-butylphenolは非照射試料からも検出され，製品の製造時にも生成するものと考えられた．ポリスチレン製品では，添加剤は含有されておらず，残存していた不純物のスチレンダイマー及びトリマーについては，ガンマ線照射による含有量の変化は認められなかった．

Keywords: gamma irradiation, food contact plastic, additives

河村葉子，左山佳代，山田 隆：**食品用ポリエチレン，ポリプロピレン及びポリスチレン製品へのガンマ線照射の影響－揮発性物質**

食品照射，**35**, 15-22 (2000)

市販ポリエチレン，ポリプロピレン及びポリスチレン製品に10～50kGyのガンマ線を照射した．全てのポリエチレン及びポリプロピレンにおいて，照射により酸，アルデヒド，ケトン，アルコール等の揮発性物質が生成し，特に，酢酸及びアセトンの生成量が多かった．また，ポリプロピレンでは，分枝した化合物など生成物の種類が多く，しかも生成量も高く，ポリエチレンよりも照射分解を受けやすいことが示された．一方，ポリスチレンは，非照射時に残存していた原料モノマーであるスチレン及びエチルベンゼンの含量が照射により減少し，照射分解物と思われる揮発性物質の生成もわずかであり，ポリエチレンやポリプロピレンよりも照射耐性があることが示された．

Keywords: gamma irradiation, food contact plastic, volatiles

河村葉子，前原玉枝，和久井千世子，山田 隆：**ポリ塩化ビニル製手袋中の可塑剤及びノニルフェノールの溶出**

食品衛生学雑誌，**41**, 330-334 (2000)

ポリ塩化ビニル(PVC)製手袋に残存するフタル酸ジ(2-エチルヘキシル)(DEHP)，フタル酸ジイソノニル(DINP)，アジピン酸ジ(2-エチルヘキシル)(DEHA)及び4-ノニルフェノール(NP)は，水，20%エタノール及び4%酢酸では0.005～0.416 μg/cm²の溶出であったが，*n*-ヘプタン(25℃60分間)ではDEHPが1,410～2,500 μg/cm²，DINPが720 μg/cm²，DEHAが137～841 μg/cm²，NPが2.72～36.4 μg/cm²と極めて高い溶出を示した．ナタネ油への溶出量は，60℃30分間で薄手手袋では*n*-ヘプタンの1/2～1/4，やや厚手手袋では1/4～1/10に相当し，試験温度が高く，時間が長いほど溶出量は多くなったが，低温や短時間でもかなりの溶出が見られた．以上より，PVC製手袋を脂肪性食品に使用すると，残存するDEHP，DINP，DEHA，NPが大量に食品へ移行することが示唆された．

Keywords : polyvinyl chloride glove, migration test, di (2-ethylhexyl) phthalate

河村葉子，井之上浩一*，中澤裕之*，山田 隆，米谷民雄：**飲料缶からのビスフェノールA移行原因の解明と改良缶の評価**

食品衛生学雑誌，**42**, 13-17 (2001)

ビスフェノールA(BPA)含有量が高かったコーヒー及び紅茶飲料の相当缶を検討したところ、いずれもエポキシ樹脂コーティングに由来したが，サイドシームや底蓋部でBPA濃度が極めて高かったり，胴部がやや高いため缶全体の残存量が高いなど原因部位は様々であった．水60及び95℃，20%エタノール，*n*-ヘプタンではBPAは溶出しなかったが，水120℃30分間では35～124ng/mL溶出した．相当缶のBPA溶出量は材質中の残存量とほぼ一致し，缶入飲料のBPA含有量とも近い値であった．以上よりBPAの移行は，コーティング中のBPA残存量と飲料の加熱条件に依存することが示された．一方，改良缶ではコーティング中のBPA量が大幅に減少し，溶出量は1/10以下に低減された．

Keywords: bisphenol A, can for drink, epoxy resin

*星薬科大学

Fukuhara, K., Hara, Y., Nakanishi, I., Miyata, N.: **Hydroxylation of Nitrated Naphthalenes with KO_2/Crown Ether**

Chem. Pharm. Bull., **48**, 1532-1535(2000)

Superoxide radical anion ($O_2^{\cdot-}$), generated by KO_2/crown ether, is effective for hydroxylation of nitronaphthalenes. When mono- and di-nitronaphthalenes are treated with KO_2/crown ehter, hydroxylation results at the electron-deficient site caused by the electron withdrawing effect of the substituted nitro group. Kinetic experiments suggest that the hydroxylation proceeds by two different mechanisms dependent on the first one-electron reduction potential of nitronaphthalenes.

Key words : superoxide; nitroarene; hydroxylation

Kurihara, M., Tanaka, M.*1, Oba, M.*1, Suemune, H.*1, Miyata, N.: **Computational study on conformation of oligopeptides prepared from α,α-disubstituted amino acids**

Peptides 2000, **427-428** (2001)

Recently the conformation of peptides constituted by non-proteinogenic amino acids (β -amino acid, α , α -disubstituted amino acid, etc.) has received considerable attention. α , α -Disubstituted amino acids have two alkyl substituents at the α -position of normal α -amino acids and are conformationally restricted. To predict the conformation of these peptides presents an interesting challenge. We report the conformational analysis of oligopeptides prepared from α , α -disubstituted amino acids

(isovaline, diethylglycine, butylethylglycine) by computational study. Conformational energy computations on oligopeptides of α, α-disubstituted amino acids were performed using molecular mechanics. Conformational search calculations were carried out by the Monte Carlo method of MacroModel® (ver. 6.5, Schrodinger, Inc.). When AMBER* was used as the force field, the global minimum energy conformations were found to be 3_{10}-helix. These results are in agreement with their conformational properties in the solid state determined by X-ray crystallographic analysis. In the case of the MMFF force field the global minimum energy conformations of diethylglycine and butylethylglycine peptides are planar structures, which are in agreement with their conformations in solution.

Key words: α, α-disubstituted amino acid, oligopeptode, molecular mechanics calculation

*1 九州大学薬学部

Nakanishi, I., Yamakoshi, Y., Ohkubo, K.*1, Fujita, S.*1, Fujitsuka, M.*2, Ito, O.*2, Fukuzumi S.*1 and Miyata, N.: **Superoxide Generation in C_{60}-Photosensitized oxidation of NADH and an Analogue by Oxygen**

Fullerenes, **8**, 242-255 (2000)

Visible light irradiation of poly(vinylpyrrolidone) (PVP)-solubilized C60 in water in the presence of NADH and molecular oxygen results in the formation of superoxide anion ($O_2^{\cdot-}$), which was detected with use of 5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide (DEPMPO) as a $O_2^{\cdot-}$-trapping agent. Formation of $O_2^{\cdot-}$ having a characteristic *g//* value of 2.18 was also evidenced by the direct observation with use of a low-temperature ESR technique at 77 K. Photoinduced $O_2^{\cdot-}$ formation was also observed for the N-methyl-2-pyrrolidone (NMP) solution of C_{60} and 1-benzyl-1,4-dihydronicotinamide (BNAH) in the presence of O_2, whereas C_{60} radical anion ($C_{60}^{\cdot-}$) was formed in the absence of O_2 under otherwise the same experimental conditions. These results suggest that C_{60}^{2-} formed in the photoinduced electron-transfer reduction of C_{60} by BNAH acts as an electron donor to molecular oxygen to give $O_2^{\cdot-}$ in NMP.

Key words: Fullerene, superoxide, photosensitization,

*1 Osaka University
*2 Tohoku University

Fukuhara, K., Miyata, N.: **Electrochemical studies of quinone and nitroarene in generation and quenching of superoxide.**

Environ. Mutagen Res., **22**, 155-162(2000)

Quinones are common in several natural products and endogeneous biochemicals or generated through metabolism of aromatic hydrocarbons. Some quinones are potent redox active compounds which can undergo enzymatic redox cycling with their corresponding semiquinone anion radicals and as a result generate superoxide anion radicals.

In this paper, we review the catalytic activity of quinones and nitroarenes, as mediators in the reductive activation of molecular oxygen and in the oxidative quenching of superoxide, examined by electrochemical method.

Keywords: quinone, nitroarene, superoxide

栗原正明, 近藤一成, 鈴木 隆, 豊田正武, 宮田直樹：**カテキンの抗酸化作用機構：半経験的分子軌道法による解析**

磁気共鳴と医学, **12**, 114-117 (2001)

Catechins are a group of polyphenolic compounds abundantly contained in green tea. It is well known that catechins have multiple biological activities including anticarcinogenic and antiinflammatory effects. These protective effects are due to their antioxidative activities by scavenging free radicals. The antioxidative activity of catechins has been considered to arise from the potential to scavenge free radicals by donating hydrogen atoms from phenolic O-H. However the effectiveness of catechins for peroxyl radical scavenging cannot be explained by the donation of the phenolic hydrogen atom. In this paper we calculated bond dissociation enthalpies (BDE's) of all C-H and O-H bonds in catechins ((-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate) by semiempirical molecular orbital calculation (PM3) using the *SPARTAN* program(ver. 5.0, Wavefunction Inc.) and found that the BDE's of benzyl hydrogens (C-2 position in catechins) were quite low compared with O-H BDE's at phenolic sites. This result suggests that the abstraction of benzyl hydrogen plays an important role in antioxidant activity. This is also supported by the reported results of LC/MS and spectrophotometric analysis of the reaction intermediate from catechins treated with 2,2'-azobis(2-aminopropane)hydrochloride (AAPH).

Key words: catechin, Antioxidation Mechanism, semiempirical molecular orbital calculation

Kobayashi, S.*1, Kobayashi, H.*1, Yamaguchi, T.*1, Nishida, M*1., Yamaguchi, K.*2, Kurihara, M., Miyata, N., Tanaka, A.*1: **Synthesis, conformation, and chemical properties of new mini parallel double-stranded peptides conjugated with -Phe-Phe- and -Phe-Phe-X- sequences**

Chem. Pharm. Bull., **48**, 920-934 (2000)

To investigate the chemical conformations and functions of the -Phe-Phe-Val- or -Phe-Phe- sequences contained in the Alzheimer's disease related β-amyloid peptide, a series of mini parallel double-stranded peptides conjugated with two peptide residues to one spacer were designed and prepared. The structure of the compounds was elucidated by circular dichroism (CD) spectrum and NMR two dimensional (2D) nuclear overhauser enhancement and exchange spectroscopy (NOESY) measurments. The structure of 1,2-ethano-bis(L-Phe-L-Phe-L-Leu), 1, 12-dodecano-bis(L-Phe-L-Phe-L-Leu), 1,12-dodecano-bis(L-Phe-L-Phe-L-Val), and 1,12-dodecano (D-Phe-D-Phe-D-Leu) conjugated with L-Leu and L-Val residues show a β-turn-like nucleation. The dihedral angles (θ =+75°, +180°, ω =+90°, Φ =-87°, Ψ =+180°) obtained from experimental coupling constant (J) data, *etc.* support that 1,12-dodecano-bis(L-Phe-L-Phe) adopts β-turn mimic nucleation. The 1,12-dodecano-bis(L-Leu-L-Leu-L-Phe), 1,12-dodecano-bis(L-Ile-L-Phe-L-Leu), and 1,12-dodecano-bis(L-Phe-L-Val-L-Leu), *etc.* adopt most probably a random structure by CD studies. It was found by titration spectrum that an inclusion complex of 1 : 1 ratio (association constant; Ka=1.0x10^{4}M^{-1}) is formed between 1,12-dodecano-bis(L-Phe-L-Phe-L-Leu) and azobenzene (guest,

$[L_0]=1.758x10^{-5}M^{-1}$). Moreover, the stability of the complexes was increased in order of 1,12-dodecano-bis(L-Phe-L-Phe-L-Leu)・azobenzene>1,12-dodecano-bis (L-Phe-L-Phe-L-Val)・azobenzene>1,12-dodecano-bis(L-Phe-L-Val-L-Leu)・azobenzene. The data show that X-Phe-L-Phe-L-spacer (S)-L-Phe-L-Phe-X (X=amino acids; S=1,2-ethano- and 1,12-dodecano-) plays an important role as a binding site of the artificial receptor. The hydrophobic interaction of the four Phes in the two strands is a very interesting issue in the physiological action of proteins as wellas the conformation of the backbone of X-L-Phe-L-Phe-spacer(S)-L-Phe-L-Phe-X.

Key words: double-stranded peptide, β-turn mimetics, β-amiloid peptide

*1 昭和薬科大学
*2 千葉大学分析センター

Konno, K.*1, Fujishima, T.*1, Maki, S.*1, Liu, Z.*1, Miura, D.*2, Chokki, M.*2, Ishizuka, S.*2, Yamaguchi, K.*3, Kan, Y.*4, Kurihara, M., Miyata, N., Smith, C.*5, DeLuca, H. F.*5, Takayama, H.*1: **Synthesis, biological evaluation, and conformational analysis of A-ring diastereomers of 2-methyl-1,25-dihydroxyvitamin D3 and its 20-epimers.**

J. Med. Chem., **43**, 4247-4265 (2000)

All eight possible A-ring diastereomers of 2-methyl-1, 25-dihydroxyvitamin D_3 (**2**) and 2-methyl-20-epi-1, 25-dihydroxyvitamin D_3 (**3**) were convergently synthesized. The A-ring enyne synthons 19 were synthesized starting with methyl (*S*)-(+)- or (*R*)-(-)-3-hydroxy-2-methylpropionate (**8**). This was converted to the alcohol **14** as a 1:1 epimeric mixture in several steps. After having been separated by column chromatography, each isomer led to he requisite A-ring enyne synthons **19** again as 1:1 mixtures at C-1. Coupling of the resulting A-ring enynes **20a-h** with the CD-ring portions **5a,b** in the presence of a Pd catalyst afforded the 2-methyl analogues **2a-h** and **3a-h** in good yield. In this way, all possible A-ring diastereomers were synthesized. The synthesized analogues were biologically evaluated both in vitro and in vivo. The potency was highly dependent on the stereochemistry of each isomer. In particular, the alpha alpha beta-isomer **2g** exhibited 4-fold higher potency than 1 alpha,25-dihydroxyvitamin D_3 (**1**) both in bovine thymus VDR binding and in elevation of rat serum calcium concentration and was twice as potent as the parent compound in HL-60 cell differentiation. Furthermore, its 20-epimer, that is, 20-epi-alpha alpha beta **3g**, exhibited exceptionally high activities: 12-fold higher in VDR binding affinity, 7-fold higher in calcium mobilization, and 590-fold higher in HL-60 cell differentiation, as compared to 1 alpha,25-dihydroxyvitamin D_3 (**1**). Accordingly, the double modification of 2-methyl substitution and 20-epimerization resulted in unique activity profiles. Conformational analysis of the A-ring by ^{1}H NMR and an X-ray crystallographic analysis of the alpha alpha beta-isomer **2g** are also described.

Key words: vitamin D_3, conformational analysis, MacroModel

*1 帝京大学薬学部
*2 帝人
*3 千葉大学分析センター
*4 サントリー生有研
*5 University of Wisconsin-Madison

Kittaka, A.*1, Suhara, Y.*1, Takayanagi, H.*1, Fujishima, T.*1, Kurihara, M., Takayama, H.*1: **A concise and efficient route to 2 α -(ω -Hydroxyalkoxy)-1 α , 25-dihydroxyvitamin D_3: remarkable high affinity to vitamin D receptor**

Org. Lett., **2**, 2619-2622 (2000)

A convenient and potentially valuable synthetic approach to the novel 2alpha-functionalized 1alpha,25-dihydroxyvitamin D_3 [1alpha,25$(OH)_2D_3$] derivatives (1a-c), which are the C2-epimer of ED-71 and its analogues, has been developed. The C2alpha-modified ring A precursors (1,7-enynes 16, n = 0, 1, and 2) were constructed stereoselectively starting from D-glucose in high yield. In the synthesized 2alpha-(omega-hydroxyalkoxy)-1alpha, 25$(OH)_2D_3$ derivatives, 1a and 1b showed a greater binding affinity to vitamin D receptor (VDR), up to 1.8 times that of the native hormone.

Key words: vitamin D_3, vitamin D receptor, MacroModel

*1 帝京大学薬学部

Tanaka, M.*1, Oba, M.*1, Imawaka, N.*1, Tanaka, Y.*1, Kurihara, M., Suemune, H.*1: **Cnoformational study of heteropentapeptides containing an α -ethylated α , α -disubstituted amino acids: (S)-butylethylglycine (= 2-amino-2-ethylhexanoic acid) within dimethylglycine (= 2-aminoisobutyric acid) residues**

Helv. Chim. Acta., **84**, 32-46 (2001)

Heteropentapeptides containing the α -ethylated α , α -disubstituted amino acid (S)-butylethylglycine and four dimethylglycine residues, i.e., CF_3CO-[(S)-Beg]-$(Aib)_4$-OEt (4) and CF_3CO-$(Aib)_2$-[(S)-Beg]-$(Aib)_2$-OEt (7), were synthesized by conventional solution methods. In the solid state, the preferred conformation of 4 was shown to be both a right-handed (*P*) and a left-handed (*M*) 310-helical structure, and that of 7 was a right-handed (*P*) 3_{10}-helical structure. IR, CD, and ^{1}H-NMR spectra revealed that the dominant conformation of both 4 and 7 in solution was the 3_{10}-helical structure. These conformations were also supported by molecular-mechanics calculations.

Key words: α , α -disubstituted amino acid, oligopeptode, molecular mechanics calculation

*1 九州大学薬学部

Tanaka, M.*1, Imawaka, N.*1, Oba, M.*1, Kurihara, M., Suemune, H.*1: **Conformational study of peptides containing α -ethylated α , α -disubstituted amino acid: (*s*)-Buthylethylglycine.**

Peptides 2000, 441-442 (2001)

α , α -Disubsituted amino acids and their peptides have attracted considerable attention since these amino acids and peptides show unique biological activities and very stable secondary structures. The conformation of the peptides prepared from achiral α , α -disubstituted amino acids such as 2-aminobutyric acid: Aib, dialkylglycine has been studied extensively because the achiral α , α -disubstituted amino acids could be easily prepared. The property of Aib is known to be a 3_{10}-helical structure, and that of dialkylglycine such as diethylglycine and dipropylglycine is proved to be a fully planar C_5-conformation. Recent develop-

ment of asymmetric reaction enables the peptide chemists to synthesize several chiral α , α -disubstituted amino acids, and the properties of α -methylated α , α -disubstituted amino acids are known to be 3_{10}-helical structure.

We studied the conformation of peptides prepared from a chiral α -ethylated α , α -disubstituted amino acid; (*S*)-butylethylglycine (=2-amino-2-ethylhexanoic acid: Beg). The homopeptides containing (*S*)-Beg (up to hexapeptide) were prepared by using solution-phase methods employing an ethyl ester as the C-terminal protection and a trifluoroacetyl group as the N-terminal protection. The heteropentapeptides containing (*S*)-Beg as a guest molecule in the Aib sequence were also prepared in the similar manner. The conformations of these peptides in the solid state were studied using X-ray crystallographic analysis, and those in solution were studied using IR, ^{1}H NMR, and CD spectra. The dominant conformation of homopeptides containing (*S*)-Beg was a fully planar C5-structure, and that of heteropeptides containing (*S*)-Beg and Aib was a 3_{10}-helical structure.

Key words : α , α -disubstituted amino acid, oligopeptode, molecular mechanics calculation

*1 九州大学薬学部

Ohnishi, S.*1, Murata, M.*1, Fukuhara, K., Miytata, M., Kawanishi, K.*1: **Oxidative DNA damage by a metabolite of carcinogenic 1-nitropyrene**

Biochem. Biophy. Res. Comm., **280**, 48-52(2001)

Nitropyrenes are carcinogenic pollutants. Adduct formation following nitro-reduction is considered to be a major cause of nitropyrene-mediated DNA damage. We investigated the role of 1-nitrosopyrene, a metabolite of 1-nitropyrene, in causing oxidative DNA damage, using ^{32}P-5'-end-labeled DNA.1-Nitrosopyrenewas found to facilitate Cu(II)-mediated DNA damage in the presence of NADH. Catalase and a Cu(I)-specific chelator attenuated DNA damage, indicating the involvement of H_2O_2 and Cu(I). Typical ¥OH scavenger did not have a significant effect. These results suggest that the main reactive species is probably a DNA-copper-hydroperoxo complex. We also measured 8-oxo-7,8-dihydro-2'-deoxyguanosine formation by 1-nitorosopyrene in the presence of Cu(II) and NADH, using an electrochemical detector coupled to a high-pressure liquid chromatograph. We conclude that oxidative DNA damage, in addition to DNA adduct formation, may play an important role in the carcinogenesis of nitropyrenes.

Keywords : Nitropyrene, Nitrosopyrene, DNA damage, Copper, Hydrogen peroxide

*1Mie University School of Medicine

Hachisuka, A., Nakajima, O., Yamazaki, T., Sawada, J. : **Developmental expression of opioid-binding cell adhesion molecule (OBCAM) in rat brain**

Dev.Brain Res., **122**, 183-191 (2000)

OBCAM, a neuron-specific protein, has been presumed to play a role as a cell adhesion/recognition molecule, but its function has not been fully elucidated. We investigated the developmental expression of OBCAM in rat brain by using a monoclonal anti-OBCAM peptide antibody (OBC53). OBCAM was clearly detectable on embryonic day 16 (E16) as assessed by immunoblotting. The expression level increased by the second postnatal week and was maintained at a constant level until week 17. During the early developmental period OBCAM was found to be expressed on postmitotic neurons and to be strongly expressed in at the fiber tracts containing expanding axons, in contrast to the adult brain, in which OBCAM is principally expressed in the gray matter. These findings suggest that the function of OBCAM involves axonal outgrowth.

Keywords: OBCAM, development, immunohistochemistry

Suzuki R., Furuno T., Teshima R., Nakanishi M.: **Bi-directional relationship of in vitro mast cell-nerve communication observed by confocal laser scanning microscopy.**

Biol. Pharm. Bull., **24**,291-294 (2001)

Communication between nerves and mast cells is a prototypic demonstration of neuro-immune interaction. Recently, we used an in vitro co-culture approach comprising cultured murine superior cervical ganglia (SCG) and rat basophilic leukemia (RBL) cells to study this interaction. In the present work, we studied the communication from mast cells to neurites. We observed that binding of anti-IgE receptor antibodies to mast cells increases calcium ion concentration $[Ca^{2+}]_i$ in SCG neurites. This indicates that mast cell-nerve communication is bi-directional. Confocal fluorescence microscopic images indicated that $[Ca^{2+}]_i$ in neurites increased after an increase of $[Ca^{2+}]_i$ in mast cells. The lag-time of neurite activation was several times longer than that of mast cell activation.

Keywords:nerve-immune interaction, co-culture,rat basophilic leukemia cells

Aketani S.*, Teshima R., Umezawa Y.*, Sawada J.: **Correlation between cytosolic calcium concentration and degranulation in RBL-2H3 cells in the presence of various concentrations of antigen-specific IgEs.**

Immunol. Lett.,**75**,185-189 (2001)

We studied the dependence of β -hexosaminidase release from RBL-2H3 cells on the antigen-specific IgE concentrations. The cells were sensitized with DNP-specific IgE (0.5-5000 ng/ml) or OVA-specific IgE (5-50 ng/ml) and stimulated with DNP_{35}-HSA (10^{-2}-100 ng/ml) or OVA (10^{-1}ng/ml-10 μg/ml). It was found that the β-hexosaminidase release increased in a dose-dependent manner with the concentration of the IgEs added to the mast-cell suspension. The percentage of β-hexosaminidase release from the cells was well correlated with $[Ca^{2+}]_i$ increase, and the correlation coefficient was 0.88 for DNP-specific IgE and 0.99 for OVA-specific IgE. Therefore, the $[Ca^{2+}]_i$ monitoring system is a sensitive marker of degranulation from RBL-2H3 cells and can be used to measure even low amounts of antigen-specific IgE.

Keywords:degranulation,calcium signal, IgE

*The University of Tokyo

Okunuki H.,Teshima R., Sakushima J., Akiyama H., Goda Y., Toyoda M., Sawada J.: **Induction of active systemic anaphylaxis by oral sensitization with ovalbumin in mast-cell-deficient mice.**

Immunol. Lett.,**74**,233-237 (2000)

Mast-cell-deficient W/W(v) mice were sensitized by oral administration of 0.1 and 1.0 mg ovalbumin (OVA) by gavage every day for 9 weeks, and active systemic anaphylaxis (ASA) was induced by intraperitoneal injection of OVA. The production of OVA-specific IgE and IgG1 by oral immunization of the W/W(v) mice was high, and the production of IL-4 by splenocytes re-stimulated with OVA in vitro was increased. In contrast, production of OVA-specific IgG2a and IgG2b was low, and production of IFN-gamma by splenocytes after re-stimulation with OVA in vitro was rather decreased. These findings suggest that Th2-dominant helper T-cell activation had occurred. The plasma platelet-activating factor(PAF) levels of the mice sensitized with 0.1 and 1.0 mg OVA by gavage increased significantly. W/W(v) mice seems to be a good model for studying induction of food allergy.

Keywords : Mast cell-deficient mice, PAF, food allergy

Aketani S.*, Teshima R., Sawada J., Umezawa Y.*: **A screening method for antigen-specific IgE using mast cells based on intracellular calcium signaling.**

Anal.Chem., **72**, 2653-2658 (2000)

A simple screening method is presented for the measurement of antigen-specific IgEs in sera in which mast cells are used. This method is based on the intracellular calcium signal in mast cells induced by cross-linking the surface high-affinity Fc receptors (Fc εRIs) with IgEs and multivalent antigens. Two kinds of rodent mast cells, RBL-2H3 cells and mouse BMMCs, were used. Two antigen-specific IgEs (DNP-specific IgE, OVA-specific IgE) was used. It was found that $[Ca^{2+}]_i$ increased linearly with IgE concentrations ranging from 25 to 5000 ng/mL for DNP-specific IgE and from 5 to 50 ng/mL for OVA-specific IgE. By monitoring the increase of $[Ca^{2+}]_i$ in mast cells, we could determine the antigen-specific IgEs. The present immunological assay based on the Ca^{2+} signal transduction in mast cells offers new possibilities for efficient screening of antigen-specific IgEs.

Keywords : calcium signal, RBL-2H3 cells, BMMC

* The University of Tokyo

Teshima R., Onose J., Okunuki H., Sawada J.: **The effect of Ca^{2+}-ATPase inhibitors on the stimulation of mast cells.**

Recent.Res.Develop.Immunol., **2**, 141-151 (2000)

The effects of two Ca^{2+}-ATPase inhibitors, thapsigargin (TG) and cyclopiazonic acid (CPA), and three hydroquinone-antioxidants, DTBHQ, DTAHQ, MTBHQ on the production of various cytokines and degranulation and LTC4 release from RBL-2H3 cells and BMMC (bone marrow-derived mast cells) were investigated. TG, CPA, DTBHQ and DTAHQ, all of which induce intracellular free Ca^{2+} concentration($[Ca^{2+}]_i$) increase, induced degranulation and TNF- α release in the presence of TPA in a dose-dependent manner. In contrast, MTBHQ, which does not induce increase in $[Ca^{2+}]_i$ did not induce the release of histamine and TNF-α. LTC4 production and IL-4 and MCP-1 release were increased by CPA and DTBHQ in a dose-dependent manner without TPA. There were two types of mediator release process in RBL-2H3 cells; 1.($[Ca^{2+}]_i$) increase itself is sufficient, 2.($[Ca^{2+}]_i$) increase and PKC work synergistically.

Keywords : Ca^{2+}-ATPase inhibitors, TNF-a, mast cells

Teshima R., Akiyama H., Okunuki H., Sakushima J., Goda Y., Onodera H., Sawada J., Toyoda M.: **Effect of GM and non-GM soybeans on the immune system of BN rats and B10A mice.**

J.Food Hyg.Soc.Japan, **41**, 188-193 (2000)

Subchronic animal feeding studies to examine the effect of glyphosate-tolerant soybeans, which contain the bacterial 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp. strain CP4, on the immune system were conducted with BN rats and B10A mice. The studies were designed to compare the feeding value of a line of genetically modified glyphosate-tolerant soybeans (GM soybeans) to that of closely-related and one-parent same cultivar (non-GM soybeans). Heat-treated soybean meal was incorporated into the diets of the rats and mice at a concentration of 30%. The study duration was 15 weeks. Growth, food intake and weights of the liver and the spleen were compared between animals fed the non-GM and GM lines. Growth, feeding value, and the histopathology of immune-related organs showed no significant differences between animals fed GM and non-GM lines. No immunotoxic activity was found in GM-soybean-fed rats or mice.

