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MORE HEPATIC LYSOSOMES AND DECREASE OF BILIARY EXCRETION OF LYSOSOMAL ACTIVITY IN CIRRHOTIC LIVERS.

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Lysosomes, the cellular digestive system, are involved in restructuration of tissue e.g. in hepatic cirrhogenesis. We therefore investigated the activity of lysosomal enzymes in liver homogenate, hepatic lysosomal fractions, plasma and bile of rats with cirrhosis induced by chronic exposure to CCl 4/Phenobarbital (cirrhotics:CIR n=16, untreated controls:CTR n=17). The activity of arylsulfatase (8±SD 2 vs 18±6 nmol/min/mg; $p<0.001$) and β-Hexosaminidase (10±2 vs 25±8; $p<0.001$) were significantly increased in liver homogenates in CTR and CIR respectively; the corresponding plasma levels were: 7±1 vs 12±3 ($p<0.01$) and 7±1 vs 12±1 ($p<0.001$), respectively. No significant increases were found in lysosomal fractions. Arylsulfatase activity was significantly decreased in bile: 47±16 nmol/min/mg liver vs 26±11 ($p<0.02$). Electron microscopy of lysosomes (Gomori) showed an increased number of lysosomes in hepatocytes of cirrhotic livers. These data suggest that lysosomes, which normally are excreted in bile, are retained in cirrhotic livers. This retention may be important in cirrhogenesis.

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EFFECT OF TRIMETHOPRIM (T) OR TRIMETHOPRIM-SULFAMETHOXAZOLE (TS) ON ZIDOVUDINE (Z) DISPOSITION

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T or TS are frequently prescribed in AIDS patients. Since Z and T are metabolized and excreted by glomerular filtration and tubular secretion, their coadministration could give rise to drug interaction which, considering the low therapeutic range of Z, might be clinically relevant. The kinetics of Z (3 mg/kg 1h i.v. infusion) were therefore evaluated in 9 AIDS patients in an open randomized crossover 3-leg study at a one-week interval: on Z alone and with T (150 mg) and TS (160-800 mg). Z and its glucuronide (GZ) were measured by HPLC in plasma and urine collected for 9 hr. Among the calculated kinetic parameters (Z: AUC, CL, T½, Vz, Vss, CLr, CLnr, MRT; GZ: AUC and CLr; AUC ratio, urine metabolic ratio), only the urine metabolic ratio and the renal clearance (CLr) of Z were significantly altered by the administration of T (-47% and -56% resp.) and TS (-42% and -47% resp.). This drug interaction, apparently due only to T, could become clinically relevant in patients with hepatic failure.

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"ENDOGENOUS" LITHIUM : A NEW TOOL IN NEPHROLOGY.

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In renal physiology, lithium (Li) clearance is used as a marker of proximal tubular function. With the present methods, administration of exogenous Li is necessary to evaluate Li clearance. Since the Li load might affect the function under study, determination of "endogenous" Li may be more accurate. Accordingly a sensitive atomic absorption spectrometric method has been developed. Li was measured at 670.8 nm with ammonium nitrate as a matrix modifier without background correction. Since Li measurement is influenced by the biological matrix, with recoveries of 95-105% in diluted urine but only 75-80% in plasma, the use of standard additions is required. Under these conditions, concentrations as low as 0.03 umol/L can be measured accurately. The intra/interday variability is <10%. Li has been measured in plasma and urine of healthy subjects (0.1-0.26 and 0.6-4.2 umol/L) and in patients with acute renal failure (0.06-1.7 and 0.03-4.2 umol/L). Thus, endogenous Li clearance can be determined successfully in humans. This approach represents a new investigational and diagnostic tool in renal pathophysiology.

