# THE IV$^{TH}$ MORAVIAN MORPHOLOGICAL DAY

The Medical Faculty of the Masaryk University in Brno was the site of the IV$^{th}$ Moravian Morphological Day that took place on June 11$^{th}$, 2003. This traditional meeting was organised by the Department of Anatomy under the auspices of the Dean of the Medical Faculty *Prof. MUDr. Jan Žaloudík, CSc*.

More than 50 morphologists, from the Czech Republic as well as Slovak Republic, took part in this event. The programme involved 18 lectures and 16 poster presentations that covered fast all branches of macro- and micromorphology.

The meeting was sponsored by the dairy co-operative Mlékárna Olešnice.

*Svatopluk Čech*

# ABSTRACTS

*K. Belej, L. Bošelová, E. Ochodnická, E. Fuseková* (Department of Histology and Embryology, Comenius University, Jessenius Faculty of Medicine, Martin, Slovak Republic)**: New digital record in documentation of observations in light and electron microscope**.

We have had to replace, from the technical reasons, usually used means of documentation of tissue structure and ultrastructure observations in the form of preparations or electronmicrographs, with new approaches. It was based upon improving departmental technical equipment and availability of computer software.

Light microscopy: Quality light microscope NIKON Phase contrast – 2; Digital video – camera SONY CDC/RGB; Camera adaptor CMA – C 1CE; Computer digital record by software Dr Kamera, version 3.00, or Morpholog, version 3.10.1.0.

Electron microscopy: TEM TESLA BS 500; CAMEDIA digital camera C – 720 Ultra – zoom; Panorama card with panoramatic function, 64/128 MB card memory; Reading and recording apparatus for Compact Flash Smart, media card with connector for data transmission between memory card and computer; Record into computer memory.

Documentation: Adobe Photoshop 5.0/5.5 software; Photoshop Album software; Downloading into computer memory by floppy disk, or by CD – ROM

*M. Bezděková, M. Kopecký, M. Hřivnová* (Department of Anthropology and Health Science, Faculty of Pedagogy, Palacky University, Olomouc): **Relation of blood pressure and heart rate to BMI children weight category aged 7–15 years**.

We detected basic somatometric measurements, body weight and body height by 1167 children (608 males, 559 females). For a consideration these we selected BMI categories in relation to percentiles norms. As following we examined blood pressure and heart rate in an idle state.

It was proved that by the children aged 7–15 years values of blood pressure and heart rate in idle state increased according to increasing of BMI weight category. In comparison between group of males and females we established higher values of blood pressure and lower values of heart rate in idle state by males compared with same age category of females.

*M. Bezdíčková, A. Jakubec* (Department of Functional Anthropology and Physiology, Faculty of Physical Culture, Palacky University, Olomouc): **Application of EMN (Electrophoretical Mobility of Nuclei) method**.

The method of EMN is a new strategy to evaluate balance of organism's inner milieu. The value of EMN index (in percentage) shows response of organism to the inside and outside conditions (e.g. stress, psychological strain, alcohol, smoking, and etc.).

The electrophoretic activity of cell nuclei was firstly observed and proved in plant and animal material (*Badr & Waldman*, 1973; *Shakbazov, Popa & Atramentova*, 1976). The EMN phenomenon, still only partly explained, is related to the biochemical composition and physiology of cellular structures as well as to the properties of physical and chemical nature and other cellular structures undergoing changes within age.

The aim of our pilot study was to provide basic information about values of EMN index in different endogenous and exogenous conditions. We mainly pursued relation to the physical activity and examined individuals in conditions of test Vita maxima (n = 16) and following recovery phase. Next study observed sample of probands (n = 63, control group 65) in certain psychic stress and finally population of smokers (n = 36) and non-smokers (n = 42) was compared.

Our observations established that EMN index in relation to physical activity shows decreasing tendency of the EMN index after certain physical exercise and during the recovery phase as well. Values in conditions of psychic strain and by smokers in comparison with non-smokers shows trend of decline, too.

*Ľ. Bošelová, K. Belej, E. Fuseková, E. Ochodnická* (Department of Histology and Embryology, Comenius University, Jessenius Faculty of Medicine, Martin, Slovak Republic): **Correlation peripheral nerve damage after single and repeated ischemia**.

The aim of present paper was to correlate some morphological changes of myelinated nerve fibres of the peripheral nerve after a single and repeated ischemia-reperfusion. Reaction of peripheral nerves onto repeated short-term ischemia is not sufficiently revealed and documented even if its significant clinical relevance is well known.

Using transmission electron microscope, changes in the myelinated nerve fibres were studied in guinea pigs subjected to 40 min of single ischemia and the results were compared with those after 15–15–10 min of repeated ischemia followed by 1 h recirculation. The ischemia was induced by ligature of abdominal aorta. The time of survival of the animals was 1, 2 and 3 days. The distal parts of the sciatic nerve were fixed in situ with phosphate buffered 3 % glutaraldehyde and Millonig's fixative for postfixation was used. Stained sections were examined and taken by electron microscope Tesla BS 500.

Obtained observations revealed only negligible changes in myelinated nerve fibres after a single ischemia in interval of 40 min. The ischemia caused changes of some membrane structures of axon and Schwann cells. The degree of ischemic injury was considerably increased if the ischemia has been repeated three times at periods of 15–15–10 min and at 1 h of interischemic recirculation intervals. The main pathomorphologic changes observed after repeated ischemia with a short interischemic interval were irregular intramyelinic dilatations, the vesicular disruption of the myelin sheath and more pronounced injury of the membrane structures of axon, namely of Schwann cells.

*M. Buchtová*[1]*, F. Tichý*[1]*, P. Matulová*[2]*, I. Míšek*[1,2] ([1]Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno and [2]Laboratory of Genetics and Embryology, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Brno): **Proliferation of epithelial cells forming hamster palate during prenatal period**.

Epithelial cell proliferation of the hamster palate during prenatal period was examined immunohistochemically using anti–PCNA antibody (PCNA marks cycling cells). Oral surface of the mammalian hard palate typically possesses a series of transversal ridges (rugae palatinae = RP, in adult hamster form 6 to 7 ridges), which probably assist the tongue to transport food during mastication.

