THE III[RD] MORAVIAN MORPHOLOGICAL DAY

The Faculty of Veterinary Medicine of the University of Veterinary and Pharmaceutical Sciences in Brno was the site of the III[rd] Moravian Morphological Day that took place on 19[th] September 2001. This already traditional meeting of Czech and Slovak morphologists was organised by the Department of Anatomy, Histology, and Embryology under the auspices of the Dean of the Faculty of Veterinary Medicine Prof. MVDr. M. Svoboda, CSc., in collaboration with the Czech Anatomical Society and the Brno branch of the Czechoslovac Biological Society. 44 morphologists, 30 from the Czech Republic and 14 from the Slovakia, took part in this event. The accompanying programme included presentations of four commercial companies (*OLYMPUS C&S Ltd., MIKRO Ltd.,* representative of *LEICA, ROCHE Ltd.* and the dairy co-operative *MLÉKARNA OLEŠNICE*).

The programme of the III[rd] Moravian Morphological Day, consisting of 13 lectures and 33 poster presentations, was divided into one forenoon and two afternoon sections and offered opportunities first and foremost to young scientists. The lectures and posters covered the respiratory and cardiovascular systems, central and peripheral nervous system, locomotor apparatus, splanchnology, skin and skin appendages, mucosae, embryology, and last but not least morphological methodology. Thanks to rich participation of Slovakian morphologists the event acquired international character. The all presented contributions have shown that the array of morphological methods and techniques has extended considerably including, in addition to the conventional macroscopic and microscopic procedures, also advanced methods of scanning electron microscopy, computer-assisted image processing, prospective plastination anatomical procedures, and molecular biological methods. The III[rd] Moravian Morphological Day showed that, like in other biomedical branches, the interest of the Czech and Slovak morphologists shifts progressively to subcellular structural entities.

Ivan Míšek

ABSTRACTS

K. Belej, E. Ochodnická, E. Fuseková, Ľ. Bošelová (Department of Histology and Embryology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia): **Functional morphology of the paranodium myelinated nerve fibre**.

Lateral loops of the myelin sheath are the only places, where the myelin sheath is connected directly to the axolemma. With help of horseradish peroxidase was found out the communication of the periaxonal space with the nodium area through triangle-like gaps between lateral loops. Paranodium is the place of active metabolic cooperation between the axon and the Schwann cell.

This is very important for the axon as well as for the Schwann cell. The position changes are connected with the change of the lateral loops slope and with them joined incompact lamellae of the myelin sheath. Above-mentioned area on the nerves is artificial.

By the retracted nerve fixation in situ we achieved the normal appearance of the loops and the incompact part of the myelin sheath lamellae. The terminal loops in the paranodium area were regular and connected to axolemma by 45–60° angle. By the nerve stretching during the fixation this area reacts with the longer loops in the direction of stretching and with the change of the slope of incompact lamellae.

According to our results obtained on released and stretched sciatic nerve of the guinea pig we can conclude that the paranodium and the whole nerve fibre are „constructed" very cleverly. The harder parts (covered by the myelin sheath) are interrupted by the flexible parts (paranodal incompact lamellae), which allow the mechanical movements as a result of contractions of surrounding muscles bending of joints and stretching of extremities.

Ľ. Bošelová, K. Belej, E. Fuseková, E. Ochodnická (Department of Histology and Embryology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia): **Axoplasmic organelles at nodes of Ranvier after the partial ischemia.**

Using electron microscopy we have studied the structure and distribution of axoplasmic organelles in myelinated nerve fibres of the sciatic nerve of the guinea pigs after partial ischemia with special reference to the paranode – node – paranode regions.

The ischemia was induced by ligation of the abdominal aorta. The time of survival of the animals was 2, 4 and 8 days. The distal parts of the sciatic nerves were prefixed in situ with 3 % glutaraldehyde; the Millonig's fixative was used to postfixation. Sections stained with uranylacetate and lead citrate were observed and taken by electron microscope Tesla BS 500.

Electron microscopy revealed dilatation and vacuolation of axonal organelles and their accumulation. Most organelles present in an axon were accumulated in the paranode–node-paranode regions. The organelles showed a mutual proximo–distal segregation. The dense lamellar and multivesicular bodies prevail in the distal part, while the vesiculo–tubular membranous organelles were dominant in the proximal part. The vesiculo–tubular organelles formed distinct longitudinal strands arranged into the middle part of the constricted axon segment. In animals 8–days after ligation have been found accumulations of electron dense glycogen–like granules that were in the lateral loops or near the axolemma.

M. Buchtová, F. Tichý, I. Putnová, V. Procházka[1] (Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic, [1]Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Prenatal development of palatal surface structures: a scanning electron microscopic study**.

The development of palatal surface structures was investigated in embryos and fetuses of Microtus subterraneus (Arvicolidae, Rodentia) using scanning electron microscopy. Examined individuals were taken between days 13 to 21 of the prenatal development, their crown-rump length (CRL) varied from 6.0 to 21.0 mm. The „staging and ageing" method according to Štěrba (Acta vet. Brno, 64: 83–89, 1995) was applied to the age estimation of embryos and fetuses under study.

The formation of microplicae on the surface of epithelial cells began at the early fetal period (CRL 13.0–16.0 mm). These superficial structures looked like irregularly arranged lines (microplicae) in rugal areas and mostly short microvilli-shaped structures in interrugal areas. During father development microplicae composed massive and dense nets.

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L. Burianová, J. Škarda, M. Mrazíková (Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Prague 10 – Uhříněves, Czech Republic): **Bioassay of androgens, antiandrogens, antiprogestins, antiestrogens and glucocorticoids by quantitative mammary histology.**

The weak structure–activity relationship of compounds having biological effects of steroid hormones precludes an accurate prediction of hormonal or antihormonal activities on the basis of chemical structure or radioimmunoassay, and thus suggests that there is not an alternative to special tests on living animals. The effective bioassay could be developed by combining some existing techniques with growth response of mammary glands in mice. In the present experiments mammary growth response, uterine, seminal vesicles and spleen weights in C3H/Di mice were used to assess hormone activities. All tested compounds were injected s.c. daily for 10 or 15 days. In males norethindrone acetate (NA) increased the area of mammary fat pad occupied by epithelial structures to more than 40% similarly as in animals treated with a combination of 17β-estradiol (E) plus progesterone (Prog). Antiestrogens and antiprogestins decreased the effect of E, Prog and NA. E plus Prog or NA stimulating mammary growth was inhibited by testosterone (T) in adult gonadectomized mice. However, the weight of uterine and seminal vesicles was increased by T. All these effects of T were decreased or disappeared by simultaneous application of antiandrogens–flutamide or chlormadinone acetate or casodex. Cyproterone acetate (CA) had antiandrogenic effects in uterus and seminal vesicles but not in the mammary gland. The effect of CA on mammary growth was inhibitory; CA decreased size of mammary lymph nodes and spleen weights. An inhibitory activity of CA cannot be explained by its androgenic activity but rather by its glucocorticoid activity as the size of mammary lymph nodes and spleen weights were reduced by CA as in animals treated with cortisol. Cortisol also decreased E or E + Prog stimulated mammary growth.

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J. Danko, F. Dorko[1] (Department of Anatomy and Histology, University of Veterinary Medicine, Košice, Slovakia, [1]Department of Anatomy, University of Pavol Jozef Šafárik, Košice, Slovakia): **Silicone and its use in anatomy.**

The described method enables the making of casting preparations, which fulfil the conditions of quality and visual teaching aids and thus can to a great extent help to be better oriented in topographic relations, structures and also in inter-generic differences. For making casting preparations authors used single composition of acetate silicone that is a paste-like substance of different colour shades. By vulcanise humidity, a thin superficial film is formed almost after 10 minutes and it becomes dry to the depth of 6 mm during 24 hours.

Fresh organs are suitable to make the forms. It is recommendable to fix the organs in their natural position by perfusion of 4% of formaldehyde solution or saline under pressure. After fixative washing the opening for the applicator must be prepared. As the silicone is relatively viscous, the entering the applicator deep into the empty space gives the best results. If the organ cavity is filled with it, the applicator must pull out slowly and gradually. The organ is then left in its physiological position. The method allows visualizing of individual parts of the cavity system with colours; different coloured substances are used in sequences to this purpose. The specimens are then macerated in potassium hydroxide solution (c = 3–5%) at the temperature of 70°C, washed carefully with tape water and leaved to dry.

E. Dorko[1]*, F. Dorko*[2]*, J. Danko*[3] ([1]Department of Physiology, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia, [2]Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia, [3]Department of Anatomy, University of Veterinary Medicine, Košice, Slovakia): **Candidal leukoplakias – histopathology.**

Oral leukoplakia is considered to be a precancerous lesion that occurs in the oral cavity as either a homogeneous variety that is usually asymptomatic or as non-homogeneous varieties that may be associated with burning and stinging sensations during food intake.

