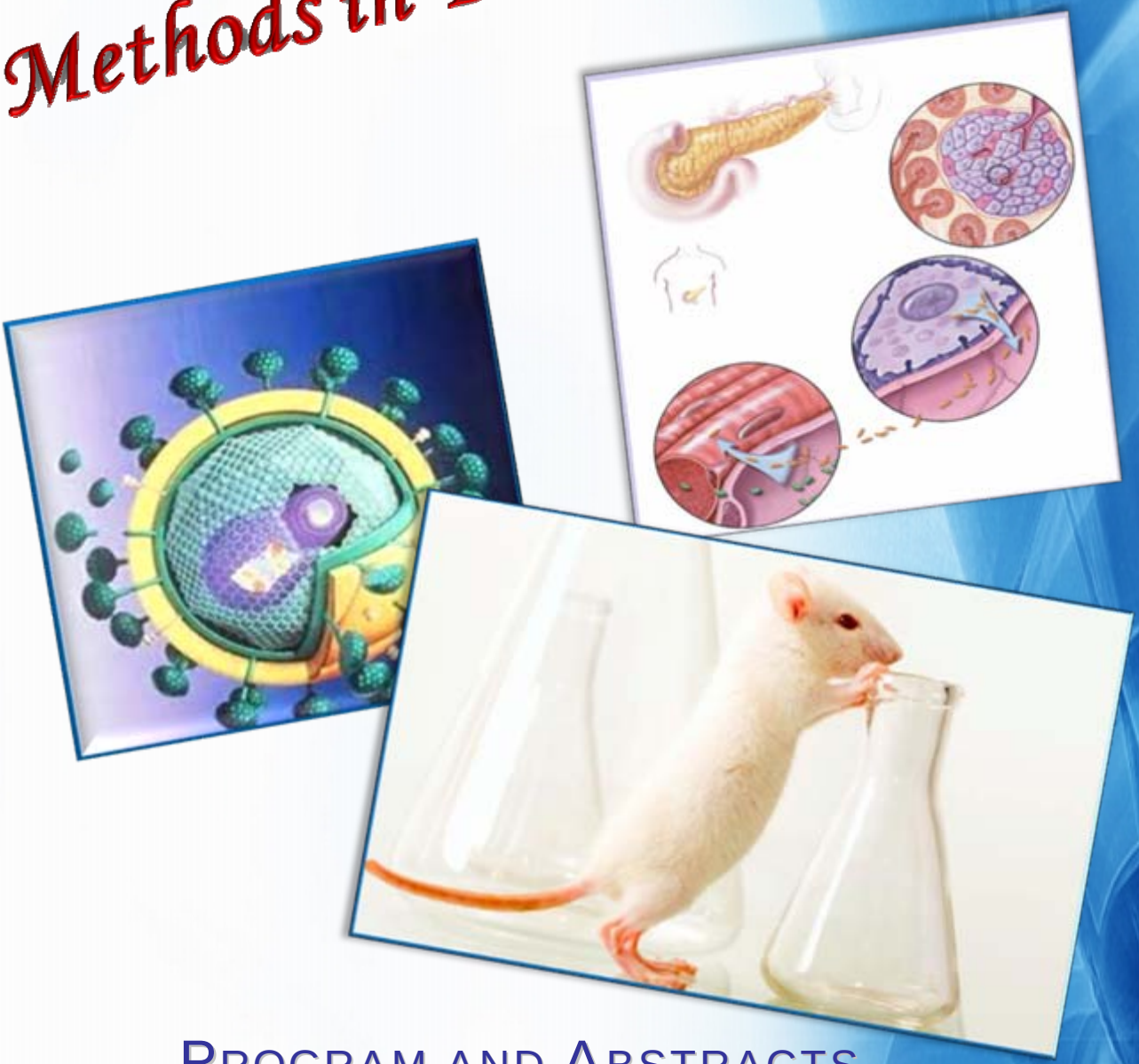
Experimental Models and Methods in Biomedical Research



Program and Abstracts

May 16th-18th, 2011

Sofia, Bulgaria

THE SECOND WORKSHOP

"EXPERIMENTAL MODELS AND METHODS IN BIOMEDICAL RESEARCH"

IS ORGANIZED BY THE INSTITUTE OF EXPERIMENTAL MORPHOLOGY, PATHOLOGY AND ANTHROPOLOGY WITH MUSEUM (IEMPAM)

UNDER THE AUSPICES OF
THE BULGARIAN ACADEMY OF SCIENCES

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- *Institute of Experimental Morphology, Pathology and Anthropology with Museum, BAS*

Monday, 16 May 2011

10.00 – 10.10 – Opening Remarks

Session A

Chairpersons:

Assoc. Prof. Ludmil Kirazov, IEMPAM, BAS

Assoc. Prof. George Miloshev, Institute of Molecular Biology, BAS

10.10 – 10.30

COMPLEX EXPERIMENTHAL APPROACH FOR STUDYING THE MECHANISMS OF ANDROGEN / ESTROGEN ACTION IN MAMMALIAN TESTIS

N. Atanassova, E. Pavlova

10.30-10.50

DROSOPHILA AS AN EXPERIMENTAL MODEL TO STUDY THE FRAGILE X CHROMOSOME SYNDROME

M. Georgieva, G. Genova, G. Miloshev

10.50-11.10

STUDYING AGING OF THE GENOME IN THE MODEL SYSTEM *SACCHAROMYCES CEREVISIAE*

K. Uzunova, M. Georgieva, G. Miloshev

11.10 – 11.30

Coffee Break

10.30 - 11.50

YEAST *SACCHAROMYCES CEREVISIAE* – AS A MODEL SYSTEM FOR DETECTION OF GENOTOXIC AGENTS

E. Peycheva, R. Alexandrova, G. Miloshev

11.50 – 12.10

МОЛЕКУЛЯРНО КЛОНИРАНЕ, ХЕТЕРОЛОЖНА ЕКСПРЕСИЯ И АНАЛИЗ НА НОВ ТИП МЕТАЛОПРОТЕИН TS-PCHTP ОТ *T. SPIRALIS*

А. Йовева, Г. Радославов, Д. Теофанова, П. Христов

12.10 – 12.30

Discussion

Session B: *"DIABETES – KNOWN AND UNKNOWN"*

Chairpersons:

Assoc. Prof. Reneta Toshkova, IEMPAM, BAS

Assoc. Prof. Anna Tolekova, Medical Faculty, Trakia University

14.00 – 14.20

EXPERIMENTALLY INDUCED RODENT MODELS OF TYPE 2 DIABETES

M. Yakovlieva, R. Iliev, A. Tolekova

14.20 – 14.40

SMOOTH MUSCLE DYSFUNCTION IN EXPERIMENTAL DIABETES MELLITUS

St. Mihaylova, Ts. Georgiev, P. Hadzhibozheva, A. Tolekova

14.40 – 15.00

VIRUSES AND DIABETES

R. Alexandrova, T. Zhivkova, L. Dyakova, B. Andonova-Lilov**a**

15.00-15.20

STEM CELLS IN THE TREATMENT OF DIABETES – FROM DREAMS TO REALITY

B. Andonova-Lilova, T. Zhivkova, L. Dyakova, R. Kalfin, R. Alexandrova

15.20 – 15.40

Discussion

Tuesday, 17 May 2011

Session C

Chairpersons:

Assoc. Prof. Mashenka Dimitrova, IEMPAM, BAS

Assoc. Prof. Marin Alexandrov, IEMPAM. BAS

9.45 – 10.05

DISTRIBUTION AND DYNAMIC OF PATHOGENIC STRAINS *ESCHERICHIA COLI* ISOLATED FROM PIGS IN BULGARIA FOR THE PERIOD 2000 - 2005

A. Dimitrova, M. Dragoycheva, S. Yordanov

10.00 – 10.25

REVIEW OF THE METHODS FOR DIAGNOSIS OF ENZOOTIC PNEUMONIAE (EP) IN PIGS (survey)

R. Pepovich, S. Yordanov

10.25 – 10.35

BIOLOGICAL METHODS FOR QUICK FIELD DETECTION OF ANTI-CHOLINESTERASE INSECTICIDES

E. Arnaudova, B. Georgiev

10.35 – 10.55

***IN VIVO* CHRONIC TREATMENT WITH COBALT(II) COMPOUNDS**

Y. Gluhcheva, M. Madzharova, R. Nizamova, V. Atanasov, Ju. Ivanova, E. Pavlova, M. Mitewa

10.55 – 11.05

AN EXPERIMENTAL MODEL OF ACUTE LITHIUM INTOXICATION IN MICE – PRELIMINARY DATA

E. Petrova, Y. Gluhcheva, V. Ormandzhieva, I. Ivanov, V. Atanasov, M. Svetoslavova, B. Eremieva, L. Kirazov, E. Kirazov, D. Deleva, M. Dimitrova, D. Kadiysky

Session D

Chairpersons:

Assoc. Prof. Evgeni Kirazov, IEMPAM, BAS

Assoc. Prof. Reni Kalfin, Institute of Neurobiology, BAS

14.00-14.20

THE AMYLOID β PEPTIDE AFFECTS THE ELECTRICAL ACTIVITY OF NEURONAL CELLS

L. Kirazov, E. Kirazov, D. Kadiyski, E. Vassileva, E. Petrova

14.20 – 14.30

SODIUM NITRITE-INDUCED HYPOXIA – EFFECT ON THE FREE FATTY ACID CONTENT IN RAT BRAIN SYNAPTOSOMES

E. Petrova, A. Dishkelov, E. Vasileva, T. Gramatikova

14.30-14.50

EFFECT OF AMINO ACID DERIVATIVE ON COGNITIVE FUNCTION OF SOCIALLY ISOLATED RATS AFTER MATERNAL DEPRIVATION

E. Encheva, M. Novoselski, L. Tancheva, V. V. Petkov, R. Klisurov

14.50 – 15.10

Coffee Break

15.10 – 15.30

NEW DESIGN FOR EXPERIMENTAL INVESTIGATIONS OF STATIC BIOMECHANICAL CHARACTERISTICS AND AXIAL FORCE OF BLOOD VESSELS

M. Antonova, S. Stoytchev

15.30 – 15.50

ANIMAL LIVER PERFUSION TECHNIQUE

A. Alexandrova, L. Petrov

15.50 – 16.00

ЛУМИНОМЕТРИЧНО ДОКАЗВАНЕ НА АКТИВНИ ФОРМИ НА КИСЛОРОДА В ЧОВЕШКИ ЕЯКУЛАТ

Г. Ненкова

Wednesday, 18 May 2011

Session E

9.45 – 11.30 YOUTH SEMINAR *"VIRUSES THAT SHAKE THE WORLD"*

HAPPY ANNIVERSARY, VIRUS Mc29 !

R. Alexandrova

THE BULGARIAN STORY OF EPSTEIN BAR VIRUS

A. Abudalleh

THE GREAT VICTORY OVER SMALL POX

L. Dyakova

POLIOMIELITIS – IT IS NICE TO CLOSE THIS PAGE FOREVER !

T. Zhivkova

MORBILLI VERSUS PEOPLE OR PEOPLE VERSUS MORBILLI

J. Kojumdjian-Ivanova

THE TRUE STORY OF MARBURG AND EBOLA

L. Dyakova

THE LESSONS OF SARS

B. Andonova-Lilova

RABIES STILL "BITING PEOPLE"

J. Kojumdjian-Ivanova

INFLUENZA VIRUS – THE VIRUS WITH A HISTORY !