Keywords : GM soybeans; CP4-EPSPS; immune system

Matsui, S.*, Adachi, R., Kusui, K., Yamaguchi, T., Kasahara, T.*, Hayakawa, T. and Suzuki, K.: **U73122 inhibits the dephosphorylation and translocation of cofilin in activated macrophage-like U937 cells**

Cell. Signalling, **13**, 17-22 (2001)

We found that U73122, an inhibitor of phospholipase C (PLC), suppressed both opsonized zymosan (OZ)-induced dephosphorylation and translocation of cofilin in macrophage-like U937 cells. OZ triggered an increase in inositol 1,4,5-trisphosphate (IP3), and U73122 inhibited it. U73343, which was employed as an inactive analogue, had no such inhibitory activities as did U73122. Furthermore, herbimycin A, an inhibitor of src-type tyrosine kinase, also inhibited OZ-triggered IP3 formation. These results suggest that the activity and localization of cofilin are regulated by PLC at the downstream of src-family tyrosine kinase.

Keywords: cofilin, U73122, phosholipase C

*共立薬科大学

Adachi, R., Matsui, S.*, Kinoshita, M.*, Nagaishi, K.*, Sasaki, H., Kasahara, T.*, and Suzuki, K.: **Nitric oxide induces chemotaxis of neutrophil-like HL-60 cells and translocation of cofilin to plasma membranes**

Int. J. Immunopharmacol., **22**, 855-864 (2000)

We investigated chemotaxis of neutrophil-like HL-60 cells induced by NO, as well as the influence of NO on cofilin, an actin-binding phosphoprotein. Two NO donors were shown to cause chemotaxis, and a NO-specific scavenger inhibited the chemotaxis. Inhibitors of soluble guanylate cyclase inhibited this NO action. We also found that NO caused translocation of cofilin to the cell periphery, though dephosphorylation of cofilin was not detected. These results demonstrated that NO has chemotactic activity for neutrophils and caused the translocation of cofilin to

the plasma , embrane regions without its dephosphorylation.
Keywords : nitric oxide, chemotaxis, cofilin
*共立薬科大学

安達玲子, 楠井 薫, 鈴木和博: **食細胞の機能発現におけるコフィリンの役割**
炎症, Vol.20, No.6, 667-674 (2000)

Phagocytes play a central role in the host defense system. We have previously reported that cofilin, an actin- and PIP2-binding phosphoprotein , is dephosphorylated upon activation of phagocytes. We investigated the relationship between cofilin and phagocyte activation. Opsonized zymosan (OZ) induced dephosphorylation and translocation to the plasma membrane regions of cofilin. Both an inhibitor of Src family tyrosine kinase and an inhibitor of phospholipase C (PLC) inhibited OZ-induced superoxide production and phagocytosis as well as dephosphorylation and translocation of cofilin. It was shown that the activation of phagocytes and the change on the condition of cofilin are significantly related. The signaling pathway from OZ stimulation to cell response including Src family tyrosine kinase and PLC is discussed.
Keywords : phagocyte activation, signaling pathway, cofilin

Schmidt, A.G.*1, Kadambi, V.J.*1, Ball, N.*1, Sato, Y., Walsh, R.A.*2, Kranias, E.G.*1, Hoit, B.D.*2
Cardiac-specific overexpression of calsequestrin results in left ventricular hypertrophy, depressed force-frequency relation and pulsus alternans in vivo
J. Mol. Cell. Cardiol., **32**, 1735-1744 (2000)

The purpose of the present study was to determine the effects of calsequestrin (CSQ) overexpression on basal cardiac function and the force-frequency relation in vivo. CSQ overexpressing mice and their isogenic controls were studied with an integrative approach using transthoracic echocardiography, stress-shortening relations, and invasive hemodynamics in intact closed-chest mice. Our findings indicated that: (i) although increased levels of CSQ result in decreased myocardial contractility and a depressed force-frequency relation, LV wall stress is reduced and chamber function is normal, and (ii) an increase in SR Ca2+storage capacity induces pulsus alternans in the intact anesthetized mouse.
Keywords: calsequestrin, hemodynamics, transgenic mice
*1 University of Cincinnati, USA
*2 Case Western Reserve University, USA

Sato, Y., Kiriazis, H.*1, Yatani, A.*1, Schmidt, A.G.*1, Hahn, H.*1, Ferguson, D.G.*2, Sako, H.*1, Mitarai, S.*1, Honda, R.*1, Mesnard-Rouiller, L.*1, Frank, K.F.*1, Beyermann, B.*1, Wu, G.*1, Fujimori, K., Dorn, G.W. II *1, Kranias, E.G.*1
Rescue of contractile parameters and myocyte hypertrophy in calsequestrin overexpressing myocardium by phospholamban ablation
J. Biol. Chem., **276**, 9392-9399 (2001)

To test the hypothesis that inhibition of phospholamban activity may rescue myocardial abnormalities due to defects in sarcoplasmic reticulum, calsequestrin overexpressing mice were crossbred with phospholamban-knockout mice. Phospholamban ablation in calsequestrin overexpressing mice led to reversal of the depressed cardiac contractile parameters, restoration in the ability of ICa to trigger SR Ca2+ release, and normalization of ventricular myocyte size. These results indicate that attenuation of phospholamban function may prevent or overcome functional and remodeling defects in hypertrophied hearts.
Keywords: phospholamban, transgenic mice, cardiac hypertrophy
*1 University of Cincinnati, USA
*2 Case Western Reserve University, USA

Ken-ichi Tanamoto, Hitomi Kato, Yuji Haishima and Satoko Azumi: **Biological property of lipid A isolated from Flavobacterium meningosepticum.**
Clin. Diagn. Lab. Immunol., **8**, 522-527 (2001)

The lipid A from *Flavobacterium meningosepticum* exhibited generally moderate activity compared to *Salmonella abortus equi* LPS used as a control in all the assay systems tested. The moderate activity of the lipid A may be explained by the unique fatty acid composition and the lack of a phosphate group in position 4'. Noticeably, the lipid A apparently induced TNF-a release from peritoneal macrophages in LPS-unresponsive C3H/HeJ, and the activation was suppressed by the LPS-specific antagonist. Taken together with the previous results concerning *Porphyromonas gingivalis* lipid A, which has high structural similarities to that of *F. meningosepticum*, and the induction of TNF-a release in macrophages from C3H/HeJ mice, the lipid A having novel fatty acids may possibly play an role for the activation of C3H/HeJ macrophages.
Key words: lipid A, C3H/HeJ mice, Flavobacterium meningosepticum

Ken-ichi Tanamoto, Takatoshi Iida, Yuji Haishima and Satoko, Azumi: **Endotoxic property of lipid A from *Comamonas testosteroni.***
Microbiology, **147**, 1087-1094 (2001)

The lipid A from *Comamonas testosteroni* was characterized by its relatively short-chain length (C10) of the 3-hydroxy fatty acid components directly bound to the glucosamine disaccharide backbone by either amide or ester linkage. The lipid A exhibited endotoxic activity in all of the assay systems tested to the same extent with those of *Salmonella* lipid A or *E. coli* lipopolysaccharide used as a control. The strong endotoxic activity of the lipid A indicates that the composition of 3-hydroxydecanoic acid is not responsible for the low endotoxicity of lipid A. Furthermore both the defect of second acylation of 3-hydroxy fatty acid attached to the position 3', and the substitution of a hydroxyl group to the 3-hydroxy fatty acid attached at position 2 do not affect the manifestation of endotoxic activity or species specificity.
Keywords: LPS, TNF-α, NO

Fujihara, M.*1, Wakamoto, S.*1, Ito, T.*1, Muroi M, Suzuki, T*2 Ikeda, H.*1, Ikebuchi, K.*1: **Lipopolysaccharide-triggered desensitization of TNF-α mRNA expression involves lack of phosphorylation of IκBα in a murine macrophage-like cell line, P388D1.**
J. Leukoc. Biol., **68**, 267-76 (2000)

We investigated the role of NF-κB in lipopolysaccharide (LPS)-induced desensitization of TNF-α gene expression in P388D1 cells. Gel-shift assays revealed that nuclear localization of p65/p50, c-rel/p50 and p65/c-rel, and p65 homodimers was reduced in LPS-tolerant cells, whereas that of p50 homodimers was only slightly increased. Western analysis showed that the phosphorylation of Ser32 on IκB α and its degradation did not occur in LPS-tolerant cells. These results suggest that desensitization of TNF-α gene expression in LPS-tolerant cells is closely associated with down-regulation of transactivating NF-κB and may involve a defect in the LPS-induced IκBα kinase pathway.

Keywords: NF-κB, signal transduction, Toll-like receptor 4

*1 日本赤十字社北海道支部
*2 Department of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center

Yukiko Hara-Kudo*1, Michiko Miyahara, and Susumu Kumagai *1 : **Loss of O157 O Antigenicity of Verotoxin-Producing Escherichia coli O157:H7 Surviving under Starving Conditions**

Appl. Environ. Microbiol., **66**, 5540-5543 (2000)

Verotoxin (VT)-producing *Escherichia coli* O157:H7 was culturable on agar media after being left in water for 21 months. However, there were a number of colonies which had lost O157 O antigenicity. These colonies produced VTs, which are pathogenic to humans. These observations suggest that the immunologic methods based on O157 O antigenicity are unable to detect and isolate VT-producing *E. coli* in foods and other enviroonments if the organism has been under starvation conditions for a long period.

Keywords: *Escherichia coli* O157:H7; O-antigenicity; verotoxin

*1 国立感染症研究所

Michiko Miyahara, Makoto Miyahara : **Effects of Gammaand E-beam Irradiation on Survival of Anaerobic and Aerobic Bacteria**

On line ; *12th International Meeting on Radiation Processing Extended Synopsis*

http://www.atriplei.com/pdf/IMRP_3-P05_3-231.pdf(2001.3~)

Extended approvals of irradiated foods are requested, due to increase of food poisoning cases in the world. There are some discussions on effects on survival of bacteria by both irradiations (gamma rays and electron beam). Some anaerobic and aerobic bacteria irradiated will be tested on survival effects on agar in packaged atmosphere. We may propose preferable irradiation for reduction of some bacteria population.

Keywords : gamma irradiation; electron beam irradiation; bacteria

Yukiko Hara-Kudo*1, Susumu Kumagai*2, Takashi Masuda*3, Koukichi Goto*4, Kayoko Ohtsuka*5, Hiroyuki Masaki*5, Hiroyuki Tanaka*6, Kenji Tanno*6, Michiko Miyahara, and Hirotaka Konuma : **Detection of *Salmonella* enteritidis in shell and liquid eggs using enrichment and plating**

Intl. J Food Microbiol., **64**, (2001), 395-399

Detectrion methods using various enrichment and plating media and immunoconcentration for Salmonella enteritidis in shell and liquid eggs were evaluated. For liquid egg samples naturally contaminated with *S.* enteritidis, pre-enrichment in 225 ml of buffered peptone water with cysteine followed by selective enrichment in 10 ml of tetrathionate broth was the superior, resulting in the detection of *S.* enteritidis in all samples on six of the seven types of selective agar substrate investigated. This enrichment procedure also enabled detection of *S.* enteritidis in most of artificially inoculated shell egg and pasteurized liquid egg samples.

Keywords : *Salmonella* enteritidis; shell Egg ; Detection

*1 国立感染症研究所
*2 東京大学院農生命
*3 静岡県環境科学研究所
*4 新潟県食肉衛生検査センター
*5 埼玉県衛生研究所
*6 日本食品分析センター

工藤由起子*1, 小西良子*1, 春日文子*1, 伊藤嘉典*1, 岩城正昭*1, 斉藤典子*1, 小沼博隆, 熊谷 進*1 : **腸管出血性大腸菌O157:7によって実験的に汚染した野菜種子に関する研究**

日食微誌, **17**, 201-205(2000)

野菜種子が食中毒細菌に汚染され, 生育した野菜が食中毒を引き起こすことが知られている. 腸管出血性大腸菌O157:7 (*E. coli* 0157:H7) による実験的汚染種子の冷蔵保存中の菌の消長を調べた結果, カイワレ大根種子10 g当たり約10^3cfuの汚染濃度の場合は36週間以上生存し, 約10^2cfuの場合は20週間以上生存した. 小松菜種子において本菌はカイワレ大根よりも速く死滅した. また, *E. coli* 0157:H7で実験的に汚染させたカイワレ大根種子を栽培し, 得られた可食部における*E. coli* 0157:H7の分布を電子顕微鏡下で観察し, 気孔周辺に多くの本菌が存在することが判明した.

Keywords : *Escherichia coli* O157:H7, radish sprout, seed,

*1 国立感染症研究所

Nakagawa, H*1, Hara-Kudo, Y.*2, Kojima,T., Ikedo, M*3, Kodaka, H.*4, Konuma,, and Kumagai, S.*2 : **Detection of freeze-injured *Escherichia coli* O157:H7 from foods by resuscitation prior to selective enrichment**

Int. J. Food Microbiol., **60**, 107-110(2000)

We tried to detect *Escherichia coli* O157:H7 in food samples artificially contaminated with freeze-injured *Escherichia coli* O157:H7 using an enrichment method with modified EC broth supplemented with novobiocin. When the samples were cultured for enrichment immediately after inoculation of freeze-injured cells, *E. coli* O157 was not detected in 13 out of 18 samples. Ground beef or radish sprouts inoculated with 6 colony forming units of *E. coli* O26 were homogenized in 225ml of various broths. After static incubation at 37℃ or 42℃ for 6h or 18h, we isolated the inoculated bacterium by plating onto Rainbow Agar O157 with novobiocin. In combination with the immunomagnetic separation methd, *E. coli* O26 was isolated from all samples by using enrichment in tryptone soy broth at 37℃ for 6h and in modified E. coli broth with novobiocin(mEC+n) at 42℃ for 18h ground beef and radish sprouts, respectively. Enrichment *E. coli* O157 from both ground beef and radish sprouts.

Keywords : *Escherichia coli* O26, *Escherichia coli* O157:H7,

ground beef
*1財団法人東京顕微鏡院
*2国立感染症研究所
*3栄研化学株式会社
*4日水製薬株式会社

後藤公吉*1, 渡 昭博*2, 瀬ノ口芳文*3, 春口真一*4, 増田高志*5, 塚本定三*6, 小沼博隆, 品川邦汎*7：**食肉のサルモネラモニタリング**
日本獣医師誌, **53**, 473-477(2000)

わが国の対米輸出食肉と畜場では米国農務省の改正により, サルモネラのモニタリングを行うことが義務づけられた. このサルモネラの検査法は, 米国農務省の食品安全検査局(FSIS)より検出感度97%以上, 検出特異性96%以上の方法であることが規定されている. 今回, サルモネラの検査として, わが国で通常行われている食品衛生検査指針に示されている方法（食衛法と）FSISの方法(FSIS法)について比較検討した. その結果、硫化水素（H2S）生産サルモネラの検出では食衛法はFSIS法と同等の成績であったが, H2S非生産サルモネラでは食衛法はFSIS法に比べ明らかに低い検出率であった.

Keywords : HACCP, H2S, *Salmonella*
*1新潟県食肉衛生検査センター
*2群馬県中央食肉衛生検査所
*3宮崎県食肉衛生検査所
*4鹿児島県末吉食肉衛生検査所
*5静岡県環境衛生科学研究所
*6大阪府公衆衛生研究所
*7岩手大学農学部

Sakai, A., Teshima, R.: **2,5-Di-tert-butyl-1,4-hydroquinone enhances cell transformation accompanied by an increase in intracellular free calcium ion concentration**
Cancer Lett., **168**, 183-190 (2001)

Two hydroquinones with similar structures, 2,5-di-*tert*-butyl-1,4-hydroquinone (DTBHQ) and 2-*tert*-butyl-1,4-hydroquinone (MTBHQ), are used as antioxidants in the environment. DTBHQ and MTBHQ were examined for their ability to induce cell transformation using BALB/3T3 cells. DTBHQ at concentrations of 2.5-15 μM enhanced cell transformation initiated by a subthreshold dose of 3-methylcholanthrene (MCA) in a two-stage cell transformation assay. Because DTBHQ is known to act as a calcium ion mobilizing agent in other cells, we examined the effects of DTBHQ on intracellular free calcium ion concentration ($[Ca^{2+}]_i$) in BALB/3T3 cells. DTBHQ elevated $[Ca^{2+}]_i$ with a dose dependency similar to that of its enhancing effect on the MCA-initiated cell transformation. MTBHQ neither enhanced cell transformation nor induced increase of $[Ca^{2+}]_i$. Aberrant calcium signaling produced by DTBHQ might contribute to the enhancement of MCA-initiated transformation in BALB/3T3 cells.

Keywords : 2,5-di-*tert*-butyl-1,4-hydroquinone, cell transformation, calcium ion

Sakai, A.: ***p*-Nonylphenol acts as a promoter in the BALB/3T3 cell transformation**
Mutation Res., **493**, 161-166 (2001)

p-Nonylphenol (NP) has attracted attention as an estrogenic contaminant, and the environmental pollution by NP has been found to be extensive. NP is classified as a phenolic antioxidant. Some phenolic antioxidants are known to induce and/or enhance carcinogenesis. We examined the effects of NP on the two-stage transformation of BALB/3T3 cells, a model of two-stage carcinogenesis. The treatment by NP in the promotion phase markedly enhanced the transformation of the cells pretreated with a subthreshold dose of a carcinogen, 3-methylcholanthrene (MCA), but not that of non-pretreated cells. The promoting activity of NP was approximately one hundredth of that of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The treatment by NP in the initiation phase did not induce cell transformation with and without post-treatment by TPA. These results indicate that NP acts as a pure promoter of cell transformation. The enhancement by NP of MCA-initiated transformation was suggested not to be mediated by estrogen receptors in BALB/3T3 cells because 17 β-estradiol did not promote cell transformation in our experiments, and it has been reported that BALB/3T3 cells do not express estrogen receptors at a detectable level.

Keywords : Nonylphenol; cell transformation; tumor promotion

村松芳多子*1, 太田利子*2, 相原真紀*3, 朴鍾吉吉*4, 徐闊*4, 鈴木明子, 成田紀子, 高鳥浩介：**真菌定量試験における適培養期間**
防菌防黴, **28**(4):225-230(2000)

真菌定量試験の基本的条件として, 培養期間の問題がある。国内にある真菌試験の規格をみてもかなり条件が異なる。そこで, 今後の規格設定するための基礎実験として, 多種真菌を用いて, 適培養期間と定量性をあわせて検討した。

Keywords : optimal growth period, quantitative test, CFU
*1千葉県立衛生短期大学
*2相模女子大学
*3お茶の水女子大学
*4 Yonsei University, Korea

相原真紀*, 田中辰明*, 高鳥浩介：**居住環境壁面にみる真菌の垂直分布**
防菌防黴, **28** (7) : 421-426 (2000)

居住環境にみる真菌の生態を研究しており, 微気象と真菌の関係を検討した。真菌の分布をみると最も影響するであろう湿度の観点から研究する必要がある。そこで, 同一居住環境での壁面にみる真菌垂直分布を検索した。環境条件として, 温湿度を測定したところ, 温度変化は少ないが, 湿度は下方ほど高い傾向にあった。そこで真菌分布をみたところ, 下方ほどCFUが有意に高かった。さらに主要真菌にも特徴がみられた。

Keywords : fungal CFU and distribution,vertical point of dwelling, micro meteorology
*お茶の水女子大学

小菅旬子*1, 後藤義隆*1, 新城敏晴*1, 安斉了*2, 高鳥浩介：**ウマ飼育環境における敷料からの喉嚢炎原因真菌Emericella nidulansの検出とその意義**
真菌誌, **41** : 251-256 (2000)

真菌性喉嚢炎の起因真菌であるEmericella nidulansは

ウマ特有の感染症であり，いままで本菌の起病性，疫学を調査してきた。ここでは，衛生学的観点からE. nidulansの環境での生息性を敷料で検討し，さらに分布する因子を研究した。さらに本菌の分布意義について考察を加えた。
Keywords : *Emericella nidulans*, horse bedding,gattural pouch mycosis
*1 宮崎大学
*2 日本中央競馬会

高橋淳子*1, 土谷正和*2, 田中重則*3, 中瀬崇*4, 山口英世*5, 高鳥浩介：**環境水と浄水にみる真菌とその化学的背景**
秦野研報，**23** : 18-23(2000)
水環境での真菌は，環境汚染のひとつのバロメーターとなりうる。同時に水質の程度を知るうえでも重要である。ところが，こうした水環境での真菌に関する規格基準は必要であるにもかかわらず成文化されていない。そこで環境水として河川，湖沼，浄水，工業用水にみる真菌と水質の分析をおこない，今後の規格基準の基礎資料とする。
Keywords : water, fungi, chemical analysis
*1 (財)食品薬品安全センター
*2 和光純薬(株)
*3 生化学工業(株)
*4 理化学研究所
*5 帝京大学

Toru Anzai*1, K.Takatori,J.Kosuge*2, M.Akai*1 and T. Higuchi *3 : **Distribution of *Emericella nidulans* in the environment of Thoroubred stables**
J.Equine Sci., **11**:119-21 (2000)
In order to understand the distribution of *Emericella nidulans*, the main causative agent of guttural pouch mycosis, in the environment of stable, fungal isolation was carried out from samples collected from a racehorse training center and breeding farms. *E. nidulans* was mainly isolated from bedding straw at the training center stables and from straw at the breeding farms. The main habitat of *E. nidulans* in the stables included straw and hay for bedding or feeds and that these materials were often contaminated with fungi before use.
Keywords : *Emericella nidulans*, guttural pouch mycosis, horse environment
*1 Japan Racing Association
*2 Miyazaki University
*3 Hidaka Agriculture Mutual Aid Association

Aihara, Maki*, Tanaka T.* and K. Takatori : ***Cladosporium*, as the main fungal contaminant of locations in dwelling environments.**
Biocontrol Science **6** : 49-52 (2001)
A total of 75 locations in 26 houses were examined for fungal contamination. Sixteen gerera from 68 locations were deteced. *Cladosporium* was highly isolated from them. A high frequency of contamination was seen to involve *Cladosporium*, of which *C. sphaerospermum* and C. *cladosporioides* were detected frequently at rates of 63.6 and 14.6% ,respectively.
Keywords : *Cladosporium*, fungal contamination, dwelling environments
*Ochanomizu University

Jong-Chul Park*, Han D-W*,Park B-J*,Lee D-H*, K.Takatori and Hwal Suh* : **Effective screening medium for the biodegradation of oleic acid by *Aspergillus niger*.**
Biocontrol Science **6** : 37-41 (2001)
To investigate oleic acid biodegradation, 7 *Aspergillus niger* strains were tested with 3 different types of Czapek broth medium containing oleic acid, and their metabolic abilities to decompose the fatty acid into carbon dioxide and water were compared. When the fungal strains were grown in the Czapek broth with both ^{14}C-labeled and non-labeled oleic acid, 2 strains of *A. niger* oxidized more than 58% of the supplied substrate within 72 hours.The addition of saccharose as an additional carbon source substantially reduced the biodegradation of oleic acid to the point tha all the strains showed less than 4% degardation.
Keywords : Oleic acid, *Aspergillus niger*, biodegradation
* Yonsei University,Korea

Kaminuma, T., Nakata, K., Nakano, T. and Takai-Igarashi, T.: **Pharmacoinformatics Infrastructure for Genome-based Personalized Medicine.** CBI Journal, **1**, 1-17 (2001)
Personalized medicine is an idealized medical practice aiming to give the right drugs to the right patients at the right times. It has been widely admitted that many projects for finding Single nucleotide polymorphisms (SNPs) are the basis for such practice. New informatics is also needed in order to utilize these data and knowledge effectively. Such an informational environment, i.e., data and knowledge bases and computational tools, would be called the infrastructure for personalized medicine. The infrastructure would also be useful in pharmaceutical research for finding leads and analyzing detailed mechanisms of drug actions. As the only national institution for pharmaceutical research in Japan, we have started to implement some components of the infrastructure and put their prototypes on the Web. Further research initiatives are being discussed in the Chem-Bio Informatics Society as of its Grand Challenge Projects.
Keywords : Informatics, Genome, Infrastructure

山本 都：**化学物質のデータベース構築ー地研との交流**
公衆衛生，**64** (6), 405-409 (2000)
1990年代半ばから，サリン事件，重油流出事故，和歌山ヒ素カレー事件など化学物質が係わる重大事故・事件が相次ぎ，「健康危機管理」や「情報ネットワーク」の重要性がクローズアップされてきたことから，化学物質情報部で作成している化学物質情報のデータベース等の紹介と共に，分野・機関横断的な情報ネットワークおよび情報の共同利用を目指したシステムの構築について考察した．

山本 都：**化学物質情報へのアクセスーその３：化学物質の情報検索ー実践編**
食品衛生学雑誌，**41** (4), J-259-263 (2000)
化学物質の安全性に係わる情報が収載されている各種データベースや資料等の情報源を調査し，情報収集の際に情報の信頼性，検索の簡便性，コストなどの点から有用と思われる情報源，媒体等を考察した．

中野達也，高島一[*1]，長嶋雲兵[*2]：**タンパク質の第一原理電子状態計算－生体化学反応の理論的解析を目指して－**
シミュレーション，**19**, 271-281 (2000)

Information of electronic state of biomolecules such as protein is indispensable to understanding of property of biomolecules and mechanism of biochemical reactions. Molecular orbital calculation is applied to simulate its electronic states. However molecular orbital calculation for large scale system as biosystem is very difficult to execute even on supercomputer because the amount of calculation is proportionally increased with the fourth power of system size. In order to realize low cost and personal execution of ab initio MO, we are developing a special purpose computer for molecular orbital calculation: MOEngine to increase calculation power. A novel method: the fragment MO method was also developed to decrease the amount of computation in MO calculation. We report that the calculation of electronic state of biomolecule will be inexpensively and personally executable using MOEngine and the fragment MO method showing some examples.

Keywords : Fragment MO method, MOEngine, biomolecules

[*1] 大正製薬（株）
[*2] 産業技術融合領域研究所

T. Nakano, T. Kaminuma, T. Sato[*1], Y. Akiyama[*2], M. Uebayasi[*3], and K. Kitaura[*4] : **Fragment Molecular Orbital Method: Application to polypepties**
Chem. Phys. Letters, **318**, 614-618. (2000)

Recently we have proposed the fragment molecular orbital method for calculating large molecules such as proteins. The method, with some modifications for a practical convenience, was applied to the model peptides of (Gly)n and (Ala)n (n=5~20), [Met5]enkephalin (YGGFM), and the synthetic designed peptide ALPHA-1 (Acetyl-ELLKKLLEELKG). The calculated total energies were well compared with those from the conventional ab initio MO method; the errors were within about 2 kcal/mol. It indicates that the fragment MO method is sufficiently accurate and useful to study electronic properties of large molecules.

Keywords : Fragment MO method, ab initio MO method, polypeptides

[*1] Fuji Research Institute Corporation
[*2] Electrotechnical Laboratory
[*3] National Institute of Bioscience and Human-Technology
[*4] Osaka Prefecture University

T. Nakano, T. Kaminuma, M. Uebayasi[*1], Y. Nakata[*2]: **3D Structure Based Atomic Charge Calculation for Molecular Mechanics and Molecular Dynamics Simulations**
CBI J., **1**, 35-40 (2001)

We propose a new charge equilibration approach that depends upon molecular 3D structure. Nishimoto - Mataga equation is used to express the shielding effect. With the present approach, it is not necessary to iterate simultaneous equations for evaluating charge equilibration, although that is required in the QEq method. Atomic charge calculations were carried out for several organic molecules. Calculated charge distributions are in good agreement with experimental values.

Keywords : atomic charge, QEq, Nishimoto - Mataga equation

[*1] National Institute of Bioscience and Human-Technology
[*2] Gunma University

Sekizawa J., Suter, G.[*1], Vermeire T.[*2] and Munns W.[*1] : **An Example of Integrated Approach for Health and Environmental Risk Assessment: Case of Organotin Compounds**
Water Science & Technology, **41**, 305-313 (2000)

Because environmental decision making based solely on simple compilation of toxicological data on either wild life or humans in isolation can not give effective answers about the nature and levels of risk, an integrated approach for risk assessment of adverse effects of chemicals is required. Integration of available information on health and environmental effects, from in vitro to the level of humans, across various species, across different endpoints, and combination with integrated exposure data, permits enhanced estimation of the potential risks posed by various agents. Mechanistic and quantitative consideration are the keys in this process. The value and utility of the integrated approach is shown using the example of organotin compounds.

