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CULTURE OF ANIMAL CELLS AS ALTERNATIVE APPROACH IN BIOMEDICAL RESEARCH

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The technique of isolation of living organs, tissues as well as separate cells from animal organism, followed by their maintaining and cultivation *in vitro* for relatively long periods, was introduced at the beginning of the 20th century. The reason was to obtain a relatively simple and well defined experimental system free of a wide spectrum of variable and unexpected factors that might arise in the whole organism, especially under stress during experimental procedures (for review see [1]).

Explosive expansion of this technique into multiple scientific fields is typical for the second half of the 20th century and recent years. Cell culture technology has been adopted into many routine applications in fundamental research, medicine, pharmacy as well as agriculture and industry. *In vitro* cultivation enables relative precise control of physical and chemical cell environment, such as pH, temperature, osmotic pressure, tension of O_2 and CO_2. Another advantage is the possibility of better economy and mechanization of experiments. The cell samples can be usually considered as more homogeneous than corresponding tissue samples taken *ex vivo*, so that the need for statistical analysis of variance of these virtually identical samples is reduced. In addition, tested reagents added to cell culture media have a direct access to cells. Consequently, lower amounts of reagents are required than for injections *in vivo*, and cells can be directly exposed to a wide range of better defined concentrations. Thus, screening tests with many variables and replicates are cheaper, and the legal, moral, and ethical questions of animal experimentation are avoided. New developments in multiwell plates and robotics also have introduced significant economies in time and scale. The development of histotypic and organotypic cultures (i.e., three-dimensional structures formed by reaggregation of cells and resembling original tissues or organs) also increases the accuracy of *in vivo* modeling (for review see [1]).

Limitations of cell culture technique are represented by production of relatively little amount of tissue, usually less than 10g. In addition, this technique must be carried out under strict aseptic and sterile conditions, because the danger of contamination with bacteria, mold, yeast, mycoplasma etc. is relatively high. In conventional cell culture systems, most cells still require supplementation of the medium with serum or other poorly defined constituents. Moreover, the cells are propagated on a two-dimensional support, which is less physiological that the substrate with three-dimensional geometry. Many specific cell-cell and cell-matrix interactions are lost which results to higher growth activation, phenotype and genome changes and loss of differentiated phenotype, e.g., modulation of vascular smooth muscle cells from contractile to synthetic phenotype [2], or changes known as transformation, immortalization or senescence of cultured cells. Thus, all these limitations imply high level of skill, highly specialized, time consuming and disciplined work, relatively expensive instruments, special tools, chemically defined culture media as well as laboratory space.

Despite of these limitations, cell cultures enabled exciting progress in understanding of molecular mechanisms controlling physiological cell functions, pathogenesis of various diseases as well as in development of therapeutic strategies. Cell culture technique facilitated genome analysis, studies on replication and transcription of DNA, transfer of cell nuclei, chromosomes and genes, research of energy and drug metabolism, studies on membrane transport of various ions and molecules, on ligand-receptor interactions, drug and hormone action, cell-cell and cell-matrix adhesion cell fusion, cell migration, proliferation and differentiation, metabolic cooperation, tests on cytotoxicity, mutagenesis, carcinogenesis, understanding of cell invasion and tumor metastasis and many other research fields [1, 3-7]. In clinical and pharmaceutical practice, cell cultures are used e.g. for chromosomal analysis of cells derived from the womb by amniocentesis in order to reveal genetic disorders in unborn children, determination of toxic effects of pharmaceutical compounds, production of antiviral vaccines, human growth hormone, insulin, interferon or antibodies for immunochemical research [1].

In our laboratory, cell cultures have been used for studies of the sex- and extracellular matrix-dependent growth activation of vascular smooth muscle cells, which plays an important role in the onset and development of vascular diseases [2, 8] or for development of strategies for therapy of brain tumors [9]. In addition, by experiments related to construction of artificial replacements of vascular and bone tissue, we tried to contribute to advanced interdisciplinary scientific field of "tissue engineering", which aims at creation of so-called "hybrid bioartificial tissues and organs". The artificial component of these constructs, usually based on synthetic polymers, is designed as a three-dimensional scaffold promoting controlled ingrowth and maturation of cells. It could be colonized under *in vitro* conditions with patient's own cells obtained by biopsy prior to the planned surgery, or even with stem cells guided to a certain differentiation pathway. In ideal case, the artificial support should be reorganized and resorbed by growing cells and gradually replaced by the newly formed extracellular matrix and differentiated cells, i.e. fully functional native tissue existing in the organ prior to damage (for review see [10]).