B. Andonova-Lilova

(UN)STABLE VIRUSES AND SOME OTHER GOSSIPS

R. Alexandrova

ХУМБОЛТОВ СЪЮЗ В БЪЛГАРИЯ

HUMBOLDT-UNION IN BULGARIEN

ИНСТИТУТ ПО ЕКСПЕРИМЕНТАЛНА МОРФОЛОГИЯ, ПАТОЛОГИЯ И АНТРОПОЛОГИЯ С МУЗЕЙ (ИЕМПАМ) ПРИ БЪЛГАРСКА АКАДЕМИЯ НА НАУКИТЕ

организират

ИНФОРМАЦИОННА СРЕЩА

на тема:

СТИПЕНДИИ ЗА НАУЧНИ ИЗСЛЕДВАНИЯ НА ФОНДАЦИЯ "АЛЕКСАНДЪР ФОН ХУМБОЛТ"- ГЕРМАНИЯ

Лектор:

Проф. дбн Илза Пъжева - учен-представител на фондация "Александър фон Хумболт" в България

Поканват се **млади научни работници и докторанти**, както и техните научни ръководители, ръководители на научни звена и хумболтови стипендианти

Срещата ще се състои на 18 май (сряда) 2011 г. от 11.45ч. в Заседателната зала на ИЕМПАМ, ул."Акад. Г. Бончев", блок 25, София

Session F

Chairpersons:

Prof. Radka Argirova, National Centre of Infectious and Parasitic Diseases, Sofia

Assist. Prof. Radostina Alexandrova, IEMPAM. BAS

14.00 – 14.20

STUDY OF GLYCOSYLATION – A CHEMICAL APROACH FOR UNDERSTANDING THE BIOLOGICAL ACTIVITY IN VIRUS – CELL INTERACTION

R. Gavazova, D. Ivanov, K. Borissov, P. Genova-Kalou, S. Raleva, D. Dundarova, R. Argirova

14.20-14.40

EFFECT OF SOME INHIBITORS ON THE SIALYLTRANSFERASE ACTIVITY OF MCF-7 CELLS

D. Ivanov, R. Alexandrova, T. Zhivkova, R. Gavazova

14.40 – 15.00

BRIEFLY ABOUT TUMOR ANTIGENS AND TUMOR MARKERS

R. Alexandrova, K. Timcheva, I. Gavrilov

15.00 – 15.20

Coffee Break

15.20 – 15.30

ROLE OF TAU PEPTIDE IN THE ONCOGENES AND TUMOR-SUPPRESSOR GENES BALANCED FUNCTIONAL ACTIVITY

I. Sainova, I. Vavrek, T. Daneva, E. Nikolova

15.30 – 15.40

SOME ADVANTAGES AND DISADVANTAGES OF STEM CELLS

D. Martinov

15.40-16.00

MESENCHIMAL STEM CELLS AND BONE TISSUE ENGINEERING

R. Alexandrova, B. Andonova-Lilova, T. Zhivkova, L. Dyakova, O. Alexandrov, S. Tepavitcharova

16.00 – 16.15

General Discussion and Closing Remarks

ABSTRACTS

***Each author is responsible for the content of his/her abstract**

SESSION A

AO1. COMPLEX EXPERIMENTHAL APPROACH FOR STUDYING THE MECHANISMS OF ANDROGEN / ESTROGEN ACTION IN MAMMALIAN TESTIS

N. Atanassova, E. Pavlova

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bontchev Str., Bl.25, 1113, Sofia, Bulgaria
E-mail: e_bankova@yahoo.com

Androgens (A) and their derivates estrogens (E) play an important role in regulation of testis development and function through specific nuclear receptor – androgen receptor (AR), estrogen receptors (ER) alpha and beta that are differentially distributed in testicular cell types. The cellular and molecular mechanisms of A/E regulation are investigated by experimental hormonal manipulation of A and E levels as well as by transgenic knockout of AR totally (ARKO) or selectively in Sertoli cells (SCARKO). We developed complex system of morphological and functional criteria for characterization of A/E action in the testis that was applied for: 1) evaluation of specific role of A and E in germ cell apoptosis; 2) precise estimation of common and specific pathways of A and E in control of germ, Sertoil and Leydig cell number and their functional properties (spermatogenic capacity, Inhibin-B and testosterone production); 3) expression of Sertoli and Leydig cell markers. Using these criteria we distinguished direct and indirect (gonadotropin-mediated) mechanism of E action in the testis and evaluated the importance of A/E balance for differentiation of main testicular cell types. Morpho-functional characterization of germ and somatic cells of the testes from ARKO and SCARKO mice enable us to evaluate the cell-autonomous action of AR in Sertoli cells for complete spermatogenesis and the size of adult Leydig cell but not Sertoli cell populations. Our complex approach provides recent understanding on the cellular mechanisms of A and E as well as on the close relationships between action of both hormones and their receptors in the male. This could contribute for treatment of male infertility and development of male contraception programs.

Acknowledgements: The studies were funded by The UK Royal Society Postdoctoral Fellowship/2000; The UK Wellcome Trust IRDA/065187/2004; NF Scientific Research B-815/2002

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AO2. *DROSOPHILA* AS AN EXPERIMENTAL MODEL TO STUDY THE *FRAGILE X CHROMOSOME SYNDROME*

M. Georgieva[1], G. Genova[2], G. Miloshev[1]

[1]Molecular Genetics Lab, Institute of Molecular Biology "R. Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Department of Genetics, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

Fragile X syndrome is the leading heritable cause of mental retardation worldwide. One out of every 4,500 males suffers cognitive impairment due to alterations in the fragile X gene. The gene has a complicated biology. The overriding clinical manifestation of this disease is mild to severe cognitive impairment, autism etc.

In most cases the disease is caused by the methylation-induced transcriptional silencing of the *fragile X mental retardation 1* (*FMR1*) gene. This is due to the expansion of a CGG repeat in the gene's 5′UTR and leads to the loss of protein product - the fragile X mental retardation protein (FMRP). Chromatin structure is also important in the propagation of the FMR1 gene expression. According to the recent data the nucleosome occupancy is a contributor to CGG repeat instability. An artificially obtained transgene mouse bearing as much as 26 CGG repeats has an open chromatin structure compared to the stable Fmr1 in the endogenous mouse.

FMRP is an RNA binding protein that shuttles between the nucleus and the cytoplasm. It binds to several mRNAs including its own mRNA and to translating polyribosomes modulating the translation of different RNAs. At a cellular level pathological studies from the brains of patients show abnormal dendritic spines implicating FMRP in synapse formation and function. Evidence from both *in vitro* and *in vivo* neuronal studies indicates that FMRP is located at the synapse and the loss of FMRP alters synaptic plasticity. As synaptic plasticity has been implicated in learning and memory, analysis of synapse abnormalities in patients should prove useful in studying the pathogenesis of fragile X syndrome and understanding learning and cognition in general.

Recent work in *Drosophila melanogaster* has shown that the fly homolog of FMR1 (dFMR1) plays an important role in regulating neuronal morphology, which may underlie the observed deficits in behaviors of dFMR1 mutant flies. The *Drosophila* genome encodes a single dFMR1 gene with close sequence homology to the human gene. FMRP is required during *Drosophila* brain development to control the exit from quiescence and proliferative capacity of NB as well as neuron production. A novel assay to examine social interactions in *Drosophila* has been developed and in its first attempt of application the authors examine the behavior of Drosophila Fragile X Mental Retardation gene (dfmr1) mutants. The results show that *Drosophila* that lack the dFMR1 gene (dfmr1 null mutants) recapitulate MR-associated molecular, cellular and behavioral phenotypes, suggesting that FMR1 function has been conserved. Importantly, hFMR1 can fully rescue dFMR mutation phenotype, both at the

molecular and cellular levels in neurons. More surprisingly, biochemical analysis has revealed that dFMR1 forms a complex that includes ribosomal proteins and, surprisingly, Argonaute2 (AGO2), an essential component of the RNA-induced silencing complex (RISC) that mediates RNA interference (RNAi) in Drosophila. dFMR1 also associates with Dicer, another essential processing enzyme of the RNAi pathway. Moreover, both a micro-RNA (miRNA) and short interfering RNAs (siRNAs) can coimmunoprecipitate with dFMR1.

In this study we are intending to reveal several important molecular genetics mechanisms which lie in the basis of mental retardation fragile X syndrome. We plan the following tasks: as a first step in the research - new contacts of dFMRP with different target mRNA and/or proteins to be searched. Concomitantly, the chromatin structure of brain cells taken from mutant flies tol be thoroughly investigated. By developed by us special technique Chromatin Comet Assay (ChCA) to be compared the higher-order chromatin organization (chromatin loops) in wild type and mutant brain cells.

The initial results about higher-order chromatin organization of fragile X chromosome cells will be presented and the consequences for the organisms will be discussed.

Because the core mechanisms of complex behaviors such as learning and memory and circadian rhythms appear to be conserved, studies of Fragile X syndrome using *Drosophila* as a model provide an economy-of-scale for identifying biological processes that likely underlie the abnormal morphology of dendritic spines and behavioral disturbances observed in Fragile X patients.

AO3. STUDYING AGING OF THE GENOME IN THE MODEL SYSTEM *SACCHAROMYCES CEREVISIAE*

K. Uzunova, M. Georgieva, G. Miloshev

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Aging, a problem that thrills all of us, is manifested by a progressive decline in vitality over time, leading to age-related diseases and finally to death. Notably, aging is regulated by a specific genetic network. Certain experimental results show that the causes of aging depend on the interplay between genetic background and environment, i.e epigenetic factors highly influence the way we all age. While in humans the following of normal pathways of aging requires quite a long time and does not allows genetic manipulations, studies of aging in different model systems has proved to be useful. The model systems can be invaluable for understanding of many age-related diseases, such as cancer, dementia, cardiovascular diseases, Werner syndrome, Hutchinson-Gilford Progeria, etc.

An useful model organism for studying of aging is the yeast *Saccharomyces cerevisiae*. This single-cellular organism holds some very important characteristics that grant it with quite a big advantages in many kinds of aging studies. These characteristics can be summarized as follows:

1. Yeast cells are easy to be manipulated genetically.
2. They possess fully sequenced and well characterized genome.
3. Their short generation time allows uncomplicated and easy culturing.
4. Moreover, yeast cells are eukaryotic and all basic cellular, molecular biology and metabolism processes and factors involved in them are conserved among yeast and human cells.

Two different forms of aging in yeast cells are studied: replicative and chronological aging. Yeast "replicative life span" is measured by the number of cell generations, not by the calendar time. This kind of replicative aging of a cell population based on asymmetric cell divisions is investigated as a model for aging of a stem cell population in higher organisms. A primary cause of yeast replicative aging stems from genomic instability at the rDNA locus and formation of extrachromosomal ribosomal circular DNAs (ERCs). Replicative stress has complex effects upon yeast cells which influences genome stability in the high repetitive ribosomal locus and the result is formation of ERCs. Much evidence suggests the role of *SIR2* as a key longevity protein that extends life span by suppressing rDNA recombination.

The other form of aging in yeast cells, the so-called "Chronological life span", is the length of time during which a population of yeast cells remains viable in a non-dividing state following nutrient deprivation. Chronologically aging yeast has proven to be a valuable model for studies of oxidative damage in the post-mitotic tissues of higher eukaryotes.

We shall discuss the involvement of different genes implicated in chronological aging and the association between stress response pathways and chronological longevity. Our interest is mainly aimed to age-related changes in higher-order chromatin structures in certain yeast strains. Among them are yeast mutants without a linker histone which is responsible for the organizing and maintenance of the higher-order chromatin structures in *S. cerevisiae.* A discussion on the problem of calorie restriction extension of replicative and chronological life span will also be presented.

Acknowledgements: M.G. is supported by Bulgarian Science Fund, Grant № DMU 02/8.

AO4. YEAST *SACCHAROMYCES CEREVISIAE* – AS A MODEL SYSTEM FOR DETECTION OF GENOTOXIC AGENTS

E. Peycheva[1], R. Alexandrova[2], Гл Miloshev[1]

[1]Molecular Genetics Lab, Institute of Molecular Biology "Acad. R. Tsanev", Bulgarian Academy of Sciences, Acad. G. Bontchev Str., Bl.21, 1113, Sofia, Bulgaria,

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Genotoxins is a term generally used for physical and chemical agents that induce damages in DNA molecule. They can be chemical compounds or physical agents like UV, X-rays, gamma rays etc. Genotoxins could execute their effect by different mechanisms, however the result is that part of the molecule of DNA is chemically modified. It can be cut, chemical groups can be modified or integrated with its structure. Moreover, in DNA molecule internal and external covalent bonds can be created. These damages in the DNA are very dangerous for the cell and the whole organism. They can lead to cell death or to malignant transformation followed by destructive uncontrolled cell divisions. In addition, the damages in DNA can increase recombination levels inducing genome arrangements, which can be inherited by progeny.

The deleterious effect of some DNA damaging agents is undoubtedly proven, but supposedly thousands compounds polluting the environment possess undetected genotoxic effect.