The aim of this study was to detect Candida hyphae present in bioptic samples originating from the buccal leukoplakias and to describe their histopathological characteristics. Biopsy specimens of buccal leukoplakias (n = 64) for histopathological study were fixed in neutral formalin and embedded in paraffin. Transverse 5 μm sections were stained with PAS, and Grocott's silver staining method.

The average thickness of the buccal epithelium was 330 μm (from 270 μm to 370 μm). Light microscopy of sections cut from the white patches revealed signs of keratosis, hyperkeratosis or hyperplasia (combined with parakeratosis, orthokeratosis) and other marked atypia. Some modified epithelium showed, apart from parakeratosis, acanthotic changes as well. The basal and parabasal cells were hyperplastic with increased mitotic figures and loss of nuclear palisading.

The inflammatory reaction was characterized by an early phase during which PMNs predominated: PMNs were observed in large numbers in dilated vessels and migrating from the lamina propria to accumulate in the superficial epithelial layers. The formation of subcorneal microabscesses was a regular feature of the early inflammation. Accumulations of mononuclear cells and PMNs were detected in the connective tissue. The inflammation, which sometimes reached the muscle layer in the cheeks, involved a large proportion of the oral mucosa. Sites of mycelial proliferation resulted in a massive recruitment of neutrophils in the superficial layers while mononuclear cells predominated in those sites where few or no mycelial elements remained. The skeletal muscle fascicles found adjacent to the infected epithelium showed striking degeneration and atrophy associated with a marked infiltration of chronic inflammatory cells.

E. Dorko[1], *F. Dorko*[2], *M. Zibrín*[3] ([1]Department of Physiology, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia, [2]Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia, [3]Department of Histology, Embryology and Electron Microscopy, University of Veterinary Medicine, Košice, Slovakia): **Histological study of the development of chronic candidiasis of the rat tongue.**

The purpose of the study was to determine the invasion of Candida albicans strains on rat tongue. Sixty female Sprague-Dawley rats, with an average weight of 200 g, were randomly assigned into twelve groups comprised of five animals each. Inoculation was accomplished by introducing 0.1 ml of the Candida suspension ($3x10^8$ cells/ml) into the oral cavity of a rat by means of a syringe with needle. After 1, 2 and 3 weeks, the animals were killed using ether anaesthesia and tongues were taken and examined. Histological sections were stained with haematoxylin-eosin, PAS and Grocott's staining procedure.

Microanatomical observation of the tongue sections revealed: (1) hyphal penetration through the keratin layer into the giant conical papillae and filiform papillae of dorsal tongue and these aggregated as microabscess within the keratin layer, (2) the mucosal lesions had a flattened surface morphology with prominent parakeratin production, (3) the connective tissue immediately subjacent to the lesional epithelium showed increased cellularity due to infiltration by chronic inflamatory cells, (4) the skeletal muscle fascicles immediately subjacent to the infected epithelium showed striking degeneration and atrophy.

F. Dorko, M. Kočišová, J. Danko[1], *E. Dorko, A. Jenča, E. Švický*[1] (Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia, [1]Department of Anatomy, University of Veterinary Medicine, Košice, Slovakia): **Innervation of the rat thymus in physiological and experimental conditions.**

The direct thiocholin method based on detection of ACHE in situ was used in study of the innervation of the thymus in the rat. Results obtained can be summarized as follows: The acetylcholinesterase (ACHE)- positive nerve fibres supply the thymus of the rat. They were found either in the interlobular septa as a part of the perivascular plexuses and or in the corticomedullary junction from where they extended into the organ parenchyma. The number and density of ACHE-positive nerves become to increase up to the 3rd month of age while a reduction in thymic parenchyma and ACHE-positive nerves was usually observed in 12-month-old rats.

Moreover, it has been shown that the thymus of surgically gonadectomized old rat regenerated on the 10th – 13th week after operation but in a chemical castrate (induced by long – time application of LHRH already on the 4th week of the hormone administration. The density of ACHE-positive nerve fibres was obviously higher in regenerated thymuses than in those of control animals. The ACHE- positive nerve fibres were prevailingly found in the medulla; only solitary nerve fibres are contained in the cortex and/or in periarterial and periarteriolar nerve plexuses.

A low density of ACHE-positive nerves was typical of thymuses of old rats.

L. Dubská[1], *E. Matalová*[2], *I. Míšek*[2,3] ([1]Department of Genetics and Molecular Biology, Faculty of Science, Masaryk University, Brno, Czech Republic, [2]Laboratory of Genetics and Embryology, Academy of Sciences CR, Brno, [3]Department. of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic): **Morphological criteria for identification of apoptosis in histological sections.**

Apoptosis was originally defined as a distinct mode of cell death on the basis of its characteristic ultrastructural features. More recently, internucleosomal DNA cleavage, membrane alteration, specific gene expression, and characteristic pathway activation have been used as markers of apoptosis. However, morphological changes still provide one of the most reliable criteria.

With increasing interest in apoptosis a lot of kits and assays to prove apoptotic cell death in cell populations and also in individual cells has emerged in the last ten years. However, their exploitation in histological sections is often limited, mainly in archived tissues. The paper reports on some easy available methods for apoptosis detection based mainly on morphological criteria with minimal request on special equipment so that useful in standard histological laboratories.

Three influencing factors were investigated with regards to reliability of methods under study: 1) type of fixation (formol, methacarn, Bouin's mixture) 2) type of tissue (different cell density – ovary, thymus), 3) duration of sample archiving (3 months to 10 years).

The effect of pre-treatment on false negative and positive findings, respectively, was tested when TUNEL test was used. TUNEL assay enables to evaluate both apoptotic criteria – biochemical and morphological, however, advantages and disadvantages of this method are often discussed mainly regards to results reliability in archived histological sections. Influence of five different pre-treatment procedures was tested in this study: 1) proteolytic digestion by proteinase K, 2) microwave irradiation (MW), 3) detergent permeabilisation (by TRITON X-100), 4) proteolytic digestion after MW, 5) detergent permeabilisation after proteinase K treatment.

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B. Erdösová, L. Hlávková, J. Procházková (Department of Histology and Embryology, Faculty of Medicine, Palacký University, Olomouc, Czech Republic): **Detection of CD68+ cells in connexion with the occurrence of apoptosis in the developing kidney of human embryos.**

The aim of the study was to detect macrophages in mesenchymal interstitium of the neogenous zone in the human metanephros. According to recent research on mice, lesser on samples of the human, cells responsible for clearing away apoptotic elements during development are not only non-professional phagocytes but also tissue-fixed macrophages.

Histologically normal kidneys were collected from 7 embryos and fetuses ranging from the 8th–21st week of IUD. Tissues were routinely processed and the standard indirect three-step immunohistochemical method using mouse monoclonal antibody NCL-CD68-KP1 to the antigen CD68 being localized on lysosome membranes was applied. Quantitative evaluation was carried out by means of graphic analysis system ACC 4.0 and labelling indices were determined (LI = the rate of CD68 positive cells and all the cells in observed area).

CD68 positive cells were quantified in the mesenchymal interstitium of the neogenous zone in metanephroi where apoptotic cells were found as referred in our previous papers. CD68+ macrophages appeared dispersal in all age groups under study. Values of LI reached the maximum in the 11th and 13th weeks of IUD, only single positive cells occurred between the18th and 21st weeks.

The presence of CD68 positive cells indicates their involvement in the clearance of apoptotic cells. However, this issue requires further investigation (e.g. double staining for the proof of apoptotic bodies engulfed by macrophages).

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E. Fuseková, K. Belej, Ľ. Bošelová, E. Ochodnická (Department of Histology and Embryology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia): **Complete and incomplete Schmidt-Lantermann incisures in the regenerated myelinated nerve fibres.**

In previous project authors have described the Schmidt-Lantermann incisures in isolated nerve fibres by light microscopy. As object of the present paper served internodia with incisures that are studied on longitudinal sections using the transmission electron microscope. The observed incisures were divided into two groups in respect to the fact whether they passed throughout the entire thickness of the myelin sheath (Group 1 – complete incisures) or only through its inner or outer part (Group 2- incomplete incisures).

The regenerating and very thin nerve fibres occurred in low density in the experimental animals and they often contained the incomplete myelin lamellae in a longer part of internodia. The Schmidt-Lantermann incisures have never been found in them.

Only the myelinated nerve fibres composed of more than 15 lamellae appeared to be suitable to description of the shape and orientation of Schmidt-Lantermann incisures.

N. Ghalib, P. Dubový (Department of Anatomy, Medical Faculty, Masaryk University, Brno, Czech Republic): **Formation of the minifascicles and their perineurial sheaths in the acellular nerve grafts prepared from motor and cutaneous nerves.**

Nerve fibres are usually organized into fascicles surrounded by the perineurium, a connective tissue sheath. The perineurium protects the fascicles from mechanical stimuli and acts as a diffusion barrier to substances with specific permeability to protect intrinsic ionic conditions for normal nerve function. Stimuli for formation of the minifascicles and their perineurium are not clear.

