Yoshioka, S., Aso, A., Kojima, S., Sakurai, S.,* Fujiwara, T.* and Akutsu, H.*: **Molecular Mobility of Protein in Lyophilized Formulations Linked to the Molecular Mobility of Polymer Excipients, as Determined by High Resolution ^{13}C Solid-state NMR**

Pharm. Res., **16**, 1621-1625 (1999)

The mobility of bovine serum γ-globulin (BGG) molecules in lyophilized formulations was compared with the mobility of dextran molecules used as an excipient based on the spin-lattice relaxation time (T_1) of each molecule determined by high resolution ^{13}C solid-state NMR. The correlation time (τ_c) of dextran methin carbon in BGG-dextran formulations exhibited a temperature dependence with a distinct break at the critical temperature of appearance of Lorentzian relaxation due to liquid BGG and dextran protons (T_{mc}). The τ_c of BGG carbonyl carbon exhibited a similar temperature dependence to the τ_c of the dextran methin carbon and substantially decreased at temperatures above T_{mc} in the presence of dextran. The results indicated that the molecular motion of BGG was enhanced above T_{mc} in association with the increased global segmental motion of dextran molecules.

Keywords: NMR relaxation time, molecular mobility, storage stability.

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Yoshioka, S., Aso, Y., Kojima, S. and Tanimoto, T.: **Effect of Polymer Excipients on the Enzyme Activity of Lyophilized Bilirubin Oxidase and β-Galactosidase Formulations**

Chem. Pharm. Bull., **48**, 283-285 (2000)

The effects of excipients on the protein stability during lyophilization as well as the storage stability of lyophilized bilirubin oxidase (BO) and β-galactosidase (GA) formulations were studied using four polymer excipients: dextran, polyvinylalcohol (PVA), poly(acrylic acid) (PAA), and α, β-poly(N-hydroxyethyl)-L-aspartamide (PHEA). Denaturation of BO and GA during lyophilization largely depended on the excipient used. Dextran appeared to cause severe damage to proteins, whereas PHEA protected proteins effectively from denaturation. Storage stability of BO and GA formulations also depended on the excipients, such that the formulations containing dextran and PAA were relatively unstable. Storage stability was improved by absorption of a small amount of water for all the formulations studied. Absorption of a larger amount of water, however, decreased the storage stability of the formulations containing PVA, PAA or PHEA , but not that of the formulation containing dextran. This may be because formulations containing dextran have a higher glass transition temperature.

Keywords: lyophilization, enzyme activity, protein stability

Aso, Y., Yoshioka, S., Nakai Y., Kojima S.: **Thermally controlled protein release from gelatin-dextran hydrogels**

Rad. Phys. Chem., **55**, 179-183 (1999)

Biodegradable hydrogels in which drug release was controlled by sol-gel transition were prepared. Gelatin was used as a component because it exhibits sol-gel transition in response to temperature changes. Glycidyl methacrylated (GMA) dextran was crosslinked by low dose γ-irradiation in the presence of gelatin and the model drugs, β-galactosidase (β-GA), bovine serum albumin (BSA) or 5-fluorouracil (5-FU). The enzyme activity of β-GA remained greater than 95% after irradiation. Temperature-responsive release of β-GA and BSA resulted from the sol-gel transition of gelatin. Sol-gel transition was confirmed by the temperature dependence of the spin-spin relaxation time of the gel polymer protons. The protein release rate was affected by both the degree of GMA substitution and the gelatin concentration. Desired release rate could be achieved by adjusting these factors. The release rate of 5-FU was not affected by the sol-gel transition of gelatin.

Keywords: hydrogel, controlled release, protein

Aso, Y., Yoshioka S., Kojima S.: **Relationship between the crystallization rates of amorphous nifedipine, phenobarbital and flopropione, and their molecular mobility as measured by their enthalpy relaxation and 1H-NMR relaxation Times**

J. Pharm. Sci., **89**, 408-416 (2000)

Isothermal crystallization of amorphous nifedipine, phenobarbital and flopropione was studied at temperatures above and below their glass transition temperatures (T_g). A sharp decrease in the crystallization rate with decreasing temperature was observed for phenobarbital and flopropione, such that no crystallization was observed at temperatures 20-30°C lower than their T_g within ordinary experimental time periods. In contrast, the crystallization rate of nifedipine decreased moderately with decreasing temperature, and considerable crystallization was observed at 40°C below its T_g within 4 months. The molecular mobility of these amorphous drugs as measured by the enthalpy relaxation time and ^1H-NMR relaxation time suggests that nifedipine has higher molecular mobility than phenobarbital and flopropione at temperatures below T_g. The faster crystallization of nifedipine than that of phenobarbital or flopropione observed at temperatures below its T_g may be partly ascribed to its higher molecular mobility at these temperatures.

Keywords: Crystallization, Mobility, Relaxation

Izutsu, K., Kojima, S.: **Phase separation of polyelectrolytes and non-ionic polymers in frozen solutions.**

Phys. Chem. Chem. Phys., **2**, 123-127 (2000)

タンパク質など高分子凍結乾燥製剤の物性制御を目的として，高分子電解質と非イオン性高分子を含む水溶液の凍結濃縮による相分離現象について検討した．DEAE-dextran や poly（acrylic acid, sodium salt）等高分子電解質と polyvinylpyrrolidone（PVP），dextran等非イオン性高分子を含む単相の水溶液を凍結・熱分析すると単一または複数の最大濃縮相ガラス転移T_g'が現れ，組合せにより混合アモルファス状態で氷晶間に濃縮される場合と複数相に分離する場合のあることが示された．塩など共存物質は静電的親和力の抑制など分子間相互作用に影響を与えることにより凍結溶液中における高分子の混合性を変化させた．

Keywords: phase separation, miscibility, freeze-concentration

石橋無味雄：**日本薬局方の参照吸収スペクトルに関する研究 その1 参照赤外吸収スペクトルに関する研究(I)**

医薬品研究，**30**，547-552(1999)

日本薬局方（JP）では，確認試験法として赤外吸収スペクトル測定法が多用されている．しかし，この方法は，試料を測定して得られるスペクトルの波数と医薬品各条に数字で規定された波数を比較することにより確認する方法である．このため標準品と試料のスペクトルを比較する方法よりも精度が低くなる．しかし，標準品を用いて試験を行う方法は，標準品数の増加をまねくため，実現が不可能な

方法である．そこで参照スペクトルをJPに収載し，確認試験を行うこととし，検討を行った．その結果，原薬製造メーカの違いによるスペクトルの変動，測定雰囲気の影響（湿度等），塩酸塩の原薬に臭化カリウム錠剤法を用いた場合に起こる塩交換によるスペクトル変化等について検討を行い，JPに参照スペクトル法を収載することができた．
Keywords: Identification. IR. JP

Kikura, R., Nakahara, Y. and Kojima, S.: **Simultaneous determination of dimethylamphetamine and its metabolites in rat hair by gas chromatography-mass spectrometry**
J. Chromatogr. B, **741**, 163-173 (2000)
擬似覚せい剤として問題となっているdimethylamphetamine（DMAP）について，DMAP及びヒトにおける主代謝物であるDMAP N-oxide, methamphetamine（MA），amphetamine（AP）の同時定量法を確立するとともに，DMAPをラットに投与して，これらの化合物の血液，尿，毛髪への分布を検討した．DMAP N-oxideはGC/MSによる分析中に一部が熱分解してDMAPに，また試料をアシル誘導体化する際に試薬と反応してMAのアシル化体に変化する．そのため，血漿中，尿中，毛髪中のDMAP，DMAP *N*-oxide, MA, APの4化合物のGC/MSによる定量分析を行うために，各生体試料を強アルカリ条件下で液・液抽出をして*N*-oxide体を他の化合物と分離した後，*N*-oxide体をアシル誘導体化剤と反応させてMAの誘導体として測定した．一方，DMAP投与4週間後に採取したラット毛髪試料中からDMAP（4.82ng/mg），DMAP *N*-oxide（0.45ng/mg），MA（3.25ng/mg）及びAP（0.89ng/mg）が検出され，投与後長期を経ても毛髪中から親化合物であるDMAPを検出することが可能であった．また，DMAP *N*-oxideは血漿中，尿中からは高濃度検出されたものの，毛髪中からは低濃度しか検出されず，この化合物の血中から毛髪への移行性が極めて低いことが示唆された．
Keywords: hair analysis, dimethylamphetamine, GC/MS

Nakahara, Y.: **Hair Analysis for Abused and Therapeutic Drugs**
J.Chromatogr. B, **733**, 161-180 (1999).
This review focuses on basic aspects and recent studies of hair analysis for abused and therapeutic drugs and is discussed with 164 references. Firstly, the pretreatment methods of hair analysis have been commented on and analytical methods for each drug have been discussed. The outcomes of hair analysis studies have been reviewed by dividing into 6 groups; morphine and related, cocaine and related, amphetamines, cannabinoids, the other abused drugs and therapeutic drugs. In addition, reports on stability of drugs in the living hair and studies on drug incorporation into hair and dose-hair concentration relationships have been reviewed. Applications of hair analysis to the estimation of drug history, discrimination between OTC drug use and illegal drug use, drug testing for acute poisoning, gestational drug exposure and drug compliance, have also been reviewed. Finally, the promising prospects of hair analysis have been described.
Keywords: Hair analysis, abused drugs, therapeutic drugs

Nakahara, Y. and Hanajiri, R.: **Hair analysis for Drugs of Abuse: XXI. Effects of functional groups on benzene on the incorporation of methamphetamine analogs into rat hair.**
Life Sci., **66**, 563-574 (2000)
In order to study the effect of para-substituents on the benzene ring of methamphetamine on drug incorporation into hair from blood, the plasma AUCs and hair concentrations of 8 methamphetamines in DA rats was determined after intraperitoneal injection at 5 mg/kg. Drug incorporation rates into hair (ICRs) were calculated by dividing each hair concentration by each plasma AUC. Comparing the highest value to the lowest one, the ICR of p-nitro compound was 31.7 times larger than that of p-hydroxy compound. Based on the ICR of methamphetamine, nitro, bromo, methylenedioxy, methoxy and amino groups raised the drug incorporation into rat hair in this order. On the other hand, hydroxy substitution showed a negative effect on the ICR. Our results showed that there is a elatively strong effect of the functional groups on drug incorporation into hair.
Keywords: methamphetamine homologs, hair root, drug incorporation into hair

Toyooka, T.*, Kanbori, M.*, Kumaki, Y.*, Oe, T.*, Miyahara, T.* and Nakahara, Y.: **Detection of Triazolam and Its Hydroxy Metabolites in Rat Hair by Reversed-Phase Liquid Chromatography with Electrospray Ionization Mass**
Spectrometry. J.Anal.Toxicol., 24: 194-201 (2000).
A sensitive method using LC-ESI-MS for simultaneous determination of triazolam, and its hydroxy metabolites in hair has been developed. After the addition of deuterated hydroxytriazolam as an internal standard, hair samples were extracted with CH_2Cl_2/MeOH/NH_4OH(20:80:2) and analyzed LC-MS. The method was applied to determine the hair concentrations of triazolam and its metabolites in rat hair. Triazolam, 1-hydroxy- and 4-hydroxy-triazolam were incorporated in the rat hair. The method has been found to be useful as a screening procedure of triazolam intake in humans.
Keywords: hair analysis, triazolam, LC-MS
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Kawakami, N.*1, Takemasa, H.*1, Okamura, N.*2, Hayakawa, T., Shimohama, S.*3, and Fujimoto, S.*1: **Participation of cytosolic protein phosphatase in regulation of NADPH oxidase in polymorphonuclear leukocytes**
Biol. Pharm. Bull., **22**, 556-60 (1999)
When calyculin A, a protein phosphatase inhibitor, was added to the PMA-stimulated guinea pig polymorphonuclear leukocytes (PMNs) after the addition of H-7, a protein kinase C inhibitor, O_2- production and translocation of p47phox to the membrane fraction were occurred. The activity of NADPH oxidase and the amount of phosphorylated p47phox in the membrane of activated PMNs, were reduced by the cytosol fraction from unstimulated PMNs. These results indicate that the active form of the NADPH oxidase in PMNs can be reconstituted after the active complex of the enzyme has disappeared once, and that one of the regulation mechanisms of this enzyme activity involves the phosphorylation of p47phox in the cyotosol and dephosphorylation of the p47phox in the oxidase complex by protein kinase and protein phosphatase, respectively.
Keywords: NADPH oxidase, protein phosphatase, PMNs
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Kawakami, N.*1, Hayakawa, T,. Shimohama, S.*2, and Fujimoto, S.*1: **The hydroxyl radical formation system in**

polymorphonuclear leukocytes
Biol. Pharm. Bull., **22**, 1034-1037 (1999)
When phenylalanine was incubated with the alpha, beta and gamma fractions prepared from pig polymorphonuclear leukocytes (PMNs) and H_2O_2, significant levels of formation of m- and o-tyrosine were observed in the alpha and beta fractions, but not in the gamma fraction. With the heat-treated beta fraction (having little tyrosine formation activity) and myeloperoxidase (MPO) preparations from human neutrophils in the presence of H_2O_2, the amount of tyrosine formation increased with the addition of increasing amounts of heat-treated beta fraction. Tyrosine formation by the beta fraction in the presence of H_2O_2 was significantly reduced by hydroxyl radical (OH*) scavengers. The above results suggest the existence of an OH*-generating system in which MPO and H_2O_2 participate in the granules of PMNs and, especially, in specific granules, there may exist some factors that cause more effective OH* generation.
Keywords: PMNs, myeloperoxidase, hydroxyl radical
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Hayakawa, T.: **Japanese Perspective with Respect to Quality Control of Biotechnological/Biological Products**
Pharmaeuropa, Special Issue, Proceedings Biologicals Beyond 2000, 261-262 (2000)
バイオテクノロジーで生産される医薬品について，一般的な留意点を考察した．また，新たな医療技術として開発が進められている組織・細胞加工医薬品・医療用具の品質・安全性等の確保に向けて，どのような問題点があるかと，わが国での取り組み状況を明らかにした．さらに，本問題に対応できる国際的枠組み作り，具体的には国際調和ガイドライン作成に向けて，日・欧・米が積極的に取り組むべきことを提唱した．
Keywords: Biotechnology products, Cellular and tissue therapy

Hayakawa, T., Ohta, M., and Kawasaki, N.: **Current Analytical Procedures for Glycosylated Proteins**
Pharmeuropa, Special Issue, Proceedings Biologicals Beyond 2000, 87-102 (2000)
This paper mainly describes current analytical procedures for glycosylated proteins, including the following 3 topics: 1) a brief overview of currently available analytical methods for characterization of the structural features of the carbohydrate moiety of glycoproteins; 2) Japanese experience with the structural characterization of the carbohydrate moieties of various glycoprotein products, especially the use of two- dimensional oligosaccharide mapping of 2-aminopyridine derivatives; and 3) utilization of liquid chromatography with electrospray ionization mass spectrometry (ESI-LC/MS) and liquid chromatography with tandem electrospray ionization mass spectrometry (ESI-LC/MS/MS) for analysis of the size, sugar sequences, and branch structure of closely related oligosaccharides in glycoproteins and for elucidation of carbohydrate heterogeneity at each glycosylation site. Relevant application of these analytical methods to product characterization for research, development, quality control, and the comparability of therapeutic glycoprotein(s) is discussed.
Keywords: Glycoproteins, Carbohydrate analysis, ESI-LC/MS

Kinoshita, M.*, Murakami, E.*, Oda, Y.*, Funakubo, T.*, Kawakami, D.*, Kakehi, K.*, Kawasaki, N., Morimoto, K., and Hayakawa, T.: **Comparative studies on the analysis of glycosylation heterogeneity of sialic acid-containing glycoproteins using capillary electrophoresis**
J. Chromatogr. A., **866**, 261-271 (1999)
キャピラリー電気泳動法を用いてシアロ糖タンパク質のグリコフォームを分析するための比較検討を行った．表面がコーティングされた市販のガスクロマトグラフィー用のキャピラリーと，等電点に近いランニングバッファーを用いることによって，シアロ糖タンパク質のグリコフォームを分離することに成功した．分析時間は50分以内で，再現性も良好であった．この分析法を用いて血清中のα1-acid glycoproteinのグリコフォームを分析することができた．本分析技術は，糖タンパク質製剤の不均一性評価に有用であると思われる．
Keywords: glycoproteins, capillary electrophoresis
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Kawasaki, N., Lee, Y. C.*, Hashimoto, O., Yamamoto, M., Kawanishi, T., and Hayakawa, T.: **Fluorometric determination of aminopolycarboxylates using fluo-3**
Anal. Biochem., **270**, 329-331 (1999)
時間分解蛍光法に用いるタンパク質等のランタノイド標識化に，EDTA, DTPA及びEGTA等のaminopolycarboxylateがよく用いられる．しかし，微量標識タンパク質を精製する際に，未反応物のaminopolycarboxylateや関連物質はUV吸収が弱いために検出することができない．そこで，蛍光プローブfluo-3を用いた高感度aminopolycarboxylate定量法を開発した．さらに，時間分解蛍光法を用いた抗血栓治療薬トロンボモジュリン（TM）の活性測定法の開発を目指してTMを無水DTPAで標識し，Fluo-3法を用いることによって僅かなサンプル量で標識TMの精製ができることを確認した．
Keywords: fluo-3, aminopolycarboxylate, lanthanide
* Department of Biology, Johns Hopkins University

Hyuga, S., Yamagata, S.*1, Takatsu, Y.*1, Hyuga, M., Nakanishi, H.*2, Furukawa, K.*3, and Yamagata, T.*1: **Suppression by ganglioside GD1a of migration capability, adhesion to vitronectin and metastatic potential of highly metastatic FBJ-LL cells**
Int. J. Cancer, **83**, 685-691 (1999)
これまでに，低転移性FBJ-S1細胞に高発現していたガングリオシドGD1aが高転移性FBJ-LL細胞の運動能を抑制することを報告したが，さらに，GD1aがFBJ-LL細胞のビトロネクチンに対する接着を特異的に抑制することを明らかにした．また，FBJ-LL細胞にGM2合成酵素遺伝子を導入して作成したGD1a高発現性のトランスフェクタントは運動能及びビトロネクチンに対する接着が低下し，*in vivo*の転移能を完全に喪失した．これらの結果から，GD1aは細胞運動及び細胞接着を抑制することにより，転移を抑制していることが示唆された．
Keywords: GD1a, vitronectin, metastasis
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Hyuga, S., Kawasaki, N., Hashimoto, O., Hyuga, M., Ohta, M., Yamagata, S.*, Yamagata, T.*, and Hayakawa, T.: **Possible role of hepatocyte growth factor/scatter factor and activin A produced by the target organ in liver metastasis**
Cancer Lett., **153**, 137-143 (2000)
癌細胞の臓器特異的な転移の分子機構はほとんど解明さ

れていない．しかし，癌細胞の増殖や運動能をパラクラインで促進するような増殖因子を標的臓器が産生していれば，これらの因子は臓器特異的な転移を誘導する可能性がある．そこで，肝転移を起こしやすい癌細胞を用いて，肝臓で合成されるHGF及びアクチビンAの効果を調べた．HGF及びアクチビンAはこの癌細胞の運動能を相乗的に促進し，この細胞はこれらの因子の受容体を発現していることが明らかになった．従って，HGF及びアクチビンAは癌細胞の運動能を促進することにより，肝臓特異的な転移を誘導していることが示唆された．

Keywords: HGF, activin A, metastasis

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Kanayasu-Toyoda, T., Yamaguchi, T., Uchida, E., and Hayakawa, T.: **Commitment of Neutrophilic Differentiation and Proliferation of HL-60 Cells Coincides with Expression of Transferrin Receptor**

J. Biol. Chem., **274**, 25471-25480 (1999)

HL-60細胞を用いて，好中球分化の制御機構の解析を行った．DMSOによってHL-60細胞の好中球分化を誘導するとトランスフェリンレセプター（Trf-R）の発現量の異なる細胞が出現することを見出した．このTrf-R陰性細胞は分化型であり，Trf-R陽性細胞は増殖型であること，G-CSFはそれぞれのコミットメントを変えることなく分化や増殖能を亢進することを明らかにした．

Keywords: neutrophilic differentiation, STAT3

Kishi, K., H.[*1], Mikawa, T.[*2], Seto, M.[*1], Sakai, Y.[*1], Kanayasu-Toyoda, T., Yamaguchi, T., Imamura, M.[*1], Ito, M.[*1], Karaki, H.[*3], Bao, J.[*1], Nakamura, A.[*1], Ishikawa, R.[*1], and Kohama, K.[*1]: **Stable transfectants of smooth muscle cell line lacking the expression of myosin light chain kinase and their characterization with respect to the actomyosin system.**

J. Biol. Chem., **275**, 1414-1420 (2000)

平滑筋細胞の運動能におけるミオシン軽鎖キナーゼの役割を明らかにすることを目的として，平滑筋細胞株SM3にミオシン軽鎖キナーゼに対するアンチセンスヌクレオチドを恒常的に発現するプラスミドを導入した変異株を樹立した．変異株では，ミオシン軽鎖キナーゼの発現が低下していた．さらに，変異株ではPDGFに対する走化性やlamellipodia形成が認められなかった.従って，平滑筋の運動性においてはミオシン軽鎖キナーゼが中心的な役割を担っていることが示された．

Keywords: MLCK, cytoskeleton

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Takahashi, M.[*1], Misawa, Y.[*1], Watanabe, N.[*1], Kawanishi, T., Tanaka, H.[*2], Shigenobu, K.[*2], and Kobayashi, Y.[*1]: **Role of P-glycoprotein in Human Natural Killer-Like Cell Line-Mediated Cytotoxicity**

Exp. Cell Res., **253**, 396-402 (1999)

Natural killer (NK) cells express the highest amount of P-glycoprotein (Pgp) among lymphoid cells, and our previous studies demonstrated that Pgp is required for NK cell-mediated cytotoxicity. In this study we examined the role of Pgp in NK cell-mediated cytotoxicity using a human NK-like cell line, YTN cell, and two MDR reversing agents, nicardipine and its structural analog, AHC-93. These two agents inhibited the Pgp function as well as cell-mediated cytotoxicity. AHC-93 did not inhibit the increase in the intracellular Ca^{2+} concentration upon binding to target cells, whereas nicardipine did. The two reagents relocated acridine orange dye from lysosomes to the cytoplasm at concentrations similar to those required for the inhibition of cell-mediated cytotoxicity. These results suggest that Pgp is directly or indirectly involved in pH regulation in lysosomes, but not in calcium homeostasis.

Keywords: P-glycoprotein, natural killer cells, pH

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Tanaka, H.*, Ichikawa, T.*, Matsui, S.*, Okazaki, K.*, Masumiya, H.*, Kawanishi, T., and Shigenobu, K.*: **Calcium Channel Antagonistic Effects of AH-1058, a Novel Antiarrhythmic Drug, on Guinea-Pig Myocardium**

Res. Communi. Pathol. Phrmacol., **104**, 13-21 (1999)

Effects of AH-1058, a novel cyproheptadine derivative with high antiarrhythmic activity in *in vivo* arrhythmia models, were studied in guinea-pig myocardium. In coronary- perfused right ventricular tissue preparations, AH-1058 (10^{-5}M) shortened the action potential duration with little effect on the resting membrane potential, maximum rate of rise and overshoot. AH-1058, 10^{-7}M to 10^{-5}M, concentration-dependently decreased the contractile force. The increase in contractile force by Ca^{2+} was markedly inhibited by 3 x 10^{-6}M AH-1058 while that by isoproterenol was only slightly affected. In isolated ventricular myocytes, AH-1058 concentration-dependently decreased the nicardipine sensitive transient inward current with no effect on steady state currents, and decreased the amplitude of the evoked Ca^{2+} transient. These results suggest that AH-1058 has Ca^{2+} channel antagonistic effects which may contribute to its antiarrhythmic activity.

Keywords: Ca^{2+} channel antagonist, myocardium, Ca^{2+}

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Mizuguchi, H., and Kay, M. A.*: **A simple method for constructing E1 and E1/E4 deleted recombinant adenovirus vector**

Hum. Gene Ther., **10**, 2013-2017 (1999)

One of the limitations of recombinant adenovirus (Ad) vectors is the time and labor involved in their construction. In this study, we have developed the system to improve the simplicity of vector construction and in addition, allow for production of an E1/E4 deleted vector.

Keywords: adenovirus vector, gene therapy

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Mizuguchi, H., Zhili, H., Ishii-Watabe, A., Uchida, E., and Hayakawa, T.: **IRES-dependent second gene expression is significantly lower than cap-dependent first gene expression in a bicistronic vector**

Mol. Ther., **1**, 376-382 (2000)

This study was undertaken to characterize the relative expression of IRES-dependent second gene in a bicistronic vector, which was derived from the 5'-untranslated regions of the encephalomyocarditis virus (EMCV). IRES-dependent second gene expression was compared with cap-dependent first gene expression in several cultured cell lines and in mouse liver *in vivo*. The expression of the IRES-dependent second gene

ranged from 6-100 % (in most cases between 20 and 50%) that of the first gene. This finding has important implications for the use of IRES, i.e., care should be taken regarding the decreased capacity of IRES-dependent downstream gene expression as well as in determining which gene should be positioned as the first or second gene in a bicistronic vector.
Keywords: IRES, EMCV, gene therapy

Mizuguchi, H., Hosono, T., and Hayakawa, T.: **Long-term replication of Epstein-Barr virus-derived episomal vectors in the rodent cells**
FEBS Letter, **472**, 173-178 (2000)

Plasmids containing the origin of replication, oriP, of the Epstein-Barr virus (EBV) and EBV nuclear antigen-1 (EBNA-1) genes replicate extrachromosomally in primate cells. However, these plasmids have been believed not to replicate in rodent cells. We demonstrate here that these plasmids can replicate in some types of rodent cells over a long period. This result should offer not only the new insight into the mechanisms of species-specific replication of EBV, but also the possibility that an EBV-based vector can be used for gene transfer experiments in non-primate cells and animal experiment regarding human gene therapy.
Keywords: Epstein-Barr virus, episomal vector, gene therapy

Nakanishi, M.[*1], Mizuguchi, H., Ashihara, K.[*2], Senda, T.[*3], Eguchi, A.[*1], Watabe, A., Nakanishi, T.[*2], Kondo, M.[*2], Nakagawa, T.[*2], Masago, A.[*1], Okabe, J.[*1], Ueda, S.[*1], Mayumi, T.[*2], and Hayakawa, T.: **Gene delivery system using the Sendai virus**
Mol. Membrane Biol., **16**, 123-127 (1999)

Fusogenic liposome (FL) is a delivery system that can transfer encapsulated materials into living cells directly through membrane fusion. FL is a promising approach for gene therapy because it can deliver various genetic materials much more efficiently than other non-viral vectors without damaging the cell. This review has described a detailed analysis of these fusion phenomena and discussed possible applications of FL-mediated gene delivery to human gene therapy.
Keywords: Sendai virus, fusogenic liposome, gene therapy

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Ishii-Watabe, A., Uchida, E., Mizuguchi, H., and Hayakawa, T.: **On the Mechanism of Plasmin-Induced Platelet Aggregation: Implications of the Dual Role of Granule ADP**
Biochem. Pharmacol., **59**, 1345-1355 (2000)

Plasmin-induced platelet aggregation has been considered to be a cause of reocclusion after thrombolytic treatment with plasminogen activators. However, little is known regarding its mechanism and regulation. In this study, we demonstrated that plasmin causes the degranulation of platelets, and that ADP released from granules plays a crucial role in the induction of platelet aggregation. We also demonstrated that pretreatment of platelets with ADP makes the platelets more sensitive to plasmin, and plasmin-induced platelet aggregation is therefore observed at lower concentrations where no aggregation occurs in quiescent platelets. The effect of ADP was inhibited by AR-C69931, a selective antagonist for the P2T*AC* subtype of P2 receptor, but not by the P2Y1 receptor-selective antagonist. P2X1 receptor agonist did not mimic the action of ADP. These data indicate that ADP potentiates plasmin-induced platelet aggregation via the P2T*AC* receptor. These results demonstrate that, in plasmin-induced platelet aggregation, ADP is secreted from platelet granules, and concomitantly works in conjunction with plasmin in a P2T*AC* receptor-mediated manner.
Keywords: plasmin, ADP, platelet

Kobayashi, T., Niimi, S., Hashimoto, O., and Hayakawa, T.: **Expression of Inhibin βA, βB, and Follistatin mRNAs in the Carbon Tetrachloride Induced Rat Liver Regeneration Model**
Biol. Pharm. Bull., **23**, 755-757 (2000)

Follistatin (FS, an activin-binding protein) and activin A (homodimer of inhibin βA chain) promote and inhibit cell proliferation in rat liver, respectively. The roles of activin AB (heterodimer of inhibin βA and βB) and activin B (homodimer of inhibin βB) in rat liver have not been elucidated yet. In this study, we examined, by RT-PCR analysis, whether the levels of FS, inhibin βA and βB mRNAs change in the carbon tetrachloride induced rat liver regeneration model. There are 2 types of FS mRNA, FS-288 and FS-315, and the levels of both had begun to increase at 3 h, were maximal at 6 h, remained constant up to 12 h, and thereafter gradually decreased. The inhibin inhibin βA mRNA had started to decline at 3 h, reached its lowest level at 6 h, partly returned at 12 h, and remained constant up to 48 h. The inhibin βB mRNA level had begun to increase at 1 h, was maximal at 3 h, remained constant up to 24 h, and returned to the original level at 48 h. These results indicate that FS and Activin A may act reciprocally in liver regeneration, and also suggest that activin AB and B may play roles in liver regeneration that differ from that of activin A.
Keywords: activin, follistatin, liver regeneration

Yukihiro Ozaki, Jing Rui* and Yuan Tai Tang*: **Antiinflammatory Effect of *Forsythia suspensa* Vahl and Its Active Principle**
Biol. Pharm. Bull., **23**, 365-367 (2000)

This study was carried out to elucidate the antiinflammatory active principles obtained from 70% methanol extract of the dried fruit of *Forsythia suspensa* Vahl (*F. suspensa*). The methanol extract was then partitioned between n-hexane and water, and then the n-hexane fraction was evaporated to dryness under vacuum. The *n*-hexane fraction was chromatographed (Frs. I-V), Fr. IV was rechromatographed (Frs. VI-VIII), and then Fr. VII was rechromatographed (Frs. IX-XI) by silica gel column chromatography. The antiinflammatory activity of these fractions was investigated on acetic acid-induced vascular permeability in rats. The *n*-hexane fraction showed an antiinflammatory effect and these activities shifted successively to Fr. IV, Fr. VII and Fr. X. The chemical structure of the active principle obtained from Fr. X was inentified as 3β-acetoxy-20, 25-epoxydammarane-24-ol. These results suggest that the antiinflammatory and an analgesic effect of 70% methanol extract of *F. suspensa* may be the result of the compound that it contains.
Keywords: antiinflammatory effect, *Forsythia suspensa* Vahl, active principle

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Hosoe, T.*1, Noxawa, K.*1, Kawahara, N., Fukushima, K.*2, Nishimura, K.*2, Miyaji, M.*2, Kawai, K.*2: **Isolation of a new potent cytotoxic pigment along with indigotin from the pathogenic basidiomycetous fungus *Sohizophyllum commune***

Mycopathologia, **146**, 9-12 (1999)

アレルギー性気管支肺真菌症患者から単離された *Sohizophyllum commune* IMF 46788（monokaryon）の成分研究を行い，schizocommunin と命名した新規インドール誘導体を単離・構造決定した．本化合物は murine lymphoma cell に対して強い細胞毒性を示した．

Keywords: *Sohizophyllum commune*, human allergenci bronchopulmonary mycosis, schizocommunin, cytotoxicity

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Kawahara, N., Nozawa, M., Kurata, A.*, Hakamatsuka, T.*, Sekita, S., Satake, M.: **A. novel Sesterterpenoid, Nitiol, as a Potent Enhancer of IL-2 Gene Expression in a Human T Cell Line, from the Peruvian Folk Medicine "Hercampuri" (*Gentianella nitida*)**

Chem. Pharm. Bull., **47**, 1344-1345 (1999)

ペルーにおいて民間薬として肝炎等の治療の目的で用いられている薬用植物 Hercampuri（*Gentianell nitida*）の成分検索を行った．本植物の全草ジクロロメタン抽出エキスより nitiol と命名した新規セスタテルペノイド誘導体を単離し，各種スペクトルデータ解析よりその立体構造を決定した．本化合物はヒトT細胞における IL－2 遺伝子発現増強活性が認められた．

Keywords: Hercampuri, *Gentianella nitida*, nitiol, sesterterpenoid

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Shirota, O.*, Nakanishi, K.*, and Berova, N.*: **Phytosphingosines –A facile synthesis and spectroscopic protocol for configurational assignment**

Tetrahedron, **55**, 13643-13658（1999）

ファイトスフィンゴシンの考えられる8つの配置異性体のうち4種の簡便な合成法を確立した．さらにこれら4種の異性体を用いて，化学誘導／CD／NMR プロトコールによるファイトスフィンゴシン全異性体の相対および立体配座の決定法を開発した．このプロトコールにより，未知のファイトスフィンゴシンの立体配置を数ナノモルレベルで決定することが可能である．

Keywords: phytosphingosine, sphingolipids, circular dichroism, relative and absolute configurations

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吉富史郎*，渡辺正純*，川西史明**，佐竹元吉：**カノコソウの品質と鎮静作用**

Natural Medicines, **54**, 55-60（2000）

The sedative effects of 30％ EtOH extracts of the two Japanese valerian roots, "Kameba-Kisso" and "Hokkai-Kisso", were examined. These extracts significantly prolonged the pentobarbital-induced sleep in mice at doses of 11.5 g/kg and 5.8 g/kg, respectively. The spontaneous motor activity of mice in an open field test was significantly suppressed after oral administration of Kameba-Kisso (16.7g/kg) and Hokkai-Kisso (19.4g/kg). Both of these extracts showed neither inhibition of the pentetrazol-induced convulsion nor restoration of reserpine-induced hypothermia. These result indicate that both of the valerian extracts have similar sedative effects through the suppression o fht central nervous system.