A large number of methods using prokaryotic or eukaryotic cells have been developed to detect genotoxicity. The usefulness of these tests is defined from their sensitivity, but their applications depend on expenditures and time for their performance. Importantly, in these

tests damages in the structure of DNA and hence the future prospects for the cells can be easily underestimated. Many of these techniques are difficult to perform, they are expensive, require a large number of cells and their sensitivity is limited.

Yeast *S. cerevisiae* shows homology with the higher eukaryotic cells in the molecular mechanisms of the general cellular processes. In addition, yeast and human cells possess many homologous in their genes. Yeast genetics is well studied, which gives a possibility using yeast to collect data for certain processes like cell cycle, glucose fermentation, amino acid metabolism etc. On one hand, the yeast cells are typical eukaryotic organisms, but on the other hand, they present a lot of advantages of microorganisms.

In the recent years, one of the methods, which acquires popularity for investigation of DNA damages is the Single Cell Gel Electrophoresis (SCGE) also known as Comet assay. The technique allows easy, fast and reproducible visualization and measurement of DNA damages at a single-cell level. The obtained results can easily be quantified by visual scoring of the comets or automatically by software.

The method of Comet assay adapted by us for use on yeast cells was called Yeast Comet Assay (YCA). Currently we upgraded the technique and developed different conditions for its implementation in order to improve its sensitivity and the reproducibility of the obtained results.

The goal of this study is to optimize Yeast Comet Assay as a sensitive method for detection of small concentration of genotoxic agents.

In the study, we have tested several substances used in food industry and pharmacology for genotoxic activity. They were investigated for genotoxicity by Yeast Comet assay and standard Comet assay. In addition, some of them were analyzed as suppressors of yeast cultures growth and cytotoxicity. Importantly, the tested compounds used in food and pharmaceutical industry were applied at concentrations ten times lower than used in practice. The results clearly demonstrated their genotoxicity. In order to better characterize the sensitivity of YCA for detection of genotoxic activity we have compared it with the standard Comet Assay (CA) on mammalian cells. Remarkably, *Saccharomyces cerevisiae* cells turned out to be 10 to 100 times more sensitive than mammalian to the action of different genotoxic agents. Therefore, YCA proved to be highly sensitive, cheap and easy and could be used in local laboratories for environmental protection.

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АО5. МОЛЕКУЛЯРНО КЛОНИРАНЕ, ХЕТЕРОЛОЖНА ЕКСПРЕСИЯ И АНАЛИЗ НА НОВ ТИП МЕТАЛОПРОТЕИН TS-РСНТР ОТ *T. SPIRALIS*

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Паразитните нематоди са в основата на голям брой заболявания по домашните, дивите животни и човека. Те водят до икономически загуби в глобален мащаб. Смята се, че повечето патологични промени, наблюдавани при инвазии с нематоди се дължат на модифициране гостоприемниковите физиологични процеси от определени белтъци на паразита. Това налага необходимостта от откриване и изучаване на паразитните компоненти, които са отговорни за появата на съответните промени (Andersen, 1992).

Trichinella spiralis е паразитен нематод от семейство *Trichinelidae* с голямо стопанско и медицинско значение. Инфектира прасета, плъхове, мишки, диви животни и хора, като причинява заболяването трихинелоза. В доклад на Международната Комисия по Трихинелоза за 2004г. са установени повече от 1 100 трихинелозни случая в Европа, от които 984 в Сърбия, Хърватия, Румъния и България.

Ts-PCHTP (poly-cysteine and histidine-tailed protein) е металопротеин, експресиран в мускулни ларви на *T. spiralis*. Той е първият изследван представител на ново протеиново семейство PCHTP - поли-цистеинови протеини, уникални за Разред Trichocephalida. Генът, кодиращ Ts-PCHTP включва 1896нд, организирани в шест екзона и пет интрона (GenBank^{TM} GQ497342). Нуклеотидната секвенция на мРНК се състои от 1576нд. Транскриптът има единична отворена рамка на четене, състояща се от 1272нд. Аминокиселинната последователност на металопротеина е съставена от 424 аминокиселини, на които отговаря молекулна маса от 47744 Да. Белтъчната верига показва високо съдържание на няколко аминокиселини - цистеин (8,8%), хистидин (6,4%), тирозин и триптофан, и включва два хомоложни поли-цистеинови домена, поли-хистидинов домен (хистидинова опашка) в С-края и сигнален пептид за екстрацелуларна локализация в N-края си. Белтъкът свързва двувалентни метални йони и вероятно има отношение към транспорта и съхранението им в паразита (Radoslavov G et al., 2010).

Извършен беше сравнителен анализ на нуклеотидната и аминокиселинна последователности на PCHTP при шест вида от род *Trichinella - T. spiralis , T. britovi, T. nativa, T. nelsoni, T. pseudospiralis, T. murrelli.* За целта беше изолирана геномна ДНК и амплифицирана с две двойки ген-специфични праймери. Резултатите показаха висока степен на хомология (94 – 99 %) между сравняваните нуклеотидни последователности, като най-голямо бе сходството при *T. spiralis* и *T. britovi*. Основните различия бяха отчетени в интронните части, а повечето нуклеотидни замени в екзоните на гена се оказаха "nonscense" (безсмислени) точкови мутации.

PCR-амплификацията на гДНК и кДНК на Ts-PCHTP с ген-специфични праймери (прав-S 5'- GGGAATTCCATATGGCTTTCTCAACTATTG–3' и обратен праймер-AS 5'- CGCGGATCCTTATCAATGATGATGATGATGATGATG - 3') показа отклонения от очакваните резултати. Наблюдаваха се три значително по-къси фрагмента, представени от ~800нд, ~600нд и ~300нд, но не и такива с очакваната дължина. След клониране и секвенционен анализ се установи, че фрагментите с дължина ~ 800нд съдържат пълната рамка на четене за *ts*-pchtp –1272нд, а PCR продуктите с дължина ~600нд и ~300нд представляват два различни варианта, при които средната част на гена липсва (от 112нд до 1121нд и 245нд до 1002нд). Възможно обяснение е ДНК self-splicing вследствие формиране на конформационни структури (hairpin loops) в едноверижната ДНК, поради наличието на няколко обърнати повторени елемента (William, 2007). Опитно беше изследвана PCR-амплификацията при различни изходни концентрации на фрагмента, кодиращ Ts-PCHTP (от 1.10^{2} ng до 1.10^{-6} ng на PCR-реакция). Резултатът показа, че с намаляване концентрацията на ДНК процесът на амплификация се измества в посока формиране на по-късита фрагменти. Възможно обяснение на получените резултати е възпрепятстване формирането на споменатите конформационни структури при по-високи концентрации на ДНК фрагмента.

За хетероложна експресия на Ts-PCHTP се извърши изолиране на паразитна РНК от мускулни ларви на *T. spiralis*, последвано от синтез и амплифициране на кДНК с ген-специфични праймери -S и -AS. Амплифицираният фрагмент беше клониран в експресионен вектор pJC20 (Clos, 1994). Компетентни E.coli клетки щам BLD DE3 бяха трансформирани с получения рекомбинантен плазмид. Експресията на рекомбинантния белтък беше индуцирана с IPTG (4 часа на 37 ̊C), а пречистването му се извърши посредством Ni-афинитетна хроматография по протокол на производителя (QIAGEN). Чистотата и молекулната маса на изолирания протеин бяха анализирани на 12% полиакриламиден гел, а за доказването му се извърши Western blot анализ.

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SESSION B: DIABETES – KNOWN AND UNKNOWN

BO1. EXPERIMENTALLY INDUCED RODENT MODELS OF TYPE 2 DIABETES

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Type 2 diabetes mellitus (T2D) is a complex and heterogenous metabolic disorder with high influence of the environmental factors, characterized by a relative insulin deficiency resulting from a reduced sensitivity of tissues to insulin and impairment of pancreas insulin secretion. The T2D is the most common endocrine disease with increasing incidence. It represents more than 80% of the DM cases in human.

In order to improve our knowledge of pathogenesis of T2D, its consequent complications, and testing of new anti-diabetic agents have been developed various animal models. Most experiments are carried out on rodents. Rodent models are classified in the following main categories: 1) spontaneous or genetically induced derived, 2) diet/nutrition induced, 3) chemically induced , 4) surgical diabetic animals, 5) transgenic/knock-out diabetic animals. These categories have different experimental characteristics, gender differences, advantages and disadvantages. In this review we will present in detail models, induced by

diet/nutrition, chemically induced models and combination diet and chemically induced (streptozotocin) models.

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BO2. SMOOTH MUSCLE DYSFUNCTION IN EXPERIMENTAL DIABETES MELLITUS

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Diabetes mellitus is a socially significant metabolic disorder with a tendency for an increasing incidence - from 171 million people in 2000 to 366 million by 2030. With progression of the disease number of complications are developed, which seriously reduce the quality of life and the life expectancy of patients. In order to reveal different aspects of etiology and pathogenesis of diabetes many experimental models have been developed. They are useful in studying the intimate mechanisms of the diabetic complications, which could help to improve a treatment. Some of the manifested complications are associated with changes in contractile activity of the smooth muscles. The main gastrointestinal complications are: dilatation of the stomach, slowing down of the intestinal passage and development of megacolon. Also, in the models of experimental diabetes are observed an increase in muscle mass and urothelium, the volume capacity and compliance of the urinary bladder as well as changes in its contractile responses to different pharmacological agents and electrical stimulation. Smooth muscle of the blood vessels also show abnormal function in experimentally induced diabetes. This overview presents the changes in smooth muscle of the gastrointestinal, circulatory and urinary systems as long-term complications of experimentally induced diabetes mellitus. There is not enough knowledge regarding the precise mechanisms of smooth muscle dysfunction, as well as its treatment, which requires a detailed study of the problem.

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BO3. VIRUSES AND DIABETES

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Type I diabetes (T1D, initially termed insulin-dependent diabetes mellitus) is an organ-specific autoimmune disease characterized by a deficit in insulin production as a result of selective and massive destruction of islet β cells or of their functional impairment. During the last decades, the incidence of T1D has increased significantly, reaching percentages of 3% annually worldwide. These data suggest that besides genetical factors environmental perturbations are also involved in pathogenesis of the disease. At least ten viruses have been reported to be associated with the development of T1D in humans and/or similar syndromes in animals.

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BO4. STEM CELLS IN THE TREATMENT OF DIABETES – FROM DREAMS TO REALITY

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SESSION C

CO1. DISTRIBUTION AND DYNAMIC OF PATHOGENIC STRAINS *ESCHERICHIA COLI* ISOLATED FROM PIGS IN BULGARIA FOR THE PERIOD 2000 - 2005

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Introduction

At a meeting in November 1974 in Leipzig, the Veterinary Department at the Council for Mutual Economic Assistance definition appears that porcine diseases in the etiology of participating *Escherhia coli* (*E. coli*) and are serious economic problem for the pig and are defined as colibakteriosis or colibakterioses (Vachev, 1977). In the 60 years of XX century in Bulgaria colibakteriosis (CB) in its different forms is registered in 52% of the all 2438 swinefarms (SF) and in 70 years in 72% of existing 1350 farms. The colibacteriosis occupy second place in the structure of morbidity and mortality in pigs and can be explained by the transition from extensive to intensive and hibride pig husbandry (Dimitrov et al., 1977). For the next period to 2000 there is evidence that CB is one of constant diseases in all (100%) surveyed pigfarms (Yordanov et al., 1990; Yordanov and Motovski, 1995, Yordanov et al., 1995 and Yordanov et al . 1996). Vachev (1977) and Stojanov (1977) reported that CB are due by 20 to 50% of the mortality in pigs during the period from 1960 to 1975. According Yordanov (1982) the mortality from CB in the industrial pigfarm Popovo for the period 1975 to 1981 was a move within 41 to 71% of total mortality, but evidence of Jordanov and Motovski (1995), Yordanov et al. (1995 and 1996) during the last years of the century, this mortality was reduced to 9.9 and 8.5%.