Palatal ridges, as specific localized thickenings, were starting to develop in the hamster before the elevation of palatal shelves. Formation of the RP anlagen was observed in embryos at day of ontogenesis (DO) 11.5. During the RP formation, PCNA–positive cells were detected in the epithelium of the interrugal region (interprotruding areas) or anterior and posterior slopes of rugae mainly. At DO 13.5–14.5, the number of PCNA–positive cells was decreased in both areas under study. At DO 15.5–16, the proliferating activity was increased. The PCNA–positive cells were distinguishable in basal epithelial cell layer. In the mesenchyme, PCNA–negative cells were prevailed (except for DO 12.5).

Palatine glands developed from tubular invaginations of the oral epithelium lining caudal hard and soft palate, and they grew by pouching. The development of glands started at DO 14 and canalization of epithelial cord appeared at DO 15.5. At birth (DO 16), the palatine glands consisted of ducts and immature acini. Gland cells stained by immunohistochemistry showed higher proliferating activity in prenatal period. Obtained results suggest that the cell proliferation was involved in the hamster palate development.

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*P. Čech* (Department of Anatomy, 3rd Faculty of Medicine, Charles University, Prague): **Karel Žlábek's life and work in the light of documents from Prague archives**.

The career of *Prof. Karel Žlábek* (Ž) had culminated in Brno where he eventually died twenty years ago (May 22, 1983); Prague, on the other hand, was the scene of his youth. Two sets of documents from Prague archives illustrate some stages of both the life periods.

Most of them are personal documents filed in the Archives of Charles University:

Ž applies for demonstrator with Janošík's reference (Nov 23, 1925) and the board of professors votes for Ž's demonstratorship (Nov 25, 1925). The dean applies for the demonstrator grant (Dec 9, 1925); the Provincial Political Administration bestows the grant (Dec 18, 1925). The dean applies for the grant for the next year (Oct 22, 1926); done by the Prov. Polit. Adm. (Nov 5, 1926). Applying for habilitation, Ž notifies his programme of lectures: 1) statics and dynamics of the locomotor system, 2) structure of the human body in the light of comparative anatomy, 3) selected chapters of systematic anatomy; Weigner guarantees provision of rooms and teaching aids (Oct 13, 1932). The 1st session of the habilitation commission (com.) recorded (Oct 20, 1932). The dean demands for the habilitation com. proposal (Oct 26, 1932) and invites its members to the 2nd session (Nov 2, 1932), which results in the proposal for admission of Ž to the further habilitation steps (Nov 3, 1932). Asking Ž to announce 3 topics for the habilitation lecture, the dean invites him to the habilitation colloquium (Nov 25, 1932). Ž announces: 1. On the origin of the human chin, 2. On the phylogenetic development of vertebrate cervical muscles, 3. On the importance of muscle varieties in the individual anatomy (Nov 31, 1932). After the habilitation lecture the board of professors decides to bestow the „venia docendi" on Ž (Dec 17, 1932) and requests the ministerial confirmation (Dec 21, 1932); done by the minister of education (Mar 10, 1933); the dean informs Ž (Mar 18, 1933). Borovanský asks to delegate stand-in of the department of topographical and surgical anatomy to Ž, sending a private letter to the dean (Mar 29, 1939); he later must resend both letters (Sep 7, 1939). The ministry of education gives consent to the delegation of stand-in to Ž (Oct 27, 1939); the dean eventually informs Ž (Nov 7, 1939).

6 letters from Brno (Pellicova 3a, 1947; Komenského square 2, 1948–1952) to Prof. Studnička in Prague, filed in the Archives of Academy of Sciences, show Ž as asking for Studnička's bibliography as well as for identification of slides found in Brno, repeatedly thanking for offprints and, in return, offering particular data concerning the journal Experientia or sending the 2nd edition of his Přehled anatomie.

*E. Fuseková, K. Belej, Ľ. Bošelová, E. Ochodnická* (Department of Histology and Embryology, Comenius University, Jessenius Faculty of Medicine, Martin, Slovak Republic): **Pathological changes in peripheral nerve after experimental nerve injury**.

The compression of the peripheral nerve may occur as a result of different circumstances (overgrow of malignant cells, traumatic impairment, incidental compression during an operation). The experimental mechanical damage of the peripheral nerve was induced by single short-term compression.

The aim of the study was to obtain the knowledge concerning the origin and development of changes in the axon and its myelin sheath.

Guinea pigs (Cavia aperea var. porcellus) were used as experimental animals. The left sciatic nerve was crushed for 30 seconds with fine watchmaker's forceps while the contralateral nerve served as control. After defined time period (1, 2 days) the operated animals were killed and both sciatic nerves taken for transmission electron microscopy.

The most constant early changes were found in the axon and consisted in accumulation of mitochondria, multivesicular bodies, lamellar bodies and small tubular and vesicular profiles. Mitochondria were situated centrally in the paranodal region.

Alterations of the myelin sheath occurred later than the axonal ones. The nodal gap was widened. The lateral myelin loops became separated from the axolemma and then undergo vesicular breakdown. The dilatation of the Schmidt–Lantermann incisures was seen as an early response to injury. It presages the later segmentation of the myelin into ovoid fragments. Dilatations of Schmidt–Lantermann incisures were constantly found in all fibres at day 1 after experimental injury.

*G. Hešková, A. Holomáňová*[1], *Y. Mellová, D. Výbohová, L. Kunertová, M. Marčeková, M. Mello* (Department of Anatomy, Comenius University, Jessenius Faculty of Medicine, Martin and [1]Department of Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic): **Anatomical and histological changes in the nasal septal deformation**.

Nasal septum deformation is a very common clinical problem. It is estimated that 75–95 % of the population has septal deformation. The nasal septum plays an important role in the development and growth of the facial skeleton. Septal deformities may be caused by unequal development of the nose and they can affect the growth and development of the maxilla and vice versa.