In the present study authors demonstrate the minifascicle formation and their perineurium of the motor axons that regenerate into acellular nerve grafts prepared from motor and cutaneous nerve segments. Sixteen female adult rats (Wistar) were used for experiments. The saphenous nerve, the femoral nerve, and its main motor branch were exposed on both sides under deep anaesthesia. A nerve segment (10 mm) was dissected from the motor and cutaneous branch of the femoral nerve and repeatedly treated by freezing and thawing. The nerve segment was applied instead of segment removed from the motor branches of the femoral nerve on both sides. Rats were euthanased after one (n = 8) and two (n = 8) months after the surgery. Following the period of time, samples of grafts were dissected, fixed and embedded in Durcupan ACM according to standard procedure for light and electron microscopy.

One month after surgery, the motor axons regenerated into the motor nerve graft were organized into minifascicles separated each other and from the blood vessels by the perineurium. The motor axons regenerating into the cutaneous nerve graft were not divided into minifascicles after the same time. Moreover, the axons were not separated from the blood vessels by the perineurial lamellae. Two months after surgery, number of perineurial layers increased around the minifascicles of motor axons regenerated into the motor graft, while a lamellar formation without typical structural features of the perineurium was observed in the cutaneous nerve graft.

Obtained results suggest that the formation of motor axon minifascies and their perineurium is stimulated by original extracellular cues present in motor grafts but rather not by intrinsic axonal signals.

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J. Hanzlová, J. Hemza[1] (Department of Anatomy, Medical Faculty, Masaryk University, Brno, Czech Republic, [1]Department of Neurosurgery, St. Anna's Faculty Hospital, Brno, Czech Republic): **The lamina cribrosa – the anatomical problem of the anterior skull base fossa.**

The present paper extends previous author's observations of the ethmoid lamina cribrosa by use histological examination. The authors have usually found arachnoidal villi accompanying nerve fibres into bony structures on ordinary stained sections. In addition, clusters of cavities within the lamina were observed; they are supposed to contain other arachnoidal villi pervading into the skull base. Junctions between the osseous tissue of the skull base and structures passing through it were usually very delicate in this area. The dura mater and the arachnoid merge into the loose connective tissue of the vessel adventitia and completely disappear. The external – periosteal sheet of the dura mater was not practically discernible and remaining layer of both contained multiple lacunae of the subarachnoidal space and olfactory cistern. The ratio between nerve canals and the rest of the lamina varied from 1:5 to 1:9.

Z. Haviarová, P. Weismann, D. Pavlíková[1]*, Š. Durdík*[2]*, P. Kováč*[3]*, V. Štvrtinová*[4]*, P. Mráz*[4] (Department of Anatomy, [1]Department of Histology and Embryology, [2]Institute of Surgical Clinic, [3]Institute of Forensic Medicine, [4]2nd Internal Clinic, Faculty of Medicine, Comenius University, Bratislava, Slovakia): **Presence of mast cell infiltration in varicose vein wall.**

Varicose veins of the lower limbs are abnormally dilated, tortuous and elongated veins. The exact cause of the vein dilatation has still not been established. Mast cells produce, store and release various kinds of vasoactive substances (histamine, tryptase, prostaglandins, leukotrienes, cytokines). Histamine enhances local vasopermeability and smooth muscle cell proliferation, leading to the intimal thickening. Tryptase can attribute to local vascular injury and subsequent weakness of the vascular wall causing varix formation.

10 varicose long saphenous vein samples taken by "stripping surgery" were compared with 10 "healthy" (non-dilated) vein samples. Toluidine blue was used for the differentiated histological staining of mast cells. Histological sections were scanned with colour CCD camera (Jay, Japan) connected with inverted microscope Olympus IMT-4 (Japan). Mast cells were counted manually and vein sections were analysed with morphometric software analysis (Soft Imaging Systems, GmbH Germany) by using PC Pentium 150.

It is believed that mast cells might play an important role in the development of the varices; study of factors released by the mast cells is in progress.

The work was supported by Grant No. 39/2001/UK of the Comenius University.

D. Horký, R. Autrata[1]*, L. Ilkovics, V. Procházka* (Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic [1]Institute of Scientific Instruments, Academy of Sciences CR, Brno, Czech Republic): **Electron microscopy of biological specimens at their natural state.**

The idea of observation of biological specimens at their natural state is connected with the very beginning of electron microscopy. In the 40 years of the past century different preparation techniques were tested with the intention to separate a specimen with high content of water from destructive influence of vacuum by convenient means. Satisfactory results were obtained as late as in the eighties.

The principle of environmental scanning electron microscopy is based on separation of high vacuum space from the specimen chamber by a differential chamber placed between the specimen chamber and high-vacuum part of the electron-optical column. The detection of secondary electrons and backscattered electrons at pressure about 1000 Pa is provided by ionisation or scintillation detector.

Observation of specimens in their natural state was carried out at the Department of Histology and Embryology of the Faculty of Medicine of Masaryk University in Brno. For the purposes of observation the AQUASEM environmental microscope, which is a product of the TESCAN firm in collaboration with the Institute of Scientific Instruments of the Academy of Sciences of the Czech Republic, was used.

The achieved results showed that the success of tissue preservation is very much dependent on type of biological material. Excellent results were obtained in such objects whose significant components were quinine and keratin. Satisfactory results gave also specimens of plant origin. Examination of the softer water-saturated tissue was however substandard. One of the causes consists in very difficult control of a balanced state of saturated vapours. If this is not possible to be kept at defined bounds, the specimen either loses water and thus its surface is deformed, or, on the contrary, it absorbs water and thus its surface structure is covered with water. Partial solution of this problem in case of wet soft tissues is their freezing by the Peltier's element.

L. Ilkovics, D. Horký, V. Procházka (Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **The new scanning electron microscope VEGA UniVac TS 5140.**

The VEGA UniVac TS is a compact fully computer-controlled scanning electron microscope intended for work in both high-vacuum and low-vacuum modes. The unique design of the electron optic and high-quality detection systems allows resolution at 3.5/4 nm, which is fully comparable with that of SEM offered by companies with world-wide reputation. All its functions are controlled with mouse, trackball, and keyboard. All data on topical configuration and state of all parameters are currently displayed on the monitor. Putting into operation does not take more than 5 minutes. Automatic control of the vacuum system and electronically controlled setting and centring of the optical system have replaced almost all mechanical control elements. The control of software, such as jet setting, optimisation of track size for the given magnification, optimisation of scanning speed, contrast and brightness setting, focussing, or astigmatism correction, is not overly demanding.

Author's attention was focused on the possibility to utilise its top parameters in studies of biological objects. Samples, prepared by the conventional procedure of fixation, dehydration, drying in CPD, and coating, were viewed in the high-vacuum mode. The obtained results showed surprisingly good. Recently, the low-vacuum mode that allows viewing of nonconductive samples at pressures within the range from 6 to 600 Pa, is preferred. The benefit of the mode consists in the fact that special processing of some biological samples can be avoided. The success of the work in the low-vacuum mode closely depends on sample characteristics. Most important is the ability to retain the original shape at reduced pressure for a sufficient period. For most biological objects, particularly soft materials, the resolution of the microscope in the low-pressure mode cannot be fully utilised. Fine surface structures soon lose their shapes and disappear from the moderately over-radiated nonconductive surface.

P. Kalanin, J. Danko[1] (General Physician Ambulance, Košice, Slovakia, [1]Department of Anatomy and Histology, University of Veterinary Medicine, Košice, Slovakia): **Protection by VIP against tissue injury in neuronal cells.**

During the past several years it has been reported that VIP prevents tissue injury and improves survival in a variety of experimental models of the acute distress syndrome (Šmajda and Jalč, Psychiatrie /Suppl./ 2: 133–134, 2001). In brain and neuronal cells:

1) The VIP promotes survival and differentiation in several neuronal cell types,

2) The VIP protects neuronal cells against death due to electric impulse blockade with tetrodoxin, NGF deprivation, or HIV glycoprotein 120 toxicity,

3) The specific VIP antagonist induces microcephaly in the fetal mouse,

4) The VIP acting through the cyclic AMP-independent mechanism protects the mouse developing brain against exocytotoxic cell death induced by the glutamatergic agonist ibotenate (Gressens et al., J. Clin. Invest., 100: 390–397, 1997),

5) VIP protects cortical neuronal cells against cell death, and PC-12 cells against NMDA-mediated glutamate toxicity.

The obtained findings seem to be important for understanding the nature of neurodegeneration and neuronal cells protection by VIP.