Studying the etiology of colienteritis and oedema disease Vachev and Stoyanov (1975), Stoyanov (1977) and Vachev (1977) reported that persistent large numbers of haemolytic (O8, O18, O45, O138, O139, O141, O147 and O149) and nonhaemolytic (O20, O54, O78, O101) strains of *E. coli*, isolated from 1960 to 1970. This autors and Motovski et al. (1977) and Minev (1977) published data that in the period 1971 - 1980 predominated representatives of O149 (100 to 20%), O 139 (41.4%), O138 (32.2%), O141 (5.7%), O147 (3.5%) and O8 (21.5%) of the haemolytic, and O78 (from 100 to 21.4%), O54 (35.7%) and O101 (21.4%) of the nonhaemolytic *E. coli*. Since 1971, already isolated strains O18, O20 and O45 and from 1976 - O8, O54, O78 and O101 non are isolated. During the period 1981 - 1990, were registered strains only from the haemolytic groups O138, O139 and O149, but from O157 was isolated for the first time and was not isolated more of these O141 and O147. This trend continued in the period from 1991 to 2000, and strains only from O101 again appear.

We set the goal to investigate the distribution and dynamic of the pathogen for swine *E. coli* strains for the period 2001 - 2010 year for which data were missing at home.

Materials and methods

To achieve ours goal using the methods of the epizootiological study in 16 pigfarms and microbiological examination of the 1446 materials from live and dead swine and pigs with clinical symptoms of infection disease connected with *E. coli* during the period 2000-2005.

Results

The epizootiological studies on 8 pigcomplexes (PC) and 8 pigfarms (PF) found that all farms are fixed for colibakteriosis (CB). The disease occurs with expressive clinical signs and typical pathological changes in the piglets and weaners. Of bacteriological tests performed on material from pigs originating from PC, PF and personal farm (PF) and subsidary farms (SF) per years, we found that positive for *E. coli* samples increased from 2000 to 2004 in the range

of 9.0 to 38.5%. This increase, however, is characteristic of PC (from 7.0 to 47.1 %) and PF (from 5.3 to 34.3 %), while for PF and SF the tendency is in the conversely direction (from 40.0 to 10.0 %). During the following 2005 year the percentage of positive for *E. coli* samples decreases noticeable, as follows: for PC - 16.1%, for PF - 12.2% and for PF and SF - 15.1%. In more the commonly registered in the pigs 13 "O" serogroups of *E. coli*, while the study found the following distribution by years: **2000** - O101, O138, O139, O149 and O157, **2001** - O101, O139, O149 and O157, **2002** - O54, O101, O138, O139, O141, O147, O149 and O157, **2003** - O54, O101, O138, O139, O147, O149 and O157, **2004** - O8, O54, O101, O138, O139, O149 and O157, **2005** - O54, O78, O138, O139, O147, O149 and O157. As overall, for the period the proportion of the serogroups is: O8 - 1.6%, O54 - 10.6%, O78 - 1,1% O101 - 11.7%, O138 - 8.5%, O139 - 17% , O141 - 2.7%, O147 - 6.4%, O149 – 12,8 % and O157 - 27.6%.

Discussion

These results give us grounds to assume that the colibakteriosis in the pigs is a widespread disease in Bulgaria, which confirms the attitude of Vachev (1977), Dimitrov et al. (1977) and Yordanov et al. (1995). The proportion of tha disease in the pathology of the pigs is grew significantly from 2000 to 2004 and in 2005 diminish rapidly. This is explained by the massive appearance and subsequent decay of immunosuppressive viral diseases PRRS and PCVD, affect stronger industrial and semi-industrial farms (Yordanov and Motovski, 1995). For the period 2000-2005 in the etiology of the colibakteriosis participated strains of 10 serogroups, including haemolytic and nonhaemolytic from which the highest share occupied O157 (27.6%), O139 (17%), O149 (12.8%), O101 (11.7%) and O54 (10.6%), which is consistent with read Stoyanov (1977), Motovski et al (1977), Yordanov et al. (1996). Make an impression that representatives of the O18, O20 and O45 are not registered, and since 2002 has been appeared by such single from O141 (2.7%) and O147 (6.4%) and during 2004 - O8 (1.6%) and O78 (1.1%), which repeats the established periodicity in the circulating of the strains of these groups in 1971-1976 from Motovski et al. (1977).

Conclusion

The widespread of the colibakteriosis in the our country is maintained, with variable part in the pathology of the pigs that is connect with its associated running with PRRS and PCVD. Registered a significant dynamic of the strains from different serogroups *E.coli*, both over time and depending on the technological nature of the husbandrys.

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CO2. REVIEW OF THE METHODS FOR DIAGNOSIS OF ENZOOTIC PNEUMONIAE (EP) IN PIGS (survey)

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Enzootic pneumonia is prevalent in the world and occupies a large proportion of the pathology of pigs, which makes it a problem for veterinary science and practice. As the cause of EP is considered *Mycoplasma hyopneumoniae* (*M. hyo*) (Androsik, 1989; Abiven and Pommier, 1993). The analysis of domestic and foreign results on diagnosis of the disease, show that different laboratory and out of laboratory methods can be applied successfully.

Out of laboratoty methods of administration are epizootiological studies, clinical and pathological studies. The disease has the characteristics of stationary disease that occurs throughout the whole year in which the incidence rate reached 80-100% and the lethality is within 20% (Pustovar, 1991). Source of infection appears to be sick pigs, regardless of whether the disease is as clinical or sub-clinical (latent) infection (Androsik, 1989). The incubation period lasts 8 to 40 days. Two weeks after birth in piglets appear cough and serous leak from the nasal cavities (Dushuk et al., 1987; Shafiev, 2002). According Androsik (1989), Pustovar (1991), Kuzymin (1999) and Subramaniam (2000), the first stage of the disease lasts up to 14 days and is characterized by remitting fever (40,5 - 40,8 ºC) and dry cough of seizures and animals occupy the stand of "dog sitting". In the second stage, which lasts 25 days and more, it appears signs of pneumonia. Pigs are half-hearted, with curvature of the spine, appears on the serous-purulent conjunctivitis and leak from the nose, coughing and grunting. Coughing was observed in 50% of patients pigs (Dushuk et al., 1987; Jacks et al., 1980; Romanenko et al., 1988; Berdnik, 1991; Shafiev, 2002). Postmortem changes in enzootic pneumonia were localized predominantly in the apical, diaphragmatic and intermedial shares of the lungs in the form of serous-catarrhal and interstitial pneumonia, as well as in bronchial lymph nodes. The disease is sometimes complicated with fibrinous pleuritis and pericarditis (Jensen et al., 1995; Dimitrova, 2009).

By laboratory methods of administration are pathohystological, bacteriological, serological and molecular biological research. By histological examination establishes lymphohistocytaris peribronchitis and perivaskulitis, edema and cell infiltration of interalveolaris septis, moderate accumulation of mononuclear and other cells in the alveoli, alveolar-cell pneumonia and catarrhal or catarrhal-purulent (Goodwin et al., 1965; Bulnes et al., 1999; Gonzalez et al., 1999). In the recovery period it could be found alveolar emphysema (Ross et al., 1999). According to Androsik (1989) the only reliable method of diagnosis of enzootic pneumonia is bacteriological, but isolation of *M. hyo* from diseased pigs is associated with considerable difficulties and do not always get positive results. Being "membrane parasites, mycoplasma include in its cytoplasm membrane integrated proteins of guest cells, and in that way lose their ability to cultivate on food environments. Moreover, they adapt very poorly to artificial nutrient media and that impede their isolation. The reliability of this method is 32.6% to 52.9%, which is why in many countries it is hardly ever used (Androsik, 1989; Berdnik, 1991). By means of the spotted and exponent reaction of agglutination (RA) can be find of 20% to 87% of infectious animals with micoplasma. The reaction is difficult to account due to the formation of small complexes agglutination (Dushuk et al., 1987; Androsik, 1989; Pustovar, 1991). Immunodiffusion reaction (IDR) is an insensitive method and is of little diagnostic significance (Stipkovits et al., 1988; Androsik, 1989). Complement fixation reaction (CFR) is highly sensitive, specific and promising method for diagnosis of EP (Mori et al., 1983; Berdnik, 1991), but there are reports of insufficient sensitivity of CFR in the diagnosis of EP (Young et al. , 1983). In recent years, have been tested and new methods, including the most noteworthy enzyme-linked immunosorbent assay (Enzyme Linked Immunosorbent Assay, ELISA). Principle of the test is based on antigen-antibody interaction. This reaction is characterized by high specificity and sensitivity and it's one of the most modern methods for diagnosis of EP caused by *M. hyopneumoniae* (Yordanov et al., 2007; Yordanov, 2009; Dimitrova, 2009). Polymerase chain reaction (Polymerase Chain Reaction, PCR) amplification method allows using specific sections of DNA to detect the presence of antigen in the pathological material. The method is much faster than cultural. The same is high specific and can be used both for while still living diagnosis and postmortem (Ruiz et al.,

2002). PCR method was first used in 1991 for the diagnosis of EP in pigs (Harasawa et al., 1991).

In practice, laboratory diagnosis of enzootic pneumonia in pigs is difficult and only a holistic approach based on a different, modern and highly sensitive methods holistic approach based on a different (Pustovar, 1991; Kovalishin et al., 2001; Dimitrova, 2009).

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CO3. BIOLOGICAL METHODS FOR QUICK FIELD DETECTION OF ANTI-CHOLINESTERASE INSECTICIDES

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Cholinesterase insecticides are among the most frequently used chemicals in agriculture and everyday life. They are phosphor-organic or carbamate salts. They possess a strong and immediate anti-cholinesterase activity and produce high and quick insect mortality.

Experiments:

1. On the bottom of a 5 – 10 l desiccator 3 or 4 drops of honey are mixed with the same quantity of the studied sample. Separately on the bottom only honey and only sample drops are spilled upon. Then 5 – 6 flying flies are introduced into the desiccators and their behavior (flying and consumption) in the experimental and control specimens are watched.

Result:

In the presence of phosphor-organic or carbamate salts a prompt paralysis and death of flies takes place.

2. Method to provoke secretion of bloody (pink) tears in rats by phosphor-organic or carbamate salts

6 white rats are used in this experiment.

Two of them are injected subcutaneously with 2 – 3 ml of 0.5 % solution of NaCl (control).

Two are injected with 2 – 3 ml of 0.1 % solution of the studied sample.

The last two are injected with 2 – 3 ml of 1 % of the studied sample.

Results

After 10 – 15 minutes eyes of all rats were wiped with gauze or soft paper. In the presence of the used salts the experimental rats leave pink spots from their eyes as a result of "bloody" tears.

3. Influence of phosphor-organic or carbamate salts on the width of the pupil

In this experiment 3 rabbits were used. The width of their pupils in mm was measured against a constant source of light or the day light. After that the eyes of the first rabbit were spilled upon with 3 – 4 drops of 0,9 % NaCL solution (control); one eye of the second animal received the same amount of 0,1 % solution of the sample, the third one – the same procedure using a 1 % solution of the sample in one of its eyes.

After 10 minutes the eyes treated with sample salts showed shrinkage of the pupil by 40 – - 70 %.

CO4. *IN VIVO* CHRONIC TREATMENT WITH COBALT(II) COMPOUNDS

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Cobalt(II) compounds exhibit both beneficial and harmful effects on humans and animals upon treatment. Long-term treatment, age, organ distribution, morphological and biochemical changes are important parameters which should be considered when evaluating the effect of heavy metals.

Experimental design. In the present study an in vivo model for Co(II) treatment of mice from different stages of development is established. Pregnant balb/c mice are subjected to CoCl2 or Co-EDTA via drinking water. The animals have access to food ad libidum. Day 25 mice are placed in individual cages and weighed weekly to adjust the dose. At certain time periods (day 18, 30, 45, 60 and 90) the newborn mice are sacrificed. Mice are also injected with 5-bromo-2-deoxyuridine (BrdU) for immunohistochemical investigation the labeling index of cell proliferation under the influence of the metal compounds. All changes are compared to control samples of age-matched mice drinking the same quantity tap water. The model is used for both morphological and biochemical studies.

1. Morphological investigations

The morphological investigations include cytological analysis of peripheral blood smears and histological studies of paraffin-embedded samples of bone marrow, spleen, liver,

kidney and testes. The cellular and ultrastructural changes in the hematopoietic tissues due to the influence of CoCl2 and Co(II)-EDTA are analyzed by light and electron microscope investigations.

The type, shape, size and number of the cells from the peripheral blood are analyzed by light microscope on smears stained with May-Grünwald-Giemsa.

Spermatozoa count is also determined in mature mice to elucidate the effect of Co(II) compounds on spermatogenesis.

Organ indices (calculated as a ratio of organ weight to body weight) are used to evaluate the effect of heavy metal treatment.