Keywords : integration, organotin compounds, risk assessment

[*1] US Environmental Protection Agency
[*2] RIVM, the Netherlands

関澤 純：**化学物質のリスクにおける不確実性の評価**
日本リスク研究学会誌，**12** (2), 4-9 (2000)

リスクと不確実性とは本来不可分の関係にあるが良く理解されていない．本論文は筆者が日本リスク研究学会の春期シンポジウム（2000年6月）で講演した内容を基に書かれた．リスク評価の枠組みから解きおこし，リスク評価における不確実性の要因としてメカニズムの未解明による「真の不確実性」と，分布や変動のために一定の値をとれない「不確定で幅があることによる不確実性」の区別について解説した．より具体的にリスク評価における不確実性と問題点について，推測の必要性と情報における不確実性の原因，感受性の多様性や環境条件における分布と変動，データ取得の困難による不確実性，パラメータの不確実性とサンプリングや測定法の問題点と誤差，シナリオやモデルにおける不確実性について論じた．さらに「内分泌撹乱化学物質のリスク評価における不確実性分析」についての筆者の研究を踏まえて，健康リスク評価における不確実性の検討の問題を，動物データから人への外挿における不確実性（データに基づく不確実性係数の選択）と，健康へのリスクと環境へのリスクの統合的な評価の観点から論じた．

Keywords : uncertainty and its components, chemical risk, endocrine disruptor, International Programme on Chemical Safety, uncertainty factor

関澤 純：**環境リスクの評価**
環境と測定技術，**27** (5), 61-69 (2000)

「環境と測定技術」誌が環境リスクの評価をシリーズとしてとりあげるという企画の導入として，化学物質による人の健康と環境中生物へのリスクの評価において，筆者らが国際協力により積み重ねてきた成果の一部を紹介しつつ，現在発展しつつある環境リスク評価の基礎的な枠組みとその背景，および今後の課題について概説した．ＩＰＣＳによる評価とその仕組みを基本に，リスク評価の目的とあり方について，

データの組織化と解析手法の体系的研究, 健康リスク評価の手順およびリスク管理との関係を論じ, 環境リスク評価についてUS EPAの環境リスク評価のスキームと欧州連合で化学物質の健康と環境への影響の初期評価に用いられているEUSESの概念を紹介した. さらに, 情報が不十分な中での評価と不確実性の解析, CICAD計画の紹介, 今後の課題として確率論的扱いと不確実性解析, 統合的リスク評価, 透明なプロセスと批判的検討, 枠組みと判定の基準となる手法の確立, リスクの比較による優先順位付けと社会の判断, その基礎としてリスク評価とリスク対応のリンクおよび, そのサイクリックな関係による両者の改善について提示した.
キーワード:データの組織化と解析手法の体系的研究, 統合的リスク評価, 不確実性の解析

関澤 純:**環境管理におけるリスク・コミュニケーション**
水環境学会誌, **23** (7), 406-411 (2000)

環境問題におけるリスク・コミュニケーションの国内外の事例から, 環境ホルモン・ダイオキシン問題, 0157病原菌健康被害への対応, 消費者団体・行政および業界の代表が同席したワークショップでの応答, 日本化学会における環境庁と通産省の委託による3年間にわたる国内の化学物質リスクコミュニケーションの現状分析と今後のあり方の検討, 米国における「地域住民の知る権利法」などを分析し, リスク・コミュニケーションにおけるマスコミ, 専門家, 住民, NGOの役割について論じた. 今後のあり方については, 独自に行ったアンケートの結果, ＩＰＣＳのCICAD (国際簡潔評価文書) 計画, 有害廃棄物埋立地近傍の住民を対象としたATSDRの健康影響評価, 健康影響モニタリング, 地域公衆衛生の指針の作成と情報の提供, 米国大統領府・議会諮問委員会の「リスク管理の新たな枠組み」も参考にして, リスク・コミュニケーションが21世紀社会における共存の必須の基盤であることを示した.
キーワード:科学への興味と信頼性, 評価プロセス・判断基準と決定根拠の明示, リスクの予測と未然防止

関澤 純:**化学物質管理とリスクコミュニケーションのあり方**
高圧ガス, **37** (10), 12-17 (2000)

化学物質は危険なもの, やっかいなものというイメージが広くある. しかしそのイメージは一面では当っており, 正しくリスクを予測して管理しないと, とんでもないことになる. したがってリスクを予測して, よりリスクを少なくする手段を講じたり, より良い手法を模索するため, 関係者がそれぞれの立場から知恵をしぼり, 意見を交換しあって適切に運営してゆくことが求められている.

すなわち, 考えられるリスクを分析し, リスクの予防と管理のための方策を検討することが, まずなされなければならない. この段階でも, できうる限り関係者がいっしょになって可能な問題点を探り, さまざまな方策を検討する必要がある. それぞれの自主性を尊重し違った考え方や手法を比較し, その根拠や特質を討論し最善の対策を選び選択がなされたら, 実行し, 手順を踏んでみて, 不都合があればさらに改善を重ねていかなければならない.
キーワード:科学的推測と価値判断, 化学物質への不安, リスクコミュニケーションの発展段階

関澤 純:**リスクコミュニケーションと情報公開**
環境技術, **29** (10), 52-57 (2000)

情報公開とリスク・コミュニケーションとは違うこと, なぜリスク・コミュニケーションについて研究されるようになってきたか?「地域住民の知る権利法」制定の背景や, 環境ホルモン・ダイオキシン問題と市民の不安の分析を進めると同時に, リスクと有害性, コミュニケーションと情報開示・説明責任・情報公開・情報提供・情報交換の違いについて解説した. さらに科学的なリスク評価, 価値判断とリスク対応の意思決定の関係, 公衆のリスク受容性に影響を及ぼす要因と, 違いの尊重, 違いの理由や原因の相互理解の重要性, 透明性と批判的検討の関係では仮定の立て方や予測の道筋の明示と, 批判的検討を許すプロセスについて記し, 危機管理とリスクコミュニケーションの違いと関係, 日常的な対応や緊急時の即応, リスクコミニュケーションの枠組みの構築の必要性, 社会の各グループの役割について論じた.
キーワード:リスク・コミュニケーションの専門能力, 立場の違いの尊重と自主的な判断, 双方向の意見交換

関田清司, 梅村隆志, 斉藤 実, 小川幸男, 上野克典, 金子豊蔵, 内田雄幸, 松島裕子, 川崎 靖, 黒川雄二, 井上 達:**F344ラットによるフクロノリ抽出物90日間反復混餌投与毒性試験**
食品衛生学雑誌, **42**, 96-101 (2001)

食品添加物, フクロノリ抽出物 (FE) の0, 0.5, 1.5及び5.0%添加飼料を雄雌のラットに90日間摂取させる毒性試験を行った. 結果, 雄雌の1.5%群以上で摂餌量の増加が. また, 雄5.0%群で血清脂質 (総コレステロール, 中性脂肪) の弱い減少傾向が認められた. しかし, いずれの変化も毒性変化とは考察されなかった. 以上のことから, FE5.0%添加飼料摂取, 平均FE摂取量mg/kg/day, 雄で3, 362mg, 雌で3, 594mgは毒性変化が認められない用量であると結論した. また, FEの一般毒性は弱いものと考察された.
Keywords : fukuronori (gloiopeltis furacata) extract, 90-day toxicity study, funoran

Takahashi, Y., Koizumi, K.*, Takagi, A., Kitajima, S., Inoue, T., Koseki, H.*and Saga, Y.: **Mesp2 initiates somite segmentation through the Notch signalling pathway.**
Nat Genet., **4**, 390-396 (2000)

The Notch signaling pathway plays an important role in establishing metameric pattern during somitogenesis. In mice, the lack of either of MesP2 or Presenilin-1 (PS1) results in contrasting phenotypes, caudalized vs. rostralized vertebra. We adopted a genetic approach to analyze the molecular mechanism underlying the establishment of rostro-caudal polarity in somites. By focusing on the fact that expression of a Notch ligand, Dll1 is critically important for prefiguring somite identity, we found that MesP2 plays an important role to initiate establishment of rostro-caudal polarity by controlling two Notch signaling pathways. MesP2- and PS1-dependent activation of Notch signaling pathways might differentially regulate Dll1expression resulting in the establishment of the rostro-caudal polarity of somites.
Keywords : Somitogenesis, Notch signaling, Molecular genetics
*Chiba University School of Medicine

Kitajima, S., Takagi, A., Inoue, T. and Saga, Y.: **MesP1 and MesP2 are essential for the development of cardiac mesoderm**
Development, **127**, 3215-3226 (2000)

The transcription factors, MesP1 and MesP2, sharing an almost identical bHLH motif, have an overlapping expression pattern during gastrulation and somitogenesis. To understand the cooperative functions of MesP1 and MesP2, either a deletion or sequential gene targeting strategy was employed to inactivate both genes. The double-knockout (dKO) embryos died around 9.5 days postcoitum (dpc) without developing any posterior structures such as heart, somites or gut. The major defect in this double-knockout embryo was the apparent lack of any mesodermal layer between the endoderm and ectoderm. In the chimeric embryos, dKO cells were scarcely observed in the anterior-cephalic and heart mesoderm, however, they did contribute to the formation of the somites, notochord and gut. These results strongly indicate that the defect in the cranial-cardiac mesoderm is cell-autonomous, whereas the defect in the paraxial mesoderm is an non-cell-autonomous secondary consequence.

Key words : MesP1, MesP2, heart morphogenesis

Sawada, A.[*1], Fritz, A.[*2], Jiang, Y-J.[*3], Yamamoto, Y.[*4], Yamasu, K.[*5], Kuroiwa, A.[*1], Saga, Y. and Takeda, H.[*1]: **Zebrafish Mesp family genes, mesp-a and mesp-b are segmentally expressed in the presomitic mesoderm, and Mesp-b confers the anterior identity to the developing somites**
Development, **127**, 1691-1702 (2000)

We have characterized zebrafish mesp-a and mesp-bgenes that are segmentally expressed in the somite primordia. Observation in fused somites (fss) embryos suggests that these genes are downstream targets of fss at the segmentation stage. Ectopic expression of Mesp-b in embryos causes a loss of the posterior identity within the somite primordium, leading to a segmentation defect. These observations suggest that mesp genes are involved in anteroposterior specification within the presumptive somites.

Key words : Somitogenesis, Notch signalling, Zebrafish

[*1] Graduate School of Science, Nagoya University
[*2] Department of Biology, Emory University, USA
[*3] Imperial Cancer Research Fund, UK
[*4] Department of Biological Chemistry, University of California, USA
[*5] Faculty of Science, Saitama University

Yoon, BI., Hirabayashi,Y., Kawasaki, Y., Kodama, Y., Kaneko, T., Kim, DY.*and Inoue, T.: **Mechanism of action of benzene toxicity: Cell cycle suppression in hemopoietic progenitor cells (CFU-GM)**
Experimental Hematology, **29**, 278-285(2001)

Objective: The aim of this study was to clarify previously reported controversial data and hypotheses concerning the effect of benzene on the cell cycle of hemopoietic stem cells. Materials & Methods: In this study, the bromodeoxy-uridine UV (BUUV) suicide assay was performed in normal C57BL/6 and p53 knockout (KO) C57BL/6 mice during and after expo-sureto 300 ppm of benzene for 2 weeks. Conclusions: Our present study revealed the mechanism of action of benzene hematotoxicity. Benzene suppresses the cell cycle by p53-mediated over-expression of p21, a cyclin-dependent kinase inhibitor, resulting not simply in suppression of hemopoiesis but rather in a dynamic change of hemopoiesis during and after benzene exposure. Thus, the controversies raised by previously reported data are resolved by our present findings of hemopoietic stem cell kinetics.

Keywords : Cell cycle, CFU-GM, Benzene

*ソウル国立大学獣医学部

Iwama, T.*, Kamikawa, J.*, Higuchi, T.*, Yagi,K. *, Matuzaki, T.*, Kanno ,J.and Maekawa, A.*: **Development of invasive adenocarcinoma in a long-standing diverted ileal J-pounch for ulcerative colites:report of a case**
Dis Colon Rectum, **43** (1), 101-4 (2000)

We report a case of a 50-year-old male with ulcerative colitis who developed well-differentiated adenocarcinoma in the ileal J-pouch, which had been defunctioning for 18 years. The extension of the carcinoma in the pouch suggested that it had recently appeared in the pouch. Monitoring by endoscopic examination and biopsy or pouch excision seems to be an appropriate action if a pouch is out of the fecal stream.

Keywords : Ulcerative colitis, Ileal J-pouch, Adenocarcinoma

* Kyoundou Hospital and Department of Pathology, Sasaki Institute

Fujiwara M[*1], Okayasu I[*2], Oritsu M[*1], Komatsu J[*1], Yoshitsugu M[*1], Katoh Y[*1], Bandoh T[*1], Toyoshima H[*1], Kase Y[*3], Sugihara K[*4], Kanno J, Hayashi Y[*5].: **Significant increase in prostaglandin E-main urinary metabolite by laxative administration: comparison with ulcerative colitis**
Digestion. **61**,201-6(2000)

OBJECTIVE: To assess the production of prostaglandin E(2), an important chemical mediator in diarrhea induced by laxative administration, a prostaglandin E-main urinary metabolite (7alpha-hydroxy-5,11-diketotetranor-prosta-1,16-dioic acid, PGE-MUM) was measured in healthy volunteers and compared with the values of patients with ulcerative colitis. METHODS: PGE-MUM was determined by a simplified immunoassay of bicyclic PGE-MUM. CONCLUSION: Laxative administration induces production of prostaglandin E(2) as one of the chemical mediators, although its production grade is relatively low as compared with ulcerative colitis in the active phase.

Keywords : Laxatives, ProstaglandinE, Ulcerative colitis

[*1] Japanese Red Cross Medical Center
[*2] Kitasato University School of Medicine
[*3] Central Laboratory, Tsumura & Co
[*4] Kudanzaka Hospital
[*5] Kitasato University School of Pharmacy

根本哲夫*, 森山美樹*, 坂田康子*, 山本嘉子*, 江石義信*, 菅野 純：**家族性大腸腺腫に合併した甲状腺乳頭腺癌の細胞像**
日本臨床細胞学会雑誌, **39**, (5) (2000)

背景：家族性大腸腺腫（Familial adenomatous polyposis以下FAP）はAPC遺伝子のgermline mutationに起因する遺伝性疾患であり，若年女性の甲状腺に乳頭腺癌をしばし

ば合併することが知られている．近年，甲状腺乳頭癌に細胞の篩状配列や充実性増殖などの組織学的特徴を有するcribriform-morular variant（CMV）が提唱され，FAPに伴う甲状腺乳頭癌は，多くがその範疇に入るものと考えられる．われわれは家族性大腸腺腫症に合併した甲状腺癌の２例を経験し，穿刺吸引細胞像を検討した．症例：症例はいずれも２０歳女性．甲状腺腫瘍に対し吸引細胞診が行われた．通常の乳頭癌にみられるコロイド，核の切れ込みなどの他に，１）細長い核を有する高円柱状細胞の柵状配列，２）多角形細胞の充実性胞巣，３）立方状胞の篩状配列が特徴的な細胞所見であり，穿刺吸引細胞像からCMV甲状腺癌の推定が可能と考えられた．結論：CMVは非FAP患者では比較的まれであることから，甲状腺穿刺吸引細胞診でこれらの特徴的な細胞像を認めた場合には，甲状腺腫瘤が初発症状として気付かれたFAPである可能性を考慮する必要があると考えられると述べた．

Keywords : Thyroid papillary carcinoma, Familial adenomatous polyposis, Aspiration cytology

*東京医科歯科大学

樋口哲郎*，岩間毅夫*，家城和夫*，金仁燮*，松崎　淳*，菅野純：**長期の経過観察中に回腸癌が発生した家族性を示す若年性ポリポーシスの１例**

胃と腸，**35**，(3) (2000)

要旨　患者は49歳，女性．長期間に亙って追跡を行っているJSPの１家系の発端者である．腹痛，嘔吐を主訴し，腸重積の診断で開腹術を施行した．回腸癌（発達度mp）を先進部とする腸重積であった．腫瘍はp53の過剰発現をfocalに認め，JSPに発生する回腸癌に関しても一般の大腸癌と同じくp53遺伝子異常が関係している可能性を示唆していた．JSPの責任遺伝子と言われるSMAD4，更にJSPに関連する可能性のあるSMAD1，SMAD2，SMAD3，SMAD5，PTEN，PTCHを検索したが異常は認めなかった．JSPの原因遺伝子はSMAD4のみではない可能性が高く，更に関東が必要であると述べた．

Keywords：若年性ポリポーシス，回腸癌，遺伝子診断

*佐々木研究所付属杏雲堂病院

Kamikawa, Y.*1, Shibukawa, A.*1, Uchida, K.*1, Kojima, S.*1, Sakuma, A.*1, Kubota, K.*1, Ohno, Y. and Okuda, H.*2: **Comparison of motor reactivity of the human colon cold-stored in different preservative solutions for carbachol and noradrenaline *in vitro***

Polish J. Pharmacol. **52**, 299-305 (2000)

冷蔵保存ヒト結腸輪状標本の収縮性についてカルバコールをもちいて検討したところ，MEM培地及びKrebsリンゲル液で保存した標本の収縮はリン酸緩衝液中で保存した標本より低下が大きかった．同様の結果はノルアドレナリンによる弛緩反応においても認められた．

Keywords : preservation, smooth muscle, contractivity

*1 獨協医科大学
*2 日本製薬工業協会

Sakai, T.*1, Takahashi, M.*2, Mitsumori, K.*3, Yasuhara, K., Kawashima, K.*4, Mayahara, H.*5 and Ohno, Y.: **Collaborative work to evaluate toxicity on male reproductive organs by 2-week repeated dose toxicity studies in rats**

J. Toxicol. Sci. **25**, special issue, 1-21 (2000)

薬物等の雄性生殖臓器毒性を検出するために必要なげっ歯類での反復投与毒性試験の期間と観察方法について明らかにするために，28社の協力のもとで，雄性生殖臓器へ影響することの知られている24種の薬物について，その毒性発現をラットを用いた２週間および４週間の反復投与毒性試験で比較した．その結果，適正な用量設定を行い，精子のステージ分析を含む詳細な病理組織学的観察を行うことにより，２週間でも検出できることが明らかになった．

Keywords : validation, male fertility test, repeated dose toxicity study

*1 山之内製薬
*2 昭和大学
*3 東京農工大学
*4 化合物安全性研究所
*5 ラビトン

Takahiko, B.*1, Touchi, A.*1, Yamaguchi, Y.*1, Ito, K.*2, Yamazoe, Y.*3, Sugiyama, Y.*4 and Ohno, Y.: **Can in vitro metabolism and enzyme inhibition be explained by unbound drug concentration?**

Drug metabolism Reviews, **32** (suppl.2), p.222 (2000)

薬物速度論的解析においては蛋白等と結合していない，遊離型の薬物が代謝を受けたり，阻害活性を有する事を前提にしている．すなわち，反応系における結合蛋白の有無にかかわらず遊離型の薬物濃度を基準にして求めたKm値やKi値は変わらないはずである．しかし，薬物によってはアルブミンや肝可溶性分画の反応系への添加によりそれらの値が変化することを示した．

Keywords : pharmacokinetics, protein binding, free form

*1 塩野義製薬
*2 北里大学
*3 東北大学
*4 東京大学

Hide, I.*1, Tanaka, M.*1, Inoue, A.*1, Inoue, K., Nakajima, K.*2, Kohsaka, S.*2 and Nakata, Y.*1: **Extracellular ATP triggers TNF-a release from rat microglia**

J.Neurochem., **75**, 965-972 (2000)

The TNF-a release was maximally elicited by1 mM ATP and also induced by a $P2X_7$ selective agonist, 2'- and 3'-O-(4-benzoylbenzoyl)-adenosine 5'-triphosphate (BzATP), suggesting the involvement of $P2X_7$. ATP-induced TNF-α release was Ca^{2+}-dependent and a sustained Ca^{2+} influx in response to ATP was correlated with the extent of TNF-α release. TNF-α release induced by ATP was inhibited by PD98059, an inhibitor for MEK1 which activates extracellular signal-regulated protein kinase (ERK), and SB203580, an inhibitor for p38 MAP kinase. However, both ERK and p38 were rapidly activated by ATP even in the absence of extracellular Ca^{2+}. These results indicate that extracellular ATP triggers TNF-α release in rat microglia via P2 receptor, likely $P2X_7$, by a mechanism, which is dependent on both the sustained Ca^{2+} influx and ERK/p38 cascade regulated independently from Ca^{2+} influx.

Keywords: ATP, microglia, TNF-α

*1 Department of Pharmacology, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine

*2 Department of Neurochemistry, National Institute of Neuroscience

Honda, S.*, Imai, Y.*, Ohsawa, K.*, Nakamura, Y.*, Inoue, K., Kohsaka, S.*: **Extracellular ATP or ADP induce chemotaxis of cultured microglia through Gi/o-coupled P2Y receptors.**

J.Neurosci., **21**, 1975-1982 (2001)

Extracellular ATP and ADP induced membrane ruffling and markedly enhanced chemokinesis in Boyden chamber assay. Further analyses using the Dunn chemotaxis chamber assay, which allows direct observation of cell movement, revealed that both ATP and ADP induced chemotaxis of microglia. The elimination of extracellular calcium or treatment with pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid, suramin, or adenosine-3'-phosphate-5'-phosphosulfate did not inhibit ATP- or ADP-induced membrane ruffling, whereas AR-C69931MX or pertussis toxin treatments clearly did so. As an intracellular signaling molecule underlying these phenomena, the small G-protein Rac was activated by ATP and ADP stimulation, and its activation was also inhibited by pretreatment with pertussis toxin. These results strongly suggest that membrane ruffling and chemotaxis of microglia induced by ATP or ADP are mediated by G (i/o)-coupled P2Y receptors.

Keywords : ATP, microglia, chemotaxis

* Department of Neurochemistry, National Institute of Neuroscience

Tsuda M, Koizumi S, Kita A, Shigemoto Y, Ueno S, Inoue K.: **Mechanical allodynia caused by intraplantar injection of P2X receptor agonist in rats: involvement of heteromeric $P2X_2/3$ receptor signaling in capsaicin-insensitive primary afferent neurons.**

J. Neurosci., **20**, RC90 (2000)

Here, we found a novel nociceptive response induced by ATP, mechanical allodynia. Injection of α , β -methylene ATP (α β meATP), an agonist to P2X receptor, into plantar surface in rats produced the mechanical allodynia along with previously described nocifensive behavior and thermal hyperalgesia. This allodynic response was blocked by pretreatment with the P2 receptor antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate. Interestingly, only the mechanical allodynia evoked by α β meATP selectively remained in neonatal capsaicin-treated adult rats that had selectively lost the capsaicin-sensitive neurons. Taken together with our previous finding that the α β meATP-activated slow desensitizing current in DRG neurons is mediated by heteromeric $P2X_{2/3}$ ($P2X_2$ and $P2X_3$) receptors, it is hypothesized that activation of heteromeric $P2X_{2/3}$ receptors in peripheral terminals of capsaicin-insensitive primary afferent fibers leads to the induction of mechanical allodynia.

Keywords : P2X receptors, Mechanical allodynia, Capsaicin sensitivity

Sato, K., Matsuki, N.*, Ohno, Y., Nakazawa, K.: **Extracellular ATP reduces optically monitored electrical signals in hippocampal slices through metabolism to adenosine**

Eur. J. Pharmacol., **399**, 123-129 (2000)

Electrical signals in rat hippocampal slices were optically monitored using a voltage-sensitive dye to determine whether extracellular ATP exhibits direct effects through its own receptors or indirect effects after its hydrolysis to adenosine. The dentate gyrus was stimulated and electrical signals in the CA1 and the CA3 region were analyzed. ATP, ADP and AMP inhibited the excitation in the CA1 region. The inhibition by ATP was antagonized by adenosine receptor antagonists and α , β -methylene ADP, an inhibitor of 5'-nucleotidases. The results suggest that extracellular ATP inhibits neuronal electrical signals in hippocampal slices after its metabolism to adenosine.

Keywords : ATP, Hippocampus, Optical recording

*東京大学薬学部

Frame,L.T.*1, Ozawa, S., Nowell, S.A.*1, Chou, H.C.*2, DeLongchamp, R.R.*2, Doerge, D.R.*1, Lang, N.P.*1, Kadlubar, F.F.*2 : **A simple colorimetric assay for phenotyping the major human thermostable phenol sulfotransferase (SULT1A1) using platelet cytosols**

Drug Metabolism Disposition, **28**,1063-1068(2000)

A thermostable phenol sulfotransferase, SULT1A1, has been implicated in numerous detoxification and bioactivation pathways. A simple endpoint colorimetric assay is described that can be used for rapid phenotyping of SULT1A1 activity in human populations. This reaction concomitantly results in generation of p-nitrophenol that can be quantified colorimetrically at 405 nm (epsilon = 18,200 M(-1)) to give an indirect measure of sulfotransferase activity.

Keywords : phenol sulfotransferase assay, human, genetic polymorphism

*1 National Center for Toxicological Research, USA
*2 Arkansas Cancer Research Center, USA

Ozawa, S., Ohta, K.*, Miyajima, A., Kurebayashi, H., Sunouchi, M., Shimizu, M.*, Murayama, N., Matsumoto, Y.*, Fukuoka, M.*, Ohno, Y. : **Metabolic activation of o-phenylphenol to a major cytotoxic metabolite, phenylhydroquinone: role of human CYP1A2 and rat CYP2C11/CYP2E1**

Xenobiotica, **30**, 1005-1017 (2000)

The in vitro metabolic activation of o-phenylphenol has been evaluated as yielding a toxic metabolite, 2,5- dihydroxybiphenyl (phenylhydroquinone), by p-hydroxylation in liver microsomes of rat and human. The involvement of rat CYP2C11, CYP2E1 and human CYP1A2 in the p-hydroxylation of o-phenylphenol is suggested.

Keywords : o-phenylphenol, phenylhydroquinone, cytochrome P450

*昭和薬科大学

Nowell, S.*1, Ambrosone, C. B.*2, Ozawa, S., MacLeod, S.L. *1, Mrackova, G.*2, Williams, S.*1, Plaxco, J.*1, Kadlubar, F.F.*2, Lang,N. P.*1: **Relationship of phenol sulfotransferase activity (SULT1A1) genotype to sulfotransferase phenotype in platelet cytosol**

Pharmacogenetics, **10**,789-97 (2000)

To date, one genetic polymorphism (Arg213His) has been iden-

tified that is associated with reduced platelet sulfotransferase activity. A simple colorimetric phenotyping assay, in conjunction with genotyping, was employed to demonstrate a significant correlation ($r = 0.23$, $P < 0.01$) of SULT1A1 genotype and platelet sulfotransferase activity towards 2-naphthol.

Keywords : Phenol sulfotransferase, genotype, food-derived carcinogen

*1 Arkansas Cancer Research Center, USA
*2 National Center for Toxicological Research, USA

Gaubatz, S.*2, Lindeman, G.J.*2, Ishida, S., Jakoi, L*1, Nevins, J.R.*1, Livingston, D.M.*2, Rempel R.E.*1: **E2F4 and E2F5 play an essential role in pocket protein-mediated G1 control**
Mol. Cell., **6**, 729-35 (2000)

E2F transcription factors are major regulators of cell proliferation. The diversity of the E2F family suggests that individual members perform distinct functions in cell cycle control. E2F4 and E2F5 constitute a defined subset of the family. Until now, there has been little understanding of their individual biochemical and biological functions. Here, we report that simultaneous inactivation of E2F4 and E2F5 in mice results in neonatal lethality, suggesting that they perform overlapping functions during mouse development. Embryonic fibroblasts isolated from these mice proliferated normally and reentered from G0 with normal kinetics compared to wild-type cells. However, they failed to arrest in G1 in response to p16INK4a. Thus, E2F4 and E2F5 are dispensable for cell cycle progression but necessary for pocket protein-mediated G1 arrest of cycling cells.

Keywords : cell cylcle, E2F, knock out

*1 Dep. of Genetics, HHMI, Duke University Medical Center, USA.
*2 The Dana-Farber Cancer Institute and Harvard Medical School, USA

Rempel, R.E.*, Saenz-Robles, M.T,*, Storms, R.*, Morham, S.*, Ishida, S., Engel, A.*, Jakoi, L.*, Melhem, M.F.*, Pipa,s J.M.*, Smith, C., Nevins, J.R.*: **Loss of E2F4 activity leads to abnormal development of multiple cellular lineages**
Mol. Cell., **6**, 293-306 (2000)

We have generated mice deficient in E2F4 activity, the major form of E2F in many cell types. Analysis of newborn pups deficient in E2F4 revealed abnormalities in hematopoietic lineage development as well as defects in the development of the gut epithelium. Specifically, we observed a deficiency of various mature hematopoietic cell types together with an increased number of immature cells in several lineages. This was associated with an increased frequency of apoptotic cells. We also found a substantial reduction in the thickness of the gut epithelium that normally gives rise to crypts as well as a reduction in the density of villi. These observations suggest a critical role for E2F4 activity in controlling the maturation of cells in a number of tissues.

Keywords ; cell cycle, E2F, knock out

* Dep. of Genetics, HHMI, Duke University Medical Center, USA.