A. Kiss (Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia): **Effect of alpha 1-adrenergic receptor stimulation or inhibition on the function of corticoliberinergic neurons of the rat hypothalamus.**

The role of the stress and intracerebroventricular (i.c.v.) administration of a specific alpha 1-adrenergic agonist–methoxamine or antagonist-prazosin on the corticoliberin (CRH) secretion from the median eminence (ME) was studied in colchicine treated rats using avidin-biotin-peroxidase immunohistochemistry. In one set of experiments marked decrease in CRH immunoreactivity from the ME was observed 6 hours after i.c.v. injection of colchicine and subsequent 2 hr of immobilization stress or i.c.v. injection of methoxamine (100 μg) that indicates release of the neuropeptide into the portal circulation. This release was partially prevented by prazosin i.c.v. injection. In other experimental model, the animals were sacrificed 48 hr after colchicine injection, i.e., in the time when colchicine acts as a stressor a causes marked depletion of CRH from the ME. This stress effect of colchicine was also reduced by prazosin treatment (i.c.v. injection followed by continuous minipump infusion) indicating that alpha 1-adrenergic stimulation contributes to the action of colchicine. In addition, the typical increase of CRH-perikaryonic immunoreactivity observed 48 hr after colchicine treatment was also markedly reduced by prazosin suggesting that alpha 1-adrenergic stimulation is involved not only in the CRH release but also in its synthesis at the level of the hypothalamic paraventricular nucleus.

The results obtained indicate that central alpha 1-adrenergic mechanisms probably play very important role in the regulation of hypothalamic CRH secretion during stress.

V. Konrádová, J. Uhlík, L. Vajner, J. Zocová[1] (Department of Histology and Embryology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic, [1]Department of Applied Mathematics and Computer Science, Faculty of Science, Charles University, Prague, Czech Republic): **Ultrastructure of tracheal epithelium in rabbits exposed to normobaric hypoxia.**

The ultrastructure of the tracheal epithelium was studied in rabbits exposed for 96 hours to normobaric hypoxia in a chamber containing an atmosphere with 10% of O_2, humidity 100% and temperature 23°C. Rabbits' tracheae were lined with a slightly altered pseudostratified columnar ciliated epithelium.

The target cells for the function of hypoxia were the goblet cells. They were over-stimulated and their distribution in the epithelium was significantly ($\alpha = 0.01$) changed compared with controls. 60 ± 5% of the goblet cells were arranged in groups forming thus tiny intraepithelial mucous glands. 13 ± 2% of secretory elements discharged secretion. The mechanism of mucus evacuation was accelerated and signs of apocrine type of secretion were encountered. Only 10% of cells contained in their cytoplasm isolated small secretory granules of various electron densities. The exhausted degenerated goblet cells amounted to 7 ± 2%. The percentages of non-stimulated, mucus discharging and degenerated goblet cells differed significantly ($\alpha = 0.01$) from those found in the epithelium of healthy control rabbits.

The ciliated cells revealed only mild signs of pathological alteration. In their cytoplasm, an increased number of tiny vacuoles and lysosomes, the dilated cisternae of granular endoplasmic reticulum and of Golgi complex were sometimes found. The ciliary border was slightly impaired. A significant ($\alpha = 0.01$) decrease in number of kinocilia /μm^2 to 7.1 ± 0.5 was accompanied by a significant ($\alpha = 0.01$) increase in the number of altered cilia to 2.8 ± 1.2%. Among the altered kinocilia, the pathological cilia were the most numerous. In the area of the ciliary border, tiny clumps of condensed mucus represented signs of impairment of the self-cleaning ability of the airway epithelium. According to our classification, the injury to the tracheal epithelium due to the normobaric hypoxia was mild to moderate.

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M. Krajničáková, J. Danko[1]*, I. Maraček* (Research Institute of Veterinary Medicine, Košice, Slovakia, [1]Department of Anatomy and Histology, University of Veterinary Medicine, Košice, Slovakia): **Relation between uterine and ovary weight and ovary hormone level after treating the ewe in early puerperium.**

The aim of the paper was to observe the dynamic of 17-beta oestradiol (E_2) and progesterone (P_4) level and to compare it with the uterine and ovary weight changes in milking ewes during the puerperium and in animals repeatedly treated with carbetocin (*Depotocin*, Léčiva). In addition, the correlation analysis of obtained results was applied.

Thirty milking Merino ewes that had lambed in the first ten days of February were put in experiments. Animals of the control group (n = 15) were treated only with saline whereas 0.07 mg of *carbetocin i.m.* and *s.c.* at the 24 and 72 h after lambing was administered to animals of the experimental group. The level of E_2 and P_4 was determined in blood withdrawn from the jugular vein at the 36 h and on the 4th, 7th, 14th, 17th, 21st, 25th and 34th days after lambing. The RIA-test-ESTRA and RIA-test-PROG kits (*ÚRVJT-Košice*) were used for this purpose. Both the control and the experimental group animals were slaughtered in different time (n = 3) at the 36 h and on the 7th, 17th, 25th and 34th days after lambing. The weight of the uterus and of pregnant and non-pregnant horns with the respective ovary was estimated.

The evaluation of E_2 level revealed an inter-group significance of differences ($P < 0.05$ or $P < 0.01$) at the 36 h and on the 7th day after lambing. With the increased P_4 level in the experimental group significance of inter-group differences were found on the 17th day after lambing ($P < 0.05$). In experimental animals, the weight of the uterus body and the horn on the 17th and 34th postpartum days were increased if compared to those of the control group. The weight of the ovary constantly increased in both pregnant and non-pregnant horns of Depotocin-treated animals but no statistically significant differences between both groups were observed. Significant correlation was found in the E_2: G- relation (non-pregnant horn) of control animals on the 7th day ($P < 0.05$).

It is supposed that the increased oestrogen production in the ovarian follicles during recruition and selection shows a feedback effect on the adenopituitary gland with subsequent influence on the uterus, creating thus presuppositions for cyclic functioning of the ovaries.

D. Kylarová, J. Procházková, B. Erdösová, P. Havelka, P. Vranka[1]*, V. Lichnovský* (Department of Histology and Embryology, Faculty of Medicine, Palacký University, Olomouc, Czech Republic, [1]Department of Zoology and Ecology, Faculty of Science, Masaryk University, Brno, Czech Republic): **Statistic comparison of evaluation of embryonic tissues stained for apoptosis and its regulating proteins using Image Analysing Systems ACC 4.0 and LUCIA M 3.5.**

The task of the work was to confront two systems for image analysis, LUCIA M 3.5 (Laboratory Imaging) and ACC Image Structure and Object Analyser 4.0 (SOFO) and to determine whether they produce comparable results (apoptotic or labelling indices). Regarding this comparison it was performed the quantitative assessment of the level of apoptosis detected by three different techniques as well as regulatory proteins in selected tissues of human embryos.

Organs undergoing extensive rearrangement during morphogenesis – the metanephros, anlage of limbs, and axial skeleton and primitive intestine of 58 routinely processed embryos (i.e. fixed in methacarn or 4% buffered formaldehyde and embedded in paraffin) served as experimental model. The TUNEL, Apostain, and Lamin B techniques (using AP-NBT/BCIP for visualisation) were used to visualization of apoptosis. Apoptosis regulating proteins Bcl-2 and p53 were stained by standard three-step immunohistochemistry based on the POD-DAB system.

Statistic comparison involved the set of tests – mainly ANOVA and the two-tailed Student's t-test for independent arrangement. T-tests confirmed in most of samples (42 from 52) that there is no significant difference between data obtained by both image analysing systems. Regarding the structure, the most of parallel results were present in structures of developing axial skeleton and intestine. The smallest accordance was demonstrated in structures of metanephros, which were often changed by autolysis, but results obtained in both analysing systems changed in dependence of structure similarly. In the case of immunohistochemical detection of Bcl–2 and p53 proteins

accordance in the all structures under study was confirmed. ANOVA test proved that variations between both analysing systems are statistical important; authors decided data obtained by LUCIA M do not use in next studies.

The study was supported by Grant No: 304/01/0485 of the Grant Agency of the Czech Republic.

S. Laichman (Department of Normal Anatomy, Faculty of Medicine, Palacký University, Olomouc, Czech Republic): **Transposition possibilities of pelvic muscles.**

The most part of the sphincter mechanisms of the distal bowel are formed by striated musculature. Replacement of them by a striated muscle tissue is a logical consequence of the fact. Although practically the all muscles of the buttocks and the inner aspect of the thigh have been used for reconstruction of sphincters, no substantial success has been achieved in solving the problems of faecal incontinence. Apart from the first-rate problem of the functional importance of the striated muscular coat of the sphincters in the process of continence, the main cause of failure is the disturbed nutrition of the muscle transported into the new surroundings around the anus. Therefore, the familiar question has been raised anew, viz. which of possible muscles fulfils the criteria of its transposition in the function of the anal neosphincter.

In total 270 muscles have been used and 45 specimens of each muscle tested in this study. Author's attention was centred to the following muscles: the sartorius muscle (ventral group of femoral muscles), the gracilis muscle (adductors of the thigh), the semimembranous and semitendinous muscle (dorsal group of femoral muscles), the greatest gluteal muscle (external hip muscles) and the internal obturator muscle (pelvitrochanteric muscles). The preparations were collected from the autopsy material of the Department of Normal Anatomy, Faculty of Medicine of the Palacký University in Olomouc. The basic criterion for each muscle studied was the preservation of the neurovascular stalk in the transported muscle. Assessments were made of (1) the length variants of individual muscles, (2) the variants of the blood and nerve supply of the muscle in situ.