Keywords: Kameba-Kisso, Hokkai-Kisso, pentobarbital-induced sleep, spontaneous motor activity

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Kaniwa, M.: **A profile of haptenic allergens in indoor environment**

Environmental Dermatology, **5**(Suppl.2), 78-84 (1998)

アトピーの発症・増悪の要因となりうる室内環境中のハプテンアレルゲンとして，衣類中の分散染料，プラスチック眼鏡フレーム中の着色剤，椅子のポリ塩化ビニル製側地中の抗菌剤，防虫剤のパラジクロロベンゼン等について，市販の家庭用品における使用実態，健康影響の種類（毒性）と強度について情報収集し，家庭用品中のハプテンアレルゲンの分布実態を明らかにするとともに，それらによる健康影響の大きさについて概説した．

Keywords: haptenic allergen, indoor environment, household product

Hayakawa, R.*1, Sugiura, M.*1, Tanaka, S.*2, Miyachi, Y.*3, Kanbe, N.*3, Horio, T.*4, Mitsuya, K.*4, Ichihashi, M.*5, Bito, T.*5, Shimizu, R.*6, Nakamura, T.*6, Higashi, N.*7, Natsuaki, M.*8, Ito, M.*9, Takahashi, K.*9, Nishioka, K.*10, Shimada, S.*11, Tsukamoto, K.*11, Ikezawa, Z.*12, Osuna, H.*12 and Kaniwa, M-A.: **Group study on latex allergy**

Environmental Dermatology, **6**, 10-16 (1999)

日本における天然ゴム手袋等によるラテックスアレルギー（I型タイプ）の発生状況を把握するとともに，新規の症例の発生防止策を講じるために，8大学病院，4総合病院，国立医薬品食品衛生研究所療品部が共同研究を行った．プリック／スクラッチテスト（I型アレルギーの確認法），パッチテスト（IV型アレルギーの確認法）による検討の結果，接触じんましん（I型タイプ）22例，接触じんましん（I型タイプ）と接触皮膚炎（IV型タイプ）の併発2例，アナフィラキシー（I型タイプ）2例を確認した．

Keywords: group study, latex allergy, natural rubber glove

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*10 山口赤十字病院

*11 山梨医大

*12 横浜市立大医

Sugiura, M.*1, Hayakawa, R.*1, Kato, Y.*1, Sugiura, K.*2, Ueda, H.*2 and Kaniwa, M-A.: **Five cases of latex allergy**

Environmental Dermatology, **6**, 153-160 (1999)

ラテックスアレルギー患者5名について，プリック／スクラッチテスト（I型アレルギーの確認法），パッチテスト（IV型アレルギーの確認法）により検討した．その結果，患者1名では，プリック／スクラッチテスト，パッチテストいずれにおいても天然ゴムラテックスに陽性反応を示し

たことから，天然ゴムラテックスに対してI型タイプとIV型タイプの二つのアレルギーが併発されていたことを確認した．

Keywords: latex allergy, immediate type(type I), delayed type(type IV)

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Shono, M.* and Kaniwa, M-A.: **Allergic contact dermatitis from a perinone-type dye C.I. Solvent Orange 60 in spectacle frames**

Contact Dermatitis, **41**, 181-184 (1999)

眼鏡フレームのプラスチック製の先セル部分による耳背部に発生したアレルギー性接触皮膚炎事例を検討した結果，先セルに配合されていた着色剤中のペリノン系染料Solvent Orange 60が新規の染料アレルゲンであり，今回の事例の原因化学物質となっていたことを明らかにした．

Keywords: Solvent Orange 60, allergic contact dermatitis, spectacle frame

*しょうの皮膚科

Ikarashi, Y., Tsuchiya, T. and Nakamura, A.: **Effect of heat treatment of poly(L-lactide) on the response of osteoblast-like MC3T3-E1 cells**

Biomaterials, **21**, 1259-1267 (2000)

骨固定用整形材料のポリL-乳酸（PLLA）は加熱成形される．そこで，加熱処理したPLLAが骨形成に影響を及ぼすかどうか，PLLA上で培養した骨芽細胞様MC3T3-E1細胞のタンパク含量，DNA含量，アルカリホスファターゼ（ALP）活性及びヒドロキシプロリン（HYP）含量を指標として検討した．PLLA（分子量1000000）を高温度，長時間で加熱した場合，細胞活性は著しく増加した．PLLAの分子量は20000程度まで低下し，L-lactide及びL-乳酸も生成した．これらの分解生成物と細胞を培養したところ，低分子量PLLAが最も活性を示し，L-乳酸では反応が表れなかった．よって，PLLAの加熱処理による骨形成作用の増強は，溶解性のL-乳酸よりもむしろ低分子量PLLAによるものと推測された．

Keywords: poly(L-lactide), heat treatment, osteoblast

Nakaoka, R., Tsuchiya, T. and Nakamura, A.: **Studies on the mechanisms of tumorigenesis induced by polyetherurethane in rats: Production of superoxide, tumor necrosis factor, and interleukin 1 from macrophages cultured on different polyetherurethanes**

J. Biomed. Mater. Res., **49**, 99-105 (2000)

*In vivo*で発癌性が異なる3種類のポリエーテルウレタンが炎症系の細胞であるマウス腹腔マクロファージにそれぞれどのような影響を与えるのかを，そのマクロファージから産生されるインターロイキン1（IL-1），腫瘍壊死因子（TNF）および活性酸素の量を測定することで評価した．その結果，最も発癌性の弱かった材料上では活性酸素の産生量が大きくIL-1の産生量が最も少ないことが認められた．これらのことから，このポリウレタンを体内に埋入した場合には，炎症が他のものと比べて弱いこと，さらに活性酸素による癌細胞除去が起こっている可能性があり，それらの結果，発癌率が低下していることが考えられた．なお，TNFの産生量には差が認められず，TNFはポリウレタンによる発癌に関係しないことが示唆された．

Keywords: polyetherurethane, tumorigenesis, superoxide

Nakaoka, R., Tsuchiya, T. and Nakamura, A.: **Studies on tumor promoting activity of polyethylene immobilized with various proteins**

Biomedical Materials Research in Asia (IV), 122-123 (2000)

生体内埋入後の副作用が数多く報告されているポリエチレンを種々のタンパク質で表面修飾し，その材料が生体に与える影響を*in vitro*で評価することを試みた．細胞の恒常性維持に重要な機能である細胞間連絡機能に着目して，それぞれの材料上での細胞機能をFluorescence re-distribution after photobleaching assay（FRAP法）で測定したところ，未処理の材料ではその機能が阻害され，コラーゲンを修飾した場合にはその機能が回復することが認められた．その機能を担うタンパク質の細胞内での状態を免疫染色で観察したところ，未処理の材料上では細胞質全体に，コラーゲン修飾した場合には正常の細胞と同様に細胞膜上に局在することが認められた．すなわち，ポリエチレン上での細胞機能阻害はその機能を担うタンパク質の局在性が乱されるためであり，タンパク質の局在性が回復するように材料を表面修飾することで機能が回復することが示された．

Keywords: polyethylene, gap junction, protein immobilization

Shintani, H: **Selective analysis of compounds in body fluids and how to avoid artifact formation**

J. Liquid Chromatogr., **21**, 2297-2312 (1998)

歯科材料の一つである義歯床の主たる材料はポリメチルメタクリレート（PMMA）である．PMMA合成にはメチルメタクリレート（MMA），N,N-ジメチル-p-トルイジン（DMPT）並びにベンゾイルパーオキサイド（BPO）等が出発原料として用いられる．DMPTとBPOはお互いに反応して新規化合物（アーチファクト）を生成する．またその新規化合物あるいはBPOは唾液と反応してさらに異なるアーチファクトが生成される．出発原料並びに生成したアーチファクトなどは体液中に溶出してくる．それらの成分をHPLCでのグラジエント溶出法で分離，分析した．またそれらの成分の選択的な前処理法を開発した．その溶出液組成が従来報告されている組成と異なるためその科学的根拠を確認した．

Keywords: dental material, artifact, solid phase extraction

Shintani, H: **Validation of D value by different SCD culture medium manufacturer and/or different SCD culture medium constituent**

PDA J. Pharm. Sci. Technol., **54**, 6-12 (2000)

培地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生物指標の抵抗値の差の原因について調べた．

Keywords: culture medium, biological indicator, variation

森川鉄朗*，林　譲：**化学教育における平衡をめぐる理解と誤解について**

上越教育大学研究紀要，**19**，461-471（2000）

中等高等化学教育における平衡の理解をすすめ指導法を改良するため，平衡に関連してみられる疑問や誤解や思い違いをひろくとりあげ，検討した．

Keywords: chemical equilibrium, education, chemistry

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Hiratsuka, H.*1, Satoh, S.*2, Satoh, M.*3, Nishijima, M.*4, Katsuki, Y.*4, Suzuki, J.*4, Nakagawa, J.*4, Sumiyoshi, M.*5, Shibutani, M., Mitsumori, K., Tanaka-Kagawa, T., Ando, M.: **Tissue distribution of cadmium in rats given minimum**

amounts of cadmium-polluted rice or cadmium chloride for 8 months

Toxicol. Appl. Pharmacol., **160**, 183-191 (1999)

To investigate the relationship between cadmium (Cd) toxicity, intestinal absorption, and its distribution to various tissues in rats treated orally with minimum amounts of Cd, 14 female rats per dose group per time point were given diets consisting of 28% purified diet and 72% ordinary rice containing Cd-polluted rice (0.02, 0.04, 0.12, or 1.01 ppm of Cd) or $CdCl_2$ (5.08, 19.8, or 40.0 ppm of Cd) for up to 8 months. At 1, 4, and 8 months after the commencement of Cd treatment, seven rats per group were euthanized for pathological examinations to determine the Cd concentrations in the liver and kidneys and metallothionein (MT) in the liver, kidneys, intestinal mucosa, serum and urine. One week before each period of 1, 4, and 8 months, the remaining seven rats in each group were administered a single dosage of ^{109}Cd, a tracer, to match the amounts of the designated Cd doses (about 1.2 to 2400 μg/kg body wt). They were euthanized 5 days later to determine the distribution of Cd to various tissues. No Cd-related toxic changes were observed. The concentrations of Cd in the liver and kidneys at any time point and MT in the liver, kidney, serum, and urine at 4 and 8 months increased dose-dependently, whereas MT in the intestinal mucosa did not alter markedly at any time point. The distribution rats of Cd to the liver increased dose-dependently (40% at lower doses to 60% at higher doses), whereas those to the kidney decrease dose-dependently (20% at lower doses to 10% at higher doses). The Cd retention rates 5 days after ^{109}Cd administration (amounts of Cd in various tissues/amounts of Cd administered) ranged from 0.2 to 1.0% at any time point. these results suggest that the distribution of Cd to the liver and kidneys after the oral administration vary depending on the dosage levels of Cd. The difference of the distribution pattern of Cd to the liver and kidney is probably due to the difference in the form of the absorbed Cd, i.e., free ion or Cd-MT complex, although not closely related to the MT in the intestinal mucosa.

Keywords: Cadmium, Tissue Distribution, Renal Toxicity

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日本建築学会計画系論文集, **522**, 45-52 (1999)

地方都市と首都圏に建立する50戸を対象に居住環境内におけるHCHOとVOCs (32種類)について，季節変動，住宅種別等の実態調査を行い，以下の結論が得られた．全平均値はHCHO濃度が0.048 ppm，TVOCsが3640 μg/m^{3}であった．集合と戸建住宅を比較するとHCHO及びTVOCsとも集合住宅の濃度が高い傾向を示していた．冬期と夏期を比較するとHCHO及びTVOCsとも冬期に濃度が高い傾向を示していた．温度，湿度とHCHO及びTVOCsとの間には相関が認められた．室内VOCs濃度は建材が支配する時期と生活行動が支配する時期のあることが判った．新築住宅に入居することにより，HCHOとTVOCsが極端に減少することが明らかとなった．

Keywords: volatile organic compounds, residence, field measurement

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佐々野僚一*, 古庄義明*, 松村年郎：**デイスク型固相捕集とGC大量注入法を用いた室内空気中の有機リン化合物の分析**

空気清浄, **37**, 50-55 (1999)

固相捕集-溶媒抽出-GC/MS法による室内空気中の有機リン化合物 (Tributyl phosphate, Triphenyl phosphate, Tricresyl phosphate, Tris(2-chloroethyl)phosphate, Diazinon, Fenitrothion, Fenthion, Chlorpyrifos, Pyridaphention)の測定法の開発を行った．本法はC18デイスク型固相に試料空気を毎分10 Lで2時間サンプリングした後，溶媒抽出アットカラム濃縮大量導入で測定する高感度測定法である．本法の検出限界は0.3 ng/m^3，添加回収率も90%以上，繰り返し精度も5%以下と良好である．本法を室内空気の実測に適用した結果，トリブチルホスフェート (12.3 ng/m^3)やクロルピリホス (4.8 ng/m^3)が検出された．

Keywords: Indoor environment, measuring technology, organophosphorus compounds

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徳永裕司, 鄭 然孫, 内野 正, 安藤正典：**乳液およびクリーム中のエストラジオールおよびエチニルエストラジオールの定量**

日本化粧品技術者会誌, **33**, 163-169 (1999)

化粧品の乳液およびクリーム中のエストラジオールおよびエチニルエストラジオールの定量法は衛生試験法追補1995に既に設定されている．しかしクロロホルムおよびジクロロメタンのような生体に有害な試薬が分析操作に使われている．有害試薬を除き，同時に，ほとんどの化粧品に含まれ，エストラジオールおよびエチニルエストラジオールの定量を妨害するパラベン類の影響を受けない新しく，簡便な定量法を検討した．乳液あるいはクリームを水浴中で濃縮し，残留物中のエストラジオールあるいはエチニルエストラジオールをエタノール抽出した．抽出物をODSカラム (TSKgel ODS80TM, 4.6×150mm)，移動相として水／アセトニトリル／メタノール混液 (3:1:1) および蛍光検出器 (励起および蛍光波長：290および310nm) 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，われわれは乳液およびクリーム中のエストラジオールおよびエチニルエストラジオールを防腐剤のパラベン類あるいは他の賦形剤の影響もなく定量することができることを明らかにした．

Keywords: estadiol, ethinylestradiol, cosmetic preparations

徳永裕司, 鄭 然孫, 内野 正, 安藤正典：**CHO細胞に及ぼす界面活性剤の影響**

日本香粧品科学会誌, **24**, 14-20 (2000)

界面活性剤のcell viablityへの影響を調べるため，9種類のカチオン性界面活性剤，8種類のアニオン性界面活性剤，12種類のノニオン性界面活性剤および異なるEO鎖を持つpolyoxyethylene nonylphenyl ether (POE.NPE), polyoxyethylene lauryl ether (POE.LE), polyoxyethylene castor oil and polyoxyethylene oleyl etherの影響をChinese Hamster Ovary (CHO) 細胞を用いて研究した．CHO細胞の1x104 cells/0.1

mlを37℃のCO_2インキュベータで1日間培養した後，10μlの界面活性剤溶液を96穴マイクロプレートに添加し，更に37℃のCO_2インキュベータで1日間培養した．cell viabilityは 1-Methoxy PMS/WST-1混液で測定した．CHO細胞の50%の残存率を示す界面活性剤の濃度（IC50）が検量線より求められた．各界面活性剤から得られたIC50を界面活性剤でモルモットの剥離皮膚を処理した後のメチルパラベン（MP）あるいはサリチル酸（SA）の透過速度（%）あるいはウサギの赤血球を50%溶血させる界面活性剤の濃度（EC50）と比較検討した．カチオン性界面活性剤の脂肪族炭化水素鎖の炭素数10～18への増加は$1/IC_{50}$の増加に依存しており，$1/IC_{50}$と$1/EC_{50}$の間に良い一致を示した($p < 0.01$)．これらの結果はカチオン性界面活性剤が細胞膜への障害を与え，脂肪族炭化水素鎖の炭素数の伸長に伴い細胞膜への負の影響が増加することが示唆された．cell viabilityに対する異なるEO鎖を持つPOE.NPEおよびPOE.LEの影響はそれらのhydrophobic/lipophilic balanceに依存していた．EO鎖の異なるPOE.NPEおよびPOE.LEでCHO細胞および赤血球を処理した時，$1/IC_{50}$および$1/EC_{50}$あるいは1/IC50およびMPあるいはSAのflux(%)の間に良い一致が見られた ($p < 0.05$)．これらの結果から，nonylphenyl基あるいはlauryl基のようなPOE.NPEあるいはPOE.LEの脂溶性の部分は生体膜に同じ様な影響を与えることが分かった．

Keywords: cell viability, excised abdominal skin, permeation

Uchino, T.,Tokunaga, H. and Ando, M.: **ProstaglandinE_2 Release, Squalene Monohydroperoxide Production and Cell Toxicity of Skin^{2TM} ZK1301 as a Human Skin Model in the Presence of Haematoporphylin and Ultraviolet-A Irradiation**

Toxicol. in Vitro, **13**, 483-489(1999)

For the clarification of the mechanism of ultraviolet-A (UVA)-induced cytotoxicity to skin, the prostaglandinE_2 (PGE_2) release and squalene monohydroperoxide (SQOOH) production in a human skin model (Skin^{2TM} ZK1301) in the presence of haematoporphylin (HP) as a photosensitizer were investigated. The PGE_2 release and SQOOH production were significantly increased depending on both the irradiation time of UVA (350-380 nm) and the HP concentration. In addition, concentration-dependent inhibitions of cytotoxicity, PGE_2 release and SQOOH production were observed when a UVA sunscreen was applied to the surface of Skin^{2TM}. These results suggest that the mediator of inflammation and lipid hydroperoxide production induced by UVA-HP photosensitization cause cytotoxicity to skin, and that the test method of UVA-HP photosensitization using the Skin^{2TM} ZK1301 model is a useful in vitro model for studying UVA phototoxicity and for UVA sunscreen evaluations.

Keywords: UVA, cytotoxicity, Skin^{2TM}, haematoporphylin, PGE_2

Hanioka, N., Jinno, H., Chung, Y.-S., Tanaka-Kagawa, T., Nishimura, T. and Ando, M.: **Inhibition of rat hepatic cytochrome P450 activities by biodegradation products of 4-tert-octylphenol ethoxylate**

Xenobiotica, **29**, 873-883 (1999)

The effects of some biodegradation products of 4-*tert*-octylphenol ethoxylate (OPEO), namely 4-*tert*-octylphenol (OP), 4-*tert*-octylphenol diethoxylate (OP2EO) and 4-*tert*-octylphenol monocarboxylate (OP1EC) on the kinetics of cytochrome P450 (P450)-dependent monooxygenases in rat liver microsomes have been studied. Testosterone 16β-hydroxylase (TS16BH), testosterone 2α-hydroxylase (TS2AH) and testosterone 6β-hydroxylase (TS6BH) activities were extensively inhibited by OP at 100 μM (56.0-90.3%). Inhibition was competitive for all P450-dependent monooxygenases. The K_is of TS16BH, TS2AH and TS6BH from Lineweaver-Burk plots were 6.37, 3.38 and 34.8 μM respectively. The activities of acetanilide 4-hydroxylase (AA4H), 7-ethoxycoumarin *O*-deethylase (ECOD) and bufuralol 1'-hydroxylase (BF1'H) were also effectively inhibited by OP at 100 μM (48.6-56.0%). The inhibition of these P450-dependent monooxygenases was non-competitive, and K_is (50.1-63.9 μM) were higher than those of TS16BH, TS2AH and TS6BH. OP2EO also inhibited AA4H, ECOD, TS16BH, TS2AH, BF1'H and TS6BH activities by 38.7-69.3% at 100 μM, although the inhibition rates were slightly lower than those for OP. K_is were 14.4-106 μM, and the inhibition was of mixed type (AA4H and ECOD), competitive (TS16BH, TS2AH and TS6BH) and non-competitive (BF1'H). Testosterone 7α-hydroxylase (TS7AH), 4-nitrophenol 2-hydroxylase (4NP2H) and lauric acid ω-hydroxylase (LAOH) activities were only slightly affected by OP and OP2EO. The ability of OP1EC to inhibit P450-dependent monooxygenase activities was generally weaker than that of OP and of OP2EO: the K_i > 200 μM. These results suggest that OPEO biodegradation products interact with constitutive P450 isoforms, CYP1A2, CYP2A2, CYP2B2, CYP2C11 and CYP3A2 in rat liver *in vitro* (OP > OP2EO > OP1EC), and that the mechanism of this interaction differs depending on the compound and P450 isoform.

Keywords: 4-tert-Octylphenol ethoxylate; Cytochrome P450; Inhibition

Hanioka, N., Jinno, H., Tanaka-Kagawa, T., Nishimura, T. and Ando, M.: ***In vitro* metabolism of simazine, atrazine and propazine by hepatic cytochrome P450 enzymes of rat, mouse and guinea pig, and oestrogenic activity of chlorotriazines and their main metabolites**

Xenobiotica, **29**, 1213-1226 (1999)

The *in vitro* metabolism of chlorotriazines, simazine (SIZ), atrazine (ATZ) and propazine (PRZ) in liver microsomes from rat, mouse and guinea pig and the oestrogenic activity of chlorotriazines and their main metabolites have been studied. The formation rates of products in chlorotriazine metabolism were determined by HPLC. The principal reactions catalysed by the cytochrome P450 (P450) system were *N*-monodealkylation and isopropylhydroxylation in all liver microsomes. As a result, 2-chloro-4-ethylamino-6-amino-1,3,5-triazine (M1) (SIZ-M1 for SIZ and ATZ-M1 for ATZ) and 2-chloro-4-amino-6-isopropylamino-1,3,5-triazine (M2) (ATZ-M2 for ATZ and PRZ-M2 for PRZ), and 2-chloro-4-ethylamino-6-(1-hydroxyisopropylamino)-1,3,5-triazine (M3) (ATZ-M3 for ATZ) and 2-chloro-4-isopropylamino-6-(1-hydroxyisopropylamino)-1,3,5-triazine (M4) (PRZ-M4 for PRZ) were detected as the metabolites. N-bidealkylation was not found in this system. The formation rates of *N*-deethylated metabolites (SIZ-M1 and ATZ-M2) were generally higher in mouse than in rat and guinea pig. The formation rates of *N*-deisopropylated metabolites (ATZ-M1 and PRZ-M2) in guinea pig were the lowest among the three animal species. The formation rates of isopropylhydroxylated metabolites (ATZ-M3 and PRZ-M4) were remarkably low in mouse compared with rat and guinea pig. The enzyme kinetics of chlorotriazine metabolism were examined by Eadie-Hofstee analyses. Some species differ-

ences in Michaelis-Menten parameters for each metabolite were observed, and the ranking orders were varied among the metabolites. The binding affinity of chlorotriazines (SIZ, ATZ and PRZ) and their metabolites (M1-4) for recombinant human estrogen receptor-α was assayed using the fluorescence polarization method. The binding affinity of M2 was significantly higher than those of parent compounds and other metabolites, although the oestrogenic activity was remarkably low compared with that of 17β-oestradiol (E2). These results suggest that the pattern of metabolism of SIZ, ATZ and PRZ by the P450 system differs extensively among rat, mouse and guinea pig, and that M2 may be an activated metabolite of chlorotriazines.
Keywords: Chlorotriazine; Cytochrome P450; Oestrogenic activity

Hanioka, N., Jinno, H., Chung, Y.-S., Nishimura, T., Tanaka-Kagawa, T. and Ando, M.: **Effect of 4-tert-octylphenol on cytochrome P450 enzymes in rat liver**
Arch. Toxicol., **73**, 625-631 (2000)
The effects were studied of 4-tert-octylphenol (OP) on hepatic cytochrome P450 enzymes in rats. Rats were treated intraperitoneally with OP twice, at doses of 5, 10 and 20 mg/kg. Among the cytochrome P450-dependent monooxygenase activities, testosterone 2α-hydroxylase activity, which is associated with CYP2C11, was significantly decreased by OP at all doses. The level relative to control activity was 67-22%. CYP3A2-dependent monooxygenase, testosterone 6β-hydroxylase activity was also decreased by 51% by OP at 20 mg/kg. Furthermore, immunoblotting showed that OP (10 or 20 mg/kg) significantly decreased CYP2C11/6 and CYP3A2/1 protein levels. However, the reduction ratio of CYP2C11/6 and CYP3A2/1 protein levels by OP treatment was lower than that of testosterone 2α-hydroxylase and testosterone 6β-hydroxylase activities. The Cl_{int} (V_{max}/K_m) value for testosterone 2α-hydroxylase was significantly decreased by OP at all doses, whereas the Clint value for testosterone 6β-hydroxylase was only decreased by OP at 20 mg/kg. In addition, 7-ethoxycoumarin *O*-deethylase activity was significantly decreased by 32% by the highest dose of OP. By contrast, CYP1A1-, CYP1A2-, CYP2A1-, CYP2B1/2-, CYP2D1-, CYP2E1- and CYP4A1/2/3- dependent monooxygenase activities were not affected by OP at any dose. These results suggest that OP changes the male-specific cytochrome P450 isoforms in rat liver, and that these changes closely relate to the toxicity of OP.
Keywords: 4-tert-Octylphenol; Cytochrome P450; Suppression

Hanioka, N., Jinno, H., Tanaka-Kagawa, T., Nishimura, T. and Ando, M.: **Interaction of bisphenol A with rat hepatic cytochrome P450 enzymes**
Chemosphere, **41**, 973-978 (2000)
The effect of bisphenol A (BPA) on the kinetics of cytochrome P450 (P450)-dependent monooxygenases in rat liver microsomes was studied. Testosterone 16b-hydroxylase (TS16BH) and testosterone 2a-hydroxylase (TS2AH) activities were extensively inhibited by BPA at 100 mM (69 and 74%, respectively). The inhibition type was mixed for both P450-dependent monooxygenases. The K_i of TS16BH and TS2AH from Lineweaver-Burk plots was 25.9 and 24.9 μM, respectively. The activities of acetanilide 4-hydroxylase (AA4H), 7-ethoxycoumarin O-deethylase (ECOD), bufuralol 1'-hydroxylase (BF1'H), chlorzoxazone 6-hydroxylase (CZ6H) and testosterone 6β-hydroxylase (TS6BH) were also effectively inhibited by BPA at 100 μM (43-52%). The inhibition type of these P450-dependent monooxygenases was mixed or uncompetitive, and the K_is (50.5-88.5 mM) were higher than those of TS16BH and TS2AH. By contrast, the values of IC50 and Ki of testosterone 7α-hydroxylase (TS7AH) and lauric acid ω-hydroxylase (LAOH) for BPA were > 1000 μM. These results suggest that BPA interacts with rat hepatic CYP1A2, CYP2A2, CYP2B2, CYP2C11, CYP2D1, CYP2E1 and CYP3A2 *in vitro*.
Keywords: Bisphenol A; Cytochrome P450; Interaction

佐々木久美子，高附　巧，根本　了，今中雅章*1，衛藤修一*2，村上恵美子*2，豊田正武：**食品中のアルキルフェノール及び2,4-ジクロロフェノールの分析**
食品衛生学雑誌，**40**, 460-472 (1999)
11種のアルキルフェノール及び2,4-ジクロロフェノールをＧＣ/ＭＳで同時測定する系を確立し，米，野菜，果実，魚介類，畜肉類，乳製品及び缶詰について汚染実態調査を実施した．
その結果，魚介類，肉類及び野菜・果実から4-ノニルフェノールがそれぞれ10～723 ng/g，9～180 ng/g及び7～131 ng/g検出された．また，一部の野菜から2,4-ジクロロフェノールが2～17 ng/g検出された．
Keywords:alkylphenol, 2,4-dichlorophenol, nonylphenol
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高附　巧，根本　了，松田りえ子，佐々木久美子，豊田正武：**フォトダイオードアレイ検出HPLCによる農作物中の21農薬の一斉分析**
食品衛生学雑誌，**40**，314-319(1999)
フォトダイオードアレイ検出HPLCによる農作物中の21農薬の一斉分析法を確立した．試料をアセトニトリル抽出し，ゲル浸透クロマトグラフィーで精製後，シリカゲルミニカラムで4画分に分けて農薬を溶出し，フォトダイオードアレイ検出HPLCで定性及び定量を行った．6作物に各農薬を0.4 μg/g添加したときの回収率はキンクロラック及びバナナのベンタゾンを除いて，平均62.1～116.7％，標準偏差0.1～17.9％であった．オレンジでは，妨害が多いため4農薬の測定ができなかった．本法の検出限界は0.005～0.02ppmであった．
Keywords: pesticide residue, HPLC

松田りえ子，林譲，四方田千佳子，田頭洋子，勝峰万里*1，岩木和夫*1：**クロマトグラフィー分析における適切なデータ取り込み間隔とデータ処理に関する考察**
分析化学，**49**，233-238　(2000)
Various kinds of digital processing methods are used for obtaining the highest possible amount of information from digital data. This paper treats the effects of some digital processings (boxcar, trimming, moving average method and exponential smoothing) on the precision (RSD) of measurements. The following rules of thumb concerning an A/D converter, multi-channel detector and smoothing are obtained: 1) The data collecting time of an A/D converter should be set at a large value near or equal to the sampling intervals of the A/D converter; 2) The smaller is the number of frequencies detected in the multi-channel detector, the higher is the precision of measurements; 3) The smoothing (moving average method and exponential smoothing) is effective for height measurement, and not for area measurement. The original data should be stored for another analysis.

Keywords: chromatography, precision; sampling intervals of data
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堀江正一*1, 城戸靖雅*2, 村山三徳, 豊田正武, 中澤裕之*3：**HPLCによる畜水産食品中のスピラマイシンI及び主代謝物ネオスピラマイシンIの定量**
食衛誌, **40**, 401-406 (1999)

A simple and rapid method using HPLC for the determination of spiramycin I (major and most important component) and its metabolite, neospiramycin I, in meat, fish and milk has been developed. Neospiramycin I was obtained by acidic treatment of spiramycin I. The drugs were extracted with 1.2% metaphosphoric acid-methanol (5:5), and the extracts were cleaned up on a Bond Elut SCX (500 mg) cartridge. The HPLC separation was performed on a Puresil 5C18 column (150 × 4.6 mm i.d.) using 0.05 mol/L phosphate buffer (pH 2.5)-acetonitrile (76:24) as the mobile phase at a flow rate of 0.5 mL/min. The drugs were detected at 235 nm. The calibration graphs were rectilinear from 2 to 50 ng for both drugs. The recoveries of the drugs from meat, milk and fish at the level of 0.2 μ g/g were 80.3?85.3%, and detection limits were 0.05 μ g/g for both drugs.
Keywords: macrolides, spiramycin, residual analysis
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Suzuki, T., Kondo, K., Uchiyama, M., Murayama, M.: **Some Sulfur-Containing Metabolites of Tri-n-butyltin Chloride in Male Rats**
J. Agric. Food Chem., **47**, 4791-4798 (1999)

In an attempt to elucidate metabolic destination of TBTO, sulfur-containing metabolites were investigated in the urine. Tri-n-butyltin chloride (TBTC), tri-n-butyltin oxide (TBTO), and their in vitro metabolites in rat liver microsomal enzyme systems, di-n-butyl(3-hydroxybutyl)tin chloride (T3OH), di-n-butyl(3-oxobutyl)tin chloride (T3CO), dibutyltin dichloride (DBTC), and monobutyltin trichloride (MBTC), were intraperitoneally administered to rats. In particular, administration of T3OH and T3CO gave higher amounts of mercapturic acid derivatives, such as *N*-acetyl-*S*-(3-oxobutyl)-*L*-cysteine (3CO-MA) and *N*-acetyl-*S*-(3-hydroxybutyl)-*L*-cysteine (3OH-MA), than TBTC or TBTO. On the other hand, DBTC and MBTC did not yield measurable amounts of 3CO-MA and/or 3OH-MA. The appearance of organotin metabolites in urine indicates that T3OH, T3CO, and hypothesized secondary metabolites, such as *n*-butyl(3-hydroxybutyl)(3-oxobutyl)tin chloride, *n*-butyl-(3-hydroxybutyl) (4-hydroxybutyl)tin chloride, etc., are subject to the action of glutathione *S*-transferase to give mercapturic acid derivatives. These sulfur-containing metabolites (3CO-MA and 3OH-MA) were also found in control rat urine.
Keywords: tributyltin, mercapturic acid, glutathione conjugate

Kawasaki, M.*1, Ono, T.*2, Murayama, M., Toyoda, M., Uchiyama, S.*1: **Determination of Thiabendazole and 5-Hydroxythiabendazole in Livestock Foods by HPLC-UV**
J. Food Hyg. Soc. Japan, **40**, 481-487 (1999)

Thiabendazole (TBZ) is a benzimidazole antibacterial agent used as an anthelminthic for livestock. 5-Hydroxythiabendazole (5-OH TBZ) is a metabolite formed by hydroxylation of TBZ in an animal's body. The recovery of 5-OH TBZ from animals by extraction in conventional TBZ analysis, which employs a strongly alkaline (pH 11.0) buffer, is as low as 50%. In order to establish a method for simultaneous analysis of TBZ and 5-OH TBZ with high recovery, we examined the optimum pH of a buffer for extraction, a suitable solvent for extraction, and a clean-up column. As a result, it was found that the optimum pH of the buffer was 9.0 and that suitable solvents were ethyl acetate (AcOEt) for pig muscle and liver, and MeCN for pig fat and cow's milk. Among the three types of solid phase extraction (SPE) clean-up columns investigated, the highest recovery for both chemicals was achieved by using an alumina N (ALN) column.
Keywords: thiabendazole, 5-hydroxythiabendazole, solid-phase extraction
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食衛誌, **41**, 149-153 (2000)

A biological method for determination of residual benzylpenicillin (PCG) in livestock products was evaluated. PCG was extracted from a sample with distilled water. The extract was deproteinized by adding tungstic acid, followed by a Bond Elut C18 cartridge clean-up procedure. The cylinder method was employed for quantitative determination, and microbioautography for identification of PCG. The average recovery at the maximum residue limits recommended by the Codex committee was 80.7% and the determination limits were 5 ppb (muscle, liver, kidney) and 1 ppb (milk).
Keywords: benzylpenicillin, microbioassay, microbioautography
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Kondo,K., Kurihara,M., Miyata,N., Suzuki,T. and Toyoda, M.: **Scavenging mechanisms of (-)-epigallocatechin gallate and (-)-epicatechin gallate on peroxyl radicals and formation of superoxide during the inhibitory action**
Free Radical Biol. Med. **27**, 855-863 (1999)

The scavenging effects of (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG) on peroxyl radicals and their mechanisms were studied by investigating the products formed during the first stages by 2,2'-azobis(2-aminopropane) hydrochloride (AAPH)-induced oxidation, without any isolation, using LC/MS, spectrophotometry, chemiluminescence analyses, and semiempirical molecular orbital (MO) calculations. The results show that EGCG can be converted to an anthocyanin-like compound followed by cleavage of the gallate moiety by oxidation. On the other hand, ECG can be converted to an anthocyanin-like compound after cleavage of the gallate moiety. The calculated C-H bond dissociation enthalpies (BDE's) for EGCG and ECG at the C-2 position were quite low (62.7 and 66.8 kcal/mol, respectively) compared to O-H BDE' s at the phenolic sites (ca. 70 kcal/mol), suggesting that the C-2 hydrogen can be abstracted by free radicals. The addition of superoxide dismutase (SOD) decreased the chemiluminescence in EGCG by one-half during the inhibitory action. Active oxygen including superoxide (O^{2-}) would be produced in EGCG, but not in ECG. The authors proposed the antioxidative mechanisms of EGCG and ECG depending on the experimental results and theoretical calculations.