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DRUG INDUCED ARTERIAL HYPOTENSION ASSOCIATED WITH METAMIZOL (DIPYRONE/NOVAMINSULFONE)

Results of the Comprehensive Hospital Drug Monitoring (CHDM)

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In the years 1974-1988, among 34'838 consecutively admitted patients, 9'720 were treated with metamizol. Arterial hypotension considered to be drug induced, probably with a pharmacological mechanism, was observed in 18 of 3'922 patients with intravenous and 1 of 4'181 with oral application. A further patient reacted with an anaphylactic shock and urticaria following oral use. For comparison, arterial hypotension due to other drugs was also documented for the years 1974-1982.

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RENAL EFFECTS OF DOPEXAMINE, A NEW DOPAMINERGIC AGONIST, IN THE NEWBORN RABBIT.

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Dopamine is frequently used in premature infants. When given at high doses, it may induce a deleterious vasoconstriction due to α-adrenergic vascular receptor stimulation. The renal effects of dopexamine, a new dopaminergic agonist with marked β_2-adrenergic and devoid of any α-adrenergic effect has been studied in 8 anaesthetized mechanically-ventilated newborn rabbits. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were assessed by the clearance of inulin and PAH, respectively. After a control period, dopexamine was infused intravenously at a rate of 4 µg/kg per min for 40 min; following a 20 min washout period, the same drug was again infused at a higher rate of 10 µg/kg per min for 40 min. Blood gases and pH remained stable throughout the experiment, while the hematocrit decreased slightly. Dopexamine, 4 µg/kg per min, did not induce changes in renal functions, heart rate or mean arterial pressure. When given at a rate of 10 µg/kg per min, a significant increase in urine flow rate (+ 25±5%; $p<0,01$), urine sodium excretion (+77±17%; $p<0.01$) and fractional excretion (+ 69±25%; $p<0.05$) was observed. GFR (ml/kg per min), RPF (ml/kg per min) and RVR (mmHg/ml per kg·min) remained stable with values of 2.49, 16.8 and 1.39, respectively. Heart rate increased slightly but significantly (+8±3%; $p<0.05$), without change in mean arterial pressure. The natriuretic and diuretic effect of dopexamine in the absence of changes in RPF or GFR, is probably mediated by a direct action of this agent on the dopaminergic tubular receptors.

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POSTPRANDIAL METABOLIC, ENDOCRINE, CARDIO-RESPIRATORY AND MUSCLE ACTIVITY RESPONSES TO β-ADRENERGIC AGONISTS (β-AG) IN CALVES

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Postprandial responses to two β-AG, po administered for 28 d, were studied in milk-fed calves. In β-AG treated animals, on d 1, blood insulin (I), glucose (G), lactate and nonesterified fatty acids levels, 0_2-consumption, CO_2-production and heart rate increased above, whereas blood growth hormone (GH) levels decreased below values of controls. On d 14 and 28, pre-and postprandial values of these parameters were similar to and only insulin-like growth factor I levels were below controls. After iv insulin injection, G and I remained elevated on d 1 in β-AG treated calves, whereas on d 14 and 28, I and G decreased as in controls. Muscle tremor was only seen on d 1 in β-AG treated animals. Thus, early and late posprandial responses to β-AG differed considerably and there was rapid development of desensitization to β-AG.

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CARDIORESPIRATORY, ENDOCRINE AND METABOLIC CHANGES IN EXERCISED CALVES DURING EARLY AND LATE β-ADRENERGIC AGONIST (β-AG) TREATMENT

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Cardiorespiratory, metabolic and endocrine changes were studied before, during and after 12-min treadmill exercise of calves daily p.o. administered the β-AG clenbuterol for 4 wk. On d 1 preexercise blood glucose, lactate (L), insulin, respiratory rate (RR) and volume (RV) and heart rate (HR) were elevated in β-AG treated animals. During exercise, L, cortisol, RR, RV and HR increased markedly and remained longer elevated afterwards in treated calves than controls, whereas O_2 consumption was similar because of reduced extraction rate in β-AG treated animals. After 2 wk, all values before and during exercise in β-AG treated animals were similar to controls. HR increase after Isoproterenol injection was much smaller in treated than in control calves. 7 d after β-AG withdrawal (wk 4), the β-AG evoked the same effects as on d 1. The data demonstrate rapid changes in sensitivity to the β-AG, leading to markedly different reactions to exercise load during β-AG administration.

