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# 論文發表摘要

中山醫學院營養學系

翁玉青、陳暉雯、歐珠琴\*

RELATIONSHIPS BETWEEN MATERNAL AND NEONATAL  
VITAMINS, RBP, AND FATTY ACIDS NUTRITIONAL STATUS  
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College

We have studied the changes in the fatty acid profiles of red blood cell membrane phospholipid and in the concentrations of vitamin E, vitamin A and RBP of plasma in 29 pairs of moth and their term infants.

Lipid of RBC was extracted according to the method of Folch et al. Phospholipids were separated from other lipid classes by TLC and fatty acid composition was determined by GC. Vitamins A and E of plasma were analysed by HPLC. RBP was determined by the RID Kit.

Maternal RBC stearate and arachidonate at delivery were significantly greater than those in the cord blood ( $p < 0.05$ ), however, oleate was significantly lower in cord blood than in maternal RBC at delivery ( $p < 0.05$ ). Maternal RBC docosahexaenoic acid was significantly lower in the first trimester than that at delivery and cord blood ( $p < 0.05$ ). The levels of vitamin A and E and RBP were, however, significantly lower in the cord blood than in the maternal blood ( $p < 0.05$ ).

It was suggested that discriminating placental transport mechanisms existing for certain long-chain polyunsaturated fatty acid and fat-soluble vitamins.

## 中山醫學院解剖學科

鄭敏雄\*、王旭明、楊世忠

### DISCRETE LOCALIZATION OF CELLULAR UPTAKE BY FLUORESCENT DYES IN CSL-1, GOTO AND MULTIDRUG-RESISTANT TGW TUMOR CELLS AFTER CDDP AND /OR RICIN TREATMENT

M.-H. Cheng\*, S.-M. Wang, S.-C. Youn

Department of Anatomy, Chung Shan Medical and Dental College

The localization of the mitochondrial uptake by rhodamine 123 and the AO-RNA or AO-DNA complexes by acridine orange in the CSL-1 (Human large cell undifferentiated lung carcinoma), GOTO (Human neuroblastoma) or the multidrug-resistant TGW (Human neuroblastoma) cells after the treatment of CDDP in combination with or without ricin would be detected and measured by the 3D (three-dimensional reconstruction) manipulation of the confocal laser scanning microscope (Zeiss LSM 410) was desirable.

According to the current experiments, we found the result of the in vitro chemosensitivity of TGW was similar to that of CSL-1 cells, measured by the microplate fluorometer assay using a computer-driven CytoFluor 2300, and the achroplan object of LSM 410 did not fit for the determination of fluorescence.

Then we used the plan-neofluor object instead of achroplan to get a good fluorescent observation. We have tried to establish the drug-resistant human carcinoma sublines in culture by progressively increasing the concentration of the anticancer drug and to detect the uptake of rhodamine 123 and the localization of p-glycoprotein 170 of the cells by the confocal LSM 410 after the treatment of the drug and the staining with the C219 immunofluorescence labelling.

中山醫學院病理科、生化研究所

許振東\*、林玉玲、黃惠珮、王朝鐘

TUMORGENICITY OF NO-NTA DERIVED FROM REACTION OF MAILLARD PRODUCTS WITH SODIUM NITRITE

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Department of Pathology\*, Institute of Biochemistry, Chung Shan Medical and Dental College

N-nitroso-N-(3-keto-1,2-butanediol)-3'-nitrotyramine (NO-NTA) is a product of model browning system in the presence of sodium nitrite. In this study, the chemical structure is confirmed by spectral studies, including UV, mass, nuclear magnetic resonance and infrared. NO-NTA is strongly genotoxic to the rat hepatocyte and is moderately cytotoxic to mouse C3H10T1/2 cells. Results obtained in this study indicate that NO-NTA inflicted DNA damage by the formation of DNA adduct. Additionally, C3H10T1/2 cells are treated with NO-NTA and, following an addition of 12-0-tetradecanoylphorbol-13-acetate(TPA) as promotor, the increase of transformed foci indicates that NO-NTA could possible be and inhibitor of TPA tumor promotion. Transformed cell line from NO-NTA initiated and TPA promoted foci had increased saturation density and growth ability in soft agar reactive to control line. These results suggest that the formation of genotoxic agent of nitroso-derivatives may take place in nitrite-containing food or in vivo by nitrosation following ingestion of tyrosine and glucose. Tumorigenicity of NO-NTA-transformed cells inoculated into ICR mice are now in progress.

## 中山醫學院營養學系

陳肅霖、王進崑\*

### THE FORMATION AND MUTAGENICITY OF N-NITROSOGUVACOLINE IN BETEL QUID

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Alkaloids of areca nut contributed some physiological activities, including stamina and general well-being. Arecoline was the main alkaloid of areca nut. N-nitrosoguvacoline, nitrosating product of arecoline, was the only one nitrosamine found in the betel quid chewing juice in Taiwan. To understand the role of N-nitrosoguvacoline in human health. The formation and mutagenicity of N-nitrosoguvacoline was discussed in this study.