Keywords: catechins, APCI-LC/MS, photodiode array, MO calculation, superoxide, chemiluminescence

Kondo, K., Kurihara, M., Fukuhara, K., Tanaka, T., Miyata, N., Suzuki, T. and Toyoda, M.: **Conversion of procyanidin B-type (catechin dimer) to A-type: Evidence for abstraction of C-2 hydrogen in catechin during radical oxidation.**
Tetrahedron Lett. **41**, 485-488 (2000)

Procyanidin B-1 and B-2 were converted to A-1 and A-2 by radical oxidation using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals under neutral conditions, respectively. Transformation of procyanidin B-type into A-type certainly shows abstraction of the hydrogen atom at the C-2 position during radical oxidation.
Keywords: polyphenols, antioxidant, radicals, bond dissociation enthalpy

Goda, Y., Hoshino, K.[*1], Akiyama, H., Ishikawa, T., Nakamura, T., Otsuka, H.[*2], Takeda, Y.[*3], Tanimura, A.[*1], Toyoda, M.: **Constituents in watercress: inhibitors of histamine release from RBL-2H3 cells induced by antigen stimulation**
Biol. Pharm. Bull., **47**, 1319-1326 (1999)

Histamine release inhibitors in watercress (*Nasturtium officinale*) were isolated using a monitoring system with antigen-stimulated RBL-2H3 cells. Of the 15 compounds isolated, flavonols and megastigmanes significantly inhibited histamine release. Two flavonols, 3-*O*-sophorosides of rhamnetin and rhamnazin, were new compounds. To investigate the inhibitory mechanism, the effects of rhamnethin, rhamethin 3-*O*-sophoroside and an isolated megastigmane glucoside on the increase in the intracellular free calcium concentration were examined at a concentration providing 60% inhibition of histamine release. The results suggest that these compounds did not affect the calcium influx at that concentration. The structure-activity relationships of the megastigmanes on histamine release were also investigated.
Keywords: *Nasturtium officinale*, histamine release inhibitor, flavonol

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Terahara, N.[*1], Kato, Y.[*2], Nakamura, M.[*2], Maitani, T., Yamaguchi, M.-a.[*1], Goda, Y.: **Six diacylated anthocyanins from storage roots of purple sweet potato, Ipomoea batatas**
Biosci. Biotech. Biochem., **63**, 1420-1424 (1999).

Eight acylated anthocyanins were isolated from the storage roots of the purple sweet potato, *Ipomoea batatas* cv Yamagawamurasaki, which is the source of the food colorant "purple sweet potato color." Of these, six pigments were identified as diacylated anthocyanins, cyanidin and peonidin 3-*O*-(6-*O*-(*E*)-caffeyl-2-*O*-(6-*O*-acyl-β-D-glucopyranosyl)-β-D-glucopyranoside)-5-*O*-β-D-glucopyranoside, in which each acyl substituent was a p-hydroxybenzoyl, (*E*)-caffeyl or (*E*)-ferulyl residue, mainly by NMR analyses.
Keywords: *Ipomoea batatas*, diacylated anthocyanin, purple sweet potato color

[*1] College of Horiculture, Minami-Kyushu University
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穐山浩，菊池裕，成田紀子，鈴木明子，合田幸広，高鳥浩介，一戸正勝*，豊田正武：**国産穀物等から分離された Fusarium moniliforme 及び F. proliferatum のフモニシン産生能及び新規フモニシン類の検出**
食衛試, **41**, 30～37 (2000)

国産農作物，ニュギニア産トウモロコシ等から単離したFusarium菌12株について，コーングリッツ上のフモニシン産生能を，ポストカラムHPLC法で調べたところ，12株中9株がフモニシンを産生することが判明した．次にフモニシン産生能が高い3株が産生するフモニシン類についてLC/MSを用い，詳細に解析した．その結果，国産玄米から単離した*Fusarium proliferatum*株の培養抽出液からフモニシンB1モノメチルエステル体が天然より初めて検出された．またグレープフルーツから単離した*Fusarium moniliforme*株の培養抽出液から新規フモニシンB1異性体が検出された．
Keywords: fumonisins, *Fusarium* spp, Japanese agricultural commodities

* 東京家政大学

穐山浩，奥貫晴代，都筑智子，荒見真一郎*，三浦裕仁*，佐久嶋順一郎，手島玲子，合田幸広，日野明寛*，澤田純一，豊田正武：**シキミ酸リン酸化酵素II発現系の構築とシキミ酸3－リン酸の調製**
食衛試, **40**, 438～443 (1999)

除草剤グリホサート耐性ダイズの5-enolpyruvylshikimic acid 3-phosphate synthaseの基質であるshikimic acid 3-phosphate (S-3-P)を得るために，shikimate kinase II (SK-II) 遺伝子を，エレクトロポレーション法で大腸菌BL21株に形質導入した．発現されたSK-IIの酵素活性は^{32}Pのシキミ酸への取り込み等で確認した．生成されたS-3-Pはカーボンカラムを用いたLC/MS等で同定した．カーボンカラムを用いるHPLCは不揮発性溶媒を用いず，S-3-Pを分離できるので，S-3-Pの簡便な調製が可能となった．
Keywords: shikimic acid 3-phosphate, shikimate kinase II, carbon column HPLC

* 農水省食品総合研究所

Akiyama, H., Hoshino, K.[*1], Tokuzumi, M.[*1], Teshima, R., Mori, H.[*2], Inakuma, T.[*2], Ishiguro, Y.[*2], Goda, Y., Sawada, J.-i., Toyoda. M.: **The Effect of feeding carrots on Immunoglobulin E production and anaphylactic response in mice**
Biol. Pharm. Bull. **22**, 551-555 (1999).

Carrot juice was administered orally to BALB/c mice immunized intraperitoneally with dinitrophenylated (DNP)-OVA for about 1 month. The titers of DNP-specific IgE, DNP-specific IgG, and the levels of total IgE in mouse sera were determined. The DNP-specific IgE production by mice fed carrot juice was significantly inhibited. On the other hand, the DNP-specific IgG production and the level of total IgE in mice fed carrot juice were not significantly different from those in control mice. We also examined the effect of feeding carrots on immediate-type hypersensitivity. One hour after antigen stimulation, the ears of mice fed carrots swelled less than those of control mice. Furthermore, the rise in serum histamine in the mice fed carrots under active systemic anaphylaxis was lower than in controls. We then examined the pattern of cytokine production by spleen cells from mice followed by restimulation with DNP-OVA *in vitro*. The spleen cells from the mice fed carrots produced more interferon-γ than those from the control group. In contrast, the spleen cells from

the mice fed carrots produced less interleukin-4 than those from the control group. Furthermore, the interleukin-12 production of the spleen cells from mice fed carrots was also higher than that of the control group. These findings suggest that feeding carrots improves the helper T cell (Th)1/Th2 balance, inhibiting specific IgE production and antigen-induced anaphylactic response.

Keywords: anti-allergic activity, carrot, Immunoglobulin E

*1 Showa Women's University
*2 Research Institute, Kagome Co., Ltd.

Kusaka, T.*1, Matsufuji, H.*1, Chino, M.*1, Kato, Y.*2, Nakamura, M.*2, Goda, Y., Toyoda, M., Takeda, M.*1: **Isolation, identification, and determination of a magenta subsidiary color in Food Blue No.1 (Brilliant Blue FCF)**

Food Additives and Contaminants, **16**, 501-507 (1999).

A magenta subsidiary colour was isolated from commercial Food Glue No. 1 (B-1; brilliant Blue FCF). The absorption maximum for this subsidiary colour at 580 nm is outside of the range of 614-628 nm found for other subsidiary colours and m,m-B-1. On the basis of MS and NMR analyses, the structure of the subsidiary colour was elucidated as the disodium salt of 2-[[4-[*N*-ethyl-*N*-(3-sulfonatophenylmethyl) amino]phenyl] [4-oxo-2,5-cyclohexadienylidene]methyl]benzenesulfonic acid. HPLC analyses revealed that 24 batches of commiercial Food blue No. 1 (three manufactures) contain 0.1-0.8% (average: 0.5%) of the magenta subsidiary colour.

Keywords: Food Blue No. 1, Brilliant Blue FCF, subsidiary colour

*1 College of Bioresource Science, Nihon University
*2 San-Ei Gen FFI. Inc.

神蔵美枝子，義平邦利，合田幸広：**食用赤色104号中の副成色素の構造と定量**

食衛誌，**40**，455-459 (1999)

食用赤色104号（R104）中の2種の副成色素（P1，P2）を単離し，各種機器分析を用い構造決定を行った．その結果，P1は，R104のキサンテン部の2位，7位の臭素が脱離した4',5'-ジブロモ-4,5,6,7-テトラクロロ-3',6'-ジオキシドスピロ［イソベンゾフラン-1（3H），9'-［9H］キサンテン］-3-オン，P2は，7位の臭素が脱離した2',4',5'-トリブロモ-4,5,6,7-テトラクロロ-3',6'-ジオキシドスピロ［イソベンゾフラン-1（3H），9'-［9H］キサンテン］-3-オンであることが判明した．さらに，HPLCを用い市販R104（4社9試料）中の混在量を調べた．その結果，全ての試料でP2が検出され，0.08-5.21%の存在であった．他方，P1は，5試料で検出されず，最大検出値は，0.06%であった．

Keywords: food red No. 104, subsidiary color, xanthene color

Kojima, T.*, Akiyama, H., Sasai, M.*, Taniuchi, S.*, Goda, Y., Toyoda, T., Kobayashi, Y.*: **Anti-allergic effect of apple polyphenol on patients with atopic dermatitis: A pilot study**

Allergology International, **49**, 69-73 (2000)

The aim of the present study was to evaluate the anti-allergic effect of apple condensed tannins (ACT) in patients with atopic dermatitis (AD) as a pilot study. An ACT supplement given to patients at oral doses of 10 mg/kg per day for 8 weeks reduced the inflammation, lichenification, cracking, itching, sleep disturbance and peripheral blood eosinophil counts. Itching and sleep disturbance score after ACT supplement even for 2 weeks were significantly decreased compared with control group. The results suggest that ACT has an anti-allergic effect and that its use improved the symptoms AD.

Keywords: anti-allergic effect, apple polyphenol, atopic dermatitis

* Department of Pediatrics, Kansai Medical University

Matsuoka, T.*, Kawashima, Y.*, Akiyama, H., Miura, H.*, Goda, Y., Kusakabe, Y.*, Sebata, T*., Isshiki, K.*, Toyoda, M., Hino, A.*: **A method of detecting recombinant DNA from genetically modified maize**

J. Food Hyg. Soc. Japan, **41**, 137-143 (2000)

A method using polymerase chain reaction (PCR) was designed for the detection of genetically modified maize (GM-maize). There are four lines of GM-maize imported from the United States, and the presence of recombinant deoxyribonucleic acid (DNA) in the maize could be detected with four pairs of specific oligonucleotide primers designed from the sequences of the newly introduced genes. The maize zein gene was also detected as an internal control. This method allows specific detection of each of Bt11, Event176, MON810 and LIBERTY by using pairs of specific primers designed to amplify the segment including part of the intrinsic maize. The detection sensitivity was about 0.05% for Event176, MON810 and LIBERTY, and about 0.01% for Bt11. To distinguish among three insect resistant GM-maize lines, we designed a multiplex PCR method. These three GM-maize lines were distinguishable on the basis of the expected length of their amplicons.

Keyword; genetically modified maize, recombinant DNA, PCR

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

宮原誠，長沢妙子*1，伊住慶子*2，北村真弓*2，豊田正武，斉藤行生：**新規レーザー蛍光検出HPLCによるガンマ線照射フェニルアラニン水溶液及び豚肉中のチロシンの分析**

食品照射，**34**，3-8（1999）

New analytical procedure for o-tyrosine was studied to investigate effects of gamma irradiation on aqueous phenylalanine solution and pork. The process includes extraction and hydrolysis of protein, derivatization of the free amino acid by fluoro-reagent, and finally separation and detection by LASER fluorometric HPLC. The detection limit was 25ng.

To study how the procedure works, irradiated phenylalanine solution and pork were analyzed. The samples were irradiated at doses up to 10kGy at room temperature.

Three tyrosine isomers were detected in phenylalanine solution, and 2 isomers (o-and p-tyrosine) were found in pork. Dose response was found in the formation of the isomers both in phenylalanine solution and in pork. O-tyrosine peak obtained from irradiated pork was separated from interference successfully. Those findings illustrate the procedure may be applicable to detection of irradiated food.

Keywords: gamma-irradiation, ortho-tyrosine, NBD-F, fluorometric, HPLC

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Miyahara, M., Ito, H.*1, Nagasawa, N.*2, Kariya, M.*2, Izumi, K.*3, Kitamura, M.*3, Toyoda, M., and Saito, Y.: **Determination of o-Tyrosine Production in Aqueous Solutions of Phenylalanine Irradiated with Gamma Ray, Using High Performance Liquid Chromatography with Automated Pre-column Derivatization and LASER**

Fluorometric Detection.

J.Health Sci., 46, 192-199 (2000).

Tyrosine isomers produced by gamma radiation of aqueous phenylalanine solutions at mid dose levels (1-10 kGy) were examined to obtain basic information for irradiated food detection using a new high performance liquid chromatography (HPLC) analytical procedure. The procedure was established using an automated pre-column derivatization with 4-fluoro-7-nitro 2,1,3-benzoxadiazole (NBD-F) followed by reverse phase HPLC and LASER fluorometric detection. The limit of detection (LOD) was 0.06 ng on-column and the linear range for calibration was 0.06 to 50 ng for the tyrosine derivatives. The relative standard deviation was 10% to 12%. The amounts of the tyrosine isomers increased with levels of irradiation. Irradiation at low temperature with reduced oxygen decreased the yields of tyrosine isomers. In the pH range of 5 to 7, the amount of product was not changed significantly by pH. Outside of the range, the pH did have an effect on product generation. At constant dose levels the yields of tyrosine isomers initially increased with phenylalanine concentration. However, with further increases in phenylalanine a reduction in the absolute amounts of tyrosine isomers was observed.

Dose rates varying from 0.5 kGy/h to 10 kGy/h had no significant effect on tyrosine isomer formation if a total of 10 kGy was used in each case. In addition, demonstrating the usefulness of this new analytical technique for o-tyrosine determination, these studies suggest that the presence of o-tyrosine as another parameter that is indicative of gamma irradiation.

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

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Toyoda, M., Uchibe, H.*1, Yanagi, T.*2, Kono, Y.*2, Hori. T.*3, and Iida, T.*3: **Decreased Daily Intake of PCDDs, PCDFs and Co-PCBs from Foods in Japan from 1977 to 1998**

J. Food Hyg. Soc. Japan, **40**, 494-499(1999)

Since 1977, pooled total diet samples (13 food group composites) from one district of Japan were analyzed for PCDDs, PCDFs and co-PCBs. Daily intakes of PCDDs+PCDFs decreased from 3.8 (4.7) pgTEQ/kg bw/day in 1977 to 0.92 (1.8) pgTEQ/kg bw/day in 1998, calculated at ND (not detected)=0 (and ND=half the detection limit). Over the same period, daily intake of co-PCBs decreased from 4.4 (4.7) pgTEQ/kg bw/day to 1.8 (2.1) pgTEQ/kg bw/day, calculated at ND=0 (and ND=half the detection limit). This amounts to a decrease of 76% for PCDDs+PCDFs and 59% for co-PCBs. In contrast, total dioxin intake decreased by approximately one-third over the same period. A strong correlation between decreasing dioxin intake and dioxin concentration in breast milk in the same district was found (r=0.969, total dioxin). These results show a downtrend in the dietary intake of dioxins in Japan.

Keywords: dioxin, daily intake, PCDDs, PCDFs, coplanar PCBs, total diet study

*1 Japan Food Research Laboratories, *2 Japan Food Research Laboratories Tama Laboratory, *3 Fukuoka Institute of Health and Environmental Sciences

Ishiwata, H., Sugita, T., Kawasaki, Y., Takeda, Y., Yamada, T., Nishijima, M.*1, and Fukasawa, Y.*2: **Estimation of preservative concentrations in food and their daily intake based on official inspection results in Japan in fiscal year 1996**

J. Food Hyg. Soc. Japan, **40**, 246-258 (1999)

1996年度の全国自治体の行政検査結果を基に，保存料（安息香酸，デヒドロ酢酸，*p*-ヒドロキシ安息香酸，プロピオン酸）の使用実態と摂取量を求めた．総検査件数は112,131検体であった．食品中の濃度は上記の順に使用基準の7.8，0.4，3.2，1.7，14.1％，摂取量は各々11.0，0.0474，1.06，5.43，26.0 mg/日/人で，ADIの4.4％以下であった．摂取量に対し関与率の高い食品は上記の添加物の順に，清涼飲料水（摂取量の82.7％），菓子（56.7％），醤油（58.9％），魚介製品（41.2％），魚肉練り製品（30.1％）であった．

Keywords: food additive, preservative, daily intake

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Ishiwata, H., Sugita, T., Kawasaki, Y., Takeda, Y., Yamada, T., Nishijima, M.*1, and Fukasawa, Y.*2: **Estimation of antifungal agent concentrations allowed as food additives in food and their daily intake based on official inspection results in Japan in fiscal year 1996**

J. Food Hyg. Soc. Japan, **40**, 407-416 (1999)

1996年度の全国自治体の行政検査結果を基に，防かび剤（ジフェニル，イマザリル，オルトフェニルフェノール，チアベンダゾール）の使用実態と摂取量を求めた．総検査件数は6,369検体であった．食品中の濃度は上記の順に使用基準の0.007，8.0，3.0，3.2％，摂取量は各々0.0395，11.1，8.79，26.2 μg/日/人で，それぞれADIの0.0016，0.89，0.090，0.52％であった．1994年度の同様の結果と比べ，ジフェニルの摂取量が約1/50であったことを除き，ほぼ同様であった．

Keywords: food additive, antifungal agent, daily intake

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Ishiwata, H., Sugita, T., Kawasaki, Y., Takeda, Y., Yamada, T., Nishijima, M.*1, and Fukasawa, Y.*2: **Estimation of inorganic food additive (nitrite, nitrate, and sulfur dioxide) concentrations in foods and their daily intake based on official inspection results in Japan in fiscal year 1996**

J. Food Hyg. Soc. Japan, **41**, 79-85 (2000)

1996年度の全国自治体の行政検査結果を基に，無機食品添加物（亜硝酸塩，硝酸塩，二酸化硫黄）の使用実態と摂取量を求めた．総検査件数は15,569検体であった．食品中の濃度は上記の順に使用基準の17.2，30.9，14.4％であった．摂取量は各々1.07，1.43，1.45 mg/日/人で，それぞれADIの35.7，0.8，4.1％であった．使用基準の定められている食品のうち，摂取量に対し関与率の高い食品は，硝酸塩，亜硝酸塩では食肉製品（各々摂取量の11.6％，43.1％），二酸化硫黄ではかんぴょう（23.0％）であった．

Keywords: food additive, inorganic salt, daily intake

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*2 山梨県衛生公害研究所

Ishiwata, H., Fukushima, A., Abe, Y., Yamada, T., Nishijima, M.*1, and Fukasawa, Y.*2: **Estimation of BHA, BHT, propylene glycol, and sodium saccharin concentrations in foods**

and their daily intake based on official inspection results in Japan in fiscal year 1996

J. Food Hyg. Soc. Japan, **41**, 86-93 (2000)

1996年度の全国自治体の行政検査結果を基に，BHA，BHT，プロピレングリコール，サッカリンナトリウムの使用実態と摂取量を求めた．総検査件数は28,428検体であった．食品中の濃度は上記の順に使用基準の3.9，0.7，16.8，5.7％であった．摂取量は各々0.105, 0.220, 41.4, 7.64 mg/日/人で，それぞれADIの0.4, 1.5, 3.3, 3.1％であった．摂取量に対し関与率の高い食品は，上記の順に，魚介乾製品（摂取量の75.9％），菓子（57.0％），生麺（70.3％），清涼飲料水（33.0％）であった．

Keywords: food additive, concentration, daily intake

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山田真記子*1，加藤喜昭*1，中村幹雄*1，神谷恒夫*2，関川富士雄*3，米谷民雄：**二酸化チタンの定量法に関する研究**

日本食品化学学会誌，**6**, 34-37 (1999)

食品添加物二酸化チタンの定量法について検討した．第六版食品添加物公定書に収載されている重量法では，規格の上限値100.5%を越える製品があった．一方，アルミニウム還元法を用いた滴定法は，指示薬法及び電位差滴定法とも良好な結果を示し，従来法で上限値を超えた製品でも，規格内におさまった．特に，アルミニウム還元法（指示薬法）は簡便かつ迅速な方法であり，食品添加物二酸化チタンの定量法として最適と考えられた．

Keywords: titanium dioxide, aluminum-reduction, gravimetric method

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*2 東邦チタニウム株式会社
*3 フロイント産業株式会社

Nagaoka, M., Yamada, T., and Maitani, T.: **High resolution ICP-MS connected directly with HPLC for the study of metal-transferrin binding in aluminum and iron.**

Metal Ions in Biology and Medicine, 6, 386-388 (2000)

HPLC connected directly with high-resolution ICP-MS (HPLC/HR-ICP-MS) was applied to study the chemical states of Al and Fe bound to human serum transferrin in vitro and in vivo. HPLC/HR-ICP-MS chromatograms detected by ^{27}Al and ^{56}Fe levels were obtained without polyatomic isotope interference. This method seems effective for investigating the binding of metal and biological materials from the metal side.

Keywords: ICP-MS, aluminum, iron

Kubota, H., Sato, K., Yamada, T. and Maitani, T.: **Phytochelatin homologs induced in hairy roots of horseradish**

Phytochemistry, **53**, 239-245 (2000)

When exposed to excess heavy metals, plants induce phytochelatins and related peptides (all designated as PCAs). Thus, when hairy roots of horseradish (*Armoracia rusticana*) were exposed for 3 days to cadmium (1 mM) along with reduced glutathione (2 mM), PCA induction occurred. Moreover, a new family of thiol peptides was detected as well as the previously known PCAs, as revealed by postcolumn-derivatization HPLC. Two were isolated and their structures were identified as $(\gamma\text{-Glu-Cys})_n\text{-Gln}$ (n = 3 and 4) by electrospray ionization-mass spectrometer spectra, this being confirmed by chemical synthesis of the peptides. These new analogs constitute the sixth PCA family identified to date.

Keywords: phytochelatin, cadmium, horseradish

Sato, K., Sasaki, S. S., Goda, Y., Yamada, T., Nunomura, O.*1, Ishikawa, K.*2 and Maitani, T.: **Direct connection of supercritical fluid extraction and supercritical fluid chromatography as a rapid quantitative methods for capsaicinoids in placentas of Capcicum**

J. Agric. Food Chem., 47, 4665-4668 (1999)

The pungent components of Capsicum annuum, which are irritants, are called capsaicinoids (CAPS). The principal components are capsaicin and dihydrocapsaicin. To analyze CAPS in the placentas of Capsicum fruits rapidly and safely, we used a directly connected system of supercritical fluid extraction and supercritical fluid chromatography (SFE/SFC). The CAPS contents in placentas of C. annuum determined by the SFE/SFC method agreed well with those in the range of 0 - 13.8 mg/g fr. wt determined by the usual extraction-HPLC method. The SFE/SFC method has the advantages of no need for pretreatment and no (or minimal) need for organic solvents.

Keywords: capsicum, capsaicinoid, SFE/SFC

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*2 千葉大学園芸学部

佐藤恭子，杉本直樹，山田 隆，米谷民雄：**天然着色料中のアスタキサンチンの光学異性及びファフィア色素の主色素成分に関する研究**

食衛誌，**41**, 44-47 (2000)

天然着色料中のアスタキサンチン(AST)の化学形について検討した．まず，天然着色料ファフィア色素の主色素成分をHPLC及びLC/APCI-MSにより調べた結果，既存添加物名簿収載品目リストの記載通り，主色素成分は非エステル化体ASTであった．ついで，ファフィア色素，ヘマトコッカス藻色素及びオキアミ色素中のASTの光学異性について光学活性カラムを用いたHPLCにより検討した結果，ファフィア色素のASTは，(3*R*,3'*R*)，けん化したヘマトコッカス藻色素及びけん化したオキアミ色素のAST部分は，それぞれ(3*S*, 3'*S*)及び(3*R*,3'*R*)であった．

Keywords: phaffia color, astaxanthin, optical isomerism

佐藤恭子，坂元（佐々木）史歩，米谷民雄，山田 隆：**エビ中のコウジ酸の残存量に対する調理等の影響**

食衛誌，**41**, 122-125 (2000)

既存添加物のコウジ酸が甲状腺腫瘍を引き起こすことが報告されている．そこで，コウジ酸製剤を食品に使用した場合の残存量に寄与すると考えられる種々の要因，浸漬時間，洗浄，保存，煮沸等の影響について，殻付きの甘エビを用いて検討した．その結果，冷凍，解凍の操作により，殻及び身中のコウジ酸は減少し，解凍時の水分の流出とともに溶出するものと考えられた．また，殻ごと洗浄した場合は，身中のコウジ酸含量は洗浄しない場合と変わらず，洗浄では溶出しないと考えられた．冷蔵では，冷凍に比べ，身中の残存量が多かった．さらに，煮沸により，殻及び身中のコウジ酸の8～9割が溶出すると考えられた．

Keywords: kojic acid, northern shrimp, cooking

秋山卓美，小嶋裕美，米谷民雄：**天然着色料イカスミ色素の試験法に関する研究**

日本食品化学学会誌，**6**, 88-92 (1999)

天然着色料イカスミ色素の確認試験法，色素含量を色価

で表現する方法，及びたんぱく質含量について検討した．確認試験法については，塩基性条件下での過酸化水素によるユーメラニンの分解反応を利用した方法について検討した．試料量として5 mg，観察時間として反応開始後5分が適当と考えられた．イカスミ色素の色価測定法としては，イカスミ色素を過酸化水素と加熱下反応させた時の350 nmにおける吸光度から色価をもとめる方法を検討した．吸光度の相対標準偏差は約2％であり，また吸光度0～0.85の範囲で試料量と吸光度の間に良好な直線性が得られた．たんぱく質含量については，イカスミ色素を塩酸で加水分解した後，遊離したアミノ酸をHPLCで分析し，その定量値から推定したところ，全体の10%程度であると推定された．

Keywords: Sepia color, color value, protein content

Akiyama, T., Washino, T.*, Yamada, T., Koda, T.* and Maitani, T.: **Constituents of enzymatically modified isoquercitrin and enzymatically modified rutin (extract)**
J. Food Hyg. Soc. Japan, **41**, 54-60 (2000).

Enzymatically modified isoquercitrin and enzymatically modified rutin (extract) were analyzed to determine the structures and contents of their constituents. NMR analysis revealed that the 4-hydroxyl group of glucose was glucosylated in the manufacture of enzymatically modified isoquercitrin. LC/MS analysis elucidated the number of additional glucose moieties of each constituent. It was suggested that two additional enzymes, as well as cyclodextrin glucanotransferase, play roles in the manufacture of one sample. HPLC was employed to evaluate the contents of quercetin glycosides, which should determine solubility and antioxidative activity.

Keywords: enzymatically modified isoquercitrin, enzymatically modified rutin (extract), LC/MS

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Sugimoto, N., Kiuchi, F.*1, Mikage, M.*1, Mori, M.*2, Mizukami, H.*2, Tsuda, Y.*1: **DNA profiling of Acorus calamus chemotype differing in essential oil composition**
Biol. Pharm. Bull., **22**, 481-485 (1999).

The phylogenetic relationship of *Acorus gramineus* (**AG**) and three types of *A. calamus* (**AC**) was analyzed by comparing the 700 bp sequence of a 5S-rRNA gene spacer region. Although there was no intra-specific variation in the essential oil profile of **AG** which contained *Z*-asarone, **AC** could be classified into two chemotypes: chemotype A in which *Z*-asarone is a major constituent and chemotype B which contained sesquiterpenoids predominantly. An intermediate type (M) of these two chemotypes in various ratios was also observable. The NJ tree constructed based on the sequences revealed that **AG** was clearly distinguished from any of the chemotypes of **AC** and that the phylogenetic relationship predicted by the spacer region data correlated well with the chemotypes of **AC**.