It was concluded that the muscle most suitable for transposition and reconstruction of the anal neosphincter is the internal obturator muscle.

A. Marciniaková, P. Havelka, V. Lichnovský (Department of Histology and Embryology, Faculty of Medicine, Palacký University, Olomouc, Czech Republic): **The role of programmed cell death and the genes involved in regulation of programmed cell death in differentiation of human embryo myocard.**

During the last 10 years the research attention was focused on defining basic principles of apoptosis, the process complementary to proliferation, even if not antagonistic to it. The apoptosis or programmed cell death assumes that genetic equipment of each cell involves information for system of molecules whose co-operation results in removal of an undesirable cell.

The aim of paper was to compare sensitivity of methods for detection of apoptosis and expression of its regulatory proteins and to detect macrophages in embryonic myocard by immunohistochemistry. 9 human embryos and foetuses aged 6 to 22 weeks were examined. Apoptotic cells were detected by TUNEL, APOSTAIN, and LAMIN B techniques; the presence of proliferation proteins Ki-67 and PCNA and apoptosis regulatory proteins p53 and BCL-2 were determined by a standard three-step immunohistochemical assay. The same technique was used for the demonstration of macrophages by the antibody NCL-CD68-KP1 against antigen CD68 in lysosomal membranes. In addition, the labelling and the apoptotic indices (LI, AI) were assessed.

The expression of PCNA showed an increase in two periods (weeks 8 to 11, 18 to 20), especially in the compact layer (LI 0.2–0.3). The Ki-67 expression was always increased well but LI achieved lower values (0.02–0.17). In general, in all stages the LI was very low in case of proteins p53 and BCL-2. The AI showed quite high values (0.55), especially in spongy layer of ventricles in individuals aged 9 weeks. In foetuses aged 14 and 18 weeks the AI was always higher in the spongy (0.53) than in the compact layer (0.35). The high level of apoptosis and expression of proliferation proteins are in a good accordance with the fundamental reconstruction of wall in the developing human myocard.

Cells containing antigen CD68 in lysosomal membranes were constantly detected. The cells are supposed to collaborate in removal of apoptotic cells.

The study was supported by Grant No. 1470/2001and Grant No. 151100001of the Ministry of Education, Youth and Sport of the Czech Republic.

M. Martiniaková, M. Vondráková (Department of Zoology and Anthropology, Faculty of Science, Constantine the Philosopher University, Nitra, Slovakia): **Ground preparations drawing up methodics of anthropologic skeletal material from Dubovany's cemetery.**

Only a few papers dealing with microscopic structure of human fossil bones have been published in last years. The aim of present paper was to draw up brief scope of methods used in preparation of ground sections of fossil bones and to compare results obtained by their use.

The anthropological skeletal material, collected in Dubovany's cemetery (Trnava district), came from the 8th–9th century (the Early Middle Ages). The locality was object of archaeological studies in 1992–1995 and in 1998. Findings on skeletal remains of 35 humans from 36 graves obtained by M. Vondrakova have not been published up to date.

Authors verified four modifications of three methods depending on material conditions. The right femur bone of 40–50 years old man (the grave No. 20/1995) was ground in diaphyse section and 12 ground preparations were utilized in description of its several quantitative characteristics.

P. Matulová, K. Witter, I. Míšek (Joint Department of Laboratory of Genetics and Embryology, Academy of Sciences CR, Brno, Czech Republic and Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic): **Proliferating cell populations during prenatal development of tooth anlagen of the field vole (Microtus agrestis, Rodentia).**

Immunohistochemical detection of endogenous proliferation marker PCNA (Proliferating Cell Nuclear Antigen) is used to determine proliferation activity of tissues. In comparison with other detection ways of proliferating cell populations, the method has the advantage that it does not require experiments and can be applied without any problems also to collection material. Authors applied immunostaining on single sections of embryos and fetuses and preliminary found that a large part of cells of the developing dentition showed positive immunoreactivity with the anti-PCNA-antibody. Fully differentiated cells, cells of rudimental tooth buds and cells of the inner enamel epithelium in the enamel-knot-region were PCNA-negative. Non-proliferating cell populations are larger than regions with higher apoptotic activity (e.g. enamel knots).

The location of immunopositive cells was specific from the temporal point of view and along the antero-posterior axis of the individual primordia of functional and rudimental teeth. Assessing of gene expression and the level of his product in the developing dentition seems to be nearly impossible without precise knowledge on temporo-spatial pattern of development. A study dealing with immunostaining of serial sections of tooth anlagen is in progress.

S. Mazánek, H. Černý[1] (Zoo Brno, Brno, Czech Republic, [1]Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic): **Comparative microscopic anatomy of the digestive tract mucous membrane in parrots, domestic fowl and pigeon.**

It was the aim of present study to compare the structure of the digestive tract mucous membrane in parrots and some domestic birds.

Digestive tracts of 18 parrots, 6 domestic fowls, and 4 pigeons were collected and examined using transverse and longitudinal section cut at various levels (cervical oesophagus, thoracic oesophagus, junction between the oesophagus and proventriculus, proventriculus, pars intermedia gastris, ventriculus, small intestine) by optical microscopy. Samples were fixed in 10 % neutral formalin and Karnovsky solution (pH 7.2), embedded in paraffin and stained according Mallory's and Masson-Goldner's procedures.

Authors found that the mucous membrane of the cervical oesophagus contained no tubular glands both in parrots and pigeons. In all species studied the mucous membrane of the thoracic oesophagus contained numerous *glandulae esophageales*. The stomach of examined animals was of the type 2. *Glandulae proventriculares* occupied a considerable portion of the proventricular wall. Their lobules were separated by fibrous septa and, except parrots, were organized in several rows. A hard and chemically resistant *cuticula gastris* originating from glandular secretion of the gizzard and pars pylorica covered the inner aspect of the ventriculus.

Concerning the intestinal mucous membrane, in parrots it projected in high enteric villi and its epithelium contained more unicellular mucous glands among the enterocytes than in the domestic fowl and pigeon.

The fibrous layer of the mucous membrane, the *lamina propria mucosae*, does not contain lymphoreticular tissue.

M. Miklošová (Department of Anatomy, Faculty of Medicine, University of Pavol Jozef Šafárik, Košice, Slovakia): **Our experiences with the standard silicone S 10 plastination technique.**

In anatomy and other morphological disciplines play the illustration teaching tools great importance. The anatomy uses anatomical specimens that are prepared by various ways. Plastination developed by Gunther von Hagens (1987) is one of the latest invented methods of conservation of the biological material.

Plastination is a process during which water and fat from specimens are replaced by curable polymers (silicone, epoxy, polyester), which are subsequently hardened, resulting in dry, odourless and durable specimens. The plastinated specimen is dry to touch, odourless and non-toxic, yet it maintains its original shape and in many cases, is reasonably close in colour and consistency. It resists deterioration and can be stored at room temperature indefinitely.

In this work the silicone plastination method S 10 was presented to prepare the different organs (heart, stomach, small intestine, large intestine, lungs etc.) of human body. Plastinated specimens showed perfect quality. Experiments with plastination by using other methods such as P 35 and E 12 are planned in the next future.

J. Mokrý, J. Karbanová, S. Filip[1]*, J. Vávrová*[2]*, M. Bláha*[3] (Department of Histology and Embryology, Faculty of Medicine, Charles University, Hradec Králové, Czech Republic, [1]Department of Oncology and Radiotherapy, University Hospital, Hradec Králové, Czech Republic, [2]Department of Radiobiology and Immunology, Purkyně Military Medical Academy, Hradec Králové, Czech Republic, [3]Department of Haematology, University Hospital, Hradec Králové, Czech Republic): **Generation of non-neural cells by neural stem cells.**

Stem cells (SCs) are the first cell types produced in the course of ontogeny. They represent very primitive cells endowed with ability to give rise to specialised cells that form multicellular organisms. Organ-specific SCs were believed to generate only few types of cells. However, recent data suggest that multipotent SCs have broader and perhaps unrestricted potential for generation of cells that can participate in formation of a variety of organs irrespective of their different origins.

To test developmental potential of organ-specific stem cells, authors used neural SCs isolated from the forebrain of E14 Balb/c fetuses in the form of neurospheres. Spontaneous differentiation of multipotent neural SCs (induced by neural transplantation or by replacement of growth factors from culture medium with serum) resulted in production of neuronal and glial cells.

To find out whether neural SCs can be stimulated to produce non-neural cell types, neural SCs were exposed to different experimental conditions. For in vivo tests, neural SCs were injected into sublethally irradiated recipient Balb/c mice. Counting CFU-GM colonies yielded from the spleen and bone marrow of irradiated animals confirmed that mice injected with neural SCs revealed increased haematopoiesis when compared with untreated irradiated animals. In in vitro experiments, neural SCs labelled with bacterial beta-galactosidase were mixed with embryonic stem cells in hanging drops to form chimerical embryoid bodies. Cystic embryoid bodies were harvested after 9–25 days, stained with X-Gal (to identify labelled neural SCs) and processed for histology. Our

results indicate that neural SCs exhibit features of remarkable plasticity and they can produce non-neural cell types including haematopoietic and endodermal cells. Chimerical embryoid bodies represent a novel in vitro model for testing stem cell plasticity.

