POLISH SOCIETY OF PATHOLOGISTS

ABSTRACTS

SYMPOSIUM ON ELECTRON MICROSCOPY

MEETING OF THE MICROSCOPY COMISSION OF THE COMMITTEE OF HUMAN GENETICS AND MOLECULAR PATHOLOGY OF THE POLISH ACADEMY OF SCIENCES AND DIVISION OF THE ELECTRON MICROSCOPY OF THE POLISH SOCIETY OF PATHOLOGY AND CHAIR AND DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY, MEDICAL ACADEMY IN WROCŁAW AND INSTITUTE OF ZOOLOGY WROCŁAW UNIVERSITY AND CHAIR AND DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY, POZNAŃ UNIVERSITY OF MEDICAL SCIENCES

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MORPHOLOGICAL ASPECTS OF THE AGING PROCESS IN PERIPHERAL NEURAL TISSUE

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We investigated the morphological expression of aging in two neighboring peripheral neural tissues of fundamentally different functional and physical attributes: the carotid body (CB) – containing receptor neurons that detect changes in oxygen concentration, the sensory organ of high metabolism and the highest blood flow in the body, and the superior cervical ganglion (SCG), containing neurons that supply sympathetic innervation to the face, an organ of low blood flow and metabolism; both placed in the vicinity of the internal carotid artery.

Rats of two contrasting age groups were used for the study: young – 3 months old and senescent – 24 months old. The animals were euthanized by perfusion through the left heart with 2.5%paraformaldehyde/2% glutaraldehyde, and both organs were rapidly dissected and postfixed in the aldehydes. Ultrathin sections were made, dehydrated in a series of increasing concentrations of ethanol, and embedded in Spurr raisin. The specimens were viewed under an electron microscope (JEM 1200, Japan). We found substantial and grossly similar age-changes across all parenchymal components of both tissues examined. The old CB were characterized by fewer receptor neurons that were surrounded by proliferating connective tissue. These cells were at varying stages of degeneration, up to a dark variant of necrosis occasionally observed. The most prominent changes were damage to the mitochondrial matrix, vacuolization of the cytoplasm, fewer secretory vesicles, the appearance of phagolysosomes. Interestingly, the number of sustentacular, glial-like cells was increased. As the latter cells were loaded with phagolysosomes, they could have an active role in the cleansing of degenerating receptor neurons. In addition, perivascular fibrosis, increased number of fibroblasts, and platelet aggregation were visible in CB parenchyma. Age-changes also were present in the SCG. There were deposits of lipofuscin, swollen mitochondria, and hyperactive Golgi area in ganglion neurons. Interestingly, we found aggregation of long spacing-type of collagen fibrils of 200 nm, as opposed to the usual ~67 nm collagen fibrillar periodicity, i.e, pathologically formed proteins of the extracellular matrix in the SCG. We conclude that neural tissue is subject to the aging process, which has some common characteristics, irrespective of the functional and anatomical attributes of tissue.

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IMMUNOLOCALIZATION OF ENOLASE IN KLEBSIELLA PNEUMONIAE CELLS

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Enolase is an enzyme of the glycolysis/glukoneogenesis pathway, catalyzing reversible conversion of 2-phosphoglycerate (2-PGA) to phosphoenolpyruvate (PEP). It has been found in almost all organisms in several isoforms and is differently regulated. Mammals and human enzyme is homo- or heterodimer consisting of α, β and γ subunits, encoded by three distinct loci.α-Enolase is found in a variety of tissues including liver and kidney, whereas β-isoform is almost exclusively found in muscle tissues and γ-enzyme appears in neuron and neuroendocrine tissues. Enolase, although traditionally considered for glycolytic enzyme localized in cell cytoplasm, proved to be a multifunctional protein, acting also as the cell-surface factor for binding plasminogen. Pericellular promotion of plasminogen activation in Gram-positive *Streptococcus pneumoniae* bacterial strain plays a critical role in degradation of extracellular matrix and appears one of important factors of the cell transmigration and host tissue colonization. In our previous studies we obtained rabbit polyclonal antibodies specific against *Klebsiella pneumoniae* enolase. We demonstrated interaction of these antibodies with purified cytosol enolase and enolase-like protein from cell wall outer membrane fraction of *Klebsiella pneumoniae* cells in SDS-polyacrylamide gel electrophoresis and immunoblotting assay. Immunobloting experiments allowed to prove interaction of plasminogen only with purified enolase-like protein from cell wall outer membrane of *Klebsiella pneumoniae*. Moreover, we observed enolase catalytic activity of intact bacterial cell.

The aim of the present report was to identify enolase in the cytoplasm and cell wall of *Klebsiella pneumoniae* cells and show its ability to bind plasminogen using immune electron microscopy. Subcellular localization of surface and cytosol enolase and detection of plasminogen binding on *K. pneumoniae* cells was examined in transmission electron microscope (Tesla BS 540). Post-embedding labelling of ultrathin sections revealed the reactivity of the anti-bacterial enolase antibodies with *K. pneumoniae* cytoplasmic and cell-wall-exposed enolase. Human plasminogen is localized only on the surface of *K. pneumoniae* cells.

ULTRASTRUCTURAL EVALUATION OF CELLS OF THYROID PAPILLARY CARCINOMA

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For the present studies there were used samples of: a) papillary carcinomas larger than 1 cm (taken from 20 patients), papillary microcarcinomas, as well as c) from colloid goiter patients in whom in 2 cases papillary carcinomas were diagnosed. All samples were from female patients aged 21 to 65 years. In none case there were no hyperthyroidism nor elevated TSH levels.

After histological studies we found, that in 20 cases there were papillary carcinomas and in next 2 cases papillary-follicular carcinomas. Parallely there samples fixed and processed for a routine transmission electron microscopy. After ultrastructural studies we found that not all cancer cells were well-differentatied and matured and not all formed typical papillary projections. In all cases there found groups of cells which might be not fully differentiated, as they were small and not polarized, with centrally placed single regular nucleus which occupied majority of the cell territory. In the cytoplasm of such cells we found single channels of rough endoplasmic reticulum, small aggregates of polyribosomes, single mitochondria and small vacuoles with thyreoglobulin (used as a marker of differentiation). Comparing those cells to typical cells of papillary carcinomas which had morphological

polarity and elaborated free and lateral surfaces. These cells contained several hormonal vacuoles of different diameters, with well developed Golgi apparatus, with multiple channels of smooth and rough endoplasmic reticulum as well as numerous mitochondria. The aforementioned differences were so impressive that they could be used as a prognostic marker. The second in order of importance morphological finding in the whole studied material was the presence of large nuclei (corresponding to size of 2 or 3 normal nuclei) in well differentiated carcinomas. Such nuclei were oval, euchromatic without typical for papillary carcinomas nuclear membrane grooves. Those just mentioned nuclei in immunohistochemical studies had high levels of cyclin D. Third morphological feature which was found in the whole material it was presence of intracytoplasmic glandular lumina which were already described as typical for papillary carcinomas (and as a marker of inappropriate differentiation). Such phenomenon in our material was found not only in papillary carcinomas but also in follicular variant of this cancer. Concluding our results it could be suggested that presence of cells abnormalities in differentiation and maturation as well as presence of dividing cells might be responsible for progression of the disease.

THYMIC EPITHELIAL CELL DIFFERENTIATION: AN UPDATE

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The thymus provides an optimal and essential microenvironment for maturation of thymocytes and their differentiation into self-restricted, self-tolerant T cells. This microenvironment is formed, first of all, by several distinct epithelial cell types, classified on the basis of their phenotypic traits and anatomical location into superficial, cortical and medullary epithelial cells, the lineage relationships of which are unclear. The thymic epithelium not only supplies a three-dimensional network, it also provides the combination of cellular interactions, growth factors and chemokines typical for the cortex or operating within medulla to promote program of thymocytes differentiation or apoptosis for the generation of non-autoreactive T cell repertoire. In turn, signals from developing thymocytes determine the fate of epithelial cells and also the spatial organization of epithelial network. The processes are initiated at the earlier stage of the thymus organogenesis and involve epithelial mesenchymal interactions in the absence of thymocytes. The patterning and epithelial cell differentiation at the later stage organogenesis are followed by immigration of the thymocyte precursors and initiated through epithelial-thymocyte interaction. Many transcription factors have been demonstrated to be critical for thymus organogenesis and epithelial cell differentiation. However, the molecular mechanisms regulating function of epithelial cells and the lympho-epithelial interaction are not well defined. The controversy regarding thymic epithelial cells involved its origin, physiology and pathology. Although the origin of the thymic anlage in the third pharyngeal pouch endoderm is at present preferentially accepted, it remains controversial (i) whether these morphologically and functionally distinct epithelial cell types arise from common bipotent progenitor/stem cells; (ii) whether the common stem cells for both cortical and medullary epithelium reside in the adult thymus.