Keywords: molecular phylogeny, 5S-rRNA gene, *Acorus calamus*

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河村葉子，互井千恵子，前原玉枝，山田 隆：**ポリ塩化ビニル中の添加剤の一斉分析法**
食衛誌，**40**, 189-197 (1999)

既報のポリエチレン製品中の添加剤一斉分析法の，ポリ塩化ビニルへの適用を検討した．試料はシクロヘキサン－2-プロパノール混液に37℃で一晩浸漬して抽出し，濃縮によりオリゴマーを除去し，HPLC及びGC/MSにより測定した．これまで対象としていた酸化防止剤，紫外線吸収剤，滑剤50種類のほか，フタル酸エステル類，アジピン酸エステル類などの可塑剤やノニルフェノール，ビスフェノールAを含む39種類の添加剤も分析可能であった．複数のピークをもつ化合物については，ピークの形状に応じて定量した．添加回収率は，酸化防止剤等で53.2～118.0％，可塑剤で69.3～119.5％とほぼ良好であった．また，定量限界はGC/MSで確認可能な50～500μg/gとした．

Keywords: polyvinyl chloride, additives, simultaneous determination method

河村葉子，互井千恵子，前原玉枝，山田 隆：**ポリ塩化ビニル及びポリ塩化ビニリデン製品中の残存添加剤**
食衛誌，**40**, 274-284 (1999)

ポリ塩化ビニル(PVC)及びポリ塩化ビニリデン(PVDC)製品について，添加剤89種類の残存量を調べた．PVC製ラップフィルムの可塑剤残存量は14～38％で，アジピン酸ジイソノニル等のアジピン酸エステル類が主であったが，容器類では残存添加剤は少なかった．手袋では可塑剤が34～55％に達し，全検体からフタル酸ジ(2-エチルヘキシル)とアジピン酸ジ(2-エチルヘキシル)が検出された．玩具類は可塑剤が8.7～45％で，フタル酸ジイソノニル及びフタル酸ジ(2-エチルヘキシル)が多用されていた．一方，PVDC製フィルム類は可塑剤が2.7～7.8％で，主にクエン酸アセチルトリブチル及びセバシン酸ジブチルが使用されていた．またPVC製ラップフィルム，手袋等からノニルフェノールが530～5,500μg/g検出された．

Keywords: polyvinyl chloride, wrapping film, gloves

河村葉子，米澤里香，前原玉枝，山田 隆：**食品用ポリプロピレン製品中の添加剤の分析**
食衛誌，**41**, 154-161 (2000)

既報のポリエチレン及びポリ塩化ビニル中の添加剤一斉分析法のポリプロピレン製品への適用を検討した．84種類の添加剤を分析対象とし，そのうち29種類の添加回収試験を行ったところ，回収率は63.1～114.1％とほぼ良好であった．食品用ポリプロピレン製器具・容器包装39検体中の残存添加剤を測定したところ，酸化防止剤のIrganox 1010が最も高頻度に検出され，次いでIrgafos 168であった．その他，酸化防止剤のBHT，滑剤のオレアミド，ステアミド，エルカミド，ステアリン酸なども高頻度に検出された．更に，界面活性剤のモノパルミチン，モノステアリンや可塑剤のDEHP，BBP，DINP等も検出された．また，滑剤として添加されたと推定される3種類の脂肪族炭化水素群が見いだされた．

Keywords: food contact polypropylene, additives, simultaneous determination method

栗原正明，田中正一*，今若直人*，末宗 洋*，宮田直樹：**α,α-ジ置換アミノ酸イソバリンおよびジエチルグリシンより構成されるホモペプチドの分子力学法によるコンフォメーション解析**
JCPE Journal, 11, 185-190 (1999)

α,α-ジ置換アミノ酸であるイソバリンおよびジエチルグリシンよりそれぞれ合成したホモペプチドの分子力学法によるコンフォメーションサーチを，MacroModel®のモンテカルロ法を用いて行った．力場としてAMBER*を用いた場合，両ペプチドとも最安定構造として3_{10}-ヘリックスを与

えた．これはX線構造解析の構造とよい一致を示した．また，力場としてMMFFを用いた場合に，ジエチルグリシンのホモペプチドの最安定構造はプラナーな構造を与えた．これは，溶液中での構造がプラナーであることと一致した．

Keywords: molecular mechanics, α,α-disubstituted amino acid, conformational analysis, homooligopeptides

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M. Kurihara, M. Tanaka*, N. Imawaka*, H. Suemune* and N. Miyata: **Molecular mechanics study on conformational analysis of homooligopeptides prepared from α,α-disubstituted amino acids.**

Peptide science 1999, 329-330 (2000)

イソバリン，ジエチルグリシン，エチルブチルグリシンより合成したホモペプチドの分子力学法によるコンフォメーションサーチを行った．力場としてAMBER*を用いた場合，3_{10}-ヘリックスを与えた．これは結晶中の構造とよい一致を示した．また，力場としてMMFFを用いた場合に，ジエチルグリシン，エチルブチルグリシンのホモペプチドの最安定構造はプラナーな構造を与えた．これは，溶液中での構造がプラナーであることと一致した．

Keywords: molecular mechanics, α,α-disubstituted amino acid, conformational analysis, homooligopeptides

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M. Tanaka*, N. Imawaka*, M. Kurihara, H. Suemune*: **Conformation of peptides prepared from α-ethylated α,α-disubstituted amino acids**

Peptide science 1999, 327-328 (2000)

エチル基を有するα,α–ジ置換アミノ酸ジエチルグリシン，エチルブチルグリシンのホモペプチドを合成し，X線構造解析，NMRによりコンフォメーション解析を行った．その結果，ジエチルグリシンのホモペプチドは結晶中では3_{10}-ヘリックス構造，溶液中ではプラナーな構造であった．エチルブチルグリシンのホモペプチドは結晶中，溶液中でいずれでもプラナーな構造であった．

Keywords: α,α-disubstituted amino acid, conformational analysis, homooligopeptides

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宮田直樹，山越葉子，末吉祥子，増水章季[*1]，河野雅弘[*1]，笠 明美[*2]，荒金久美[*2]，梅沢直樹[*3]，長野哲雄[*3]：**光励起フラーレンの生物作用発現に関与する酸化活性種の解析**

磁気共鳴と医学，**11**，9-12 (2000)

優れた光増感剤であるフラーレンは，光照射条件下種々の生物作用を示す．今回，生物作用発現に関与する活性種の解析研究を行った．一重項酸素やスーパーオキシド消去剤存在下でのDNA切断実験結果の解析，一重項酸素の発光による近赤外スペクトルの観測，スピントラップ剤を用いるEPRスペクトルの測定などにより，電子欠損型の光増感剤である[60]フラーレンは，光照射によりスーパーオキシドならびにヒドロキシルラジカルを発生することがわかった．これらの活性種が生物作用発現に関与していると考える．

Keywords: fullerene, C60, hydroxyl radical, superoxide, photosensitizer

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D.Desai [*1], J.Krzeminsky[*1], J.-M.Lin [*1], A.Chada [*2], N.Miyata, H.Yagi[*2], D.M.Jerina[*2], S.Amin[*1]: **Syntheses and identification of benzo[c]chrysene metabolites**

Polycyclic Aromatic Compounds, **16**, 255-264 (1999)

発ガン性ならびに変異原性を有することが知られているベンゾ[c]クリセン（B[c]C）は，構造的にbay領域とfjord領域を有することからその代謝活性化機構が興味深い．今回，予想代謝物を合成するとともに，それらを用いて代謝物の解析を行った．その結果，代謝物の一つ1,2-ジヒドロ体がB[c]Cよりもより強い活性を示し活性代謝物であることが明らかになった．

Keywords: benzo[c]chrysene, in vitro metabolism, mutagenicity, polycyclic hydrocarbon

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

Brain Res., **842**, 482-486 (1999)

We investigated the tissue distribution and brain localization of opioid-binding cell adhesion molecule (OBCAM) in the adult rats by immunoblotting and immunohistochemistry using a monoclonal anti-OBCAM peptide antibody that is specific for OBCAM. OBCAM was preferentially expressed in the central nervous system (CNS) and at a very low level in the spleen. Within the brain, OBCAM was distributed in almost all the gray matter, but little or no immunoreactive OBCAM was found in the white matter. Morphologically, distribution pattern of OBCAM immunoreactivity was very similar to that of synaptophysin, suggesting a role in the synaptic machinery.

Keywords: OBCAM, rat brain, immunostaining

Saito, Y., Takagi, K., Teshima, R., Ikebuchi, H., Yamazaki, T., Sawada, J.: **Role of ecto-kinase in phorbol ester-enhanced growth hormone-binding protein release from human IM-9 cells**

Mol. Cell. Endocrinol. **152**, 65-72 (1999)

The mechanisms of the phorbol ester-enhanced human growth hormone (hGH)-binding protein release were further investigated. hGH-BPs released by PDBu stimulation are derived from IM-9 cell surface hGH receptors and not generated within the cells. Protein kinase inhibitors with broad specificities, K-252a and K-252b, inhibited the PDBu-enhanced release with almost the same dose-dependency although only a trace amount of K-252b was incorporated into IM-9 cells than K-252a, suggesting that K-252b probably inhibits an ecto-kinase extracellularly. Taken together, these results suggest that, in addition to intracellular PKCa, activation of an undefined ecto-kinase may be also involved in the PDBu-enhanced hGH-BP release.

Keywords: IM-9,growth hormone-binding protein,ecto-kinase

Ihara, Y.*, Cohen-Doyle, M.F.*, Saito, Y., Williams, D.B.*: **Calnexin discriminates between protein conformational states and functions as a molecular chaperone *in vitro***

Mol. Cell, 4, 331-341 (1999)

Using purified components *in vitro*, calnexin effectively prevented the aggregation not only of glycoproteins bearing monoglucosylated oligosaccharides but also proteins lacking N-

glycans, an effect enhanced by ATP. It also suppressed the thermal denaturation of non-glycosylated proteins and enhanced their refolding in conjunction with other cellular components. Calnexin formed stable complexes with unfolded conformers of these proteins but not with the native molecules. Therefore, in addition to being a lectin, calnexin functions as a bona fide molecular chaperone capable of interacting with polypeptide segments of folding glycoproteins.

Keywords: calnexin, chaperone, folding

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Saito, Y., Ihara, Y.*, Leach, M.L.*, Cohen-Doyle, M.F.*, Williams, D.B.*: **Calreticulin functions in vitro as a molecular chaperone for both glycosylated and unglycosylated proteins**

EMBO J., **23**, 6718-6729 (1999)

Calreticulin (CRT) is thought to be a molecular chaperone that interacts with glycoproteins exclusively through a lectin site specific for monoglucosylated oligosaccharides. We show that CRT suppresses the aggregation not only of a glycoprotein bearing monoglucosylated oligosaccharides but also that of non-glycosylated proteins. CRT also confers protection against thermal inactivation and maintains substrates in a folding-competent state. We conclude that in addition to being a lectin CRT possesses a polypeptide binding capacity capable of discriminating between protein conformational states and that it functions in vitro as a classical molecular chaperone.

Keywords:calreticulin,molecular chaperone

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Takagi, K., Saito, Y., Nakajima, O., Sawada, J.: **Characterization of an antagonist monoclonal antibody, GHBP116, specific for human growth hormone receptors**

Biol. Pharm. Bull., **22**, 734-737 (1999)

To obtain an antagonist antibody against human growth hormone receptors (hGHRs), we prepared monoclonal antibodies against the recombinant hGHR extracellular domain. One of the clones, GHBP116, exhibited binding activity to intact human IM-9 cells and effectively immunoprecipitated the receptors in cell lysate. GHBP116 competitively inhibited 125I-human growth hormone (hGH) binding to the cells, ligand-induced receptor internalization, degradation, and phosphorylation of signal transducer and activator of transcription (STAT) 5 used as an indicator of JAK-STAT signaling. These results suggest that GHBP116 acts as a specific antagonist of hGH.

Keywords: growth hormone receptor, monoclonal antibody, immunoprecipitation

Suzuki, R.*1, Furuno, T.*1, McKay, D. M.*2, Wolvers, D.*2, Teshima, R., Nakanishi, M.*1, and Bienenstock, J.*2: **Direct neurite-Mast cell communication in vitro occurs via the neuropeptide substance P.**

J.Immunol., **163**, 2410-2415(1999)

We used an in vitro coculture approach comprising cultured murine superior cervical ganglia and rat leukemia basophilic-cells(RBL-2H3 cells). Following loading with the calcium fluorophore, Fluo-3, neurite-RBL units were examined by confocal laser scanning microscopy. Addition of bradykinin, or scopion venom, dose-dependently elicited neurite activation and, after a lag period, RBL Ca2+ mobilization. Addition of a neutralizing substance P Ab or a neurokinin (NK)-1 receptor antagonist dose-dependently prevented the RBL activation that resulted as a onsequence of neural activation by either bradykinin or scopion venom.

Keywords: neurite, RBL-2H3 cells,substance P

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Teshima, R., Onose,J., Okunuki, H. and Sawada, J.; **Effect of Ca^{2+}-ATPase inhibitors on MCP-1 production from BMMC and the involvement of p38 MAP kinase activation**

Int. Arch. Allergy Immunol.,**121**, 34-43(2000)

The effect of two Ca^{2+}-ATPase inhibitors, cyclopiazonic acid (CPA) and 2,5-di-(tert-butyl)-1,4-hydroquinone (DTBHQ), on the release of MCP-1 from BMMCs (bone- marrow-derived mast cells) was investigated. CPA and DTBHQ increased the intracellular free Ca^{2+} concentration ([Ca2+]i), and they induced MCP-1 release in a dose-dependent manner. These Ca^{2+}-ATPase inhibitors induced MCP-1 release in the absence of phorbol ester, in contrast to their induction of TNF-a. MCP-1 release reached a maximum at 6-9 hr. It was inhibited by treatment with actinomycin D and the immunosuppressant cyclosporine A. MCP-1 release was also dose-dependently inhibited by the p38 MAP kinase inhibitor SB202190. Therefore, transcriptional activation and its release seems to be dependent on NF-AT and p38 MAP kinase activation.

Keywords: Ca^{2+}-ATPase inhibitors, MCP-1, BMMC

Teshima, R., Onose, J., Saito Y., Ikebuchi H., Kitani S.* and Sawada J.; **Casein-kinase-II-like ectokinase activity on RBL-2H3 cells**

*Immunol.lett.***68**,369-374 (1999)

We studied the properties of the ectokinase activity on the outer cell-surfaces of RBL-2H3 cells and examined the phosphorylation of exogenous substrates to clarify the substrate specificity of the ectokinases on RBL-2H3 cells. Among the several protein substrates tested, casein was the most strongly phosphorylated with [γ-32P]ATP, and casein kinase II peptide was also phosphorylated. Phosphorylation of casein and casein kinase II peptide was also obserbed by [γ-32P]GTP. Western blot analysis using anti-casein kinase II antibody revealed a 44kDa casein kinase band in the membrane fraction and FceRI complexes. This is the first report about the existence of ectokinase on mast cells.

Keywords: ectokinase, casein kinase, RBL-2H3 cells

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Miyazaki, C.*1, Iba, N.*2, Yamada, Y.*1, Takahashi, H.*1, Sawada, J. and Kurosawa, Y.*2; **Changes in the specificity of antibodies by site-specific mutagenesis followed by random mutagenesis.**

Protein Engng.,**12**, 407-415, 1999

The specificity for 11-deoxycortisol (11-DOC) of a monoclonal antibody (mAb), designated SCET, was changed to specificity for cortisol (CS) by site-specific mutagenesis followed by random mutagenesis. The Fab form of SCET was expressed on the surface of a phage. During the first step, mutations were introduced at 14 amino acid positions in three complementarity-determining regions (CDRs) of the VH domain that seemed likely to form the steroid-binding pocket. A clone, DcC16, was isolated from the resultant library with multiple mutations and this clone was shown to have CS-binding activity but also to retain high 11-

DOC-binding activity. During the second step, mutations were introduced randomly into the entire VH-coding region of the DcC16 clone by an error-prone polymerase chain reaction, and CS-specific mutant antibodies were selected in the presence of 11-DOC as a competitor. Three representative clones were analyzed with the BIAcore instrument, and each revealed a large increase in the binding constant for CS and a decrease in that for 11-DOC. Structural models, constructed by computer simulation, indicated the probable molecular basis for these changes in specificity.

Keywords: monoclonal antibody, steroid, mutagenesis

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Nagaishi, K., Adachi, R., Matsui, S., Yamaguchi, T., Kasahara, T.*, Suzuki, K.: **Herbimycin A inhibits both dephosphorylation and translocation of cofilin induced by opsonized zymosan in macrophage-like U937 Cells**

J. Cell. Physiol., **180**, 345-354 (1999)

We have reported that a 21kDa phosphoprotein may play an important role in superoxide production by neutrophil-like differentiated HL-60 cells through dephosphorylation. The phosphoprotein was identified as cofilin, an actin-binding protein, and activation-induced changes in its intracellular distribution have been described. However, the physiological roles of cofilin in the phagocytes remain to be established and the regulatory mechanisms for dephosphorylation and translocation of cofilin are unknown. In this study we investigated the roles of cofilin in the opsonized zymosan (OZ)-activated macrophage-like U937 cells using herbimycin A, an inhibitor for protein tyrosin kinase. In the individual adherent phagocytes OZ induced various events: (1) production of superoxide, (2) phagocytosis of the insoluble particles OZ, (3) dephosphorylation of cofilin, (4) translocation of cofilin from cytosol to plasma membrane regions, (5) decrease in intracellular pH from 7.4 to about 6.8, and (6) rapid and transient increase in filamentous actin (F-actin) at the cell periphery. All of these events were inhibited or reduced significantly by herbimycin A. OZ increased phosphorylation of tyrosine of 110, 50, 34, and 29kDa proteins and herbimycin A inhibited it. These results suggest that a tyrosine kinase plays an essential role at upstream of these events through phosphorylation of such proteins. Furthermore, microinjection of anti-cofilin antibody to the differentiated U937 cells caused inhibition of the phagocytosis. These results suggest that cofilin plays critical roles in the phagocyte functions through changes in cytoskeletal organization.

Keywords: herbimycin A, cofilin, dephosphorylation

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Azumi S., & K. Tanamoto. **Anti-endotoxin properties of cinnamon bark-derived compound and its effect on the endotoxin shock model.**

J. Endotoxin Res., **5**, 109-117 (1999)

シナモン由来のエンドトキシン活性抑制物質について，活性中心の化学的性状の解明と，抗LPS活性について検討を行った．化学分析の結果から活性中心は脂質部分にあることがわかった．LPS分子と混合することによって抑制物質はLPSの種々の細胞からのTNF-α及びNO産生誘導，さらにはリムルス活性を抑制した．また，致死毒性及び発熱作用はそれぞれ1/1000及び1/100に減少した．一方致死量及び発熱量のLPSをマウスもしくはウサギに抑制物質と共に投与することにより大部分のマウスは生存し，また発熱活性は有意に抑制された．以上の結果，この物質は細菌内毒素の有望な抑制物質であることが示された．

Keywords: cinnamon, endotoxin, anti-endotoxin substance

Tanamoto K & Azumi S. **Salmonella-type heptaacylated lipid A is inactive and acts as an antagonist of LPS action on human line cells.**

J. Immunol., **164**, 3149-3156 (2000)

3種の異なるサルモネラ由来リピドA及びサルモネラ型化学合成リピドA 516は，強いリムルス活性，マウス腹腔マクロファージ，及びJ774細胞からのTNF-a産生誘導活性を示した．しかしながら*E. coli* LPS及び大腸菌型化学合成リピドA 506がヒト細胞にも同様に強いTNF-α産生活性を示したのに対して，サルモネラ由来リピドAはほとんど活性を示さず，516は10 mg/mlの高濃度においてもまったくTNF-α産生誘導を起こさなかった．同様の結果はNF-κBの活性化においても見られた．さらにヒト細胞に対して不活性な516は，506の作用にアンタゴニストとして作用した．一方，サルモネラLPSはリピドAと異なり，ヒト細胞に対しても*E. coli* LPSと同様の強い活性を示した．以上の結果からサルモネラ型リピドAは種特異性を示すこと，ヒト細胞は大腸菌型とサルモネラリピドAの一本の脂肪酸の構造差を認識していること，LPSの多糖部分がヒト細胞の活性化に関与していることが明らかになった．

Keywords: Salmonella lipid A, species specificity, endotoxin antagonist

Michiko Miyahara, Hirotaka Konuma: ***Escherichia coli* O157 Strains Which Caused Japanese Outbreaks Have Residues of Bacteriophage Sequences**

Bull. Pharm. Bull., 22, 11372-1375 (1999)

Twelve strains of *Escherichia coli* O157 which caused outbreaks in Japan were used as DNA sources. The seqeunces of the gene encoding the Shiga toxin 2 in all 12 strains were almost identical and the sequences downstream of this gene were similar to that of bacteriophage 933W.

Keywords: *Escherichia coli* O157; bacteriophage 933W; Shiga toxin 2

小高秀正，水落慎吾，小沼博隆：ふき取り検査におけるおけるふき取る強さによる菌数の挙動に関する検討

日食微誌，**16**, 131-133 (1999)

環境や食品検体の表面を検査する方法としてふき取り法が一般的に用いられている．しかしながら，ふき取り法による表面の微生物のサンプリング方法にはふき取る力について記載されたものはない．そこで，ふき取る力を変えて，微生物の回収性を調べたところ，ふき取る圧力が弱いと菌数がばらつくこと突き止めた．この結果を踏まえて，ふき取る力が圧力表示でわかるトルクピンセットを考案し，ふき取る力を150g／cm^{2}と300g／cm^{2}で比較したところ，300g／cm^{2}でふき取った方がバラツキが少なく，拭き取り法の信頼性を高めることができた．

Keywords: swab method, environmental monitoring, torque forceps

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Hara-Kudo, Y.*1, Konuma, H., Nakagawa, H.*2, and Kumagai, S.*1: ***Escherichia coli* O26 detection from foods using an enrichment procedure and an immunomagnetic separation**

method

Letters Applied Microbiol.,**30**, 151-154 (2000)

We found effective enrichment procedures for detecting *Escherichia coli* O26 in foods using methods that are used for E. coli O157. 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* brothwith 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

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Sakai, A., Yamakoshi, Y. and Miyata, N.: **Visible light irradiation of [60]fullerene causes killing and initiation of transformation in BALB/3T3 cells**

Fullerene Sci. Technol., 7, 743-756 (1999)

Cell transformation in vitro is a model of carcinogenesis in vivo. Two-stage transformation assay increases the sensitivity of cells to chemicals and permits detection of carcinogens acting as initiating agents. [60]Fullerene (C_{60}) was cytotoxic in BALB/3T3 cells when it was irradiated by visible light, but not without light irradiation. Under conditions when C_{60} was cytotoxic, it acted as an initiating agent for cell transformation, but it did not act as a complete transforming agent. The initiating activity of visible-light-irradiated C_{60} was statistically significant in a modified two-stage transformation assay including a procedure for replating cells treated by C_{60} and light, but it was equivocal in the standard two-stage transformation assay.

Keywords: fullerene, visible light irradiation, cell transformation

Suganuma, M.*1, Okabe, S.*1, Marino, M.W.*2, Sakai, A., Sueoka, E.*1 and Fujiki, H.*1: **Essential role of tumor necrosis factor α(TNF-α) in tumor promotion as revealed by TNF-α-deficient mice**

Cancer Res., **59**, 4516-4518 (1999)

To examine the hypothesis that tumor necrosis factor (TNF)α is an essential cytokine in carcinogenesis, we conducted two-stage carcinogenesis experiments with an initiator, 7,12-dimethylbenz(*a*)anthracene (DMBA), plus either of two tumor promoters, okadaic acid and 12-*O*-tetradecanoylphorbol-13-acetate (TPA), on the skin of TNF-α-deficient ($TNF^{-/-}$) mice. $TNF^{-/-}$ mice treated with DMBA plus okadaic acid developed no tumors for up to 19 weeks, and at 20 weeks, the percentage of tumor-bearing $TNF^{-/-}$ mice was 10 %, whereas the percentage of tumor-bearing $TNF^{+/+}$ mice was 100 %. In $TNF^{-/-}$ mice treated with DMBA plus TPA, tumor onset was delayed 4 weeks, and the time to development of small tumors in 100 % of mice was 9 weeks later than that seen in $TNF^{+/+}$ CD-1 mice. The average number of tumors in TPA-treated $TNF^{-/-}$ mice was 2.8, compared with 11.8 for $TNF^{+/+}$ CD-1 mice. To understand the residual tumor-promoting activity in $TNF^{-/-}$ mice, we also investigated the possible significance of interleukin (IL) 1 as an additional cytokine in tumor promotion. A single application of TPA and okadaic acid increased IL-1α and IL-1β gene expression in $TNF^{-/-}$ mice. All of our results demonstrate that TNF-α is the key cytokine for tumor promotion in mouse skin and, very possibly, for carcinogenesis in humans as well.

Keywords: tumor promotion, tumor necrosis factor α, interleukin 1

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Tsuchiya, T.*1, Umeda, M.*2, Nishiyama, H.*3, Yoshimura, I.*3, Hayashi, M., Sakai, A., et al.: **An interlaboratory validation study of the improved transformation assay employing Balb/c 3T3 cells: Results of a collaborative study on the two-stage cell transformation assay by the non-genotoxic carcinogen study group**

ATLA, 27, 685-702 (1999)

The Non-genotoxic Carcinogen Study Group of the Environmental Mutagen Society of Japan organized the first step of an interlaboratory validation study on an improved cell transformation assay employing Balb/c 3T3 A31-1-1 cells. Nineteen laboratories participated in this study. The modified transformation assay was evaluated for its responsiveness, its interlaboratory reproducibility and its transferability. In this study, a mixture of Dulbecco's modified Eagle's medium and nutrient mixture F12, supplemented with insulin-transferrin-ethanolamine-sodium selenite and 2 % fetal bovine serum (FBS) was used during the period of expression of transformed foci, instead of the usual minimum essential medium with 10% FBS. 20-Methylcholanthrene (MCA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) were selected as a prototype initiator and a tumor promoter, respectively. Two series of experiments were conducted. In the first series, the transformation activity of MCA was examined at various concentrations. In the absence of the promoting treatment with TPA, exposure to MCA only weakly induced transformed foci. In the presence of 0.1 μg/ml TPA, all laboratories observed significant dose-dependent increases in the number of transformed foci with increasing MCA concentrations. In the second series of experiments, various concentrations of TPA were tested. In the absence of initiating treatment with MCA, exposure to TPA weakly induced transformed foci in about half of the laboratories. In the presence of 0.2 μg/ml MCA, all the laboratories observed significant dose-dependent increases in the number of transformed foci with increasing TPA concentrations. The results from this study support the usefulness of this modified two-stage transformation assay with Balb/c 3T3 cells.

Keyword: cell transformation, Balb/c 3T3 cells, interlaboratory validation study

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相原真紀*, 田中辰明*, 高鳥浩介：**住環境にみる付着真菌の月別変動**

防菌防黴, **28** (1), 3-8(2000)

同一住環境106ヶ所における付着真菌の月別変動を1996年7月～1997年12月にわたり調査した．住居内の温・湿度は，夏期に高温多湿傾向にあり，特に相対湿度は平均して70%以上になり，また冬期は50％以下であった．同一住居内付着真菌を月別にみたところ，総真菌数は秋から初冬にかけてピークを示した．同一住居内で特に特徴ある変

動を示した付着真菌は *Cladosporium, Penicillium, Eurotium*, 酵母であった．*Cladosporium* は，春から夏にかけてはほとんど検出されず，秋以降急激に検出数の多くなる傾向がみられた．

Keywords: indoor environment, relative humidity,fungi

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太田利子[*1]，Jong-Chul PARK[*2]，Hawl SUH[*2]，村松芳多子[*3]，高鳥浩介：**真菌試験における培養温度の影響**

防菌防黴，**27**，309-313(1999)

真菌発育に影響をおよぼす培養温度の検討をした．培養温度20, 25, 28, 30, 32, 34, 36℃でCFU，集落形成性，胞子形成性を10真菌で検討した結果，いずれの真菌においても良好な温度域は，20, 25, 28℃域であり，特に25, 28℃は至適培養温度であった．培養温度が30℃より高くなるにつれCFU，集落形成性，胞子形成性とも低下する傾向が認められた．したがって真菌の培養温度は30℃以上は必ずしも適条件とはいえず，30℃以下の培養温度とすることが望ましいものと結論された．

Keywords: incubation temperature, fungi , optimal growth temperature

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村松芳多子[*1]，高鳥浩介，大田利子[*2]，Jong-Chul PARK[*3]，Hawl SUH[*3]，秋山 茂[*4]：**薬剤配合による抗真菌活性評価および形態変化**

防菌防黴，**27**，287-293(1999)

衛生上常用される薬剤4種（BC，TBZ，GC，SLS）を用いて薬剤配合による抗真菌活性を5種真菌を用いチェッカーボード方式で測定した．薬剤の2種等量配合によりカチオン系薬剤のBC，GCはアニオン系製剤のSLSと強く拮抗し，同濃度の配合では高濃度域まで失活した．TBZはとくにBCとの併用により黒色系真菌に対し，有効な作用を認めた．薬剤配合によるそれぞれの真菌の形態変化をみたところ，菌糸の不均一化，膨化，厚膜胞子産生が確認され，明らかに薬剤抵抗性を示した．

Keywords：antifungal activities, checker board test, morphological changes

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村松芳多子[*1]，大田利子[*2]，李 憲俊[*3]，Jong-Chul PARK[*4]，Hawl SUH[*4]，鈴木明子，成田紀子，高鳥浩介：**真菌用培地による生菌数の測定と培地評価**

防菌防黴，**27**，707-711(1999)

各種真菌用培地（PDA，SDA，M40YA，DG-18A，CZA）によるCFU測定結果を検討した．単一真菌でのCFUをみる限り，多くの真菌は各培地でほぼ一致する傾向にあった．しかし，培地性状（胞子産生性，集落性状）を併せて観察すると真菌の種類により，培地適性が認められた．単一真菌による培地特性は，PDAとM40YAにおいて胞子産生が高く，SDA，DG-18AとCZAにおいて菌糸形成が高く，また小集落も多かった．試料中の真菌培養による培地特性はPDA，SDAで広範な真菌を培養することが可能であった．一方，CZA，M40YAとDG-18Aは特定な真菌の培養に適していることが認められた．

Keywords: fungal media, CFU, mycological value

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小菅旬子[*1]，高鳥浩介，安斉 了[*2]：**ウマ喉嚢炎病巣由来Emericella nidulansの生物学的性状**

真菌誌，**40**，169-173(1999)

ウマ喉嚢炎病巣由来 *Emericella nidulans* 分離株7株は，いずれも一般的な5種類の培地において同程度の発育速度であり，形態にも顕著な差がないこと，幅広い温度やpH条件下で発育可能であったが，至適発育温度はウマの平均体温である38℃付近に，至適発育pHは酸性側にあること，およびそのような発育に適した条件下では無性胞子を多量に産生することが明らかとなった．また，全ての菌株が蛋白分解能や溶血能を有していることが明らかになった．以上の結果から *E. nidulans* は幅広い環境下で発育可能であり，ウマ生体に対する病原性を持つことも示唆された．

Keywords: *Emericella nidulans*, guttural pouch mycosis, biological characteristic

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Park, J-C*., Han,D-W*., Hwang, Y-S.*, Lee, H. J**, Takatori, K. and Suh, H*.: **Determination of a favorable medium for detection of fungal extracellular protease**

Biocontrol Sci., **4**,91-95(1999)

To determine a favorable medium for detecting fungal extracellular protease (EP), a plate method was examined. In this study, 12 fungal strains were tested with 4 different types of Czapek Dox agar medium containing skim milk, and the clear zones around the colonies were compared on the different types of medium. Among them, 9 strains produced EP. Triton X-100 was required to detect an EP from *Alternaria alternata*, while in case of penicillium frequentans, saccharose was required. It was found that the media supplemented with both tritonX-100 and saccharose gave acceptable results in terms of EP detection.