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DETERMINATION OF CITALOPRAM AND ITS DEMETHYLATED AND DEAMINATED METABOLITES IN PLASMA BY GC-MS

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Citalopram, an antidepressant, is a highly selective inhibitor of serotonin reuptake. It is metabolized by demethylation to form desmethyl- and didesmethylcitalopram. Deamination leads to the propionic acid derivative. So far, HPLC and TLC methods were used for the determination of citalopram and its metabolites. In order to increase sensitivity and specificity in the study of low plasma levels following therapeutic doses, we have developed a gas-chromatographic mass-spectrometric (GCMS) procedure with single ion detection. Citalopram and the demethylated metabolites are extracted from plasma with heptane at pH 10 and derivatized with TFAA prior to analysis by GCMS. The propionic acid metabolite is extracted from the same plasma with diethylether at pH 4, methylated with iodomethane and separately determined by GCMS. Mass spectra of the compounds have been obtained by electron impact (EI) or positive chemical ionization (PCI) mode. EI is found to give the best sensitivity and is used to analyze plasma extracts. Selected-ion monitoring is carried out at m/z 58 for citalopram and at m/z 238 for desmethyl-, didesmethylcitalopram and the propionic acid metabolite. Internal standards are methylmaprotiline, maprotiline and desmethylmaprotiline . The method is tested in a group of depressive patients treated with a daily dose of 20 to 60 mg of citalopram.

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STABILIZATION OF XENOBIOTIC METABOLISM IN RAT MONOLAYER HEPATOCYTE CULTURE

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The activity of enzymes involved in xenobiotic metabolism is essential for toxicity testing in vitro. In cultured rat monolayer hepatocyte cultures, up to 50% of the activity is lost within the first few hours after liver perfusion. Agents, known to stabilize xenobiotic metabolism were added to the culture medium during the first 3, 24 or 48 hours after seeding. Attachment (cell density), maintenance of cytosceleton (morphology) and proteins (cytochrome P-450, protein content), membrane integrity (LDH content/release), and function (aldrin epoxidase activity) were determined. Phenobarbital (3mM) and dimethylsulfoxide (0.04%v/v) stabilized xenobiotic metabolism and protein content, fetal calf serum (2% and 10%) increased attachment, decreased cellular protein content but stabilized xenobitotic metabolism and glycerol (0.03-3%) was most effective in the 3h attachment period. It was concluded that each of the additives affected specific steps during the in vivo to in vitro transition of hepatocytes.

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XENOBIOTIC METABOLISM IN 'MICROLIVER', A TRIDIMENSIONAL CULTURE OF ADULT RAT LIVER CELLS

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Conventional cultured hepatocytes (monolayers) lose their xenobiotic metabolic capacity within 1-2 days. 'Microliver', a three dimensional culture of hepatocytes and sinusoidal cells obtained from adult rats (6 months of age), might improve the maintenance of this metabolism in vitro. The activity and inducibility of selected enzymes important in xenobiotic metabolism were investigated in microliver cultures. After 6 days in culture, exposure to methylcholanthrene (25μM) and/or phenobarbital (3.2mM) increased several fold cytochrome P-450 dependent class I and II enzymes: ethoxycoumarin deethylase, ethoxyresorufin deethylase and aldrin epoxidase. Levels of induction are similar to those obtained in vivo and will allow comparison to short term monolayer cultures. Microliver, i.e. spheroid cultures of liver cells appears a suitable in vitro model for long term exposure to xenobiotics.