HISTOLOGY AND ULTRASTRUCTURALY STUDY OF GROWING AND MATURE ANTLER IN RED DEER (CERVUS ELAPHUS)

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Every year in red deer stag (*Cervus elaphus*) the old set of antlers is shed and replaced with a new one. The periodicity of these processes makes antlers a unique material for research underlying the basic regeneration of mammal tissues. This study aims at presenting histology of terminal fragments growing and mature antlers in red deer. The cross-section of terminal fragments of regenerating antlers depicts 4-fold organisation of an antler. Microscopically, from the outside we can identify: hairy skin, perichondrium, mesenchyme and chondroprogenitors area as well as a central shaft. Epidermis is keratinised stratified squamous epithelium built from five layers of cells: basal, squamous, granular, clear and horny. Dermis consists of proper connective tissue and has two layers: papillary and reticular. The wave-like organisation of the first layer constitutes the basis of good connection with epidermis while the second layer possesses numerous bundles of collagen fibres, hair roots and sebaceous glands. The subcutaneous layer has numerous blood vessels, collagen fibres and adipose tissue cells. Below the skin region, there is a darker staining zone of tissue constituting the perichondrium. The outer part of it is fibrous and the inner part contains cells. Below the perichondrium there is a wide layer of undifferentiated cells constituting mesenchyme of the antler. It surrounds the so-called central shaft built from numerous blood vessels stretching from base to apex of the growing antler. Among vessels there are chondrocytes organised in characteristic columns. In electronographs numerous cells were observed – these were fibroblasts, squamous epithelium cells participating in keratinisation processes and chondroblast cells producing collagen fibres. By performing immunochistochemistry, we found that cell expressing Ki-67 antigen and proliferative cell nuclear antigen (PCNA) antigen were localized in basal layer of epidermis, skin glands and beneath their secretory sections, mesenchyme as well as within and in the vicinity of central blood vessels. Unlike growing antlers, mature antlers are built from mineralised lamellar osseous tissue. The architectural antler units are osteons built from Haver's ducts around which two or three systemic plates are concentrically arranged. Both in and among plates, there are osseous lacunae which possess osteocytes. All osseous lacunae within an osteon are connected by a network of bone canaliculi in which osteocyte processes are located. On the basis of previous study we are already certain that antlerogenic stem cells in the nearest future will find a practical application in broadly defined regenerative medicine.

ACUTE MORPHOLOGIC CHANGES IN RAT LUNGS AFTER EXPOSITION ON HIGH DOSE OF TOBACCO SMOKE

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Tobacco use has accompanied human being for two millennia. First notes announcing negative impacts of the use of nicotine were

reported in the 16th century. Since than consecutive reports have been published. However, majority of reports emphasize results of long term smoking habit. Based on current publications, the most negative consequence of smoking is cancerogenesis of the head and neck, lung, gastrointestinal and others. Furthermore, there are convincing data related to influence of smoking on the development of atherosclerosis and its consequences. There are not enough studies on organ pathology relevant to short term smoking of tobacco. The aim of this study was analysis of changes in upper and lower respiratory tract after exposure to short term inhalation of tobacco smoke. As a model for the experiment, Wistar rats were inhaling tobacco smoke containing 1000mg/m3 of carbon monoxide for 8 hours a day, 5 days a week for 3 weeks in specially controlled toxicological chamber. Subsequently, all respiratory tracts underwent light and electron microscopic study.

Results obtained revealed numerous data indicating acute hypoxic and hypo-perfussive changes in bronchial mucosa and lung parenchyma. Bronchial wall contraction lead to diminishment of its lumen, accompanied by large accumulation of mucous in which epithelial cells, RBC, macrophages were present. Changes were observed in both ciliated cells and mucous producing cells. Many cilia did not have dyneine arms and microtubules in many cilia were singlet instead of doublets. WBC were focally located below the epithelium. Capillaries were also not properly located. In lung parenchyma broadening of inter-alveolar septa by evidently enlarged congested septal capillaries were observed. Additionally transudation/ exudation, broadening of lymphatic spaces and lysis of single cells were present. Single RBC and lymphocytes, and more numerous macrophages in alveolar lumen were observed. Results obtained by this experiment revealed that short lasting inhalation of tobacco smoke induced evident pathologic changes in the lungs. Upper respiratory airway contraction and improper removal of the increased amount of mucous changed the airway's normal function. Peripheral part of pulmonary vessel congestion lead to hypoxia, transudation/exudation, cell lysis and increased production of lung surfactant.

SZKIELET AKTYNOWY W KOMÓRKACH PŁOMYKOWYCH CERKARII DIPLOSTOMUM PSEUDOSPATHACEUM. BADANIA ULTRASTRUKTURALNE I FLUORESCENCYJNE

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Celem pracy było zbadanie występowania i rozmieszczenia F-aktyny w komórkach płomykowych cerkarii *Diplostomum pseudospathaceum* Niew. 1984 (Trematoda, Plathelminthes). Materiał do badań pobierano z naturalnie zarażonych ślimaków *Lymnaea stagnalis* L. pochodzących z jeziora okolic Warszawy. Zwierzęta do badań ultrastrukturalnych przygotowywano wg rutynowych metod stosowanych w mikroskopii elektronowej, natomiast do badań fluorescencyjnych zastosowano metodę z użyciem falloidyny i rodaminy (Czubaj, Niewiadomska, 1997). Badania ultrastrukturalne wykazały obecność gęsto upakowanego, włóknistego materiału w części szczytowej komórki płomykowej oraz kanalikach wydalniczych układu protonefrydialnego. W komórkach płomykowych filamenty występowały w tzw. żeberkach – cienkich wypustkach przypominających stereocilia i tworzących aparat filtracyjny. Były one gęsto upakowane i tworzyły rdzeń w środku każdego żeberka. Średnica filamentów wynosiła ok. 7 nm i odpowiadała średnicy filamentów aktynowych występujących w komórkach innych zwierząt. Potwierdzeniem obecności filamentów aktynowych były badania fluorescencyjne, które wykazały wyraźną fluorescencję świadcząca o dużej zawartości F-aktyny w komórkach. Szczególnie silną fluorescencję stwierdzono w części szczytowej komórki płomykowej odpowiadającej lokalizacji aparatu filtracyjnego. W tej części komórki wykazano wcześniej, przy użyciu TEM, obecność silnie upakowanego materiału włóknistego. Badania ultrastrukturalne i fluorescencyjny wykazały również obecność filamentów aktynowych (F-aktyny) w komórkach nabłonkowych układu protonefrydialnego tworzących ściany kanalików wyprowadzających. Włókienka aktynowe były rozmieszczone obwodowo i tworzyły sieć filamentów podścielających błonę komórkową. Wydaje się, że obecność filamentów aktynowych w komórkach płomykowych, w szczególności w aparacie filtracyjnym, odgrywa istotną rolę w usztywnienie i stabilizacji cytoarchitektury protonefrydium, natomiast wykazanie obecności F-aktyny w komórkach nabłonkowych kanalików wyprowadzających sugeruje udział filamentów aktynowych krążeniu płynu wydalniczego.

Czubaj A, Niewiadomska K: 1997, Acta Parasitologica, 42, 199--218.

ELECTRON MICROSCOPY IS NECESSARY FOR PRECISE DIAGNOSIS OF MEMBRANOUS GLOMERULOPATHY

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The etiology in the majority of membranous glomerulopathy is unknown and the term idiopathic or primary membranous glomerulopathy is used. The mean age of affected patients is typically between 40 and 50 years. Approximately two-thirds of the membranous glomerulopathy patients had nephrotic syndrome at the time of renal biopsy, thus membranous glomerulopathy is the most common cause of idiopathic nephrotic syndrome in adults. Hematuria accompanies proteinuria in 27 to 71% of these cases. By light microscopy the characteristic finding in membranous glomerulopathy is thickening of the capillary walls in the glomeruli. However, in the early stages this thickening is slight, and distinction from minimal change disease may be very difficult. Although minimal change disease is primarily a disease of childhood, this glomerulopathy accounts for around 20-25% of adult nephrotics with well known progressive decline in incidence with age. Moreover, adults show an increased incidence of atypical clinical features such as hypertension, renal function impairment, non-selective proteinuria and microscopic hematuria. These cases of minimal change disease may be clinically very similar to those with membranous glomerulopathy. In view of the above, the role of electron microscopy in distinguishing early cases of membranous glomerulopathy and minimal change disease in adults seems to be crucial, especially when immunoflurescence is not available. Furthermore, mesangiocapillary glomerulonephritis may show capillary wall thickening suggestive of membranous glomerulopathy by light microscopy, because in some cases of mesangiocapillary glomerulonephritis small mesangial expansion and scant hypercellularity is noted. Moreover, as described by Churg and Ehrenreich, four stages of membranous glomerulopathy could be recognized depend on the density and distribution of the electron dense deposits. The size of the electron dense deposits and the thickness of the basement membranes are significantly correlated with clinical parameters such as proteinuria, serum creatinine as well as with prognosis. Therefore,

the electron microscopy seems to be fundamental method in the diagnostic process of the membranous glomerulopathy both to avoid misdiagnosing and to evaluate progression of glomerular basement membrane damage.

ALTERATIONS IN THE NEUROVASCULAR UNIT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS FOLLOWING SURGICAL INJURY

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The blood-brain barrier is the regulated interface between the peripheral circulation and the central nervous system. The anatomical substrate of the blood-brain barrier is the cerebral microvascular endothelium, which together with astrocytes, pericytes, neurons, and the extracellular matrix constitute a neurovascular unit that is the essential for the health and function of the CNS. This study investigated the effects of streptozotocin-induced diabetes following surgical injury on the neurovascular unit in the rat. Electron microscopic ultrastructural, immunohistochemical and immunochemical analysis was performed.