The work was supported by Grant No. 304/00/0338 of the Grant Agency of the Czech Republic.

M. Mrazíková, L. Burianová, J. Škarda (Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Prague 10 – Uhříněves, Czech Republic): **Bioassay of estrogenic and progestantional activities of compounds on the mouse mammary gland in vivo.**

Estrogens and progesterone are essential for normal mammary development. The mammary gland of male mice is equipotent with the female in its response to a combination of estradiol plus progesterone. This makes mammary gland a suitable system for the routine evaluation of natural and man-made endocrine disrupting chemicals.

In our experiments the mice of C3H strain were daily-administered hormones for 10 days in females and for 15 days in males. In 17β estradiol (E) treated females, mammary glands showed a progressive lengthening and branching of duct system from a dose 0.001 μg/d. The maximum effective dose of E was 0.01–0,1 μg/d. However, high dose of E showed the opposite effect. Uterine weights were increased from a dose 0.01 μg/d E. Progesterone (Prog) alone increased mammary duct growth and branching. Uterine weight was not affected by Prog in young intact animals but in adult OV-X ones was increased. Mammary growth in E treated males was increased from dose 0.01 μg/d and the maximum effective dose was 0.1 μg/d. High dose of E 10 μg/d decreased mammary growth. Prog alone had no effect on mammary growth in adult castrated males. E acted synergistically with Prog to produce a higher mammary growth rate than that observed in animals treated with E alone.

The seminal vesicles weights were decreased both by E alone and Prog alone. The effects of diethylstilbestrol (DES) in males were similar to that of E; genistein (G) alone had no effect on mammary growth, however in combination with Prog mammary growth was stimulated by G from dose 10 μg/d. The sensitivity of E assay on female mammary gland was at least 10 x higher than that on the uterus.

The specificity of the assay was higher in the male mammary gland than that in female.

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E. Ochodnická, M. Ochodnický[1], *K. Belej, E. Fuseková, Ľ. Bošelová* (Department of Histology and Embryology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia, [1]Department of Internal Medicine I, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia): **Ultrastructural changes in axons of myelinated nerve fibers of peripheral nerve in experimental streptozotocin diabetes mellitus.**

The aim of the study was further to investigate ultrastructural changes of axons in peripheral nerves in early stages of experimental diabetes mellitus.

17 week aged male Wistar rats (Rattus norvegicus var. alba) with an average starting weight 352 g were used in the study. Diabetes mellitus in experimental animals was induced by an intraperitoneal injection of streptozotocin (40 mg/kg body weight, *Zanosar/ Upjohn*). Samples of common peroneal nerve from diabetic rats (4, 8 and 14 weeks after induction of diabetes mellitus) and age–matched control animals were removed under diathylether anaesthesia, processed and examined by standard transmission electron microscopy.

The first ultrastructural changes in axons of peripheral nerve were noted after 14 weeks of duration of diabetes mellitus. The accumulations of mitochondria and dense glycogen–like granules surrounded by membrane, as well as finger–like invaginations of adaxonal Schwann cell cytoplasm penetrating the axon, were found in diabetic rats. The ultrastructural changes observed are probably a reaction of Schwann cells to the axonal damage and are supposed to correlate with early axonal damage.

The study showed that development of experimental diabetic neuropathy in streptozotocin rats was accompanied by the appearance of a variety of ultrastructural abnormalities in axons of myelinated nerve fibers of the peripheral nerve.

L. Páč (Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **2001 – Year of important anniversaries of heads of Anatomical Department in Brno.**

This year the anatomists in Brno have opportunity to remember the life anniversaries of practically all heads of Department of Anatomy at Medical Faculty of Masaryk University. The historically first of them was Carl Lintz (1711–1788), head of the Collegium anatomicum (1753). Professor MUDr. Otomar Völker, MVDr.h.c. (1871–1955) was the builder and the first head of Department of Anatomy of the Masaryk University Medical Faculty (1919–1939). The next one, Professor MUDr. Karel Hora (1901–1942), was head of department only during very short period in 1939. Professor MUDr. Přemysl Poláček, DrSc. (1921–1980) took up leadership of the anatomical department in year 1962 (1962–1970). Professor MUDr. Lubomír Malinovský, DrSc. (1931–1996) was department head in years1970–1990.

L. Páč (Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Important morphologists at the postage stamps.**

Although medical philately has received considerable attention, the philately of anatomy has been neglected. The author attempted therefore to collect the postage stamps with portraits of important morphologists. 16 stamps with anatomists were found and described (some of anatomists occurred even at two stamps). There were as follows: Hippocrates (460–377 BC.), Leonardo da Vinci (1452–1519), Francois Rabelais (1490–1553), Andreas Vesalius (1514–1564), William Harvey (1578–1657), Franciscus de la Boe Sylvius (1614–1672), Niels Stensen (Steno) (1638–1686), Anthony van Leeuwenhoeck (1632–1723), Albrecht von Haller (1708–1777), Jan Evangelista Purkyně (1787–1869), Jozsef Hyrtl (1811–1894), Theodor Meynert (1883–1892), Ferdinand von Arlt (1812–1888), Theodor Billroth (1829–1894), Camilo Golgi (1847–1926) and Ramon y Cajal (1852–1934). The portraits of prominent anatomists at the postage stamps can serve as the basis of an attractive introduction to history of human anatomy.

Z. Pirník, M. Schwendt, D. Ježová (Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia): **Effect of acute morphine treatment on mRNA levels of selected neuroactive substances in the rat brain.**

It is known that many exogenous substances including drugs may modify regulation of gene expression. The aim of the study was to investigate possible influence of morphine (μ-opioid agonist) on gene expression of proopiomelanocortin (POMC) in the anterior pituitary. Moreover, the action of morphine treatment on gene expression of NR1 subunit of N-methyl-D-aspartate (NMDA) receptor in the hippocampus was also investigated. POMC is the precursor of several peptides, which participate in the activity of hypothalamo-pituitary-adrenal (HPA) axis. POMC is produced mainly during stress, the triggering factors being corticotropin-releasing hormone and other neuropeptides released from the hypothalamus, followed by enhanced synthesis and secretion of glucocorticoids in the adrenal gland. NMDA receptor is one of several subtypes of receptors for endogenous excitatory amino acids. These amino acids were found to be involved in the control of HPA axis function, particularly via NMDA receptors (Ježová et al., 1995).

In situ hybridisation was used to measure POMC mRNA concentrations, reverse transcription-polymerase chain reaction for quantification of NR1 subunit of NMDA receptor and radioimmunoassay to measure plasma corticosterone. Authors found that acute injection of morphine (10 mg/kg s.c.) did not change expression of the genes investigated 4 h and 24 h after drug administration. Comparison of the morning and the afternoon concentrations of plasma corticosterone revealed that morphine had impact on daily rhythm of corticosterone secretion.

Present findings indicate that the action of morphine on HPA axis function may be mediated also through other mechanisms than affecting POMC and NMDA receptor gene expression.

The study was supported by the SK-VEGA 2/608 -Grant

J. Procházková, B. Erdösová, D. Kylarová, V. Lichnovský (Department of Histology and Embryology, Faculty of Medicine, Palacký University, Olomouc, Czech Republic): **Detection of apoptosis and its regulation by Bcl-2 family members in human embryos.**

During intrauterine development the role of apoptosis is crucial. Permeabilization of mitochondrial membranes, a decisive feature of early cell death, is regulated by members of the Bcl-2 family. Present study is focused on the four members of Bcl-2 family. There were as follows: Anti-apoptotic proteins Bcl-2 and Bcl-X_L can form ion channels, which increase the rate of proton extrusion from mitochondria and proteins with pro-apoptotic function – Bcl-X_S that inhibits death suppressor activity of Bcl-2, and Bax, which if over-expressed, accelerates apoptosis. The decision, if a cell leaves to die or not to die, depends on the relative level of expression of all participating proteins that interact on the principle of dimerisation.

Objects of the study were some embryonic organs undergoing substantial morphologic rearrangement (anlagen of limbs, axial skeleton, heart, metanephros) in early developmental periods. Expression of observed proteins was semiquantitatively assessed by using standard three-step indirect immunohistochemical assay. The level of apoptosis was assessed as a comprehensive median of data obtained by three detection techniques (TUNEL, Apostain, Lamin B).

Authors confirmed the strong anti-apoptotic effect of Bcl-2 in morphogenesis, but the level of Bax, Bcl-X_L Bcl-X_S was rather moderate and equal during all observed developmental periods. It is supposed that in the course of human morphogenesis the stress is rather given on the apoptosis-suppressing role of Bcl-2. Obtained results also proved that Bcl-X_S is in some cases capable of counteracting the Bcl-2 ability to block apoptosis.

The paper was supported by Grant No. 304/01/0485 of the Grant Agency of the Czech Republic.