The content of nitrite decreased during the nitrosation of arecoline at pH 3 condition significantly. But the formation of N-nitrosoguvacoline favored at pH 7 condition. It's evident that the addition of slaked lime, promote the formation of N-nitrosoguvacoline. Crude phenolic extracts from *Piper betle* inflorescence and leaf inhibited the formation of N-nitrosoguvacoline effectively. However, high concentration of crude phenolic extract from areca nut increased the formation of N-nitrosoguvacoline. The mutagenic results showed that arecoline induced weak mutagenicity on *Salmonella typhimurium* TA100, and N-nitrosoguvacoline was mutagenic to strains of both TA98 and TA100.

## 中山醫學院附設醫院婦產科部

蔡宏志

### THE EFFECT OF TCDD (DIOXIN) ON ENDOMETRIOSIS IN FEMALE RATS AFTER ACUTE INTOXICATION

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At recent, it has described an association of endometriosis in rhesus monkeys following chronic exposure to 2,3,7,8-tetrachlorodibenzo- $\beta$ -dioxin (TCDD), and tissue distribution and toxicokinetics of TCDD in rats after intravenous injection has been investigated. At this study we chose female rats (250-290g) to receive TCDD intravenously (acute intoxication). TCDD 25  $\mu\text{g}/\text{kg}$ , 50  $\mu\text{g}/\text{kg}$  or 75  $\mu\text{g}/\text{kg}$  in DMSO were injected intravenously to rats, and the animals were euthanized by decapitation 2,8,24hr and 2,8,16 days. Control rats were injected with DMSO only, treated same way 2 hr and 2 days. Trunk blood was collected, and the serum was prepared to assay the concentration of estradiol ( $E_2$ ), progesterone ( $P_4$ ), testosterone (Testo), and androstenedione (ADD) by RIA. The liver, brain, lungs, gall bladder, lymph nodes, ovary, uterus, and peritoneum were removed respectively and frozen then prepared for histopathologic study. Preliminary result of our study showed that TCDD 50  $\mu\text{g}/\text{kg}$  in DMSO treatment to rats had estrogenic and progestational effect after acute intoxication. This phenomenon may be one of the etiologic factors in the pathogenesis of endometriosis.

## 中山醫學院牙醫系

黃明發、楊惠雯\*、呂毓修

Effect of glazing, grinding and polishing treatment on the properties of dental porcelain surface

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In clinical practice, it is occasionally necessary to modify the occlusal relationships and contour of ceramic restorations. This adjustment procedure will break the glazed surface. Based on the equipments, time and appointments, most dentists usually used some grinding and polishing instrument to smooth the surface instead of reglazing. The purpose of this study is to compare the effects of glazing, grinding and polishing on the properties of the ceramic restorations, in order to recommend an efficient and effective sequence for the polishing of the ceramic restoration which have had their autoglazed surface altered. Six groups, each consisting of five specimens were subjected to glazing, polishing sequence of Shofu Porcelain Adjustment Kit and diamond polishing paste. The surfaces following each treatment were evaluated for average roughness(Ra) with a surface roughness tester. The results indicated that the glazed surface is the smoothest, while the surface adjusted with the Dura-White Stone is the roughest. Significant difference is existed between the sequential polishing method of Shofu Porcelain Adjustment Kit and the glazing( $P < 0.01$ ). No significant difference is found in the resulting roughness values between the glazing and the diamond polishing paste( $P > 0.05$ ).

中山醫學院營養科學研究所

蘇國雄、張淑君\*

THE ANTI-GROWTH EFFECTS OF SEVERAL EDIBLE PLANTS ON A. NIGER AND C. ALBICANS.

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Chung Shan Medical and Dental College.

The infection of *A. niger* and *C. albicans* has occurred in immune-suppressed patients. The drug for fungi infection therapy shows strong cell toxicity and is not practical for clinical application. In this research, the anti-growth effects of several edible plants on *A. niger* and *C. albicans* were studied in vitro. The plants used in this research were :garlic ball, leek white ,leek green ,green onion( white), green onion( green) , hot pepper, old ginger , young ginger, basil, and chinese parsley.

*A. niger* and *C. albicans* were incubated on Sabouraud agar. The disc with plant extract were placed on the agar. After 48 hours, the circle area around the disc and without any visible fungus growth were measured. Garlic ball, leek green, leek white , green onion( green) , and green onion ( white), showed anti-growth effects on both *A. niger* and *C. albicans* . Then, the influence of temperature (heat) and salt on the anti-growth effect of the five plants on both fungi were also studied. The results showed that the anti-growth effect of each plant decreased with increasing temperature. The salt at the regular concentration we consumed did not show any interfere on the anti-growth effect of each plant on both fungi.

Furth studies may be necessary to investigate the influence of pH. This research provides the information regarding the clinical application of several edible plants for immune-suppressed patients to prevent the infection of *A. niger* and *C. albicans*.