Ultrastructural evaluation of the cerebral specimens using electron microscopy showed changes in all components of the neurovascular unit. Capillaries in cerebral parenchyma showed unusual structure, mostly composed of several layers of endothelial cells. Pericytes contained mostly cytoplasmic phagolysosomes filled with membrane material and lipid droplets suggesting degeneration of these cells. The abluminal surface of the endothelial cells was surrounded by solid, markedly thickened, often branched basal membrane formed from amorphic substance of moderate electron density. Astrocytes in the perivascular zone contained numerous lysosomes and phagolysosomes and markedly dilated cisternae of the Golgi apparatus. Ultrastructural changes were also seen in the neurons contributing to the neurovascular unit. These neurones showed morphological signs of neurodegeneration, and often ultrastructural features of cellular death. Numerous phagolysosomes and autophagic vacuoles were seen in the cytoplasms of many neurons. In addition, some neurons contained electron dense cytoplasm with nucleus separated by a lucid rim from the remainder of the cell. These were so called dark neurons. In addition to dark neurons and neurons undergoing autophagy, the analyzed specimens contained neurons with necrotic features, characterized by electron lucent cytoplasm with distinct nucleus containing loose, electron lucent chromatin. These data were confirmed and extended by immunohistochemical and morphological studies. The light and fluorescent microscopy were used. The early and late degeneration and death of nervous cells were detected in the injured crebral cortex. In the early postlesion time points numerous necrotic neurons were observed. A massive neuronal apoptosis were detected in the late postlesion phase. Apoptotic neurons were localized not only within injured area, but also in the perilesion cortical regions. The lesion caused the glia response. Two phenomena: a massive but temporary astrogliosis and the immediate formation of glia scar were detected in the injured cortex. Reactive astroglial cells showed hypertrophic appearance. In the late postlesion phase degenerative chnages in the astrocytes were detected. Immunochemical studies confirmed this observation and showed decrease of GFAP level in the late time points.

THE EFFECT OF CHEMICAL COMPOUNDS (ARSENIC TRIOXIDE AND G-CSF) ON ACTIN REORGANIZATION IN HL-60 PROMYELOCYTIC LEUKEMIA CELLS

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The main object of this study was the analysis of actin reorganization in whole cells and nuclei isolated from the human leukemia cell line (HL-60) after treatment. Three different doses of anticancer drug arsenic (0,6 μg/ml, 1,2 μg/ml, 2,4μg/ml) and two different doses of the second agent – G-CSF (Granulocyte-Colony Stimulating Factor) (5 ng/ml, 10ng/ml), were applied for 24 hours in culture. The aim of the study was to investigate the rearrangement of actin, using classical fluorescence microscopy, transmission electron microscopy and flow cytometry. After isolation and purification of nuclei in gradient of glycerol, purity and integrity were determined spectrophotometrically and by means of transmission electron microscopy. Changes in actin organization were reflected by fluorescent staining with phallacidin-BODIPY, whereas changes in the protein level were assessed using flow cytometry. Some symptoms of apoptotic cell death occurred even at the lowest dose of arsenic. Moreover, at higher drug concentrations there was a tendency toward an increase in the number of cells displaying apoptotic features. The treatment with arsenic also resulted in changes of intracellular actin localization. Fluorescence and electron microscopy observations revealed characteristic apoptotic morphology. The presence of the cytokine in the cell environment was not only accompanied by increased F-actin labeling in the cytoplasm, but also by a weaker intensity of peripheral ring staining in comparison with control. In spite of the fact that HL-60 cells exposed to G-CSF contained different F-actin forms such as aggregates and the network, the rate of actin polymerization was not significantly enhanced. Moreover, alterations relied mainly on considerable changes in the relative proportion of these varying structures. These might be related to specific features of the differentiation process observed by us, including low apoptosis frequency, the G1 to S phase transition in the cell cycle, as well as possible alternative ways of the cell death. Thus, the results obtained in our experimental conditions re**veal** unique characteristics of actin reorganization, which is related to both the proapoptotic and the differentiating effect of arsenic trioxide and G-CSF, respectively.

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CYTOSKELETON REORGANIZATION IN CHO AA8 CELL LINE AFTER APOPTOSIS AND MITOTIC CATASTROPHE INDUCTION

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The aim of this study was to examine reorganization of the actin cytoskeleton in CHO AA8 cell line after induction of cell death by apoptosis and mitotic catastrophe. In this experimental model we used hyperthermia – non-invasive and toxic-free method. Materials and Methods: Chinese hamster ovary cells, (CHO AA8) were cultured in minimum essential medium eagle (MEM). The effects of elevated temperature exposure on morphology and actin cytoskeleton was analyzed by fluorescent microscopy. Electron microscopy (including immuno-gold technique for actine localization) was also used. Flow cytometry (TUNEL and Annexin V-FITC/propidium iodide assays) were used for identification of cells death. Results: Hyperthermia caused inhibition of cell proliferation and changes in cell morphology. We observed two types of the cells: rounding and detached from the substratum as well as flattened and giant multinucleated. There were also the shrinked cells with bubbles on their surface. Characteristic fluorescence staining pattern of F-actin organization was seen in controls. F-actin was demonstrated as extensive arrays network and stress fibers in giant and flattened cells. There were also seen cells with strong labeling in which the enlarged nuclei showed increased F-actin labeling comparing to other cells. In the rounded, shrinking ones strong labeling was observed in the centre of the cells as well as in the buds on the surface. On the ultrastructural level, in the nucleus of hyperthermic treated CHO AA8 cells, actin was localized especially in the areas of chromatin condensation. Cell cycle analysis revealed increase cell polyploidy and significant increase in the percentage of cells with DNA fragmentation estimated by TUNEL assay. Conclusions: CHO AA8 cells following elevated temperature exposure underwent two different mode of cell death: apoptosis and mitotic catastrophe. It is also suggested that reorganization of actin system is involved in both processes.

APPLICATION OF SCANNING ELECTRON MICROSCOPY IN DIAGNOSTICS OF PLANT EPIDERMIS

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The epidermal surfaces of aerial plant organs (e.g. leaves, stems, flowers, fruits, seeds) are covered by epicuticular waxes that form an important physiological and ecological interface between a plant and its environment. Epicuticular waxes have diverse crystallization patterns, chemical compositions, and relative abundance that change with plant age, development, and environment. The physical and chemical properties of these surface waxes play role as barriers to a variety of biotic and abiotic stresses, including those caused by fungal pathogens, phytophagous insects, drought, solar radiation, freezing temperatures, mechanical abrasion, and anthropogenic influences. It is generally assumed that epicuticular wax layer forms the true surface of epidermal cells and therefore they tend to be diagnostic for environmental condition. Today, scanning electron microscopy (SEM) is the most powerful technique for investigating the fine structure of plant surface waxes. It application has helped to improve our knowledge of the wide range of forms of epicuticular wax aggregates and their spatial distribution. In botanical literature, classification of epicuticular waxes is based on detailed SEM study of over 13,000 angiosperm species that identified 23 wax types (Barthlott et al, 1998). This system has been used in the characterization of leaf waxes of various plant taxa. In cultivated *Brassica* species, we are not aware of any comparative SEM studies on the structure of their epicuticular wax. The objective of the present study was to investigate the structure of the epicuticular leaf waxes of selected *Brassica* species (*B. campestris*, *B. oleracea* and their hybrid *B. napus*) using scanning electron microscopy (SEM) and to study the natural reflectance of the wax and the auto-fluorescence of the underlying layers of epidermal cell walls using confocal laser scanning microscopy (CLSM). Macroscopically, waxes were visible on *Brassica* primary surfaces of leaves as a bluish-white colored coating, called glaucousness or waxy bloom. The SEM analysis of the leaf wax coating showed differences between the examined *Brassica* species. The epicuticular wax was organized in three layers, i.e. a continuous sheet, flat crystals and upright crystal forms. These wax crystals were composed of rods, tubes, vertical plates, and dendritic-like structures. SEM observations of plant surface topography revealed that the wax pattern of all accessions of *B. napus* was intermediate between the putative parents, suggesting its hybrid nature. In conclusion, the present study supported the use of epicuticular wax pattern as an important diagnostic parametr for identification of *Brassica* species and provided more robust information about phylogenetic affinities between these species.

EFFECTS OF COMBINED THERAPY WITH DOXORUBICIN PLUS TEMPOL IN EXPERIMENTAL MODEL OF CAERULEIN-INDUCED ACUTE PANCREATITIS: AN ULTRASTRUCTURAL STUDY

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The aim of this study was to limit autodestructive processes in acute pancreatitis (AP) by lowering the excessive production of pancreatic enzymes and preventing oxidative stress. For this reason doxorubicine (Dox) and 4-hydroxy-TEMPO (tempol) were applied. This study was conducted on adult male Wistar rats weighing 340±30 g of body mass. Before the experiment, the rats were deprived of food for approximately 24 hours, water was available in unrestricted quantities. All experimental procedures are in compliance with currently accepted guidelines for care and use of animals. AP was induced by four-fold s.c. injection of caerulein with 60 min. intervals in the dose of 15 μg/kg. Rats were randomised to control or caerulein-induced pancreatitis groups, treated with saline or tempol (250 mg/kg) and/or Dox (4 mg/kg). After 6 hours, the animals were sacrified and pancreas was removed for ultrastructural studies.

Results: In control animals treated with physiological saline or tempol, the ultrastructure of rat's pancreas was normal, but after application of Dox, a small edema and vasodilatation were observed. There were a few acinar cells with widening rough and smooth endoplasmic reticulum channels. In rats receiving caerulein injection, pancreas was considerable edemic with broaden vessels, sometimes with necrotic endocytes and with polimorphonuclear infiltration. There were numerous acinar cells with deformation of RER (rough endotelium reticulum). Secretory cells had swollen mitochondria, very large vacuoles with myeline figures and many lysosomes, zymogen and condensing granules diversified in size and density. Inside interlobular areas there were squamoused acinar cells with degenera-

tive organelles. The erythrocyte in the extracellular parenchyma testified to a more permeability of capillary walls.In Dox administered rats accompanied with AP a moderate edema and vasodilatation was observed in pancreas. Inside intralobular areas there were number of active macrophages and fibroblasts with collagen fibres without leukocytes. Tempol injection to rats with AP resulted in small edematous changes and appearance of many small capillaries. In the interstitium there were numerous fibroblasts, macrophages and apoptotic bodies. Some acinar cells had slightly delated RER, vacuoles, swallen mitochondria, autophagosomes and sometimes nuclei with partly compacted chromatin on theirs edges. In rats with AP receiving Dox and tempol, pancreas cells had almost normal ultrastructure, but in cytoplasm there were numbers of lysosomes, moderate widen RER channels and large vacuoles with phagocyted apoptotic bodies. Some secretory cells had attributes of apoptosis and in the parenchyma there were many macrophages and apoptotic cells.