2. Biochemical analysis

Microassays on organ homogenates are performed to evaluate cobalt accumulation in them.

Whole blood collected in heparinized tubes is used for biochemical analysis of hemoglobin content and hematocrit. Cobalt, iron and erythropoietin concentrations are measured in blood plasma. Plasma samples are also used for creatinine and NO2/NO3 analysis.

3. Results

Results show increased organ weight indices when mice are exposed to Co(II) compounds compared to control samples. Red blood cell count is reduced in immature mice. Enhanced extramedullar erythropoiesis is observed in spleens of mature mice.

More cobalt(II) was measured in the kidneys and liver compared to the spleen. The obtained results show an increase in hemoglobin content after Co(II) treatment, decrease in iron concentration, while the creatinine value remained within the normal range.

Results show that exposure to cobalt(II) affects key processes such as hematopoiesis and spermatogenesis.

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CO5. AN EXPERIMENTAL MODEL OF ACUTE LITHIUM INTOXICATION IN MICE – PRELIMINARY DATA

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Background and aim

Lithium has been the first line drug for the past five decades, since its introduction, in treating bipolar mental disorders. During these years its application in psychiatry has become important especially in the treatment and prophylaxis of mania and depressions (1). The precise mechanism of its mode of action remains unknown. In certain instances, however, neurotoxic effects of the lithium ion at "therapeutic" levels have been reported (2).

The severity of neurotoxic symptoms correlates with serum lithium ion levels. Acute lithium intoxication has several neurological manifestations such as encephalopathy, tremor, ataxia, dyskinesia, seizures. These symptoms are usually reversible, however, a number of cases of persistent neurological sequelae have been reported (3). A likely association between lithium toxicity and cerebellar degeneration has been suggested and neuropathological studies have demonstrated a reduction of Purkinje cells in the cerebellar cortex and spongiform changes in the white matter of the cerebellum (4).

Animal models have been insufficiently employed to study the neurotoxicity of lithium. Cerebellar spongiform degeneration is reported in a rat model of acute lithium intoxication and these data correlate well with findings in humans (5). Chronic lithium treatment markedly decreases the density of serotonin receptor subtypes in the frontal cortex, hippocampus and choroid plexus of rat brain (6). Congestion, haemorrhage and hyperplasia of the choroid plexus and of neurons are the most striking phenomena after long-term administration of lithium in rats (7). Experimental data for the neurotoxic effect of lithium salts in mice are scarce.

In this study, we present our preliminary results employing a mouse model of lithium-induced intoxication. Morphological studies on different parts of the mouse central nervous system (CNS) were carried out. Biochemical and rheological analysis of blood samples and enzyme activity studies have also been performed.

Experimental model

Three-month old male mice were subjected to lithium intoxication. Mice were housed under standard conditions on a 12-h light/dark cycle and had free access to laboratory chow and tap water. In brief, a single toxic but not lethal dose of lithium chloride (250 mg/kg body weight, 0.2 ml dosing volume in saline) was administered intraperitoneally. Control mice were injected with the same volume of saline.

Lithium treated and control mice were anaesthetized with diethyl ether and decapitated 24 h after the administration.

Different regions of the CNS were studied – cerebellum, cerebral cortex, thalamus, medulla oblongata, choroid plexus, ependyma and the cervical part of spinal cord.

For the histochemical and immunohistochemical investigations the material obtained was used frozen or fixed in Carnoy solution.

For the study of enzyme activities and silver staining of neuronal degeneration the samples were fixed, embedded in optimal cutting tissue medium and frozen sections were prepared.

Material for electron microscopy was fixed in Karnovsky's aldehyde mixture, postfixed in OsO4 and embedded in Durcupan.

Blood samples for biochemical and rheological analysis were collected in heparinized tubes.

Preliminary results

1. At the 24th hour after acute intoxication we have found that lithium chloride provokes changes in the mouse CNS.
2. Rapid vacuolization of the brain tissue and subsequent formation of the zones of spongiosis is one of the earliest morphological signs of this intoxication.
3. Similar histopathological changes in CNS are specific for brain tissue degeneration.
4. A species-specific vulnerability of the mouse CNS could have played a role in the distribution of spongiosis in this model explaining why more intensive vacuolization out of cerebellum is found.
5. More intensive compact areas with spongiform changes in mouse brain are found in thalamic region and less intensity is registered in the cortex. Vacuolization in the mouse cerebellum seems to be minimal.
6. An open question for further studies employing this model remains the reversibility of the early histopathological changes in mouse CNS after lithium intoxication and their eventual restoration at later stages.

7. Studies of blood samples showed increased glucose level suggesting a possible affect of lithium chloride on the pancreas. Furthermore, elevated blood viscosity was measured indicating changes in the properties of the erythrocyte membrane.

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CP1. EFFECT OF ZINC AND IRON COMPLEXES ON VIABILITY AND PROLIFERATION OF HUMAN CERVICAL CARCINOMA CELLS

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Zinc and iron are essential elements that take part in many key biochemical processes supporting life. Zinc is required for the biological function of more than 200 enzymes and is known to play an important role in the development and maintenance of the immune system. Iron is known to be essential for fundamental cell functions, such as DNA synthesis, transport of oxygen and electrons, and cell respiration. It has been found in our previous experiments that some Zn(II) and Fe (II,III) complexes with various ligands (Mannich bases, cholic acids) express time-and concentration dependent cytotoxicity against animal and human permanent cell lines.

In order to continue the investigations in this field, the following zinc (II) and iron (III) complexes were synthesized and their physical and chemical characteristics were determined: $Zn(MorfBig)Cl_3$,$Zn(Metf)_2Cl_2.3CH_3OH$,[Zn(MorphBig)(Metf)Cl]Cl,$[Fe(Metf)_3](NO_3)_3.CH_3OH$, $[Fe(MorfBig)(Metf)(H_2O)_2](NO_3)_3$, $[Fe(MorfBig)_2(H_2O)_2]Cl_3.2C_2H_5OH$ (where MorphBig = morpholine biguanide hydrochloride and Metf = metformin hydrochloride). The aim of the study presented here was to evaluate the influence of these compounds on viability and proliferation of cultured human carcinoma HeLa cells.

The investigations were carried out by MTT test (which reflects damage to mitochondria), neutral red uptake cytotoxicity assay (indicates damage to lysosomes and Golgi apparatus), crystal violet staining (nuclear staining) and double staining with acridine orange and propidium iodide. The compounds examined were applied at concentrations of 10-200 μg/ml for 24h, 48h and 72h. The results obtained revealed that Zn(II) complexes are more pronounced cytotoxic and cytostatic agents as compared to the Fe(III) complexes.

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SESSION D

DO1. THE AMYLOID β PEPTIDE AFFECTS THE ELECTRICAL ACTIVITY OF NEURONAL CELLS

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Alzheimer's disease is the most frequent degenerative cause of dementia. According to the statistics only in the United States there are more than 5 million people suffering from this disease. The main risk factor appears to be the age, and the frequency of the illness rises with age and reaches 50% in people over 85 years. Due to the constantly increasing life expectancy the number of suffering people is constantly increasing and Alzheimer's disease becomes a serious social and economical problem.

Alois Alzheimer was the first to describe the neurophatological hallmarks of intracellular senile plaques, which core is comprised of fibrilar amyloid β peptide (Aβ), and extracellular neurofibrillary tangles (consisting of non-typically phosphorilated tau-protein). The amyloid β peptide is part of a larger integral membrane protein called amyloid precursor protein (APP). The main pathway for proteolytic cleavage of APP in healthy brain is the enzyme called α-secretase, which cleaves within the aminoacid sequence of Aβ, thus making impossible the release of Aβ and the formation of the senile plaques. There are also alternative cleavage ways of APP with much lower activity. These are the β- and γ-secretases which cleave at the amino- and the carboxy-end of Aβ liberating monomeric Aβ. In diseased brain the balance of this secretases is disturbed and the β- and γ-secretases are much more active. As a result Aβ remains intact and can aggregate as a core of senile plaques.

Aβ is thought to have a central role in the pathogenesis of Alzheimer's disease, however, the mechanism of action is not clear. A number of actions are attributed to it like inhibition of cell adhesion in neuronal cultures; inhibition of neurite outgrowth; impairment of calcium homeostasis; induction of oxidative stress; abnormal phosphorylation of the cytoskeletal tau-protein; initiation of apoptic mechanisms; effects on electrophysiologigal processes etc.

It has been shown that Aβ causes depolarization of the cell membrane; influences the synaptical activity; inhibits LTP. Our investigations were directed to the effects of Aβ on the electrophysiological activity of neurons. For this purpose frontal cortex tissue from 17-day old mouse embryos were used to obtain cells, which were then cultured on microelectrode arrays. The 64-electrode arrays were made using standard photoetching techniques. Recording of the data was performed with the Multichannel Acquisition Processor System, a computer controlled 64-channel amplifier system commercially available from Plexon, Inc. (Dalas, USA). Action potential (spike) data from active channels were recorded. Analog spike integration was used as a major scheme for extraction of burst activity from single channel spike patterns.

Aβ is secreted in two major forms one containing a 40 aminoacid sequence (Aβ1-40) and the other 42 aminoacids (Aβ1-42). In non-diseased brain the ratio is roughly 10:1. Aβ1-42 has been shown to aggregate more readily and is the major form found in the core of the senile plaques. It was found that the effects of Aβ are mediated by a fragment enclosing amino acids 25-35. Although this sequence is contained in both naturally occurring Aβ1-40 and Aβ1-42 it has been shown that they exhibit clear pharmacological differences and may play different

roles in Alzheimer's disease. Therefore, it is of importance to determine whether they affect the electrical activity of cultured neuronal networks and whether there is a difference between their effects and potency compared to the Aβ25-35 fragment.

We started our studies with the Aβ25-35 fragment and could show that it influences the electrical activity of neuronal networks rapidly (in the first 3 minutes after addition) reproducibly, in a concentration-dependent manner and reversibly (decreasing the concentration of Aβ25-35 by medium change resulted in a recovery of the electrical activity). Reduction of electrical activity is evident already at 25 nM Aβ25-35 and an almost complete shut off occurs at 50 μM Aβ25-35. We compared the effect of the biologically active fragment Aβ25-35 with the effect of the endogenously presented forms Aβ1-40 and Aβ1-42 using the most effective concentration - 50 μM. It was shown that Aβ1-40 and Aβ1-42 also affect the electrical activity rapidly and reversibly. The inhibition was strongest with the fragment Aβ25-35, followed by Aβ1-42 and Aβ1-40, which also directs the attention to the role of Aβ1-42 in Alzheimer's disease.

Aβ is known to induce peroxidation of membrane pipids of cultured neurons or isolated synaptosomes. To test the possibility that the effect of Aβ on the electrical activity of neuronal networks is caused by peroxidation of neuronal membranes we compared the effect of Aβ to that of Fe2+ ions, an agent known to induce lipid peroxidation. It was shown that Aβ affects the electrical activity rapidly and reversibly while the effect of Fe2+ ions appears after a longer time period and is irreversible. These points to cell death and a different mechanism of action.

For long time the amyloid cascade hypothesis which states that Aβ "precipitates to form amyloid and, in turn, causes neurofibrillary tangles and cell death" was the dominating one. Whether the deposition of Aβ has a central role or is only one element of the etiology of Alzheimer's disease remains a controversial issue. Most problematic is the weak correlation between the deposition of amyloid and the degree of dementia. There are conflicting reports regarding the toxicity of Aβ in different aggregation states. It begins with the monomers through dimers, low oligomers, Aβ derived diffusible ligands to fibrils. An appealing hypothesis is that "Aβ aggregation and deposition is a protective mechanism of clearing the brain of toxic Aβ". We made two preparations of Aβ1-42 one containing monomers, di- to tetramers and high ologomers and another one containing only monomers and minor amounts of di- to tetramers and compared their effect on the electrical activity of cultured neuronal networks. It was shown that the preparation containing monomers and traces of di- to tetramers has a pronounced effect on the electrical activity, whereas the preparation comprised of high oligomers hardly affected the electrical activity. The concentration of monomers and di- to tetramers of Aβ1-42 in the latter preparation is too small to have an effect on the electrical activity.