## 中山醫學院營養科學研究所

殷梅津\*

### THE DELAYING EFFECTS OF NATURAL ANTIOXIDANTS ON LIPID OXIDATION: LIPOSOMES MODELS.

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and Dental College.

The delaying effects of several natural antioxidants alone, or combined, on lipid oxidation were studied in this investigation.

The natural antioxidants used included  $\alpha$ -tocopherol,  $\beta$ -carotene and coenzyme Q. A phosphatidylcholine liposomes model was used as lipid oxidation base. These natural antioxidants based on their lipid-soluble characteristic, were incorporated into liposomes during liposomes preparation. The degree of lipid oxidation was measured by TBA method at times: 0, 12, 24, 36, 48, 60, and 72 hours.

The results showed that the delaying effects of these antioxidants on lipid oxidation followed this order: control >  $\alpha$ -tocopherol >  $\beta$ -carotene >  $\alpha$ -tocopherol plus  $\beta$ -carotene > coenzyme Q >  $\alpha$ -tocopherol plus coenzyme Q >  $\beta$ -carotene plus coenzyme Q =  $\alpha$ -tocopherol plus  $\beta$ -carotene plus coenzyme Q. The combination of any two antioxidants showed synergistic effect on delaying lipid oxidation. However, the combination of three antioxidants used in the research model didn't show better delaying effect than the group:  $\beta$ -carotene plus coenzyme Q. The application of these three antioxidants alone on medical therapy, human nutrition and food science has been studied. It is the first time to show the comparison of these three antioxidants on lipid oxidation delaying in vitro. Further studies are necessary to investigate the difference of these antioxidants on different conditions. The possible variables that may interfere the influence of antioxidants include oxygen pressure, interaction of protein and lipid.

## 中山醫學院藥理學科

施宏哲\*、劉生明、周明勇

### EFFECTS OF ANTIGASTRIC ULCER ON SIJUNZITANG IN RATS(IV)

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SiJunZiTang is the representative medicine of Chinese Tradition Medicine for strengthening digestion. From the several previous experiments. We know that SiJunZiTang can help retard psychic stress gastric ulcer by reducing the quantity of free HCl in gastric juice. The experiment of vagotomy shows that the effect of SiJunZiTang on the quantity of the free HCl in the gastric juice has nothing to do with vagus nerve of the stomach. Thus, this experiment is to judge of SiJunZiTang on influence the quantity of Neuro Chemical Transmitter and via the effect to prevent psychic stress gastric ulcer. The experiment used male wistar rat ( $200 \pm 20$ g), was divided into two groups: A-group were Continuously administer 500mg/kg by p.o. for 7 and 14 days. Group B were administration 250mg/kg, 500mg/kg and 1000mg/kg by p.o. 30 min. before test. Then follow by Sibuya's method, Waters HPLC was used to analyze the change of monoamine in the brain of rat. The experiment cleraly shows that the quantity of serotonin and dopamine has drug dependent and change by the time. From this result. We know that SiJunZiTang can prevent psychic stress gastric ulcer. Whic is connected to change of quantity of serotonin and dopamine in the brain. It will be discussed in the further research.

**Characterization of NO-NTA-transformed C3H10T/12 cell line**  
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Shan Medical College, Taichung, Taiwan, R.O.C.

N-nitroso-N-(3-Keto-1,2-butadiol)-3'-nitrotyramine(NO-NTA), is a product from the reaction of tyrosine, glucose with  $\text{NaNO}_2$  by the Maillard reaction model system. A new cloned cell line, NO-NTA cells, was derived from transformed C3H10T1/2 cells-initiated by NO-NTA and promoted by TPA. The characterization of these clones are: (1) showing a type II and type III morphology, (2) high growth rate, (3) forming colonies in soft agar, (4) strongly tumorigenic and (5) increasing DNA distribution by flow cytometric analysis. All properties obtained from NO-NTA cell were related to the passage of cell. Furthermore, we found that the high passage NO-NTA cell overexpress p53 and c-myc. These results suggest that c-myc and p53 may play a role in chemical carcinogenesis.