Leone, G.*, Nuckolls, F.*, Ishida, S., Adams, M.*, Sears, R.*, Jakoi, L.*, Miron, A*, Nevins, J.R.*: **Identification of a novel E2F3 product suggests a mechanism for determining specificity of repression by Rb proteins**
Mol. Cell .Biol., **20**, 3626-3632 (2000)

The tumor suppressor function of Rb is intimately related to its ability to interact with E2F and repress the transcription of E2F target genes. Here we describe a novel E2F product that specifically interacts with Rb in quiescent cells. This novel E2F, which we term E2F3b, is encoded by a unique mRNA transcribed from an intronic promoter within the E2F3 locus. The E2F3b RNA differs from the previously characterized E2F3 RNA, which we now term E2F3a, by the utilization of a unique coding exon. In contrast to the E2F3a product that is tightly regulated by cell growth, the E2F3b product is expressed equivalently in quiescent and proliferating cells. But, unlike the E2F4 and E2F5 proteins, which are also expressed in quiescent cells and form complexes with the p130 protein, the E2F3b protein associates with Rb and represents the predominant E2F-Rb complex in quiescent cells. Thus, the previously described specificity of Rb function as a transcriptional repressor in quiescent cells coincides with the association of Rb with this novel E2F product.

Keywords: cell cycle, E2F, Rb

*Dep. of Genetics, HHMI, Duke University Medical Center, USA.

Onoda, F.*, Seki, M.*, Miyajima, A., Enomoto, T.*: **Elevation of sister chromatid exchange in Saccharomyces cerevisiae *sgs1* disruptants and the relevance of the disruptants as a system to evaluate mutations in Bloom's syndrome gene**
Mutat. Res. **28** ; 459, 203-9 (2000)

The *SGS1* of *Saccharomyces cerevisiae* is a homologue of the Bloom's syndrome and Werner's syndrome genes. The *sgs1* disruptants show hyperrecombination, higher sensitivity to methyl methanesulfonate and hydroxyurea, and poor sporulation. In this study, we found that sister chromatid exchange was increased in sgs1 disruptants. We made mutated *SGS1* genes coding a protein proved to lack DNA helicase activity (*sgs1-hd*), having equivalent missense mutations found in Bloom's syndrome patients (*sgs1-BS1*, *sgs1-BS2*). None of the mutated genes could suppress the higher sensitivity to methyl methanesulfonate and hydroxyurea and the increased frequency of interchromosomal recombination and sister chromatid exchange of *sgs1* disruptants. On the other hand, all of the mutant genes were able to complement the poor sporulation phenotype of *sgs1* disruptants, although the values were not as high as that of wild-type *SGS1*.

Keywords: *SGS1*, sister chromatid exchange, Bloom's syndrome

* Tohoku University

Miyajima, A., Seki, M.*1, Onoda, F.*1, Shiratori, M.*1, Odagiri, N.*1, Ohta, K.*2, Kikuchi, Y.*3, Ohno, Y., Enomoto, T.*1: **Sgs1 helicase activity is required for mitotic but apparently not for meiotic functions**
Mol. Cell Biol. **20**, 6399-409 (2000)

The *SGS1* gene of *Saccharomyces cerevisiae* is a homologue for the Bloom's syndrome and Werner's syndrome genes. The disruption of the *SGS1* gene resulted in very poor sporulation, and the majority of the cells were arrested at the mononucleated stage. The recombination frequency measured by a return-to-growth

assay was reduced considerably in *sgs1* disruptants. However, double-strand break formation, which is a key event in the initiation of meiotic DNA recombination, occurred. The spores produced by *sgs1* disruptants showed relatively high viability. The *sgs1 spo13* double disruptants sporulated poorly, like the *sgs1* disruptants, but spore viability was reduced much more than with either *sgs1* or *spo13* single disruptants. The poor sporulation of sgs1 disruptants was complemented with a mutated *SGS1* gene encoding a protein lacking DNA helicase activity; however, the mutated gene could suppress neither the sensitivity of *sgs1* disruptants to methyl methanesulfonate and hydroxyurea nor the mitotic hyperrecombination phenotype of *sgs1* disruptants.

Keywords: *SGS1*, helicase, meiotic function

*1 Tohoku University
*2 The Institute of Physical and Chemical Research
*3 The University of Tokyo

Miyajima, A., Seki, M.*, Onod,a F.*, Ui, A.*, Satoh, Y.*, Ohno, Y., Enomoto, T.*: **Different domains of Sgs1 are required for mitotic and meiotic functions**

Genes Genet. Syst. **75**, 319-26 (2000)

The *SGS1* of *Saccharomyces cerevisiae* is a homologue for human Bloom's syndrome, Werner's syndrome, and Rothmund-Thomson's syndrome causative genes. Disruptants of *SGS1* show high sensitivity to methyl methanesulfonate (MMS) and hydroxyurea, and hyper recombination phenotypes including interchromosomal homologous recombination in mitotic growth. In addition, *sgs1* disruptants show poor sporulation and a reduced level of meiotic recombination as assayed by return-to-growth. We examined domains of Sgs1 required for mitotic and meiotic functions of Sgs1 by transfecting variously mutated *SGS1* into *sgs1* disruptants. The N-terminal 1-401 amino acid region was required for complementation of MMS sensitivity and suppression of hyper heteroallelic recombinations of *sgs1* disruptants in mitotic growth and for complementation of poor sporulation and of reduced meiotic recombination. Although DNA helicase activity of Sgs1 was not required for Sgs1 to complement the meiotic functions, a deletion of helicase motifs III-IV (842-1046 amino acid) abolished the complementing activity of Sgs1, indicating that a structurally intact helicase domain is necessary for Sgs1 to fulfill its meiotic functions.

Keywords : SGS1, DNA repair, DNA recombination

* Tohoku University

Onoda, F.*, Seki, M.*, Miyajima, A., Enomoto, T.*: **Involvement of *SGS1* in DNA damage-induced heteroallelic recombination that requires *RAD52* in Saccharomyces cerevisiae**

Mol. Gen. Genet. **264**, 702-8 (2001)

The *SGS1* gene of *Saccharomyces cerevisiae* is homologous to the genes that are mutated in Bloom's syndrome and Werner's syndrome in humans. Disruption of *SGS1* results in high sensitivity to methyl methanesulfonate (MMS), poor sporulation, and a hyper-recombination phenotype including recombination between heteroalleles. In this study, we found that *SGS1* forms part of the *RAD52* epistasis group when cells are exposed to MMS. Exposure to DNA-damaging agents causes a striking, Rad52-dependent, increase in heteroallelic recombination in wild-type cells, but not in *sgs1* disruptants. However, in the absence of DNA damage, the frequency of heteroallelic recombination in *sgs1* disruptants was several-fold higher than in wild-type cells, as described previously. These results imply a function for Sgs1: it acts to suppress spontaneous heteroallelic recombination, and to promote DNA damage-induced heteroallelic recombination.

Keywords : *SGS1*, *RAD52*, heteroallelic recombination

* Tohoku University

Nakajima, M.*, Takahashi, H.*, Sasaki, M.*, Kobayashi, Y.*, Ohno, Y., Usami, M.: **Comparative developmental toxicity study of indium in rats and mice**

Teratogenesis Carcinog. Mutagen., **20**, 219-227 (2000)

The developmental toxicity of indium was examined in both rats and mice using comparable experimental protocols. In rats, indium caused fetal weight decrease and fetal gross malformations. In mice, however, indium did not cause fetal gross malformations although it caused fetal weight decrease and fetal death. It was concluded from these results that rats and mice were susceptible to the embryotoxicity of indium at similar developmental stages in the early organogenetic period, but mice were less susceptible to the teratogenicity of indium than rats in terms of gross malformation.

Keywords : indium trichloride, malformation, embryotoxicity

* Institute for Life Science Research, Asahi Chemical Industry Co., Ltd., Japan

Nishikawa, A., Furukawa, F., Kasahara, K., Ikezaki, S., Itoh, T., Suzuki, T., Uchida*1, K., Kurihara, M., Hayashi, M., Miyata, N. and Hirose, M.: ***Trans*-4-hydroxy-2-nonenal, an aldehydic lipid peroxidation product, lakcs genotoxicity in *lacI* transgenic mice.**

Cancer Lett., **148**, 81-86 (2000)

In order to cast light on the significance of lipid peroxidation products for carcinogenesis, the *lacI* mutant frequency (MF), micronucleus induction and cell proliferation were analyzed in *lac*I transgenic mice treated with *trans*-4- hydroxy-2-nonenal (HNE), a typical example. Male mice were ip injected with HNE at doses of 0, 5 or 50 mg/kg bw and 48 h thereafter, peripheral blood was collected for analyzing micronucleus induction. After 14 days, the mice were sacrificed to allow tissue sampling for examination of *lac*I MF and cell proliferative activity. Sixty percent of the mice given 50 mg/kg HNE died within 5 days after the treatment, but no other mortalities were observed. Histopathologically, marked pulmonary hemorrhage was found in the 50 mg/kg HNE group mice that survived until day 14. Immunohistochemically, HNE-modified proteins were detected in their alveolar macrophages. The HNE treatment did not increase *lac*I MF in the liver, kidney and lung and no significant increase in micronucleus induction or cell proliferation in major organs was found in either treatment. Moreover, no tumors developed in the 5 mg/kg HNE-treated mice which survived until week 78. Our results thus indicate that HNE lacks in vivo genotoxicity in lacI transgenic mice even when lethal doses are applied.

Keywords : *Trans*-4-hydroxy-2- nonenal, *lacI* mutation, Micro-

nucleus

*1 Nagoya University

Furukawa, F., Nishikawa, A., Ishiwata, H., Takahashi, M., Hayashi, Y. and Hirose, M.: **Renal carcinogenicity of concurrently administered fish meal and sodium nitrite in F344 rats**
Jpn. J. Cancer Res., **91**, 139-147 (2000)

The effects of long-term concurrent administration of powdered fish meal (FM) and sodium nitrite (SN) were examined in F344 rats. Rats in groups 1-3 and 7-9 were respectively fed diets supplemented with 64%, 32% and 8% (basal diet) FM, and simultaneously given 0.12% SN in their drinking water. Groups 4-6 and 10-12 were respectively given 64%, 32% and 8% FM and tap water. At the 104th week, all surviving animals were killed and examined histopathologically. Treatment with FM dose-dependently increased the incidences and multiplicities of atypical tubules, adenomas and renal cell carcinomas in SN-treated males. Females were less susceptible than males for renal tumor induction. In males given the 64% FM diet alone, the incidence and multiplicity of atypical tubules were also significantly increased as compared with the 8% FM alone case. Nephropathy was apparent in FM-treated groups in a clear dose-dependent manner, irrespective of the SN treatment, and was more prominent in males than in females. Dimethylnitrosamine was found in the stomach contents after 4-week treatment with 64% FM plus 0.12% SN, at a level twice that in the 8% FM plus 0.12% SN group. The results clearly indicate that concurrent administration of FM and SN induces renal epithelial tumors. Further studies are required to elucidate how nephropathy and nitrosamines produced in stomach contents may contribute to the observed renal tumor induction.
Keywords : Powdered fish meal, sodium nitrite, renal cancer

Imazawa, T., Mitsumori, K., Kitajima, S., Onodera, H., Tamura, T., Takahashi, M., Hirose, M.: **Time course of ultrastructural changes and immunoelectron microscopic localization of neurocalcin in motor endplates of the lumbrical muscles of rats given a single administration of 2,5-di(tert-butyl)-1,4-hydroquinone** *Acta Neuropathol.*, **99**, 109-116 (2000)

A time-course study of ultrastructural changes and immunoelectron microscopic localization of neurocalcin was performed on motor endplates of the lumbrical muscles of female rats given a single administration of 2,5-di(tert-butyl)-1,4-hydroquinone (DTBHQ) at a dose of 120 mg/kg. At the ultrastructural level, neurotoxicity characterized by a decreases or loss of synaptic vesicles and mitochondria was observed after 24 h and at the 1 week time point. After 6 weeks, newly formed reinnervated endplates were observed. Immunoelectron microscopically, the synaptic vesicle membranes were heavily labeled for neurocalcin in the control rats, but not at 24 h after DTBHQ treatment. The results strongly suggest that DTBHQ targets the motor endplates in the rat lumbrical muscles, causing depletion of neurocalcin in the synaptic vesicles followed by their loss.
Keywords : DTBHQ, rat, motor endplate

Shibutani, M., Uneyama, C., Miyazaki, K., Toyoda, K., Hirose, M.: **Methacarn fixation, a novel tool for analysis of gene expressions in paraffin-embedded tissue specimens**
Lab. Invest., **80**, 199-208 (2000)

In order to establish a quantitative method for analysis of gene expressions in small areas of tissue after paraffin-embedding, preliminary experiments with RT-PCR were performed using methacarn-fixed rat tissues. A sufficient amount of total RNA for quantitative RT-PCR of many genes could be extracted from a deparaffinized liver section by a simple, single step extraction method. Low concentration of contaminating genomic DNA and resolution of ribosomal RNAs proved the purity and integrity of extracted RNA samples, allowing PCR-amplification of long mRNA sequence and mRNA species expressing low-copy numbers. Thus, methacarn-fixed paraffin-embedded tissue has benefits for analysis of RNAs in the cells of histologically defined areas.
Keywords : Methacarn, Paraffin-embedded tissue, Gene expression analysis

Toyoda, K., Shibutani, M., Sato, H., Uneyama, C., Takahashi, M., Hayashi, Y., Hirose, M.: **Lack of carcinogenicity and increased survival in F344 rats treated with 5-fluorouracil for 2-years**
Food Chem. Toxicol., **38**, 187-193 (2000)

The carcinogenicity of 5-fluorouracil (5-FU) was investigated in F344 rats of both sexes. 5-FU was administered to groups of 50 male and 50 female rats ad lib. for 104 weeks, added to drinking water at concentrations of 0, 62, 125 ppm. Body weight gains were slightly depressed in the 125 ppm group of both sexes. While not statistically significant in females, final survival rates at week 111 in the 125 ppm group of both sexes were higher than those in the control group, suggesting an ability of 5-FU to prolong the life span. Histopathologically, there was no significant induction of any neoplastic or non-neoplastic lesions, indicating a lack of carcinogenicity of 5-FU under the present experimental conditions using rats.
Keywords : 5-Fluorouracil, Carcinogenicity, Rat

Mitsumori, K., Onodera, H., Shimo, T., Yasuhara, K., Takagi, H., Koujitani, T., Hirose, M., Maruyama, C. and Wakana, S. : **Rapid induction of uterine tumors with p53 point mutations in heterozygous p53-deficient CBA mice given a single intraperitoneal administration of *N*-ethyl-*N*- nitrosourea**
Carcinogenesis., **21**, 1039-1042, (2000)

The sensitivity of heterozygous p53-deficient CBA mice [p53 (+/-)] to *N*-ethyl-*N*-nitrosourea (ENU) was investigated. p53 (+/-) and wild-type littermates [p53(+/+)] were given an i.p. injection of ENU and were maintained for 26 weeks. The incidence of uterine tumors and lung adenomas in p53 (+/-) mice was significantly greater than that in p53 (+/+) mice. Malignant lymphomas were only induced in p53 (+/-) mice. Gene analysis revealed GCG → GTG point mutations in codon 135 of exon 5 of the p53 allele in the uterine endometrial stromal sarcomas. Our results suggest that female p53(+/-) CBA mice are very susceptible to uterine carcinogenesis, providing a useful model for ENU- induced uterine tumors.
Keywords : p53-deficient mouse, MNU, uterine tumor

Son, H-Y., Nishikawa, A., Furukawa, F., Kasahara, K., Miyauchi, M., Nakamura, H., Ikeda, T. and Hirose, M.: **Organ-dependent modifying effects of oltipraz on N-nitrosobis (2-oxopropyl)amine (BOP)-initiation of tumorigenesis in hamsters**

Cancer Lett., **153**, 211-218 (2000)

Oltipraz is known to inhibit tumorigenesis induced by variety of carcinogens in several animal model systems. In the present experiment, the modifying effects of dietary oltipraz, given during BOP initiation of carcinogenesis, were investigated in Syrian hamsters. Groups 1-3 were thrice given subcutaneous injections of BOP at 1 week intervals and fed diets supplemented with 400 or 200 ppm of oltipraz or basal diet alone, starting 1 week prior and finishing 1 week after the carcinogen exposure. The incidences and multiplicity of adenocarcinomas of the pancreas were higher in groups 1 and 2 than in group 3 although without statistical significance. The incidence of pancreatic duct dysplasias was significantly increased in group 2 but not in group 1 as compared with group 3. While the incidences of alveolar adenomas and carcinomas were significantly decreased by the high dose, the multiplicities of hepatocellular adenomas, cholagiocellular carcinomas and gall bladder adenomas were elevated in the BOP/oltipraz groups. The results of the present study suggest that oltipraz exerts organ-dependent modifying effects on BOP-induced carcinogenesis in hamsters when given in the initiation stage.

Keywords : oltipraz on *N*-nitrosobis (2-oxopropyl)amine, hamster

Imazawa, T., Nishikawa, A., Furukawa, F., Kasahara, K., Ikeda, T.*, Takahashi, M., Hirose, M.: **Lack of carcinogenicity of gardenia blue colour given chronically in the diet to F344 rats**

Food Chem. Toxicol., **38**, 313-318 (2000)

The carcinogenicity of gardenia blue colour was examined in Fischer 344 (F344) rats.

Groups of 50 males and 50 females were given the material at dietary doses of 0 (control), 2.5 or 5% for 104 weeks and then sacrificed. The doses were selected on the basis of results from a 13-week subchronic toxicity study. A slight increase in relative organ weights of the left lung was observed in male rats of the 5% group. However, no significant differences between the control and treated groups were noted with regard to clinical signs, mortality and haematological findings. A variety of tumours developed in all groups, including the controls, but all were histologically similar to those known to occur spontaneously in F344 rats, and no statistically significant increase in the incidence of any type of neoplastic lesion was found for either sex in the treated groups. Thus, it was concluded that, under the present experimental conditions, gardenia blue colour is not carcinogenic in F344 rats.

Keywords : Carcinogenicity, gardenia blue colour, F344 rat

* Showa Women's University

Chung, F-L.*, Nath, R.G.*, Ocando, J.*, Nishikawa, A. and Zhang, L*.: **Deoxyguanosine adducts of *t*-4-hydroxy-2-nonenal are endogenous DNA lesions in rodents and humans: detection and potential sources**

Cancer Res., **60**, 1507-1511 (2000)

t-4-Hydroxy-2-nonenal (HNE) is a free radical- mediated oxidation product of polyunsaturated fatty acids. As an electrophile, HNE readily binds to proteins and yields diastereomeric cyclic $1,N^2$-propano adducts with deoxyguanosine (dG). Here, we report the detection and identification of the HNE-derived cyclic $1,N^2$-propano-dG adducts as endogenous DNA lesions in tissues of untreated rats and humans using a highly sensitive ^{32}P-postlabeling method in conjunction with high-performance liquid chromatography. These adducts were first verified by their comigration with the synthetic UV standards of HNE-dG adducts. Subsequently, their identities were unequivocally established by two independent reactions. An approximately 37-fold increase in the levels of HNE-dG adducts was observed in the liver DNA of F344 rats after treatment with CCl_4, suggesting that tissue lipid peroxidation is a likely source of their formation. Our studies in vitro further indicate that ω^6 polyunsaturated fatty acids are likely a unique class of fatty acids involved in HNE-dG adduct formation.

Keywords : *t*-4-Hydroxy-2-nonenal, adduct, endogenous DNA lesion

* American Health Foundation

Shoda, T., Mitsumori, K., Takahashi, T., Horiuchi, K., Yamazaki, Y., Suzuki, Y., Katsuda, Y., Yokomoto, Y. and Kurumi, M.: **Liver tumor promotion by β -naphthoflavone, a strong CYP1A1/2 inducer, in a 28 week two-stage rat hepatocarcinogenesis model using diethylnitrosamine as an initiator**

J. Toxicol. Pathol., **13**, 37-43 (2000)

β-naphthoflavone (BNF) is a strong inducer of cytochrome P450 (CYP) 1A but not CYP 2B. To determine its liver tumor promotion potential, a F344 rat model initiated with diethylnitrosamine (DEN) and subjected to two-thirds partial hepatectomy was used. The incidence of hepatocellular adenomas and glutathione S-transferase placental form (GST-P)-positive altered hepatocellular foci were elevated in the DEN+BNF group as compared to the DEN alone group at week 28. In conclusion, BNF promoted hepatocarcinogenesis initiated by DEN. The data of proliferating cell nuclear antigen labeling indices supported this conclusion.

Keywords : β-naphthoflavone, hepatocarcinogenesis, rat

Shinoda, K., Mitsumori, K., Uneyama, C. and Uehara, M.: **Induction and inhibition of testicular germ cell apoptosis by fluoroacetate in rats**

Arch. Toxicol., **74**, 33-39 (2000)

Fluoroacetate (FA), an inhibitor of aconitase, has been suggested to be a possible determinant of the form of cell death, apoptosis or necrosis. To investigate FA-induced testicular toxicity, adult SD rats were given a single dose of FA and sequentially analyzed. Germ cell degeneration was first found in round spermatids and in spermatogonia. Degenerating spermatogonia exhibited characteristic features of apoptosis by both electron microscopy and in situ terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), whereas spermatids did not. After that, the degenerating/TUNEL-labeled spermatogonia were drastically decreased compared to those from the control. It was concluded

that FA induces either apoptosis or necrosis of male germ cells in the early stage after dosing and subsequently inhibits spontaneous apoptosis.
Keywords : fluoroacetate, testicular toxicity, apoptosis

Yasuhara, K., Kobayashi, H., Shimamura, Y., Koujitani, K., Onodera, H., Takagi, H. Hirose, M. and Mitsumori, K.: **Toxicity and blood concentrations of xylazine and its metabolite, 2,6-dimethylaniline, in rats after single or continuous oral administrations**
J. Toxicol. Sci., **25**, 105-113 (2000)
To investigate risk of 2,6-dimethylaniline (DMA), a metabolite of xylazine, which intake may increase by ingestion of edible tissues from its treated domestic animals, the following studies were performed. Male F344 rats fed a diet containing 3000 or 300 ppm of DMA for 4 weeks. Bowman's gland atrophy and irregular arrangement of olfactory epithelial cells were observed at 3000 ppm. The plasma concentration of DMA was 0.20 to 0.36 ug/ml at the 3000 ppm, but under the detection limit at 300 ppm. So, it was suggested that the probability of nasal carcinogenic effects of DNA on consumers via ingestion of edible tissues from food-producing animals treated with xylazine is extremely low.
Keywords : 2,6-dimethylaniline, rat, plasma concentration

Ikeda, T.*, Nishikawa, A., Imazawa, T., Kimura, S.*, Hirose, M.: **Dramatic synergism between excess soybean intake and iodine deficiency on the development of rat thyroid hyperplasia**
Carcinogenesis., **21**, 707-713 (2000)
The effects of soybean and/or iodine-deficient diet feeding were investigated in female F344 rats. Rats were fed basal gluten (Group1), iodine-deficient gluten (Group 2), 20% defatted soybean (Group 3) or iodine-deficient defatted soybean (Group 4) diets. At week 10, relative thyroid weights were significantly higher in Groups 2 and 4 than in Group 1 and pituitary weights were significantly higher in Groups 3 and 4 than in Group 1. T4 was significantly lower in Groups 2 and 4 than in Group 1. Serum TSH was significantly higher in Groups 3 and 4 than in Group 1. Histologically, marked diffuse follicular hyperplasia of the thyroid was evident in Group 4 rats. Ultrastructurally, severe disorganization and disarrangement of mitochondria were apparent in thyroid follicular cells of Group 4. In the anterior pituitary, dilated rSER and increased secretory granules were remarkable in this group. Our results thus strongly suggest that dietary defatted soybean synergistically stimulates the growth of rat thyroid with iodine deficiency.
Keywords : soybean, iodine deficiency, thyroid
*Showa Women's University

Miyauchi, M., Nishikawa, A., Furukawa, F., Nakamura, H., Son, H-Y., Murakami, A.*1, Koshimizu, K.*1, Ohigashi, H.*2 and Hirose, M.: **Inhibitory effects of 1'-acetoxychavicol acetate on *N*-nitrosobis (2-oxopropyl) amine-induced initiation of cholangiocarcinogenesis in Syrian hamsters**
Jpn. J. Cancer Res., **91**, 477-481 (2000)
The influence of 1'-acetoxychavicol acetate (ACA) during the initiation stage was investigated in the *N*-nitrosobis (2-oxopropyl) amine (BOP)- initiated hamster tumorigenesis model. Ninety male 5-week-old hamsters were divided into three groups, each consisting of 30 animals, and s.c. injected with 20 mg / kg of BOP twice with a one-week interval. Groups 1 through 3 were fed diet supplemented with ACA at concentrations of 500, 100 and 0 ppm, respectively, for 3 weeks starting one week before the first carcinogen application. At the termination of experimental week 54, the total incidence and multiplicity of cholangiocellular adenomas and carcinomas in group 1 were significantly decreased as compared to the group 3 values. Our results thus indicate that ACA exerts an inhibitory effect on BOP-induced cholangiocarcinogenesis in hamsters. Taken together with previous findings of inhibited colon, oral and skin carcinogenesis in rats and mice, they suggest that ACA is a candidate chemopreventive agent with a wide spectrum of activity.
Keywords : 1'-acetoxychavicol acetate, *N*-nitrosobis (2-oxopropyl) amine, cholangiocarcinogenesis
*1 Kinki University
*2 Kyoto University

Furukawa, F., Nishikawa, A., Miyauchi, M., Nakamura, H., Son, H-Y., Yamagishi, M. and Hirose, M.: **Concurrent administration of fish meal and sodium nitrite does not promote renal carcinogenesis in rats after initiation with *N*-ethyl-*N*-hydroxyethylnitrosamine**
Cancer Lett., **154**, 45-51 (2000)
The modifying effects of concurrent administration of fish meal (FM) and sodium nitrite (SN) on the development of renal tumors after initiation with *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) were investigated. A total of 120 male 6-week-old Wistar rats were divided into six groups. Groups 1-3 were given 1000 ppm EHEN in their drinking water for 3 weeks as an initiation treatment for renal cancer induction and thereafter fed respective diets containing 64, 32, and 8% FM, and simultaneously given 0.12% SN in the drinking water for 33 weeks. Groups 4-6 were similarly treated without the prior application of EHEN. At the end of the 37th experimental week, all surviving animals were autopsied and examined histopathologically for the existence of renal proliferative lesions. The incidences of dysplastic lesions, adenomas or adenocarcinomas of the kidney were not significantly different among groups 1-3. No renal proliferative lesions were found in groups 4-6. Chronic nephropathy was slightly but significantly enhanced in the 64 and 32% FM-treated groups as compared with group 3. Our results suggest that concurrent administration of FM and SN does not affect the post-initiation phase of EHEN-induced renal carcinogenesis in the rat.
Keywords : Fish meal, sodium nitrite, renal carcinogenesis

Mori, I., Yasuhara, K., Hayashi, S., Nonoyama, T., Nomura, T and Mitsumori, K.: **Carcinogen dose- dependent variation in the transgene mutation spectrum in urethane-induced lung tumors in transgenic mice carrying the human prototype c-Ha-ras gene**
Cancer Lett., **153**, 199-209 (2000)
Genetic changes in urethane-induced lung tumors induced in

transgenic mice carrying a human prototype c-Ha-ras gene (rasH2 mice) were investigated. rasH2 mice and non-transgenic littermates (non-Tg) were i.p. injected urethane once or three times at 2-day intervals. The multiplicities of lung proliferative lesions including hyperplasias, adenomas and carcinomas in treated rasH2 mice were significantly higher than those in treated non-Tg mice. The variation of the lesions induced by different doses of urethane was not the cause of the variation of the mutation spectrum, and mutations of both transgene and mouse c-K-ras genes were not principal genetic events in urethane-induced lung proliferative lesions in rasH2 mice.

Keywords : urethane, lung tumor, rasH2 mouse

Koujitani, K., Yasuhara, K., Usui, T., Nomura, T., Onodera, H., Takagi, H., Hirose, M. and Mitsumori, K.: **Lack of susceptibility of transgenic mice carrying the human c-Ha-ras proto-oncogene (rasH2 mice) to phenolphthalein in a 6-month carcinogenicity study**

Cancer Lett., **152**, 211-216 (2000)

Phenolphthalein has carcinogenic activity, causing malignant lymphomas in B6C3F1 mice at a dietary dose of 3000 ppm in a 2-year carcinogenicity study and in heterozygous p53-deficient female mice at the same dose in a 6-month study. To examine whether phenolphthalein carcinogenic potential can be detected in male and female transgenic (Tg) mice carrying the human c-Ha-ras gene (rasH2) and their wild-type littermates (non-Tg), a diet containing 3000, 6000 or 12000 ppm was given for 6 months. Unequivocal induction of neoplastic lesions was not apparent, suggesting that rasH2 mice are resistant to the induction of malignant lymphomas by the treatment of phenolphthalein.