It can be concluded that cell culture technique is essential for progress of biology, medicine and related disciplines. Moreover, it could significantly reduce the use of experimental animals, especially in fields where this use may not be morally justifiable, e.g. in cosmetics development.

[1] Freshney R.I.: Culture of animal cells: a manual of basic techniques. Willey-Liss, New York, 2000.
[2] Bačáková L., Pellicciari C., Bottone M.G. Lisá V. Mareš V.: Histol. Histopathol. 16: 675-684, 2001.
[3] Antonická H., Floryk D., Klement P., Stratilová L., Hermanská J., Houšťková H., Kalous M., Drahota Z., Zeman J., Houštěk J.: Biochem. J. 342: 537-344, 1999.
[4] Bouřová L., Pešanová Z., Novotný J., Bengtsson T., Svoboda P.: Mol. Cell. Endocrinol. 167: 23-31, 2000.
[5] Jakubík J., Tuček S., El-Fakahany E.E.: J. Pharmacol. Exp. Ther. 308:105-110, 2004.
[6] Kopecký J., Flachs P., Bardová K., Brauner P., Pražák T., Šponarová J.: Ann. N. Y. Acad Sci. 967: 88-101, 2002.
[7] Krůšek J, Vyskočil F.: Biochim. Biophys. Acta 1646: 119-130, 2003.
[8] Bačáková L., Lisá V., Kubínová L., Wilhelm J., Novotná J., Eckhart A., Herget J: Virchows Arch. 440: 50-62, 2002.
[9] Mareš V, Krajčí D, Lisá V.: Physiol Res. 52: 629-635, 2003.
[10] Bačáková L., Filová E., Rypáček F., Švorčík V., Starý V.: Physiol. Res. 53: 5P, 2004.

POSSIBLE RELATION BETWEEN INTESTINAL INFLAMMATION AND JOINT DISEASE : AN EXPERIMENTAL STUDY IN MICE.

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There are findings suggesting a direct relationship between gut inflammation and joint diseases. Evidence is predominantly based on clinical observations in patients with inflammatory bowel disease (IBD). In our work we examined , on an animal model, a possible relation between intestinal inflammation – chronic colitis, and a joint disease, ankylosing enthesopathy (ANKENT), in mice.

ANKENT is a spontaneous joint disease affecting the ankle and/or tarsal joints of hind paws in mice and resembles serious human disease ankylosing spondylitis (AS) both in histopathology of affected tissues and factors influencing the disease development.

In our study we tested whether an intestinal inflammation can result in higher incidence of ANKENT. We induced chronic colitis in ANKENT-prone mice by administration of dextran sodium sulphate (DSS) in drinking water. The control mice received drinking water alone. Both groups were regularly checked for ANKENT by palpation.

Symptoms of colitis – diarrhea and rectal bleeding - were manifest after 5-7 days from the beginning of DSS administration and were confirmed by findings of inflammatory process in the colon in DSS-treated mice. ANKENT developed in 12.8% mice with chronic colitis and 13.6% mice in the control group. Thus no significant difference was found between males with and without chronic colitis and the occurrence of the joint disease in both group was typical for conventional conditions. It appears that generalized inflammatory process in gut itself cannot induce ANKENT.

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INFLUENCE OF RESTRICTION ON FYSIOLOGICAL PARAMETERS BY MALES AND FEMALES OF LABORATORY RAT (*RATTUS NORVEGICUS*).

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The response to dietary restriction by males and females was studied for 120 days using 24 laboratory rats stock Wistar. Twelve rats were fed *ad libitum* (6 males and 6 females) and the other twelve rats (6 males and 6 females) were dietary restricted (quality as well as time restriction was combined). Body weight gain, feed intake, relative weight of visceral organs, intensity of grow and biochemical parameters was followed at this study. There was found different body weight gain (87,6 g B.W.) between dietary restricted males and *ad libitum* fed males, on the other hand the response of DR females was not significant. In addition, the relative weight of liver, kidney, heart and also the lenght of shin were significantly different between the groups of DR males and females. However, all the biochemical parameters of the DR rats were evaluated within current fysiological standards for young rats, exclude of S-cholesterol which was slightly higher by restricted females. The result has brought consideration that males and females of rats may respond differently to the way of feeding. Moreover, dietary restriction looks to be more appropriate feeding technic for young males than females of laboratory rats.