The paper deals with the incidence of the nasal septum deformities not only in children and adolescents but also in newborns. The existence of nasal septal deviations was also confirmed in fetuses. Moreover, the authors review described histological changes in the mucous membrane of the human nasal septum and nasal mucociliary transport in relation to deviation of the septum.

Knowledge of age-related growth changes of the nose and maxilla and relations between deformation of the nasal septum and facial skeleton may be useful in planning the time of aesthetic or reconstructive nose surgery.

*I. Khadang* (Department of Anatomy, Charles University Prague, Faculty of Medicine in Pilsen): **Biomechanical properties of the human mandible**.

Structure and biomechanical properties of the human mandible is still in center of interest of stomatosurgeons and biomechanics.

In recent biomechanical studies (based on the finite element methods) mandible is understood as a bone with uniform structure and mechanical properties.

For our study we analyzed structure and selected mechanical properties (microhardness by Vickers) of 60 samples from 10 adult partially edentulated mandibles.

Results obtained show that compact architecture of mandible corresponds with the way of loading at different sites of the body and the ramus.

From biomechanical point of view the body of mandible is characterized as a beam being subjected to compression in its ventral part while the central portion of the body been elevated by contraction of masticatory muscles. The alveolar process contains not only vertically running osteons but also obliquely oriented osteons, which correspond with micromovement of teeth. The condylar process is predominantly under compression.

*K. Kikalová* (Department of Normal Anatomy, Faculty of Medicine, Palacky University, Olomouc): **Immunohistochemical proof of endothelium in laboratory rat Peyer's patches**.

In author's previous studies Peyer's patches of the pig (Sus scrofa f. domestica) were examined using REM moulds of blood vessels.

The aim of the paper was to complete published results by immunohistochemical examinations.

Considering material accessibility and specifying of antibodies, the author accomplished detection of endothelium cells of rat using Mouse anti Rat CD 31.

*The study was supported by internal grant of LF UP (Grant No.1130110).*

*L. Krejčířová*[1], *I. Lauschová*[1], *M. Doubek*[2], *D. Horký*[1], *J. Mayer*[2], *J. Doubek*[3] ([1]Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno; [2]Department of Internal Medicine – Haematooncology, Faculty of Medicine, Masaryk University and University Hospital Brno and [3]Department of Physiology and Pathophysiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno): **Changes of rat kidneys after administration of Amphotericin B colloidal dispersion and conventional Amphotericin B deoxycholate**. **Morphological Observations**.

The goal of paper was to compare the nephrotoxicity of Amphotericin B colloidal dispersion (ABCD) and conventional Amphotericin B deoxycholate (AmB), namely changes in structure of rat kidneys seen on the light and electron microscopic level because preliminary clinical findings did not show significant differences after their administration.

9 rats divided into three groups were used. Rats of group 1 served as controls. l2 mg/kg of ABCD and/or 4mg/kg of AmB were daily-administered i. p. to animals of group 2 and/or group 3 for a period of l4 days. Thereafter, both kidneys of all treated and control rats were taken and processed by light microscopy and standard electron microscopy protocol in combination with the morphometry.

In the light microscopy, obvious changes of structure implicating necrosis were observed in proximal and distal tubules of the renal cortex. An extensive damage of brush border area was mainly observed in the case of proximal tubules. Authors have never seen substantial structural alterations, especially after administration of ABCD in the medulla.

By the electron microscope, dilatations of intercellular spaces among cells of distal tubules were observed toward the basal labyrinth. Cell apices usually protruded in numerous cytoplasmic buds lacking cell organelles. While in the cortex cells of distal as well as proximal tubules constantly contained an increased number of lysosomes, mitochondria with uncommon structure, and vacuoles in bases, only proximal tubule cells showed signs of alteration in the renal medulla. Intercellular spaces among cells were conspicuously dilated and numerous lysosomes and alterated mitochondria regularly occurred in their cytoplasm. Changes of tubules described in the cortex and the medulla were frequently seen after administration of AmB. In good accordance with qualitative findings were results of morphometric observations.

Obtained results verified that ABCD and AmB cause damage of renal tubules, however, morphological alterations induced by AmB were more evident. There is an open question how observed structural changes can project in or modify the long-termed therapy.

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*L. Kunertová, Y. Mellová, J. Beňuška*[1], *G. Hešková, M. Marčeková, M. Mello, D. Výbohová* (Department of Anatomy, Comenius University, Jessenius Faculty of Medicine, Martin and [1]Department of Anatomy, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic): **Respiratory epithelial changes induced by air pollution**.

The epithelium of respiratory passages has a number of mechanical functions, including mucociliary clearance and protection against noxious agents. Epithelial cells are able to release a variety of mediators (e.g. cytokines, chemokines, and growth factors) and may control inflammatory reactions by the release of anti-inflammatory mediators or by the inactivation of pro-inflammatory substances. The normal conducting airway epithelium from the nose to the bronchioles is pseudostratified with basal cells, ciliated and secretory cells. Homeostatic

mechanisms maintain a balance of the major cell types, as cells die and are replaced. Exposure of the epithelium of respiratory passages to injurious agents alters the steady-state producing changes in the proportions of specific cell types, modifies the ultrastructure of the epithelium and the mucociliary defense mechanisms and increases cell proliferation.

Recent studies suggest, that air pollution may play an important role in the development and the clinical manifestation of acute and chronic respiratory diseases. The mechanisms and cell types involved in pollutant-mediated effects in the airways, however, are not clear. Hence, the object of the paper was to map relationships between morphology of the epithelium of respiratory passages and defined disorders of the function.

*M. Kýr, T. Kubek, P. Dubový* (Division of Neuroanatomy, Department of Anatomy, Faculty of Medicine, Masaryk University, Brno): **Morphological evidence of collateral sprouts from intact motor and afferent axons.**

End-to-side anastomosis is one of new alternative methods providing a source of motor and afferent axons for reinnervation of the target tissues. A growth of collateral sprouts from intact axons of donor nerve into the distal stump of damaged nerve is suggested to be neurobiological ground of the way of reinnervation.