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HETEROLOGOUS EXPRESSION OF HUMAN CYTOCHROME P450 IA1 AND IA2 IN S. CEREVISIAE

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Human cDNAs for P450IA1 and P450IA2 have been inserted behind a derivative of the constitutive GAPDH promoter or the inducible PHO5 promoter on a 2μ based yeast plasmid. Yeast strains transformed with these plasmids expressed considerable amounts of the heterologous enzyme which copurified with microsomal membranes. The sequence composition immediately upstream of the genes' start codon was observed to strongly influence the level of expression. On the one hand the use of the P450LI (yeast lanosterol demethylase) inhibitor ketoconazole allowed quantitative estimations of the heterologous P450 content and on the other hand ketoconazole proved to be an inhibitor of P450IA1 and P450IA2. The expression of human P450 enzymes in S.cerevisiae has shown to be a valuable tool for the study of specific enzyme activities as well as drug interactions and kinetic parameters.

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24-HOUR PLASMA ALCOHOL LEVELS IN ROMAN HIGH- AND LOW-AVOIDANCE (RHA AND RLA) RATS DURING CONSUMPTION, AND AFTER ACUTE I.P. INJECTIONS, OF ETHANOL.

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It has been reported that RHA rats drink ethanol more readily, and are less sensitive to acute ethanol, than RLA rats (Experientia 46, A60, 1990). Based on the measurements of plasma alcohol levels (Inst. f. Klin. Chemie, Univ. Zürich), the present studies show that adult, male RHA and RLA rats eat and drink (either 10% ethanol or water) mostly during the night phase of a 12hD-12hN cycle, and that increased ethanol consumption is closely correlated with increased plasma levels, which drop to zero soon after the onset of the day phase. At 15, 30, 60, 90 or 120 min after injections of ethanol there were also no differences in blood levels between RHA and RLA females during either phase, indicating that the sensitivity differences seen earlier were CNS-located. Supported by a "Schweiz. Stiftung für Alkoholforschung" grant.

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METABOLISM GENERATED DURING THE VINIFICATION PROCESS

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The wines have to go through two different fermentation processes: first the sugars of the grapes are fermented to ethanol by the alcoholic fermentation and second the, in our cool climate sometimes overrepresented, aggressive malic acid is primarily converted to the more pleasant lactic acid by the malolactic fermentation, also called biological acid degradation, respectively.
Our interests are focused on the metabolic products which are produced during the two biological processes and which either increase or decrease the quality of the wines. The metabolisms, which influence the growth of the micro-organisms as well as the flavour and the taste of the wines are very much dependent on the yeasts used in the vinification process. We have already tested different commercial available dry yeasts on their influences on the whole vinification process. Specially, we are interested in the biogene amines which are derivates of the amino acids. The biogene amines are responsible for the occurence of allergies like sike headache and itched redskin. We try to detect such wines and we want to explore the possibility to reduce- or even to prevent the occurence of biogene amines in the wines.

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NEUROTOXIC EFFECTS OF CHLOROQUINE IN VITRO

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In order to investigate the neurotoxic effects of the antimalaria drug chloroquine, embryonic chick brain, neuronal retina, retinal pigment epithelium (RPE) and meninges cells were cultured under serum-free conditions. Drug was added on day 1, and endpoints were measured on day 8. The sensitivity of the cells, measured as inhibition of viability, showed the following ranking order: RPE>brain>meninges>neuronal retina. In vivo chloroquine accumulates in the RPE. At chloroquine concentrations that correspond to those measured in plasma of patients with chloroquine retinopathy, RPE viability was inhibited by 15-30 %. Nerve cell differentiation (expression of MAP2, MAP5, neurofilament 68K and 160K antigens, PNA-lectin binding) was affected at lower concentrations than that of astroglia (GFAP). Nerve cells in brain cell cultures were more sensitive than those in neuronal retina cell cultures.