## 中山醫學院公衛系及微生物科

王凱助、劉姿妙、歐威志\*、張德卿

### GENETIC CLONING OF HUMAN POLYOMAVIRUS JCV VP1 FROM URINE AND ITS APPLICATION

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The major capsid protein VP1 gene of human polyomavirus JC virus, Taiwan-3 strain, was generated from urine of an autoimmune disease patient by polymerase chain reaction (PCR). The VP1 DNA was cloned into a prokaryotic expression vector, pGEX-4T-1, for expression in *E. coli*. The nucleotide sequences and deduced amino acid sequences were compared with the JC virus prototype, Mad-1. Thirty nucleotides were different but only six of them affected amino acid coding between these two strains. The recombinant VP1 protein was purified by electroelution and used to raise monospecific antiserum from rabbit. The antiserum was able to recognize the recombinant VP1 protein in Western blot. The concentration of the recombinant JCV VP1 antigen and the monospecific antiserum has been titrated for enzyme-linked immunosorbent assay. The recombinant VP1 protein can be used for serologic assay in the epidemiological study of JC virus. The crossreactivities of the antisera of the major capsid protein VP1 human (JC virus), murine (polyoma) and avian polyomaviruses BFDV were also investigated. The crossreactivities of anti-JCV VP1 antiserum to polyoma and BFDV VP1 were approximately 30% and 4% respectively according to the densitometry of Western blot analysis. Furthermore the VP1 genes of the three JC virus strains, CY, Taiwan-1, Taiwan-3, found in Taiwan were also sequenced. When compared to the JCV prototype, Mad-1, it was found that three were 37 nucleotide alterations among these four strains but three were only 12 amino acids affected by the nucleotide alterations. The recombinant JCV VP1 protein and the monospecific antiserum will be very important reagents for the molecular characterization to understand the physiology of the virus. In addition, the VP1 protein could possibly be developed as a subunit vaccine in the future.

## 中山醫學院解剖學科

楊世忠\*、王旭明、廖克剛、鄭敏雄

### THE MORPHOLOGICAL STUDY OF THE CONFOCAL MICROSCOPIC AND THREE DIMENSIONAL RECONSTRUCTURAL TO ASTROCYTE

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The RCR-1 cell line derived from normal newborn rat brain (JAR-2, 51 rats). In spite of long-term culture these cells preserve their basic characteristics in vivo. For examples; These cells have processes bearing appearance and the ability of glial fibrillary acidic protein (GFAP) synthesis, without cAMP added in culture medium.

The observation under phase-contrast microscope, we can see the membranous-like structure at the end of the cell's processes. To examine these cells by Giemsa stain or scanning electron microscope (SEM) have the same resemblance as above. In lower cell's density culture, the undulating movement occur at the membranous portion of the free-end of the cell process. In contrast, in higher cell's density culture the membranous portion attached from one cell to another, to make a network appearance that is called as syncytial structure of neuroglial cells.

The localization of GFAP in RCR-1 cells was detectable in perinuclear of cytoplasm and their processes. The distribution of the long fibric-like filaments of GFAP may verified by immunocytochemical stain method. The facts make the three dimensional structure may revealed and restructured by LSM-confocal microscopy.

## 中山醫學院附設醫院外科\*

林豐彥\*、蔣思澈、林偉隆、周明智

盲腸結紮針刺誘發敗血症小白鼠血中，腹腔液Endotoxin存在率及各器官病理變化的觀察。

於先前的預實驗，利用ICR及BLB/C品系的mice來作盲腸結紮針刺誘發的敗血症觀察研究中發現ICR品系mice比BLB/C品系mice較容易誘發呈現敗血症。因此，本實驗的動物model均採用ICR品系mice。在一般飼養食物與生活環境條件下。禁食8小時後以pentothal靜脈麻醉做盲腸結紮與針刺法誘發敗血症，16小時後沒有死亡的小白鼠經斷頭犧牲後，採取血清；腹腔液及各組織。在實驗組中血液及腹腔液均可培養出屬腸道革蘭氏陰性菌為多，佔約87.5%，而在腹腔液所測量到的內毒素約為血清值的百倍左右。但血清中的量仍無法測出，可能是內毒素含有值太低。在肝臟及脾臟及腹膜等組織切片觀察上也均有macrophage及白血球蓄積等發炎症狀的出現。

中山醫學院付設醫院 小兒科・檢驗科

林文豹、陳家玉、江惠玲、潘美珠、劉嘉斌

FRUCTOSAMINE AND APOLIPOPROTEINS IN OBESE PRE-SCHOOL CHILDREN

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Obesity is frequently associated with higher risk for coronary artery disease (CAD) as well as impaired glucose tolerance in adults. In CAD, apoB is increased, ApoA1 is decreased and ApoA1/ApoB ratio is reduced. Similar changes of apolipoproteins occur in obesity. It is the aim of this study to investigate the pattern of apolipoproteins, lipoprotein(a) and glucose tolerance in 5-to-6 year-old pre-school children.

Body mass index (BMI) and fasting serum cholesterol, triglyceride, glucose, protein, fructosamine, ApoA1, ApoB, lipoprotein (a) were studied in 33 obese preschool children (BMI 21.56 +/- 1.14) (mean +/- SD), and 47 control children (BMI 14.75 +/- 1.14) (mean age 5.3 years, range 5 to 6 years old). Obese children had higher concentration of protein, TG, ApoB ( $p < 0.05$ ), lower concentration of ApoA1, while there was no significant difference in glucose, cholesterol, fructosamine and lipoprotein(a) levels. Obese children demonstrated lower ApoA1/ApoB ratio (91.20 +/- 0.302 vs 1.47 +/- 0.36) and fructosamine/protein ratio (29.98 +/- 1.79 vs 32.88 +/- 5.53) than control children ( $p < 0.05$ ).