Double retrograde labeling of neurons was used for morphological evidence of collateral sprouting in our experimental model. 8 adult rats (females, Wistar strain) were anaesthetized by an intraperitoneal injection of ketamine and xylazine. The musculocutaneous (MCN) and ulnar nerves (UN) were exposed. The distal stump of transected MCN was sutured into perineurial window of the UN. After 2 months, both nerves were cut distal to the end-to-side anastomosis and Fluoro-Ruby and Fluoro-Emerald (Molecular Probe) were applied to the proximal stumps of the UN and MCN, respectively. After 1 week, the rats were perfused with Zamboni's fixation and cryostat sections were cut through the spinal cord segments ($C_6$–$C_8$) and corresponding dorsal root ganglia. The sections were viewed and digitized in a fluorescence microscope (Leica DMLB) using G/R filter. Fluorescence characterization of labeled neurons in the sections, single tracers and their mixture (1:1) were assessed using a Lucia image analysis system. The greatest number of neurons labeled with red fluorescence (Fluoro-Ruby) was related with axons of the UN that did not produce collateral branches into the MCN. A smaller amount of neurons displayed orange fluorescence (mixture of Fluoro-Ruby and Fluoro-Emerald) suggesting the growth of collateral branches into the MCN. A small number of neurons labeled with green fluorescence (Fluoro-Emerald) was only connected with the axons that regenerated directly into the stump of MCN.

Obtained results provide unequivocal morphological evidence of collateral sprouting from intact motor and afferent axons in the rat peripheral nerve. Various proportions of red and green channels of orange fluorescence in the neurons indicate a different retrograde transport of tracers in the maternal axon and its collateral branches.

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*M. Marčeková, E. Neščáková*[1]*, Y. Mellová, G. Hešková, L. Kunertová, D. Výbohová, J. Marček, M. Mello* (Department of Anatomy, Comenius University, Jessenius Faculty of Medicine, Martin and [1]Department of Anthropology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovak Republic): **Employment of anthropometry in the evaluating somatic development of ill children**.

The physical anthropology directed the attention for the watching the somatic development of the physiologic child in the past. Since 60$^{th}$ years the attention is paid to objective watching the somatic development of the sick children.

Somatic growth as individual health basic indicator is adverse affected by some congenital or obtained illnesses. Secure progress for some diseases and the need of its timely start necessitates individual watching the somatic development on healthy as well as sick children. The anthropologic objective methods confirm difference in the development and physical structure at some diseases and therefore they are classified as significant diagnostic methods.

The clinical practice indicates that the somatic growth and development, especially development of the thorax, are influenced by the respiratory diseases (cystic fibrosis, idiopathic interstitial pulmonary fibrosis, bronchial asthma) and by congenital diseases of the heart and vessels. A lot of diseases and syndromes can be related to short body height. These illnesses are different in the causes, clinical statures, therapeutic growth in effect of various possibilities and height prognosis.

The anthropometry can contribute to early reveal of serious illnesses besides traditional diagnostic methods.

*P. Matulová*[1], *K. Witter*[1,2], *I. Míšek*[1,2] ([1]Laboratory of Genetics and Embryology, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Brno and [2]Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno): **Proliferation markers used in study of embryonic development**.

Cell proliferation represents one of the most fundamental biological processes that are associated with many cellular functions including cell reproduction, development of tissues or growth of multicellular organisms. It is clear that developing organism must have highly co-ordinated mechanisms to control proliferation of its cells to suit developmental needs. Cells receive a variety of positive and negative signals from external and internal milieus and must decide to start or cease the cell cycle in response to these signals.

Several methods are available and used for study of cell proliferation. The present study brings authors' experience with their application on the example of the developing dentition of laboratory rodents.

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*M. Miklošová, R. Herich*[1] (Department of Anatomy, Faculty of Medicine, P. J. Šafárik University, Košice and [1]Department of Pathological Anatomy, University of Veterinary Medicine, Košice, Slovak Republic): **Plastinated specimens and their application in medical teaching modules**.

Plastination is a technique that permits the preservation of the anatomical specimens in a physical state approaching that of the living conditions. The „ideal" specimen would be durable, lifelike, stable and non-hazardous. The most promising method is a process of specimen plastination. The department of authors began to produce plastinated specimens in 2001 year. To date, there are over 80 specimens for gross anatomy (e.g. heart, stomach, kidney, liver, etc.) at the department.

Authors used the standard silicone method S10 according to von Hagens (1987). Specimens taken were fixed in formalin for 4 weeks and then flushed with water. They were then dehydrated in cold acetone (–25°C), which was replaced three times in one-week interval. After dehydration, specimens were impregnated with polymer reaction-mixture of S10 and S3 in the ratio of 100:1 at room temperature for 8 weeks. Finally, the impregnated specimens were drained and hardened by exposure to S6 (2 days).

A collection of plastinated specimens is being prepared for use in multipurpose learning resources facilities, which enhance the level of anatomy teaching for students. Although the production of plastinated specimens is primarily assigned for undergraduate medical teaching, they are frequently explored in teaching of nurses and students of psychology and design.

In general, learning aids used in anatomy are of different types - cadavers, dissected wet specimens, plastinated specimens and models. The wet specimens are unpleasant to the nose and fingers. The models are namely dry but not realistic. If compared plastinated specimens with former ones, they are dry and odorless, non-toxic and non-infections and do not require specific conditions of storage. Plastinated specimens have shown excellent utility and they have been well received by students.

In conclusion, plastinated specimens have definite use and preference in teaching of gross anatomy where detailed knowledge is not essential.

*I. Míšek*[1,2]*, H. Pavlíková*[2,3] ([1]Laboratory of Genetics and Embryology, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Brno, [2]Department of Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, University of Pharmaceutical Sciences, Brno and [3]Department of Anatomy, Faculty of Medicine, Masaryk University, Brno): **Innovation of Anatomy Museum at the Faculty of Veterinary Medicine UVPS Brno – a part of active teaching of anatomy.**

An active teaching of anatomy is based on numerous good prepared standing specimens exposed in anatomical collection at the Department of Anatomy. Due to complexity of the veterinary curriculum it is quite a challenge to prepare educational material in an interdisciplinary manner. Some of anatomical specimens have to be repaired and/or next new one having to be dissected. Anatomy is a morphological science thus having constant references to drawings and images of anatomical structures. Recent multimedia technologies and communications procedures may be of great interest in our field of teaching as they allow a relatively easy diffusion of a large amount of image format.