Conclusions: Caerulein impairs the pancreas through slowing down exocytosis of digestive enzymes that provokes the autolysis of acinar cells and causes polimorphonuclear infiltration with frustrated phagocytosis. Doxorubicin is the cytostatic antibiotic attenuating an activation both of acinar and inflammatory cells. Dox tends to bind to DNA and causes damage the double helix, as well as, inhibits RNA synthesis.The application of 4-Hydroxy-Tempo produces an antioxidative effect, and the combined administration of Dox and tempol inhibits infiltrating leukocytes and protects pancreatic cells and capillary walls through increasing apoptosis-"programmed cell death", which eliminates cells with destructed DNA. It names "scavengering".

ZMIANY ULTRASTRUKTURALNE HEPATOCYTÓW W PRZEWLEKŁYM DŁUGOTRWAŁYM ZAKAŻENIU WIRUSEM C ZAPALENIA WĄTROBY (HCV)

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Częstość występowania przewlekłego zapalenia wątroby typu C wynosi od 47-81% zakażonych. Zakażenie HCV jest przyczyną zwiększonego ryzyka rozwoju marskości wątroby oraz raka z komórek wątrobowych (HCC). Połączenie klasycznych technik mikroskopowych i metod biologii molekularnej pozwala na wyjaśnianie korelacji między dystrybucją wirusa i morfologicznym uszkodzeniem wątroby, immunologiczną odpowiedzią gospodarza i objawami klinicznymi w naturalnym przebiegu zakażenia. W pracy podjęto próbę określenia powtarzalnych zmian morfologicznych w wątrobie na poziomie mikroskopu świetlnego i elektronowego w przewlekłym zapaleniu wątroby typu C u dorosłych z długotrwałym (około 20 lat) zakażeniem HCV. Do badań zakwalifikowano biopunktaty wątroby 19 dorosłych pacjentów, z czynną replikacją HCV, przed leczeniem przeciwwirusowym, po wykluczeniu innych przyczyn zmian w wątrobie. Zmiany morfologiczne narządu w mikroskopie świetlnym oraz stopień aktywności zapalnej (*grading*, G1 i G2) i zaawansowania włóknienia w wątrobie (*staging,* S) oceniano w skali 4-punktowej. Średnie nasilenie zmian zapalnych (suma G1 i G2) wynosiło 4.0±0.4, *staging* - 2.6±0.3. U 11 pacjentów wykazano morfologiczne cechy marskości wątroby. Wspólną cechą w obrazie mikroskopu świetlnego było obrzmienie, zwakuolizowanie cytoplazmy (często liza), cechy stłuszczenia drobno- i wielkokropelkowego hepatocytów oraz cholestazy. Powtarzalną zmianą w całym materiale była różna wielkość, liczba i kształt jąder komórkowych. Chromatyna jądrowa ogniskowo ulegała rozrzedzeniu. Obrazowi „pustych" jąder z mikroskopii świetlnej, odpowiadał ubytek chromatyny na terenie całych jąder i gromadzenie się struktur tubularnych przy błonie jądrowej w obrazie utrastrukturalnym. Na terenie powiększonych jąder stwierdzano zwiększoną liczbę ziaren peri- i interchromatynowych. Ponadto występowały struktury fibrylarno-tubularne o dużej gęstości, niekiedy rozgałęzione. Jedynie pojedyncze hepatocyty wykazywały cechy apoptozy. Zmianom jądrowym w tych samych komórkach z reguły towarzyszyły zmiany układu błon RER. Była ona poszerzona i silnie osmofilna, zwłaszcza w obrębie jej zdegranulowanych fragmentów. Kanały ER były poszerzone, z drobnowłókienkowym materiałem w świetle. Te odcinki błon często tworzyły struktury podkowiaste. Inną modyfikacją błon ER było jej pofałdowanie lub wgłabianie się (tzw. błony „ondulowane"). W badaniu ultrastrukturalnym obserwowano także patologię mitochondriów. Miały one znacznie rozjaśnioną, drobnoziarnistą obrzmiałą macierz z krótkimi grzebieniami, których błony tworzyły liczne struktury podkowiaste. Nie obserwowano typowych cząstek wirusopodobnych w hepatocytach. Badania nasze potwierdzają zmiany w jądrach komórkowych jako najbardziej charakterystyczną cechę zarówno w mikroskopie świetlnym, jak i elektronowym także w odniesieniu do długotrwałego zakażenia HCV. Wskazują na potencjalny udział zwłaszcza tych organelli w zaburzeniu funkcji całej komórki. Zmiany cytoplazmatyczne dotyczą przede wszystkim błon ER oraz mitochondriów i wiążą się prawdopodobnie z nasiloną syntezą białek wirusowych.

ULTRASTRUCTURAL EVALUATION OF KIDNEYS IN RATS EXPOSED TO ADRIAMYCIN

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Adriamycin, an antracyklin antibiotic, has been used in oncologic chemotherapy for almost forty years. It has a wide antineoplastic spectrum and is used to treat acute leukemia, Hodgkin's disease, soft tissue sarcoma, breast cancer, lung cancer and others.However, increased doses of adriamycin to provide better therapeutic effect were found to be associated with organ toxicity.The aim of the present study was to evaluate ultrastructural changes within kidney in rats exposed to adriamycin. Moreover, the selected biochemical parameters were assessed in the serum. The examinations were performed in Wistar rats randomly divided into 10 experimental groups, 8 animals each. Animals were administered i.p. doxorubicin hydrochloride in the dose of 2 mg/kg body weight bw, 5 mg/kg body wt,15 mg/kg body wt. The control group received i.p. saline. In each experimental group, blood for biochemical examinations was sampled from the carotid arteries and kidneys were removed for histological and ultrastructural examination after 4, 48 and 96 hours.The lesions in the proximal tubules were observed under the light microscope 48 h after the administration of adriamycin in the dose of 5mg.kg body wt., which increased 96

hours after the exposure to the same dose. The lumen of proximal tubules was focally dilated, epithelial cells showed features of vacuolar degeneration. Moreover, single protein casts were found in the tubular lumen and interstitial oedema was observed. Epithelial desquamation to the lumen of proximal tubules was also visible. The changes described markedly increased after 96 hours. The ultrastructural changes in rat kidneys were visible 48 h after the administration of 5mg/kg body wt. of adriamycin. Oedema and proliferation of podocyte cells were observed within renal glomeruli accompanied by focal fusion of their foot processes, dilated canals of the rough endoplasmic reticulum and vacuoles of various sizes within the podocyte cytoplasm. The mitochondrial matrix was oedematous with total or partial loss of mitochondrial crests. The abnormalities described above increased 96 hours after the administration of 5mg/kg body wt. of adriamycin. Similar abnormalities were observed within the glomeruli 48 and 96 h after the exposure to 15mg/kg body wt. of adriamycin. However, the changes were found to be substantially more intensive and extensive. Forty-eight hours after the administration of 5mg/kg body wt. of adriamycin, numerous, bright vacuoles and enlarged lisosomes located at the base of the brush border were visible in proximal tubules. Oedematous mitochondria with partial or total loss of mitochondrial crests were observed. The above changes increased 96 h after the exposure to the same dose of adriamycin. Similar lesions were visible 48h after the administration of 15 mg/kg body wt. of adriamycin. In this group, focal flattening, loss or desquamation of the brush border to the lumen of proximal tubules were observed as well as desquamation of the epithelial cells. These changes were more prominent 96 h after the administration of the same dose of adriamycin.The histological and ultrastructural changes observed in kidneys were more severe with an increase in the adriamycin dose and indicated its nephrotoxic effects.

VARIABILITY OF HEPATOCYTES AS MORPHOLOGICAL ANSWER ON ASSOCIATED GIVING OF CEFALOSPORIN AND ETHANOL

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First generation cefalosporins are antibiotics commonly used in the treatment of infections caused by: Streptococcus spp., beta-hemolyzing Streptococcus, Streptococcus pneumoniae, Staphylococcus spp., Staphylococcus aureus, including the strains producing beta-lactamases. In addition they present variable activity in relation to the following strains: Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae. In the course of therapy using some of the drugs from this group there is observed a periodical increase in the level of liver index enzymes - ASPAT and ALAT in blood, and also a temporary hepatitis and/or cholestatic jaundice. There is a common opinion which has been confirmed by laboratory examinations that changes of biochemical indices in blood which are responsible for detecting liver functional disorders usually correlate with the range of hepatocyte damage. The aim of the study was to assess the influence of Cefalexin on the ultrastructure of hepatocyte nuclei. The experiment was conducted on male rats of Wistar strain. In the experimental model assumed in this study Cefalexin was administered in a single dose of 42 mg/24 hours, intragastrically through a tube, for two weeks to the animals with non-damaged livers from group I and group II of animals with damaged livers as a result of two-week intoxication with ethanol which preceded two-week combined administration of the drug with 20% ethanol. 20% ethanol solution used in the experiment has a hepatotoxic effect and is an additional agent harmful to the liver. The samples taken for the analyses were prepared following standard procedures used in ultrastructural examinations.