Keywords : phenolphthalein, carcinogenicity, rasH2 mouse

Furukawa, F., Nishikawa, A., Lee, I-S.*, Son, H-Y., Nakamura, H., Miyauchi, M., Takahashi, M. and Hirose, M.: **Inhibition by methionine of pancreatic carcinogenesis in hamsters after initiation with N-nitrosobis (2-oxopropyl) amine**

Cancer Lett., **152**, 163-167 (2000)

The modifying effects of dietary L-methionine in the post-initiation phase of pancreatic carcinogenesis were investigated in hamsters treated with *N*-nitrosobis(2-oxopropyl)amine (BOP). Groups consisting of 20 and 30 animals, respectively, were given BOP subcutaneously, once a week five times at a dose of 10 mg/kg body wt. and then continuously fed diet supplemented with 2% (group 1) or 0% (group 2) methionine (weeks 5-32). The incidence of pancreatic ductal adenocarcinomas was significantly lower in group 1 than in group 2. Multiplicity of adenocarcinomas was also significantly lowered. Similarly, total numbers of combined adenocarcinomas and dysplastic lesions were significantly decreased in group 1 as compared with group 2. Methionine enhanced atrophic change of pancreatic acinar cells in hamsters given BOP, indicating that the inhibitory effects on the post-initiation stage of BOP-induced pancreatic carcinogenesis in hamsters could be generally linked to suppression of growth.

Keywords : methionine, pancreatic carcinogenesis, hamster

*Keimyung University

Takegawa, K., Sakamori, M., Koremoto, M., Yamada, T., Takagi, S., Takeuchi, M., Yanai, T., Masegi, T. and Mitsumori, K.: **Large amount of vitamin A has no major effects on thyroidal hormone synthesis in two-stage rat thyroid carcinogenesis model using *N*-bis (2-hydroxyproyl) nitrosamine and thiourea**

J. Toxicol. Sci., **25**, 67-75 (2000)

Proliferative lesions in rat thyroids were induced by *N*-bis (2-hydroxypropyl) nitrosamine (DHPN) initiation followed by thiourea (TU) treatment. Simultaneous administration with a high level of vitamin A (VA) enhanced their induction. Iodine uptake and iodination of tyrosine residue in thyroglobulin, of the thyroid, were decreased by DHPN/TU treatment compared to the DHPN alone. Data from the DHPN/TU+VA and DHPN/TU animals were comparable. Therefore, the possibility that modification of hormone synthesis contributes to the enhancing effect of simultaneous treatment with VA on thyroidal tumor induction by TU is considered to be very minimal.

Keywords : thyroid tumor, rat, vitamin A

Hirose, M., Yamaguchi, T.[*1], Lin, C.[*1], Kimoto, N.[*1], Futakuchi, M.[*1], Kono, T.[*2], Nishibe, S.[*3] and Shirai, T.[*1]: **Effects of arctiin on PhIP-induced mammary, colon and pancreatic carcinogenesis in female Sprague-Dawley rats and MeIQx-induced hepatocarcinogenesis in male F344 rats**

Cancer Lett., **155**, 79-88 (2000)

Chemopreventive effects of arctiin, a lignan isolated from Arctium lappa (burdock) seeds, on the initiation or post initiation period of PhIP induced mammary carcinogenesis in female rats and on MeIQx-associated hepatocarcinogenesis in male rats were examined. In experiment 1, female SD rats were given intragastric doses of 100 mg/kg body wt of PhIP once a week for 8 weeks as initiation. Groups of 20 rats each were treated with 0.2 or 0.02% arctiin during or after PhIP initiation. Control rats were fed 0.2 or 0.02% arctiin, or basal diet alone during the experimental period. Animals were killed at the end of week 48. Although the incidence of mammary carcinomas did not significantly differ among the PhIP-treated groups, multiplicity was significantly decreased in rats given 0.2 or 0.02% arctiin after PhIP initiation as compared with the PhIP alone controls. The average number of colon aberrant crypt foci was also significantly decreased in these two groups. Pancreas acidophilic foci were induced in PhIP treated animals with slight decrease in the multiplicity with arctiin during the initiation phase. For liver carcinogenesis, groups of 15 male F344 rats were given a single intraperitoneal injection of DEN and starting 2 weeks later, they were administered 0.03% MeIQx in the diet, MeIQx together with 0.5% arctiin, 0.1% arctiin or basal diet for 6 weeks. They were subjected to two-third partial hepatectomy 3 weeks after DEN initiation and killed at the end of week 8 for glutathione GST-P immunohistochemistry. The numbers and areas of preneoplastic GST-P positive foci were elevated by the treatment with MeIQx, and further increased by the simultaneous treatment with arctiin. These results indicate that arctiin has a protective effect on PhIP-induced carcinogenesis particularly in the mammary gland in the promotion period. On the other hand, it may have a weak co-carcinogenic influence on MeIQx-induced hepatocarcinogenesis. In addition, the results suggested

that PhIP is a weak pancreatic carcinogen in female SD rats, targeting acinar cells.
Keywords : Chemoprevention, arctiin, PhIP
*1 Nagoya City University
*2 Meiji Institute
*3 Hokkaido University

Maronpot, R.R., Mitsumori, K., Mann, P., Takaoka, M., Yamamoto, S., Usui, T., Okamiya, H., Nishikawa, S., Nomura, T.: **Interlaboratory comparison of the CB6F1-Tg rasH2 rapid carcinogenicity testing model**
Toxicology., **146**, 149-159 (2000)

We have undertaken an interlaboratory comparison of the performance of the CB6F1-Tg rasH2 transgenic mouse in cancer bioassays concurrently conducted in the United States and Japan. Chemicals selected for study included known human carcinogens (cyclosporin A) and known rodent carcinogens (p-cresidine and vinyl carbamate) tested at carcinogenic doses, and non-carcinogens (p-anisidine and resorcinol) tested at appropriate high doses. All studies showed similar results between the two laboratories conducting each study. Although only five chemicals were successfully tested in this interlaboratory comparison, there was good concordance in outcome for the strong carcinogens and for the non-carcinogens.
Keywords : rasH2 mouse, carcinogenicity test, validation

Iwata, H., Hosoi, M., Miyajima, R., Mikami, S., Yamakawa, S., Enomoto, M., Imazawa, T. and Mitsumori, K.: **Morphogenesis of craniopharyngeal derivatives in the neurohypophysis of Fisher 344 rats: abnormally developed epithelial tissues including parotid glands derived from the stomatodeum**
Toxicol. Pathol., **28**, 568-574 (2000)

Morphogenesis of craniopharyngeal derivatives of the neurohypophysis found in 14 Fischer 344 rats was studied. They were composed of aberrant epithelial structures consisting of serous acinar and tubular and fusiform cell structures. Cells forming these structures were positive for cytokeratin, and basal cells of the acinar or tubular structures and some of the fusiform cells were positive for alpha-smooth muscle actin, and they were regarded to be myoepithelial cells. These findings indicate that the craniopharyngeal derivatives are a developmental aberration derived from the stomatodeum, which is known to be the origin of both nasal and oral epithelial tissues, including the parotid glands.
Keywords : craniopharyngeal derivatives, rat, diagnosis

Koujitani, T., Yasuhara, K., Ikeda, T., Imazawa, T., Tamura, T., Toyosawa, K., Shimada, A., Hirose, M. and Mitsumori, K.: **Sequential observation of 2,6-dimethylaniline-induced nasal lesions in a rat two-stage nasal carcinogenesis model after initiation with *N*-bis (2-hydroxypropyl) nitrosamine (DHPN)**
J. Vet. Med. Sci., **62**, 751-756 (2000)

Nasal lesions in male F344 rats administered 2,6-dimethylaniline (DMA) after initiation with *N*-bis (2-hydroxypropyl) nitrosamine (DHPN) were examined. Severe atrophy of Bowman's glands and epithelial disarrangement were apparent from week 4, followed by dilatation/proliferation of Bowman's glands, degeneration of epithelial cells, and undifferentiated epithelial cell proliferation. Focal glandular hyperplasias, dysplastic foci, and adenomas were observed from week 26, and carcinomas at 52 week. Carcinoma cells had ultrastructural characteristics identical to those in normal Bowman's glands. It was cocluded that Bowman's glands are the target of DMA, giving rise to nasal carcinomas after DHPN-initiation.
Keywords : nasal carcinogenesis, rat, DHPN

Liu, X.W., Katagiri, Y., Jiang, H., Gong, L.J., Guo, L.Y., Shibutani, M., Johnson, A.C., Guroff, G.: **Cloning and characterization of the promoter region of the rat epidermal growth factor receptor gene and its transcriptional regulation by nerve growth factor in PC12 cells**
J Biol Chem., **275**, 7280-7288 (2000)

To elucidate the molecular mechanism of NGF-induced down-regulation of the EGFR, we have clone da 2.7-kilobase promoter sequence of the rat EGFR from a rat P1 library. Six transcriptional start sites were identified by 5'-rapid amplification of cDNA ends and primer extension. Reporter gene assay was performed with 1.1 kilobases of the 5'-flanking sequence, and this sequence exhibited functional promoter activity in transient transfection experiments with PC12, C6, and CV-1 cells. Treatment of PC12 cells with NGF inhibited promoter activity, and TCC repeat sequence appeared to be at least partially responsible for the down-regulation. Supportive evidence for the relevance of this sequence was obtained from EMSA and by transfection of TCC mutation constructs.
Keywords : NGF, PC12, Transcriptional down-regulation of EGFR

Shoda, T., Mitsumori, K., Onodera, H., Toyoda, K., Uneyama, C., Takada, K. and Hirose, M.: **Liver tumor-promoting effect of β -naphthoflavone, a strong CYP 1A1/2 Inducer, and the relationship between CYP 1A1/2 induction and Cx32 decrease in its hepatocarcinogenesis in the rat**
Toxicol. Pathol., **28**, 540-547 (2000)

Interrelationships among induction of cytochrome P-450 (CYP) 1A1/2, decrease in connexin 32 (Cx32), and liver tumor-promoting activity by beta- naphthoflavone (BNF) in the promotion stage were examined in a 2-stage liver carcinogenesis model. Male Fischer 344 rats initiated with diethyl- nitrosamine (DEN) were fed a diet containing 2%, 1%, or 0% BNF for 6 weeks and subjected to a two-thirds partial hepatectomy at week 3. At week 8, all rats were sacrificed and examined. BNF induced adenoma-like hepatic foci and glutathione S-transferase-P foci, unlike phenobarbital, does not induce CYP 2B1/2 isozymes, and there seems to be no direct relationship between CYP 1A1/2 induction and Cx32 reduction in BNF hepatocarcinogenesis.
Keywoeds : beta-naphthoflavone, rat, hepatocarcinogenesis

Takegawa, K., Mitsumori, K., Onodera, H., Shimo, T., Kitaura, K., Yasuhara, K., Hirose, M. and Takahashi, M.: **Studies on the carcinogenicity of potassium iodide in F344 rats**
Food Chem. Toxicol., **38**, 773-781 (2000)

A carcinogenicity study, in which F344 rats were given potassium iodide (KI) in the drinking water at 10, 100 or 1000 ppm for

104 weeks, and a two- stage carcinogenicity study of application at 1000 ppm for 83 weeks following an injection of *N*-bis (2-hydroxypropyl) nitrosamine (DHPN), were conducted. In the former, squamous cell carcinoma was induced in the salivary glands at 1000 ppm, but no tumors in the thyroid. In the two-stage carcinogenicity study, the incidence of thyroid tumors derived from the follicular epithelium was increased in the DHPN+KI as compared with the DHPN alone group. The results suggest that excess KI has a thyroid tumor-promoting effect, but KI per se does not induce thyroid tumors in rats. In salivary gland, KI was suggested to have carcinogenic potential via an epigenetic mechanism, only active at a high dose.
Keywords : potassium iodide, salivary gland, thyroid tumor

Mori, I., Hayashi, S., Nonoyama, T., Yasuhara, K., Mitsumori, K. and Masegi, T.: **Point mutations of the c-H-ras gene in spontaneous pulmonary tumors of transgenic mice carrying the human c-H-ras gene**
J. Toxicol. Pathol., **13**, 165-172 (2000)

Spontaneous proliferative pulmonary lesions were found in 10 (6 males and 4 females) of 244 (122 of each sex) transgenic (Tg) mice carrying the human prototype c-H-*ras* gene (rasH2). The mutation patterns of the human c-H-*ras* codon 61 and endogenous mouse c-K-*ras* codons 12, 13, and 61 in these lesions were analyzed. Immunohisto-chemical detection for *p53* protein, *hsp70* or *mdm2* gene protein was also performed. Obtained findings suggested that, at least, a point mutation of the human c-H-*ras* trangene may be an important step in progression of spontaneous lung tumors, whereas *p53* abnormality may not play an important role of the pulmonary carcinogenesis in rasH2 Tg mice.
Keywords : rasH2 mouse, lung tumor, ras mutation

Toyoda, K., Shibutani, M., Tamura, T., Koujitani, T., Uneyama, C., Hirose, M.: **Repeated dose (28 days) oral toxicity study of flutamide in rats, based on the draft protocol for the 'Enhanced OECD Test Guideline 407' for screening for endocrine-disrupting chemicals**
Arch. Toxicol., **74**, 127-132 (2000)

To establish a test protocol for the'Enhanced OECD Test Guideline 407', we performed a preliminary 28-day, repeated-dose toxicity study of flutamide at doses of 0 (control), 0.25, 1 and 4 mg/kg b.w./day in rats of both sexes. Male rats receiving 1 and 4 mg/kg showed lobular atrophy of the mammary gland and a decrease in epididymal weight. At 4 mg/kg, increased levels of serum testosterone and estradiol and decrease of the weight of the accessory sex glands. In females, a slight prolongation of the estrous cycle was also observed at 4 mg/kg. Thus, among the parameters tested in the present experimental system, the weight of endocrine-linked organs and their histopathological assessment, serum hormone levels, and estrous cycle stage allowed the detection of endocrine-related effects of flutamide
Keywords : Enhanced OECD Test Guideline 407, Flutamide, Endocrine disrupters

Takegawa, K., Mitsumori, K., Yasuhara, K., Moriyasu, M., Sakamori, M., Onodera, H., Hirose, M. and Nomura, T.: **A mechanistic study of ovarian carcinogenesis induced by nitrofurazone using rasH2 mice**
Toxicol. Pathol., **28**, 649-655 (2000)

Tumorigenic mechanisms of nitrofurazone (NF) in mouse ovaries were evaluated in a short-term model using transgenic mice carrying the human c-Ha-ras gene (rasH2). Both rasH2 mice and their wild littermates (non-Tg) fed a diet containing 500 to 1,000 ppm NF for 7 weeks demonstrated ovarian atrophy with decreased labeling indices (LIs) for PCNA in granulosa cells. Increased atretic follicles and decreased LIs in granulosa cells were recognized in rasH2 mice at 250 or 500 ppm for 26 weeks, but no tumors. Ovarian atrophy was observed with increased serum luteinizing hormone levels at 1,000 ppm for 11 days. In conclusion, ovarian tumor induction by NF was associated with continuous stimulation of gonadotropins via a negative-feedback phenomenon secondary to ovarian atrophy.
Keywords : nitrofurazone, rasH2 mouse, ovarian tumor

Furukawa, F., Nishikawa, A., Nakamura, H., Miyauchi, M., Son, H-Y. and Hirose, M.: **Effects of octreotide, a somatostatin analogue, on initiation of pancreatic carcinogenesis in hamsters with N-nitrosobis (2-oxopropyl) amine**
Cancer Lett., **159**, 43-48 (2000)

The modifying effects of octreotide acetate, a somatostatin (SMS) analogue shown to inhibit secretion of digestive enzymes, bicarbonate and pancreatic juice, on the initiation phase of pancreatic carcinogenesis were investigated in hamsters simultaneously treated with BOP. Groups 1-3, each consisting of 20 animals, were given BOP subcutaneously once a week three times at a dose of 10 mg/kg body weight during administration of octreotide acetate for 28 days via osmotic pumps implanted subcutaneously at doses of 6 μ /day (group 1), 3 μ /day (group 2) or 0 μ /day (saline) (group 3). At the termination of experimental week 40, the incidences and multiplicities of pancreatic ductal adenocarcinomas and dysplastic lesions did not significantly differ among groups 1-3. Subcutaneous administration of octreotide acetate resulted in obviously increased plasma octreotide levels. Our results thus suggest that this SMS analogue may not modulate the initiation of BOP-induced pancreatic carcinogenesis, regardless of its pharmacological action.
Keywords : Octreotide acetate, pancreatic carcinogenesis, hamster

Son, H-Y., Nishikawa, A., Ikeda, T., Nakamura, H., Miyauchi, M., Imazawa, T., Furukawa, F. and Hirose, M.: **Lack of modifying effects of environmental estrogenic compounds on the development of thyroid proliferative lesions in male rats pretreated with *N*-bis (2-hydroxypropyl) nitrosamine (DHPN)**
Jpn. J. Cancer Res., **91**, 899-905 (2000)

The modifying effects of various environmental estrogenic compounds on thyroid carcinogenesis were investigated in a rodent two-stage carcinogenesis model. The compounds examined were a soy isoflavone mixture (SI) and genistein (GEN) as phytoestrogens, nonylphenol (NP) as a xenoestrogen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX) as a thyroid carcinogen and sulfadimethoxine (SDM) as a known thyroid tu-

mor promoter. Five-week-old male F344 rats were given a single subcutaneous injection of DHPN or the vehicle alone. Starting one week thereafter, GEN, SI, NP, MX or SDM was administered for 12 weeks. Thyroid weights were significantly increased only in the SDM treatment groups, especially with DHPN pretreatment. Kidney weights were slightly increased in the NP or MX treatment groups, albeit without statistical significance. There were no organ weight changes or histopathological lesions in the major organs including the thyroid in the GEN, SI, NP, and MX treatment groups regardless of DHPN pretreatment. Our results thus indicate that the weakly estrogenic compounds GEN, SI and NP and the environmental rat thyroid carcinogen MX do not exert any modifying effects on thyroid carcinogenesis in rats under the present experimental conditions.

Keywords : Estrogenic compounds, thyroid carcinogenesis, genistein

Son, H-Y., Nishikawa, A., Ikeda, T., Furukawa, F. and Hirose, M.: **Lack of modification by environmental estrogenic compounds of thyroid carcinogenesis in ovariectomized rats pretreated with N-bis(2-hydroxypropyl)nitrosamine (DHPN)**
Jpn. J. Cancer Res., **91**, 966-972 (2000)

The effects of environmental estrogenic compounds, soy isoflavone mixture (SI), genistein (GEN), and nonylphenol (NP), and the possible goitrogen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX), on thyroid carcinogenesis were investigated in ovariectomized (OVX) female rats. Five-week-old OVX F344 rats were given a single subcutaneous injection of DHPN. Starting 1 week later, GEN, SI, NP, MX, sulfadimethoxine (SDM), a known thyroid tumor-promoter, or beta-estradiol 3-benzoate (EB), a synthetic estrogen were administered for 12 weeks. SDM and EB were included as positive controls. Renal tubule lesions, uterine squamous metaplasia, vaginal keratinization and telangiectasia of pancreatic islets were also observed with EB. There were no organ weight changes or histopathological lesions in the major organs, including the thyroid, in the GEN, SI, MX or NP treatment groups. Our results thus indicated a lack of modifying effects on thyroid carcinogenesis in female OVX rats, in agreement with our previous finding in males.

Keywords : Thyroid carcinogenesis, ovariectomy, genistein

Nakamura, H., Nishikawa, A., Furukawa, F., Kasahara, K., Miyauchi, M., Son, H-Y. and Hirose, M.: **Inhibitory effects of protocatechuic acid on the post-initiation phase of hamster pancreatic carcinogenesis induced by N-nitrosobis (2-oxopropyl) amine**
Anticancer Res., **20**, 3423-3428 (2000)

The chemopreventive effects of protocatechuic acid (PCA) were investigated during the post-initiation stage of the BOP-initiated hamster pancreatic tumorigenesis model. Animals in groups 1-3 were given two s.c. injections of 20 mg/kg body weight of BOP with a one week interval as an initiation treatment. After the BOP injection, hamsters in groups 1 and 2 were respectively fed diet supplemented with 1000 or 500 ppm of PCA for 49 weeks. The animals in group 3 were treated with BOP alone. The animals in groups 4-6, each consisting of 10 hamsters, were given 1000 or 500 ppm PCA, or basal diet alone without prior BOP injection. At the termination of experimental week 52, the incidences and multiplicities of neoplastic lesions in the pancreas were comparable among the BOP-treated groups. However, the incidence of pancreatic tumors larger than 3 cm was significantly lower in the PCA-treated high dose groups than in the control group. Moreover the incidence of advanced pancreatic cancers which had directly invaded adjacent tissues such as the diaphragm, spleen and stomach was reduced by the PCA treatments, being significantly lower in group 2 than in group 3. Our results thus indicated that PCA can inhibit the late post-initiation or progression phase of BOP-induced pancreatic carcinogenesis in hamsters.

Keywords : protocatechuic acid, pancreatic carcinogenesis, BOP

Tamura, K., Yasuhara, K., Koujitani, T., Onodera, H., Takagi, H., Takizawa, T., Hirose, M., Hayashi, Y. and Mitsumori, K.: **Lack of modifying effects of cinnamaldehyde on development of lung prolifer- ative lesions induced by urethane in transgenic mice carrying the human prototype c-Ha-ras gene**
J. Toxicol. Pathol., **13**, 249-255 (2000)

To investigate the modifying effects of cinnamaldehyde (CNMA) on the development of lung proliferative lesions, male transgenic mice carrying the human prototype c-Ha-*ras* gene (rasH2) were given a single injection of urethane (UR) or saline, followed by a diet containing 0.5% CNMA or basal diet for 26 weeks. Lung tumors were induced in the treated groups except for CNMA-alone group without significant intergroup differences in their incidences and multiplicities. There were no intergroup differences between UR-alone and UR+CNMA groups in the PCNA labeling indices and the areas of lung tumors. It was concluded that the CNMA treatment does not influence on the development of lung proliferative lesions induced by urethane in rasH2 mice.

Keywords : cinnamaldehyde, rasH2 mouse, lung tumor

Lee, I-S.[*1], Yang, E-J.[*1], Kim, H-S.[*1], Chung, S-K.[*2], Furukawa, F. and Nishikawa, A.: **Suppressive effects of *Adenophora triphylla* extracts on *in vitro* tumor cell growth and *in vivo* gastric epithelial proliferation**
Anticancer Res., **20**, 3227-3232 (2000)

Adenophora triphylla (AT), an oriental medicinal plant, was extracted using water and several organic solvents and each fraction was assayed for its tumoricidal effects on human Jurkat T cells with 3- (4,5-dimethylthiazolyl) -2, 5-diphenyltetrazolium bromide (MTT). The influence on induction of apoptosis and G1 arrest was also examined. The ethyl acetate fraction showed the most pronounced inhibitory effects on proliferation of Jurkat T cells. Apoptosis was induced in line with up-regulation of FasL, tyrosine phosphorylation and c-fos mRNA levels. Arrest in G1 of the cell cycle was observed in A2780 cells with a wild type p53 gene but not HT-29 cells with a mutant p53 gene. Modifying effects of AT on cell turnover and glutathione(GSH) levels in vivo were also investigated in the stomach of rats given 150 mg/kg of MNNG by gavage and then fed a diet supplemented with 5% or 1% pulverized AT and 0.5% or 0.2% ethylacetate-extracted AT for 42 hours. The 5% AT and both of the ethylacetate fractions caused significant reduction in PCNA-labeling in the glandular

stomach epithelium as compared with the value for the MNNG alone group. In addition, the treatments significantly increased the gastric GSH levels. These results suggest that AT could be a chemopreventive agent against gastric cancer.

Keywords : *Adenophora triphylla*, apoptosis, FasL

*1 Keimyung University
*2 Kyungpook University

Son, H-Y., Nishikawa, A., Furukawa, F., Lee, I-S.*1, Ikeda, T., Miyauchi, M., Nakamura, H. and Hirose, M.: **Modifying effects of 4-phenylbutyl isothiocyanate on N-nitrosobis (2-oxopropyl) amine-induced tumorigenesis in hamsters**

Cancer Lett., **160**, 141-147 (2000)

The modifying effects of dietary 4-phenylbutyl isothiocyanate (PBITC), given during the initiation stage of carcinogenesis, were investigated in hamsters treated with BOP. Animals in groups 1-3, each consisting of 30 hamsters, were given BOP by two s.c. injections, 1 week apart, at a dose of 20 mg/kg body weight, plus 0, 10 or 100 micromol/animal of PBITC in corn oil by gavage 2 h prior to each carcinogen treatment. Ten animals in group 4 served as a vehicle control, and animals in groups 5 and 6, each consisting of ten hamsters, were given 10 and 100 μ of PBITC alone in corn oil. Sacrifice was 52 weeks after the first BOP injection. The PBITC treatments significantly inhibited the development of pancreatic ductal dysplasias and adenocarcinomas. Also, lung tumors (adenomas and adenocarcinomas) were significantly reduced in a dose-dependent manner. In contrast, both hepatocellular and cholangiocellular tumors tended to be or were significantly increased by PBITC. These results, taken together with our previous findings, indicate that the natural isothiocyanate, phenethyl isothiocyanate (PEITC), has a more potent chemopreventive action against BOP-induced tumorigenesis than synthetic isothiocyanates with longer alkyl chains, such as 3-phenylpropyl isothiocyanate (PPITC) and PBITC. Thus, their lipophilicity does not necessarily reflect the chemopreventive potential because the strength of lipophilicity is PEITC<PPITC<PBITC.

Keywords : 4-phenylbutyl isothiocyanate, BOP, hamster

*1 Keimyung University

Imazawa, T., Nishikawa, A., Todate, A., Furukawa, F., Onodera, H., Mitsumori, K., Hirose, M., Hayashi, Y.*: **Dual effects of prolonged ACTH stimulation on 4-hydroxy-aminoquinoline 1-oxide-induced adrenocortical lesions in rats**

Toxicol. Pathol., **28**, 535-539 (2000)

The effects of a long-acting synthetic ACTH on 4-hydroxyaminoquinoline 1-oxide (4HAQO)-induced adrenocortical lesions were investigated in female rats. A total of 140 rats were divided into 4 equal groups, given a single s.c. injection of 7 mg/kg 4HAQO or vehicle, followed by repeated sc administration of the synthetic ACTH or no further treatment. Subgroups of 10 rats in each group were sequentially sacrificed at weeks 20, 30, and 40. Adenomas and adenomatous nodules developed in the adrenal cortex of animals receiving 4HAQO and the chronic ACTH stimulation. From week 20, middle zone, cortical cystic degeneration, which mimics the age-associated degenerative change named adrenal peliosis, was frequently observed in the adrenal glands of animals treated with 4HAQO alone. Its development was inhibited by ACTH. These results indicate that long-term stimulation of ACTH promotes the development of adrenocortical tumors but suppresses the occurrence of adrenal peliosis in rats treated with 4HAQO.

Keywords : Synthetic ACTH, 4HAQO, adrenal cortex, peliosis

*Kitasato University

Takagi, H., Mitsumori, K., Nishikawa, A., Onodera, H., Furukawa, F., Kasahara, K. and Hirose, M.: **Lack of carcinogenic sensitivity of the glandular stomach in heterozygous *p53* knockout mice given *N*-methyl-*N*-nitrosourea in their drinking water for 26 weeks**

Asian Pacific J. Cancer Prev., **1**, 299-303 (2000)

Gastric tumorigenic sensitivity to *N*-methyl-*N*- nitrosourea (MNU) was examined in heterozygous *p53* knockout (*p53*(+/-)) CBA mice and their wild-type littermates (*p53*(+/+)). *p53*(+/-) or *p53*(+/+) CBA mice were given MNU in their drinking water at concentration of 50, 10, 5 or 0ppm for 26 weeks. The incidences of hyperplasias in the glandular stomach observed in *p53*(+/-) CBA mice treated with 50ppm and 10ppm MNU were significantly increased, as compared with the control group. No tumors were induced in the stomach of any treated groups. The present study suggests that *p53*(+/-) CBA mice have low susceptibility to MNU-induced gastric carcinogenesis.