The aim of the study was to describe mammalian species complexity and possibilities of anatomical education at the Faculty of Veterinary Medicine using anatomical preparations those are exposed in an anatomy museum and/or available in digitized form. So far many teachers and students have been able to find adequate anatomical data in textbooks and atlases. Recently, due increasing of number of anatomical specimens as well as for practical reasons it seems reasonable to create an easy-to-use databases of digitized images of most anatomical specimens occurring in museum, which could be used by anatomy teachers and many students using the Internet and Intranet technologies. Due to the volume of data that can be put on a single disk, they can currently use a large number of photos those are integrated under graphical environment.

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*H. Nechutová*[1,2]*, P. Dítě*[2]*, L. Páč*[1]*, J. Brázdil*[3] ([1]Department of Anatomy, Faculty of Medicine, Masaryk University, Brno; [2]Department of Internal Medicine – Gastroenterology and [3]Department of Pathological Anatomy, Faculty of Medicine, Masaryk University and University Hospital Bohunice, Brno): **The pancreatic stellate cells**.

Chronic diseases of the liver, pancreas, intestine, lungs and the skin are usually accompanied by scarring and loss of organ function, which is often progressive. Therefore, well-tolerated and sufficiently effective antifibrotic therapy is needed. Targets of our interest are activated mesenchymal cells, which synthesize (under the specific conditions) an excess of extracellular matrix proteins, first of all collagen fibres proteins. According to their characteristic cellular shape they are called stellate cells. In human the most extensively described are stellate cells of the liver. The first information about this form wrote Kupffer in 1898. In last 10 years the pancreatic stellate cells are also focused.

Hepatic stellate cells (called formerly as *Ito cells*, *vitamin A storing cells*) are localized in the space of Disse. They have two fenotypic functional forms: inactive (quiescent) and active. The main effect of acute or chronic injury of hepatic tissue is the activation of hepatic stellate cells and increased synthesis of extracellular matrix proteins, predominantly collagen type I and III. It leads to fibrosis, and potentially to cirrhosis of hepatic tissue. Through activation there appears intracellular smooth muscle alpha actin, which makes possible the contraction of stellate cell. It influences the sinusoidal blood flow and participates in portal hypertension.

Pancreatic stellate cells are found in perivascular and periacinar location. They also show two phenotypic forms: 1) Inactive (quiescent) form: round cellular shape, low mitotic and sythetic activity, intracytoplasmic storing of fatty components and vitamin A. 2) Active: stellate shaped, high mitotic and synthetic activity, loosing of fatty droplets with vitamin A by exocytosis. The main change in intermediate filaments is the presence of alpha smooth muscle actin. Cells become responsive to proliferative and profibrogenic growth factors. There is positive staining for procollagen alpha 1 (I) mRNA, which determinates pancreatic stellate cells as producent of

collagen. Ethanol or acetaldehyde induce the generation of oxidative stress within the cells, it is prevented by vitamin E.

Authors demonstrated the presence of stellate cells in the human pancreas, which were devastated by chronic pancreatitis or tumor processes.

*E. Ochodnická, M. Ochodnický*[1], *K. Belej, E. Fuseková, Ľ. Bošeľová* (Department of Histology and Embryology and [1]Department of Internal Medicine I, Comenius University, Jessenius Faculty of Medicine, Martin, Slovak Republic): **Changes of peripheral nerve in diabetes mellitus**.

Diabetic neuropathy is the most common chronic complication of diabetes mellitus. The pathogenesis of this complication is multifactorial. Among the various animal models for human diabetes mellitus, streptozotocin-induced diabetic rats are the most frequently used because this model exhibits early functional and biochemical alterations similar to those found in human diabetic patients.

The aim of the study was to examine the ultrastructural changes in myelinated fibers of peripheral nerves in experimental diabetes mellitus. Samples of n. peroneus com. taken from diabetic rats (4, 8 and 14 weeks after induction of diabetes mellitus by streptozotocin) and age-matched control animals under diethylether anaesthesia were processed by standard protocol of transmission electron microscopy.

The first ultrastructural changes in axons of myelinated nerve fibers were registered after 14 weeks of duration of diabetes mellitus. The finger-like invaginations of adaxonal Schwann cell cytoplasm penetrating the axon, accumulation of mitochondria and glycogen-like granules surrounded by membrane, were constantly observed in diabetic rats. By electron microscopy, the splitting of myelin sheaths, degenerated myelin figures within the myelin lamellae and dilation Schmidt-Lantermann incisures were also revealed. The occurrence of dense glycogen-like granules was observed within the cytoplasm of both Schwann and perineurial cells. The granules were either scattered in the cytoplasm or aggregated in clusters lacking any limiting membrane.

*J. Procházková, J. Marečková, B. Erdösová, D. Kylarová, V. Lichnovský* (Department of Histology and Embryology, Faculty of Medicine, Palacky University, Olomouc): **Ubiquitin–proteasome complex and its role in apoptotic cell death occurring in selected embryonic organs**.

Ubiquitin–proteasome 26S complex is the main extra-lysosomal proteolytic system encountered in the nucleus and/or cytoplasm of all eukaryotic cells. This system is involved in regulation of cell cycle, differentiation and plays a critical role in removing abnormal, misfolded, or damaged proteins. Therefore, its malfunction may contribute to genesis of some severe diseases (e.g. tumors, neuro- and myodegenerative disorders). It was shown that it also takes part on degradation of proteins during the initiation and execution phases of apoptotic process in animals. Proteosomal proteolysis is mechanism commonly used for degradation of many, mostly regulative proteins (*Naujokat C., Hoffmann S., 2002, Lab Invest, 82/8: 965–980*). Proteins recognized in the proteosomal complex have to be previously tagged by chain composed of small 76-amino-acids-long protein ubiquitin.