THE FULLY ANTAGONIZED ANAESTHESIA IN THE LABORATORY ANIMALS

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The main goal of recent anaesthesia is the usage of receptor specific drugs with the possibility of antagonizing. Among them are: alfa$_2$ agonists, benzodiazepins and opioids. You can cause anaesthesia in rat, mouse, guinea – pig, hamster, chinchilla, rabbit and pigeon with the combination of these drugs. We proved in our study that fully antagonized anaesthesia can be reached also in macacus rhesus (Macaca mullata) without reasonable influence of cardiorespiratory parameters. The results can be incorporated into the new techniques in analgosedation in humans, in emergency situations, in the disaster medicine and in the non –lethal weapons development.

Key words: fully antagonized anaesthesia, laboratory animals, macacus rhesus.

SEASONALITY IN REPRODUCTION IN THE LABORATORY BREEDING OF RHESUS MACAQUE *(MACACA MULATTA)*

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Rhesus macaque (Macaca mulatta) is a monkey with the season reproductive activity in the free nature. The oestral cycle with ovulations was observed regularly between June and September in India, Nepal and China. The second mating season peak (but lower) was described a half year later. It means the birth season peak was usually between January and April (June to August respectively).
BioTest Konarovice has bred rhesus macaques since 1958. The monkeys are bred using the multi-male group principle, with 3-6 males and 5 to 15 females in a breeding group. An independent ground floor building, equipped with internal breeding cages and premises, serves for breeding. The animals can move freely from internal cages to external runs. Rhesus macaques breed regularly and rear their youngs in amount 30 – 50 babies per year. The total number of breeding monkeys is currently 237.
The seasonality in reproduction was observed between 1981 and 2003. In that period 636 living babies were born. The distribution of births totally and during calendar years was evaluated, and separately in different generations, origin of mothers and sequence of gravidity as well.
Births are not distributed equally during a calendar year; the time of births is period from the beginning of the year to the end of spring. This fact corresponds with the copulation time in the late summer. The distribution of births isn't stable; it is changing in time and it is moving farther in the year. It's possible to say the mating and birth seasons are longer in captive breeding than in the wild and the fertility time interval is longer then a calendar year.
There were not observed the significant differences in the reproduction seasonality in the mothers of a different origin. On the contrary, the mating and birth season is moved to the later period of the year in each generation. The same trend was observed from the first to following gravidities in average.
Our data show the trend of domestication and an effect of the diverse climate conditions in the Konárovice macaque colony. Decrease of seasonality in females during more than twenty years supports this hypothesis. However, the results of study show that it is possible to successfully breed rhesus macaques in Central Europe conditions.

CZECH CONSENSUS PLATFORM FOR ALTERNATIVES (CZECOPA) - ACTIVITIES ON THE EU LEVEL

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Czech Consensus Platform for Alternatives (CZECOPA) is a not-for-profit organization, with an official seat at the National Institute of Public Health in Prague. The platform was established on December 10, 2001 and registered on May 15, 2003.
Recent local activities were focused on:
- support for responsible authorities in the legislative process, e.g. an update of the Animal Protection Act No.246/1992 Coll. and implementation of the 7th amendment of the Cosmetics Directive,
- promotion of education and information, e.g. broad public information initiated by Animal Welfare Organizations, introduction of alternatives into educational programs at schools and universities,
- introduction of alternatives into the practice of testing laboratories in case of medical devices, cosmetics, chemicals and other products,
- presentations of research and routine testing results.

On the EU level CZECOPA became an active member of the European Consensus Platform on Alternatives (ECOPA), seated in Brussels, participating on the following activities:
- project CONAM funded from the FP6, focused on networking of European platforms and dissemination of information,
- nomination of renowned scientists and experts for ECOPA Science Initiative to promote further alternatives development, particularly in the field of molecular biology,
- promotion of young scientists participation in research projects in the field of alternatives,
- organization of an ECOPA meeting with participation of all European national platforms in June 2004 in the Czech Republic.

3D HUMAN SKIN MODELS - EVALUATION OF LOCAL SKIN TOLERANCE WITHOUT THE USE OF ANIMALS

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Irritant contact dermatitis is a continuing problem arising from occupational and daily life exposure to chemicals, cosmetics and other materials coming into contact with the human skin. The use of 3D human skin models has become an established standard alternative screening method for toxicological hazard identification of skin irritation, corrosivity, photorirritation and for safety testing. Primary cells in an organotypic structure of 3D skin models have a functional stratum corneum that allows to model substance bioavailability. Validation studies proved good concordance of results obtained with 3D skin models and results from animal experiments in many types of individual chemicals and finished products. The reconstituted tissue models can also be used to evaluate efficacy issues, e.g. sun protection, wound healing and anti-aging. Our experiments were focused on identification of irritation and phototoxicity in selected substances using skin model (EpiDerm) and fibroblast cell culture (3T3 Balb/c). The benefit of alternative tissue culture techniques for sequential approach in hazard evaluation is documented.