Summarizing the data on the neurotoxic and electrophysiological effects of Aβ, the impact of genetic mutations and neurotransmitter system alterations on the metabolism of APP, causing enhanced production of Aβ, and the synaptic loss in Alzheimer's disease we propose the hypothesis that Aβ monomers are modulating/inhibiting the electrical activity of neurons thus interrupting interneuronal comminication and eventually leading to synapse loss and neuronal degeneration.

DO2. SODIUM NITRITE-INDUCED HYPOXIA – EFFECT ON THE FREE FATTY ACID CONTENT IN RAT BRAIN SYNAPTOSOMES

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Background and aim. Brain is of special interest for hypoxia studies as it is critically dependent on its oxygen supply. Limitation of oxygen delivery to the brain below a "critical" level quickly results in reduced oxidative phosphorylation, decreased cellular adenosine triphosphate and a consequent increase in adenosine and collapse of ion gradient, thereby affecting the function of the central nervous system (1).

Hypoxia (i.e., reduced oxygen availability) is a classical model of the metabolic encephalopathies. An understanding of how hypoxia alters brain function has implications for understanding other metabolic encephalopathies as well as aging and age-related disorders. Utilizing a variety of models of hypoxia is necessary to determine the effects of hypoxia on brain function and to test hypotheses about the underlying mechanisms of its actions. Both in vivo and in vitro models of hypoxia are produced by either limiting the oxygen availability or impairing the tissues'ability to utilize oxygen (2).

Sodium nitrite-induced anemic hypoxia refers to a reduction in hemoglobin's ability to transport oxygen. Sodium nitrite converts hemoglobin to methemoglobin and unlike ferrous form of hemoglobin, methemoglobin does not bind oxygen strongly. Thus the oxygen-carrying capacity of the blood is reduced. It is reported that the oxidation of oxyhemoglobin by nitrite to produce methemoglobin is a complex process that has been characterized by a lag phase followed by an autocatalytic phase (3).

Lipids are particularly sensitive to hypoxia, in comparison to other membrane components. Although significant efforts have been directed at evaluating changes in various metabolic and physiological functions (4) lipid metabolism in response to hypoxia in the brain subcellular fractions has not been fully evaluated. Considering the role of some free fatty acids (FFAs) in stimulation of cellular processes, establishing the changes in their content may be of basic significance for understanding the involved pathomechanism.

In this study, we explored the effect of sodium-nitrite induced hypoxia on the level of total and individual FFAs in rat brain synaptosomes.

Experimental model. Twenty male Wistar rats at the age of three months, each weighing 190-220 g, were subjected to sodium nitrite-induced hypoxia. Rats were housed under standard conditions on a 12-h light/dark cycle and had free access to laboratory chow and tap water. In brief, sodium nitrite was administered intravenously at 20 mg/kg body weight (2 ml/kg dosing volume). Hypoxic rats were lightly anaesthetized with diethyl ether and decapitated 20 h after the administration.

The synaptosomal fraction was isolated using two-step sucrose gradient (5) and the lipids were extracted (6). The fatty acids were converted to fatty acyl methylesters (FAME) by addition of methanol and hydrochloric acid. The FAME were extracted by petroleum ether, then concentrated in a rotary vacuum evaporator and subjected to gas-liquid chromatographic analysis.

Results. It is known that FFAs are the first that undergo changes in different pathological states. Our results showed that FFA changes are well pronounced in the synaptosomes of hypoxic brains, too. The concentration of total FFAs increased nearly 4 times the control value. Among the individual FFAs, stearic acid increased 19-fold, linoleic acid – 2.7-fold, arachidonic acid – 3-fold. A notable observation was the accumulation of $C_{18:1}$ and $C_{20:2}$, which were absent in control rats. There was a tendency to synthesize long-chain and polyunsaturated FFAs. Polyunsaturated FFAs have been shown to reverse excitotoxic changes triggered by glutamate (7) and thereby they are considered as neuroprotectors.

In conclusion, our data provide evidence that sodium nitrite-induced hypoxia influences FFA metabolism in rat brain synaptosomal membrane. They demonstrate that synaptosomes respond to hypoxia by synthesizing a high amount of unsaturated FFA and this is probably involved in the cell survival pathways.

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DO3. EFFECT OF AMINO ACID DERIVATIVE ON COGNITIVE FUNCTION OF SOCIALLY ISOLATED RATS AFTER MATERNAL DEPRIVATION

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Introduction

The importance of early life stress for mental health is well known, even though the underlying neurobiological mechanisms are not completely clear. The postnatal stress exposure in rodents affects critical periods of brain development that persistently alter structural, emotional and neuroendocrine parameters in the adult offspring [1], [2].
Maternal deprivation followed by social isolation is used as an animal model of damaged cognitive function. On this model we study the possibility for pharmacological modulation and protection of cognition with amino acid- application.
Newly synthesized aminoacids (L-valine derivatives) demonstrated significant neuroprotective effect on adult rodents in our earlier studies, especially the compound M6 [3]. We suggest that this preventive effect may be even stronger in young animals.

The purpose of the study was to examine the ability of M6 for pharmacological modulation of early life postnatal stress in female and male rat offspring with maternal deprivation.

Materials and methods:

Wistar male and female rats were deprived from their mothers on the 21 day after birth. Animals were hosted in isolated cages for 5 weeks (social isolation model according Valzelli (1978), modified by Petkov [4].

The control groups (after mother deprivation) were hosted in common cages - 6 rats in cage.

At the end of isolation period the half of the animals (grouped and isolated) received a newly synthesized compound (L-valine derivative, M6) in daily dose 150 mg/kg intraperitoneally (i. p.) for 3 days. The control groups of rats (male and female) received the solvent Oleum Helianthi (in the same volume). Comparative study on their cognitive functions was performed on the 24 hour after the last treatment – namely:

1. Exploratory activity (Hole board test, on the 1st, 2nd and 3rd minute)
2. Learning and memory (on the 1st and on the 24th hour) with the step through test.

Experimental data were analyzed by statistical software SPSS for Windows, ANOVA (mixed design).

Results and Discussion: The combination of 2 kind of stress factors- maternal deprivation (5 weeks) with social isolation - produced different cognitive changes (both in male and female rats) as was reported in the literature [1, 2].

Effect of the social isolation:

The social isolated rats had:

- increased exploratory activity
- increased processes of learning and memory (step through test) in comparison to the grouped animals (both on the 1st and the 24th hour)

Our data are in agreement with other similar reports, which used different models of early stress [1, 2].

The effect of sex:

We found that the effect of the social isolation in young animals (56-57 days) on the cognitive function in rats is sex- related.

Exploratory activity is bigger in male than in female grouped animals, but in isolated the difference is insignificant.

Effect of isolation on the learning and short-term memory (on the 1st hour) is more significant in male than in female animals.

The effect of M6 treatment:

Compound M6 demonstrates preventive effect on the cognitive function in rats - better in isolated than in grouped animals.

We found also that the effect of M6 on learning and memory is sex related - in male rats it is preventive on the 1st hour, but on female is the opposite- decline the memory.

Treatment with M6 in grouped animals increased the learning and short-term memory in male, but not in female (decreased it).The tendency is quite the opposite in isolated animals- M6 decreased short-term memory in males and increased it in females.

M6 does not exhibit any prolonged effect on memory, according to the step-through test (on the 24th hour after M6 treatment).

Conclusions:

- The combined postnatal stress exposure (maternal deprivation followed by social isolation) affects in different way the cognitive function of rodents and probably has a critical influence on brain development and neural plasticity.
- **The effect of M6 is sex-dependent:**

Males- in grouped animals the memory was improved and in isolated decreased.

Females- the opposite- improved memory in isolated and decreased in grouped animals.

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DO4. NEW DESIGN FOR EXPERIMENTAL INVESTIGATIONS OF STATIC BIOMECHANICAL CHARACTERISTICS AND AXIAL FORCE OF BLOOD VESSELS

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Biomechanical characteristics of the arterial wall give important information for the biomechanical behavior of blood vessels in studying the vascular damage in pathology and the vascular wall changes with the age and after drug treatment. The nonlinear stress-strain relation approach (Demiray, 1972, 1988, Fung et al., 1979) relates the elastic stiffness of the arterial wall to the corresponding physiological forces. The coupling of mathematical modeling and experimental data reveals perspectives of studying the arterial wall and for prediction of its responses to different stimuli.

In order to estimate the static characteristics of the arterial wall, we aimed to rearrange the experimental system for dynamic in vitro investigation of cylindrical blood vessels (reported previously by Antonova, 2004). The cited device (fig.1) enables to employ the method of forced oscillations, which is well known in engineering, for studying of cylindrical blood vessels preparations. The preparation is suspended in a fixed length immersed in nutrient solution, which assures the vitality of the preparation during the time of experiment. In dynamic experiments we recorded the signals for the pressure and volume of rat aorta as well as for the frequency and amplitude of the forced oscillations with the aid of an analog-to-digital-converter (ADC).

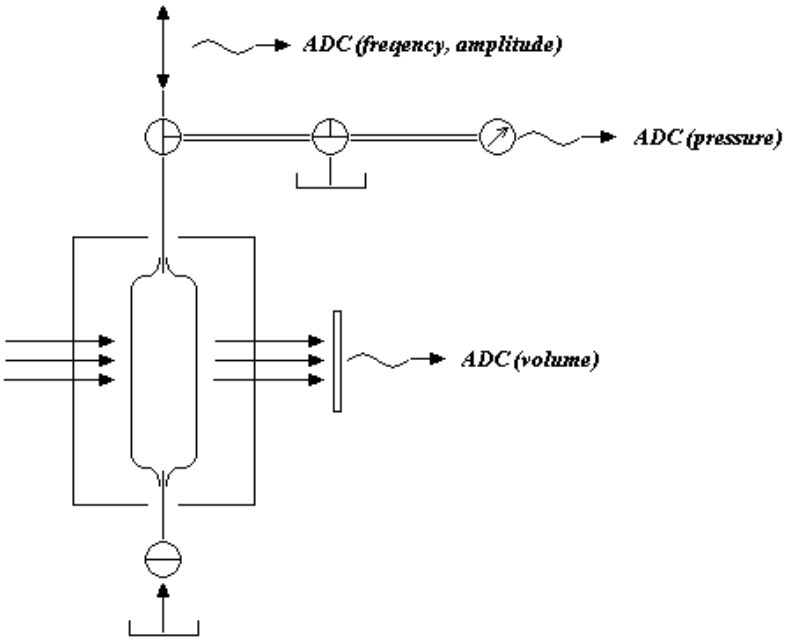

fig.1

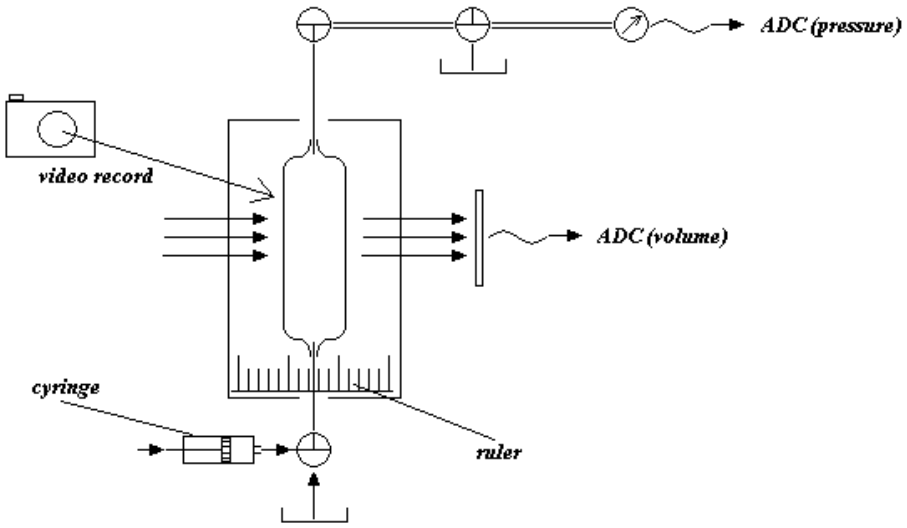

fig.2

In a new work (Antonova & Stoytchev, in preparation), for static experiments, we rearrange the device in order to record the pressure and volume of the rat aorta preparation by pushing in step-wise small portions of nutrient solution with a Hamilton-syringe (fig.2). An additional video-record of the preparation with a standard camera facilitates the estimation of the volume of preparation. The software was also worked out in order to enable a more prolonged record as well as the synchronization of the ADC and video-records. The volume-to-pressure graph can be drawn and used in the mathematical equations.