The aim of the work was to prove the activity of ubiquitin–proteasome system in apoptotic cells in selected embryonic organs undergoing substantial rearrangement during development.

Double-staining technique is based on consequent detection of apoptotic cells by TUNEL technique and the multiubiquitin chain by standard three–step immunohistochemistry. After proper pre-treatment of tissues (microwave oven, blocking of endogenous peroxidase and unspecific epitopes) the fluorescence of DNA fragments was performed by terminal dNTP transferase. Incubation with the anti-fluorescein antibody conjugated to alkaline phosphatase followed. Second cycle starts by incubation of samples with primary antibody to ubiquitinylated proteins (MAb PW 8810 or PAb UG 9510-both Affinity). Appropriate biotinylated secondary antibody was then added, succeeded by the conjugate of streptavidin–peroxidase (*BioGenex*). The last steps were visualizations of reaction products. Location of mono and double-stained cells was observed under immersion objective and evaluated semiquantitatively.

All the observed tissues contained cells positive for multiubiquitin chain bound to protein, mainly in nucleus. Although both antibody used in authors' experiments should be specific for the same kind of antigen, the intensity of staining was much higher when using antibody UG 9510, even if its concentration was hundred times lower. Authors suppose it is caused by the polyclonality of the used antibody and its better suitability to paraffin embedded tissues. The areas where the apoptosis is more abundant are also highly positive for multiubiquitin chains (e.g. hypertrophic cartilage of the anlagen of limb's long bones, epithelium of villi's tip of small intestine, liver tissue).

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*J. Riedlová* [1], *D. Palyzová* [2], *J. Zikmund* [2] ([1]Department of Anatomy, 3rd Faculty of Medicine, Charles University, Prague and [2]Clinic of Children and Adolescent, University Hospital Královské Vinohrady and 3rd Faculty of Medicine, Charles University, Prague): **Somatometric parameters and visceral intraabdominal adipose tissue in juvenile hypertonics**.

The high incidence of obesity among children and adolescents suggests that a significant portion of the population is at risk of hypertension and atherosclerosis, and, therefore, of cardiovascular disease (CVD), well before the adulthood.

These risks were studied in a sample of 115 asymptomatic hypertonics and 115 normotonics, 14–23 years old. Secondary hypertension was excluded in all patients prior to their inclusion to the study. The present study evaluated major anthropometric parameters, ultrasound measurement of the visceral adipose tissue (VAT) and the lipid metabolism in the metabolic part of the study.

The statistical evaluations of the results of the study samples support the validity of the tested hypothesis:

Significant somatotypic differences included higher body weight ($p<0.001$), larger circumferences of the trunk and extremities ($p<0.001$) and a thicker layer of subcutaneous fat ($p<0.05$) in the hypertonics than in the normotonics. The results held true not only when comparing our two samples but also when testing the hypertonics versus the general Czech population. A subgroup of patients (20 % of the sample), however, showed physical measurements within the healthy norms.

The hypertonics had a larger accumulation of VAT than the normotonics ($p=0.009$).

Proatherogenic trends were present among the hypertonics in all components of their lipid spectrum. The relationships between the studied traits were evaluated using Spearman's correlation test, which revealed a positive relation between VAT and a) BMI ($p<0.00002$), b) plasma level of T cholesterol ($p<0.0004$), c) lipid index ($p<0.00004$), d) LDL (0.0002), and e) TG ($p<0.002$).

Although the results of present study do not allow sufficient generalizations, they support, however, the hypothesis of a significant effect of the accumulation and metabolism of the adipose fat tissue in the pathogenesis of the early stages of primary hypertension and atherosclerosis.

The results identify a subset of patients who, while not fulfilling the criteria of excessive weight and obesity, carry a significant risk of clinical manifestation of CVD in adulthood.

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*M. Sedláčková, J. Šťastná, J. Žáková*[1] (Department of Histology and Embryology, Faculty of Medicine, Masaryk University and [1]Department of Obstetrics and Gynaecology, University Maternity Hospital and Faculty of Medicine, Masaryk University, Brno): **Structural changes of mitochondria and reactive oxygen species production in human gametes, early embryos, and somatic cells**.

A healthy complement of mitochondria is essential for embryonic growth and development. In spite of the fact that dysfunction of mitochondria is often associated with free radicals and reactive oxygen species generation, the submicroscopic structure of mitochondria and cytochemical detection of hydrogen peroxide production were investigated in human gametes, and early embryos and then compared with those in somatic cells.

Authors examined clinical material obtained under strict keeping of obvious ethical rules. The sample collection consisted of unfertilized oocytes, spermatozoa, *in vitro* developed embryos and

blastocysts, and at last of somatic cells from cumulus oophorus. All the samples were processed by the standard protocol for transmission electron microscopy. Simultaneously, ultracytochemical detection of hydrogen peroxide was also tested.

Mitochondria in oocytes, which failed to fertilize during in vitro insemination, and developmentally arrested or blocked early embryos, mostly produced hydrogen peroxide. Reaction product in form of cerium perhydroxide granules was localized on the outer mitochondrial membranes. The number of such granules was extremely low per mitochondria. In spermatozoa, mitochondrial hydrogen peroxide production was not usually detected. In blastocysts, as well as in cumulus cells, mitochondrial hydrogen peroxide generation was, if present, always substantially higher. This production was usually associated with altered ultrastructure of both the cells and mitochondria themselves.

If compared the morphology of mitochondria and very limited mitochondrial hydrogen peroxide generation in human gametes and early embryos on the one hand, with ultrastructure of mitochondria and substantially higher hydrogen peroxide production found out in somatic cells on the other hand, it became evident that these differences might be associated with very low intensity of oxidative phosphorylation in gametes and early embryos.

*I. Svíženská, P. Dubový, I. Klusáková* (Division of Neuroanatomy, Department of Anatomy, Faculty of Medicine, Masaryk University, Brno): **Immunohistochemical detection of IGF-I in the peripheral nerve**.