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Vitamin E inhibits drug accumulation in cultured cells

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Cationic amphiphilic drugs are pharmacokinetically characterized by a large volume of distribution and a long persisitence in the organism. These weak bases accumulate within acidic cellular organelles (lysosomes, endosomes) by the following mechanism: The uncharged base readily permeates membranes, it then gets protonated and thus trapped. As a consequence of their high affinity to the lysosomal compartment some catamphiphilic compounds cause striking morphological alterations of the cells. We were able to detect a strong increase in cellular phospholipid [PL] contents (myelinoid lysosomal inclusion bodies) after chronic administration of e.g. amiodarone, it's main metabolite desethylamiodarone and desipramine to cultured human fibroblasts.
The simultaneous administration of a-tocopherol together with these compounds allowed us to dramatically reduce the drug accumulation as well as the lysosomal PL storage. The extent of inhibition of these storage phenomena depends on the concentration of vitamin E but also on the nature of the catamphiphilic amine. Vitamin E derivatives as α-tocopherol-acetate and Trolox-C or other antioxidants like ascorbic acid and butylated hydroxytoluene did not influence drug nor PL storage. The inhibitory effect of vitamin E on the cellular accumulation of catamphiphilic drugs might reduce the incidence of adverse effects due to lysosomotropism.

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SAFETY ASSESSMENT OF HUMAN GM-CSF

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The safety of human GM-CSF was assessed in rhesus monkeys (0, 10, 30 and 100 µg/kg/day, s.c.). In the peripheral blood a distinct increase of granulocytes was observed associated with a slight increase in monocytes and eosinophils as expected. Antibodies against GM-CSF were found within two weeks in the serum. The pathological examination of the animals revealed sterile inflammation at the injection site, and on the serosae of the pericardium, thoracical and abdominal cavities at the high dose level.
These inflammatory changes were not unexpected in view that GM-CSF increases the number of terminally differentiated cells of the myeloid and monocytic lineage, which contain a variety of proinflammmatory molecules. Similar side effects were observed in patients given initially high doses of GM-CSF. It is concluded that the safety assessment in the pharmacological responsive species had a predicitive value for the clinical treatment.

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REACTIVE OXYGEN SPECIES PROMOTE COCAINE-INDUCED CYTOTOXICITY IN CULTURED RAT HEPATOCYTES

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During microsomal activation of cocaine reactive oxygen species are generated that could lead to glutathione depletion and subsequently to cell-damaging lipid peroxidation (LPO). To further explore the role of reactive oxygen species and LPO as a causative factor of cocaine-induced hepatotoxicity, we used primary short-term hepatocyte cultures from phenobarbital pretreated rats. Cocaine induced a time and concentration dependent release of LDH, which was enhanced when glutathione was depleted by diethylmaleate. Membrane damage was paralleled by LPO (TBA assay) in cultures coincubated with diethylmaleate and cocaine. However, in cultures treated with cocaine alone, LDH release preceded the rise in LPO. Reduction of LPO to control levels was achieved by both α-tocopherol and deferoxamine. However, the antioxidant α-tocopherol did not protect against cocaine-induced LDH release. In contrast, deferoxamine, which inhibits LPO induced by reactive oxygen species, reduced LDH release by 50 - 75%. These results indicate that reactive oxygen species are involved in cocaine-induced cytotoxicity but their cell-damaging effects are not a consequence of LPO.

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THE DETECTION OF NONGENOTOXIC CARCINOGENS IN RAT HEPATOCYTE CULTURES

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DNA-lesions induced by endogeneous mutagens are converted to stable mutations more efficiently during cell division. Accordingly, mitogenic compounds are considered as nongenotoxic carcinogens. For the detection of these classes of chemicals, freshly isolated and cultured rat hepatocytes were used and exposed to EGF, phenobarbital, thioacetamide, nafenopin, cyproterone acetat and dimethylsulfoxide, from day 1-3 after seeding. DNA and protein of both intact cells and of isolated nuclei were analyzed by two parameter flow cytometry. The increase of liver cells in S-phase, their degree of ploidiy, of binuclearity and the distribution of cellular and nuclear protein content were measured or calculated at each ploidy level. Each of the chemicals induced a specific pattern of alteration in the compartments analysed. It became possible to distinguish between the direct mitogenic activity and the indirect mitogenic activity due to altered homeostasis.