Keywords: extracellular protease, plate method, tritonX-100

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Nakata, K., Takai, T. and Kaminuma, T: **Development of A Receptor Database: Application to the Endocrine Disruptor Problem.**

Bioinformatics, **15**, 544-552 (1999)

受容体について構造や機能を含む種々の情報を階層的に表示するデータベースシステムを構築し，インターネット上で検索可能にした．既存のデータベース中の情報に加えてシステム内でデータを編集し高次の解析システムにかけた結果を表示できる．このシステムにより内分泌撹乱物質関連情報を解析することを試みた．

Keywords: receptor, database, endocrine disruptor, molecular structure

関沢純，大屋幸江：**植物エストロゲン物質の日本人の健康への定量的リスク・ベネフィット解析**

日本リスク研究学会誌,**11**(1), 75-82 (1999)

内分泌撹乱化学物質として多くの物質がとりざたされてきたが，実際に日本人が曝露されるレベルでどのようなリスクまたはベネフィットがあるかを定量的に評価した報告はほとんど見られない．筆者は日本人がほぼ毎日摂取しておりさまざまな証拠から実際に日本人の健康に影響を及ぼしている可能性が考えられる大豆中のエストロゲン物質の摂取量，体内レベルてを評価し，主として人で得られている知見と作用メカニズムの知見を総合して，健康へのリスクとベネフィットを現時点で入手可能な情報に基づき推定した．

Keywords: phytoestrogen, quantitative risk-benefit analysis, soybean, isoflavonoid

Saga, Y., Kobayashi, M.*1, Ohta, H.*1, Murai, N.*1, Nakai, N.*1, Oshima, M.*1 and Taketo, M. M.*2: **Impaired extrapyramidal function caused by the targeted disruption of retinoid X receptor RXRgamma1 isoform.**
Genes Cells, **4**, 219-228 (1999)

Retinoid X receptors RXRalpha, beta and gamma exert multiple functions in the genetic regulation of mammalian signalling systems . In contrast to the widespread expression of RXRalpha and RXRbeta, the expression of RXRgamma is restricted to particular tissues in which RXRgamma1 is the major isoform expressed in the mouse corpus striatum. To investigate the function of this particular isoform RXRgamma1, we generated RXRgamma1 gene-knockout mice. Independent of genetic background, the expression of choline acetyltransferase (ChAT) in the cholinergic interneurones in the striatum (caudal putamen) was markedly reduced in the RXRgamma1 gene-null mice. Furthermore, the mutant exhibited an altered response to the administration of dopamine receptor antagonists, haloperidol and chlorpromazine, which normally induce catalepsy in mice. These results strongly suggest that RXRgamma1 plays an important role in either the development or activation of cholinergic neurones in nigrostriatal extrapyramidal pathways.
Keywords: Retinoid X receptors, catalepsy, nigrostriatal extrapyramidal pathway
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Kiernan, B.W.*1, Garcion, E.*1, Ferguson, J.*1, Frost, E. E.*1, Torres, E.M.*1, Dunnett, S.B.*1, Saga, Y., Aizawa, S.*2, Faissner, A.*1, Kaur,R.*1, Franklin, R.J.*1 and French-Constant C.*1: **Myelination and behaviour of tenascin-C null transgenic mice**
Eur J Neurosci., **11**, 3082-3092 (1999)

The extracellular matrix glycoprotein tenascin-C is widely expressed during development and repair, making it surprising that few abnormalities have been found in transgenic mice lacking this molecule. We have therefore re-examined the transgenic mice described by Saga et al. We find no abnormalities of myelination or oligodendrocyte precursor distribution in adult mice, showing that local concentrations of tenascin-C are not the sole mechanism responsible for the pattern of myelination in these regions of CNS. However, we do find a number of behavioural abnormalities in these mice and show that hyperlocomotion and deficits in coordination during beam walking can be ascribed to tenascin-C deficiency. The effects on coordination are, however, not seen on a 129 genetic background. Taken together, these results significantly extend the phenotype associated with tenascin-C deficiency but argue against a role in myelination.
Keywords: tenascin-C, hyperlocomotion, myelination
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Saga, Y., Miyagawa-Tomita, S.*1, Takagi, A., Kitajima, S., Miyazaki, J.*2 and Inoue T.: **MesP1 is expressed in the heart precursor cells and required for the formation of a single heart tube.**
Development, *126*, 3437-3447 (1999)

The Mesp1 gene encodes the basic HLH protein MesP1 which is expressed in the mesodermal cell lineage during early gastrulation. Disruption of the Mesp1 gene leads to aberrant heart morphogenesis, resulting in cardia bifida. In order to study the defects in Mesp1-expressing cells during gastrulation and in the specification of mesodermal cell lineages, we introduced a (beta)-galactosidase gene (lacZ) under the control of the Mesp1 promoter. Mesp1-expressing cells in the homozygous deficient embryos stayed in the primitive streak for a longer period of time before departure. In addition, using the Cre-loxP site-specific recombination system, we could determine the lineage of the Mesp1-expressing cells. These results strongly suggest that MesP1 is expressed in the heart tube precursor cells and is required for mesodermal cells to depart from the primitive streak and to generate a single heart tube.
Keywords: Mesp1, Cre-loxP site-specific recombination, heart morphogenesis
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Kanatani, A.*1, Mashiko, S.*1, Murai, N.*1, Sugimoto, N.*1, Ito, J.*1, Fukuroda, T.*1, Fukami, T.*1, Morin, N.*2, MacNeil, D.J.*2, Van der Ploeg L.H.*2, Saga,Y., Nishimura, S.*1 and Ihara, M.*1: **Role of the Y1 receptor in the regulation of neuropeptide Y-mediated feeding:comparison of wild-type, Y1 receptor-deficient, and Y5 receptor-deficient mice**
Endocrinology, **41**, 1011-1016 (2000)

Neuropeptide Y (NPY) increaseas food intake through the action of hypothalamic NPY receptors. Although the involvement of Y1 and Y5 receptors in feeding regulation has been suggested, the relative importance of each of these NPY receptors and the participation of a novel feeding receptor are still unclear. To address this issue, we generated a Y1 receptor-deficient ($Y1^{-/-}$) and a Y5 receptor-deficient ($Y5^{-/-}$) mouse line. The icv NPY-induced food intake was remarkably reduced in $Y1^{-/-}$ mice, but was not significantly altered by inactivation of the Y5 receptor. Stimulation of feeding by moderately selective Y5 agonists [PYY-(3-36), human PP, and bovine PP] was reduced in $Y5^{-/-}$ mice, although food intake did not decrease to vehicle control levels. These results indicate that the Y5 receptor functions as one of the feeding receptors.
Keywords: NPY, feeding regulation, feeding receptor
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Sawada, A. *1, Fritz, A.*2, Jiang, Y-J.*3, Yamamoto, A.*4, Yamasu, K.*5, Kuroiwa, A.*1, Saga, Y. and Takeda, H.*6: **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)

In this study, we have characterized zebrafish mesp-a and mesp-b genes that are closely related to Mesp family genes in other vertebrates. In fused somites (fss) embryos, initial mesp-a expression remains intact, but is not detected during the segmentation period. This suggests that these genes are downstream targets of fss at the segmentation stage. Furthermore, we found that zebrafish her1 expression oscillates in the presomitic mesoderm. The her1 stripe, which first appears in the tailbud region, moves in a caudal to rostral direction, and it finally overlaps the most

rostral mesp stripe. 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 zebrafish mesp genes are involved in anteroposterior specification within the presumptive somites.

Keywords: mesp-a, mesp-b, her1

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Saitoh, M., Umemura, T., Kawasaki, Y., Momma, J., Matsushima, Y., Sakemi, K., Isama, K., Kitajima, S., Ogawa, Y., Hasegawa, R., Suzuki, T., Hayashi, M., Inoue, T., Ohno, Y., Sofuni, T., Kurokawa, Y. and Tsuda, M.: **Toxicity study of a rubber antioxidant, mixture of 2-mercapto-methylbenzimidazoles, by repeated oral administration to rats**

Fd. Chem. Toxicol., **37**, 777-787 (1999)

In this investigation, acute and subacute oral toxicity studies of 2-mercaptomethyl-benzimidazoles (MMBIs) employed industrially as rubber antioxidants in Wistar rats were conducted. The LD50 was estimated to be 330 mg/kg. In the subacute oral toxicity study, male rats administered 100 mg/kg MMBIs exhibited a 1.8-fold increase in thyroid weight associated with histopathological changes but not altered serum thyroid hormone levels. Female rats administered 100 mg/kg MMBIs exhibited significant increases of liver and kidney but not thyroid weights, and serum cholesterol level. No-observed-effect levels for male and female rats were found to be 4 and 20 mg/kg, respectively, in this subacute oral toxicity study.

Keywords: 2-mercaptomethylbenzimidazoles, antithyroid toxicity, repeated oral administration

Umemura, T., Kai, S.*1, Hasegawa, R., Sai, K., Kurokawa, Y. and Williams, G.M.*2: **Pentachlorophenol (PCP) produces liver oxidative stress and promotes but does not initiate hepatocarcinogenesis in B6C3F1 mice**

Carcinogenesis, **20**, 1115-1120 (1999)

To elucidate the mechanism of hepato-carcinogenesis of pentachlorophenol (PCP) in mice, critical effects related to carcinogenicity were studied in the livers of B6C3F1 male mice administered PCP at concentrations of 600 and 1200 ppm in the diet for 8 weeks. 8-oxodeoxy-guanosine (8-oxodG) in the liver nuclear DNA and hepatocyte cell proliferation were examined. Also, initiation and promotion were assessed in a 2-stage hepatocarcinogenesis model. Significant elevations of 8-oxodG levels and cell proliferation were observed in a dose-dependent manner. PCP promotes, but does not initiate hepato-carcinogenesis. These findings are interpreted to demonstrate that the promoting action is related to oxidative stress and compensatory hepatocellular proliferation.

Keywords: pentachlorophenol, 8-oxodeoxyguanosine, hepatocarcinogenesis

*1昭和女子大学
*2 New York Medical College

Umemura, T., Kodama, Y., Hioki, K*, Inoue, T., Nomura, T.* and Kurokawa, Y.: **Susceptibility to urethane carcinogenesis of transgenic mice carrying a human prototype c-Ha-ras gene (rasH2 mice) and its modification by butylhydroxytoluene**

Cancer Lett., **145**, 101-106 (1999)

The susceptibility of rasH2 mice to urethane lung carcinogenesis and the modifying effects of BHT on development of pulmonary lesions were examined. Single i.p. injections of urethane at 250 mg/kg in males or 500 mg/kg in females induced alveolar/bronchiolar adenomas within 6 weeks. At 4 weeks after the injection with a dose of 1000 mg/kg, adenomas occurred in both sexes. BHT administration increased the multiplicity of hyperplasias observed 3 weeks after the urethane injection and additionally caused adenomas which did not occur in the urethane alone-treated animals. The overall data suggest the possibility of rapid assays for lung carcinogens using rasH2 mice.

Keywords: rasH2 mice, in vivo rapid assay, lung carcinogenesis

*実験動物中央研究所

Umemura, T., Kodama, Y., Kurokawa, Y. and Williams, G.M.*: **Lack of oxidative DNA damage or initiation of carcinogenesis in the kidneys of male F344 rats given subchronic exposure to p-dichlorobenzene (pDCB) at a carcinogenic dose**

Arch. Toxicol., **73**, 54-59 (2000)

To examine possible mechanisms of kidney carcinogenesis, pDCB was studied for its ability to produce 8-oxodeoxyguanosine (8-oxodG) in kidney nuclear DNA and for initiating activity in a two-stage renal carcinogenesis model. As a result, pDCB did not produce oxidative DNA damage in the rat kidney or effect initiation of kidney carcinogenesis. These data suggest that oxidative stress is not involved in pDCB-induced renal carcinogenesis and the α2u-globulin-mediated chronic nephropathy probably acts as a promoter, not an initiation of renal carcinogenesis. Accordingly, pDCB is judged to have no cancer hazard to humans who are not susceptible to the α2u-globulin nephropathy.

Keywords: p-dichlorobenzene, 8-oxodeoxyguanosine, renal carcinogenesis

* New York Medical College

Iwata, T.*, Kamikawa, J.*, Higuchi, T.*, Yagi, K.*, Matsuzaki, T.*, Kanno, J. and Maekawa, A.*: **Development of Invasive Adenocarcinoma in a Long-Standing Diverted Ileal J-Pouch for Ulcerative Colitis**

Dis Colon Rectim., 43, p101-104 (2000)

潰瘍性大腸炎患者に対して全結腸切除後に行われた回腸Jポーチ造設部位に，小腸由来であるにも関わらず，潰瘍性大腸炎様の炎症変化，異型上皮巣を伴う浸潤性高分化腺癌の発生を認めた．

Keywords: ulcerative colitis, total colectomy, J-pouch adenocarcinoma

*(財)佐々木研究所付属杏雲堂病院

樋口哲朗*，岩間毅夫*，家城和男*，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一例**

胃と腸, **35**, 451-455 (2000)

回腸癌を先進部とする腸重積患者の遺伝子解析を含む症例報告

Keywords: Juvenile polyposis, ileal adenocarcinoma, invagination

*(財)佐々木研究所付属杏雲堂病院

Fujiwara, M.[*1], Okayasu, I.[*2], Orita, 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. and Hayashi, Y.[*5]: **Significant Increase in Prostaglandin E-Main Urinary Metabolite by Laxative Administration: Comparison with Ulcerative Colitis.**

Digestion, **61**, 201-206 (2000)

潰瘍性大腸炎の病勢とプロスタグランディンE2の増減が対応することを受け，下剤誘発性のプロスタグランディンE2の増加を健常人ボランティアにより検討した.

Keywords: laxatives, prostaglandin E, ulcerative colitis

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[*2] 北里大学医学部
[*3] 津村株式会社
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[*5] 北里大学薬学部

Shiraga, T.[*1] , Hata, T.[*1] , Yamazoe, Y.[*2], and Ohno, Y., Iwasaki, K.[*1]: **N-Sulphoconjugation of amines by human cytosolic hydroxysteroid sulphotransferase**

Xenobiotica, 29, 341-347 (1999)

ヒト肝可溶性分画は脂環式アミン及び芳香族アミンのN-硫酸抱合及びhydroxysteroid及びphenolの0-硫酸抱合活性を有すること及び脂環式アミン硫酸抱合活性はhydroxysteroid 0-硫酸抱合活性と良く対応するが，phenol 0-硫酸抱合活性とは対応しないことを示した．また，ヒト肝臓から脂環式アミン硫酸転移酵素を精製し，その性質を明らかにした．この酵素は34KDaで，可溶性分画中の存在量は脂環式アミン硫酸抱合活性及びhydroxysteroid 0-硫酸抱合活性と良く対応していた.

Keywords: sulfation, human liver, amine sulfotransferase

[*1] 藤沢薬品工業
[*2] 東北大学薬学部

Saitoh, M., Umemura, T., Inoue, T., Ohno, Y., Sofuni, T., Kurokawa, Y., Tsuda, M.: **Toxicity study of a rubber antioxidant, mixture of 2-mercaptomethylbenzimidazoles, by repeated oral administration to rats**

Food and Chemical Toxicology, **37**, 777-787 (1999)

ゴムの抗酸化剤のメルカプトメチルベンズイミダゾールの経口投与による一般毒性をラットで検討した．その結果100mg/kg, 4週間投与により体重減少，肝・腎重量の増加が認められるが，メルカプトベンズイミダゾールとは異なり，甲状腺重量の変化は認められないことを示した.

Keywords: antioxidant, mercaptomethylbenzimidazole, general toxicity

Shiraga, T.*, Niwa. T.*, Teramura, Y.*, Kagayama, A.*, Tsutsui, M.*, Ohno, Y., Iwasaki, K.*: **Oxidative metabolism of tacrolimus and its metabolite by human cytochrome P450 3A subfamily**

Xenobio. Metabol. And Disp. **14**, 277-285 (1999)

タクロリムスのヒト肝ミクロゾームでの代謝による主代謝物としてM-1及びM-VII, 13,15-O-脱メチル化体を得た．また，ヒト発現系P450によるM-1及びM-VIIの生成はCYP3A群によってのみ触媒されることを明らかにした.

Keywords: tacrolimus, human liver, metabolism, CYP3A

* 藤沢薬品工業

Koizumi, S., Bootman, M.D.*, Berridge, M.J.*, Lipp, P.*: **Regulation of ryanodine receptor opening by lumenal Ca^{2+} underlies quantal Ca^{2+} release in PC12 cells**

J. Biol. Chem., **274**, 33327-33333 (1999)

Mechanisms underlying graded or 'quantal' Ca^{2+} release from ryanodine receptors (RyRs) were investigated in PC12 cells. Quantal Ca^{2+} release was observed in cells stimulated with 1 to 40 mM caffeine. The Ca^{2+} load of the caffeine-sensitive stores was modulated by allowing them to refill for varying times after complete discharge with maximal caffeine. The threshold for RyR activation was sensitised ~10-fold as the Ca^{2+} load increased from a minimal to a maximal loading. In addition, the fraction of Ca^{2+} released by low caffeine concentrations increased. Our data suggest that RyRs are sensitive to lumenal Ca^{2+} over the full range of Ca^{2+} loads that can be achieved in an intact PC12 cell, and that changes in RyR sensitivity may be responsible for the termination of Ca^{2+} release underlying the quantal effect.

Keywords: Graded Ca^{2+} release, Ryanodine receptors

* Babraham Institute

Tsuda, M., Ueno, S.* and Inoue, K.: ***In vivo* pathway of thermal hyperalgesia by intrathecal administration of a,b-methylene ATP in mouse spinal cord: Involvement of glutamate-NMDA receptor system**

Br.J.Pharmacol., **127**, 449-456 (1999)

We investigated the mechanisms of the P2X receptors agonist α,β-methylene ATP (α,βmeATP)-induced modulation of acute nociception in mouse spinal cord. Intrathecal administration of α,βmeATP produced a significant and dose-dependent thermal hyperalgesic response. This response was completely blocked by a non-selective P2 receptor and a selective P2X1, P2X3 and P2X2+3 receptors antagonist. P2X1 receptor was not involved in spinal nociceptive pathway. The thermal hyperalgesia by a,bmeATP was inhibited by the intrathecal pretreatment with botulinum neurotoxin B and N-methyl-D-aspartate (NMDA) receptor antagonists. These findings suggest that the α,βmeATP-induced thermal hyperalgesia may be mediated by spinal P2X3 receptor to evoke spinal glutamate release.

Keywords: α,βmeATP, P2X3 receptor subtype, thermal hyperalgesia

* Fukuoka University School of Medicine

Tsuda, M., Ueno, S.* and Inoue, K.: **Evidence for the involvement of spinal endogenous ATP and P2X receptors in the nociceptive responses caused by formalin and capsaicin in mice**

Br.J.Pharmacol., **128**, 1497-1504 (1999)

We examined the effects of intrathecal treatment with P2X receptor antagonists on the formalin- and capsaicin-induced nociceptive behaviors in mice. Intrathecal pretreatment with general P2 receptors antagonist pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) significantly suppressed the formalin-induced nociceptive behavior. Pretreatment with selective antagonist for the P2X1, P2X3 and P2X2+3 2',3'-O-(2,4,6-trinitrophenyl)adenosine 5'-triphosphate (TNP-ATP) significantly reduced the first phase, but not second phase. Capsaicin-induced nociceptive behavior was also significantly suppressed by intrathecal pretreatment with PPADS or TNP-ATP. These findings suggest that spinal endogenous ATP may play a role in the generation of formalin- and capsaicin-induced neurogenic pain through the PPADS-sensitive receptors.

Keywords: α,βmeATP, P2X3 receptor, neurogenic pain
* Fukuoka University School of Medicine

Rhee, J. S.*, Wang, Z. M.*, Nabekura, J.*, Inoue, K and Akaike, N.*: **ATP facilitates spontanoeous glycinergic IPSC frequency at dissociated dorsal horn interneurone synapses**
J.Physiol.(Lond.), **524**, 471-483 (2000)

The ATP action on spontaneous miniature glycinergic inhibitory postsynaptic currents (mIPSCs) was investigated in rat substantia gelatinosa (SG) neurons dissociated from the 2nd layer of the dorsal horn with presynaptic glycinergic nerve terminals. ATP reversibly facilitated the frequency of the mIPSCs in a concentration-dependent manner without affecting their amplitude distribution. 2-MethylthioATP mimicked the ATP action, while ab-methylene-ATP had no effect on mIPSCs. The facilitatory effect of ATP on mIPSC frequency was abolished by suramin and pyridoxal-5-phosphate-6-azophenyl-2', 4'-disulphonic acid and in a Ca^{2+}-free external solution but not by Cd^{2+}. These results suggest that ATP enhances glycine release from nerve terminals, presumably resulting in the inhibition of SG neurons which conduct nociceptive signals to the CNS.
Keywords: ATPreceptor, dorsal horn, glycinergic neuron
* Kyusyu University School of Medicine

Shinoura, H.*, Take, H.*, Hirasawa, A.*, Inoue, K., Ohno, Y., Hashimoto, K.* and Tsujimoto, G.*: **Key amino acids of vasopressin V1a receptor responsible for the species difference in the affinity of OPC-21268**
FEBS Lett., **466**, 255-258 (2000)

A non-peptide, vasopressin V1a receptor-selective antagonist, OPC-21268, exhibited a markedly higher affinity for the rat V1a receptor (Ki = 380 nM) than for the human V1a receptor (Ki = 140 microM). To delineate the region responsible for the high affinity binding of OPC-21268 for the rat V1a receptor, we have constructed a series of chimeric human and rat V1a receptors, and examined the chimeric and point-mutated receptors by competitive radioligand binding analysis. The results showed that the transmembrane domain (TMD) VI-VII of the vasopressin V1a receptor, in particular the amino acid residue Ala-342 in TMD VII, is the major component conferring the rat-selective binding of OPC-21268 to the V1a receptor
Keywords: vasopressin V1a receptor, chimeric receptors, point-mutated receptors
* National Children's Medical Research Center

Tomioka, A.*1, Ueno, S.*2, Kohama, K.*1, Goto, F.*1 and Inoue, K.: **Propofol potentiates ATP-activated currents on recombinant P2X4 receptor channels expressed in human embryonic kidney 293 cells.**
Neurosci Lett., **284**, 167-170 (2000)

We examined the effects of a general anesthetic 2, 6-diisopropylphenol (propofol) on ATP- and a,b-methylene ATP (a,bmeATP)-activated currents in the human embryonic kidney 293 (HEK 293) cells expressing recombinant P2X receptor channels, using the whole-cell patch-clamp method. Propofol at clinical relevant concentrations (approximately 56 μM) potentiated the current responses through the P2X(4) receptor in a dose-dependent manner, whereas propofol did not affect the responses through the P2X(2) receptor or through the heterologous complex of the P2X(2) and P2X(3) (P2X(2+3)) receptor. These results suggest that activation of P2X(4) subtype in the brain and the motor neurons of the spinal anterior horn might be involved in the excitatory effect by propofol such as convulsion and unexpected movements.
Keywords: propofol, P2X4 receptor subtype, excitatory effect
*1 Gunma University School of Medicine
*2 Fukuoka University School of Medicine

Nakazawa, K., Inoue, K. and Ohno, Y.: **Block and unblock by imipramine of cloned and mutated P2X2 receptor/channel expressed in Xenopus oocytes**
Neurosci. Lett., **264**, 93-96 (1999)

アフリカツメガエルの卵母細胞に発現させた野性型および改変型ATP受容体チャネル（P2X2受容体）に対するイミプラミンの作用を検討した．イミプラミン（100 μM）は野性型のチャネルを介する電流を部分的に抑制した．高濃度（300 μM）においては抑制作用の減弱（"脱抑制"）が観察された．チャネル孔の開口部付近にある負の電荷あるいは極性を有するアミノ酸残基を中性化してイミプラミンの効果を検討した．Asp315の中性化により100 μMのイミプラミンによる抑制作用が減弱した．Thr330の中性化では，この抑制作用が促進された．Asn333の中性化では，300 μMのイミプラミンによる脱抑制作用が消失した．以上のことからイミプラミンはこれらのアミノ酸残基との相互作用によりチャネルに影響を与えることが示唆された．
Keywords: ATP receptor/channel, imipramine, site-directed mutagenesis

Nakazawa, K. and Ohno, Y.: **5-Hydroxytryptamine inhibits P2X2 receptor channel pore mutants**
Cell. Mol. Neurobiol., **19**, 665-669 (1999)

アフリカツメガエルの卵母細胞に発現させた野性型および改変型ATP受容体チャネル（P2X2受容体）に対するセロトニンの作用を検討した．セロトニン（10 μM）は野性型のチャネルを介する電流を増強した．Thr330またはAsn333をIleに置換した改変型チャネルにおいては，セロトニンによる電流抑制が観察された．以上のことからセロトニンがチャネルの開口部と相互作用することが示唆された．
Keywords: P2X2 receptor/channel, 5-hydroxytryptamine, site-directed mutagenesis

Nakazawa, K. and Ohno, Y.: **Neighboring glycine residues are essential for P2X2 receptor/channel function**
Eur. J. Pharmacol. 370, R5-R6 (1999)

P2X2受容体／チャネルのグリシンを多く含む領域の役割を改変型チャネルを作製することにより検討した．Gly247をAlaに置換した場合，ATPに対する反応性が消失した．Gly248をAlaに置換した場合にはATPに対する反応性の減弱が観察され，Valに置換した場合には反応性が消失した．以上のことから，これらの並列したグリシン残基がチャネル機能に必須であることが示唆された．
Keywords: ATP receptor/channel, ATP sensitivity, site-directed mutagenesis

Nakazawa, K. and Ohno, Y.: **Block by 5-hydroxytryptamine and apomorphine of recombinant human neuronal nicotinic receptors**
Eur. J. Pharmacol. 374, 293-299 (1999)

アフリカツメガエルの卵母細胞に発現させたヒト型ニコチン様アセチルコリン受容体／チャネルに対するセロトニンおよびアポモルフィンの作用を検討した．セロトニンお

よびアポモルフィンはこのチャネルを介するイオン電流を抑制した．抑制の度合はチャネルを構成するサブユニットの種類に依存していた．アセチルコリンの濃度-作用曲線の最大反応の低下よりこれらの薬物は非競合抑制を示すこと，また，抑制の電位依存性よりその作用点がチャネル孔であることが示唆された．

Keywords: recombinant human nicotinic acetylcholine receptor/channel, 5-hydroxytryptamine, apomorphine

Ozawa, S., Schoket, B.[*1]., McDaniel, L.P.[*2], Tang, Y.-M.[*2], Ambrosone, C. B.[*2], Kostic, S.[*3], Vincze, I.[*1] and Kadlubar, F. F.[*2]: **Analyses of bronchial bulky DNA adduct levels and CYP2C9, GSTP1 and NQO1 genotypes in a Hungarian study population with pulmonary diseases**

Carcinogenesis **20**, 991-995 (1999)

CYP2C9, GSTP1 and NQO1 遺伝子の各異型遺伝子と気管支組織の多環芳香族炭化水素のDNA付加体量との関連をハンガリー人集団について調べた．

Keywords: polycyclic aromatic hydrocarbons, genotype, drug metabolizing enzymes

[*1] National Institute of Environmental Health, Hungary
[*2] National Center for Toxicological Research. USA
[*3] Koranyi National Institute of Pulmonary, Hungary

Ozawa, S., Shimizu, M.[*1], Katoh, T.[*2], Miyajima, A., Ohno, Y., Matsumoto, Y.[*1], Fukuoka, M.[*1], Tang, Y.-M.[*3], Lang, N.P.[*4] and Kadlubar, F.F.[*3]: **Sulfating-activity and stability of cDNA-expressed allozymes of human phenol sulfotransferase, ST1A3*1 (^{213}Arg) and ST1A3*2 (^{213}His), both of which exist in Japanese as well as Caucasians**

J. Biochem. (Tokyo) **126**, 271-277 (1999)

ヒトフェノール硫酸転移酵素の異型酵素分子種(ST1A3*2, ^{213}His)の機能を野性型分子種 (ST1A3*1. ^{213}Arg)と比較し，*2型分子種は野性型分子種に比べ，熱に不安定であることを明らかにした．

Keywords: human phenol sulfotransferase, alleleic variants, heterologous cell expression systems

[*1] 昭和薬科大学
[*2] 産業医科大学
[*3] National Center for Toxicological Research. USA
[*4] Arkansas Cancer Research Center, USA

Yang, M.[*1], Katoh, T.[*2], Delongchamp, R.[*1], Ozawa, S., Kohshi, K.[*2] and Kawamoto, T.[*2]: **Relationship between NAT1 genotype and phenotype in a Japanese population**

Pharmacogenetics **10**, 225-232 (2000)

ヒトアリルアミンN-アセチル転移酵素1(NAT1)の異型野性型対立遺伝子 (*10)と野性型対立遺伝子 (*4)を有するヒトの末梢血リンパ球のNAT1依存的活性について検討した．

Keywords: human arylamine N-acetyltransferase 1 (NAT1), alleleic variants, enzymatic activities in lymphocytes

[*1] National Center for Toxicological Research. USA
[*2] 産業医科大学

Nakajima, M.*, Sasaki, M.*, Kobayashi, Y.*, Ohno, Y., Usami, M: **Developmental toxicity of indium in cultured rat embryos**

Teratogenesis Carcinog. Mutagen., **19**, 205-209 (1999)

Rat embryos at day 9.5 of pregnancy were cultured for 48 h in the presence of indium trichloride under various exposure conditions. Indium was embryotoxic to cultured rat embryos according to the embryonic age, and the exposure concentration was more critical than the exposure time. It was considered that the developmental toxicity of indium is a direct effect on the embryo or yolk sac, and that weak developmental toxicity of indium by oral administration was due to low exposure concentrations of the embryo.

Keywords: embryo culture, indium trichloride, toxicokinetics

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

Usami, M., Tabata, H., Ohno, Y.: **Effects of glutathione depletion on selenite- and selenate-induced embryotoxicity in cultured rat embryos**

Teratogenesis Carcinog. Mutagen., **19**, 255-266 (1999)

Rat embryos at day 9.5 of gestation were cultured for 48 h in the presence of Se as either sodium selenite. Embryonic GSH was depleted by the addition of L-buthionine-[S,R]-sulfoximine (BSO) without embryotoxicity. The incidence of selenite-induced malformation of the embryos was decreased with BSO, but the incidence of selenate-induced malformation was increased. It was considered that embryonic GSH was involved in the embryotoxicity of selenite and selenate.

Keywords: embryo culture, glutathione, buthionine sulfoximine

Hirabayashi, N.[*1], Matsuki, Y.[*1], Suzuki, E.[*1], Usami, M., Ohno, Y., Shimada, K.[*2]: **Toxicokinetic study of fadrozole, a non-steroidal aromatase inhibitor, in chicken eggs by the injection into the air sac**

Jpn. Poult. Sci., **36**, 382-387 (1999)

ニワトリ卵の気室にfadrozoleを投与し，胚および卵黄・卵白中のfadrozole量の経時変化および投与量との関係をHPLCにより調べた．その結果，卵黄・卵白ではほぼ投与量に依存してfadrozole濃度の上昇が認められたが，胚への分布については非線形を呈した．

Keywords: fadrozole, chicken embryo, toxicokinetics

[*1] Hatano Research Institute, Food and Drug Safety Center, Japan
[*2] Graduate School of Bioagricultural Sciences, Nagoya University, Japan

Nishikimi, H.*, Kansaku, N.*, Saito, N.*, Usami, M., Ohno, Y., Shimada, K.*: **Sex differentiation and mRNA expression of P450c17, P450arom and AMH in gonads of the chicken**

Mol. Reprod. Develop., 55, 20-30 (2000)

Effects of in ovo injection of nonsteroidal aromatase inhibitor (fadrozole) and estradiol on mRNA levels of P450c17, P450arom and anti-M殕lerian hormone(AMH) in the gonads of chicken embryos were examined. The results indicated that the expressions of P450arom and AMH are sexually dimorphic and are reciprocally regulated.

Keywords: chicken embryo, sex differentiation, mRNA

* Graduate School of Bioagricultural Sciences, Nagoya University, Japan

紅林秀雄，高橋昭江，鹿庭なほ子，高橋 悖：**安息香酸のナトリウム塩及びカリウム塩のラットにおける体内動態**

J. Health Science, **45**, 391-400 (1999)

安息香酸のナトリウム塩及びカリウム塩の各3用量を雄性ラットに経口投与しその体内動態を調べた．その結果，

これらの塩による違いは認められなかったが，投与量に依存して吸収，分布，代謝および排泄が遅くなる飽和現象が判明した．
Keywords: disposition, sodium benzoate, potassium benzoate

Sakemi, K., Usami, M., Mitsunaga, K., Ohno, Y., Tsuda, M.: **Comparative toxicokinetic study of rubber antioxidants, 2-mercaptobenzimidazole and 2-mercaptomethylbenzimidazole, by single oral administration in rats**
J. Toxicol. Sci., **24**, 399-405 (1999)
ゴムの老化防止剤として使用されている，2-メルカプトベンズイミダゾール（MBI）及びそのメチル誘導体（MMBI）をラットへ単回経口投与し，血中及び尿中の薬物濃度を経時的に測定し，TKパラメータを比較検討した．急性毒性の発現（自発運動の低下，よろめき歩行，伏臥，横臥，昏睡）と血中未変化体濃度との間に相関関係があることを明らかにした．またMMBIの尿中代謝物として脱硫体を明らかにした．
Keywords: 2-mercaptobenzimidazole, 2-mercaptomethylbenzimidazole, toxicokinetics

Maita, K.*, Kuwahara, M.*, Kosaka, T.*, Inui, K.*, Sugimoto, K.*, Takeuchi, Y.*, Hatakenaka, N.*, Harada, T.*, Yasuhara, K., Mitsumori, K.: **Testicular toxicity of thiamphenicol in Sprague-Dawley rats**
J Toxicol. Pathol., **12**, 27-34 (1999)
Group of 12 male SD rats received oral treatment with thiamphenicol (TAP) at a dose of 0, 100, or 200 mg/kg/day for 4 weeks and, then, 4 of each group were left untreated for further 13 weeks. The fertility were not affected in all treated groups except 2 males at 200 mg/kg/day. The organ weights of testes and accessory genital organs, and the serum LH and testosterone levels were decreased, but not changed at 100 mg/kg/day. Histopathologically, disorganization of seminiferous tubules at 200 mg/kg/day, and the mild decreased number of all types of germ cells in stage analysis of the seminiferous tubules at 100 mg/kg/day were observed. After withdrawal period, all testes from rats at 200 mg/kg/day showed the structure so called "Sertoli only syndrome". The present results may suggest that the role of Sertoli cells should be more highlighted in the consideration of mechanism of testicular toxicity of TAP.
Keywords: testicular toxicity, thiamphenicol, rat
* The Institute of Environmental Toxicology

Takagi, H., Mitsumori, K., Onodera, H., Takegawa, K., Shimo, T., Koujitani, T., Hirose, M.: **A preliminary study of the effect of Plantago ovata forsk on the development of 7,12-dimethylbenz[a]anthracene-initiated rat mammary tumors under the influence of hypercholesterolemia**
J Toxicol. Pathol., 12, 141-145 (1999)
To elucidate whether Plantago ovata forsk (PO) exhibits inhibiting effects on mammary carcinogenesis under a condition of hypercholesterolemia, female SD rats were first given a single oral administration of DMBA. From one week later, group 1 and group 2 received high cholesterol diets (HC) with and without 5% PO supplementation for 26 weeks, respectively. Group 3 and group 4 received basal diet (BD) with and without 5% PO supplementation for the same period, respectively. At the termination of the study, the serum levels of total cholesterol in group 1 were significantly lower than those in group 2 and the number of mammary masses was significantly decreased. Histopathologically, this decrease was due to the decreased incidences of mammary adenocarcinomas in group 1 compared with group 2, whereas due to the increased incidences of mammary adenocarcinomas in group 3 compared with group 4. The results of the present study suggest a possibility that PO exerts inhibiting effects on mammary carcinogenesis by decreasing cholesterol levels.
Keywords: mammary carcinogenesis, high cholesterol, plantago ovata forsk

Mitsumori, K., Onodera, H., Takahashi, M.*1, Funakoshi, T.*2, Tamura, T., Yasuhara, K., Takegawa, K., Takahashi, M.: **Promoting effects of kojic acid due to serum TSH elevation resulting from reduced serum thyroid hormone levels on development of thyroid proliferative lesions in rats initiated with N-bis(2-hydroxypropyl)nitrosamine**
Carcinogenesis, **20**, 173-176 (1999)
To examine whether kojic acid (KA) exerts a promoting effect on thyroid carcinogenesis, male rats were initiated with BHP and received basal diet containing 0 or 2%KA for 12 weeks. The serum T3 and T4 levels were significantly decreased and serum TSH was markedly increased in the BHP+KA group at weeks 4 and 12. Focal thyroid follicular hyperplasias and adenomas were observed in the BHP+KA group at weeks 4 and 12. These results suggest that thyroid proliferative lesions were induced by KA administration due to continuous serum TSH stimulation through the negative feedback mechanism of the pituitary-thyroid axis.
Keywords: kojic acid, thyroid tumor, TSH
*1 Division of Pathology, Sasaki Institute
*2 Safety Research Institute, Yoshitomi Pharmaceuticals

Tamura, T., Mitsumori, K., Onodera, H., Fujimoto, N.*, Yasuhara, K., Takegawa, K., Takahashi, M.: **Inhibition of thyroid iodine uptake and organification in rats treated with kojic acid**
Toxicol. Sci., 47, 170-175 (1999)
To elucidate the mechanisms of reduction of serum thyroid hormones caused by continuous administration of kojic acid (KA) and its thyroid tumor-promotion effects, male rats were given basal diet containing 0%, 0.008%, 0.03%, 0.125%, 0.5%, or 2% KA for 4 weeks. The thyroid 125I uptake was significantly decreased in the groups receiving 0.03% or more. Significant reduction of organic formation of iodine and serum T3 and T4 levels were observed in the 2% KA group along with pronounced elevation of serum TSH. Decreased colloid and follicular cell hypertrophy in the thyroid were observed in the groups given 0.03% or more. In morphometrical analysis, the ratio of the area of follicular epithelial cells to the area of colloids was significantly increased in the groups given 0.03% or more. The results suggest that KA alteration of thyroid-related hormone levels in the 2% KA group are due to inhibition of iodide uptake and iodine organification in the thyroid.
Keywords: kojic acid, thyroid iodine uptake, iodine organification
* Hiroshima University Medical School

Tamura, T., Mitsumori, K., Onodera, H., Takahashi, M.*1, Funakoshi, T.*2, Yasuhara, K., Takegawa, K., Takagi, H., Hirose, M.: **Time course observation of serum thyroid-related hormone levels and thyroid proliferative lesions in**

rats treated with kojic acid after DHPN initiation

J. Toxicol. Sci., 24, 145-155 (1999)

Time course changes in thyroid proliferative lesions as well as related hormone levels of male rats initiated with DHPN followed by basal diet containing 0, 2 or 4% kojic acid (KA) were examined at Weeks 1, 2, 4, 8 and 12. Serum T3/T4 levels in the DHPN+2%KA and DHPN+4%KA groups were significantly reduced as compared with the DHPN-alone group at each time point. Serum TSH levels in both DHPN+KA groups were significantly increased at each time point in a treatment period-dependent manner from Weeks 1 to 12. Focal follicular cell hyperplasias and adenomas of the thyroid were observed in the DHPN+2%KA group from Week 4 and in the DHPN+4%KA group from Week 8. These results strongly suggest that thyroid proliferative lesions were induced by KA administration due to continuous serum TSH stimulation through the negative feedback mechanism of the pituitary-thyroid axis.