Keywords : *p53* knockout CBA mouse, MNU, gastric tumor

Mitsumori, K., Shimo, T., Onodera, H., Takagi, H., Yasuhara, K., Tamura, T., Aoki, Y., Nagata, O. and Hirose M.: **Modifying effects of ethinylestradiol but not methoxychlor on N-ethyl-N-nitrosourea-induced uterine carcinogenesis in heterozygous p53 deficient CBA mice**

Toxicol. Sci., **58**, 43-49 (2000)

It is unknown whether endocrine-disrupting chemicals (EDCs) with estrogenic activities have any modifying effects on uterine carcinogenesis. To investigate the effects of ethinylestradiol (EE) and methoxychlor (MXC) on development of *N*-ethyl-*N*-nitrosourea (ENU)-induced uterine tumors, female p53-deficient CBA mice [p53 (+/-) mice] and their wild-type littermates [p53 (+/+) mice] received an injection of ENU, followed by a diet containing EE or MXC for 26 weeks. The present study suggests that 2.5 ppm EE, but not MXC, exerts tumor-promoting effects on stromal and epithelial proliferative lesions of the uteri in p53 (+/-) mice initiated with ENU.

Keywords : p53-deficient mouse, uterine tumor, ethinylestradiol

Shibutani, M., Mitsumori,K., Niho, N., Satoh, S.*1, Hiratsuka, H.*2, Satoh, M.*3, Sumiyoshi, M.*4, Nishijima, M.*5, Katsuki, Y.*5, Suzuki, J. *5, Nakagawa, J. *5, Ando, M.: **Assessment of *Re*nal *T*oxicity by *A*nalysis of *R*egeneration of *T*ubular *E*pithelium in *R*ats *G*iven *L*ow *D*ose *C*admium *C*hloride or *C*admium-polluted *R*ice for 22-months**

Arch. Toxicol., **74**, 571-577 (2000)

Six groups of female SD rats were fed a diet containing low amounts of CdCl2 or Cd-polluted rice at concentrations up to 40 ppm, and were killed after 12, 18, and 22 months. Animals dem-

onstrated spontaneous chronic nephropathy and fluctuation in the tubular PCNA LI, but these findings were not correlated with renal Cd levels at 22 months. PCNA LI on the other hand, appeared to be linked to the severity of chronic nephropathy. The results demonstrated that treatment with 40 ppm or less for 22 months did not influence tubular regeneration as a component of nonspecific chronic nephropathy, suggesting that long-term oral administration of low levels of Cd does not injure renal tubules in female rats.

Keywords : Cd-induced nephrotoxicity, PCNA-LI, chronic nephropathy

*1 Ina Research Inc.
*2 Mitsubishi Chemical Safety Institute Ltd.,
*3 National Institute for Environmental Studies
*4 Japan Food Research Laboratories
*5 The Tokyo Metropolitan Research Laboratory of Public Health

Hirose, M., Yamaguchi, T.*1, Lin, C.*1, Kimoto, N.*1, Futakuchi, M.*1, Kono, T.*2, Nishibe, S.*3 and Shirai, T.*1: **Effects of arctiin on PhIP-induced mammary, colon and pancreatic carcinogenesis in female Sprague-Dawley rats and MeIQx-induced hepatocarcinogenesis in male F344 rats**

Cancer Lett., **155**, 79-88 (2000)

Chemopreventive effects of arctiin, a lignan isolated from *Arctium lappa* (burdock) seeds, on the initiation or post initiation period of PhIP induced mammary carcinogenesis in female rats and on MeIQx-associated hepatocarcinogenesis in male rats were examined. In experiment 1, female SD rats were given intragastric doses of 100 mg/kg body wt of PhIP once a week for 8 weeks as initiation. Groups of 20 rats each were treated with 0.2 or 0.02% arctiin during or after PhIP initiation. Animals were killed at the end of week 48. Although the incidence of mammary carcinomas did not significantly differ among the PhIP-treated groups, multiplicity was significantly decreased in rats given 0.2 or 0.02% arctiin after PhIP initiation as compared with the PhIP alone control. The average number of colon aberrant crypt foci was also significantly decreased in these two groups. Pancreas acidophilic foci were induced in PhIP treated animals with slight decrease in the multiplicity with arctiin during the initiation phase. For liver carcinogenesis, groups of 15 male F344 rats were given a single intraperitoneal injection of DEN and starting 2 weeks later, they were administered 0.03% MeIQx in the diet, MeIQx together with 0.5% arctiin, 0.1% arctiin or basal diet for 6 weeks. They were subjected to two-third partial hepatectomy 3 weeks after DEN initiation and killed at the end of week 8 for GST-P immunohistochemistry. The numbers and areas of preneoplastic GST-P positive foci were elevated by the treatment with MeIQx, and further increased by the simultaneous treatment with arctiin. These results indicate that arctiin has a protective effect on PhIP-induced carcinogenesis particularly in the mammary gland in the promotion period. On the other hand, it may have a weak co-carcinogenic influence on MeIQx-induced hepatocarcinogenesis. In addition, the results suggested that PhIP is a weak pancreatic carcinogen in female SD rats, targeting acinar cells.

Keywords : Actiin, PhIP, MeIQx,

*1 Nagoya City University
*2 Meiji Institute of Health Science
*3 Health Sciences University of Hokkaido

Danai, T.*1, Hirose, M., Futakuchi, M.*2, Lin, C.*2, Thamavit, W.*3, Ito, N*2. and Shirai T.*2: **Enhancing effects of Thai eddible plants on 2-amino-3,8-dimethylimidazo [4,5-f] quinoxaline-hepatocarcinogenesis in a rat medium-term bioassay**

Cancer Lett., **158**, 195-201 (2000)

Boesenbergia pandurata (Zingiberaceae), *Languas galanga* (Zingiberaceae) and *Citrus hystrix* (Rutaceae) are edible plants that are commonly used as flavors or condiments in various Thai food dishes. They are known to exert strong anti-promoting activity in a test of tumor promoter-induced Epstein-Barr virus (EBV) activation. In the present study their effects on hepatocarcinogenesis were investigated in a medium-term bioassay using F344 male rats. *C. hystrix* significantly enhanced 2-amino-3,8-dimethylimidazo (4, 5-*f*) quinoxaline-associated preneoplastic liver cell focus development while *B. pandurata* and *L. galanga* had borderline effects. The results suggest that *C. hystrix* as well as B. pandurata and L. galanga may contain agents augmenting the hepatocarcinogenicity of 2-amino-3,8-dimethylimidazo(4,5-*f*) quinoxaline.

Keywords : Thai eddible plants, 2-amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline, medium-term bioassay

*1 National Cancer Institute, Thailand
*2 Nagoya City University
*3 Mahidol University

Kawabe, M.*1, Lin, C.*1, Kimoto, N.*1, Sano, M.*1, Hirose, M. and Shirai, T*1.: **Modifying effects of propolis on MeIQx promotion of rat hepatocarcinogenesis and in a female rat two-stage carcinogenesis model after multiple carcinogen initiation**

Nutr. Cancer., **37**, 179-186 (2000)

The modifying effects of the dietary administration of water- and ethanol-extracted propolis produced in Brazil (WB and EB, respectively) on MeIQx promotion of rat hepatocarcinogenesis were investigated in a medium-term liver bioassay system with use of male Fischer 344 rats. The number and area of GST-P-positive foci in rats given 0.5% WB were significantly increased compared with the group given MeIQx alone. Furthermore, the numbers of GST-P-positive foci were higher in rats given 0.1% WB or EB than in those given the basal diet alone. The modifying effects of propolis on other organs were also examined in female Fischer 344 rats given multiple carcinogens for initiation. Rats received water- and ethanol-extracted propolis produced in Brazil and Uruguay (WB, EB, WU, and EU, respectively) in the diet after exposure to three different carcinogens. The incidence of total mammary tumors was significantly lower in rats given EU than in the control group. These results indicate that a water extract of propolis exerts a cocarcinogenic effect on MeIQx hepatocarcinogenesis while promoting the effect at low dose in a two-stage hepatocarcinogenesis model. Moreover, they suggest that ethanol-extracted propolis may be an inhibitor of mammary gland carcinogenesis.

Keywords : Propolis, rat hepatocarcinogenesis, two-stage carcinogenesis

*1 Nagoya City University

Hamada, S.*1, Sutou, S.*2, Morita, T.*3, Wakata, A.*4, Asanami, S.*5, Hosoya, S.*6, Ozawa, S.*7, Kondo, K.*8, Nakajima, M.*9, Shimada, H.*10, Osawa, K.*11, Kondo, Y.*12, Asano, N.*13, Sato, S.*14, Tamura, H.*15, Yajima, N.*16, Marshall, R.*17, Moore, C.*18, Blakey, D.H.*19, Schechtman, L.M.*20, Weaver, J.L.*20, Torous, D.K.*21, Proudlock, R.*22, Ito, S.*23, Namiki, C.*24, and Hayashi, M.: **Evaluation of the rodent micronucleus assay by a 28-day-treatment protocol: Summary of the 13th collaborative study by CSGMT/JEMS・MMS**

Environ. Mol. Mutagen., **37**, 93-110 (2001)

To examine whether micronucleus tests (MNT) can be incorporated into general toxicology assays, we performed MNT applying the treatment protocols typically used in such assays. Our results indicate that the integration of the MNT into a 28-day toxicological assay is feasible. To serve this purpose, blood samples collected 4 days after the beginning of treatment and blood and bone marrow samples collected at autopsy should be examined. We propose that rats can provide biologically important and relevant information regarding potential chemical mutagens.

Keywords : UVB, epidermis, deletion

*1 SSP Company, Ltd, Japan

*2-*24 See article.

Kusunoki, Y.*, Kyoizumi, S. *, Honma, M., Kubo, Y. *, Dhnishi, H. *, Hayashi, T. * and Seyama T. *: **NK-mediated elimination of mutant lymphocytes that have lost expression of MHC class I molecules.**

J. Immunol., **165**, 3555-3563 (2000)

Mutant cells generated in vivo can be eliminated when mutated gene products are presented as altered MHC/peptide complexes and recognized by T cells. Diminished expression of MHC/peptide complexes enables mutant cells to escape recognition by T-cells. In the present study, we tested the hypothesis that mutant lymphocytes lacking expression of MHC class I molecules are eliminated by autologous NK cells.

Keywords : MHC, mutation, NK cells

*㈶放射線影響研究所

Honma, M., Momose, M., Tanabe, H., Sakamoto, H., Yu, Y.*, Little, J.B.*, Sofuni, T. and Hayashi, M.: **Requirement of wild type p53 protein for maintenance of chromosomal integrity.**

Mol. Carcinog., **28**, 1-12 (2000)

Chromosomal double strand breaks (DSBs) occurring in mammalian cells, which are responsible for initiating genomic instability, are usually repaired by end-rejoining or homologous recombination. We demonstrated here that wild-type p53 protein contributes to the maintenance of genomic integrity through recombinational DNA repair. Human lymphoblastoid cells deficient in p53 were defective in the ability to carry out recombinational DNA repair of spontaneously arising DSBs. Broken chromosomes by DSBs in p53-deficient cells triggered the breakage-fusion-bridge cycle, and were occasionally stabilized by end-rejoining or nonhomologous recombination to other chromosomes. Our results support a model in which p53 protein regulates recombinational DNA repair, providing a mechanism for the maintenance of genomic integrity in mammalian cells.

Key words : p53, DNA double strand break (DSB), recombination repair

* Harvard School of Public Health

Kohara, A., Suzuki, T., Honma, M., Hirano, N.*1, Ohsawa, K.*1, Ohwada, T.*2, Hayashi, M.: **Mutation spectrum of *o*-aminoazotoluene in the *cII* gene of lambda/*lacZ* transgenic mice (Muta™Mouse)**

Mutation Res., **491**, 211-220 (2001)

The *o*-aminoazotoluene (AAT) is carcinogenic mainly in the liver, and also in lung following long term administration. In the present report, we reveal the molecular nature of mutations induced by AAT in the lambda *cII* gene (the *cII* gene, a phenotypically selectable marker in the lambda transgene, has 294 bp, which makes it easier to sequence than the original target, the 3kb *lacZ* gene). The *cII* mutant frequency in liver and colon was five and nine times higher, respectively, in AAT-treated mice than in control mice. Sequence analysis revealed that AAT induced G:C to T:A transversions, whereas spontaneous mutations consisted primarily of G:C to A:T transitions at CpG sites.

Keywords: mutation spctra, Muta™Mouse, *o*-aminoazotuluene

*1 大正製薬㈱

*2 名古屋市立大学薬学部

Suzuki, T., Wang, X., Miyata, Y.*, Saeki, K.*, Kohara, A.*, Kawazoe, Y.*, Hayashi, M., Sofuni, T.: **Hepatocarcinogen quinoline induces G:C to C:G transversions in the *cII* gene in the liver of lambda/*lacZ* transgenic mice (Muta™Mouse)**

Mutation Res. **456**, 73-81 (2000)

Quinoline is carcinogenic to the liver in rodents, but it is not clear whether it acts by a genotoxic mechanism. In the present report, we reveal the molecular nature of the mutations induced by quinoline in the lambda *cII* gene, which is also a phenotypically selectable marker in the lambda transgene. The liver *cII* mutant frequency was nine times higher in quinoline-treated mice than in control mice. Sequence analysis revealed that quinoline induced primarily G:C to C:G transversions (25 of 34). Thus, we have confirmed that quinoline is genotoxic in its target organ, and the G:C to C:G transversion is the molecular signature of quinoline-induced mutations

Keywords : mutation spectra, Muta™Mouse, quinoline

*名古屋市立大学薬学部

Itoh, T., Suzuki, T., Nishikawa, A., Frukawa, F., Takahashi, M., Wang, X., Sofuni, T., Hayashi, M.: **In vivo genotoxicity of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline in *lacI* transgenic (Big Blue^R) mice**

Mutation Res. **468**, 19-25 (2000)

2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), a heterocyclic amine found in cooked meat, is a strong mutagen and was proven to be a hepatocarcinogen in rodents. We used the *lacI* transgenic (Big Blue^R) mouse to investigate MeIQx genotoxicity in vivo. Intragastric treatment with MeIQx (100 mg/kg) did not increase mutant frequency (MF) in liver or colon. No

apparent increase in PCNA-positive foci was observed in any of tissues analyzed 14 days after the treatment. Administration of MeIQx (300 ppm) in diet for 12 weeks, however, caused MF increases in liver and colon in male and female mice, with greater increases in the females. An increase was also obvious after 4 weeks, but only in females. These results demonstrated that in the transgenic mouse mutation assay, long-term feeding of MeIQx was more effective than single gastric exposures and that sex differences in susceptibility can also be observed.
Keywords : organ specificity, Big Blue^{R}, *lacI*

Kawasaki, K.[*1*2], Suzuki, T., Ueda, M.[*2], Ichihashi, G.[*2], Reguer, G., Yamasaki, H.[*1]: **CC to TT mutation in the mitochondrial DNA of normal skin: relationship to ultraviolet light exposure**
Mutation Res. **468**, 35-43 (2000)

In order to get a cumulative marker of UV exposure, we have established a sensitive allele-specific polymerase chain reaction (AS-PCR) assay capable of detecting one CC to TT mutation in Mt DNA among 10^7 wild-type genes using a mismatch allele-specific primer. With this assay, we found no mutation-positive samples from internal non-exposed tissue (stomach, colon, and blood) (0/50). In contrast, 17 out of 111 skin samples were positive. In normal skin tissue, the prevalence of positive samples was higher in those from exposed sites (13/51) than in those from less-exposed sites (1/26) ($p<0.05$), however, a quantitative correlation was not found. We conclude that the UV-associated CC to TT mutation in Mt DNA can be detected in normal skin, but further studies are required to develop this as a quantitative marker for UV exposure.
Keyword : allele-specific PCR, UV, mitochondria
[*1] International Agency for Research on Cancer (France)
[*2] 神戸大学医学部

Horiguchi, M.[*1], Masumura, K., Ikehata, H.[*2], Ono, T.[*2], Kanke, Y.[*1] and Nohmi, T.: **Molecular nature of UVB-induced deletions in the murine epidermis**
Cancer Res., **61**, 3913-3918 (2001)

Tumor development in the skin could be a multi-step process where various genetic alterations. We demonstrate that UVB irradiation efficiently induces deletions in the epidermis using a novel transgenic mouse *gpt* delta. In this mouse model, deletions in lambda DNA integrated in the chromosome are preferentially selected as Spi$^-$ (*s*ensitive to *P2 i*nterference) phages, which can then be subjected to molecular analysis. The mice were exposed to UVB at single doses. Four weeks later, lambda phage was rescued from the genomic DNA of the epidermis. The mutant frequencies of Spi$^-$ having large deletions in the epidermis increased more than 15-fold at a UVB dose of 0.5 kJ/m^2 over the control. Molecular sizes of most of the large deletions were greater than 1,000 base pairs. These results suggest that UVB irradiation induces deletions in the murine epidermis, and most of the deletions are generated through end-joining of DNA double strand breaks.
Keywords : UVB, epidermis, deletion
[*1] 東京農業大学
[*2] 東北大学薬学研究科

Masumura, K., Matsui, K., Yamada, M., Horiguchi, M., Ishida, K.*, Watanabe, M.*, Wakabayashi, K.* and Nohmi, T.: **Characterization of mutations induced by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in the colon of *gpt* delta transgenic mouse: novel G:C deletions beside runs of identical bases**
Carcinogenesis, **21** (11), 2049-2056 (2000)

Mutations induced by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), were characterized using *gpt* delta transgenic mice. This system has two selection methods to efficiently detect the point mutations or deletions. The mice were fed with a diet containing 400 ppm PhIP for 13 weeks and *gpt* and Spi$^-$ mutations were analyzed from the colon. Concerning the types of *gpt* mutations from PhIP-treated mice, G:C to T:A transversions and single base pair deletions at G:C base pairs predominated. Concerning Spi$^-$ mutants from PhIP-treated mice, G:C base pair deletions in monotonic G or C run sequences and beside run sequences predominated. These results suggest that PhIP induces point mutations, rather than larger deletions *in vivo* and that run sequences may play an important role in PhIP-induced G:C base pair deletions.
Keywords: PhIP, *gpt* delta, mutation spectrum
*国立がんセンター研究所

Kushida, H.*, Fujita, K.*, Suzuki, A.*, Yamada, M. Nohmi, T. and Kamataki, T.*: **Metabolic activation of *N*-alkylnitrosamines in genetically engineered *Salmonella typhimurium* expressing CYP2E1 or CYP2A6 together with human NADPH-cytochrome P450 reductase**
Carcinogenesis, **21** (11), 1227-1232 (2000)

The mutagen-activating capacity of human CYP2E1 for *N*-alkylnitrosamines was compared with that of CYP2A6 using *Salmonella typhimurium* YG7108 2E1/OR and YG7108 2A6/OR strains. Eight *N*-alkylnitrosamines, including *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosodipropylamine, *N*-nitrosodibutylamine, *N*-nitrosomethylphenylamine, *N*-nitrosopyrrolidine, *N*-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone were examined. We conclude that human CYP2E1 is mainly responsile for the metabolic activation of *N*-nitrosamines with a relatively short alkylchain(s), whereas CYP2A6 was predominantly responsible for the metabolic activation of *N*-alkylnitrosamines possessing a relatively bulky alkyl chain(s).
Keywords : *N*-alkylnitrosamines, human CYP2E1, human CYP2A6
*北海道大学薬学研究科

Kushida, H.*, Fujita, K.*, Suzuki, A.*, Yamada, M. Nohmi, T. and Kamataki, T.*: **Development of a *Salmonella* tester strain sensitive to promutagenic *N*-nitrosamines: expression of recombinant CYP2A6 and human NADPH-cytochrome P450 reductase in *S. typhimurium* YG7108**
Mutat. Res., **471**, 135-143 (2000)

We developed a new *Salmonella* tester strain highly sensitive to promutagenic *N*-nitrosamines by introducing a plasmid carrying human cytochrome P450 2A6(CYP2A6) and NADPH-cytochrome

P450 reductase (OR) cDNA into the *ada*-and *ogt*-deficient strain YG7108. The expressed CYP2A6 efficiently catalyzed coumarin 7-hydroxylation. *N*-nitrosomethylphenylamine (NMPhA) and some other chemicals were mutagenic in the new strain into the absence of any exogenous activation system. We believe that this is the first demonstration that CYP2A6 is responsible for the metabolic activation of NMPhA. The established tester strain may be useful to predict human activation of *N*-nitrosamine promutagens.
Keywords : YG7108, mutation assay, heterologous expression
*北海道大学薬学研究科

Wagner, J.*, Nohmi, T.: ***Escherichia coli* DNA Polymerase IV Mutator Activity: Genetic Requirements and Mutational Specificity**
J. Bacteriol., **182**, 4587-4595 (2000)

The *dinB* gene of *Escherichia coli* encodes a novel DNA polymerase, DNA Pol IV, which is able to dramatically increase the untargeted mutagenesis. At the amino acid level, DNA Pol IV shares sequence homologies with *E. coli* UmuC (DNA Pol V), Rev1p and Rad30p (DNA polymerase η) of *Saccharomyces cerevisiae* and human Rad30A (XPV) proteins, all of which are involved in translesion DNA synthesis. Here, we report that the DNA pol IV mutator activity clearly promotes single nucleotide substitutions as well as one base deletions in the ratio of about 1:2. The base changes were strikingly biased for substitutions toward G:C base pairs and about 70 % of them occurred in 5'-GX-3' sequences where X represents the base (T, A or C) that is mutated to G. These results are discussed in the view of the recently described biochemical characteristics of DNA Pol IV.
Keywords : *dinB*, DNA polymerase IV, untargeted mutagenesis
* IRCAD, CNRS, France

Wagner, J.*, Fujii, S.*, Gruz, P., Nohmi, T., Fuchs, R.P.P.*: **The s clamp targets DNA polymerase IV to DNA and strongly increases its processivity.**
EMBO Report, **1**, 484-488 (2000)

The recent discovery of a new family of ubiquitous DNA polymerases involved in translesion synthesis has shed new light onto the biochemical basis of mutagenesis. Among these polymerases, the *dinB* gene product (Pol IV) is involved in mutagenesis in *Escherichia coli*. We show here that the activity of native Pol IV is drastically modified upon interaction with the s subunit, the processivity factor of DNA Pol III. In the absence of the s subunit Pol IV is strictly distributive and no stable complex between Pol IV and DNA could be detected. In contrast, the s clamp allows Pol IV to form a stable initiation complex ($t_{1/2}$ approximately 2.3 min), which leads to a dramatic increase in the processivity of Pol IV reaching an average of 300-400 nucleotides. *In vivo*, the s processivity subunit may target DNA Pol IV to its substrate, generating synthesis tracks much longer than previously thought.
Keywords : *dinB*, DNA polymerase IV, processivity
* IRCAD, CNRS, France

Shinmura, K.*, Yamaguchi, S.*, Saitoh, T.*, Takeuchi-Sasaki, M.*, Kim, S.-R., Nohmi, T. and Yokota, J.*: **Adenine excisional repair function of MYH protein on the adenine:8-hydroxyguanine base pair in double-stranded DNA**
Nucleic Acids Res., **28**, 4912-4918 (2000)

Adenine paired with 8-hydroxyguanine (oh^{8}G), a major component of oxidative DNA damage, is excised by MYH base excision repair protein in human cells. We compared the repair activity of type 1 mitochondrial and type 2 nuclear MYH proteins on A:oh8G and A:G mismatches. In a reaction buffer with a low salt (0-50 mM) concentration, adenine DNA glycosylase activity of type 2 protein was detected on both A:oh^{8}G and A:G substrates. However, in a reaction buffer with a 150 mM salt concentration, similar to physiological conditions, the glycosylase activity on A:G, but not on A:oh^{8}G, was extremely reduced and the binding activity of type 2 protein for A : G, but not for A : oh^{8}G, was proportionally reduced. The glycosylase activity on A : oh^{8}G and the ability to suppress spontaneous mutagenesis were greater for type 2 than type 1 enzyme. These results indicate that human MYH protein specifically catalyzes the glycosylase reaction on A:oh^{8}G under physiological salt concentrations.
Keywords : oxidative DNA damage, 8-hydroxyguanine, base excision repair
*国立がんセンター研究所

Tanabe, H., Nakagawa, Y.*1, Minegishi, D., Hashimoto, K.*2, Tanaka, N.*1, Oshimura, M.*3, Sofuni, T., and Mizusawa, H. **Human Monochromosome Hybrid Cell Panel Characterized by FISH in the JCRB/HSRRB**
Chromosome Res. **8**, 319-334 (2000)

The human monochromosome hybrid cell panel in the Japanese Collection of Research Bioresources (JCRB) consists of 23 mouse cell clones, each containing a different human chromosome. In order to determine the state of the human chromosomes and to supply the information to investigators, we characterized the cells by fluorescence *in situ* hybridization (FISH) with corresponding human chromosome-specific painting probes. Here we report the frequency of intact human chromosomes maintained in each hybrid and the retained subregions of corresponding human chromosomes with relative frequencies estimated by fluorescent intensity. This characterization will provide valuable information to investigators using the panel.
Keywords: FISH (fluorescence *in situ* hybridization), human monochromosome hybrid cell panel, JCRB (Japanese Collection of Research Bioresources)
*1食品薬品安全性研究センター秦野研究所
*2国立感染症研究所
*3鳥取大学医学部

高田容子，増井 徹，田辺秀之，原沢 亮*，水沢 博：**培養細胞系でのマイコプラズマのPCR検出法**
Tiss. Cult. Res. Commun. **19**, 131-138 (2000)

マイコプラズマの汚染を迅速・正確に検出することを目的に，ネステッドプライマーを用いた２段階のPCR法（ネステッドPCR法）を利用した検出系を開発した．２段階のPCRは検出感度を高めると同時に，１段階目のPCRの特異性を確認できる利点がある．陽性コントロールとしてMycoplasma orale DNAを用い，テンプレートDNA濃度3～10fg/μl（１反応あたり2-6コピー）が検出限界であることが明らかに

なった．更に，PCR法で増幅されたDNAを回収し，塩基配列を決定した結果，細胞に感染しているマイコプラズマ菌種を確実に同定することができた．この一連の方法の開発により，培養細胞に汚染しているマイコプラズマの検出感度，迅速性，また，信頼性を高め，細胞バンクの品質管理を向上させることが可能となった．

Keywords : Mycoplasma, nested PCR, DNA sequencing

*東京大学医学部附属動物実験施設

Imamura, O.*1, Fujita, K.*1, Shimamoto, A.*1, Tanabe, H., Takeda, S.*2, Furuichi, Y.*1, and Matsumoto, T.*1: **Bloom helicase is involved in DNA surveillance in early S phase in vertebrate cells**
Oncogene **20**, 1143-1151 (2001)

Bloom syndrome (BS) is a recessive human genetic disorder characterized by short stature, immunodeficiency and an elevated risk of malignancy. BS cells have mutator phenotypes such as hyper-recombination, chromosome instability and an increased frequency of sister chromatid exchange (SCE). We generated *BLM-/-* mutants of the chicken B-cell line DT40. They are hypersensitive to genotoxic agents such as etoposide, bleomycin and 4-nitroquinoline-1-oxide and irradiation with the short wave length of UV (UVC) light. UVC irradiation to *BLM-/-* cells during G1 to early S phase caused chromosomal instability, leading to eventual cell death. These results suggest that BLM is involved in surveillance of base abnormalities in genomic DNA that may be encountered by replication folks in early S phase. Such surveillance would maintain genomic stability in vertebrate cells, resulting in the prevention of cellular tumorigenesis.