In the used mathematical equations for modeling the arterial wall behavior a necessity arises for estimation of the axial force at different conditions of the blood vessel preparation. To resolve this problem we created a simple experimental design (fig.3). The preparation is immersed in nutrient solution and it is suspended on an easel with fixed upper end. Different lead-pieces with known weights are hooked up on the lower end. A ruler is suspended behind the preparation to give a measure for the length. Various pictures are taken by each of the loads after drawing out the preparation from the solution for a few seconds. The force can be estimated by multiplying the mass to the standard gravity *g*. The force-to-length graph can be drawn. The axial force might be estimated from this graph for different lengths of the preparation. For the purposes of the mathematical modeling three different lengths are needed: the physiological (in situ) length and two other lengths – longer and shorter as the physiological length.

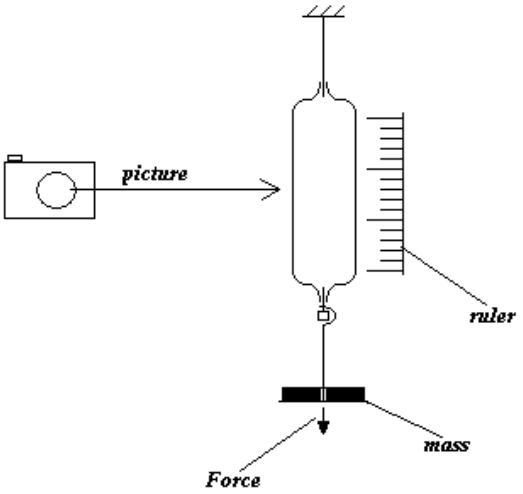

fig.3

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DO5. ANIMAL LIVER PERFUSION TECHNIQUE

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The function of the liver can be studied with different liver preparations ranging from the intact organ in vivo, through perfusion system, liver slices, isolated hepatocytes, homogenates and membrane fractions, to purified enzymes. Every model has its special advantages and disadvantages.

The isolated animal liver perfusion technique permits studies of liver function to be performed in a system that approaches normal physiology. This technique is appropriate for the study of biotransformation processes, providing information as to their rate and the product formation. Variations of data due to alternations in blood flow, blood pressure or hormonal milieu can be eliminated. Compared with *in vivo* experiments, the major advantage of the isolated perfused organ is that the experiments can be performed under precisely defined conditions. Advantages include: the intact architecture of the organ, the possibility of controlling blood and bile flow, the ease manipulation of the composition of the perfusion medium, the large number of perfusate samples that may be collected, the small number of interactions with endogenous compounds and also the possibility to use concentrations of substrates and inhibitors that would not be tolerated *in vivo* because of their toxicity. In contrast to other types of preparation, such as liver homogenates or organelles isolated from them, i.e. microsomes, it has the advantage of allowing the study of sequental metabolic pathways in an environment that included presumably physiological co-substrate levels.

The animal liver perfusion technique consists of several steps. In brief:

- Anesthetic agent application. Pentobarbital is preferred as an anesthetic agent. It can be administrated by an intraperitoneal injection (50-60 mg/kg b.w.) 20 min before surgery.
- Preparation of the working surgical area includes fur removal, a midline incision of the skin from pubis to the upper part of the chest, incision in the peritoneum on the midline, retraction of the gastrointestinal tract to the animal left.
- Cannulation of the bile duct. Two ligatures are placed around the bile duct. A cannula (PE 10-50) is inserted into the bile duct. Ligatures are tied.
- Cannulation of the portal vein. Two ligatures are placed on the portal vein. A small incision is made and a cannula (PE 240) filled with heparinized Krebs-Hnseleit buffer is introduced into the portal vein.
- In situ perfusion of the liver. Liver can be perfused in situ with 100 ml of preheated oxygenated Krebs-Henseleit buffer at a constant pressure of 12 cm in order to clear liver of blood.
- Perfusion experiment. Following its isolation, the liver is placed on the platform in the perfusion chamber. Liver can be perfused immediately or after several hours depending on the experimental protocol. The single-pass mode or a recirculation experiment could be performed. Drugs, toxins or other agents could be added into reservoir of the perfusion apparatus or various concentrations could be infused through the portal vein into the liver. Perfusate samples are taken as often as necessary. The bile can be collected into pre-weighed Eppendorf tubes and sampled. Hepatic tissue can also be sampled for determination of drug/toxin and metabolite concentrations.

During the experiment viability of the liver (appearance of the liver, perfusion flow, oxygen consumption, bile flow, redox and energy status, hepatocellular membrane integrity) and functional parameters of the liver (proteosynthetic function, sugar degradation, hepatobiliary excretory function, functional integrity of tight junctions, function and integrity of biliary epithelial cells) as well as histology evaluation of the liver can be investigated.

This method can be applied for studying: models of hepatic eliminations, in vitro and in vivo comparison of drug clearance, non linear drug elimination, proteins and drug clearance, enzyme induction studies, biosynthesis of drug metabolites by liver, etc.

DO6. ЛУМИНОМЕТРИЧНО ДОКАЗВАНЕ НА АКТИВНИ ФОРМИ НА КИСЛОРОДА В ЧОВЕШКИ ЕЯКУЛАТ

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Активните форми на кислорода – супероксиден анион, водороден пероксид и хидроксилен радикал се образуват нормално при унивалентна редукция на кислорода в организма, но при някои заболявания, както и при неблагоприятни условия на околната среда, съдържанието им може да се повиши. Доказано е, че повишените нива на активни форми на кислорода в еякулата водят до оксидативни изменения на липидите, белтъците и ДНК на сперматозоидите, респективно до стерилитет. Активните форми на кислорода в еякулата са резултат както от метаболизма на сперматозоидите, така и от метаболизма на левкоцитите. Доказано е обаче, че левкоцитите генерират 100 пъти повече активни форми на кислорода в сравнение със сперматозоидите. В семенната плазма се съдържат антиоксидантни ензими, които обезвреждат тези форми, но при обработката на семенния материал, предшестваща методите на асистирана репродукция (инвитро оплождане, изкуствена инсеминация), семенната плазма се отстранява. Ето защо в тези случаи, ако еякулатът съдържа левкоцити, ще се продуцира голям излишък от активни форми на кислорода, които няма да бъдат обезвредени.

Това може да се установи чрез луминометрично измерване нивото на активните форми на кислорода в еякулата. Към семенната проба се прибавят луминол и пероксидаза от хрян. Чрез този метод се измерва основно съдържанието на водороден пероксид. За да обезвреди водородния пероксид, пероксидазата от хрян се нуждае от редукционни еквиваленти, източник на които е луминол. Отдавайки редукционните си еквиваленти, луминол се трансформира в луминолов радикал (азасемихинон). Той от своя страна взаимодейства с O_2. Получават се азахинон и супероксиден радикал. Супероксидният радикал реагира с друга молекула луминолов радикал, в резултат на което се получава луминолов ендопероксид, излъчващ синя светлина с дължина на вълната 420*nm*. Резултатите се отчитат при 37^{o}C с помощта на луминометър. Ако има съмнения, че в семенната проба се съдържат левкоцити, към нея освен пероксидаза от хрян и луминол, се добавя и формил-метионил-левцил-фенилаланин. В случай, че луминисцентният сигнал рязко се усили, в еякулата наистина има левкоцити. Това е така, защото формил-метионил-левцил-фенилаланин ускорява метаболизма и следователно продукцията на реактивни форми на кислорода от левкоцитите, но не и от сперматозоидите (тъй като по повърхността на сперматозоидите няма рецептори за това съединение). От тук може да се направи извода, че за да са по-ефективни инвитро оплождането и изкуствената инсеминация, е необходимо левкоцитите да се отстранят от семенния материал, който ще се използва за тези процедури. Има и други, по-прости методи за доказване на левкоцити в еякулата. Най-бързият и евтин метод е оцветяването

по *Papanikolaou*, при което левкоцитите се оцветяват в синьо, а клетките на сперматогенезата – в розово. Друг метод за доказване на левкоцити, но само на пероксидаза съдържащи гранулоцити (преобладаващите левкоцити в еякулата), е тестът за пероксидазна активност. Към семенната проба се добавя орто-толуидин, който взаимодейства с водородния пероксид и под действието на пероксидаза се получава цветно съединение. Пероксидаза позитивните клетки се оцветяват в кафеникаво, а останалите не променят цвета си.

Друг метод за доказване на левкоцити е детекцията на общия за всички тях CD45-антиген. Така се установява не само наличието на гранулоцити, но също и на лимфоцити, макрофаги и моноцити. Изработват се моноклонални миши антитела срещу CD45, добавят се втори антимиши заешки антитела, към които се свързват миши антитела срещу алкална фосфатаза, предварително конюгирани с този ензим. Добавя се разтвор, съдържащ субстрат за фосфатазата и хромоген, който свързвайки се с продукта от ензимната реакция дава цветно съединение. Така клетките, по чиято повърхност има CD45 рецептори, се оцветяват.

След като е установено, че в еякулата, който ще се използва за оплождане ин витро или за изкуствена инсеминация, има голям брой левкоцити, е препоръчително те да бъдат отстранени, за да се улесни оплождането и да се намали честотата на спонтанните аборти. Отстраняването на левкоцитите може да стане с помощта на магнитни зрънца, към които са прикрепени моноклонални антитела срещу универсалния за левкоцитите CD45-антиген. Такива зрънца се прибавят към еякулата, а до стените на съответния съд се допира магнит, Магнитните зрънца се прилепват по стената на съда (към магнита), а еякулата се отлива в друг съд. Така, ако в семенната проба е имало левкоцити, те са останали прилепнали към стените на първия съд и дори да се отстрани семенната плазма (с цел инвитро оплождане, изкуствена инсеминация) няма да се стигне до оксидативен стрес, ще се улесни оплождането и ще се намали риска от спонтанни аборти, смърт на плода и раждане на деца с аномалии.

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SESSION E

YOUTH SEMINAR *"VIRUSES THAT SHAKE THE WORLD"*

EO1. HAPPY ANNIVERSARY, VIRUS Mc29 !

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Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

EO2. THE BULGARIAN STORY OF EPSTEIN BAR VIRUS

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EO3. THE GREAT VICTORY OVER SMALL POX

L. Dyakova

Institute of Neurobiology, Bulgarian Academy of Sciences

EO4. POLIOMIELITIS – IT IS NICE TO CLOSE THIS PAGE FOR EVER !

T. Zhivkova

Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences

EO5. MORBILLI VERSUS PEOPLE OR PEOPLE VERSUS MORBILLI

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[2]Institute of Experimental Morphology, Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences

EO6. THE TRUE STORY OF MARBURG AND EBOLA

L. Dyakova

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EO7. THE LESSONS OF SARS

B. Andonova-Lilova

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EO8. RABIES STILL "BITING PEOPLE"

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EO9. INFLUENZA VIRUS – THE VIRUS WITH A HISTORY !

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EO10. (UN)STABLE VIRUSES AND SOME OTHER GOSSIPS

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SESSION F

FO1. STUDY OF GLYCOSYLATION –A CHEMICAL APROACH FOR UNDERSTANDING THE BIOLOGICAL ACTIVITY IN VIRUS – CELL INTERACTION

R. Gavazova[1], D. Ivanov[1], K. Borissov[2], P. Genova-Kalou[3], S. Raleva[2], D. Dundarova[3] and R. Argirova[2*]

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The classic model of HIV-1 propagation describes cell-free virion binding to surface receptors on target cells and a consequent entry. However, HIV-1 can also spread by direct cell-to-cell transfer. For HIV-1 the relative contributions of cell-cell versus cell-free dissemination is yet unknown. DC-SIGN (Dendritic Cells Specific Intercellular Grabbing Nonintegrin) molecules promote efficient HIV infection of CD4+T cells either in cis- or in trans- by selective binding to gp120. Env differs in carbohydrate structures depending on virus producing cells so affecting binding and transmission through differential glycosylation. Studies demonstrated that trans-infection could also occur through DC-SIGN independent mechanism/s, but no molecule/s had been identified so far. The purpose of this study was to obtain the glycosylation patterns of HIV-1_{IIIB} infected/ uninfected hematopoietic cell lines (MT-2 and U937) differing by surface receptors. Preparative isoelectrofocusing (IEF) was performed for [^{14}C]-Glucosamine labeled cytosols of each cell line. Parallelly, virus attachment- and replication- assays were performed. MT-2 cells attached 80% of the virus and demonstrated the highest glycosylated peak in the acidic region (pH 4,73) known to be occupied by HIV-1 gp120. U937 exposed glycosylation peaks dispersed at wide pH range. U937 adsorbed 21,5% of the virus and further no replication occurred. Therefore, with their low adhesion efficiency and despite presence of CD4+ and CXCR4, in absence of DC-SIGN, U937 cells could only partially tether the virus. The partial binding of X4 virus to U937 cells could not exclude participation of still un-known U937 cell surface molecule(s) hampering X4 strains binding to other human (CD4+CXCR4+ and DC-SIGN -) cells.