Keywords: kojic acid, thyroid tumor, TSH

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Shinoda, K.*1, Mitsumori, K., Yasuhara, K., Uneyama, C., Onodera, H., Hirose, M. and Uehara, M.*2: **Doxorubicin induces male germ cell apoptosis in rats**

Arch. Toxicol., **73**, 274-281 (1999)

To clarify whether apoptosis is involved in doxorubicin (DXR)-induced testicular toxicity, adult rats were treated with a single intravenous dose of DXR (8 or 12 mg/kg) and euthanized at 3, 6, 12, 24, and 48 h thereafter. Germ cell degeneration was first found 6 h after dosing in meiotically dividing spermatocytes and early round spermatids of seminiferous tubules at stage I, and subsequently observed in spermatogonia at stages I-VI showing ultrastructural characteristics of apoptosis. Coincident with the appearance of morphologic changes, degenerating germ cells were shown to be undergoing apoptosis as revealed by in situ terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling. DNA laddering on gel electrophoresis was apparent 24 and 48 h after dosing. The results demonstrate that apoptosis plays an important role in the induction of testicular toxicity caused by DXR with meiotically dividing spermatocytes and type A and intermediate spermatogonia as highly vulnerable target cells.

Keywords: doxorubicin, apoptosis, testis

*1 Chemicals Evaluations and Research Institute

*2 Faculty of Agriculture, Tottori University

Ichiki, T., Onodera, H., Uneyama, C., Takegawa, K., Yasuhara K., Hirose, M., Kikuchi, M.*, Mitsumori, K.: **Liver tumor promoting effects of febantel in a two-stage hepatocarcinogenesis model of rats using d-galactosamine and diethylnitrosamine**

J. Toxicol. Pathol., **12**, 113-117 (1999)

To examine whether febantel has a tumor promoting activity, male rats were initiated with a single intraperitoneal injection of DEN. Groups 1, 2, and 3 were fed diet containing 2500, 500, and 0 ppm febantel, respectively, for 7 weeks. D-galactosamine (DGA) of 300 mg/kg was administered intraperitoneally at Weeks 1 and 6 of febantel treatment. The remaining two groups were fed diet containing 2000 or 0 ppm febantel without DGA treatment for 7 weeks. Centrilobular liver cell hypertrophy was observed in the DEN/DGA+2500 ppm and DEN+2000 ppm groups. Significant induction of CYP1A1/2, 2B1/2 and 4A1/3 was observed in the DEN/DGA+2500 ppm and DEN+2000 ppm groups, as compared to the corresponding control groups. Significant increases in GST-P positive cells and/or mini-foci in the liver were observed in the DEN/DGA+2,500 ppm group. These results suggest that febantel exerts liver tumor promotion potential.

Keywords: febantel, liver, carcinogenesis

* School of Medicine, Fukuoka University

Shoda, T., Onodera, H., Takeda, M.*1, Uneyama, C., Imazawa, T., Takegawa, K., Yasuhara, K., Watanabe, T.*2, Hirose, M., Mitsumori, K.: **Liver tumor promoting effects of fenbendazole in rats**

Toxicol. Pathol., **27**, 553-562 (1999)

To examine whether fenbendazole has tumor promoting activity, rats were initiated with a single intraperitoneal injection of DEN or given the saline vehicle alone, and given diet containing 3600, 1800, 600, 200, 70 or 0 ppm of fenbendazole for 8 weeks. After 8 weeks, periportal hepatocellular hypertrophy was observed in the groups given 600 ppm and greater. Induction of cytochrome P450 (CYP) 1A2, 2B1 or 4A1 was noted in the fenbendazole-treated groups. The numbers and areas of connexin 32 (Cx32)-positive spots per cm2 in centrilobular hepatocytes were significantly decreased with fenbendazole-treatment after DEN initiation. In situ hybridization for Cx32 mRNA revealed a remarkable decrease in its expression in the centrilobular hepatocytes in the DEN+70 ppm group. The numbers of GST-P positive foci were significantly increased in the DEN+1800 and DEN+3600 ppm groups. The present results indicate that fenbendazole may be a liver tumor promoter.

Keywords: fenbendazole, liver, carcinogenesis

*1 Institute of Environmental Toxicology

*2 Research/Development Division, Sumika Technoservice

Shoda, T.*, Mitsumori, K., Onodera, H., Toyoda, K., Uneyama, C., Imazawa, T., Takahashi, M., Hirose, M.: **The relationship between decrease in Cx32 and induction of P450 isozymes in the early phase of clofibrate hepatocarcinogenesis in the rat**

Arch. Toxicol., 73, 373-380 (1999)

To examine the relationship between the decrease in Cx32 and induction of P450 isozymes in the early phase of clofibrate hepatocarcinogenesis, male rats were initiated with a single intraperitoneal injection of DEN or given the saline vehicle alone and given diet containing 0.18, 0.09 or 0% clofibrate for 6 weeks. Diffuse hepatocellular hypertrophy with granular cytoplasmic eosinophilia was observed in the clofibrate treated rats. Positive immunostaining for anti-CYP 4A1 and CYP 2B1 were observed diffusely and centrilobularly, respectively. The numbers and areas of connexin 32 (Cx32)-positive spots per hepatocyte in the centrilobular areas in the treated rats were significantly decreased. The numbers and areas of GST-P positive foci were decreased in a dose dependent manner in the clofibrate treated groups. These results suggest that the CYP 2B1/2 induction and Cx32 decrease in centrilobular hepatocytes may also play important roles in clofibrate actions in the liver.

Keywords: clofibrate, liver, promotion

* Research Laboratories, Torii Pharmaceutical Co., Ltd.

Koujitani, T., Yasuhara, K., Kobayashi, H.*1, Shimada, A.*2,

Onodera, H., Takagi, H., Hirose, M., Mitsumori, K.: **Tumor promoting activity of 2,6-dimethylaniline in a two-stage nasal carcinogenesis model in N-bis(2-hydroxypropyl) nitrosamine-treated rats**

Cancer Lett., **142**, 161-171 (1999)

Potential promotion activity on nasal carcinogenesis of 2,6-dimethylaniline (DMA), an adrenergic agonist metabolite of xylazine which is used for food-producing animals as a sedative agent, was examined. Male F344 rats received diet containing 0 or 3000 ppm DMA for 52 weeks after initiation with DHPN. Histopathological assessment showed the incidence of carcinomas in the DHPN + DMA group (33%) to be significantly elevated as compared with that for the DHPN alone group (5%). Incidences and/or multiplicity of epithelial hyperplasias and dysplastic foci were also increased in the DHPN + DMA group. These lesions were exclusively observed in the olfactory mucosa. The lowest plasma levels of DMA in tumor- and dysplastic foci-bearing rats were 0.05 and 0.20 g /ml, respectively. These results indicate that DHPN acts as an appropriate initiator for nasal carcinogenesis and that DMA exerts a tumor promoting effect on the olfactory mucosa in the rat nasal cavity.

Keywords: 2,6-dimethylaniline, carcinogenicity, nasal cavity

*1 Research Institute of Japan Plant Protection Association
*2 The Institute of Environmental Toxicology

Koujitani, T., Yasuhara, K., Kobayashi, H.*1, Shimada, A.*2, Onodera, H., Takagi, H., Tamura, T., Hirose, M., Mitsumori, K.: **Absence of tumor promoting activity of xylazine in a two-stage nasal carcinogenesis model in N-bis(2-hydroxypropyl)nitrosamine-treated rats**

J. Toxicol. Pathol., **12**, 203-208 (1999)

Potential promotion activity of nasal carcinogenesis by xylazine, used in veterinary medicine, was examined using a DHPN-initiated two-stage nasal carcinogenesis model. Male rats received diet containing 1000 or 0 ppm xylazine hydrochloride (XZ) for 52 weeks after initiation with DHPN. Epithelial hyperplasias, dysplastic foci, adenomas, undifferentiated carcinomas, and/or a squamous cell carcinoma in the nasal cavity were observed in the groups initiated with DHPN, but the incidences of these proliferative lesions were not altered by the treatment of XZ. Plasma levels of xylazine and 2,6-dimethylaniline (DMA) were almost below the detection limit. These results suggest that XZ does not have any tumor promoting effect in the nasal tissues and its conversion to carcinogenic DMA is very low in vivo. The possibility of nasal carcinogenic effects of DMA in consumers via ingestion of edible tissues in food-producing animals treated with xylazine is extremely low.

Keywords: xylazine, carcinogenicity, nasal cavity

*1 Research Institute of Japan Plant Protection Association
*2 The Institute of Environmental Toxicology

Fujimoto, N.*, Onodera, H., Mitsumori, K., Tamura, T., Maruyama, S.*, Ito, A.*: **Changes in thyroid function during development of thyroid hyperplasia induced by kojic acid in F344 rats**

Carcinogenesis, **20**, 1567-1571 (1999)

To clarify the mechanism of tumorigenesis by kojic acid (KA), iodine uptake in the thyroid and serum levels of TSH and thyroid hormone were investigated in rats fed diet containing 2% KA. After 4 weeks, thyroid hyperplasia was apparent in males, associated with a decrease in 125I uptake into the thyroid to only 3% of that in controls. The serum T3/T4 levels dropped from the initial values and TSH increased seven times. Inhibition of organic iodination was only observed after 3 weeks treatment. On return to the control diet, normal serum T3, T4 and TSH levels become evident within 48 hr, suggesting that KA interrupts thyroid function, primarily by inhibiting iodine intake, consequently causing a decrease in serum T3 and T4. Increased TSH from the pituitary in turn stimulates thyroid hyperplasia, which is reversible on withdrawal of KA.

Keywords: kojic acid, thyroid hormone, F344 rat

* Hiroshima University

Kasahara, K., Nishikawa, A., Furukawa, F., Ikezaki, S., Tanakamaru, Z., Takagi, H., Ikeda, T., Imazawa, T., Takahashi, M.: **Suppressive effects of josamycin on the development of altered liver cell foci and chronic nephropathy in a carcinogenicity study**

Food Chem. Toxicol., **37**, 61-67 (1999)

The carcinogenicity of josamycin was examined in F344 rats. Groups of 50 males and 50 females were given the compound in their diet at concentrations of 0, 1.25 or 2.5% for 104-weeks; these dose levels were selected on the basis of the results of a subchronic study, in which animals rather rejected 5% josamycin. All surviving rats were killed at wk 106. A variety of tumours developed in all groups, including the control group, but all the neoplastic lesions were histologically similar to those known to occur spontaneously in this strain of rats, and no statistically significant increase in the incidence of any tumour was found in the treated groups of either sex. Interestingly, the josamycin treatment significantly reduced the development of altered liver cell foci and chronic nephropathy in a dose-dependent manner. Thus, it was concluded that, under the present experimental conditions, josamycin is not carcinogenic in F344 rats.

Keywords: josamycin, carcinogenicity study

Nishikawa, A., Furukawa, F., Lee, I-S.*1, Kasahara, K., Tanakamaru, Z., Nakamura, H., Miyauchi, M., Kinae, N.*2, Hirose, M.: **Promoting effects of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone on rat glandular stomach carcinogenesis initiated with N-methyl-N'-nitro-N-nitrosoguanidine**

Cancer Res., **59**, 2045-2049 (1999)

The modifying effects of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a mutagenic by-product in chlorinated water, on the development of glandular stomach cancers were investigated in male Wistar rats. After initiation with 100 ppm MNNG solution and 5% NaCl diet for 8 weeks, 30 rats each in groups 1-3 were given MX in the drinking water at concentrations of 30, 10, or 0 ppm for the following 57 weeks. Ten animals each in groups 4-6 were administered the MX without prior carcinogen exposure. The incidences and multiplicities of adenocarcinomas in the glandular stomachs were significantly higher in the initiated 30 ppm MX group than those in the MNNG/NaCl group. The incidences of atypical hyperplasias in the glandular stomachs were also significantly increased by the MX treatments. With their multiplicity, the effects were clearly dose dependent. The results of the present study thus indicate that MX exerts promoting effects when given during the postinitiation phase of two-stage glandular stomach carcinogenesis in rats.

Keywords: MX, stomach carcinogenesis, promotion

*1 Keimyung University

*2 University of Shizuoka

Miyauchi, M., Nishikawa, A., Furukawa, F., Kasahara, K., Nakamura, H., Takahashi, M., Hirose, M.: **Carcinogenic risk assessment of MeIQx and PhIP in a newborn mouse two-stage tumorigenesis assay**
Cancer Lett., 142, 75-81 (1999)

A newborn mouse two-stage tumorigenesis assay was evaluated as a possible alternative to chronic rodent carcinogenicity bioassays by investigating the carcinogenicity of two major heterocyclic amines, MeIQx and PhIP. One week after birth, CD-1 mice of both sexes were subcutaneously administered BOP at a dose of 50 mg/kg as an initiation treatment and starting 2 weeks thereafter they were fed diets supplemented with MeIQx at concentrations of 300, 30, 3 or 0 ppm or PhIP at 200, 50, 10 or 0 ppm for 23 weeks. Pulmonary adenomas and adenocarcinomas were observed in all groups with high incidences, without any significant differences between the groups. MeIQx and PhIP did not influence the multiplicity except in the group given 10 ppm PhIP where it significantly increased the number of pulmonary adenomas. Similarly, hepatocellular adenomas and carcinomas were found in all groups with high incidences, and again MeIQx and PhIP did not increase their incidences or multiplicities. These results thus suggest that the tumor-promoting effects of MeIQx or PhIP may be rather weak, if present, as far as this newborn two-stage model is concerned.
Keywords: newborn mouse, MeIQx , PhIP

Nishikawa, A., Furukawa, F., Kasahara, K., Tanakamaru, Z., Miyauchi, M., Nakamura, H., Ikeda, T., Imazawa, T., Hirose, M.: **Failure of phenethyl isothiocyanate to inhibit hamster tumorigenesis induced by N-nitrosobis(2-oxopropyl)amine when given during the post-initiation phase**
Cancer Lett., **141**, 109-115 (1999)

The chemopreventive influence of phenethyl isothiocyanate (PEITC) during the post-initiation stage was investigated in the BOP-initiated hamster tumorigenesis model. Animals in groups 1-3, each consisting of 30 hamsters, were injected twice with BOP. Starting 1 week after the second BOP injection, hamsters in groups 1 and 2 were fed diets supplemented with 6 and 3 micromol/g of PEITC, respectively, for 51 weeks. Animals in group 3 were treated as an initiation positive control. Animals in groups 4-6 were given 6 micromol/g or 3 micromol/g of PEITC alone, or were non-treated, matched negative controls for groups 1-3. Taken together with our previous finding that PEITC dramatically inhibited the initiation phase of BOP-induced pancreatic and lung tumorigenesis in hamsters, it can be concluded that PEITC specifically exerts chemopreventive effects only when given concomitantly with the carcinogen.
Keywords: phenethyl isothiocyanate, post-initiation

Ikezaki, S., Nishikawa, A., Furukawa, F., Tanakamaru, Z., Nakamura, H., Mori, H.*, Hirose, M.: **Influences of long-term administration of 24R, 25-dihydroxyvitamin D3, a vitamin D3 derivative, in rats**
J. Toxicol. Sci., 24, 133-139 (1999)

In order to examine the influences by long-term feeding of 24R, 25 dihydroxyvitamin D3[24R, 25(OH)2D3], an active form of vitamin D, male Wistar rats were fed a powder diet containing 0 or 5 ppm 24R, 25(OH)2D3 for 57 weeks. Urinary calcium levels were significantly increased by the administration of 24R, 25(OH)2D3 at weeks 3, 22 and 56, although the levels of serum calcium did not differ between the groups at week 57. In the 24R, 25(OH)2D3 group, weights of the adrenals and femurs were significantly increased. Histopathologically, this was found due to thickening of cortical bone in the femurs, and medullary hyperplasia and pheochromocytoma of the adrenals. These results indicate that 24R, 25(OH)2D3 at a dose with which serum calcium is not chronically increased causes thickening of the cortex of the femur, and development of adrenal proliferative lesions.
Keywords: 24R, 25-dihydroxyvitamin D3, long-term study
* Gifu University School of Medicine

Mori, H.*, Matsunaga, K.*, Tanakamaru, Z.*, Kawabata, K.*, Yamada, Y.*, Sugie, S.*, Nishikawa, A.: **Effects of protocatechuic acid, S-methylmethanethiosulfonate or 5-hydroxy-4-(2-phenyl-(E)ethenyl)-2(5H)-furanone (KYN-54) on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced pulmonary carcinogenesis in mice**
Cancer Lett., **135**, 123-127 (1999)

Modifying effects of dietary exposure of protocatechuic acid (PCA), S-methylmethanethiosulfonate (MMTS), and 5-hydroxy-4-(2-phenyl-(E)ethenyl)-2(5H)-furanone (KYN-54) on NNK-induced pulmonary carcinogenesis were examined in female A/J mice. Each of the test chemicals was given in diets during initiation or post-initiation phases (PCA, 1000 ppm; MMTS, 100 ppm; KYN-54, 200 ppm). All of these did not exert any preventive effect in this model when the incidence or multiplicity of pulmonary tumors (adenomas) of mice given NNK and the test chemical was compared to that of mice exposed to the carcinogen alone. In contrast, KYN-54 has a promoting effect on pulmonary carcinogenesis in mice. These data indicate an organotropic activity of these compounds and suggest that candidate compounds for cancer chemoprevention need to be carefully examined for effectiveness in multiple organs by different models.
Keywords: protocatechuic acid, S-methylmethanethiosulfonate, 5-hydroxy-4-(2-phenyl-(E)ethenyl)-2(5H)-furanone
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Koide, A.*, Fuwa, K.*, Furukawa, F., Hirose, M., Nishikawa, A., Mori, Y.*: **Effect of cigarette smoke on the mutagenic activation of environmental carcinogens by rodent liver**
Mutat. Res., 428, 165-76 (1999)

In order to assess the effect of cigarette smoke (CS) on metabolic enzymes, male hamsters and rats were exposed for two weeks to smoke produced in a Hamburg type II smoking machine. Mutagenic activities of seven heterocyclic amines (HCAs) in TA98 in the presence of rat or hamster liver S9 were elevated above controls. Enhancement of mutagenic activities of PhIP and aflatoxin B(1) was observed only in CS-exposed hamster. 7,8-Benzoflavone and/or furafylline considerably inhibited the mutagenic activation of IQ and Trp-P-1 in the presence of liver S9 from untreated hamsters and sham smoke- or CS-exposed hamsters and rats. Western immunoblot analyses of liver microsomes revealed that CS-exposure increased the levels of hamster CYP1A2 and rat CYP1A2 and CYP1A1, without significant change in the levels of CYP2E1 and CYP2B and 3A isoforms in each species. The presently observed selective induction of HCA activation and CYP isozymes due to CS supports the idea that CS may contribute to enhancing effects on initiation by carcinogens which are metabolically activated by hepatic CYP1A1/1A2.

Keywords: cigarette smoke, mutagenic activation, environmental carcinogen

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Chung, F.-L.*1, Nath, R.G.*1, Nagao, M.*2, Nishikawa, A., Zhou, G.D.*3, Randerath, K.*3: **Endogenous formation and significance of 1,N2-propanodeoxyguanosine adducts**
Mutat. Res., **424**, 71-81 (1999)

The detection of 1, N2-propanodeoxyguanosine adducts in the DNA of rodent and human tissues as endogenous lesions has raised important questions regarding the source of their formation and their roles in carcinogenesis. Both in vitro and in vivo studies have generated substantial evidence which supports the involvement of short- and long-chain enals derived from oxidized polyunsaturated fatty acids (PUFAs) in their formation. These studies collectively demonstrate that tissue lipid peroxidation is a main endogenous pathway leading to propano adduction in DNA. The possible contribution from environmental sources, however, cannot be completely excluded. The mutagenicity of enals and the mutations observed in site-specific mutagenesis studies using a model 1, N2-propanodeoxyguanosine adduct suggest that these adducts are potential promutagenic lesions. The increased levels of the propano adducts in the tissue of carcinogen-treated animals also provide suggestive evidence for their roles in carcinogenesis. The involvement of these adducts in tumor promotion is speculated on the basis that oxidative condition in tissues is believed to be associated with this process.
Keywords: 1,N2-propanodeoxyguanosine, lipid peroxidation, carcinogenesis

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Furukawa, F., Nishikawa, A., Kasahara, K., Lee, I-S.*1, Wakabayashi, K.*2, Takahashi, M., Hirose, M.: **Inhibition by beta-carotene of upper respiratory tumorigenesis in hamsters receiving diethylnitrosamine followed by cigarette smoke exposure**
Jpn. J. Cancer Res., 90, 154-161 (1999)

The modifying effects of beta-carotene at various doses on the development of upper respiratory tract tumors were investigated in Syrian hamsters treated with DEN and cigarette smoke. After a single s.c. injection of 100 mg/kg DEN, groups 1-4 hamsters were respectively administered diets supplemented with beta-carotene, at doses of 0.5%, 0.05%, 0.005% and 0% during experimental weeks 1 to 13, and simultaneously exposed to cigarette smoke. In all the groups, epithelial hyperplasias and/or papillomas were induced in the larynx and trachea. However, the incidences and multiplicities of papillomas in group 1 were significantly decreased as compared to the group 4 values. Moreover, the beta-carotene treatments significantly reduced both incidences and multiplicities of hyperplasias in a dose-dependent manner. Our results thus indicate that beta-carotene exerts inhibitory effects, even at the high dose of 0.25%, under the present experimental conditions.
Keywords: beta-carotene, cigarette smoke, respiratory tumorigenesis

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Furukawa, F., Nishikawa, A., Miyauchi, M., Nakamura, H., Son, H-Y., Hirose, M.: **Concurrent administration of fish meal and sodium nitrite does not promote glandular stomach carcinogenesis in rats after treatment with N-methyl-N'-nitro-N-nitrosoguanidine**
J. Toxicol. Pathol., **12**, 171-176 (1999)

Post-initiation modifying effects of concurrent administration of fish meal and sodium nitrite on the development of glandular stomach tumors after treatment with MNNG were investigated in male Wistar rats. Groups 1-3 were given 100 ppm MNNG in their drinking water and 10% NaCl in diet for 8 weeks as an initiation treatment for gastric cancer induction and thereafter respectively fed diets containing 64%, 32%, and 8% fish meal, and simultaneously given 0.12% sodium nitrite in the drinking water for 52 weeks. Groups 4-6 were similarly treated without the application of MNNG and NaCl. At the end of the 60th experimental week, all surviving animals were autopsied and examined histopathologically for the existence of gastric proliferative lesions. The incidences and multiplicity of atypical hyperplasias or adenocarcinomas were comparable among groups 1-3. No gastric proliferative lesions were found in groups 4-6. Our results suggest that concurrent administration of fish meal and sodium nitrite does not affect the post-initiation phase of MNNG-initiated glandular stomach carcinogenesis in the rat.
Keywords: fish meal, sodium nitrite, stomach carcinogenesis

Lee, I-S.*, Nishikawa, A., Furukawa, F., Kasahara, K., Kim, S-U.*: **Effects of Selaginella tamariscina on in vitro tumor cell growth, p53 expression, G1 arrest and in vivo gastric cell proliferation**
Cancer Lett., **148**, 81-86 (1999)

Selaginella tamariscina (ST), an oriental medicinal plant, was extracted using several solvents, and each fraction was assayed for its tumoricidal effects with MTT. Then influences on expression of p53 and induction of G1 arrest in cell cycle were respectively analyzed by northern blotting and flow cytometry. Modifying effects of pulverized ST on cell turnover in the stomach were also investigated in rats given 150mg/kg of MNNG by gavage and then fed a diet containing 5%, 1% or 0% ST. Fractions I-V showed significant tumoricidal effects against cultured human leukemia cells whereas fractions II-IV did not affect normal human lymphocytes. Among the effective fractions, the water-extracted fraction efficiently increased p53 gene expression and induced G1 arrest. The 1% ST feeding caused a significant reduction in the PCNA-labeling index of the glandular stomach epithelium as compared with the MNNG alone group value although 5% ST feeding was only associated with a tendency for decrease. These results suggest that ST could be a candidate chemopreventive as well as chemotherapeutic agent against gastric cancer.
Keywords: selaginella tamariscina, stomach cancer, cell proliferation

* Keimyung University

Tanaka, T.*1, Sugiura, H.*2, Inaba, R.*3, Nishikawa, A., Murakami, A.*4, Koshimizu, K.*4, Ohigashi, H.*5: **Immunomodulatory action of citrus auraptene on macrophage functions and cytokine production of lymphocytes in female BALB/c mice**
Carcinogenesis, **20**, 1471-1476 (1999)

The modifying effects of auraptene on macrophage and lymphocyte functions were investigated in mice. Female BALB/c mice were gavaged with auraptene at a dose of 100, 200 or 400

mg/kg once a day for 10 consecutive days. Glucose consumption of peritoneal macrophages was significantly higher than that in the control in auraptene-treated mice. Activity of acid phosphatase in peritoneal macrophages was increased at a dose level of 100 mg/kg. In addition, peritoneal macrophages in the auraptene-treated mice showed increased activity of beta-glucuronidase and increased production of interleukin (IL)-1beta and tumor necrosis factor-alpha. Stimulation indices in mice given auraptene at a dose of 200 mg/kg were significantly higher than that in the control group. However, IL-2 and IFN production stimulated by Con A were significantly increased in mice at 100 and 200 mg/kg. These findings might suggest that oral administration of auraptene effectively enhanced macrophage and lymphocyte functions in mice.

Keywords: auraptene, macrophage, cytokine

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*4 Kinki University
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Tamura, T., Shibutani, M., Toyoda, K., Shoda, T., Takada, K., Uneyama, C., Takahashi, M., Hirose, M.: **Tumor-promoting activities of hydroquinone and 1,1-dimethylhydrazine after initiation of newborn mice with 1-methyl-1-nitrosourea**
Cancer Lett., **143**, 71-80 (1999)

To clarify the suitability of a newborn-mouse assay to detect tumor-promoting activities of carcinogens, the non-genotoxic hydroquinone(HQ) and genotoxic 1,1-dimethyl-hydrazine (UDMH) were administered to mice during the promotion stage after treatment with MNU at day 9 after birth. Initiated males and females thus received either 0.8%HQ in basal diet, or UDMH, at 20mg/kg b.w. once weekly by s.c. injection, from day 14 until 30 weeks of age. HQ significantly increased the incidence and/or multiplicity of altered foci and adenomas/carcinomas in male liver, and adenomas/carcinomas in female lung. In addition, 4/11 mice in the MNU+HQ-treated males developed lung carcinomas. UDMH also exhibited a tendency to increase the incidence and multiplicity of lung adenomas in females. Thus tumor-promoting effects of HQ or UDMH were apparently exerted in the target organs and the MNU-initiated two-stage newborn-mouse carcinogenesis assay may be useful for detection of genotoxic or non-genotoxic carcinogenicity.

Keywords: new-born, two-stage carcinogenesis assay, mouse

Lee, C.C.R.*1, Wanibuchi, H.*1, Yamamoto, S.*1, Hirose, M., Hayashi, Y.*2, Fukushima, S.*1: **Molecular cytogenetic identification of cyclin D1 gene amplification in a renal pelvic tumor attributed to phenacetin abuse**
Pathol. Int., **49**, 648-652 (1999)

A phenacetin-associated renal pelvic carcinoma from an 80-year-old female patient was evaluated by molecular cytogenetic methods. Fluorescence in situ hybridization was used to identify chromosome gains or losses for the cyclin D1, p53, Rb and c-myc genes and the ploidy of their respective chromosomes. Cyclin D1 gene amplification, but normal copy numbers of p53, Rb and c-myc, and normal ploidy of chromosomes 8, 11, 13 and 17 were observed. Expression of cyclin D1 protein was confirmed by immunohistochemistry. In the absence of p53, Rb or c-myc abnormalities, the results suggested that cyclin D1 gene amplification and its protein overexpression may be involved in the genesis of renal pelvic carcinomas associated with phenacetin abuse.

Keywords: cyclin D1, p53, phenacetin

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Hagiwara, A.*1, Boonyaphiphat, P.*1, Tanaka. H.*1, Kawabe, M.*1, Tamano, S.*1, Kaneko, H.*2, Matsui, M.*2, Hirose, M., Ito, N.*3, Shirai, T.*4: **Organ-dependent modifying effects of caffeine, and two naturally occurring antioxidants alpha-tocopherol and n-tritriacontane-16,18-dione, on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced mammary and colonic carcinogenesis in female F344 rats**
Jpn. J. Cancer Res., **90**, 399-405 (1999)

Modifying effects of caffeine, alpha-tocopherol, and n-tritriacontane-16,18-dione (TTAD) on PhIP-induced mammary and colonic carcinogenesis were investigated in female F344 rats. Groups of 20 rats were given 0.02% PhIP alone, or together with 0.1% caffeine, 0.5% alpha-tocopherol or 0.1% TTAD for up to 54 weeks. The final combined incidences and multiplicity of mammary adenomas and adenocarcinomas were significantly lowered in the PhIP plus caffeine group as compared to the PhIP alone value. On the other hand, rats given PhIP plus caffeine exhibited an elevated incidence of colon tumors. Metabolic activation of PhIP was inhibited by addition of caffeine in an in vitro assay. The results indicate that caffeine exerts a potent chemopreventive action against PhIP-induced mammary carcinogenesis, but acts as a co-carcinogen for PhIP-induced colonic carcinogenesis.

Keywords: PhIP, caffeine, mammary and colonic carcinogenesis

*1 Daiyu-kai Institute of Medical Science
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*3 Nagoya City University
*4 Nagoya City University Medical School

Hirose, M., Hakoi, K.*1, Takahashi, S.*1, Hoshiya, T.*1, Akagi, K.*1, Lin, C.*1, Saito, K.*2, Kaneko, H.*2, Shirai, T.*1: **Sequential morphological and biological changes in the glandular stomach induced by oral administration of catechol to male F344 rats**
Toxicol. Pathol., **27**, 448-455 (1999)

Histogenesis and mechanisms of catechol-induced rat glandular stomach carcinogenesis were investigated in male F344 rats. The initial morphological changes were edema of the gastric wall, inflammatory-cell infiltration, erosion in pyloric region close to the duodenum, and considerable increase in apoptosis at 12 hr; later, changes included augmented DNA synthesis and cell proliferation on day 1. Downward hyperplasia appeared at edges of ulceration at week 2, then submucosal hyperplasia in the course of adenoma development. No increase in lipid peroxide levels was evident in gastric epithelium fed catechol for 1 wk. Amounts of catechol bound to tissue protein were not specifically high in the glandular stomach. These results indicate that regenerative cell proliferation due to toxicity plays an important role in catechol-induced glandular stomach carcinogenesis. Protein binding and free radicals may not be largely responsible for the toxicity.