Keywords : RecQ-type DNA helicase, Bloom syndrome, DT40

*1エイジーン研究所
*2京都大学医学部

増井 徹，祖父尼俊雄，石井美智子*1，今西由紀夫*2，安井英明*2，高田容子，林 真，水沢 博：**厚生省細胞バンクにおけるヒト組織・細胞取り扱い倫理問題への取り組み**
Tiss. Cult. Res. Commun. **19**, 1-15 (2000)

医学・生物学研究の中でヒトの組織・細胞を利用した研究が重要な位置をしめるようになってきた．そして，社会意識の変化に対応するために，ここにきてヒト試料を利用する際の倫理問題に関する複数ガイドラインが並列に存在する状況となる．研究現場では，いくつかのガイドラインに跨る形で研究を行わなければならないことになり，倫理審査が困難な状況となることも予想されている．そこで，この分野の社会認知の得るためにも，研究体制が整えられようとしている今こそ，基本的事柄に立ち還えることが重要であると考えている．我々は，厚生科学審議会先端医療技術評価部会の1998年12月の答申と日本組織培養学会の倫理問題検討委員会報告を踏まえて，ヒト試料の公的研究資源化のプロセスについて検討を試みてきた．本稿では厚生省細胞バンク（JCRB細胞バンク）の取り組みをケーススタディーとして報告する．

Keywords : human material, guideline, public research resource bank

*1東京都立大学法学部
*2関東中央病院

長谷川隆一，小泉睦子，広瀬明彦，前川昭彦*：**アルキルフェノールのエストロゲン様作用と生殖器系への影響**
日本食品化学学会誌，**7**, 1-9 (2000)

内分泌かく乱化学物質の一つとして注目されているアルキルフェノール類として，nonylphenol およびtert-octylphenolについて，文献情報を収集・整理するとともに，評価を行った．両物質ともに，in vitroおよびin vivoでエストロゲン受容体を介したエストロゲン様作用が認められる．経口投与によっては雌の生殖器官，乳腺，雄の生殖器官への強い影響は認められていないが，nonylphenolを出生直後の雄に腹腔内投与することにより，顕著な精巣及び副生殖器官重量の低下，停留睾丸及び受胎率の低下が引き起こされている．生体内動態は迅速で，半減期は数時間以内と短い．ラットの多世代混餌投与試験で，nonylphenolの生殖毒性に関する無毒性量は15mg/kg/dayである．この値を人の最大推定1日摂取量と比較したところ，安全域は3,000倍以上と推定され，人への危険性は現在のところ低いと考えられた．

Keywords : nonylphenol, octylphenol, estrogenic activity

*佐々木研究所

小泉睦子，江馬 眞，広瀬明彦，長谷川隆一：**フタル酸エステルの生殖および発生に対する毒性影響についての最近の研究：主としてDi(2-ethylhexyl) phthalateおよびDi-n-butyl phthalateについて**
日本食品化学学会誌，**7**, 65-73 (2000)

フタル酸エステルのうち，主にdi(2-ethylhexyl) phthalateおよびdi-n-butyl phthalateについて，げっ歯類の生殖および発生に対する毒性影響およびその推測されるメカニズムについて，最近の文献を中心に整理した．雄に対しては精巣萎縮や精子形成阻害を，雌に対しては性周期の延長や排卵阻害を引き起こす．生殖毒性としては，雌雄どちらに対しても不妊を，器官形成期の投与で口蓋裂，頸椎，胸椎，肋骨の奇形および腎盂拡張等の催奇形作用を，妊娠後期および哺乳期の母動物への投与では，次世代雄で肛門生殖突起間距離の短縮，尿道下裂，停留睾丸，精巣および副生殖器官の萎縮を引き起こす．毒性発現の殆どはモノエステル体によるものと考えられ，雄の精巣毒性はセルトリ細胞に対する影響を介して，また次世代雄への影響はアンドロゲン受容体を介さない抗アンドロゲン作用と考えられている．

Keywords : di(2-ethylhexyl) phthalate, di-n-butyl phthalate, reproductive/developmental toxicity

小泉睦子，江馬 眞，広瀬明彦，黒川雄二，長谷川隆一：**フタル酸エステルの生殖・発生無毒性量，精巣毒性の週齢差，種差およびDEHPの1日耐容摂取量**
日本食品化学学会誌，**8**, 1-10 (2001)

フタル酸エステルの雄生殖器に対する影響，雌生殖器に対する影響，生殖毒性および発生毒性について，構造活性相関に注目して，毒性発現の種類と程度（無毒性発現投与量）を比較，整理した．また，フタル酸エステルの精巣毒性に関する週齢差および種差の発現機構に関する情報を解析し，考察した．最後に，平成12年に設定されたDEHPのTDI誘導の根拠となって情報を整理，解説するとともに，その適正，情報の欠落等について考察した．

Keywords : NOAELs of phthalate esters, Species and age differ-

ences of testicular toxicity, TDI of DEHP

広瀬明彦, 鎌田栄一, 西川秋佳, 紅林秀雄, 江馬 眞, 安藤正典, 黒川雄二, 長谷川隆一: **ホルムアルデヒドの経口および吸入暴露による毒性と水道水における安全性の評価**

水環境学会誌, **24**, 308-316 (2001)

飲料水の消毒副生成物の 1 つであるホルムアルデヒドは, 経口摂取されるのみならず, 水道水の様々な使用形態により気化することにより, 吸入暴露を受けることが想定される. しかし, WHOや日本における水質基準の設定には, 吸入による暴露評価がほとんど考慮されていない. 本報告では, これらの基準設定の根拠となった毒性情報に最新の知見を加え, ホルムアルデヒドの毒性評価と水道水における安全性評価を, 経口摂取と吸入暴露に分けて行った.

Keywords : formaldehyde, disinfectant by-product, risk assessment

奥田秀毅[*1], 吉川一正[*1], 中西昭雄[*2], 松原俊彦[*2], 岡田敏史: **容量分析用標準液標定への電気滴定法の適用**

医薬品研究, **32**, 140-144 (2001)

日局 1 3 一般試験法の容量分析用標準液の標定は, 「0.1mol/L 亜硝酸ナトリウム液」を除けば, すべて指示薬法により行われているが, 医薬品各条の定量法における滴定法では電気滴定法, 主として電位差滴定法の採用が増加してきている. 各条で電気滴定法が採用されている標準液の標定法として, 指示薬法だけでなく電位差滴定法を適用するために, 15種類の標準液 (濃度違いを含めて) の標定結果が, 両法により一致するか否かについての共同実験の結果をまとめたものである. この共同実験結果に基いて, 日局容量分析用標準液に対する改正提案が行われた.

Keywords : volumetric analysis, potentiometric titration method, indicator method

[*1] 大阪医薬品協会技術研究委員会
[*2] 東京医薬品工業協会技術委員会

Inukai, M.,* Jin,Y.,* Yomota,C. and Yonese, M*: **Preparation and characterization of hyaluronate-hydroxy acrylate blend hydrogel for controlled release device**

Chem. Pharm. Bull., **48**, 850-854 (2001)

Hyaluronate-hydroxyethyl acrylate blend hydrogels were investigated as materials for controlled release devices. Glycidyl methacrylate(GMA) derivatized HA(GMS-HA) was synthesized by coupling of GMA to HA in the presence of a suitable catalyst. These hydrogels were prepared by a free radical copolymerization of GMA-HA and hydroxyethyl acrylate. The water content of these hydrogels was 0.978; however, these hydrogels was mechanically tough and could be used as disk shape.The hydrogels swelling were found to depend on ionic strength and pH. The dried hydrogel quickly regained their original condition in water, and they swelled to more than 90% of its initial water contents after 30 min. This swelling-deswelling behavior was reproducible. The release of chlorpromazine HCl as a model cationic drug from the gels was suppressed significantly in water. The release increased with increasing the ionic strength and decreasing pH of bulk solutions.

Keywords : hyaluronic acid, hydroxyethyl aclylate, gel

*名古屋市立大学薬学部

Miyazaki, T., Yomota, C. and Okada, S.: **Hyaluronate depolymerization following thermal decomposition of oxytetracycline**

Chem. Pharm. Bull., **49**, 118-122 (2001)

Depolymerization of hyaluronate (HA) by oxytetracycline (OTC) was investigated. On mixing with OTC and incubating at 37℃, HA was gradually depolymerized. OTC is known as a photosensitizer, however, HA depolymerization required no irradiation. As time passed, OTC solution incubated at 37℃ got colored reddish brown, even in the dark. With reversed-phase HPLC separation, several peaks derived from decomposed OTC appeared. One of the peaks had an absorbance in the visible range. A quantitative correlation between the discoloration and the HA depolymerization rate was obtained. On the other hand, when samples were incubated below 25℃ change of color was slight, and practically no HA depolymerization was observed after up to 4 hours. Oxygen depletion by nitrogen saturation or addition of mannitol also prevented the depolymerization. Under anaerobic conditions, the color of the solution did not change, whereas it turned red under aerobic conditions in the presence of mannitol. The mannitol did not inhibit the OTC decomposition, but it preserved HA from damage. On the basis of the known decomposition of OTC and the results of HPLC separation, anhydrooxytetracycline can be proposed as the derivative causing HA depolymerization.

Keywords : hyaluronate, oxytetracycline, molecular weight

Kudo, K.,* Iwaya, K.,* Yomota,C., Morris, S.* and Saito, M.*: **Determination of Enantiomeric Purity of Hyoscyamine from Scoporia Extract using HPLC-CD system without chiral separation**

Enantiomer, **5**, 369-375(2000)

Enantiomer ratio of hyoscyamine from Scopolia extract was determined by chiral HPLC-CD analysis. It was found that circular dicroism(CD) detection allowed the analysis of the sample without any special pretreatment whereas UV detection required ammonia-ether extraction. To obtain a shorter analysis time for the determination, reversed-phase HPLC-CD analysis was applied by using a g-factor calibration curve (EE% vs. CD/UV). The analysis time was shortened from 35 to 18 min. EE% values obtained were consistent with those by chiral HPLC analysis.

Keyword: Hyoscyamine, CD detector, Chiral

*日本分光株式会社

Saito, H., Arimoto I,*, Tanaka, M.*, Sasaki T.*, Tanimoto, T., Okada, S. and Handa, T.* : **Inhibition of lipoprotein lipase activity by sphingomyelin: role of membrane surface structure**

Biochim. Biophys. Acta., **1486**, 312-320(2000)

We have recently shown that sphingomyelin (SM) strongly inhibits lipoprotein lipase (LPL)-mediated lipolysis in monolayers and emulsion particles. To further evaluate how SM modulates LPL activity on the emulsion surface, the relationship between membrane surface structure and LPL activity was investigated. We measured fluorescence anisotropy of 1-palmitoyl-2-[3-(diphenylhexatrienyl)-propionyl]-sn-3-phosphatidylcholine, prob-

ing surface acyl chain fluidity, and fluorescence lifetime of N-(5-dimethyl-aminonaphthalene-1-sulfonyl)dipalmitoyl-phosphatidylethanolamine in H2O and D2O buffer, assessing the degree of hydration in the head group region. The results revealed that incorporation of egg SM into triolein-egg phosphatidylcholine emulsions markedly increased acyl chain order and decreased head group hydration of the surface monolayers. In contrast, cholesterol was shown to increase head group hydration despite a strong increase in acyl chain order. The close correlation between the apparent Km values of LPL and the degree of head group hydration indicated that LPL interacts with the head group region rather than with the hydrophobic interior of the surface monolayers. However, apparent Vmax did not show a simple correlation with any surface structure, and the finding in which SM had no effect on apparent Vmax of medium-chain triglyceride emulsions suggested that the hydrophobic interaction between acyl chains of SM and triglyceride at the emulsion surface is important for determining the apparent Vmax. These results showed conclusively that SM inhibits LPL activity mainly by changing the emulsion surface structure and not by a specific interaction between SM and LPL.

Keywords : sphingomyelin, lipoprotein lipase, cholesterol

*京都大学大学院薬学研究科

Saito, H., Okuhira, K.*, Tsuchimoto, N.*, Vertut-Doi, A.*, Matsumoto, C.*, Tanimoto, T., Okada, S. and Handa, T.* : **Modulation of Apolipoprotein E-Mediated Plasma Clearance and Cell Uptake of Emulsion Particles by Cholesteryl Ester**

Lipids, **36**, 27-33 (2001)

Cholesteryl ester, along with triglyceride (TG), is the major core component of plasma lipoproteins. We investigated the effect of core composition on the physical state and metabolic behavior of lipid emulsions, as model particles of lipoproteins. Fluorescence studies using 1,6-diphenylhexatriene analogs showed that although cholesteryl oleate (CO) significantly decreased core mobility, the surface rigidity of phosphatidylcholine (PC) monolayers was independent of core composition. When intravenously injected into rats, the increased amount of core CO tended to retard TG emulsion removal from plasma, and the initial clearance rate was correlated with the amount of apolipoprotein E (apoE) bound from plasma. In addition, PC liposomes with a similar emulsion particle size showed negligible binding of apoE and were cleared at a slower rate compared to all emulsions. Furthermore, the effect of CO on the binding behavior of apoE to the emulsion surface and the emulsion uptake by hepatocytes was assessed in vitro. Replacing core TG with CO was found to decrease the apoE binding capacity to emulsions markedly without changing the binding affinity and thereby to reduce the cell uptake of emulsion particles by HepG2 cells. These results indicate that the physical state of core lipids, which can be modulated by CO content, plays a role in emulsion metabolism through the alteration in apoE binding.

Keywords : apolipoprotein E, cell uptake, cholesteryl ester

*京都大学大学院薬学研究科

Saito, H., Tanaka, M.*1, Okamura, E.*2, Kimura, T.*2, Nakahara, M.*2 and Handa, T.*1 : **Interaction of Phosphatidylcholine Surface Monolayers with Triglyceride Cores and Enhanced ApoA-I Binding in Lipid Emulsions**

Langmuir, **17**, 2528-2532 (2001)

The binding maximum of apoA-I (N) in triolein (TO)-egg yolk phosphatidylcholine (PC) emulsions was 10-fold larger than that in PC large unilamellar vesicles (LUV) of similar size (100 nm) with no significant difference in the affinity. Replacement of the long-chain triglyceride, TO, by medium-chain triglycerides or cholesteryl oleate in emulsion cores significantly decreased the N value. The 13C NMR chemical shifts of the PC carbonyl carbon at the surface layers indicated that PC polar headgroups are more separated and exposed to water molecules in emulsions than in vesicles. The N values were satisfactorily correlated with the chemical shift, that is, the degree of separation between the carbonyl groups at the surface. Although apoA-I binding to the PC monolayers of emulsions brings about bending of the surface layers and creates local defects in the hydrocarbon regions in a similar manner as PC LUV, the surface-core interaction seems to fill the defects with the core neutral lipids, compensates for the bending stress, and eventually increases the N value. Dependence of the core effect upon the acyl chain length of triglycerides implied important roles of the acyl chains in the surface-core interaction between PC and triglycerides.

Keywords : apolipoprotein A-I, NMR, emulsions

*1 京都大学大学院薬学研究科
*2 京都大学化学研究所

Maekawa, K., Tanimoto, T., Okada, S., Suzuki, T.*1, Suzuki, T.*1, Yabe-Nishimura, C.*2, : **Expression of Aldose Reductase and Sorbitol Dehydrogenase Genes in Schwann Cells Isolated from Rat: Effects of High Glucose and Osmotic Stress**

Mol. Brain Res., **87**, 251-256 (2001)

To investigate the polyol pathway activity in Schwann cells, we determined the mRNA levels of Aldose reductase (AR) and sorbitol dehydrogenase (SDH) in cultured cells under hyperglycemic or hyperosmotic conditions using competitive RT-PCR technique. The expressions of AR and SDH mRNAs in Schwann cells were unaltered by high (30 mM) glucose content in the medium. On the other hand, osmotic stress elicited significant increases in AR mRNA without any effect on SDH mRNA expression. These findings suggest that in contrast to the induction of AR expression by osmotic stress, high glucose per se does not up-regulate expression of the enzymes constituting the polyol pathway in Schwann cells. The RT-PCR system developed in this study may be a useful tool in ascertaining the relative contributions of AR and SDH to the metabolic derangements leading to diabetic complications.

Keywords : polyol pathway, diabetic neuropathy; competitive RT-PCR

*1 三和化学研究所
*2 京都府立医科大学

小出達夫，岩田美保，前川京子，斎藤博幸，谷本 剛，岡田敏史：**国立医薬品食品衛生研究所スウェルチアマリン標準品の新規設定のための品質評価**

医薬品研究，**32 (3)**, 118-123 (2001)

スウェルチアマリン標準品の新規設定のための品質評価試験を行った．試験成績は以下のとおりである．1）元素分析：理論値と一致した．2）NMR：構造を支持した．3）紫外吸収スペクトル：236.2nmに極大吸収が認められ，比吸光度はそれぞれ257.2(236nm)．4）赤外吸収スペクトル:3346, 1697, 1619, 1282, 1068, 1013cm-1に特性吸収がみられた．5）水分含量：3.42%．6）液体クロマトグラフ法による純度試験：複数個の不純物が検出され，不純物総量は約0.3%であった．7）ガスクロマトグラフ法による残留溶媒試験：ブタノールが約3.96%検出された．以上の試験成績から，本標準品原料は，国立医薬品食品衛生研究所スウェルチアマリン標準品に適した品質を有することを認めた．

Keywords : Swertiamarin, NIHS Reference Standard, Quality evaluation

斎藤博幸，岩田美保，小出達夫，前川京子，谷本　剛，岡田敏史：**国立医薬品食品衛生研究所ニコチン酸トコフェロール標準品の新規設定のための品質評価**

医薬品研究，**31 (11)**, 818-823 (2000)

ニコチン酸トコフェロール標準品の新規設定のための品質評価試験を行った．試験成績は以下のとおりである．1）元素分析：理論値と一致した．2）NMR：構造を支持した．3）紫外吸収スペクトル：264nmに極大吸収が認められ，比吸光度は83.5であった．4）赤外吸収スペクトル：1742, 1590, 1462, 1241, 1099cm-1に特性吸収がみられた．5）水分含量：0.03%．6）融点：43.9℃．7）液体クロマトグラフ法による純度試験：4個の不純物が検出され，不純物総量は約0.91%であった．以上の試験成績から，本標準品原料は，国立医薬品食品衛生研究所ニコチン酸トコフェロール標準品に適した品質を有することを認めた．

Keywords : Tocopherol nicotinate, NIHS Reference Standard, Quality evaluation

斎藤博幸，岩田美保，前川京子，谷本　剛，岡田敏史，鎌倉浩之，川原信夫，中根孝久，関田節子，佐竹元吉，横田洋一[*1]，津野敏紀[*1]，鈴木英世[*1]，岩嶋　浄[*2]，松浦敬一[*2]：**国立医薬品食品衛生研究所バイカリン標準品の新規設定のための品質評価**

医薬品研究，**31 (7)**, 465-470 (2000)

バイカリン標準品の新規設定のための品質評価試験を行った．試験成績は以下のとおりである．1）元素分析：理論値と一致した．2）紫外吸収スペクトル：277.2nmと317.0nmに極大吸収が認められ，比吸光度はそれぞれ609(277nm)と384(317nm)．3）赤外吸収スペクトル：3385, 1728, 1662, 1611, 1575cm-1に特性吸収がみられた．4）水分含量：3.07%．5）融点：210.4 ℃．6）液体クロマトグラフ法による純度試験：1個の不純物が検出され，不純物総量は約0.5%であった．以上の試験成績から，本標準品原料は，国立医薬品食品衛生研究所バイカリン標準品に適した品質を有することを認めた．

Keywords : Baicalin, NIHS Reference Standard, Quality evaluation

*1 富山県薬事研究所
*2 松浦薬業株式会社

谷本　剛，八木澤守正[*1]，藤原　博[*2]：**日本抗生物質医薬品基準の日本薬局方への移行における問題点とその対応（その1）—医薬品各条—**

医薬品研究，**31** (9), 674-680 (2000)

日局13（第二追補を含む）に収載されている抗生物質医薬品108品目についての日抗基規格の構成内容を調査し，その他の日局医薬品各条での規格内容との差異を比較検討するとともに，適否の判定基準等に対する日局と日抗基間での考え方の相違点について検討した．更に，これらの検討結果に基づいて，原薬たる抗生物質医薬品を日局に規定する際に日局医薬品各条との整合を図るために必要な措置や留意点などについて考察した．

*1 日本抗生物質学術協議会
*2 国立感染症研究所

Tsumura, Y., Ishimitsu, S., Saito, I[*1]., Sakai, H.[*2], Kobayashi, Y.[*2], Tonogai, Y.: **Eleven phthalate esters and di(2-ethylhexyl) adipate in one-week duplicate diet samples obtained from hospitals and their estimated daily intake**

Food Add. Contam., **18**, 449 - 460 (2001)

Plasticizers in one-week total diet samples were determined for the purpose to estimate daily intake. The phthalate esters were as follows: diethyl, dipropyl, dibutyl, dipentyl, dihexyl, butylbenzyl, dicyclohexyl, di(2-ethylhexyl), dioctyl, diisooctyl (mixture of isomers) and diisononyl (mixture). Di(2-ethylhexyl) adipate was also determined. For analysis, homogenized meal sample was extracted with acetonitrile, lipids were removed by extraction into n-hexane and the acetonitrile layer was cleaned using Florisil^{R} and Bondesil PSA^{R} dual layer column. Phthalates were determined by GC/MS (SIM). Phthalate recovery from fortified meal mixture by this methods was 62.5 to 140.8 %. Quality assurance as assessed by three laboratories indicated coefficient of variance in the levels of detected phthalates in same lot samples as below 10 %. Detection limits were 0.1 to 23 ng/g for each phthalate. One - week diet samples provided at three hospitals in three remote prefectures of Japan were analyzed for individual meal. In all 63 samples, DEHP was the highest among all phthalates, 10 to 4400 ng/g. Daily intake of phthalates estimated from all samples was 519 μg DEHP / day, 86 μg DEHA / day, 65 μg DINP /day, and 4.7 μg BBP / day. Calculated DEHP in two - day samples out of 21 days exceeded EU TDI for a person of 50 kg body weight (1850 μg per day). Disposable PVC gloves used during preparation of meals were suspected as the source of high DEHP content. One-day intake of the other phthalates and DEHA was below 7% of TDI in all cases. High concentration of DEHP (5990 ng/g) was found in baby food used in quality assurance. The source of contamination was PVC-tube in production and effectively reduced by replacing the tube to stainless steel one.

Keywords : phthalate, DEHP, DEHA, total diet sample, hospital food, GC/MS.

*1 愛知県衛生研究所
*2 新潟県保健環境科学研究所

津村ゆかり，石光進，中村優美子，吉井公彦，開原亜樹子，外海泰秀：**調理用PVC製手袋使用規制後における市販弁当中のフタル酸エステル類及びアジピン酸ジ（2-エチルヘキシル）濃度**

食品衛生学雑誌，**42**, 128-132 (2001)

調理用PVC製手袋の使用が規制されて2か月経過した2000年8月に，市販弁当（いわゆるコンビニ弁当）10検体中のフタル酸エステル類(PhE)11種及びアジピン酸ジ(2-エチルヘキシル)(DEHA)を測定し，規制前と比較した．試験期間中の検出下限値は，フタル酸ジ（2-エチルヘキシル)(DEHP)が14.9ng/g，フタル酸ジn-ブチル(DBP)が18.6ng/gであった．市販弁当中の各PhEの濃度は，DEHPが45～517ng/g（平均198ng/g)，DEHAが不検出～90ng/g，BBPが不検出～10.0ng/g，フタル酸ジイソノニル(DINP)は1検体のみで検出され，その濃度は76ng/gであった．DEHP濃度は平均で前年調査時の22分の1に減少し，その他の化合物も減少した．DBPは全ての試料で不検出であった．

Yoshii, K, Kaihara, A, Tsumura, Y, Ishimitsu, S, Tonogai, Y., **Liquid chromatographic determination of emamectin, milbemectin, ivermectin and abamectin in crops and confirmation by liquid chromatography-mass spectrometry.**
J Chromatogr A, **896**, 75-85 (2000)

Emamectin, milbemectin, ivermectin and abamectin are similar macrocyclic lactone chemicals used as an acaricides or parasiticides. We developed a simultaneous analytical method for determining the residual amounts of these compounds and emamectin metabolites in crops. A sample extracted with acetone was cleaned up with Bond Elut C_{18} and NH_2. The sample was then fluorescence-derivatized with trifluoroacetic anhydride and 1-methylimidazole in acetonitrile. The analyte was measured by HPLC with fluorescence detection using an octadecylsilyl column with 3 microm particle size and gradient elution. In most crops, their recoveries by the developed method were ca. 80-110%. The detection limits of the analytes in vegetables were 0.1-0.3 ppt. Using the developed method, we surveyed the residues of these compounds in 20 commercial crops in Osaka, Japan. The result of the surveillance was that emamectin benzoate of 0.2-6.7 ppb was detected in nine cases and milbemectin of 16.7-279.3 ppb was detected in four cases. The detected samples were confirmed by LC-electrospray ionization (ESI) MS. The limit of detection by LC-ESI-MS was similar to the fluorescence detection level of 0.1-0.3 ppt in vegetables except for milbemectin.
Keywords : emamectin, LC/MS, determination, derivatization

Yoshii K, Tsumura Y, Ishimitsu S, Tonogai Y, Nakamuro K., **Degradation of malathion and phenthoate by glutathione reductase in wheat germ.**
J. Agric Food Chem, **48**, 2502-5 (2000)

Residual malathion in wheat was estimated at a lower value when analysis was performed by extraction with acetone after addition of water to swell the wheat, according to the Japanese Bulletin Method. The supernatant of the wheat homogenate showed degradation not only of malathion but also of phenthoate. Malathion and phenthoate were not degraded by the boiled supernatant of the wheat homogenate. It was presumed for this reason that glutathione reductase (GR; EC 1.6. 4.2) in the wheat degraded malathion. The following results were obtained: (1) GR originating in wheat could degrade malathion and phenthoate. (2) The degradation of malathion by the GR was inhibited by excessive GSSG. (3) There was a high correlation between GR activity and malathion degradation activity of the supernatant of wheat homogenates. It is likely that GR acted on the specific structure of malathion and phenthoate, the S=P-S bond, and the blanch structure bonding with the sulfur atom. Following the above, extraction with acetone after addition of water (the Japanese Bulletin Method) should be replaced by extraction with pure organic solvent and without addition of water for swelling.
Keywords : wheat, malathion, degradation, phenthoate, enzyme, glutathione reductase

吉井公彦，津村ゆかり，中村優美子，石光進，外海泰秀：**ＨＰＬＣによる農作物中ベンタゾン，イナベンフィド，フルスルファミドの定量及びＬＣ／ＭＳによる確認試験**
食衛誌，**41**, 268-273 (2000)

農作物中ベンタゾン，イナベンフィド及びフルスルファミドのＨＰＬＣによる分析法を作成した。上記３種農薬を試料からメタノール抽出し，酢酸エチルで再抽出，シリカゲルカラムでクリーンアップした後，ＨＰＬＣで同時に定性・定量した。また，試料からの検出ピークをＬＣ／ＭＳで確認する方法を確立した。３種農薬を各種農作物に0.1μg／ｇ添加したときの回収率は75%以上であり，ＨＰＬＣによる検出限界は試料中いずれも0.001μg／ｇであった。
Keywords : crops, pesticide, determination, HPLC, LC/MS

Kaihara A, Yoshii K, Tsumura Y, Nakamura Y, Ishimitsu S, Tonogai Y,: **Multiresidue Analysis of Pesticides in Fresh Fruits and Vegetables by Supercritical Fluid Extraction and HPLC**
J. Health Sci., **46** (5), 336-342 (2000)

A screening method was established for the determination of 27 pesticides in fresh fruits and vegetables by a super critical fluid extraction (SFE), cleaned up with cartridge columns and HPLC. The multiresidue and semiautomatic analysis was useful for a screening examination, because the determination methods for pesticides under the Japanese Food Sanitation Law are mostly individual determinations. Reported methodologies for multiresidue analysis by HPLC were not adequate to regulated pesticides in Japan. In this report, multiresidue determination of pesticides and their metabolites are discussed using SFE and HPLC. Details of the proposed method are as follows: Wet samples such as fruits and vegetables were not suitable for the SFE instrument, so the water in the samples was removed with an absorptive polymer (Arasorb® S-310) prior to SFE. The pesticides were extracted by SFE, the extracts trapped with Extrelut® NT+ Bond Elut® C_{18} and then eluted with acetonitrile. The eluate was cleaned up with Sep-Pak® Florisil+Bond Elut® PSA cartridges. After washing with n-hexane, the pesticides were eluted from the cartridges with 15% ether/n-hexane, 15 and 50% acetone/n-hexane. These three fractions were individually determined by HPLC with a photodiode-array detector. The pesticides spiked in samples at 0.5 ppm showed satisfactory recoveries except for thiabendazole, imazalil and clofentezine. Detection limits were 0.005~0.01 ppm for the 27 pesticides.
Key words : pesticide, super critical fluid extraction (SFE), HPLC

辻 澄子, 松村 郁子, 中村優美子, 外海 泰秀: **HPLCによる食用黄色5号（サンセットイエローFCF）中の副成色素, 未反応原料及び反応中間体の分離・定量の検討**
食衛誌, **41**, 367-363 (2000)

食用黄色5号 (Y-5) 中の副成色素, 未反応原料及び反応中間体などの有機性不純物の分離・定量にあたりHPLC条件を検討した. その結果, 0.02mol/l酢酸アンモニウム溶液とアセトニトリルー水混液 (7:3) との濃度勾配系を用いるHPLC条件を変化させることにより, 4, 4'-（ジアゾアミノ）ジベンゼンスルホン酸＝二ナトリウム塩 とスルファニル酸アゾG塩色素との分離定量を確立した. 本HPLC条件を用いて平成10年度Y-5製品検査合格品39検体中の有機性不純物の実態調査を行った. その結果, 検体中の副成色素の総量は規制値の五分の一未満であり, 未反応原料及び反応中間体の総量は規制値の半分以下であった.