These findings indicated that there were significant metabolic changes in childhood obesity that had already occurring in pre-school children.

中山醫學院附設醫院牙科部牙周病科 微生物學科

何全城、錢佑、簡宏堅

ANTIBIOTICS IN PERIODONTAL THERAPY IN TAIWAN

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The purpose of this study was to determine the susceptibility of 15 species in 156 clinical isolates from periodontal lesions to twelve antibiotics by using agar dilution technique. This is means of determining which antimicrobial agents were inhibitory for bacteria frequently associated with periodontal diseases. The result showed that most of the tested clinical strains were relatively susceptible to  $\beta$ -lactam groups. The inhibitory activity was noted greater with amoxicillin than with others, e.g., penicillin or ampicillin. The antibacterial activities of minocycline were significantly higher than with tetracycline for *Actinobacillus actinomycetemcomitans* and *Streptococcus* spp., however, it is pretty much the same antibacterial activities of minocycline and tetracycline for other anaerobic gram negative bacilli. Clindamycin demonstrated excellent activity against most periodontal pathogens with the exception of *Eikenella corrodens*. Metronidazole was considerably less active than other antibiotics against the majority of the periodontal isolates but was effective against *A. actinomycetemcomitans*. Antibacterial activity obtained with erythromycin was relatively susceptible to all of the penicillin sensitive strains, but was less effective to against facultative microorganisms.

In conclusion, the antibiotic susceptibility in this study indicated that several different antimicrobial agents had good to excellent activity against many of the clinical isolates of periodontal bacteria frequently associated with diseased sites. However, non of antibiotics emerged as being inhibitory for all the clinical strains that are suspected of being possible periodontal pathogens.



中山醫學院生化所

謝易修\*、陳凌雲、劉哲育、陳玉美

EVALUATION OF ANTITUMOR POTENCY OF TRYPSIN INHIBITORS

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Studies have indicated that both naturally occurring and synthetic protease inhibitors have ability to inhibit carcinogenesis *in vivo*. *In vitro* studies have demonstrated that protease inhibitors are also capable of preventing transformation of culture cells induced by chemical carcinogens. The aim of this study was performed bacterial mutagenesis assay, transforming of cell cultures, animal study, and using two type of trypsin inhibitors (Bowman Birk type & Kunitz type) to evaluate the antitumor potency of these trypsin inhibitors.

Our preliminary data shown that transforming of cell cultures were more effectively suppressed by the Bowman Birk type trypsin inhibitors.

**LOW-FREQUENCY ELECTROMAGNETIC FIELDS AND CANCER**  
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In industrial countries, the erection of high-voltage transmission lines and stations for power supply has been popular. In Taiwan, due to its dense and broad population distribution, lots of residences are located in nearby these facilities. Residents in the vicinity, therefore, have been suffering from being exposed to low-frequency (50-60 Hz) electromagnetic fields induced by the above high-voltage electricity. In addition, the use of electric products (e.g. shavers and cellular phones, etc.) may also create strong low-frequency electromagnetic fields. There have been an increasing number of investigations concerning the biological effects of low-frequency fields. Some of the results show that low-frequency fields may cause a risk of getting cancer. In this project, we review the studies of low-frequency fields and cancer up to date, and make an evaluation that takes these information into account.

中山醫學院病理學科

林偉隆\*、蔣思澈、鄭敏雄

THE EFFECT OF FREE RADICAL ON FIBRONECTIN  
DEPOSITION IN CSL-1 CELL

W.L.Lin\*, S.T.Jiang, M.H.Cheng

We established a cell line (CSL-1) from a chinese patient with large cell undifferentiated lung carcinoma metastasis to right chest wall in previously study. This cell line reveals a poorly differentiated and pleomorphic large carcinomatous cell. The tumorigenicity of this cell had been identified by xenograft experiment to nude mice.

Fibronectin is especially abundant in basal laminae, and play an important role in cell adhesion, proliferation, differentiation and migration. Many types of cancer cells lose their ability to synthesize fibronectin, and at the same time lose their normal cell shape, surface morphology, cytoskeletal organization, and attachments to the extracellular matrix.

In this study, fibronectin deposition in CSL-1 cell is only 1/3000 in amount comparatively with NIH-3T3 cell line. Furhtermore, fibronectin mRNA expression is confirmed by RT-PCR assay. In cancer chemotherapy, many antibiotics affect cell to produce free radical to kill cell. But the side effect of the antibiotics is very serious, its seem to be evaluate small amount of free radical, under lethal dosage effect on cancer chemotherapy.

We aim at the effect of free radical on cellular biochemical characterization especially fibronectin synthesis.