Keywords: catechol, cell proliferation, cytotoxicity

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Kimoto, N.*1, Hirose, M., Kawabe, M.*1, Satoh, T.*2, Miyataka, H.*2, Shirai, T.*1: **Post-initiation effects of a super critical extract of propolis in a rat two-stage carcinogenesis model in female F344 rats**
Cancer Lett., **147**, 221-227 (1999)

Post-initiation modifying effects of dietary administration of a super critical extract of propolis on major organs were examined. After initiation with 4 carcinogens, female rats were administered diet containing 0.1 or 0.01% propolis for 33 weeks. The incidence and multiplicity of mammary carcinomas were significantly decreased by the 0.1 and 0.01% propolis treatments. In the urinary bladder, the incidence of PN hyperplasia but not tumors was, in contrast, significantly increased by 0.1% propolis. Similarly, the number and area of GST-P positive liver foci were significantly elevated with this high dose. The results indicate that a low dose of a super critical extract of propolis may find application as a potent chemopreventor of mammary carcinogenesis.

Keywords: chemoprevention, mammary gland, propolis, F344 rat

*1 Nagoya City University Medical School
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Futakuchi, M.*, Hirose, M., Ogiso, T.*, Kato, K.*, Sano, M.*, Ogawa, K.*, Shirai, T.*: **Establishment of an in vivo highly metastatic rat hepatocellular carcinoma model**
Jpn. J. Cancer Res., **90**, 1196-1202 (1999)

Diethylnitrosamine exposure followed by a 16-week treatment with N-nitrosomorpholine was found to be a most efficient method for the induction of hepatocellular carcinoma metastasizing to the lung. Loss of cadherin, demonstrated immunohistochemically, occurred in an early stage of carcinogenesis, and this was reflected in malignant conversion of primary lesions. This model, with its essential similarities to malignant tumor behavior in man, should find application not only for elucidation of the mechanisms underlying metastasis, but also in the development of anti-metastatic agents.

Keywords: hepatocarcinogenesis, lung metastasis, N-nitrosomorpholine

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Hirose, M., Takahashi, S.*, Ogawa, K.*, Futakuchi, M.*, Shirai, T.*: **Phenolics: blocking agents for heterocyclic amine-induced carcinogenesis**
Fd. Chem. Toxicol., **37**, 985-992 (1999)

Chemopreventive effects of synthetic and naturally occurring antioxidants on heterocyclic amine (HCA)-induced rat carcinogenesis and mechanisms of inhibition were assessed. In a medium-term liver bioassay, combined treatment with 0.03% MeIQx and synthetic antioxidants such as HTHQ, BHA, BHT, TBHQ or propyl gallate, each at a dose of 0.25%, inhibited development of GST-P positive foci, after initiation with DEN. 8-OHdG and malonedialdehyde and 4-hydroxynonenal levels were not largely influenced by the treatment with MeIQx or antioxidants, either alone or in combination. On the other hand, quercetin, rutin, curcumin, daidzin, ferulic acid and genistein all exerted significant enhancing effects. The incidence of mammary carcinomas in female F344 rats induced by oral administration of 0.02% PhIP for 52 weeks was significantly decreased by simultaneous treatment with 0.5% HTHQ. These results indicate that synthetic antioxidant HTHQ is a very strong chemopreventor of HCA-induced carcinogenesis and that depressed metabolic activation rather than antioxidant activity is responsible for the observed effect.

Keywords: phenolic antioxidant. heterocyclic amine, chemoprevention

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Hirose, M., Fukushima, S.*1, Imaida, K.*2, Ito, N.*3, Shirai, T.*2: **Modifying effects of phytic acid and gamma-oryzanol on the promotion stage of rat carcinogenesis**
Anticancer Res., **19**, 3665-3670 (1999)

The modifying effects of phytic acid and gamma-oryzanol on the promotion stage of carcinogenesis were investigated. In a multi-organ carcinogenesis model, although the appearance of hepatic tumors was suppressed, the incidence of urinary bladder papillomas was increased by 2% phytic acid. In addition, the incidence and multiplicity of lung tumors were significantly increased by 1% gamma-oryzanol. Enhancing effects on DHPN-induced lung tumor were observed only at a dose of 1% gamma-oryzanol. When the modifying effects of phytic acid, and its salts were further examined in rats pretreated with BBN, a clear increase in the incidences of bladder tumors was noted only in sodium-PA. Examination of the modifying potential of phytic acid and gamma-oxyzanol on DMBA-induced mammary carcinogenesis in female SD rats revealed that the average tumor diameter was significantly reduced and the average survival time was increased with phytic acid. These results indicate that phytic acid inhibits hepatic and mammary carcinogenesis, while its sodium-salt is a promoter of bladder carcinogenesis. The effect of phytic acid itself on urinary bladder carcinogenesis is equivocal. Gamma-oryzanol is a promoter of lung carcinogenesis but its effect is weak and exerted only at a very high dose level.

Keywords: chemoprevention, phytic acid, gamma-oryzanol

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Hirose, M., Takahashi, S.*1, Ogawa, K.*1, Futakuchi, M.*1, Shirai, T.*1, Shibutani, M., Uneyama, C., Toyoda, K., Iwata H.*2: **Chemoprevention of heterocyclic amine-induced carcinogenesis by phenolic compounds in rats**
Cancer Lett., **143**, 173-178 (1999)

Chemopreventive effects of synthetic and naturally occurring antioxidants on heterocyclic amine (HCA)-induced rat carcinogenesis and mechanisms of inhibition were assessed. In a medium-term liver bioassay, combined treatment with 0.03% MeIQx and synthetic antioxidants such as HTHQ, BHA, BHT, TBHQ and propyl gallate, each at a dose of 0.25%, and troglitazone at doses 0.5 and 0.1%, potently inhibited development of GST-P positive foci as compared with MeIQx alone values, while quercetin, rutin, curcumin, daidzin, ferulic acid and genistin all exerted significant enhancing effects. HTHQ also inhibited PhIP-induced colon carcinogenesis in a two stage colon carcinogenesis model. Methoxyresorfin O-demethylase activity in rat liver microsomes in vitro was clearly inhibited by the addition of synthetic antioxidants, with particularly strong inhibition being observed in HTHQ. These results indicate that synthetic antioxidants, HTHQ in particular, is a very strong chemopreventor of HCA-induced carcinogenesis. Depression of metabolic activation is responsible for the observed effect.

Keywords: heterocyclic amine, antioxidants, chemoprevention, carcinogenesis

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Hiratsuka, H.*1, Satoh, S.*2, Satoh, M.*3, Nishijima, M.*4, Katsuki, Y.*4, Suzuki, J.*4, Nakagawa, J.*4, Sumiyoshi, M.*5, Shibutani, M., Mitsumori, K., Tanaka-Kagawa, T., Ando, M.: **Tissue distribution of cadmium in rats given minimum amounts of cadmium-polluted rice or cadmium chloride for 8 months**

Toxicol. Appl. Pharmacol., **160**, 183-91 (1999)

To investigate the relationship between cadmium (Cd) toxicity, intestinal absorption, and its organ distribution in rats treated orally with minimum amounts of Cd, female rats were given diets consisting of 28% purified diet and 72% ordinary rice containing Cd-polluted rice or CdCl2 for up to 8 months. As a result, no Cd-related toxic changes were observed. The concentrations of Cd in the liver and kidneys and MT in the liver, kidney, serum, and urine increased dose-dependently, whereas MT in the intestinal mucosa did not alter markedly at any time point. The distribution rates of Cd to the liver increased dose-dependently, whereas those to the kidney decreased dose-dependently. The Cd retention rates 5 days after 109Cd administration ranged from 0.2 to 1.0% at any time point. These results suggest that the distribution of Cd to the liver and kidneys after the oral administration vary depending on the dosage levels of Cd. The difference of the distribution pattern of Cd to the liver and kidney is probably due to the difference in the form of the absorbed Cd, i.e., free ion or Cd-MT complex, although not closely related to the MT in the intestinal mucosa.

Keywords: cadmium, tracer study, tissue distribution

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*2 Ina Research Inc.
*3 National Institute for Environmental Studies
*4 The Tokyo Metropolitan Research Laboratory Public Health
*5 Japan Food Research Laboratories

Nishikawa, T.*, Haresaku, M.*, Adachi, K.*, Masuda M.* and Hayashi, M.: **Study of a rat skin *in vivo* micronucleus test: data generated by mitomycin C and methyl methanesulfonate**

Mutat. Res., 444, 159-166 (1999)

We have developed an *in vivo* micronucleus (MN) test that uses rat skin as the target organ. Sample preparation involves cold-treating the epidermis with trypsin, peeling it off with a fine forceps, treating it in hypotonic solution, and staining it with acridine orange. We evaluated the assay using mitomycin C (MMC) and methyl methanesulfonate (MMS) as model clastogens, applying them as single and repeat treatments. Both chemicals induced a significant, dose-dependent increase in micronucleus frequency in basal cells. One treatment per day for three days was optimal for MN induction.

Keywords: skin, genotoxicity, micronucleus test

*Life Science Research Center, Lion

Tsuchiya, T. *, Hayashi, M., 他38名: **An interlaboratory validation study of the improved transformation assay employing Balb/c 3T3 cells: Results of a collaborative study on the two-stage cell transformation assay by the non-genotoxic carcinogen study group**

ATLA, **27**, 685-702 (1999)

The Non-Genotoxic Carcinogen Study Group of the Environmental Mutagen Society of Japan organized the first step of an interlaboratory validation study on an improved cell transformation assay employing Balb/c 3T3 A31-1-1 cells. Nineteen laboratories participated in this study. The modified transformation assay was evaluated for its responsiveness, its interlaboratory reproducibility and its transferability. In this study, a mixture of Dulbecco's modified Eagle's medium and nutrient mixture F12, supplemented with insulin-transferrin-ethanolamine-sodium selenite and 2% fetal bovine serum (FBS) was used during the period of expression of transformed foci. The results from this study support the usefulness of this modified two-stage transformation assay with Balb/c3T3 cells.

Keywords: cell transformation assay, Balb/c 3T3 cells, interlaboratory validation study

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Saotome, K.*, Sofuni, T. and Hayashi, M.: **A micronucleus assay in sea urchin embryos**

Mutat. Res., **446**, 121-127 (1999)

We have developed a micronucleus assay for use in sea urchin embryos. Treatment embryos at the early blastula stage (about 256 cells) were exposed to genotoxic treated with chemicals overnight until control embryos reached the gastrula stage. Then all embryos were suspended in 1 M urea, dissociated by pipetting, and fixed with methanol:acetic acid (9:1). The preparations were air-dried and stained with acridine orange. The test chemicals (mitomycin C, vinblastine and 1-β-D-arabinofuranosylcytosine) induced clear micronuclei dose-dependently. The maximum frequency induced with mitomycin C was 2-3% in *Clypeaster japonicus* and 1-2% in *Hemicentrotus pulcherrimus*.

Keywords: micronucleus assay, Sea urchin embryos, Air-drying method

*Yokohama City Institute of Health

Hothorn, L.A.*, Hayashi, M. and Seidel, D.*: **Dose-response relationships in mutagenicity assays including an appropriate positive control group: a multiple testing approach**

Environ. Ecol. Stat., 7, 27-42 (2000)

The objective of mutagenicity assays in regulatory toxicology is the decision on non-mutagenicity or mutagenicity. An inherent problem of statistical tests is the possibility of false decisions, i.e., a mutagenic substance will be falsely labeled as non-mutagenic or a non-mutagenic substance will be falsely labeled as mutagenic. These probabilities of false negative and/or false positive decision can be limited by using suitable testing procedures as well as a design including an appropriate positive control. Using the proof of hazard concept the well-known many-to-one procedures with total order restriction for increasing effect differences are used, while using the proof of safety concept procedures on equivalence with total order restriction are discussed. Both approaches are demonstrated on a real data example.

Keywords: dose-response analysis, positive control, mutagenicity studies

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Matsushima, T.*1, Hayashi, M., Matsuoka, A., Ishidate, M. Jr.*2, Miura, K.F.*2, Shimizu, H.*3, Suzuki, Y.*3, Morimoto, K.*4, Ogura, H.*4, Mure, K.*4, Koshi, K.*5 and Sofuni, T.: **Validation study of the in vitro micronucleus test in a Chinese hamster lung cell line (CHL/IU)**

Mutagenesis, **14**, 569-580 (1999)

We conducted a collaborative validation study, under the auspices of the Japanese Ministry of Labour, on the *in vitro* micronucleus test to see if it could be used as an alternative to the *in vitro* chromosome aberration test for evaluation of chemical safety. Concordance between was satisfactorily high (88.7%), and we concluded that the *in vitro* micronucleus test could be used in place of the chromosomal aberration test as a simple and rapid method for detecting clastogens and aneugens *in vitro*. We also propose a protocol for the test.

Keywords: *in vitro* micronucleus test, Chinese hamster lung cell line, validation study

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*3 The Jikei University School of Medicine
*4 Osaka University School of Medicine
*5 National Institute of Industrial Health

Suzuki, T., Itoh, S.*1, Nakajima, M.*2, Hachiya, N.*3 and Hara, T.*4: **Target organ and time-course in the mutagenicity of five carcinogens in Muta™Mouse: a summary report of the second collaborative study of the transgenic mouse mutation assay by JEMS/MMS**

Mutat. Res., **444**, 259-268 (1999)

Groups of mice were injected intraperitoneally with *N*-nitroso-di-*n*-propylamine (NDPA), propylnitrosourea , 7,12-dimethylbenz [*a*]-anthracene, 4-nitroquinoline-1-oxide (4NQO), or procarbazine. *LacZ* mutant frequencies of various organs, sampled 7, 14 and 28 days after treatment, were analyzed by positive selection. All chemicals, except NDPA, induced micronuclei. All chemicals increased *lacZ* MF in all of their target organs for carcinogenesis. Positive responses were also observed in a part of non-target organs although the increases were smaller than those in the target. These results suggest that a positive response in MF means the organ is susceptible for carcinogenesis but it does not always happen. The time-course of MF increases differed among tissues.

Keywords:organ specificity, Muta™Mouse, *lacZ*

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Suzuki, T., Uno, Y.*1, Idehara, K.*2, Baba, T.*2, Maniwa, J.*3, Ohkouchi, A.*2, Wang, X., Hayashi, M., Sofuni, T., Tsuruoka, M.*4, Miyajima, H.*4 and Kondo, K.*4: **Procarbazine genotoxicity in the Muta™Mouse; strong clastogenicity and organ-specific induction of *lacZ* mutations**

Mutat. Res., **444**, 269-281 (1999)

We analyzed the mutagenicity of procarbazine, a drug used for cancer chemotherapy, in various organs and the clastogenicity of the drug in hematopoietic cells of the *lacZ* transgenic Muta™Mouse. At 50 mg/kg, procarbazine induced micronuclei in hematopoietic cells, but it did not increase the *lacZ* mutant frequency (MF) in bone marrow, liver, testis, spleen, kidney, and lung. Five daily administrations of 150 mg/kg yielded highly positive MF responses in the drug's target organs for carcinogenesis (lung, bone marrow, and spleen). Liver showed only a slight increase in *lacZ* MF and brain showed no increase. The testis MF more than doubled suggests that procarbazine is mutagenic to germ cells.

Keywords: procarbazine, Muta™Mouse, *lacZ*

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Matsuoka, A., Matsuura, K., Sakamoto, H., Hayashi, M. and Sofuni, T.: **A proposal for a simple way to distinguish aneugens from clastogens in the *in vitro* micronucleus test**

Mutagenesis, **14**, 385-389 (1999)

We hypothesized based on the previous study in the *in vitro* micronucleus (MN) test that the frequency of polynuclear (PN) and mitotic (M) cells induced can distinguish aneugens from clastogens. To test the hypothesis, we conducted the MN test with well-known aneugens and clastogens. The clastogens induced MN but more than 200 PN or M cells. The aneugens induced MN, PN and M cells. We proposed that in addition to the increase in MN cells, chemicals that induce ≧200 PN cells /1000 cells and a statistically significant increase in M cells are aneugens and chemicals that induce <100 PN cells/1000 cells and do not increase M cell frequency are clastogens.

Keywords: aneugens, the in vitro micronucleus test

Matsuoka, A., Sakamoto, H., Tadokoro, S., Tada, A.*1, Terao, Y.*2, Nukaya, H.*2 and Wakabayashi, K.*1: **The 2-phenylbenzotriazole-type water pollutant PBTA-2 has cytochalasin B-mimetic activity**

Mutat. Res., **464**, 161-167 (2000)

2-[2-(Acetylamino)-4-[*N*-(2-cyanoethyl)ethylamino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2*H*-benzotriazole (PBTA-2) has been identified in samples from the Nishitakase River in Kyoto, Japan, and shows potent mutagenic activities in *S. typhimurium* with S9 mix. We conducted the *in vitro* micronucleus (MN) test on PBTA-2 in a Chinese hamster cell line CHL. PBTA-2 induced MN cells weakly, but PN cells strongly and dose dependently. PBTA-2 predominantly induced equal-sized binucleated cells. Rhodamine phalloidin staining revealed that PBTA-2 caused actin filament abnormalities similar to those caused by cytochalasin B. Cytochalasin B induced PN cells predominantly and almost all the cells were equal-sized and binucleate. The results suggest that PBTA-2 has cytochalasin B-mimetic activity, although agents affecting actin filaments, such as cytochalasins, phallotoxins and chloropeptide, have been derived only from molds so far.

Keywords: PBTA-2, cytochalasin B-mimetic activity

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*2 University of Shizuoka

Masumura, K., Matsui, M., Katoh, M.*1, Horiya, N.*2, Ueda, O.*3, Tanabe, H., Yamada, M., Suzuki, H.*3, Sofuni, T. and Nohmi, T.: **Spectra of *gpt* mutations in ethylnitrosourea-treated and untreated transgenic mice.**

Environ. Mol. Mutagen., 34(1), 1-8 (1999)

We have established a new transgenic mouse mutagenicity assay for the efficient detection of point mutations and deletions *in vivo*. In this assay, the *gpt* gene of *Escherichia coli* is used as a reporter for the detection of point mutations. Treatment of mice with ENU (150 mg/kg) enhanced the *gpt* mutant frequency in bone marrow. In the ENU-treated mice, more than 90% of the *gpt* mutations were base change mutations; the predominant types were A:T to T:A and G:C to A:T. We also report the establishment of homozygous transgenic mice that have transgene lambda EG10 DNA in both chromosome 17 of C57BL/6J mouse.

Keywords: transgenic mouse, *gpt*, ethylnitrosourea
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*2 Hatano Research Institute
*3 Chugai Phamaceutical Co. Ltd.

Nohmi, T., Suzuki, M., Masumura, K., Yamada, M., Matsui, K., Ueda, O.*1, Suzuki, H.*1, Katoh, M.*2, Ikeda, H.*3 and Sofuni, T.: **Spi⁻ selection: An efficient method to detect gamma-ray-induced deletions in transgenic mice.**
Environ. Mol. Mutagen., 34(1), 9-15 (1999)

To facilitate the detection and molecular analysis of deletion mutations *in vivo*, we established a transgenic mouse model using Spi⁻ (sensitive to P2 interference) selection. We showed nucleotide sequences of 41 junctions of deletion mutations induced by gamma-ray-irradiation. More than half of the large deletions occurred between short homologous sequences. The remaining junctions had no such homologous sequences. Two Spi⁻ mutants had P (palindrome)-like nucleotide additions at the breakpoints, which are observed in the coding junctions of V(D)J recombination, suggesting that broken DNA ends with hairpin structures can be intermediates in the repair of radiation-induced double-strand breaks.

Keywords: Spi⁻ selection, transgenic mouse, deletions
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Masumura, K., Matsui, K., Yamada, M., Horiguchi, M.*1, Ishida, K.*2, Watanabe, M.*2, Ueda, O.*3, Suzuki, H.*3, Kanke, Y.*1, Tindall, K.R.*4, Wakabayashi, K.*2, Sofuni, T. and Nohmi, T.: **Mutagenicity of 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP) in the new *gpt* delta transgenic mouse.**
Cancer Lett., **143**(2), 241-4 (1999)

Gender differences and organ specificity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced mutagenesis were examined. The *gpt* delta transgenic mouse model employed the two distinct selections, i.e., 6-thioguanine selection for point mutations and Spi⁻ selection for deletions, respectively. In both selections, the highest mutant frequencies were observed in colon, followed by in spleen and liver. No significant differences in mutant frequencies were observed in colon and liver between male and female mice. The correlation between PhIP-induced mutagenesis and carcinogenesis in colon is discussed.

Keywords: PhIP, 6-thioguanine selection, Spi⁻ selection
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Horiguchi, M.*1, Masumura, K., Ikehata, H.*2, Ono, T.*2, Kanke, Y.*1, Sofuni, T. and Nohmi, T.: **UVB-induced *gpt* mutations in the skin of *gpt* delta transgenic mice.**
Environ. Mol. Mutagen., **34**(2-3), 72-9 (1999)

Ultraviolet light B (UVB)-induced mutagenesis was studied in *gpt* delta transgenic mice. The mice were exposed to UVB at single doses of 0.3, 0.5, 1.0, 1.5, and 2.0 kJ/m^2. At 4 weeks after irradiation, the mutant frequencies (MF) of the *gpt* gene were determined in the epidermis and the dermis. The epidermis exhibited a higher sensitivity to UVB than the dermis at doses of 0.3 and 0.5 kJ/m^2 UVB. The UVB-induced mutation spectrum in the epidermis was dominated by G:C to A:T transitions at dipyrimidine sites, such as 5'-TC-3', 5'-CC-3', and 5'-T/C-CG-3'. Tandem transitions such as CC to TT were also observed. In contrast, G:C to A:T transitions at CpG sites and deletions were observed in nonirradiated mice. Hot spots of transitions were observed at different sites in UVB-irradiated and nonirradiated mice. These results indicate that *gpt* delta transgenic mouse is a suitable model for *in vivo* UVB-induced mutations at the molecular level.

Keywords: ultraviolet light B (UVB), *gpt* delta transgenic mouse, epidermis
*1 Tokyo University of Agriculture
*2 Tohoku University School of Medicine

Okada, N.*, Masumura, K., Nohmi, T. and Yajima, N.*: **Efficient detection of deletions induced by a single treatment of mitomycin C in transgenic mouse *gpt* delta using the Spi⁻ selection.**
Environ. Mol. Mutagen., 34(2-3), 106-11 (1999)

Spi⁻ selection has an advantage to preferentially identify deletions because only lambda phages deficient in both the *red* and *gam* gene functions are allowed to form phage plaques. We examined whether *in vivo* deletions induced by mitomycin C (MMC) are detectable by the Spi⁻ assay in the *gpt* delta transgenic mouse model. The mice were treated with MMC (0.5, 1.0, 2.0, and 4.0 mg/kg, single i.p.) and sacrificed 14 days after the dosing. The treatment at 4.0 mg/kg approximately doubled the Spi⁻ mutant frequency in the bone marrow. The molecular analyses indicated that seven Spi⁻ mutants at 4.0 mg/kg group had deletions with sizes from 0.8 to 8.5 kb, whereas no such deletions were observed in the control group. The results suggest that deletions induced by MMC in the bone marrow are efficiently detectable by Spi⁻ selection and also that the molecular analyses are useful to evaluate the significance of a marginal increase in mutant frequency in the transgenic rodent mutation assays.

Keywords: mitomycin C, *gpt* delta mouse, deletion
* Snow Brand Milk Products Co. Ltd.

Sui, H.*, Suzuki, M., Yamada, M., Hara, T.*, Kawakami, K.*, Shibuya, T.*, Nohmi, T. and Sofuni, T.: **Effects of O^6-alkylguanine-DNA alkyltransferase deficiency in *Escherichia coli* as the host for the detection of mutations in *lacI* transgenic mice**
Environ. Mol. Mutagen., **34** (2-3):221-6 (1999)

It is known that no mutagenicity of methyl methanesulfonate (MMS) is detected in epididymal sperm in various transgenic mice assays. To investigate whether DNA lesions in mature sperm can be transformed into mutations during replication of lambda phage in *E coli* cells, we developed strain YG5152, which is a derivative of SCS-8 but is deficient in the genes encoding *O*^{6}-alkylguanine-DNA alkyltransferases. Big Blue™ mice were treated with MMS or ENU and the phages rescued from mature sperm were infected to the *E. coli* strains. The MF of ENU-treated mice in YG5152 was two times higher than that in SCS-8. No increase was observed in the MMS-treated mice even in YG5152. These results suggest that, although YG5152 efficiently detects *ex vivo* mutations, no detectable levels of mutagenic methyl adducts are present in mature sperm of MMS-treated mice.

Keywords: sperm, *O*^{6}-alkylguanine-DNA alkyltransferases, methyl methanesulfonate,
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Minowa, O.*1, Arai, T.*2, Hirano, M.*1, Monden, Y.*2, Nakai, S.*1, Fukuda, M.*1, Itoh, M.*1, Takano, H.*1, Hippou, Y.*3, Aburatani, H.*3, Masumura, K., Nohmi, T., Nishimura, S.*2 and Noda, T.*1: **Mmh/Ogg1 gene inactivation results in accumulation of 8-hydroxyguanine in mice.**

Proc. Natl. Acad. Sci. U S A, **97**(8), 4156-61 (2000)

The major mutagenic base lesion in DNA caused by exposure to reactive oxygen species is 8-hydroxyguanine (8-OH-G). Products of the human *MMH/OGG1* gene are known to catalyze *in vitro* the reactions repairing this DNA lesion. To analyze the function of *Mmh in vivo*, we generated a mouse line carrying a mutant *Mmh* allele. *Mmh* homozygous mutant mice were found to have lost nicking activity in liver extracts for substrate DNA containing 8-OH-G. Substantial increase of spontaneous mutation frequencies was identified in *Mmh* mutant mice bearing transgenic *gpt* genes. These results indicate that exposure of DNA to endogenous oxidative species produces the mutagenic adduct 8-OH-G in mice, and *Mmh* plays an essential role in repair of this DNA damage.

Keywords: *OGG1*, 8-oxoguanine, transgenic mouse

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Kamataki, T.*1, Suzuki, A.*1, Kushida, H.*1, Iwata, H.*1, Watanabe, M.*2, Nohmi,T. and Fujita,K.*1: **Establishment of a *Salmonella* tester strain highly sensitive to mutagenic heterocylic amines.**

Cancer Lett., **143**, 113-116 (1999)

A co-expression plasmid (p1A2OR) carrying human CYP1A2 and NADPH-P450 reductase cDNAs and an expression plasmid (pOAT) carrying *Salmonella* TA1538/ARO cells were constructed. The CYP and OAT expressed in the *Salmonella* cells showed catalytic activity. The TA1538/ARO strain exhibited high sensitivity to heterocyclic amines (HCAs) such as 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,8-dimenthylimidazo[4,5-*f*]quinoline (MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) as compared with the parent Ames tester strain TA1538. It is suggested that the intracellular expression of drug-metabolizing enzymes makes the Salmonella cells highly sensitive to the HCA.

Keywords: heterocyclic amines, CYP, Ames test

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Wagner, J.*, Gruz, P., Kim, S.-R., Yamada, M., Matsui, K., Fuchs, R.P.P. * and Nohmi, T.: **The *dinB* gene encodes a novel *E. coli* DNA polymerase, DNA pol IV, involved in mutagenesis.**

Mol. Cell, 4, 281-286 (1999)

In *Escherichia coli*, the *dinB* gene is required for the SOS-induced lambda untargeted mutagenesis pathway and confers a mutator phenotype to the cell when the gene product is overexpressd. Here, we report that the purified DinB protein is a DNA polymerase. This novel *E. coli* DNA polymerase (polIV) is shown to be strictly distributive, devoid of proofreading activity, and prone to elongate bulged (misaligned) primer/template structures. Site-directed mutagenesis experiments of *dinB* also demonstrate that the polymerase activity of DinB is required for its *in vivo* mutagenicity. Along with the sequence homologies previously found within the UmuC-like protein family, these results indicate that the uncovered DNA polymerase activity may be a common feature of all these homologous proteins.

Keywords: DNA polymerase, mutagenesis, *Escherichia coli*

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Iwashita, S.*, Nobukuni, T.*, Tanaka, S.*, Kobayashi, M.*, Iwanaga, T.*, Tamate, H.B.*, Masui, T., Takahashi, I.* and Hashimoto, K.*: **Partial nuclear localization of a bovine phosphoprotein, BCNT, that includes a region derived from a LINE repetitive sequence in Ruminantia.**

Biochim Biophys Acta, **1427**: 408-16 (2000)

BCNT, named after Bucentaur, contains a 324-amino-acid region derived from LINE repetitive sequence in Ruminantia. We at first examined cellular localization and biochemical characteristics of bovine BCNT which is missing in human and mouse BCNTs. A significant amount of bovine BCNT is localized in the nuclei, while the major portion is present in the cytosol. Furthermore, bovine BCNT are phosphorylated by casein kinase II *in vitro*.

Keywords: LINE repetitive sequence, nuclear localization, BCNT

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Oomizu, S.*, Honda, J.*, Takeuchi, S.*, Kakeya, T.*, Masui, T. and Takahashi, S.: **Transforming growth factor-alpha stimulates proliferation of mammotrophs and corticotrophs in the mouse pituitary.**

J. Endocrinol., **165**: 493-501 (2000)

This study pursued the role of transforming growth factor-alpha (TGF-alpha) in the oestrogen-induced growth of mouse pituitary cells in vitro. Oestradiol-17beta (OE(2)) and TGF-alpha stimulated mammotrophic and corticotrophic cell proliferation. RG-13022, an EGF receptor inhibitor, inhibited the cell proliferation induced by EGF or OE(2). Treatment with antisense TGF-alpha oligodeoxynucleotide (ODN) inhibited the cell proliferation induced by OE(2), but treatment with antisense EGF ODN did not. OE(2) stimulated TGF-alpha mRNA and EGF receptor mRNA expression. These results indicate that TGF-alpha mediates the stimulatory effect of oestrogen on the pituitary cell.