M. Siroťáková[1]*, F. Dorko, J. Danko*[2]*, E. Dorko* (Department of Anatomy, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovakia, [1]Department of Experimental Medicine, Faculty of Medicine, University Pavol Jozef Šafárik, Košice, Slovakia, [2]Department of Anatomy and Histology, University of Veterinary Medicine, Košice, Slovakia): **Sympathetic innervation of the renal hemolymph node in rats.**

The authors studied the adrenergic innervation of the renal hemolymph node in rats using the histochemical fluorescent method with glyoxylic acid. Obtained results may be concluded: 1) Adrenergic nerve fibres enter the nodes in a common bundle with arteries in the hilus; the penetration of them through the fibrous capsule on the other sites of the organ surface was sporadically observed. 2) The highest density of adrenergic nerve profiles was always found in the periarterial or periarteriolar topography, both in medullary vessels or arterioles lying on junction between paracortex and medulla, and arterioles penetrating from the organ surface into the medulla among the small islands of the cortical lymphoid tissue.

The authors conclude that adrenergic nerves supply the same structural and functional parts of the renal hemolymph nodes as in other lymph ones. Total number of fibers was, however, conspicuously higher in the hemolymph nodes.

I. Svíženská, P. Dubový, I. Klusáková (Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Immunofluorescence staining of the insulin-like growth factor-I in the ventral and dorsal spinal roots.**

Insulin-like growth factors (IGFs) are members of a supergene family whose encoded polypeptides are functionally related not only as growth factors but also as neuroactive substances. These agents have profound effects on central as well as peripheral neurons. They may play a role in neuronal development, maintenance and regeneration.

IGF-I is synthesized and released by Schwann cells and could be a signal for the regeneration process. It is picked up by the axons and transferred to the cell body via retrograde axonal transport. Some differences are supposed between the sensitivity of motor and sensory axons to neurotrophins.

To reveal whether there are differences in appearance of insulin-like growth factor-I (IGF-I) in microenvironment of efferent and afferent axons authors have analysed immunohistochemically the expression of the IGF-I molecules in the intact ventral and dorsal roots of the spinal cord.

Four adult Wistar rats were sacrificed by overdose of ethylether inhalation and perfused with Zamboni's fixative solution through the left heart chamber. The Th_{12}-L_2 dorsal root ganglia (DRG) with short segments of dorsal and ventral spinal roots were removed from both sides. The transverse cryostat sections (10 μm) through both roots were incubated simultaneously with the polyclonal primary antibody for 240 min. It was followed by incubation with the secondary rhodamine-conjugated antibody at room temperature for 90 min. The results of immunostaining were viewed and digitised in a Leica-DMBL fluorescence microscope equipped with a DC-100 camera. A brightness of fluorescence immunolabeling was measured by means of an image analyzing system (Lucia G).

The results revealed a higher IGF-I immunoreactivity in the dorsal roots than in the ventral ones ($p < 0.01$).

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J. Šťastná, M. Sedláčková, J. Žáková[1] (Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic, 1[1st] Department of Obstetrics and Gynaecology, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Differences in the occurrence of peroxisomes in human and mouse oocytes and their cumuli oophori.**

According to some evidence, peroxisomes must be present in oocytes to be delivered to early embryos. Since no information is available concerning the occurrence of peroxisomes in these germ cells of mammals, the object of the paper was to identify the peroxisomes by means of selective cytochemical staining for catalase in human and mouse oocytes including their cumulus cells.

Freshly ovulated cumulus-oocyte complexes of mouse, isolated samples of human cumuli oophori and resting, unfertilised human oocytes were processed for electron microscopy by a standard protocol. Catalase activity was demonstrated using 3,3-diaminobenzidine according to Novikoff and Goldfisher (J. Histochem. Cytochem. 17: 675–680, 1969).

In cumulus cells of both the human and mouse oocytes, morphologically uniform, strongly stained population of microperoxisomes was clearly visualised using cytochemical procedure for catalase activity. However, great differences in peroxisomes morphology and cytochemistry were found out between mouse and human oocytes. Peroxisomes were abundant forming extensive clusters of more or less closely attached bodies (previously termed as "jigsaw" bodies) in mouse oocytes. After staining for catalase activity, strongly stained microperoxisomes (0.15 μm in diameter) and moderately or weakly stained larger peroxisomes (up to 1.0 μm in diameter) could be distinguished. In human oocytes, the peroxisomes were of typical appearance consisting of the limiting membrane, dense matrix and crystalline core. They occurred sparsely and were catalase negative.

It is supposed that catalase negative peroxisomes are „old" and therefore devoid of enzyme, since only unfertilised and developmentally arrested human oocytes could be used for the study from ethical reason.

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M. Toropila, I. Ahlers[1]*, E. Ahlersová*[1]*, M. Ondrašovič, K. Beňová, J. Danko, E. Švický, M. Vargová, F. Dorko*[1]*, E. Dorko*[1] (University of Veterinary Medicine, Košice, Slovakia, [1]Faculty of Science, University of Pavol Jozef Šafárik, Košice, Slovakia): **Changes in activities of adaptive enzymes in laboratory rats during continuous gamma irradiation.**

Changes in liver activities of selected adaptive enzymes were investigated in male rats of Wistar strain SPF breeding subjected to long-term continuous gamma irradiation with a daily dose of 0.6 Gy. The activity of tyrosine aminotransferase and tryptophan-2-3-dioxygenase in the liver of irradiated animals increased in two phases and correlated with changes in serum corticosterone. The activity of aspartate aminotransferase in the liver of irradiated animals decreased with the exception of day 14 lasting 90 days. The decrease was significant starting from day 21 up to the end of the investigated period. The activity of alanine aminotransferase in rat liver was increased significantly on days 14, 21 and 60 of irradiation and decreased on days 7 and 90 of irradiation. During the irradiation, the animals consumed less feed and their body weight decreased, too.

J. Uhlík, V. Konrádová, L. Vajner, J. Zocová[1] (Department of Histology and Embryology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic, [1]Department of Applied Mathematics and Computer Science, Faculty of Science, Charles University, Prague, Czech Republic): **Ultrastructure of the epithelium of terminal bronchioles after inhalation of mineral water aerosol.**

Inhalation of mineral water aerosols belongs to usual procedures in the treatment of chronic respiratory disorders. Authors decided to extend their experimental studies dealing with the effect of the short-time inhalation of this aerosol on the tracheal epithelium by evaluation of the epithelial reaction in terminal bronchioles.

Rabbits inhaled the aerosol of special mineral water for 10 min and material was collected immediately after inhalation for the electron microscopic examination. The ultrastructure of the epithelium of terminal bronchioles was evaluated quantitatively with the special emphasis to the cytoplasm of Clara cells, where computer image analysis Lucia G was used.

The terminal bronchioles of rabbits after 10 min inhalation of the mineral water aerosol were lined with a simple columnar epithelium composed of ciliated and Clara cells. On the apical surfaces of ciliated cells, formation of cytoplasmic protrusions that sometimes led to degeneration of free cilia was ascertained. In deeper area of ciliated cells' cytoplasm, mild marks of the pathological alteration were observed. Secretory Clara cells revealed the tendency of intensified proliferation. Their relative number increased significantly ($\alpha = 0.05$) compared to the control values. The proliferation was not accompanied with a marked liberation of the secretion. The relative number of Clara cells containing granules did not differ from findings in control animals. Only isolated Clara cells revealed marks of the pathological alteration of their cytoplasm but marks of the degeneration of these cells were not observed.

The findings of pathological alteration of epithelial cells' cytoplasm and their increased proliferation bring evidence of the non-physiological intervention to the homeostasis of the epithelium and warn against the exaggerated indication even of such simple and apparently harmless therapeutic methods.

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J. Umlauf, M. Horký (Masaryk Memorial Cancer Institute, Brno, Czech Republic)**: Doxorubicin induced cardiomyopathy is associated with nucleolar disintegration and activation of caspase 3.**

The anthracycline doxorubicin is a widely used antineoplastic agent. A severe, dose-dependent chronic cardiac toxicity is the major limitation of the anthracycline therapy. The irreversible loss of cardiac myocytes in doxorubicin-induced cardiomyopathy is probably caused by "biosynthetic shut off" and subsequent programmed cell death (PCD). Rapid proteolysis, a critical initiation step of apoptosis in cardiac myocytes, has been shown to be executed by activation of caspases (e.g. caspase 3). The nucleolus, previously thought to be solely involved in ribosome biogenesis, is now redefined as a plurifunctional nuclear compartment (e.g. regulation of cellular life span etc).

Authors studied the nucleolar morphology of left ventricular cardiac myocytes (CM) in rats treated with doxorubicin (1 mg/kg body weight) for 6 weeks. The TUNEL assay was used to confirm the presence of in situ DNA fragmentation. Polyclonal sera against active form of caspase 3 were also employed for immunohistochemistry.