Comparative observations focusing on the ultrastructure of hepatocyte nuclei, which were conducted in relation to the samples obtained from the control group animals, revealed the most advanced changes in ultrastructural image in the samples taken from the group of animals receiving ethanol and the examined drug. These changes were mainly in the structure and distribution of heterochromatin. There were found multiple plications of nuclear membrane and the presence of false nuclear inclusions. Changes of lower intensity were present in the examined samples obtained from the animals receiving only Cefalexin.

SCANING ELECTRON MICROSCOPE STUDY OF DISTRACTION OSTEOGENESIS

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In the process of distraction osteogenesis, several processes facilitate the proliferation and protection of the areas surrounding new bone regenerate formation. The length of the process is relevant to the extent of the injury performed at the beginning of the process.

The process of the entire regeneration of the bone can depend upon the numerous factors as age, sex, hormonal and vitamin status and many others. While the bone formation usually spans the entire duration of the healing process, in some instances, bone marrow within the fracture having healed two or fewer weeks before the final remodeling phase. There are three major phases of bone regeneration, 1. reactive phase, 2. distraction phase with final callus formation and trabecular bone formation, 3. remodeling phase. Today use of scanning electron microscope give us opportunity to study not only localization of bone cells and changes in bone fibroarchitecture but also composition of bone matrix. We have observed changes in calcium and phosphorus deposit within bone matrix in regard to bone biomechanical properties. We found that there exist on both periods important balance between increased bone stiffness end elastic deformations which- allow necessary adaptations to the acting forces and final adhesion of broken ends. This make possible the accommodation and revision of bone regenerate formation through the entire period of distraction osteogenesis.

DOES THE POSTMENOPAUSAL OVARY PLAY AN IMPORTENT PART IN A WOMAN'S LIFE?

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The ovary undergoes several changes after menopause. The structure of the postmenopausal ovary significantly differs from that of reproductive-age. These differences are related to changes

which concern both medulla and cortex. It is known that after menopause, ovaries are not replaced by hormonally inactive connective tissue. They produce about 50% of androgens circulating in blood, especially testosterone and androstendione, and also estrogens, however their number is much smaller than before menopause. It has not been examined yet, what is the dynamics of androgen receptor (AR), estrogen receptor (ER), progesterone receptor (PR) and gonadotropin receptors (FSHR) and (LHR) distribution during this period, and how it depends on the time from the last menstruation. The aim of this study was to investigate the role of the lapse of time from the last menstruation on the ovarian morphology in postmenopausal women and the assessment of immunolocalization and immunoexpresion of steroid receptors: AR, ERα, PR and FSHR, LHR in the ovaries of postmenopausal women. The study involved 50 postmenopausal women whose ovaries were removed by laparotomy because of non-neoplastic diseases of the uterus. The women were divided into 3 groups depending on the time which passed from the last menstruation. Group A consisted of the women who had the last menstruation no more than 5 years earlier. Group B was composed of the women whose menopause appeared 5 to 10 years earlier. Group C included the patients whose last menstruation was over 10 years earlier. The paraffin-embedded specimens fixed in 4% buffered formalin were used for immunohistochemical expression of AR, ERα, PR and FSHR, LHR. Next, the sections were incubated with monoclonal mouse anti-human antibodys for: androgen, estrogen α, progesterone receptors (Dako, Denmark) and follitropin and luteotropin receptors (Sigma, USA). In conclusion, immunohistochemical nuclear expression of AR and PR was detected in all three groups: in ovarian surface epithelium, epithelial inclusion cysts and in stromal cells; it seems to be similar in the ovaries of women examined no more than 5 years after menopause and more than 10 years. Immunohistochemical nuclear expression of ERα seems to decrease in the ovaries of women after 5 and 10 years from menopause. In the ovaries of postmenopausal women FSHR and LHR were detected in all three groups. Their expression decreased with age and depended on the time from menopause. The weaker expression of these receptors was in postmenopausal women's ovaries, the higher FSH and LH concentrations were in blood serum.

MORPHOLOGICAL EVALUATION OF BRAIN'S BLOOD VESSELS IN SSPE

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In SSPE –viral and inflammatory disease of CNS, characterised by late manifestation of persistent infection by a mutant form of measles virus, around vessels were visible inflammatory infiltrations. Numerous and rowed infiltrations were observed in acute cases and the most changed area of brains. Histological and immunohistochemical examinations showed lymphocytes T, plasma cells and microglia in perivascular inflammatory cuffs. At ultrastructural level, different morphological forms of microglia including the macrophages with numerous vacuoles in abundant cytoplasm were found. Inflammatory cells were also located in vessel wall, between lamellae of basement membrane. In acute cases electron microscope revealed the diversified damages of endothelial and smooth muscle cells. We found the viral nucleocapsids in nuclei of endothelial and some inflammatory cells. In chronic cases the most destroyed vascular smooth muscle cells as well as collagen accumulation in thickened basement membrane were visible. Clusters of collagen fibres in external layer of degenerated vascular smooth muscle cells were also observed. Our results revealed that degree of damage of cells in vascular wall may be associated with duration of SSPE.

ORGANIZATION OF SEMINIFEROUS EPITHELIUM AND EXPRESSION OF GHRELIN RAT TESTIS. AN IMMUNOHISTOCHEMICAL AND HYBRIDOCYTOCHEMICAL STUDY

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The objectives of the study was an analysis of distribution of immunohistochemical and hybridocytochemical reaction for ghrelin, its receptor and their transcripts taking into account functional compartments and the cycle of seminiferous epithelium and phases of spermatogenesis.

Ghrelin and its receptors in the rat male gonads was demonstrated immunohistochemically, in paraffin sections fixed in Bouin fluid or formaldehyde, using antibodies (anti-ghrelin and anti-GHSR1a *Phoenix-Pharmaceuticals*); peroxidase and alkaline phosphatase were used as markers of the immune reaction. For positive control an expression of the hormone in ghrelin cells of stomach of the same subjects was chosen and for the negative control IgG and complete rabbit serum were used instead antibodies. Expression of genes for ghrelin and their receptors in rat testis was demonstrated with RT-PCR technique and also with *in situ* hybridisation technique in formaldehyde fixed sections using digoxygenin-labelled oligoprobes (5'-TTA GCT GGC GCC TCT TTG ACC TCT TC-3') specific for rat ghrelin and (5'-ACA CCA CCA CAG CAA GCA TCT TCA C-3') for rat ghrelin receptor mRNA; ovine anti-digoxygenin monoclonal antibodies (Fab fragments) were used to visualize the reaction site immunohistochemically.In seminiferous epithelium the expression of ghrelin, its receptors and their transcripts was found localized at site of nuclei of spermatogenetic cells and specifically at selective steps of spermatogenesis. Expression of ghrelin and its receptor occurs in some spermatogonia and leptotenes, disappears in pachytenes and reappears in spermatids at advanced acrosome step of spermiogenesis. Finally, expression of these proteins declines in maturation phase of the process and disappears again when elongation of spermatids was progressed. In turn, the transcripts for ghrelin and its receptor were found to be expressed in some spermatogonia and from meiotic pachytenes up to acrosome phase of spermiogenesis.

Basing on the the results we suggest an existence of ghrelin signaling system, neuropeptide and its functional receptor, in the rat seminiferous epithelium as a member of the local regulatory mechanism for spermatogenesis that seems to interact with c-kit signaling system.

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MORPHOLOGICAL CHANGES IN - AMANITIN EXPOSED HEPATOCYTES

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Death in "death cap" (*Amanita phalloides*) poisoning is caused principally by acute failure of the liver, which takes up 57 % of circulating α-amanitin, the main toxin of this mushroom. The α-amanitin uptake by hepatocytes is mediated by organic anion-transporting polypeptide located in plasma membrane. Subsequently, it binds to RPB1 subunit of RNA polymerase II, thereby blocking synthesis of cellular enzymes and leading to cell death. In *Amanita phalloides* poisoning clinical symptoms of intoxication (diarrhea, abdominal pain, vomiting) occur usually 10-12 h after toadstool ingestion, but clinical and laboratory symptoms of acute liver damage develop usually on 2-3 day after death cap ingestion. Little is known about the morphological changes in hepatocytes in the first phase (in the latent period) of *Amanita phalloides* intoxication. The aim of present experiment was to study the early ultrastructural changes in α-amanitin exposed hepatocytes using canine hepatocyte cultures.

α-Amanitin was added to the cell culture at concentration 10 μM and incubated for 6h. Cells were prepared for electron microscopy routinely. Our EM studies revealed clearly that amanitin causes ultrastructural alterations in cultured hepatocytes. In cells of the experimental group the remarkable alterations were observed in mitochondria (mild to severe swelling and/or disappearance of cristae, collection of amorphous material in a very bright matrix, presence of focal brakes of the outer membrane) and in endoplasmic reticulum (vacuolization, dilatation and defragmentation of ER). In many cells the appearance of pale, foamlike cytoplasm ("empty cytoplasm") was observed. α-Amanitin exposed hepatocytes displayed the both form of cell death, necrosis (swollen and ruptured intracellular organelles, and cytoplasm blebs) and apoptosis (cell fragmentation into apoptotic bodies). In this study we have demonstrated by electron microscopy that the ultrastructural changes in hepatocytes are apparent within 6 h after administration of α-amanitin at concentration 10 μM.