EXPERIMENTAL STUDY OF ANKYLOSING ENTHESOPATHY IN MICE

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Ankylosing spondylitis (AS) is a serious joint disease with as yet unknown pathogenesis but with well-recognized risk factors including gender (higher frequency in males), genetics (association with HLA-B27) and age (young adulthood); a possible role of environmental factors (e. g. microorganisms) has been suggested, too. AS causes back pain, stiffness and loss of functional capacity in the most serious cases.
Experimental models for AS mostly rely on HLA-B27 transgenic rodents. However, a spontaneously occurring joint disease – **ank**ylosing **ent**hesopathy (**ANKENT**) – exists in mice and resembles AS by its pathogenesis as well as risk factors. ANKENT is affecting the ankle joint(s) in H-2 congenic mouse strains with the C57BL background. Analysis of ANKENT frequency in B10 (H-2^b), B10.A (H-2^a) and B10.BR (H-2^k) mice suggests that the MHC class-associated gene(s) for ANKENT susceptibility map into the H-2K locus. In conventional B10.BR males the prevalence of ANKENT of 15 - 20 % has been observed. In general, the cleaner the better rule applies with 5% sick and no affected males under specific-pathogen-free and germ-free (GF) conditions, respectively.
To study ANKENT ethiology in terms of microbial triggers and immune mechanisms involved.
B10.BR GF mice were associated with cocktails of bacteria isolated from the intestine of diseased males. Pathogenicity of the cocktails was tested in immunodeficient mice. In ANKENT-triggering cocktails, bacteria have been characterized by 16S rDNA analysis.
Two cocktails of commensal, non-pathogenic bacteria have been identified that can induce ANKENT development. In mice co-associated with *Veillonella* and *Streptococcus*, a single case of ANKENT has been recorded so far. However, a cocktail consisting of < 20 anaerobic species was as effective as conventional flora in ANKENT triggering as 16 % of males became sick before one year of age. FISH analysis has revealed that *Bacteroides* species and members of the *Eubacterium rectale* group represent dominant components in the latter cocktail. Both 16S rDNA analysis of individual bacterial species and experiments with more limited cocktails in gnotobiotic mice are in progress in our laboratory.
In contrats to the concept on a triggering role of pathogens in autoimmune disease development we have proved that commensal bacteria play the most important role. More pronounced differences in lymphatic tissue composition were found between CV, GF and gnotobiotic mice then between healthy and diseased males in the same group. Although we are close to revealing the ANKENT triggering agent(s), the immunopathology of the disease remains enigmatic.
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EFFECT OF β- GLUCANS CONTENT ON TOTAL CHOLESTEROL IN LABORATORY RATS BLOOD

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The aim of the growth model experiment with laboratory rats is determination a food value of new spring barley lines - naked feet barleys KM 1771 (group 3), KM 1057 (4), naked food barleys KM 2082 (10), KM 2092 (11), KM 2062 (12), naked food *waxy* lines (Wabet x Washonubet (17), Wabet x Krona (18) and Wabet x Kompakt (19) and line Kompakt x Krona (9) and Nordus (20) as standards. Grain samples were taken to analyse content of organics nutrients in dry matter (g/kg), contain of brutto energy (MJ/kg), essencial amino acids (g/kg) and β-glucans (g/kg).
We determinated live weight (g) of the rats, feet conversion (g/g), average gain (g) and coefficients of degestibility (%). At the beginning and the end of the experiment was monitored contain of total cholesterol (mmol/l) in animals blood (*waxy* lines 17, 18, 19, and control group 3).
Highest body daily gain was observed in group 4 (9,19g), lowest in group 17 (7,89g). Consumption of feeding mixtures for body daily gain was lowest in group 4 (2,29g/g) and highest in group 17 (2,54g/g).
Dependence of β-glucans intussusception and contain of cholesterol in blood is expressed by correlation coefficient $R^2 = 0{,}5387$, $y = 0{,}0061x^2 - 0{,}419x + 8{,}9265$.
We believe that observation of hypo-cholesterolemic efect of β-glucans, that are contained in food barleys lines, brings results of prevention human civilizing disease. This lines are not right for monogastric animals feeding indeed.

Key words: barley, nutritive value, growth, organic nutrients, β-glucans