Attention was focused to the distribution of argyrophylic nucleolar proteins (AgNOR) in left ventricular CM of doxorubicin treated rats. Authors detected dispersed nucleoli in a number of small dots and clustered in rod-shaped tortuous large structures. The nuclei were caspase 3 as well as TUNEL positive. In controls, compact nucleoli and TUNEL negative nuclei were found.

Authors revealed a distinctive nucleolar phenotype in CM of doxorubicin treated rats that coincides with the presence of active form of caspase 3 and DNA fragmentation. Obtained data support the hypothesis that nucleolus is involved in signalling leading to apoptosis and that nucleolar segregation (previously designated as micronucleoli) is linked to doxorubicin-induced inhibition of transcription.

L. Vajner, V. Konrádová, J. Uhlík, J. Zocová[1] (Department of Histology and Embryology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic, [1]Department of Applied Mathematics and Computer Science, Faculty of Science, Charles University, Prague, Czech Republic)**: Comparison of the effect of inhaled aerosols of mineral water and saline on the glycoconjugate composition in tracheal goblet cells.**

In patients suffering from recurrent and chronic respiratory disorders, repeated inhalations of mineral water represent constituent part of therapeutic procedures.

To evaluate the effect of inhaled aerosols on the glycoconjugate content in tracheal goblet cells, six rabbits (SPF New Zealand White males, body weight 1610–2200 g, Charles River, Sulzfeld, Germany) were placed successively for 10 minutes into a plastic cage connected with the inhalation device PARI Master and nebuliser PARI LL (Pari GmbH, Starnberg, Germany). Three rabbits inhaled an aerosol of saline, three others of natural hypertonic iodinated spring water (osmolarity 331 mOsm, pH 6.48). Four other rabbits served as untreated controls. The material for histochemical examinations was collected under general anaesthesia immediately post exposure. In the formalin-fixed paraffin-embedded material, the methods of conventional histochemistry i.e. Alcian Blue (AB) pH 2.5 – PAS and AB pH 1.0 as well as of in situ lectin histochemistry were employed. Maackia amurensis agglutinin MAA, Sambucus nigra agglutinin SNA (Boehringer, Mannheim, Germany), and Tritrichomonas mobilensis lectin TML (Calbiochem, La Jolla, USA) were used.

Of the two aerosols, only the mineral water induced significant changes in the composition of glycoconjugates in the secretion of the goblet cells. Significant decrease in acid sulphated glycoconjugates was accompanied by an increase of acid sialylated ones. Recently, Miyata et al. (Eur. Respir. J. 11: 480–491, 1998) described the protective effects of sialic acids on the airway epithelium. From this point of view, the changes induced in the goblet cells' secretion by inhalation of mineral water could help to protect the airway mucous membrane.

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L. Vargová, L. Horáčková (Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Morphological and palaeopathological analyses of human bone remains from the 9th –10th centuries (Olomouc–Nemilany, Czech Republic).**

In the course of archaeological investigations of a burial-place from the 9th–10th centuries at Olomouc-Nemilany, skeletal remains of 54 individuals (15 males, 16 females, 8 (the sex not determined), 3 young individuals about 16–18 years and 12 children) were revealed.

The skulls that could be measured were mesocranic, orthocranic, metriometopic, leptoprosopic, mesorrhin and mesoconchic. Body length was on average: men 177.2 cm, women 166.0 cm.

Pathological changes were found in the 39 skeletons (trauma, infectious diseases, metabolic and endocrine diseases, the case of secondary cancer have been diagnosed, degenerative changes of the joints were normal finding at group of older individuals). Paleopathological diagnoses were supported by macroscopic, histological and X-ray examinations. The reliability of these traditional diagnostic procedures was verified by the detection of the Mycobacterium tuberculosis DNA with the aid of the PCR (in some indicated bone samples). Inflammatory lesions shared in the 5% of the total number of detected pathological cases, only.

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L. Veverková, J. Kalač, L. Páč[1] (I[st] Surgical Clinic, Masaryk University, Brno, Czech Republic, [1]Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic): **Anatomy of the saphenous nerve – relations of the long saphenous vein and saphenous nerve.**

Considerable controversy exists over the need to strip the whole Long Saphenous Vein (LSV) during operation the varicose veins. Some surgeons recommend that LSV should be stripped to just below the knee. Proponents of the stripping argue that stripping procedure have better immediate results and lower long-term recurrence rate. Opponents argue that there is greater morbidity associated with stripping of the vein owing to bleeding, pain, wound infection, increased incidence

of injury to the Sphenoid Nerve (SN) and stripping of the LSV results in the loss of a possible conduit suitable for arterial or venous reconstruction. As authors are proponents of the stripping of the whole LSV by the operation of the stem varicose veins, they decided to clarify anatomic relations between LSV and SN in the region of the shin.

Description of the anatomical course of the NS is not identical in various anatomical atlases and differences are especially in:

– the level of the branching of SN,
– the presence of one or more branches of the SN,
– the position of the nerve in relation to LSV.

86 lower extremities of the cadavers were dissected in the region from ankle to the knee. LSV and SN were visualised and meticulously examined. The course of the both structures and their relations were studied and documented by photographs. The clinical part of the study involved 215 patients, operated with diagnosis varicose veins.

This anatomical study of the LSV and NS apparently showed that relation of the structures could be very variable. It is impossible to predict the course of the nerve in every operated patient. Only by meticulous preparation the operator can separate the LSV and SN and avoid the damage of the nerve during stripping of the LSV.

M. Zibrín, J. Kočišová, E. Tomajková, T. Komorová, K. Boďa[1]*, V. Sabo*[2] (Department of Anatomy and Histology, University of Veterinary Medicine, Košice, Slovakia, [1]Research Institute of Veterinary Medicine, Ivanka pri Dunaji, Slovakia, [2]Institute of Biochemistry, Slovak Academy of Sciences, Ivanka pri Dunaji, Slovakia): **The long-term experimental hypodynamy mimicking microgravity during the space flight affects the structure of long bones of the Japanese quails.**

Bone remodelling is a homeostatic process that is the result of activities of osteoclasts and osteoblasts. Under physiological conditions some 25% trabecular bone volume is renewed per year. Microgravity in space affects the bone remodelling. However, space experiments are too expensive, therefore several authors try to model these experiments on the Earth.

In present paper the effect of experimental hypodynamy on the structure of the two long bones (femur and tibiotarsus) of adult Japanese quails was studied. After 28, 56 and 84 days of hypodynamy the animals were euthanised. Small pieces of bones were fixed in formaldehyde or glutaraldehyde, decalcified in EDTA and further processed routinely for the light and transmission electron microscopy.

Structural changes in spongy bone and in the osteoclasts were found in all experimental animals, compact bone has not been affected. The osteoclasts reacted in biphasic mode. After 28 days of hypodynamy, the number of osteoclasts drops, then the osteoclasts gradually recover, activate and become more numerous than in the control animals. In animals exposed 84 days of hypodynamy, the osteoclasts covered nearly the whole surface of the bone spicules.

In addition, the results showed that the experimental hypodynamy of Japanese quails could serve as a suitable model to study of the bone remodelling not only in the space biology but also in clinical medicine.

M. Zibrín, T. Komorová, V. Cigánková, N. Lukáčová[1]*, J. Kočišová, E. Tomajková, K. Boďa*[2]*, V. Sabo*[3]*, T. Dadaševa*[4]*, O. Guryeva*[4] (Department of Anatomy and Histology, University of Veterinary Medicine, Košice, Slovakia, [1] Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovakia, [2]Research Institute of Veterinary Medicine, Ivanka pri Dunaji, Slovakia, [3] Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, Ivanka pri Dunaji, Slovakia, [4]State Science Centre, Institute of Biomedical Problems, Moscow, Russia): **The morphometry of liver and lungs of Japanese quails hatched in the space lab Mir.**

Japanese quail is an important link in artificial closed ecosystem such as orbital station or spacecraft during long flight (Boďa, Acta vet. Brno, 62 /Suppl.6: 91–94, 1993). The system is to operate in weightlessness or in microgravity conditions.

The present work is a part of an extensive project, the aim of which was to study the effect of microgravity during a brief space flight on embryogenesis, hatching and postincubation

development of Japanese quail chicks. Embryonic anomalies, changes in bones and vestibular apparatus were found by other authors after long space flight. However, a short space flight did not cause serious damage to observed organs and tissues of 3 male Japanese quail chicks that hatched in space lab Mir and returned to Earth alive.

Some changes have been found in liver, bone marrow, adrenal cortex and skeletal muscle. Considering the physiological accumulation of lipids in the liver of birds at the end of incubation and shortly after hatching, there has been a necessity to objectify our previous light and electron microscopical observations on lipid droplets in liver of 3 male Japanese quail chicks hatched in space lab mentioned above (*Zibrín et al*, Folia Vet. 45/ Suppl.: 17–22, 2001). The morphometric study of liver revealed the overall amount of lipid droplets in the liver of experimental flight quails significantly much more higher than in the control animals of the same age incubated and hatched on the Earth. Lung histomorphometry has not revealed any significant change in the number, diameter and overall surface of air capillaries.

Compiled and revised by *S. Čech*