DOUBLE PITUITARY ADENOMAS – ELECTRON MICROSCOPY AND IMMUNOHISTOCHEMICAL EVALUATION

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Pituitary adenomas are benign tumors arising from adenohypophyseal cells. They cause clinical syndromes attributed to overproduction or insufficient secretion of pituitary hormones and/or local mass effects. Double pituitary adenomas are very rare and the most common clinical feature in a previously reported patients has been acromegaly. Among 847 cases of pituitary adenoma surgically treated between 2003-2008 we encountered four patients with clearly separated double pituitary adenomas. In all cases preoperative MR imaging suggested the presence of two different tumors in the sella. Two patients (1 man and 1 woman) manifested acromegalic symptoms, 1 man presented acromegaly and subclinical Cushing's disease and in 1 woman mild hyperprolactinemia was diagnosed.On histological, immunohistochemical and ultrastructural examination of the surgical specimen a double adenoma was found, consisting of: (1) – densely granulated somatotroph and gonadotroph adenomas, (2) – sparsely granulated and densely granulated somatotroph adenomas, (3)] – silent densely granulated somatotroph and sparsely granulated lactotroph adenomas and (4) – silent corticotroph adenoma of type 2 and densely granulated somatotroph adenoma, all separated by unaffected pituitary tissue. In the presented cases detailed morphologic studies with immunohistochemical and electron microscopic examination to establish the presence of two distinct tumors composed of two different cell types with multidirectional phenotypic differentiation were important. Therefore, we suggest that all double adenomas must be examined not only by conventional light microscopy, but also by electron microscopy to achieve a correct morphological diagnosis.

ROLE OF AN ELECTRON MICROSCOPY IN THE EVALUATION OF CLINICALLY NONFUNCTIONING PITUITARY TUMORS

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Pituitary adenomas originating in adenohypophysial cells represent the most common neoplasms of the sella turcica. They cause clinical syndromes attributed to overproduction or insufficient secretion of pituitary hormones and/or local mass effects. About 30% of surgically treated pituitary adenomas are not associated with hormonal hypersecretion (so called clinically nonfunctioning pituitary adenomas). The development of immunohistochemical techniques has allowed the demonstration of a subgroup of tumors nonfunctioning but immunopositive for pituitary hormones or/and subunits. These tumors has been described as silent adenomas. There is a group of tumors showing heterogenous morphological features at ultrastructural level.

Immunohistochemical evaluation is considered as the "gold diagnostic standard" in the current WHO classification of pituitary adenomas. However, immunohistochemical profiles may overlap. Ultrastructural features of these tumors are essential to reach a correct diagnosis of adenomas with specific biologic behavior and prognosis. Electron microscopy remains the only tool to resolve diagnostic problems in some unusual cases. The aim of this study was the evaluation of immunohistochemical and ultrastructural

methods utility in prognostic evaluation of clinically nonfunctioning pituitary adenomas.Material and methods: Among 1263 pituitary tumors diagnosed between 1998 and 2008 at the Department of Pathology of Cancer Center in Warsaw there were 1087 adenomas. About of 35% of all cases were clinically nonfunctioning pituitary adenomas. All cases were studied by light and electron microscopy and immunohistochemistry. Selected cases were examined using a postembedding immunogold technique. The proliferative index was determined by MIB-1 immunohistochemistry. Results: Among of 418 clinically nonfunctioning pituitary adenomas in most cases gonadotroph adenomas were diagnosed, usually silent. Remaining 83 cases were diagnosed as silent adenomas: 30 of silent corticotroph adenomas (I, II and Crooke's type), 11 of silent thyrotroph, 23 of silent lactotroph, 13 of silent somatotroph adenomas. Silent adenoma III type were revealed by electron microscopy in 4 cases. All tumors were diagnosed as macroadenomas, in 26% with invasion of the surrounding structures. The proliferative index had low values (0 – 3%; in exceptions >3%). Conclusions: Immunohistochemical evaluation permits the diagnosis of silent adenomas associated with poor prognosis. In the cases of overlapping immunohistochemical profiles ultrastructural analysis is valuable to provide a useful information. Morphologic diagnosis of some subtypes of pituitary adenomas by electron microscopy provided essential data to classify and to introduce proper postoperative management. Proliferative markers, as MIB-1 may be helpful in diagnosis of low differentiated invasive tumors.

INVOLVEMENT OF DENDRITIC CELLS IN ORGANIZATION OF HASSALL'S CORPUSCLES IN RAT THYMUS

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Manifestation and organization of Hassall's corpuscles (Hc) was studied in thymuses of rat foetuses and in the neonatal period. Particular attention was paid to ultrastructural traits of cells within these structures, among which, apart from medullary epithelial cells (m-TEC), dendritic cells (Dc) were identified. The first Hc were noted in 17th day after birth. Attention was given to stages in which Hc were formed by m-TEC. At the early stage of Hc organization, clusters of medullary TEC were surrounded by Dc and ultrastructural traits of interaction were detected between the latter cells. At subsequent stages of Hc formation, Dc projections formed laminae which surrounded the central cell of the structure. In ME observations Dc were characterised by an electron-dense cytoplasm which formed numerous branches with a clearly marked cortex on the periphery and presence of Birbeck's granules. M-TEC forming Hc demonstrated ultrastructural traits of secretory cells and contained numerous electron-dense vesicles, some in a direct contact with the cell membrane. At subsequent stages processes of apoptosis/proliferation of thymocyte in vicinity of Hc were analysed. Moreover, Dc isolated from the thymuses were characterized in respect to their CD11c, MHC II, langerin phenotype.

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ULTRASTRUKTURA OSIERDZIA U DZIECI Z WRODZONYMI WADAMI SERCA

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Nieprawidłowe odżywienie wielu tkanek u dzieci z wrodzonymi wadami serca sprzyja częstym infekcjom i przewlekłym stanom zapalnym, które mogą swym zasięgiem obejmować osierdzie dodatkowo upośledzając pracę serca. Zakres zmian morfologicznych jakie występują w osierdziu u dzieci poddawanych rekonstrukcyjnym zabiegom kardiochirurgicznym nie został w pełni poznany, chociaż może mieć istotne znaczenie dla prowadzenia terapii w okresie rekonwalescencji. Badania przeprowadzono na fragmentach osierdzia pobranych w czasie trwania zabiegów chirurgicznych od dzieci w wieku do lat 3 cierpiących z powodu wrodzonych ubytków w ścianie przegrody międzyprzedsionkowej lub międzykomorowej. Pobierano zawsze przednie osierdzie nad aortą zgodnie z ustaleniami komisji etycznej. Osierdzie od pacjentów kontrolnych, bez wad wrodzonych serca, otrzymano podczas autopsji. Uzyskany fragment osierdzia dzielono na trzy części z przeznaczeniem do badań biochemicznych, immunohistochemicznych i ultrastrukturalnych. Tkanki utrwalone zatapiano w eponie lub parafinie. U wszystkich dzieci z wadami serca zmiany morfologiczne występowały w nabłonku (mesothelium) i części włóknistej osierdzia oraz w warstwie podosierdziowej. Nabłonek osierdzia tworzył wielowarstwowe układy komórek, wpuklające się do światła komór w postaci pseudo–kosmków. Większość bardziej powierzchownie leżących komórek ulegała zwyrodnieniu i złuszczeniu. W wielu miejscach, nabłonek był nieobecny odsłaniając włóknistą część osierdzia, często silnie sfałdowaną W warstwie tej podobnie jak w warstwie podosierdziowej obserwowano liczne naczynia krwionośne szczelnie wypełnione krwią.

W ich otoczeniu obecne były liczne komórki krwiopochodne. Wśród nich wykryto tryptazowo-immunododatnie komórki tuczne oraz ściśle przylegające do ścian tych naczyń, komórki tłuszczowe. Całość obrazu morfologicznego osierdzia dzieci z wadami wrodzonymi serca sugerowała, że toczył się w nim przewlekły proces zapalny. Polegał on przede wszystkim na zwyrodnieniu i upośledzeniu zdolności regeneracyjnych nabłonka, nie obserwowano natomiast zmian wytwórczych prowadzących do włóknienia osierdzia.

ZMIANY ULTRASTRUKTURALNE I OBECNOŚĆ BIAŁKA AMYLOIDOWEGO W SPLOCIE NACZYNIÓWKOWYM U OSÓB ZE ZWYRODNIENIAMI PARANOWOTWOROWYMI MÓŻDŻKU

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Prawidłowe funkcjonowanie ośrodkowego układu nerwowego (OUN) związane jest ściśle z homeostazą płynu mózgo-

wo-rdzeniowego produkowanego przez splot naczyniówkowy (SP).

Komórki splotu syntetyzują i uwalniają do płynu szereg substancji biologicznie czynnych oraz uczestniczą w procesie oczyszczania mózgu z produktów metabolizmu, między innymi z różnych fragmentów białka amyloidowego. Rola SP oraz zmiany jakie powstają w nim w przebiegu różnych procesów patologicznych toczących się w organizmie jest stosunkowo mało poznana. Celem obecnych badań było prześledzenie zmian pojawiających się w SP u osób z paranowotworowymi zwyrodnieniami móżdżku (PZM). Badania przeprowadzono na mózgach pobranych podczas autopsji pacjentów z chorobą nowotworową narządów wewnętrznych, u których, w czasie choroby, obserwowano kliniczne objawy uszkodzenia móżdżku lub u których typowe paranowotworowe zmiany zwyrodnieniowe móżdżku wykryto podczas badań mikroskopowych. W odpowiednich wiekowo grupach kontrolnych badano mózgi osób, u których nie znaleziono zmian zwyrodnieniowych lub u których nie wykryto choroby nowotworowej. Pobrane fragmenty mózgu utrwalano i zatapiano w parafinie lub w eponie a skrawki odpowiedniej grubości użyto do badań morfologicznych lub immunocytochemicznych.