## 中山醫學院解剖學科

劉春蘭、廖克剛

### THE DISTRIBUTION OF TWO CALCIUM- BINDING PROTEINS, PARVALBUMIN AND CALBINDIN-LIKE IMMUNOREACTIVITIES IN THE RED NUCLEUS OF RATS

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Neurons immunoreactive for calbindin (CB) antiserum were found to concentrate in the caudal part of the red nucleus as well as in a small ventromedial area of the middle red nucleus. They were large-soma cells. Not all retrogradely labeled rubrospinal neurons in this portion of the nucleus contain CB. Rubrospinal neurons scattered in the rostral red nucleus usually had medium-sized somata and contains no CB. The dendritic arbors of retrograde tracer labeled rubrospinal neurons were revealed by intracellular injection of Lucifer yellow using a fixed tissue dye injection technique. Staining of these injected tissue with CB antiserum revealed that CB was confined to the soma and proximal portion of the dendritic trunks.

Sections processed with an antiserum to parvalbumin (PV) shows that fibers traversing the red nucleus and those in the ventral tegmental decussation contained the immunoreactivity. The large rubral neurons were not stained. To find out whether rubrospinal axons contain PV they were first labeled with an anterograde tracer dextran conjugated with fluorescein from the red nucleus using a live brain slice tracing technique. Staining of such-treated tissue with PV antiserum revealed that at least some labeled axons contain PV-like substance. Immunoreactivities were found at the cut ends or swellings, presumably boutons of the larger caliber axons. In summary, our data suggest that two different calcium-binding proteins, calbindin and parvalbumin, may be present in the soma-dendritic and axonal compartment of some rubrospinal neurons, respectively. They may relate separately to the receiving and output function of these two compartment.

# 中山醫學院附設醫院免疫風濕科及醫學院微生物科

蔡榮泰\*、王梅林、張德卿

## INFECTION STUDY OF HUMAN POLYOMAVIRUSES IN AUTOIMMUNE DISEASE PATIENTS

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We have assayed for the presence of human polyomaviruses in urine of autoimmune disease patients, such as systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), rheumatoid arthritis (RA), or dermatomyositis/polymyositis (DM/PM), by PCR. The results indicate that approximately 40% of patients were JCV positive and 15% of the JCV positive patients were also infected by BKV at the same time according to Southern blot and DNA sequencing of the PCR products. Interestingly, the JCV present in autoimmune diseases patients were Taiwan-1, Taiwan-2, and Taiwan-3 strains with pentanucleotide-A (GGGAA) and/or B (AAAGC) deletions within the regulatory region. In addition, BKV found in the examined samples were Taichung-1 and Taichung-2 strains. Taichung-1 had two nucleotides alteration and Taichung-2 had six nucleotides differences within the regulatory region when compared to WW BKV archetype. Although the examined autoimmune diseases patients included RA, SLE, PM, DM and SS patients, there appears to be no correlation between disease and virus strains. However, Taiwan-2 strain JCV with 2 copies of pentanucleotide A deletion was present in the patient with the longest periods of immunosuppressive medication.

Stratotype Events	None
Rotation Events	NA[3](3,30)=3.88, 1-0.029
Heating Events	None
Distance (cp)	NA[3](3,30)=4.02, 1-0.016
Behavior Response	NA Group

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## EFFECTS OF NICOTINE ON AMPHETAMINE-INDUCED BEHAVIORAL RESPONSE OF ACUTE NICOTINE-TOLERANT RATS.

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Nicotine alone can promote or depress behavioral response that is according to dose, recorded time, type of behavioral response, tolerant or nontolerant, etc. Amphetamine is a indirect dopamine agonist that increases motor activity. The synergistic effects of nicotine and amphetamine on acute nicotine-tolerant rats are studied.

Experiments were performed on 40 Wistar rats weighing 200-280g. Rats should be adapted for experimental cage (Activity Monitor Video Path Analyzer, 50×50×35cm) 1.5 hours once each day for a week, and be induced acute tolerant (nicotine 0.3mg/kg, IP, 2 hours before treatment). 40 rat were divided into two treatment group, N (nicotine) group and NA (nicotine and amphetamine) group. N group was divided into 4 subgroup: control, N1, N2, N3 (inject saline, 0.15, 0.3, 0.6mg nicotine/kg, IP, respectively). NA group also divided into 4 subgroup: control, NA1, NA2, NA3 (inject amphetamine 1mg/kg, 0.15, 0.3, 0.6mg nicotine/kg, IP, respectively). The recorded behavioral response including distance, rearing, stereotype and rotation.

The results were summarized in the table below. Only those which one-way ANOVA revealed significant difference were reported.

Behavior Response	Treatment	
	N group	NA group
Distance (cm)	N3[F(3,36)=4.62, P=0.016]	None
Rearing Events	None	NA3[F(3,36)=4.0, P=0.026]
Rotation Events	N3[F(3,36)=3.88, P=0.029]	NA3[F(3,36)=3.25, P=0.049]
Stereotype Events	None	None

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**Pyridoxine effect on Premenstrual Syndrome-a randomized controlled clinical trial**

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**Objective:** Treatment of PMS remains a clinical uncertainty in spite of a more than 40 years dedication of research. However, few controlled clinical trials indicated that pyridoxine is a hopeful resolution. The aim of this study was to evaluate the treatment effect of Pyridoxine in PMS among college women.