Keywords : Food Yellow No. 5, subsidiary color, HPLC

辻 澄子, 天倉 吉章, 岡田 舞, 外海 泰秀: **5種の食用アゾ色素中の未反応原料, 反応中間体及び副成色素の定量用HPLC条件の改良**
食衛誌, **42**, 114-121 (2001)

第7版食品添加物公定書に定められた5種の食用アゾ色素中の未反応原料, 反応中間体及び副成色素などの有機性不純物の分析法は, 個々の色素毎に異なったHPLC条件が採用されている. そこで, 多数の検体を迅速かつ簡便に測定するために, HPLC条件を検討した. その結果, 0.02mol/l酢酸アンモニウム溶液を10分間保持した後, アセトニトリルー水混液 (7:3) との直線濃度勾配系を利用したHPLC条件により, 5種すべてのアゾ色素中の有機性不純物を定量できることが明らかになった. 本法により, 平成11年度の製品検査試料のうちアゾ色素163検体中の有機性不純物の含有量を測定した結果, すべて規格限度内であった.

Keywords : azo color, subsidiary color, HPLC

Nakamura, Y., Tsuji, S., Tonogai, Y.: **Determinartion of the levels of isoflavonoids in soybeans and soy-derived foods and estimation of isoflavonoids in the Japanese daily intake**
J. AOAC Int., **83**, 635-650 (2000)

The levels of 6 kinds of isoflavonoids found in 11 domestic and imported soybeans, and 12 kinds of soybean-based processed foods in Japan were systematically analyzed, and the Japanese daily intake of isoflavonoids from those foods was estimated. The total isoflavonoids (daidzein, glycitein and genistein) were analyzed with acid hydrolysis and the intact isoflavonoids (daidzein, glycitein, genistein, daidzin, glycitin and genistin) were analyzed without hydrolysis. This was followed by clean up with ODS cartridge column and determined by LC with a diode array detector (DAD). The highest content of isoflavonoids was found in kinako (a roasted soybean powder) and the lowest was found in soy sauce. The contents and composition of the isoflavonoids in the 11 soybeans varied by species and country of origin. The level of isoflavonoids found in the processed foods varied by manufacturing method or ingredients. The percentage of aglycon tended to be higher in miso (fermented soybean paste) and soy sauce, which are heated and fermented during the manufacturing process. Japanese daily intake of isoflavonoids from soybeans and soybean-based processed foods was estimated as 27.80 mg per day (daidzein 12.02 mg, glycitein 2.30 mg and genistein 13.48 mg).

keywords : Isoflavonoids, Soybeans

Nakamura, Y., Ishimitsu, S., Tonogai, Y.: **Effects of quercetin and rutin on serum and hepatic lipid concentrations, fecal steroid excretion and serum antioxidant properties**
J. Health Sci., **46**, 229-240 (2000)

Effects of quercetin and rutin on serum and hepatic lipid concentrations, fecal steroid excretion and their antioxidant properties were investigated in rats by oral administration. No toxic symptom was observed even at the dose of 1.0 g/kg of quercetin or rutin. Serum and hepatic lipid concentrations and fecal steroid excretion was not influenced remarkably, but serum thiobarbituric acid reactive substances (TBARS) decreased dose-dependently with the administration of quercetin or rutin. The decrease of serum TBARS was significantly correlated with the increase of serum free flavonoids ($p<0.05$-0.001). Serum flavonoid concentrations, especially free quercetin, were higher in rutin-administered rats than in quercetin-administered rats at doses of 1.0 g/kg for 10 days ($p<0.05$-0.001). When 1.0 g/kg of quercetin or rutin was administered in a single dose, they remained in the blood as aglycone or their conjugates of quercetin and isorhamnetin, even three days after administration. Recovered flavonoids were only 0.13% and 0.89% in urine for 3 days and 0.03% and 0.13% in serum on day 3 by administration of quercetin and rutin, respectively. Thus, some part of the administered quercetin or rutin was metabolized and showed antioxidant property, but had no remarkable influence on serum or hepatic lipid concentrations or fecal steroid excretion in rats.

keywords : Quercetin, Rutin

Nakamura, Y., Kaihara, A., Yoshii, K., Tsumura, Y., Ishimitsu, S., Tonogai, Y.: **Effects of the oral administration of green tea polyphenol and tannic acid on serum and hepatic lipid contents and fecal steroid excretion in rats**
J. Health Sci., **47**, 107-117 (2001)

Green tea polyphenol (Polyphenon) or tannic acid was administered orally to rats at a dose of 0.01-1.0 or 0.1-1.0 g/kg for 23 days, and changes both in serum and hepatic lipid concentrations and in fecal steroid excretion were examined. The administration of 0.2-1.0 g/kg of Polyphenon caused a significant decrease in levels of serum HDL-cholesterol, whereas tannic acid had no significant effect on serum lipid concentrations. The hepatic triglyceride concentration was significantly higher than controls in rats given more than 0.5 g/kg of Polyphenon, whereas both hepatic triglyceride and phospholipid concentrations were significantly higher after tannic acid administration. Serum thiobarbituric acid reactive substances were significantly low in rats given either 1.0 g/kg of Polyphenon or more than 0.1 g/kg of tannic acid. Fecal neutral steroid excretion increased significantly in rats given a dose of 1.0 g/kg of either Polyphenon or tannic acid. The excretion of fecal bile acids increased significantly in rats given 0.2 g/kg of tannic acid, but then tended to decrease at higher doses; however, excretion of fecal bile acids did not change after

Polyphenon administration. We found that alterations in the compositions of fecal neutral steroids and bile acids were independent of the tannic acid or Polyphenon dose: the ratio of coprostanol to cholesterol decreased significantly in rats given 0.05-0.2 g/kg of Polyphenon or 0.5 g/kg of tannic acid; and the ratio of cholic-acid-derived bile acids to chenodeoxycholic-acid-derived bile acids decreased significantly after administration of 0.05, 0.2 and 0.5 g/kg of Polyphenon or 0.1 and 0.5 g/kg of tannic acid. Primary bile acid excretion increased significantly only in rats given a dose of 0.1 g/kg of Polyphenon. This is the first report that documents the changes occurring in fecal steroid excretion induced by oral administration of green tea polyphenol or tannic acid.
Keywords : Green tea polyphenol ; Tannic acid, Fecal steroid excretion

天倉吉章, 岡田 舞, 辻 澄子, 外海泰秀 : **HPLCによる生鮮並びに果実加工品に含まれるエラグ酸の定量**
食衛誌, **41**, 206-211 (2000)
各種果実中のエラグ酸について, メタノール還流抽出, Sep-Pak® tC18カートリッジにより精製後, フォトダイオードアレイ検出器を用いたHPLCによる簡便かつ迅速な分析法を検討した. イチゴ, パイナップル, ラズベリーについて, それぞれエラグ酸各25, 50 μg/gずつ添加した時の回収率は90.1～98.3%で, 定量限界は0.05 μg/gであった. エラグ酸の同定には, フォトダイオードアレイ検出器によるUV吸収スペクトルを用いた. また本法を用いて, 市販生鮮果実及び果実加工品中におけるエラグ酸含有量について調査した結果, 同化合物はイチゴ, ラズベリーなどのベリー類に含まれ, 中でもブラックベリーが最も高い含有量 (87.66mg/g) を示した. また, パイナップル, フェイジョア, ヤマモモについても今回新たにエラグ酸の含有を認めた.
Keywords : HPLC, fruits, ellagic acid

Amakura., Y., Okada, M., Tsuji, S., Tonogai, Y.: **Determination of phenolic acids in fruit juices by isocratic column liquid chromatograpy**
J. Chromatogr. A, **891**, 183-188 (2000)
A simple and rapid analytical method of five phenolic acids, gallic, chlorogenic, caffeic, ellagic and ferulic acid, which are naturally occurring bioactives, were determined in fruit juices by isocratic LC using photodiode array UV detection. The sample was pretreated by solid-phase extraction (a combination of Sep-Pak Plus tC_{18} and Bond Elut PSA).
Keywords : phenolic acids, fruit juices, solid-phase extraction

Amakura., Y., Okada, M., Tsuji, S., Tonogai, Y.: **High-performance liquid chromatographic determination with photodiode array detection of ellagic acid in fresh and proccessed fruits**
J. Chromatogr. A, **896**, 87-93 (2000)
A high-performance liquid chromatographic (HPLC) procedure based on an isocratic elution with photodiode array detection has been developed for a simple and rapid determination of ellagic acid (EA) in fresh and processed fruits. The homogenized sample was refluxed with methanol, and the extract was refined using a solid phase cartridge before HPLC. We analyzed EA in 40 kinds of fresh fruits and 11 kinds of processed fruits by the developed method. EA was found in several berries, fueijoa, pineapple and pomegranate. This is the first occurrence of the detection of EA in bayberry, fueijoa and pineapple.
Keywords : HPLC, food, ellagic acid

Amakura., Y., Okada, M., Tsuji, S., Tonogai, Y.: **Influence of jam processing on the radical scavenging activity and phenolic contents in berries**
J. Agric. Food Chem., **48**, 6292-6297 (2000)
Selected six phenolic (aglycons; caffeic and ellagic acids, kaempferol, quercetin, myricetin and morin) contents and their changes in nine berries influenced by jam processing have been evaluated using optimized HPLC with diode array detection. The samples, fresh and after jam processing from the berries, were analyzed, and the total amounts of selected phenolics as aglycons were identified and determined by acid hydrolysis of them. Their contents in fresh and jam samples did not indicate appreciable changes; therefore the influence of jam processing on selected phenolics in berries was suggested to be small and mostly present in berries as several conjugated forms glycosylated, esterified, etc., in the samples. The total phenolic contents of each sample also were determined by the Folin-Ciocalteu method. Three samples, namely fresh, jam and acid hydrolysate of the berry, had similar contents. On the other hand, the scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was measured, and acid hydrolysates produced many aglycons showed stronger activity than that of the fresh and jam processed samples as a whole.
Keywords : berry, phenolics, jam.

Murai, T., Nakagawa, Y., Maeda, H., Terada, K.: **Altered regulation of cell cycle machinery involved in interleukin-1-induced G1 and G2 phase growth arrest of A375S2 human melanoma cells**
J. Biol. Chem., **276**, 6797-6806 (2001)
Interleukin-1 (IL-1) inhibits the growth of A375S2 human melanoma cells by arresting them at G1 and G2 phases of the cell cycle. The arrests are preceded by a rapid decrease in kinase activities of cyclin E-Cdk2 and cyclin B1-Cdc2, which are critical for G1-S and G2-M progression, respectively. IL-1 quickly enhances the protein expression of the CDK inhibitor p21Cip1. The induced p21 binds preferentially to cyclin E-Cdk2, and the increase in p21 binding parallels the decrease in cyclin E-Cdk2 activity. Thus, p21 is likely to be responsible for the inhibition of cyclin E-Cdk2 activity and G1 arrest. Coinciding with the decrease in cyclin B1-Cdc2 activity, there is an increase in tyrosine phosphorylation of Cdc2, suggesting that an increase in the inactive Tyr-15-phosphorylated form of Cdc2 is involved in the decrease in cyclin B1-Cdc2 activity and G2 arrest. Furthermore, we found that IL-1 causes rapid dephosphorylation of p107, but not of pRb or p130, while the total protein levels of p130 are increased. Thus, IL-1 may exert its growth-arresting effects via p107 and p130 pathways rather than through pRb.
Keywords : IL-1, cell growth arrest, cell cycle machinery

Ema, M., Harazono, A.: **Adverse effects of dibutyltin dichlo-**

ride on initiation and maintenance of rat pregnancy
Reprod. Toxicol., **14**, 451-456 (2000)

The present study was conducted to evaluate the adverse effects of dibutyltin dichloride (DBTCl) on initiation and maintenance of pregnancy after maternal exposure during early pregnancy in rats. Following successful mating, female rats were given DBTCl by gastric intubation on days 0 to 3 or on days 4 to 7 of pregnancy at 0, 3.8, 7.6, or 15.2 mg/kg. Food-restricted pregnant rats were given an amount of feed equal to the feed intake of female rats treated with DBTCl at 15.2 mg/kg on days 0 to 3 or on days 4 to 7 of pregnancy. Female rats were sacrificed on day 20 of pregnancy and pregnancy outcome was determined. After administration of DBTCl on days 0 to 3, the rate of nonpregnant females and the incidence of preimplantation embryonic loss in the 7.6 mg/kg group were significantly higher than those in the control group, and those in the 15.2 mg/kg group were significantly higher than those in the control and pair-fed groups. In females with implantations, the numbers of implantations and live fetuses and the incidence of postimplantation embryonic loss in the groups given DBTCl on days 0 to 3 were not significantly different from those in the control group. The incidence of postimplantation embryonic loss in the groups given DBTCl on days 4 to 7 at 7.6 and 15.2 mg/kg was significantly higher than that in the control and pair-fed groups. It can be concluded that DBTCl adversely affects initiation and maintenance of pregnancy when administered during early pregnancy and that the manifestations of the adverse effects of DBTCl vary with the gestational stage at the time of maternal exposure.
Keywords : Dibutyltin dichloride, pregnancy failure, early embryonic loss

Ema, M., Miyawaki, E.: **Adverse effects on development of reproductive system in male offspring of rats given monobutyl phthalate, a metabolite of dibutyl phthalate, during late pregnancy**
Reprod. Toxicol., **15**, 189-194 (2001)

The objective of this study was to determine the adverse effects of monobutyl phthalate (MBuP), a major metabolite of dibutyl phthalate (DBP), on development of reproductive system in offspring following maternal administration during late pregnancy, and to assess the role of MBuP in the antiandrogenic effects of DBP. Pregnant rats were given MBuP by gastric intubation at 250, 500, or 750 mg/kg on days 15 to 17 of pregnancy. Maternal body weight gain and food consumption during the administration period were significantly decreased at 500 mg/kg and higher and at 750 mg/kg, respectively. A significant increase in the incidence of postimplantation embryonic loss was found at 500 mg/kg and higher. The body weights of male and female fetuses were significantly lower at 750 mg/kg. A significant increase in the incidence of fetuses with undescended testes was found at 250 mg/kg and higher. A significant decrease in the anogenital distance (AGD) of male fetuses was observed at 250 mg/kg and higher. The AGD/body weight ratio and AGD/cube root of body weight ratio in male fetuses was also significantly reduced at 250 mg/kg and higher. The AGD, AGD/body weight ratio and AGD/cube root of body weight ratio in female fetuses in the MBuP-treated groups were comparable to those in the control group. The data of the present study indicate that MBuP on days 15 to 17 of pregnancy produced adverse effects on the development of reproductive system in male offspring and suggest that MBuP may be responsible for the induction of the antiandrogenic effects of DBP.
Keywords : Monobutyl phthalate, anogenital distance, undescended testes

Ema, M.: **Reproductive and developmental toxicity of triphenyltin chloride in rats**
Cong. Anom., **40**, 8-13 (2000)

Reproductive and developmental toxicity of triphenyltin chloride (TPTCl) was evaluated in rats. Although no significant increase in the incidence of fetuses with malformations was observed following administration of TPTCl during organogenesis, a significant increase in the incidence of postimplantation embryonic loss was found. TPTCl during early pregnancy, especially on days 0-3 of pregnancy, caused implantation failure, i. e., preimplantation embryonic loss. The effects of TPTCl on the uterine function, as a cause of implantation failure, were determined using pseudopregnant rats. TPTCl was given on days 0-3 of pseudopregnancy and the decidual cell response was induced on day 4 of pseudopregnancy. Rats was sacrificed on day 9 of pseudopregnancy and the uterine weight served as an index of the uterine decidualization. A significantly lower weight of the uterus, which indicates the suppression of uterine decidualization, was found at the doses which induced implantation failure. These doses of TPTCl also caused a significant decrease in the progesterone levels, which indicates reduced ovarian function. These findings suggest that TPTCl exerts adverse effects on uterine decidualization correlated with the reduction in serum progesterone levels and these effects are responsible, at least in part, for the implantation failure induced by TPTCl.
Keywords : Triphenyltin, diphenyltin, Embryonic loss, decidualization

Ema, M., Harazono, A.: **Developmental and reproductive toxicity of tributyltin and its metabolite, dibutyltin, in rats**
Cong. Anom., **40**, S108-S120 (2000)

Developmental and reproductive toxicity of tributyltin chloride (TBTCl) and dibutyltin dichloride (DBTCl) was evaluated in rats. Tributyltin chloride (TBTCl) was teratogenic when administered on day 8 and days 11-14 of pregnancy, and the most pronounced effect was seen after administration on day 13 of pregnancy. Cleft palate was predominantly observed. DBTCl was teratogenic when administered on days 7-8 of pregnancy, and day 8 of pregnancy was the most susceptible to the teratogenicity of DBTCl. Cleft jaw, ankyloglossia, omphalocele, anomaly of the tail, and deformity of the vertebral column and ribs were frequently observed. TBTCl and DBTCl on days 0-3 of pregnancy caused implantation failure, preimplantation embryonic loss. TBTCl and DBTCl on days 4-7 of pregnancy affected the viability of the implanted embryos. The effects of TBTCl on uterine function, as a cause of early embryonic loss, were determined using pseudopregnant rats. TBTCl was given on days 0-3 or days 4-7 of pseudopregnancy and the decidual cell response was induced on day 4. The uterine

weight on day 9 served an index of the uterine decidualization. A significantly lower weight of the uterus, which indicates suppression of uterine decidualization, was found at the doses that induced early embryonic loss. These doses of TBTCl also caused a significant decrease in the serum progesterone levels. The findings suggest that TBTCl exerts adverse effects on uterine decidualization correlated with a reduction in serum progesterone levels, and that these effects are responsible, at least in part, for the early embryonic loss induced by TBTCl.
Keywords : Tributyltin, dibutyltin, reproductive and developmental toxicity

Harazono, A., Ema, M.: **Effects of 4-tert-octylphenol on initiation and maintenance of pregnancy following oral administration during early pregnancy in rats**
Toxicol. Lett., **119**, 79-84 (2001)
4-tert-Octylphenol (OP) is an alkylphenol that is an intermediate in the production of alkylphenol ethoxylates. OP has been reported to be the most potent estrogenic alkylphenol in vitro. In the present study, the effects of OP on initiation and maintenance of pregnancy were investigated in rats. Inseminated female rats were orally given OP at 0, 15.6, 31.3, 62.5 and 125 mg/kg on day 0 through day 8 of pregnancy. Female rats were sacrificed on day 20 of pregnancy, and pregnancy outcome was determined. Decreases in body weight gain and food consumption on days 0-9 were found at 31.3 mg/kg and above, and at 15.6 mg/kg and above, respectively. The pregnancy rate was not adversely affected by OP administration during early pregnancy even at 125 mg/kg. The incidence of post-implantation loss per litter at 31.3 mg/kg and above was significantly higher than that in the control group. The body weights of live fetuses in the OP-treated groups were not significantly different from those in the control group. No increase in the incidence of fetuses with external malformations was found in any OP-treated group. We concluded that OP during early pregnancy caused post-implantation embryonic loss at doses that showed maternal toxicity.
Keywords : 4-tert-octylphenol, early pregnancy, rat

Harazono, A., Ema, M.: **Suppression of decidual cell response induced by tributyltin chloride in pseudopregnant rats: a cause of early embryonic loss**
Arch. Toxicol., **74**, 632-637 (2000)
In our previous studies, tributyltin chloride (TBTCl) at doses of 16.3 mg/kg and above caused implantation failure (preimplantation embryonic loss) and postimplantation embryonic loss in rats following administration on gestational day (GD) 0 through GD 3 and GD 4 through GD 7, respectively. This study was designed to assess the effects of TBTCl on uterine function, as a cause of early embryonic loss in pseudopregnant rats. TBTCl was given orally to pseudopregnant rats at doses of 4.1, 8.1, 16.3 and 32.5 mg/kg on pseudopregnant day (PPD) 0 to PPD 3 or 8.1, 16.3, 32.5 and 65.1 mg/kg on PPD 4 to PPD 7. The decidual cell response was induced by bilateral scratch trauma on PPD 4. The uterine weight on PPD 9 served as an index of uterine decidualization. Uterine weight and serum progesterone levels on PPD 9 were significantly decreased after administration of TBTCl at doses of 16.3 mg/kg and above on PPD 0 to PPD 3 or PPD 4 to PPD 7. Administration of TBTCl at doses of 8.1 mg/kg and above on PPD 0 to 3 also significantly decreased serum progesterone levels on PPD 4. TBTCl had no effect on ovarian weight and number of corpora lutea. It can be concluded that TBTCl suppresses the uterine decidual cell response and decreases progesterone levels, and these effects are responsible for early embryonic loss due to TBTCl exposure.
Keywords : tributyltin chloride, decidual cell response, pseudopregnancy

草野源次郎*1, 芝野真喜雄*1, 鈴木直樹*1, 渡辺 斉*2, 尾崎和男*2, 柴田敏郎, 畠山好雄, 飯島 泉*3 : **甘草屋敷のウラルカンゾウ復活**
Natural Medicines, **54** (4), 199-203 (2000)
山梨県塩山市にある高野家 (別称, 甘草屋敷) では, 江戸幕府の命により1720年頃からカンゾウの栽培が行われてきたが, 近年管理がゆきとどかず絶滅寸前であった. 今回, 現地で株を回復させると同時に筑波試験場の圃場に株を移植して増殖させ, また, 茎頂栽培により増殖も進み, 完全復活に成功した. この株は, 形態や成分の比較によりウラルカンゾウであることを確認した.
Keywords : *Glycyrrhiza uralensis*, Kanzo Yashiki
*1 大阪薬科大学
*2 武田薬品工業
*3 塩山市教育委員会

Touno, K., Harada, K.*1, Yoshimatsu, K., Yazaki, K.*2 and Shimomura, K. : **Histological observation of red pigment formed on shoot stem of *Lithospermum erythrorhizon***
Plant Biotechnology, **17** (2), 127-130 (2000)
Shikonin production on the stem of shoot cultures of *Lithospermum erythrorhizon* was controlled by use of various culture media and light irradiation. Microscopic analysis of the shoot cultures revealed that the red pigment formation was only observed on the stem surface and the stem hairs of shoots cultured in the dark. Cross section of the shoot stem which formed red pigment in the dark was morphologically similar to that of shoots cultured under illumination. Red pigment accumulation was strictly localized in the outer surface of epidermal cells. The localization of these pigments was similarly observed in root tissues generated from the cultured shoots as well as field-grown roots. Northern blot analysis indicated that LEDI-2 gene, which is one of the candidates for the regulatory element of shikonin biosynthesis and specifically expressed in the root system of the intact plants, was also expressed in the stem of shoot cultures when producing shikonin.
Keywords : *Lithospermum erythrorhizon*, shoot cultures, pigment localization
*1 千葉大学園芸学部
*2 京都大学大学院生命科学研究科

Nakanishi, F.*1, Sasaki, K.*2 and Shimomura, K. : **Kinetics of littorine content in various developing stages of regenerates of *Atropa belladonna***
Plant Cell Reports, **19** (10), 1021-1026 (2000)

Aseptically propagated regenerates were cultivated in hydroponic apparatus, phytotron or field, and their growth and littorine contents were investigated. No littorine was detected in aseptic regenerates cultured on solidified MS medium, nor in leaves through any condition tested. In roots, it was common features to all conditions used here that littorine increased dramatically after transplantation from culture tubes, and was a major alkaloid up to week 4. After then, the littorine contents varied in different cultivation conditions. The roots cultivated in the field showed marked thickening and rapid disappearance of littorine: those cultivated in hydroponic apparatus were fine and maintained littorine at high level for long. In a plant cultivated for 16 weeks in a pot, the littorine content in roots decreased with increasing their diameter.

Keywords : *Atropa belladonna*, regenerates, littorine

*1 東京学芸大学生物
*2 青森大学工学部

Touno, K., Harada, K.*1, Yoshimatsu, K., Yazaki, K.*2 and Shimomura, K. : **Shikonin derivative formation on the stem of cultured shoots in *Lithospermum erythrorhizon***

Plant Cell Reports, **19** (11), 1121-1126 (2000)

Shoot cultures of *Lithospermum erythrorhizon*, which are capable of producing red pigments, have been established. The red pigments were formed on the stem of *L. erythrorhizon* shoots cultured both on solid and in liquid media without phytohormone at 25℃ in the dark. TLC and HPLC analyses revealed that the red pigments observed on the shoot cultured on those media were shikonin derivatives. The effects of various basal media and phytohormones (IAA, IBA and kinetin) on the growth and the formation of shikonin derivatives were investigated. When the shoots were cultured on Murashige and Skoog solid medium, the addition of kinetin remarkably enhanced shikonin derivative accumulation in the shoots. However, these effects of kinetin were not observed in the liquid culture when cultured in Gamborg B5 medium. The maximum content of shikonin derivatives (2.3 % as dry weight, *ca.* 1.5 mg / flask) was observed in the shoots cultured in phytohormone-free B5 liquid medium for 5 weeks.

Keywords : *Lithospermum erythrorhizon*, shikonin derivatives, shoot culture

*1 千葉大学園芸学部
*2 京都大学大学院生命科学研究科

Yazaki, K.*1, Matsumoto, H.*1, Shimomura, K., Bechthold, A.*2 and Sato, F.*1 : **A novel dark-inducible protein, LeDI-2, and its involvement in root-specific secondary metabolism in *Lithospermum erythrorhizon***

Plant Physiology, **125**, 1831-1841 (2001)

Lithospermum erythrorhizon produces red naphthoquinone pigments that are shikonin derivatives. They are accumulated exclusively in the roots of this plant. The biosynthesis of shikonin is strongly inhibited by light, even though other environmental conditions are optimized. Thus, *L. erythrorhizon* dark-inducible genes (LeDIs) were isolated to investigate the regulatory mechanism of shikonin biosynthesis. LeDI-2, showing the strict dark-specific expression, was further characterized by use of cell suspension cultures and hairy root cultures as model systems. Its mRNA accumulation showed a similar pattern with that of shikonin. In the intact plants LeDI-2 expression was observed solely in the root, and the longitudinal distribution of its mRNA was also in accordance to that of shikonin. LeDI-2 encoded a very hydrophobic polypeptide of 114 amino acids that shared significant similarities with some root-specific polypeptides such as ZRP3 (maize) and RcC3 (rice). Reduction of LeDI-2 expression by its antisense DNA in hairy roots of *L. erythrorhizon* decreased the shikonin accumulation, whereas other biosynthetic enzymes, e.g. p-hydroxybenzonic acid:geranyltransferase, which catalyzed a critical biosynthetic step, showed similar activity as the wild-type clone. This is the first report of the gene that is involved in production of secondary metabolites without affecting biosynthetic enzyme activities.

Keywords : *Lithospermum erythrorhizon*, shikonin derivatives, LeDI-2

*1 京都大学大学院生命科学研究科
*2 Pharmazeutisches Institut, Universitat Tubingen

高上馬希重，李 宜融，飯田 修，関田節子，佐竹元吉，牧野由紀子* : **大麻 *Cannabis sativa* L. の DNA 解析**

DNA 多型， **8**, 87-90 (2000)

大麻 *Cannabis sativa* L. の DNA 解析を行い，葉緑体 DNA 上の tRNA 遺伝子をコードする *trnL*(UAA) と *trnF*(GAA) 間の intergenic spacer 領域に *C. sativa* の種内変異が存在することを明らかにし，さらに SSCP (Single Strand Conformation Polymorphism) 分析により，塩基配列の解読を行わなくても，簡便に大麻とホップを識別できることを明らかにした．

Keywords : *Cannabis sativa* L., chloroplast DNA, PCR-SSCP

*関東信越地区麻薬取締官事務所

Kohjyouma, M., Lee, I., Iida, O., Kurihara, K., Yamada, K., Makino, Y.*, Sekita, S., and Satake, M.: **Intraspecific variation in *Cannabis sativa* L. Based on Intergenic Spacer Region of Chloroplast DNA**

Biol. Pharm. Bull., **23**, 727-730 (2000)

We analyzed the nucleotide sequences of the non-coding region of chloroplast DNA: the intergenic spacer between *trnL* (UAA) 3'exon and *trnF* (GAA). Two kinds of sequence, "type-1" and "type-2", were detected in 33 populations of *Cannabis sativa*. The length of the "type-1" fragment was 354bp. In contrast, the "type-2" fragment from 3 population was 353 bp long, with only one base deletion compared to "type-1". The fragment length from *Humulus lupulus* was 353bp with a 1-bp deletion, and ten 1-bp substitutions compared to the sequences from *C. sativa* " type-1". Furthermore, we could cleary identify differences between *C. sativa* and *H. lupulus* using single-strand conformation polymorphism of PCR products (PCR-SSCP) analysis.

Keywords : *Cannabis sativa* L., chloroplast DNA; intergenic spacer ; intraspecific variation ; PCR-SSCP

* Kanto-Shin'etsu Regional Narcotic Control Office