U wszystkich osób z PZM obserwowano rozległe uszkodzenia SP. Nasilenie tych uszkodzeń wykazywało wyraźny związek z wiekiem badanej osoby. W grupie do 20 roku życia, zmiany dotyczyły głównie nabłonka splotu i polegały na gromadzeniu się w cytoplazmie tych komórek obfitych złogów lipofuscyny. Złogi te wypełniały często ponad 70% cytoplazmy, spychając jądro na obwód komórki. Połączenia ścisłe między komórkami nabłonka ulegały rozpadowi. Błona podstawna była pofałdowana i pogrubiała a od strony nabłonka widoczne były liczne pęcherzyki pinocytarne. W wielu miejscach pod błoną podstawną gromadziły się włókna tkanki łącznej. U osób powyżej 50 roku życia lipofuscyna w komórkach nabłonka ulegała charakterystycznym zmianom morfologicznym, które polegały na zaniku centralnych części „lipidowych" składowych lipofuscyny i tworzeniu się „rogalików" lub regularnego kształtu „obręczy". Przypominały one kształty tzw. Pierścieni Biondiego, które są obecne w komórkach nabłonka splotu u osób z obfitymi złogami białka amyloidowego w mózgu (blaszki dyfuzyjne, blaszki amyloidowe). Przeprowadzone przez nas badania immunocytochemiczne, wykazały obecność fragmentów białka amyloidowego w opisanych powyżej częściach lipofuscyny. Wykryto je również w naczyniach macierzy splotu oraz na powierzchni błony podstawnej nabłonka. Najcięższe uszkodzenia splotu obserwowano u pacjentów powyżej 60 roku życia, u których do opisanych już zmian dołączały się rozległe zmiany włókniste w obrębie macierzy i w ścianach naczyń krwionośnych z tworzeniem się ognisk martwiczych i rozpadowych. Znaczne części powierzchni splotu były pozbawione nabłonka, który ulegał zwyrodnieniu, rozpadowi lub złuszczeniu do światła komór. W podsumowaniu, należy podkreślić, że wyniki naszych badań wykazały wyraźny związek między nasileniem zmian zwyrodnieniowych w splocie naczyniówkowym a obecnością zmian morfologicznych charakteryzujących paranowotworowe zwyrodnienia móżdżku. Natomiast obecność białka amyloidowego w splocie potwierdza obserwacje o udziale splotu w kliransie tego białka w obrębie bariery krew-płyn mózgowo-rdzeniowy i sugeruje nasilony jego metabolizm u osób z chorobą nowotworową.

WPŁYW PODAWANIA EKSTAKTU Z KORY UNCARIA TOMENTOSA NA ULTRASTRUKTURĘ KOMÓREK L1210 – BADANIA IN VIVO

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Uncaria tomentosa (Wild.) D.C. znana na świecie jako „una de gato", czy „cat's claw" a w Polsce pod nazwą „vilcacora". Jest zdrewniałą lianą należącą do rodziny *Rubiaceae*, rosnącą naturalnie w wilgotnych lasach Ameryki Środkowej i Południowej. Należy do najpopularniejszych peruwiańskich roślin leczniczych. Preparaty przygotowywane z kory, liści czy korzeni były i są podstawą naturalnej medycyny tamtych rejonów świata do dnia dzisiejszego. Właściwości lecznicze warunkuje bardzo bogaty skład chemiczny ekstraktu zawierający liczne, aktywne biologicznie metabolity wtórne takie jak: tetracykliczne i pentacykliczne alkaloidy indolowe i oksoindolowe, triterpeny, glikozydy, flawonoidy, katechiny, procjanidy i sterole. Wodny ekstrakt z kory *U. tomentosa* posiada właściwości cytostatyczne, antykoncepcyjne, przeciwzapalne, antymutagenne i przeciw wirusowe. Celem badań było ustalenie wpływu podawania wodnego ekstraktu z kory *Uncaria tomentosa* na ultrastrukturę komórek L1210 w badaniach *in vivo*. Wodny ekstrakt przygotowano poprzez ekstrakcję suchej, sproszkowanej kory *Uncaria tomentosa* w wodzie (37^0C, 24 godz.). Całkowita zawartość alkaloidów w ekstrakcie wynosiła 0.43 % suchej masy. Głównymi alkaloidami były unkaryna C i izomitrafilina. Do eksperymentu użyto samic wsobnego szczepu myszy Balb/c i hybryd F1 Balb/c x DBA2 w wieku 7-9 tygodni o masie ciała ok. 20 g. Myszy karmiono przez 7 dni ekstraktem w dawce 10 mg/kg oraz wodą (kontrola). Po tym czasie wszczepiano komórki L1210 w okolice podłopatkową i hodowano w formie guza przez 7 dni. Po tym czasie myszy uśpiono a guzy wypreparowano, zmierzono i zważono oraz utrwalono i przygotowano preparaty do transmisyjnego mikroskopu elektronowego wykorzystując standardową procedurę.Wykonane pomiary masy guzów wykazały, że po 7 dniach od wszczepienia komórek L1210 guzy u myszy otrzymujących wyciąg z *U. tomentosa* były o połowę mniejsze niż u osobników kontrolnych przyjmujących wodę.Na podstawie analizy ultrastruktury utrwalonych guzów stwierdzono, że pod wpływem podawania ekstraktu komórki L1210 hodowane podskórnie miały zmienioną ultrastrukturę w porównaniu do komórek kontrolnych. Pod wpływem ekstraktu dochodziło do wyraźnej kondensacji chromatyny jądrowej, głównie w okolicy pod otoczką jądrową. Na terenie cytoplazmy występowały liczne wakuole nie obserwowane w komórkach kontrolnych. Uszkodzeniu ulegały również mitochondria, które miały zaburzony układ grzebieni oraz na terenie matriks często obserwowano przejaśnienia w formie dziur. Na podstawie tych wyników można stwierdzić, że podawanie wodnego ekstraktu z kory *Uncaria tomentosa* hamuje proliferację komórek mysiej białaczki L1210.

MITOCHONDRIAL BIOGENESIS DURING INSULIN-DEPENDENT MYOGENESIS FROM CLONAL MYOGENIC CELLS – THE EFFECTS OF METABOLIC INHIBITORSA

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Several studies including our own (unpublished data) suggest that metabolic differentiation of skeletal type muscles occurs at the late phase of foetal growth [1, 2, 3]. In addition, it was shown that oxidative type muscle fibers are associated with a higher sensitivity to insulin [4]. Quantitative differences in the expression of nuclear and mitochondria coded genes of mitochondrial steplimiting enzymes of respiratory chain: subunit IV of cytochrome c oxidase (COX), and beta subunit of ATP synthase (ATPase) were demonstrated by us. They indicate that the period from 180 to 260 d.p.c in bovine fetuses is characterized by metabolic differentiation and that foetal plasma insulin concentration positively correlates with tissue contents of COX and ATPase subunits. Thus, in bovine fetal muscles the expression of mitochondrial proteins of electron transport chain (ETC) and ATP synthase is increased during last trimester of pregnancy. Moreover, the *in vitro* study has also shown that mitochondrial activity raised in response to insulin in differentiating muscle cells [5]. Additionally, protein kinase kinase/extracellular-signal-regulated kinase (MAPKK/ERK - MEK) inhibitor PD98059 accelerated, whereas either the phosphatidylinositol 3-kinase (PI3-K) inhibitor LY294002 or blockade of mitochondrial respiration both abrogated insulin-mediated myogenesis. Apparently, mitochondrial transmembrane protein called hyperplasia suppressor gene/mitofusin2 (HSG/Mfn2) regulates both mitochondrial fusion (demonstrated by perinuclear mitochondria clustering in electron microscopy) and insulin-dependent myogenesis [6]. Ultrastructural examination did not give any evidence of increased mitochondrial length and interconnectivity after inhibition of PI3-K activity with LY294002. Insulin induced both Mfn2 and subunits I and IV of cytochrome-*c* oxidase (MTCOI and NCOIV) in L6 myoblasts. We found, that inhibition of MEK-dependent signalling pathway elevated Mfn-2 protein level. The molecular mechanism of this phenomenon is unknown, although interaction of Ras with Mfn2 took place unless insulin was present and was blunted after PD98059 co-treatment. It indicates that insulin-mediated myogenesis is increased by inhibition of MEK, most likely by the lack of mitogenic signals opposing muscle differentiation. We conclude that insulin-mediated myogenesis depends on PI3-K activity which results in stimulation of mitochondrial activity and extensive fusion of mitochondria. We suggest, that insulin stimulates Mfn2 protein expression which in turn binds to Ras and inhibits MEK-dependent signalling pathway. At the same time PI3-K-dependent signalling pathway is boosted, the rate of mitochondrial biogenesis is exaggerated resulting in accelerated myogenesis.

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ULTRASTRUCTURAL EVIDENCES OF PROAPOPTOTIC EFFECTS OF BISINDOLYLMALEIMIDE-IX IN TNF-α-RESISTANT COLON ADENOCARCINOMA COLO 205 CELLS

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Human COLO 205 colon adenocarcinoma cells are immune to extrinsic apoptosis induced by immunomodulatory cytokines. Among the antiapoptotic mechanisms responsible for the immune escape, the overexpression of the cFLIP protein seems to be critical. cFLIP appears to inhibit the TNF-α-induced death receptor signal. The application of the metabolic inhibitor bisindolylmaleimide IX (Bis-IX), known as a potent PKC repressor, sensitized COLO 205 cells to TNF-α-mediated apoptosis. Apoptosis was demonstrated by several methods, however, spectacular view was obtained from ultrastructural examination with electron microscopy. Moreover, the Western-blot analysis revealed that the susceptibility of human COLO 205 cells to apoptogenic stimuli resulted from time-dependent reduction in $cFLIP_L$ and TRADD protein levels. At the same time, the level of FADD protein was up-regulated. Additionally, the combined TNF-α and Bis-IX treatment caused cleavages of Bid and procaspase-9, as well as cytochrome *c* release. Thus, the evidence of this study indicates that Bis-IX facilitates the death receptor signal mediated by TNF-R1. Moreover, Bis-IX alone initiated intrinsic apoptosis, which could be abolished by Bcl-2 delivery. It heralds the involvement of mitochondria in caspase-8-independent intrinsic apoptosis. In turn, the treatment with bisindolylmaleimide III (Bis-III) did not assist TNF-α-dependent apoptosis.

