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In this work we have studied the application of mass spectrometry for the detection and characterisation of radicals adducts produced from oxidation reactions of linolic acid using DMPO as a spin trap. We have identified several different spin adducts although the FAB-MS spectra alone does not permit to locate the DMPO bond position in these spin adducts.

An important corollary of this work is that the study of the main fragmentation patterns in the CA-MIKES spectrum of the spin adducts of linolic acid allows, however, to suggest probable locations for the spin adduct bond. No characteristic charge remote fragmentation of fatty acids spin adducts is observed. Based on this assumption we have suggested different structures for the fragment ions seen in the CA-MIKES spectrum of the spin adduct, allowing to propose different spin trapped adducts of linolic acid. Additionally, we have found that the use of the neutral loss scan of 113 allows us to identify the spin adducts of DMPO in complex mixtures.

POTH-028

Expression of hexose transporters GLUT1 and GLUT3 in isolated male germ cells

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The expression of different isoforms of glucose transporters (GLUTs) has been largely visualized as an adaptation to the requirement of substrates for cell metabolism. Thus, the expression of GLUTs by human, rat and bull spermatozoa is not restricted to the high-affinity glucose transporter GLUT3 and the fructose transporter GLUT5, but they also express GLUT1 and GLUT2. Suggesting that an adaptation to the seminiferous tubule and seminal fluid environment could be the goal for such diversity of GLUT expression. One hour incubation in glucose containing medium produced a marked rise in $[Ca^{2+}]i$ in round spermatids. In pachytene spermatocytes, this effect of glucose was not observed, suggesting that it could have a differentiation-related meaning, and that substrate delivery to the microenvironment of spermatogenic cells could be a mechanism of regulation of $[Ca^{2+}]i$ and development in these cells. This differential glucose-induced changes in $[Ca^{2+}]i$ can be regulated either by a differential transport of glucose or a differential glucose metabolism by these cells. In order to explore these possibilities, we analyzed the expression of hexose transporters (GLUT1 and GLUT3) in rat spermatogenic cells. Immunocytochemical and immunoblotting analyses demonstrated that round spermatids and pachytene spermatocytes express GLUT1 and GLUT3. Deoxyglucose transport experiments showed the functional expression of GLUTs in spermatogenic cells. Our data indicate that hexose fluxes and the expression of GLUT1 and GLUT3 did not differ significantly. Hence, our data suggest that the differential response of $[Ca^{2+}]i$ to glucose in these cells is not regulated at the level of GLUTs.

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POTH-029

On the role of protease inhibitors from buckwheat seeds in protection against pathogens

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Inhibitors of proteolytic enzymes are widely presented in plant tissues and attract attention by ambiguity of their functions. High qualitative diversity of these proteins, their inability to inhibit endogenous proteolytic enzymes and high activity towards enzymes of animals, insects and pathogenic microorganisms may point to protective role played by the inhibitors. A group of protease inhibitors was obtained from the seeds of buckwheat *Fagopyrum esculentum* by chromatography of the seed extract on trypsin-Sepharose and Mono-Q and Mono-S ion-exchangers. The molecular masses of the inhibitors range from 5.2 to 7.7 kDa. Besides animal proteases (trypsin, chymotrypsin) the studied inhibitors suppressed activity of proteinases secreted by filamentous fungi *Alternaria alternata, Fusarium oxysporum, Botrytis cinerea* and some of them (BWI-3c and BWI-4c) – of bacterial subtilisins and subtilisin-like proteinases. The direct evidence for the toxic effect of the studied inhibitors is the ability of the individual preparations of the buckwheat seed protease inhibitors to suppress spore germination as well as growth and development of filamentous fungi *A. alternata, F. oxysporum, Trichoderma harzianum, Ulocladium artrum, Aspergillus penicillosa* and, etc. It was also demonstrated that *in vivo* the studied inhibitors can diffuse into environment during seed imbibition and germination as well as in the case of seed damage. It is suggested that investigated protease inhibitors are a part of the defense system in buckwheat seeds protecting them from attack of pathogens.

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POTH-030

Interaction between glutathione and protopine alkaloid

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In previous studies, glutathione decreased the inhibition of mitochondrial NADH dehydrogenase by protopine alkaloid. This fact suggests the possibility of formation of a complex between glutathione and protopine, decreasing the effective concentration of inhibitor in the reaction medium. Absorption spectra of glutathione in the absence and in the presence of protopine corroborate this hypothesis. Changes in the UV spectrum of glutathione in the presence of protopine were detected, and the rate of change with time was monitored.

POTH-031

Sequencing and analysis of the genomic system responsible for the antibiotic A201A biosynthesis in *Streptomyces capreolus* NRRL 3817

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A201A is a nucleoside antibiotic with a chemical structure homology to aminoglucoside antibiotic hygromycin A and nucleoside antibiotic puromycin. A201A antibiotic is synthetized by the Gram positive microrganism *Streptomyces capreolus* NRRL 3817. We have isolated four cosmids from that microrganism genomic library with overlaping inserts that may constitute the whole gene cluster responsible for the antibiotic biosynthesis (cluster *a2a*). Resistance genes are also included in this gene cluster. This continuous genomic DNA fragment is approximately 35 Kb long and its sequencing revealed the existence of 28 putative open reading frames (ORFs). The amino acid sequences deduced from these ORFs have been compared with the proteins in the databases and significant homologies for most of them have been found. In this way, their possible function in A201A biosynthesis has been proposed; we have also elaborate a putative biosynthesis pathway for the antibiotic.

Among these sequenced ORFs we have located proteins homologous to several of the *Streptomyces alboniger* puromycin biosynthesis pathway enzymes: Pur3, Pur4, Pur5, Pur7 and Pur10. In our laboratory some *S. alboniger* deletion mutants defective in puromycin production are available; we have used these mutants to assay their heterologous complementation using cluster *a2a* genes: *A2A-pur4*, *A2A-pur5*, and *A2A-pur10*. For this purpose we have cloned them in the *Streptomyces* multicopy vector pIJ702. In this way, we have restored antibiotic production in these *S. alboniger* mutants.

POTH-032

Age dependent effects on cholesterol metabolism in the thymus gland

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Mammalian cells require cholesterol for normal cell function. This requirement can be fulfilled by endogenous biosynthesis, from acetyl CoA by de novo pathway they may obtain it from the blood stream by receptor mediated endocytosis. Inside the cell, cholesteryl esters are hidrolized by cholesterol ester hydrolase (CEH) to free cholesterol which is either incorporated in cell membranes, used for steroidogenesis by some specialized cells or reesterified by acyl-CoA: cholesterol acyltransferase(ACAT) for storage as lipid droplets.

The thymus is an important central lymphoid organ that begins to involute at around puberty until becoming a vestigial organ. During this process the cells undergo pronounced morphological and biochemical changes that are accompained by different needs for cholesterol.

There is strong evidence that cultured thymic nonT-cells produce soluble pregnenolone and deoxycorticosterone and immunohistochemestry as demonstrated steroidogenic enzymes in radioresistant thymic epithelial cells but not in thymocytes. It was identified a role for endogenously produced glucocorticoids in thymocyte development related with thymocyte apoptosis after this hormone synthesis inhibition.

Thymus ACAT activity is increased until complete growth and cholesterol ester content as a maximum at puberty. Thymus CEH activity increase until sexual development as cholesterol ester content. After sexual maturity we observed lower cholesterol ester and cholesterol content and a stabilization of CEH and ACAT activity in the thymus gland, probably related to a reduction in endogenous steroid biosynthesis, consistent with an elevation of the apoptotic index of the thymic cortex in aduit.