**Subjects and design:** A randomized controlled double-blind cross-over clinical trial for 106 college women was conducted from September 1994 through July 1995. Moos' MDQ score system was used in measurement of PMS intensity. The trial scheme was designed for 3 cycles in each course and 1 cycle for washout in between. One hundred mg Pyridoxine per day for 10 days premenstrually was used in treatment arm and starch was used for placebo.

**Results:** There were more women showed treatment effect of Pyridoxine (58 vs 47). The odd ratio (OR) was 1.84 with 95% confidence interval of 1.07 - 3.17.

The mean of Moos MDQ score reduction of pyridoxine treatment is 4.28 (SD 21.95) while placebo arm showed adverse effect with 1.26 (SD 17.84) increase. No drug complication.

**Conclusion:** Our finding suggest that Pyridoxine virtually demonstrated a clinical effect in the treatment of PMS and no side effect had been seen.

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ANTIHEPATOTOXIC EFFECT AND MOLECULAR MECHANISM OF  
CAPILLARISIN ON CHEMICAL CARCINOGEN INDUCED INJURY IN  
RAT HEPATOCYTES

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Capillarisin is a naturally occurring constituents of *Artemisiae Capillaris Flos* which is used as a refreshing and diuretic folk medicine commonly, and tert-butyl hydroperoxide is an organic carcinogen. In the present study we examine the effect of capillarisin on tert-butyl hydroperoxide-induced hepatic toxicity and DNA damage in rat hepatocytes. It was found that the hepatic function marker ALT and LDH were significantly suppressed by capillarisin (0.5 mM) and that the elevation of malondialdehyde activity induced by tert-butyl hydroperoxide (1.5 mM) was also suppressed by capillarisin. To further elucidate the hepatoprotective mechanism of capillarisin, the tert-butyl hydroperoxide-induced DNA repair synthesis in rat hepatocytes was studied.



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SEASON VARIATIONS OF HOUSE DUST MITE ALLERGEN

(Der p 11) IN TAICHUNG AREA

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In Taiwan one of three children have related allergic diseases, such as asthma, rhinitis, and so forth. It is found that house dust mite (HDM) is the most important allergen in Taiwan, especially for asthmatic children. Moreover *Dermatophagoides pteronyssinus* (Dp) was recognized as the dominant species of HDM in Taiwan area. More than 90 % children with asthma had positive skin test responses to Der p1 and Der p11. The objective of this investigation was to assess the Der p11 amounts of surface samples in the houses of 15 HDM-allergic children. Dust samples were collected monthly by vacuuming a 1m<sup>2</sup> area for 2 min. from the homes of 15 asthmatic children from October 1994 to September 1995. The assay of samples is not finished completely, we will show the results in the symposium.

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## EFFECT OF AGE ON THE MODULATION OF INTRACELLULAR CALCIUM BY THYROTROPIN-RELEASING HORMONE (TRH) IN MALE RAT LACTOTROPHS

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The thyrotropin-releasing hormone (TRH)-stimulated prolactin secretion by modulating intracellular calcium release was investigated. The release of intracellular calcium was measured by fluorescence confocus microscope, and the amount of prolactin secretion was determined by SCIBA. The result have found that the increased secretion of prolactin from individual lactotroph stimulated by TRH was correlated with the change of intracellular calcium release. It is confirmed that the secretion of prolactin was promoted by calcium mobilization.

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DETECTION OF CYTOMEGALOVIRUS IN HEALTHY  
BLOOD DONORS AND INFANTILE AUTISM  
PATIENTS BY POLYMERASE CHAIN REACTION

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Cytomegalovirus (CMV) is a ubiquitous human viral pathogen belonging to the family of herpes viruses. Infection with CMV is usually asymptomatic and often gives rise to undetected latent infection and reinfection. Certain groups are at risk of developing far more severe symptoms, such as leukopenia, pneumonia, retinitis, and, occasionally, encephalitis. Those considered at greatest risk are neonatal infants and immunocompromised or immunosuppressed individuals. Transmission of the virus may occur via blood or blood components received during transfusion or transplantation. Virus transmission also occurs via the placenta and via ingestion of breast milk or contact with urine and other bodily fluids.

In this study, a PCR assay was developed and optimized to detect CMV DNA in the blood of 150 donors, urine of 50 infantile autism patient, and to compare its sensitivity with CMV cell culture.