Insulin-like growth factor-I (IGF-I) is a member of the insulin gene family with neurotrophic properties. Schwann cells are a significant source of IGF-I molecules in a peripheral nerve.

The aim of the study was to find out quantitative differences of immunohistochemical staining for IGF-I in the Schwann cells alongside afferent and motor axons in the femoral nerve of adult rat. Intensity of immunofluorescence was compared between myelin-forming Schwann cells accompanying intact axons and undifferentiated Schwann cells of transected nerve stumps.

8 adult rats (Wistar strain) were divided into experimental and control groups. Transection of both right and left femoral nerve was performed in rats of the experimental group (n=4). The control group (n=4) was used for removal of intact cutaneous and motor branches of the femoral nerve. Animals of the experimental group were allowed to survive for 2 weeks after surgery. All animals were sacrificed by overdose of anesthetics and perfused with Zamboni's fixative solution through the left heart chamber. The transverse cryostat sections (10 mm) cut simultaneously through cutaneous and muscular branches of either intact or transected femoral nerve were incubated with goat polyclonal antibody raised against IGF-I for 4 hours. It was followed by treatment with affinity purified donkey anti-goat antibody conjugated with rhodamine 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 (*Dubový P. et al., 2002, Histochem Cell Biol 117:473–480*).

The results revealed a higher IGF-I immunostaining of the Schwann cells in both intact and transected afferent than motor branches of the femoral nerve ($p<0.01$). Undifferentiated Schwann cells of the distal nerve stumps expressed higher level of IGF-I molecules than myelin-forming Schwann cells of corresponding intact segments ($p<0.01$). The results indicated a higher synthesis of IGF-I molecules by the Schwann cells of afferent than motor axons. The increased immunofluorescence of undifferentiated Schwann cells suggests a significance of IGF-I molecules for regenerating axons but in dissimilar rate for afferent and motor axons.

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*O. Štěrba* (University of Veterinary and Pharmaceutical Sciences, Brno): **The ganglionic nervous system: A pattern for the truncus sympathicus**?

The author is concerned with the coincident arrangement of the nervous system in invertebrates (Annelids and Arthropods) and the sympathetic trunk in vertebrates. Common characteristics of

both systems were examined from the comparative, evolutionary and ontogenetic point of view. The idea is supported by facts as follow: 1) Both systems arise from the nervous plate. 2) The fundamental arrangement and its pattern, i.e. the paired ganglia arranged segmentally and united by paired longitudinal nerve cords, is practically identical in both systems. 3) Both systems show an identical position in the organisms: the nervous system of annelids and arthropods lies at the bottom of the coelom; the truncus sympathicus of vertebrates lies at the roof of the coelom, i.e. in places mutually corresponding according to the homeotic genes.

The author offered an original opinion about the origin of the sympathetic trunk vertebrates. He is made sure that it is necessary to identify the genes controlling the successive developmental stages of the nervous system formation.

*Z. Tonar*[1,2], *J. Jeník*[2], *V. Nováček*[2] ([1]Department of Histology and Embryology, Charles University, Faculty of Medicine in Pilsen and [2]New Technologies-Research Centre in Westbohemian Region, University of West Bohemia, Pilsen): **Morphology and finite element models in the biomechanics of aorta and femur**.

Our task was to create a simplified model of the aorta and its main branches in a form suitable for the computational fluid dynamics (CFD) modeling. We also had to develop a femur finite element mesh including a fixating nail for purpose of modeling the fixation of per-trochanteric fracture.

The morphology of the inner surface of the aorta was based on the digital image data sets produced by Visible Human Project™, which comprised of axial anatomical cross-sections at 1 mm intervals obtained from a male cadaver. SurfDriver software was used for the detection of geometrical edges of the aorta morphology. The data were exported via the AutoCAD to the Gambit package, where the grid for the finite element method was prepared and the pre-processing for the CFD analysis was made. The geometry of the femur was obtained from the ISB Finite Element Repository, improved in AutoCAD and the fixating nail was joined to this geometry respecting exactly the nail's proportions. The mesh was prepared for the computation in the HyperMesh and Gambit software.

The model of the aorta ranged from the aortic valve to the common iliac arteries, including also the bifurcation of the left common carotid artery, coeliac artery, superior and inferior mesenteric arteries and renal arteries. The model of femur respected both the compact and cancellous bone tissue geometry. Therefore, the element size was very small, especially in the epiphysis.

We prepared a model of the aorta for the numerical computation dealing with the simulation of the flow disturbation and with the modeling of the blood-aortic wall interaction. It was developed in order to solve the deformation of the aortic wall caused by the blood flow. We used the femur model successfully in numerical computation of the fixation of per-trochanteric fracture in the PamCrash system.

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*L. Veverková* (1st Department of Surgery, Faculty of Medicine and St. Anne's University Hospital, Masaryk University, Brno): **What you did not find in books on anatomy**.

In my paper I have concentrated on detailed description and determination of the relationship between individual structures of the venous system in the area of the shin. My research was inspired by clinical praxis and problems that the lack of knowledge of these structures causes.

The varicosity of the lower limbs is an illness that affects more than 30 per cent of the European population. Operations of varicose complexes of the lower limbs are ordinary and have a low mortality rate. The anatomic description of structures in the area of the shin is not detailed. This then results in problems that occur at operations, e.g. the damage of nervus saphenus. The specific tie-up of the perforators of the shin, various links to the VSM and Leonardo's vein are all crucial in determining when to extirpate the whole VSM and when it is sufficient to dissect the perforators. Moreover it is the determination of the junction of VSP and the occurrence of gastrocnemic veins,

which are often insufficient and simulate the insufficiency of the VSP junction. These are issues that I dealt with in the paper.

*M. Vondráková, M. Martiniaková* (Department of Zoology and Anthropology, Constantine the Philosopher University, Nitra, Slovak Republic): **Development abnormalities of the spine structure in Slovak medieval populations.**

Submitted contribution is not only review from the current knowledge of development abnormalities of the spine structure but also their application. The variations and abnormalities of the spine were evaluated morphoscopicaly.