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BENZODIAZEPINES SUPPRESS DEVELOPING AND ADULT IMMUNE RESPONSE IN THE LONG EVANS RAT

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Treatment of pregnant rat dams with 1.25 mg/kg diazepam from gestational day 14-20 resulted in offspring with depressed lymphocyte proliferative response. An involvement of the peripheral type benzodiazepine (PBDZ) receptor is indicated by the following: 1) Treatment of the pregnant dam was most effective at a time when PBDZ are present in the fetus, and 2) treatment with Ro 5-4864, a benzodiazepine with high affinity for the PBDZ receptor also inhibited cellular immune responses in offspring. 3) In adult murine lymphocytes Concanavalin A stimulated T cell, Lipopolysaccharide-induced B cell proliferation and T cell response as a consequence of recognition of alloantigens were considerably reduced in presence of micromolar concentrations of benzodiazepines. In all systems, PBDZ receptor agonists (Ro 5-4864 and PK 11195) and diazepam were most potent. 4) PBDZ receptors are present on lymphocytes; the affinity constant is significantly changed in prenatally diazepam-exposed offspring of 2 and 8 weeks.

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EFFECT OF $HgCl_2$ ON THE EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS IN MICE

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Chronic exposure to $HgCl_2$ can induce autoimmune glomerulonephritis in rats and man. Since expression of major histocompatibility complex (MHC) antigens is increased during idiopathic autoimmune diseases, we studied whether $HgCl_2$ had the same effect. A.SW mice, which are genetically predisposed to produce antinuclear antibodies in response to $HgCl_2$, and C57BL/6 mice which are not, were used. S.c. injections for 4 days with 1 mg/kg increased the expression of H-2K and I-A molecules on spleen leukocytes in both strains. In A.SW mice it had no effect on thymocytes, but in C57BL/6 mice it enhanced the level of both MHC antigens on these cells. In both strains the tubular epithelia of the kidney cortex expressed a higher number of H-2K molecules after treatment. Therefore, $HgCl_2$ increases the expression of MHC antigens on various cells in mice, independent of their genetic background. (We thank Mrs. E. Niederer for technical assistance).

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COULD YOU BE DECLARED POSITIVE FOR DOPING AGENTS AFTER RECEIVING AN EPHEDRA PHYTOTHERAPEUTIC PREPARATION ?

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Nowadays, there is a renewal of interest in phytotherapy as alternative to orthodox medicine. Plant preparations which are mostly employed to cure or to prevent disease might be also used to increase sport or working performances. Ephedra ssp which are a source of sympathomimetic amines (e.g. ephedrines) meet these different prescriptions. It is to mention that the amines are on the IOC list of forbidden substances, their misuse can result in heavy toxicity. After oral administration of a phytotherapeutic preparation of Ephedra sinica containing 2 % alkaloids, urines from two volunteers were collected during 2 days. Two different extraction and chromatographic methods were used to screen the urines for basic drugs : a) - cation exchange solid-phase extraction and gradient elution HPLC with diode-array detection b) - liquid-liquid extraction and capillary GC with NPD detection. Positive results were confirmed by GC-MS. Our results show that: - the two analytical methods allow ephedrines detection and quantification - volunteers remain positive for doping agents (i.e. ephedrines) during the 10 first hours after intake of at least one capsule (= 5 mg alkaloids) of Ephedra stems.

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A CELL CULTURE SYSTEM TO STUDY EPIGENETIC EFFECTS IN AN INHOMOGENEOUS RADIATION FIELD

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In radiation biology, effects usually are investigated in a homogeneous radiation field. However, in nature radiation is often distributed very inhomogeneous. An example is the deposition on the lung surface of originally airborne, small, radioactive particles, so called "Hot Particles", which are made up of insoluble fission products. We have developed a cell culture model with a small neutron-activated Yttrium-wire (a strong beta-emitter) as a source for an inhomogeneous radiation field. The wire was placed below the cell monolayer for several hours. The dose and doserate become very high for cells directly adjacent to the wire and decrease very fast within a few millimeters. Cells in a region of a given dose were trypsinized and replated for a colony forming tests. The colony forming ability decreases steeply between 3 and 10 mm distance of the wire. Dose rate effects were investigated using Yttrium-wires with different amounts of radioactivity.