Keywords: mouse, pituitary, TGF-alpha

* Okayama University

Tanabe, H., Takada, Y., Minegishi, D., Kurematsu, M., Masui, T. and Mizusawa, H.: **Cell line individualization by STR Multiplex system in the cellbank found cross-contamination between ECV304 and EJ-1/T24.**

Tiss. Cult. Res. Commun., **18**, 329-338 (1999)

Recent technical advances have enabled us to analyze multilocus short tandem repeat (STR) regions simultaneously by a method, called STR Multiplex system, that uses a single PCR amplification in one tube. We established a new evaluation system for the identification of cell lines based on an STR Multiplex method that uses 9 different loci: D5S818, D13S317, D7S820, D16S539, vWA, TH01, Amelogenin, TPOX, and CSF1PO. The STR profiling data from 96 cell lines were examined and an efficiency of this approach for cell standardization was found. We have analyzed the STR profiles of human cell lines, ECV304, EJ-1 and T24, and Cell Cultures to have been cross-contaminated. Our results clearly detect the cross-contamination between

ECV304 and EJ-1/T24. The cross-contamination was estimated to be derived from the T24 cells. Collectively, the STR Multiplex system provides a rapid, precise, and powerful method in cell line identification for quality control at the JCRB Cell Bank.
Keywords: STR Multiplex system, Cell line identification, Cross-contamination

長谷川隆一，広瀬明彦，西川秋佳，紅林秀雄，江馬 眞，黒川雄二：**ジクロロ酢酸の毒性評価と経口摂取による1日耐容摂取量の算定**
水環境学会誌, **22**, app 821-826 (1999)
飲料水の消毒副生成物の1つであるジクロロ酢酸は，1992年にWHO飲料水ガイドラインで水質基準が設定され，日本でも監視項目として選定された．その後，1998年には，それぞれで改訂が行なわれた．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，経口摂取に対するジクロロ酢酸の耐容一日摂取量(TDI)を再評価した．
Keywords: dichloroacetic acid, toxicity evaluation, drinking water, tolerable daily intake

Hirose A, Nishikawa A, Kinae N*, Hasegawa R: **Toxicological properties of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) and the risk assessment in drinking water**
Rev. Environ. Health, **14**, 103-120 (1999)
MX(3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone)は，水道の塩素消毒処理の過程で生じる副生成物の一つであるが，サルモネラを使ったAmes試験において強力な変異原性を示す物質としても知られている．MXは，各国で飲料水中に数十ppbオーダーで検出されていると共に，近年ラットに対して発がん性があることが報告され，ヒトの健康影響に対する評価を行うことが急務となっている．本報告ではMXの毒性影響に関する情報を毒性の種類ごとに分類し，各毒性に対してNOAEL（またはLOAEL），発がん性に関してはユニットリスクを算出した．さらに，各々に対するTDI（tolerable daily intake）とVSD（virtual safety dose）を算出し，飲料水中におけるMXの安全性を評価した．
Keywords: disinfectant by-product, carcinogen, tolerable daily intake(TDI), virtual safety dose(VSD)
* 静岡県立大学

岩上正蔵*1，山下治夫*1，岡田敏史：**液体クロマトグラフ法における「システムの再現性」について**
医薬品研究, **29**, 544-549(1998)
日局医薬品各条の定量法において，液体クロマトグラフ法が適用される品目中，絶対検量線法が用いられる品目については，操作条件中に「試験の再現性」として「相対標準偏差○○％以下」と規定されている．これらの規定にあたって，製品中の含量の変動及び含量規格などと切り離して，単純にシステムの再現精度を規定してきたきらいがあることを指摘し，その規定にあたっては，試料の前処理操作をはじめ，含量のロット間変動，含量規格幅，危険率をどのように設定するかなどを考慮して規定すべきことを考察した．
Keywords: system repeatability, system suitability, Japanese Pharmacopoeia
*1 大阪府立公衆衛生研究所薬事指導部

Miyazaki, T., Yomota, C. and Okada, S.: **Depolymerization of hyaluronate by the phototoxic drugs phenothiazines and sulfacetamide**
Colloid Polym. Sci. **278**, 84-89 (2000)
Phenothiazine and sulfacetamide, known as phototoxic drugs, depolymerized aqueous sodium hyaluronate (HA) in the light state. The depolymerization was followed by SEC equipped with a low-angle laser light scattering. The molecular weight of HA practically unchanged without UV irradiation in the presence of drugs, or with UV irradiation in the absence of drugs. It indicated that these drugs required photo energy to yield any kind of damaging chemical species for HA depolymerization. We investigated the reactive species by use of some radical scavengers and elimination of oxygen. Promazine and promethazine depolymerized HA through active oxygen radicals, because the depolymerization was inhibited under anaerobic condition. Further, since the reaction was controlled by mannitol, hydroxyl radical was supposed as the damaging active oxygen. Chlorpromazine and sulfacetamide preferably depolymerized HA under anaerobic condition, therefore, the participation of hydrated electron was proposed.
Keywords: hyaluronate, depolymerization, phototoxic drugs

Yomota, C. Tagashira, Y. Okada, S. Hayashi, Y. Matsuda, R: **Prediction of Measurement Precision by Chemometric Invalidation of Flow Pulsation in Refractive Index Detection of Liquid Chromatography**
Analytical Sciences, **15**, 549-554(1999)
The standard deviation and relative standard deviation of measurements in the refractive index detection of liquid chromatography are predicted based on 1/f fluctuation model which is made up of white noise and the markov process. First repeated measurement and Monte Calro simulation corroborate the repeatability of this prediction in the absence of flow pulsation. Pulsation, if any, can be distinguished in the freqency space from the 1/f noise which is common to most analytical instruments. In this case, the uncertainty prediction is also available without modifying the prediction theory, if a zero window is set at the time period of a fundamental tone of the pulsation.
Keywords: precision, uncertainty, refractive index detector

四方田千佳子，田頭洋子，勝峰万里*，岩木和夫*，松田りえ子，林 謙：**クロマトグラフィ分析における適切なデータ取り込み間隔について 1.適切なデータ取り込み間隔の推定**
分析化学, **49**, 225-231 (2000)
HPLCとイオンクロマトグラフにおいてデータ取り込み間隔を変化させた場合の，測定精度の変化を調べた．測定精度はFUMI理論に基づいて，繰り返し測定なしにピークの形とノイズの確率論的性質から予測した．ピークの形を正確に観測するためには，データ取り込み間隔はピーク領域で最低約30データポイントが必要であることが明らかとなった．クロマトグラムを保存するメモリ容量と測定精度を考慮すると，ピーク領域のデータポイントは30-50くらいになるように設定することが適切であると結論した．
Keywords: chromatography, precision, sampling intervals
*(株)荏原総合研究所

Saito, H., Kawagishi A.,*, Tanaka, M.*, Tanimoto, T., Okada, S., Komatsu, H.* and Handa, T.*: **Coalescence of lipid emulsions in floating and freeze-thawing processes: examination**

of the coalescence transition state theory
J. Colloid Interface Sci. 219, 129-134 (1999)
エマルションの物理化学的な安定性に及ぼす表面脂質やコア脂質の影響について，粒子表面膜の自発的曲率の点から議論した．一般にトリグリセライドーリン脂質系の脂質エマルションは物理化学的に安定であるが，アシル鎖が不飽和脂肪酸から成るリン脂質やコレステロールなどの負の曲率を持つ表面脂質をエマルションに加えると，超遠心による負荷や凍結融解過程で粒子間の凝集による粒子径の増大が観察された．また蛍光プローブによる脂質混合実験の結果，粒子の凝集は表面脂質膜の融合を伴っていることが確認された．さらに，トリグリセライドのアシル鎖長によっても粒子の安定性が変化することを見出した．これらエマルション粒子の凝集・融合過程を，Kabalnovらのcoalescence transition state theoryから議論した．
Keywords: emulsions, phospholipid, coalescence
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谷本 剛，斎藤博幸，前川京子，北島 文，岩田美保，岡田敏史，石井 清*1，海老名甲一*1，穂積 宏*2，宮本一夫*2，八重津昌孝*3，樋口 登*3，大山美宏*4，岩本正人*4：**国立医薬品食品衛生研究所組織培養ウロキナーゼ標準品新規設定のための品質評価**
医薬品研究，**30**, 289-294 (1999)
国立医薬品食品衛生研究所組織培養ウロキナーゼ標準品を新規に設定した．標準品原料は5機関の共同検定によって，免疫化学的，物理化学的，酵素化学的に評価した．二重免疫拡散法によって，標準品原料は抗組織培養ウロキナーゼ血清に対して一本の沈降線を形成し，この沈降線はヒト尿由来ウロキナーゼの沈降線と融合することを認めた．ゲル浸透HPLC法で求めた標準品原料の分子量は30,700±400と推定された．二段法で求めたウロキナーゼ力価は1バイアル当たり8,221±582単位であった．
これらの結果に基づいて，本標準品原料を初回の国立医薬品食品衛生研究所組織培養ウロキナーゼ標準品（Control 981）とすることとし，1バイアル当たり8,200単位を含有すると認定した．
Keywords: Tissue culture urokinase, Urokinase, NIHS reference standard
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*3杏林製薬株式会社，*4三菱化学株式会社

Saito, H. and Handa, T.*: **Structure and dynamics of surface monolayers in lipid emulsions and their role in apolipoprotein binding**
Curr. Topics Colloid Interface Sci. **3**, 19-33 (1999)
血漿リポ蛋白質が分泌されてから異化されるまでの過程で起こる主要な脂質組成変化は，表面コレステロールとコア中のコレステリルエステルの増加である．リポ蛋白質モデル粒子として脂質エマルションを用い，コレステロール及びそのエステルによる粒子構造の変化を詳細に検討した結果，コレステロールは表面膜アシル鎖の運動性を，コレステリルエステルはコアの流動性をそれぞれ低下させることで粒子構造に変化を与え，結果としてアポリポ蛋白質の結合性を制御していることが明らかとなった．
Keywords: emulsions, lipoproteins, apolipoprotein
*京都大学大学院薬学研究科

Nakamura, Y., Tsumura, Y., Tonogai, Y., Shibata, T.: **Fecal steroid excretion is increased in rats by oral administration of gymnemic acids contained in *Gymnema sylvestre* leaves**
J. Nutr., **129**, 1214-1222 (1999)
The effects of gymnemic acids on fecal steroid excretion were investigated in rats. Three kinds of extracts from Gymnema sylvestre leaves, extract (GSE), acid precipitate (GSA) and column fractionate (GSF), of which the gymnemagenin (an aglycone of gymnemic acids) concentrations are 58.87, 161.6, and 363.3 mg/g respectively, were administered to rats orally at the dose of 0.05-1.0 g/kg for 22 d. GSA and GSF decreased body weight gain and food intakes in a dose-dependent manner ($P<0.01$). GSF (1.0 g/kg) significantly increased fecal excretion of neutral steroids and bile acids in a dose-dependent manner ($P<0.05$). The increases in fecal steroid excretion of cholesterol, total neutral steroids, total bile acids and cholic acid (CA)-related bile acids were acute and significantly correlated with fecal gymnemagenin levels ($r^2 = 0.2316$-0.9861, $P<0.05$). These results demonstrated for the first time that a high dose of gymnemic acids increases fecal cholesterol and CA-derived bile acid excretion.
Keywords: gymnemic acids, cholesterol, bile acids

Yoshii, K., Okada, M., Tsumura, Y., Nakamura, Y., Ishimitsu, S. and Tonogai, Y.: **Supercritical Fluid Extraction of Ten Chloracetanilide Pesticides and Pyriminobac-methyl in crops: comparison with the Japanese Bulletin method**
J. AOAC Int., 82, 1239-1245 (1999)
In Japan, the maximum residue limit and an analytical bulletin method for alachlor and pyriminobac-methyl were recently published. Because this method has some problems, such as many interfering chromatographic peaks for some residues in certain crops, time-consuming sample preparation and so forth, we have developed an alternative method. In the developed method, pesticides are extracted by SFE and directly cleaned up with a trap column consisting of Extrelut and Florisil (acetone/n-hexane 3:7 as eluent) or Bond Elut SAX and PSA (acetone/n-hexane 1:1 as eluent). The test solution was quantitatively determined by GC/MS. Average recoveries of spiked samples (0.1 ppm) were between 52 and 104 % with the bulletin method, and between 68 and 106 % with the developed method.
Keywords: SFE, GC/MS, pesticide

Amakura, Y., Miyake, M.*1, Ito, H.*1, Murakaku, S.*1, Araki, S.*1, Itoh, Y.*1, Lu, C.-F.*2, Tang, L.-L.*2, Yen, K.-Y.*2, Okuda, T.*1 and Yoshida, T.*1: **Acalyphidin M1, M2 and D1, ellagitannins from Acalypha hispida**
Phytochemistry, **50**, 667-675 (1999)
Two new monomeric hydrolysable tannins, acalyphidins M1 and M2, together with fourteen known polyphenols were isolated from the leaves of A. hispida, and characterized as 1-O-galloyl-3,6-(R)-hexahydroxydiphenoyl-4-O-breviforincarboxyl-b-D-glucose, and oxidative metabolite of geraniin, respectively, by spectroscopic and chemical methods. A new hydrolysable tannin dimer, acalyphidin D1, which is composed of two moles of geraniin, was also isolated and characterized as an acetonyl derivatives.
Keywords: Acalypha hispida, Euphorbiaceae, Tannins
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Yoshida, T.*1, Amakura., Y., Yokura, N.*1, Ito, H.*1, Isaza, J.

H.[*2], Ramirez, S.[*2], Pelaez, D.P.[*2] and Renner, S.S.[*3]: **Oligomeric hydrolysable tannins from Tibouchina multiflora**

Phytochemistry, **52**, 1661-1666 (1999)

Two hydrolysable tannins, nobotanins O and P, were isolated from the leaf extract of T. multiflora and their dimeric and tetrameric structures elucidated on the basis of spectral data and chemical correlations with nobotanins B and K, respectively. Thirteen known hydrolysable tannins including nobotanins A, B, C and J, which are oligomers characteristic of the Melastomataceae, were also isolated.

Keywords: Tibouchina multiflora, Melastomataceae, Ellagitannin oligomer

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Ema, M., Miyawaki, E. and Kawashima, K.: **Suppression of uterine decidualization as a cause of implantation failure induced by triphenyltin chloride in rats**

Arch. Toxicol., **73**, 175-179 (1999)

In our previous study, triphenyltin chloride (TPTCl) was found to induce implantation failure, preimplantation embryonic loss, in rats. In this study, the effects of TPTCl on the uterine function, as a cause of implantation failure, were determined using pseudopregnant rats. Female rats were given TPTCl by gastric intubation at 0, 3.1, 4.7 or 6.3 mg/kg on pseudopregnant day (PPD) 0 to PPD 3 and the decidual cell response was induced on PPD 4. The uterine weight on PPD 9 served as an index of the uterine decidualization. A significant decease in the uterine weight, which indicates suppression of the uterine decidualization, was detected at 4.7 and 6.3 mg/kg. In our previous study, these doses induced a significant increase in implantation failure in female rats given TPTCl on gestational day (GD) 0 to 3. The ovarian weight and number of corpora lutea in the TPTCl-treated groups were comparable to the controls. A significant decrease in the serum progesterone levels after administration of TPTCl was found at 4.7 and 6.3 mg/kg. These findings suggest that implantation failure due to TPTCl may be mediated via the suppression of uterine decidualization correlated with the reduction in serum progesterone levels.

Keywords: triphenyltin, implantation failure, decidual cell response

Ema, M., Miyawaki, E. and Kawashima, K.: **Adverse effects of diphenyltin dichloride on initiation and maintenance of pregnancy in rats**

Toxicol. Lett., 108, 17-25 (1999)

Following successful mating, female rats were given DPTCl by gastric intubation at 0, 4.1, 8.3, 16.5, 24.8 or 33.0 mg/kg on days 0-3 or days 4-7 of pregnancy. Female rats were sacrificed on day 20 of pregnancy and pregnancy outcome was determined. The pregnancy rate was significantly decreased after administration of DPTCl on days 0-3 at 24.8 mg/kg and on days 4-7 at 33.0 mg/kg. The incidence of preimplantation loss was significantly increased after administration on days 0-3 at 16.5 mg/kg and above and on days 4-7 at 33.0 mg/kg. In females having implantations, the numbers of implantations and live fetuses and the incidences of pre- and postimplantation loss in the groups given DPTCl on days 0-3 were comparable to the controls. The incidence of postimplantation loss was significantly increased after administration of DPTCl on days 4-7 at 33.0 mg/kg. A pair-feeding study revealed no evidence of pre- and postimplantation embryolethality induced by food restriction. It could be concluded that DPTCl during early pregnancy causes early embryonic loss and DPTCl has greater effects on reproduction when administered during earlier than later stages of blastogenesis.

Keywords: diphenyltin, embryonic loss, implantation failure

Ema, M., Miyawaki, E. and Kawashima, K.: **Developmental effects of plasticizer butyl benzyl phthalate after a single administration in rats**

J. Appl. Toxicol., 19, 357-365 (1999)

The objective of this study was to determine the susceptible day for the developmental toxicity of butyl benzyl phthalate (BBP) by a single administration on one of the days during organogenesis. Pregnant rats were given a single dose of BBP by gastric intubation at 1000 mg/kg on one of days 13-15 of pregnancy and at 1500 mg/kg on one of days 6-16 of pregnancy. Postimplantation embryolethality was found in pregnant rats given on one of days 6-16, except for day 7. Teratogenicity was noted after a single dosing of BBP on one of days 6, 7, 9, 10, 12, 14 and 15. Deformity of the cervical vertebrae was frequently observed after administration of BBP on day 7. Cleft palate and fusion of the sternebrae were exclusively found after administration of BBP on day 15. It can be concluded that the manifestation of deviant development induced by BBP varies with the developmental stage at the time of administration and that BBP induces two discrete responses from embryos to teratogenicity during early and late organogenesis.

Keywords: butyl benzyl phthalate, teratogenicity, embryolethality

Ema, M., Miyawaki, E. and Kawashima, K.: **Effects of dibutyl phthalate on reproductive function in pregnant and pseudopregnant rats**

Reprod. Toxicol., **14**, 13-19 (2000)

In our previous studies, dibutyl phthalate (DBP) was found to be embryolethal and teratogenic in rats. In this study, the effects of DBP on reproductive function were investigated on pregnant and pseudopregnant rats. Rats were given DBP by gastric intubation at 0, 250, 500, 750, 1000, 1250 or 1500 mg/kg on days 0 to 8 of pregnancy and the pregnancy outcome was determined on day 20 of pregnancy. The same doses of DBP were given to pseudopregnant rats, with an induced decidual cell response, on days 0 to 8 of pseudopregnancy and the uterine weight on day 9 served as an index of the uterine decidualization. DBP caused significant increases in the incidences of preimplantation loss in females successfully mated at 1250 and 1500 mg/kg and of postimplantation loss in females having implantations at 750 mg/kg and above. The uterine decidualization in pseudopregnant rats was significantly decreased at 750 mg/kg and above. These findings suggest that early embryonic loss due to DBP may be mediated, at least in part, via the suppression of uterine decidualization, an impairment of uterine function.

Key Words: dibutyl phthalate, early embryonic loss, decidual cell response

Ema, M., Miyawaki, E. and Kawashima, K.: **Critical period for adverse effects on development of reproductive system in male offspring of rats given di-n-butyl phthalate during**

late pregnancy

Toxicol. Lett., **111**, 271-278 (2000)

Pregnant rats were given dibutyl phthalate (DBP) by gastric intubation at 1000 or 1500 mg/kg on days 12-14 or days 18-20 of pregnancy or at 500, 1000 or 1500 mg/kg on days 15-17 of pregnancy. A significant increase in the number of resorptions per litter was found in the groups given DBP at 1500 mg/kg on days 12-14 and days 15-17 of pregnancy. The weights of male and female fetuses were significantly decreased in the groups given DBP at 1000 and 1500 mg/kg on days 12-14 and days 18-20 and at 1500 mg/kg on days 15-17. A significant increase in the incidence of fetuses with undescended testes was found at 1500 mg/kg on days 12-14 and at all doses on days 15-17. A significant decreases in the anogenital distance (AGD) of male fetuses was observed in the groups treated with DBP regardless of the days of treatment. The AGD/body weight ratio in male fetuses was significantly reduced in the groups given DBP on days 15-17, but neither on days 12-14 nor days 18-20. The AGD of female fetuses in the DBP-treated groups was comparable to that in the control group. It was concluded that period of days 15-17 of pregnancy was the most susceptible for DBP-induced undescended testes and decreased AGD in male offspring.

Keywords: dibutyl phthalate, anogenital distance, undescended testes

村井敏美，中川ゆかり，前田秀子，川島邦夫，田中重則[*1]，田村弘志[*1]，土谷正和[*2]，高岡文[*2]，松川正之[*3]，堀内善信[*4]：**国立医薬品食品衛生研究所標準品「エンドトキシン100標準品」の新規設定**

医薬品研究，**31**，75-79 (2000)

バイアル当たりのエンドトキシン充填量をより少量としたエンドトキシン標準品を新規に設定するため，その候補品を製造し，評価した結果，当該候補品は十分な保存安定性を備えていることが確認され，5機関による共同検定で力価120 EU/バイアルと定められた．これらの成績に基づき，同候補品を国立医薬品食品衛生研究所標準品「エンドトキシン100標準品」の初回ロットとして認定した．

Keywords: Endotoxin, NIHS reference standard, Stability, Potency

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芝野真喜雄[*1]，川瀬さおり[*1]，村上理恵[*1]，喜多俊二[*1]，草野源次郎[*1]，柴田敏郎，畠山好雄，縣 功[*2]：**ウラルカンゾウ（*Glycyrrhiza uralensis*）栽培のための基礎研究(1)**

Natural Medicines, **54** (2), 70-74 (2000)

日本の環境に適応したウラルカンゾウを選抜する目的で，6系統を3年間栽培して比較した結果，北海道医療大学系統ならびに北海道薬用植物栽培試験場系統の2系統が，収量やグリチルリチン含量の面で栽培品種としての適性を備えた系統であることが判明した．

Keywords: *Glycyrrhiza uralensis*, cultivation, glycyrrhizin contents

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[*2] 北海道医療大学

Minami, M., Mia, Md W., Sakai, E., Nishi, K., Satake, M., Kondo, S.*, Nunoura, Y.* and Shibata, T.: **Variation in Flowering Date, Shoot Growth and Contents of Choleretic Substances in Capitulum in *Artemisia capillaris* Collected from Various Locations in Japan**

Plant Production Science, **2** (4), 241-246 (1999)

生薬インチンコウの基原植物であるカワラヨモギについて，日本の10地点に野生する植物を採取し，筑波にて比較栽培した結果，低緯度地方に自生する系統ほど，開花期が遅れ花穂や茎葉重量が増加するが活性成分含量には差がないこと，地上部の生育量や活性成分含量の集団内変異が大きいことが判明した．また，多変量解析の結果，4つのグループに分かれることも判明した．

Keywords: *Artemisia capillaris*, capillarisin,6,7-dimethylesculetin

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Minami, M., Mia, Md W., Sakai, E., Nishi, K., Satake, M., Kondo, S.[*1], Oka, K.[*1], Tabei, Y.[*2], Koga-Ban, Y.[*2], Kayano, T.[*2] and Shibata, T.: **The Difference of flowering date, flower head size, choleretic substances content and molecular characters between erect and prostrate-growth forms of *Artemisia capillaris* in Japan**

J. Phytogeogr. Taxon., **47** (1), 1-15 (1999)

カワラヨモギについて，日本の18地点の野生植物を比較栽培し，また，国立科学博物館所蔵の62点のさく葉標本を調査した結果，河岸に野生するものは直立型，海岸のものは多くがほふく型となり，ジメチルエスクレチン含量は直立型が有意に高いことが判明した．また，葉緑体DNAのDIG-RAPD分析の結果，河岸型と海岸型の2グループに分かれた．

Keywords: *Artemisia capillaris*, growth form, DIG-RAPD

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[*2] 農林水産省生物資源研究所

Mia, Md W., Sakai, E., Minami, M., Anetai, M.*, Aoyagi, M.* and Shibata, T.: **Root Growth of *Astragalus mongholicus* and *A. membranaceus* as Affected by Soil Compaction**

Natural Medicines, **53** (6), 302-307 (1999)

生薬黄耆の基原植物キバナオウギ，ナイモウオウギについて，塩化ビニールパイプを用いて栽培し，根の生育・成分に及ぼす土壌硬度の影響を検討した結果，両種ともに10kg cm-2以上の土壌硬度条件では，主根の伸長や分枝根の発生に影響が認められ，特にキバナオウギにおいて，その反応がより敏感であること，アストラガロサイドおよび希エタノールエキス含量は土壌硬度が低下するにつれて増加することを明らかにした．

Keywords: *Astragalus mongholicus*, *Astragalus membranaceus*, soil penetration resistance

* 北海道立衛生研究所

Shibano, M.[*1], Nukui, H.[*1], Kita, S.[*1], Kusano, G.[*1], Shibata, T., Watanabe, H.[*2] and Ohashi, H.[*3]: **Studies on Index Compound for HPLC Analysis of *Glycyrrhiza macedonica***

Natural Medicines, **53** (4), 166-172 (1999)

生薬甘草の基原植物である*Glycyrrhiza*属植物について，指標成分による同定を行う研究の一環として，今回，地上部の形質より同定した*Glycyrrhiza macedonica*のMeOHエキスのHPLC分析により，地上部から7種，地下部から5種の成分を単離同定し，その内4種を新規化合物として報告した．

Keywords: *Glycyrrhiza macedonica*, MeOH extract

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*3 東北大学理学部

Hosokawa, K., Oikawa, Y.*, Yamamura, S.*: **Clonal propagation of *Wasabia japonica* by shoot tip culture.**
Planta Medica **65** (7), 676 (1999)
ワサビの優良系統真妻の茎頂培養方法について検討した．その結果，サイトカイニンとして1 mg/l BAの条件が最も優れていることを見い出した．オーキシンとの併用ではその効果が認められなかった．増殖したシュートはホルモンフリーの条件で容易に発根し順化することができた．この最適条件では1本のシュートから1年間に50,000本のシュートを増殖できることができた．
Keywords：micropropagation, *Wasabia japonica*
*（財）岩手生物工学研究センター

Honda, K.*, Tsutsui, K.*, Hosokawa, K.: **Analysis of the flower pigments of some *Delphinium* species and their interspecific hybrids produced via ovule culture.**
Scientia Horticulturae 82, 125-134 (1999)
子房培養により作出したデルフィニューム属植物の種間雑種（*D. cardinale* x *D. grandiflorum* と *D. grandiflorum* x *D. nudicaule*）についてその花色成分であるアントシアニンを調査し，花色発現の遺伝と共にデルフィニューム属植物の育種の可能性について検討した．
Keywords: *Delphinium*, flower pigments, hybridization breeding
* 北海道大学

Hosokawa, K., Minami, M., Kawahara, K.*1, Nakamura, I.*2, Shibata, T.: **Discrimination among three species of medicinal *Scutellaria* plants using RAPD markers.**
Planta Medica 66, 270-272 (2000)
形態的に似ている薬用植物である *Scutellaria* 属植物3種（*S. baicalensis*, *S. galericulata*, *S. lateriflora*）をRAPD法を用いて識別することについて検討した．その結果，検討した10種類のプライマーの内3種類のプライマーで3種の *Scutellaria* 属植物の識別を可能にするための種特異的バンドを見いだした．
Keywords: RAPD marker, *Scutellaria*
*1 日野薬品
*2 千葉大学

Hosokawa, K. Matsuki, R.*, Oikawa, Y.*, Yamamura, S.*: **Production of transgenic gentian plants by particle bombardment of suspension-culture cells.**
Plant Cell Reports, **19** (5), 454-458 (2000)
リンドウ（品種 Polarno white）の形質転換をパーティクルガンにより行った．葉外植片からカルスを誘導し，懸濁培養細胞を調製した．この懸濁培養細胞にパーティクルガンを行い，ハイグロマイシン耐性カルスの選抜・再分化により，ハイグロマイシン耐性リンドウを作出した．形質転換リンドウの確認はサザン解析により実施した．
Keywords: Gentian, Particle bombardment, Transformation
*（財）岩手生物工学研究センター

Washida, D.*1, Shimomura, K., Nakajima, Y.*2, Takido, M.*1 and Kitanaka, S.*1: **Ginsenosides in hairy roots of a *Panax* hybrid**
Phytochemistry, **49**, 2331-2335 (1998)
Hairy roots of an interspecific hybrid ginseng (*Panax ginseng* x *P. quiquefolium*), named Pgq, were established by the infection of *Agrobacteruim rhizegenes* ATCC 15834. Growth and ginsenosides content of hairy roots cultured in various basal liquid media were measured periodically from 2 to 8 weeks. In Gamborg B5 liquid medium, the hairy roots showed best growth (5.87 g fresh weight per flask) at week 8. The highest content of ginsenoside was 2.87 % as dry weight at week 8 when cultured in 1/8 Murashige-Skoog liquid medium. The ginsenoside content of Pgq hairy roots was comparable to that of Pgq root cultivated in the field. However the highest yield of ginsenosides was obtained in B5 liquid medium (3.85 mg per flask at week 8).
Keywords: *Panax ginseng*, *P. quiquefolium*, hairy root
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*2 長野農業技術研修センター

Gondo, M.*1, Tanaka, N.*1, Tanaka, T.*2, Shimomura, K., Nakanishi, F.*3 and Ishimaru, K.: **A naphthalene glycoside from callus cultures of Diospyros *kaki***
Phytochemistry, **51**, 879-881 (1999)
Calli of *Diospyros kaki* Thunb. were induced on half-strength Murashige-Skoog solid medium supplemented with 1.0 mgl-1 IAA and 0.1 mgl-1 BA in the dark and successfully subcultured on the same medium. A new phenolic metabolite, 7-methyl-1, 4, 5-trihydroxy-naphthalene 4-O-(6'-O-β-xylopyranosyl)-β-glucopyranoside, was isolated from MeOH extract of the callus cultures and the chemical structure elucidated by NMR spectroscopic evidences.
Keywords: *Diospyros kaki*, callus, naphthalene
*1 佐賀大学
*2 長崎大学
*3 東京学芸大学

Nishikawa, K.*1, Furukawa H.*1, Fujioka, T.*2, Fujii, H.*2, Mihashi, K.*2, Shimomura, K. and Ishimaru, K.*1: **Phenolics in tissue cultures of *Scutellaria***
Natural Medicines, **53**, 209-213 (1999)
Concentrations of eight phenolics, acteoside (1), baicalin (2), wogonin 7-glucuronide (3), baicalein (4), wogonin (5), skullcapflavone I (6), skullcapflavone II (7) and chrysin (8) in shoot cultures of nine *Scutellaria* species were determined. The nine species were classified into four groups on the basis of the major phenolics in shoot cultures, i. e., (A) *S. iyoensis* and *S. ventenatii* of which major phenolics were 1, 3 and 5, (B) *S. lateriflora* of which major phenolics were 4 and 5, (C) *S. incana*, *S. orientalis* and *S. taurica* of which major phenolics were 3 and 5 and (D) *S. pontica*, *S. galericulata* and *S. alpina* of which major phenolic was 1. On the other hand, callus cultures of seven *Scutellaria* species produced 1 as the major phenolic of which the maximum concentration was 4.04 % in the light and 4.06 % in the dark in S. iyoensis callus.
Keywords: *Scutellaria*, *Lamiaceae*, shoot culture
*1 佐賀大学
*2 福岡大学

Sommer, S.*1, Köhle, A.*1, Yazaki, K.*2, Shimomura, K., Brechthold, A.*1 and Heide, L.*1: **Genetic engineering of shikonin biosynthesis hairy root cultures of *Lithospermum erythrorhizon* transformed with the bacterial *ubiC* gene**
Plant Molecular Biology, 39, 683-693 (1999)
The biosynthetic pathway to 4-hydrobenzoate (4HB), a precursor of the naphthoquinone pigment shikonin, was modified in

Lithospermum erythrorizon hairy root cultures by introduction of the bacterial gene *ubiC*. This gene of *Escherichia coli* encodes chorismate pyruvate-lyase (CPL), an enzyme that converts chorismate into into 4HB and is not normally present in plants. The *UbiC* gene was fused to the sequence for a chloroplast transit peptide and placed under control of a constitutive plant promoter. This construct was introduced into L. erythrorizon by *Agrobacteruim rhizogenes*-mediated transformation. The resulting hairy root cultures showed high CPL activity. 4HB produced by the CPL reaction was utilized for shikonin biosynthesis, as shown by *in vivo* inhibition of the native pathway to 4HB with 2-aminoindan-2-phosphonic acid (AIP), an inhibitor of phenylalanine ammonia-lyase. A feeding experiment with [1,7-13C2] shikimate showed that in the absence of AIP the artificially introduced CPL reaction contributed ca. 20 % of the overall 4HB biosynthesis in the transgenic cultures. *ubiC* transformation did not lead to a statistically significant increase of shikonin formation, but to 5-fold increase of the accumulation of menisdaurin, a nitrile glucoside which is presumably related to aromatic amino acid metabolism.

Keywords: *Lithospermum erythrorhizon*, *UbiC*, shikonin

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*2 京都大学

Tanaka, N.*, Shimomura , K.and Ishimaru, K.*: **Antioxidative and radical scavenging activities of *Cornus capitata***

Japanese. J. of Food Chemistry, **6**, 48-52 (1999)

Antioxdative and radical scavenging activities of methanol extract of *C. capitata* adventitious roots, cultured in the medium with various concentrations of Cu^{2+}, were investigated. The activities of the root extract were compared to those of gallic acid and related metabolite (galloylglucoses) standards.

Keywords: *Cornus capitata*, galloylglucose, antioxidation

* 佐賀大学

Nishikawa, K.*1, Furukawa, H.*1, Fujioka, T.*2, Fujii, H.*2, Mihashi, K.*2, Shimomura, K. and Ishimaru, K.*1: **Flavone production in transformed root cultures of *Scutellaria baicalensis* Georgi**

Phytochemistry, **52**, 885-890 (1999)

A new flavone derivative 5, 2'-dihydroxy-6, 7, 8, 3'-tetramethoxyflavone, together with two known phenolics skullcapflavone I and acteoside (3), was isolated from *Scutellaria baicalensis* transformed roots (clone C) in which β-glucuronidase gene has been integrated by the infection with *Agrobacterium rhizogenes* A13. Another transformed roots (clone W) were also induced by the infection with *A. rhizogenes* ATCC 15834 (wild type). Both clones C and W, cultured in phytohormone-free BF liquid medium, produced 3 at high content (maximum; clone C: 1.807 % and clone W: 2.962 %, dry weight) under the light and dark conditions. The contents of glucuronide-type flavonoids such as baicalin and wogonin 7-O-glucuronide in clone W were almost three-times larger than those in clone C. Compound 3, which was not detected in the intact plant roots, was also accumulated in leaf (0.233 %, dry weight) and root (0.681 %, dry weight) portions of *in vitro* cultured plantlets.

Keywords: *Scutellaria baicalensis*, β-glucuronidase, hairy root

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*2 福岡大学

Matsuura, E.*, Shimomura, K. and Ishimaru, K.*: **Flavonoid and polyacetylene from *Pratia nummularia***

Natural Medicines, **54**, 44 (2000)

From the aerial parts of Pratia nummularia, four flavonoids (diosmin, linarin, apigenin 7-*O*-rutinoside and luteolin 7-*O*-rutinoside) and a polyacetylene (lobetyolin) were isolated and their chemical structures were characterized by NMR spectroscopic data.

Keywords: *Pratia nummularia*, flavonoid, polyacetylene

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Nishikawa,K.*1, Munechika, T.*1, Ibaraki, M.*1, Fujioka, T.*2, Fujii, H.*2, Mihashi, K.*2, Shimomura , K.and Ishimaru, K.*1: **New benzoxepin glucoside from transformed root cultures of *Helianthus annuus* L.**

Natural Medicines, **54** (2), 93-96 (2000)

A new benzoxepin glucoside was isolated from *Helianthus annuus* L. root transformed with *Agrobacterium rhizogenes* MAFF 03-01724, and its chemical structure was elucidated by NMR spectroscopic analysis. From the H. annuus transformed root, two quinic acid derivatives, 3-*O*-caffeoyl quinic acid (chlorogenic acid) and 3,5-di-*O*-caffeoyl quinic acid, were also isolated.

Keywords: *Helianthus annuus*, hairy root, benzoxepin

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