FO2. EFFECT OF SOME INHIBITORS ON THE SIALYLTRANSFERASE ACTIVITY OF MCF-7 CELLS

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With the emergence of glycobiology, carbohydrates became known to play important roles in a range of biological processes such as cell-cell adhesion, tumor progression, immune function, and bacterial and viral infection [1]. Glycosyltransferases are enzymes responsible for the biosynthesis of complex oligosaccharides, glycoproteins, and glycolipids.

Glycosyltransferase inhibitors have attracted increasing interest not only as tools for fundamental studies of carbohydrate-mediated biological processes but also as the foundation for potential therapies [2].

Glycosyltransferases transfer sugar residues from activated donor (usually sugar nucleotides) to various glycosyl acceptors such as carbohydrates, peptides, and lipids [3]. Many glycosyltransferase inhibitors are designed based on donor or acceptor structures.
Inhibitors of the donor analog tipe are bound in the active site of the enzyme in competition with the natural glycosyl donor CMP-Neu5Ac, thus preventing or at least delaying glycosyl transfer. These inhibitors generally contain a cytidine part which was found to be essential for binding to the enzyme [4].

A systematic search for inhibitors of sialyltransferases has not been published up to now. Bernacki [5], Shah and Raghupathy [6] investigated the influence of nucleotides on the sialyltransferases activities of rat liver microsomes and on the sialyltransferases of rat and human sera. The inhibition of the α-2,6-sialyltransferase from rat liver, the α-2,3-sialyltransferase from porcine submandibular gland and of the galactosyltransferase from human milk were studied using monosaccharide-, nucleoside- and nucleotide-derivatives of their naturally occurring donor substrates [7].

The properties of MCF-7 cytidine 5'-monophosphate (CMP)-N-acetylneuraminic acid glycoprotein sialyltransferase were studied. Optimal enzyme activity was observed in an assay medium buffered with 0,5 M maleinate at pH 6,5 and using desialyzed fetuin as an acceptor. Enzyme inhibition was also observed in the presence of detergent Triton X-100. The reaction proceeds according to the following equation:

$$\text{CMP-sialic acid + acceptor} \longrightarrow \text{sialyl-acceptor + CMP}$$

The effects of nucleotides and nucleotide analogs on sialyltransferase were also studied. It was found that cytidine nucleotides, which act as competitive inhibitors, showed the greatest inhibitory activity. Like cytidine nucleotides its analog cytosine arabinofuranoside acted as competitive inhibitors but were not as effective in enzyme inhibition. A noncompetitive inhibition was observed for both adenosine nucleotides. The extent of inhibition for all nucleotides and their analogs increased with an increasing number of phosphate groups in the compound (71,4%, 63,5%, 39,1% inhibition for cytidine 5'-triphosphate, cytidine 5'-diiphosphate and cytidine 5'-monophosphate, respectively). These studies are important in assessing the activity of potential inhibitors of glycoconjugate biosynthesis and should aid in the design of more active and specific agents which may be useful in the chemotherapy of cancer.

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FO3. BRIEFLY ABOUT TUMOR ANTIGENS AND TUMOR MARKERS

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FO4. ROLE OF TAU PEPTIDE IN THE ONCOGENES AND TUMOR-SUPPRESSOR GENES BALANCED FUNCTIONAL ACTIVITY

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For establishment of the role of peptide Tau and Secretagogin in the oncogenes and tumor-suppressor genes balanced functional activity, malignant RIN-5F cells from rat insulinoma, containing additional copy of the Secretagogin gene, inserted by their transfection with recombinant gene construct *pGEX-1λT* (Amersham Pharmacia Biotech) of **Escherichia coli,** are used. These cells are *in vitro*-co-cultivated with malignant cell precursors, derived from laboratory-cultivated mouse embryonic stem cells in the presence of Doxyciclin (2 μg/ml - Sigma-Aldrich), probably by the known from literature sources activation of tumor-suppressor genes from *STAT*-family [2]. According to other literature findings, a calcium-dependent SCGN–TAU interaction, as well as co-appearance of both proteins is shown [1, 3, 4]. Furthermore, the so induced Secretagogin over-expression could exert protective function on the transfected Rin-5F cells [3]. These data could be confirmed by noticed by us differences in the degree of further myeloid differentiation of the derived precursor cells in their *in vitro*-co-cultivation with transfected with additional copy of the Secretagogin gene Rin-5F malignant rat insulinoma cells, in comparison with the results, obtained in their laboratory co-cultivation with human cervical carcinoma Hela cells.

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FP1. ANTIPROLIFERATIVE ACTIVITY OF NANOFIBERS CONTAINING QUATERNIZED CHITOSAN AND/OR DOXORUBICIN AGAINST MCF-7 HUMAN BREAST CARCINOMA CELL LINE

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Natural polymers are of interest as carriers of antitumor drugs because of their wide distribution, renewability and biocompatibility. Chitosan is one of the most widely used natural polymers. Chitosan is a polysaccharide, which is obtained by partial *N*-deacetylation of the natural polymer chitin. Chitosan has been reported to possess a set of valuable properties: very low toxicity, biodegradability, biocompatibility, intrinsic antibacterial properties, good antitumor activity and ability to influence the macrophage function [1,2,3]. The quaternized chitosan derivatives (QCh) exhibit higher activity towards bacteria and fungi, and have a broader spectrum of activity as compared to chitosan [4,5].

The antiproliferative activity of electrospun mats of poly(L-lactide-*co*-D,L-lactide) (coPLA) containing quaternized chitosan (QCh) and/or doxorubicin hydrochloride (DOX) was evaluated against the MCF-7 human breast carcinoma cell line. QCh- and DOX-containing nanofibrous mats possess good antiproliferative activity and decrease considerably the viability of the MCF-7 cells for the different periods of cell incubation as evidenced by the performed MTT assay. Fluorescent microscopy analyses and scanning electron microscopy observations revealed that apoptosis was one of the major mechanisms of MCF-7 cell death induced by the QCh and DOX-containing mats.

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FP2. *IN VITRO* IMMUNOBIOLOGICAL ACTIVITY OF AN ANTARCTIC STREPTOMYCES POLYSACCHARIDE

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Antarctic Streptomyces - *sp. 1010,* were obtained from the sea water samples (Livingston Island, Antarctica), during the Third Bulgarian Antarctic Scientific Expedition. A new extracellular polysaccharide has been isolated and purified from cultured broth of these cold-adapted, Gram-positive microorganisms (*psychrophiles*).

The aim of the present study is to examine the biochemical structure and *in vitro* immunobiological characteristics of an extracellular purified polysaccharide, produced by the Antarctic Streptomyces *sp. 1010* (ASMP).

Methods: The monosaccharide content of the purified ASMP has been examined by thin layer chromatography (TLC) and gas chromatography/mass spectrometry (GC/MS). The mitogenic and immunopotential properties of the ASMP have been studied *in vitro* - in short-term cultures of human peripheral blood mononuclear cells (hPBMCs) and mouse spleen lymphocytes (mSps) by ^{3}H-thymidine incorporation.

Results: The results showed that ASMP, is an amorphous, water-soluble white powder, which UV-spectra displays only end-absorption. The results obtained from TLC and GC/MS demonstrated that the purified ASMP is a heteropolysaccharide containing the following carbohydrates/monosaccharides: L-ramnose, D-ribose, L-arabinose, D-mannose, D-glucose, D-galactose, inositol and N-acetyl glucosamine, in ratio 1.5 : 1.5 : 4.5 : 5.2 : 3.2 : 2.0 : 4.0 : 2.2. The immunobiological study pointed that ASMP has a double lectin-like effect on the proliferative activity of hPBMCs similar to this of Con A on the lymphoid cells (preliminary T-lymphocytes) and to the effect of LPS on the mononuclear cells from monocyte-macrophage lineage. The mitogenic response of mSps (expressed as a proliferative index - PI) to ASMP, was also higher than PI in the negative, as well as in the positive controls (mSps, cultured in the presence of PHA, Con A and LPS).

Conclusion: From our data could be concluded and hypothesized that not only mouse spleen and human peripheral blood lymphocytes could be stimulated by ASMP. Mitogenic, phagocytic and colony-forming activity of human monocytes/macrophages could be changed (stimulated, regulated, modulated) as well in presence of ASMP in vitro and/or in vivo. In this aspect, the examined new Antarctic Streptomyces' heteropolyssacharide (ASMP) could be useful in the future as an immunomodulating biologically active substance and its extracellular production may contribute to the development of thermobiochemistry, immunomodulative drug therapy and immunopharmaceutical industry.

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FO5. SOME ADVANTAGES AND DISADVANTAGES OF STEM CELLS

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FP3. NEW MATERIALS FOR BONE IMPLANTS – SOME "TECHNICAL" PROBLEMS FROM BIOLOGICAL POINT OF VIEW …

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Bone disease is a serious health problem that directly impacts on the quality of life of sufferers. It has been predicted that the percentage of persons over 50 years of age affected by bone diseases will double by 2020. Bone and joint degenerative and inflammatory problems, bone fractures, low back pain, osteoporosis, scoliosis ant other musculoskeletal problems need to be solved by using permanent, temporary or biodegradable devices. Although autogenous bone grafts are still considered the gold standard for bone replacement and allogenic bone grafts are widely used, several biomaterials (metals, calcium phosphate ceramics, bioactive glasses, polymers, composites) have been developed with more or less clinical success. In orthopedic applications there is a significant need and demand for the development of a bone substitute that is bioactive and exhibits material properties (mechanical and surface) comparable with those of natural, heathly bone. The establishment of the appropriate in vitro model systems and experimental designs are required for the initial biocompatibility assessment of such biomaterials.

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FP4. COMBINED ADMINISTRATION OF DIFFERENT ASSAYS FOR THE ESTIMATION OF CELL VIABILITY

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The establishment of appropriate protocols for the assessment of potential cell toxicity of synthetic and natural compounds in one of the major challenges in modern biomedical investigations. There are varrious assay methods for evaluating the cytotoxic effects of chemicals on cultured cells. Thus, MTT (3-4,5-dimethylthiazol-2-(yl-2,5-diphenyl tetrazolium bromide)) test is based on the uptake and reduction by mitochondrial succinic hedydrogenase of the soluble yellow tetrasolium salt to an insoluble blue MTT formazan product. Neutral red uptake (NR) assay is based on the ability of this dye to accumulate in the lysosomes of uninjured cells whereas trypan blue dye exclusion method (TB) indicates damage to the plasma membrane. The crystal violet staining (CVS) is based on the growth rate reduction reflected by the colorimetric determination of the stained cells. The measurement of the total cell protein amount can give additional information for cell number and viability. The simultaneous performance of various cytotoxicity assays is required because of at least three reasons: i) Each of these assays has its own advantages and disadvantages; ii) The data for organelle damage can facilitate further identification of cell target(s) and drug mechanism(s) of action; iii) The most appropriate experimental design could be considered for each test system (drug xenobiotic /cell culture). The application of both – primary cultures and permanent cell lines can also improve the reliability of such investigations.

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FO6. MESENCHIMAL STEM CELLS AND BONE TISSUE ENGINEERING

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Mesenchimal stem cells (MSCs) have the potential to differentiate into various cell types including chondrocytes and osteoblasts and are expected to become candidates for bone regeneration therapy. Although this strategy seems very promising, several problems remain to be solved before any clinical applications. Better understanding of the molecular mechanisms directing the differentiation of MSCs could help us to properly manipulate these cells both in vivo and ex vivo to allow the regeneration of complex tissues and organs.

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