## 生鮮乳中免疫球蛋白(IgG)含量的比較

本篇研究之目的在利用傳統的三明治型酵素連結免疫吸附分析法(Sandwich enzyme-linked immunosorbent assay)來分析及觀察在生、鮮乳中免疫球蛋白濃度及量上的差異性，來探討此蛋白的濃度可否作為牛奶品質的一個指標。

在之前的預實驗，利用SDS-polyacrylamide gel electrophoresis電泳分析經不同濃度硫酸銨劃分所得到的生、鮮乳蛋白質成份的差異，發現其中的免疫球蛋白G(Immunoglobulin G)的色帶顏色差異相當大。由東海大學牛乳加工廠所取得的生乳經單離純化得到均一純度免疫球蛋白，由兔子免疫後所製得具有單一特異性(mono-specific)的抗血清，應用三明治型酵素連結免疫吸附分析法追蹤生、鮮乳中免疫球蛋白含量的變化。

生乳中的濃度為 $140.73 \pm 16.99 \mu\text{g/ml}$ ，在市售鮮乳濃度的變化在 $0.16 \pm 0.017 \mu\text{g/ml}$ 至 $0.28 \pm 0.04 \mu\text{g/ml}$ ，此現象可能是因為牛奶會隨著加熱、加工過程所造成的差異。

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**THE INVESTIGATION OF THE EXPRESSION  
OF PKC IN HUMAN BREAST CANCER**

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The catalytic fragment of phosphatidylserine-dependent protein kinase (protein kinase C, PKC) has been shown to correspond to tumor formation. It is referred to as phosphatidylserine-independent protein kinase (protein kinase M, PKM). These protein kinases were examined in breast cancer. Both PKC and PKM were separated by DEAE-cellulose column chromatography; PKC was eluted in 120 mM KCl fraction while PKM in 300 mM KCl fractions. The expression of both PKC and PKM specific activities were increased in breast cancer tissue, as compared with the normal adjacent tissue. These results suggest that the elevated activity of both PKC and PKM may be involved in tumor formation in breast cancer.

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EXPRESSION OF CYTOKERATINS 8, 18 AND 19 IN HUMAN OVARY  
AND TESTIS AND IN THEIR TUMORS

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The expression of cytokeratins (CKs) 8, 18 and 19 was studied in normal human ovary and testis and in certain kinds of diseases and primary ovarian and testicular tumors by immunohistochemical methods using specific monoclonal antibodies anti - CK 8 (35/H11), anti - CK 18 (DC 10) and anti - CK 19 (RCK 108). Ten percent formaldehyde solution - fixed, paraffin - embedded sections obtained from patients' without ovarian disease or testicular lesion, and patients with simple ovarian cystic lesion, tubal pregnancy, mucinous or serous cystadenoma or cystadenocarcinoma, granulosa cell tumor of benign or malignant course, Sertoli - Leydig cell tumor of ovary, and thecofibroma, and patients with cryptorchidism, and sertoli cell tumor of testis were examined. The somatic cells of germinal epithelial (coelomic epithelium) type including reactive surface mesothelium of the ovary, the simple cyst, mucinous and serous cystadenomas and their malignant counterpart were stained for CK 8 and CK 19, whereas no detectable immunoreactivity for CK 18 was obtained. The somatic cells of sex - cord stromal type including granulosa cells of the follicle and surrounding theca cells, hormone - effected luteinizing granulosa and theca cells, tumor cells of granulosa cell tumor and thecofibroma and malignant counterpart of the former, and Sertoli - Leydig cells of testis were negative for CKs, but positive for vimentin. The germ cells both in follicles and seminiferous tubules show no detectable immunoreactivity for CKs and vimentin. The epididymis, which was derived from mesonephros, was positive staining for CK 8 and CK 19, and negative for vimentin. The results may suggest a regulation of CK and vimentin synthesis insupporting cell lineage. Therefore, demonstration of CK 8 and CK 19 is useful to differentiate an epithelial tumor from a sex - cord ovarian tumor.

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### PREPARATION OF MONOCLONAL ANTIBODIES AGAINST THE STRUCTURAL PROTEINS OF HERPES SIMPLEX VIRUS TYPE 1

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Herpes simplex virus type 1 (HSV-1) has a complex structure with three distinct morphological components. A lipid bilayer containing more than 14 membrane proteins surrounds an amorphous region called the tegument which comprises of more than 10 proteins. Within this is the icosahedral capsid which contains the double-stranded DNA virus genome 152 kbp in size. Fractionation of purified virus particles has demonstrate that the membrane proteins are responsible for virus attachment and penetration and the capsid proteins maintain the integrity of the virus genome. It has been difficult to assign functions to the tegument proteins although a transcriptional activator, a protein kinase and a protein responsible for shut-off of host protein synthesis have been identified. Efforts were made to produce monoclonal antibodies against these structural proteins. Different detergents were used to treat with purified virus and virus-infected cells to solubilize the membrane and tegument proteins from capsids. The protocol of antigen preparation for production of monoclonal antibodies was optimised. This project is aimed at studying the interactions between these structural proteins and their post-translational modification utilising monoclonal antibodies. Data will be presented which report on methods for the isolation and characterisation of monoclonal antibodies against these proteins and preliminary progress on the utilisation of the antibodies to study viral structural proteins.