Investigation of Slovak medieval populations brought the information there is a ponticulus posticus atlantis and/or foramen arcuate situated in atlas. Opposite sign-foramen transversarium atlantis ante apertum (besides atlas) is often located at axis. Every of column vertebral was affected by rhachischisis posterior in some findings. Spondylolysis affected almost exclusive lumbar vertebrae. This anomaly might be accompanied by spondylolisthesis as it was also documented in studied skeletal material. For the lumbo-sacral region both sacralisation (cranial shift) and lumbalisation (caudal shift) were characteristic and often found. We were not able to make quantitative analysis of the results because of incomplete medieval skeletal material excavated from graves.

Studies of the vertebral variations and abnormalities are of considerable interest for both medicine and anthropology, not elucidated is their impact on diseases of the spine and spinal cord and sometimes different frequencies in various populations.

*D. Výbohová, K. Adamicová*[1]*, Y. Mellová, M. Marčeková, G. Hečková, L. Kunertová, M. Mello* (Department of Anatomy and [1]Department of Pathological Anatomy, Comenius University, Jessenius Faculty of Medicine, Martin, Slovak Republic): **Effect of ageing and photoageing on skin microvasculature.**

Authors review described changes of microvasculature of the ageing and photoageing skin.

In ageing skin dermal vessels are significantly reduced. One of the most typical changes of aged skin is an appearance of abnormally thin walled vessels. Veil cells surrounding these vessels are fewer in number or absent. Their synthetic activity is decreased. Endothelial cells show irregular surface and decreased number of cytoplasmic organelles.

Microvessels injured by UV radiation were characterized by thick wall. Thickening is formed by the deposition of perivascular layer of the basement membrane like material. The veil cells are increased in number, size and synthetic activity. It is proposed that veil cells are stimulated to produce basement membrane like material in response to UV rays. Endothelial cells have increased number of cytoplasmic organelles. Latest findings revealed co-existence of regressive changes and formation of new vessels. A dermal overexpression of the vascular endothelial growth factor by fibroblasts, which is induced by UV rays, may contribute to dilated microvasculature.

Above mentioned alterations in the microvasculature of the ageing and photoageing skin were observed namely in papillary dermis.

Obtained knowledge can be exploited to understand some specialties of the skin disorders in elderly and a part of photoageing in the pathogenesis of some disorders, especially skin tumors.

*M. Vystrčilová* (Department of Anatomy, Faculty of Medicine, Masaryk University, Brno): **Are microscopic ageing techniques more accurate than macroscopic ones**?

Different approaches to the estimation of age at death in mature human skeletal remains were evaluated utilizing samples from 42 historical adult individuals from medieval to modern Moravian burial grounds, of which 7 were of known age thanks to the historical sources.

Common morphological methods of estimating age at death such as the cranial suture closure, the occlusal dental wear, the extent of processus alveolaris, the sternal end of the ribs, the auricular and symphyseal faces of the pelvis, the radiographs of the proximal femur and humerus, and the combined method were used. From the microscopic methods the modification of Gustafson

procedure (on the teeth sections of one-rooted teeth) and qualitative and quantitative evaluation with osteon counting in the section of the rib cortex were performed. For age prediction according to the microscopic methods equations derived from the evaluation of dental and rib sections employing recent material of known age were used.

To sum up the results of the age estimation using various methods on the historical material we can conclude that there are no significant differences between the estimates according to morphological methods and osteon counting in the rib cortex. From the age estimates according to all morphological methods their mean value was considered. We found statistically significant differences in age estimates between morphological and dental techniques as well as between osteon counting and the teeth sections.

Since we did not know real ages of all studied individuals from the historical sample, we could not prefer the results of none of these methods. It is not possible to state that method is the most predictive. But according to the recent sample and to material of known age the histological dental technique seems to be the most effective method for age estimation. We also favor the use of standard morphological methods in their combination. Results given by the use of the rib sections were not very precise. It is suggested that combination of all available age information can provide more accurate and less biased age estimates. Teeth and their histology should become an integral part of the age estimation.

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*F. Wágner, B. Erdösová, J. Procházková, D. Kylarová* (Department of Histology and Embryology, Faculty of Medicine, Palacky University, Olomouc): **Specification of cells phagocyting apoptotic bodies during human nephrogenesis.**

We have dealt with the degradation phase of apoptosis in human metanephros. According to recent research on mice, less on human material, cells responsible for clearing apoptotic bodies away during development are, besides non-professional phagocytes, also tissue-fixed macrophages. The role of macrophages in the earlier stages of intrauterine development (IUD) was being cast doubt. Thus our aim was finding out at what time macrophages appear in metanephros during IUD.

18 intact kidneys were collected from embryos and fetuses ranging from the 8$^{th}$–28$^{th}$ week of IUD. The paraffin embedded tissues were used for immunohistochemical staining. Macrophages were detected by standard indirect three-step immunohistochemical method having used mouse Mab Ab–1 (Macrophage Marker, Oncogene) and goat polyclonal antibody to CD64 (N–19; *Santa Cruz Biotechnology, Inc.*), which is expressed constitutively on monocytes and macrophages. In addition, the same method was applied for mesenchymal cells having used Mab against matrix metalloproteinase 9 (mouse MAB NCL–MMP9–43; *Novocastra Lab.*). The results were evaluated semiquantitavely. The localization of positive cells was compared with apoptotic cells. The latter ones were detected by means of TUNEL method.

Cd64+ macrophages appear in the 12$^{th}$ week while elements labeled by Ab–1 are detectable since the 14$^{th}$ week of IUD. Obtained results are somehow contradictory in comparison with our previous outcomes ensuing from the detection of macrophages by Mab to CD68. They were detectable since the 8$^{th}$ week of IUD. The level of MMP9 was lower than we had expected. It is impossible to judge precisely the co-localization of apoptotic bodies and macrophages or phagocyting mesenchymal cells without using double staining method.

Compiled and revised by *S. Čech*