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ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF ANGIOMYOLIPOMA

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Background: Angiomyolipoma is a rare, benign neoplasm, composed of adipose tissue, thick-walled blood vessels and smooth muscle cells. It comprises 0.7 to 2.0 percent of all renal tumors. It has a high incidence in adult patients suffering from the autosomal dominant disorder tuberous sclerosis. The histogenesis of these tumors is currently unclear. It is believed that these tumors arise from perivascular epithelioid cells (PEC) with no nor-

mal counterpart convincingly demonstrated. Others neoplasms including perivascular epithelioid cell tumors (PEComa), lymphangiomyomatosis and sugar tumor of lung develop from this cells. Material and methods: We analyzed 6 cases of renal angiomyolipoma from patients treated at Cancer Center in Warsaw. All cases were examinated by histology, electron microscopy and immunohistochemistry. Results: These tumors were composed of a variable proportion of adipose tissue, spindle and epithelioid smooth muscle cells and blood vessels. Imunohistochemically, the tumor cells were strongly positive for smooth muscle markers, focally for melanocytic markers (HMB45, Melan A) and strongly for microphthalmia transcription factor (MITF). Ultrastructural analysis showed intracytoplasmic membrane-bound dense bodies as well as typical premelanosomes. In the smooth muscle cells abundant accumulation of glycogen was found. Some smooth muscle cells also contained lipids.

Conclusion: Ultrastructural and immunohistochemical analysis of our 6 cases of Angiomyolipoma show features of melanocytic and smooth muscle differentiation.

ULTRASTRUCTURAL DEFECTS OF SPERMATOZOA: GENETIC BACKGROUND OF MORPHOLOGICAL ABNORMALITIES OF MALE GAMETES

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The severe male infertility may be of genetic origin (10-15%). The most sperm submicroscopic alternations are known to reflect developmental failure during the complex spermatogenetic remodeling process. Many studies of human semen and researches using animal model reveal relationship between sperm structural malformations and mutations of genes involved in morphogenesis of spermatozoa. The genes code structural, regulatory and enzymatic proteins require for normal course of spermiogenesis. Concerning the sperm head, the candidate genes for abnormal head, *GOPC, hrb, keratin 5/S57, keratin 9, Zpbp1, Csnk2a2, azh/Hook1, nectin-2/CD155* have been reported to produce spermatozoa with abnormal size and shape of head (*e.g.* rounded head: globozoospermia), with translocated head and with reduced or without acrosome during spermiogenesis. It should be noted, that these mutations can affect not only sperm head and acrosome formation but simultaneously tail. Coiled flagellum with disorganized stratified mitochondrial sheath and cytoplasmic remnants was observed. The drastic head and tail defects may result from: 1/ disorders of vesicle transport from Golgi region to the center of acrosome and flagellum formation in differentiating spermatid, 2/ formation of abnormal acroplaxome – subacrosomal cytoskeletal plate involved in acrosome and head shaping, 3/ ectopic position of manchette – structure composed of microtubules, located in postacrosomal region of the developing spermatid head and plays a role in the elongation of the sperm head and in nucleocytoplasmic transport of vesicules and 4/ disruption of the Sertoli cell-spermatid junction – apical ectoplasmic specialization. The sperm tail defects characterized by quantitative and topographical alternations of outer dense fibers and microtubules of the axoneme, abnormal disposition of longitudinal column (periaxonemal structure) of the fibrous sheath, excess residual cytoplasm and coiled tail can be an effect of the mutation of *Spag6*, *Spem1*, *Ube2b*, *act, hTaf7L, Cnot7, Tex18, ApoER2* genes. In conclusion, the spermatozoa with described structural failures are immotile or have impaired motility and diminished ability to penetrate *zona pellucida*. Moreover, they reveal aneuploidies. These spermatozoa are not able to fertilize egg cell in natural or even in assisted procreation. Intracytoplasmic sperm injection (ICSI) is the only method to allow for reproduction. On the other hand ICSI may increase genetic risk. Therefore, ultrastructural investigation of semen is needed to identify sperm morphological defects of supposed genetic origin and to select patients particularly with asthenoteratozoospermia. Thus, the transmission electron microscopic method plays an important role during proper diagnosis and treatment of male infertility and can help to predict successful *in vitro* fertilization.

OSMOFORES ULTRASTRUCTURE IN STAPELIADS (APOCYNACEAE-ASCLEPIADOIDEAE – CEROPEGIEAE STAPELIINAE)

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The carrion flower stapeliads represent olfactory mimicry forming flowers mimic food source or oviposition sites to attract pollinators. Flowers of *Orbea variegate* and *Boucerosia indica* (Asclepiadoideae-Ceropegieae-Stapeliinae), which produce strong, unpleasant for human nose, however, attractive for flies scent, were examined using light and electron microscopy. Corolla in both species glandular has secretory epidermal epithelium + sub-epidermal secretory layers. Secretory cells are extremely rich in endoplasmic reticulum and have flocculent material in vacuole. We find diversity of adaxial corolla epidermal in both cell shape and microarchitecture of surface, not only between species but also among different part of corolla of one flower.

EARLY STAGES OF THE OOGENESIS IN ISOHYPSIBIUS GRANULIFER (TARDIGRADA: EUTARDIGRADA): PREVITELLOGENESIS

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The reproductive system of *Isohypsibius granulifer* is composed of the hermaphroditic gonad and the gonoduct which opens into the rectum (Węglarska, 1987). Two ligaments suspend the gonad to the dorsal body wall. The gonad of this species consists of three parts: germarium, vitellarium and the distal part filled with male germ cells. Germarium is a small area located in the anterior (apical) part of the gonad. It contains small, non-differentiated cells (cystoblasts) which are connected by cytoplasmic bridges. The central part of this cell occupies large, spherical nucleus with oval nucleolus. Few mitochondria, free ribosomes and

single cisterns of RER are located in the cytoplasm. The second, larger part of the gonad is vitellarium. During previtellogenesis this part is filled with clusters of cystocytes. They have the same size and shapes so it is difficult to show which cell is an oocyte. It is possible only when we count the number of cytoplasmic bridges. The cell which possesses the highest number of cytoplasmaic bridges will be oocyte. During previtellogenesis the volume of each cell increases. Spherical nucleus with oval, granular nucleolus occupies central part of the cell. Numerous free ribosomes, mitochondria and single cisterns of RER are observed in the cytoplasm. During early vitellogenesis all cells located in the vitellarium begin to synthesize reserve material.

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ULTRASTRUCTURE OF EGG CAPSULES IN EUROPEAN AND TROPICAL STONEFLIES (PLECOPTERA: PERLIDAE)

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Stoneflies are amphibiotic insects. Embryonic and larval development of the representatives of the family Perlidae, takes place in well oxygenated water. A mating period occurs on land. Females lay packets of eggs into water. Each egg is covered by egg capsule which is equipped with a specialized attachment structure. Egg capsule is multilayered, radially and regionally differentiated. Egg capsule organization facilitates crucial physiological functions, in particular efficient gas exchange both in aquatic and land environments. Numerous (multiregional) studies carried in many stoneflies species show that egg capsules of all the investigated stoneflies are species specific (Fenoglio and Rościszewska, 2003). Therefore they can serve as important taxonomical criterion (Knispel *et al*, 2002). In this contribution the organization of the egg capsules in several tropical (Equadoran, Nicaraguan and Bolivian) species belonging to the family Perlidae is described. The results and the appropriate results which concern the European species of the mentioned family are compared. Adaptations of the egg capsules to the different environments are discussed.

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DIFFERENTIATION OF THE REGENERATIVE CELLS IN THE MIDGUT EPITHELIUM OF LEPISMACHILIS NOTATA (INSECTA, ARCHEOGNATHA)

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Regeneration of insect midgut epithelium undergoes due to cyclic or continuous degeneration, while regenerative cells fulfill the functions of midgut stem cells (Rost, 2006; Rost-Roszkowska, 2008).The midgut epithelium of *Lepismachilis notata* is composed of columnar epithelial cells and regenerative cell groups. The cytoplasm of regenerative cells is poor in organelles. Intercellular junction as gap junction between regenerative cells are observed. According to degeneration which proceeds both in a continuous (during the entire life) and a cyclic manner (degeneration undergoes much intensively during the molting), all processes which enable the midgut epithelium renewal are observed. In each regenerative cell group numerous mitotic divisions are observed. Just before mitotic division the number of RER and SER cisterns and Golgi complexes in their cytoplasm increases. External cells of each regenerative cell group proceed differentiation, therefore the number of mitochondria in their cytoplasm increases gradually. The mitochondria begin to accumulate near cell membranes and especially in the apical region of such elongating cell. Regionalization in organelles distribution appears gradually, and when the apical membrane reaches the midgut lumen it forms the microvilli. Some of regenerative cells proceed apoptosis.

Rost MM: 2006. Comparative Studies on regeneration of the midgut epithelium in *Lepisma saccharina* L. and *Thermobia domestica* Packard (Insecta, Zygentoma). Ann Entomol Soc Am 99: 910-916.
Rost-Roszkowska MM: 2008. Ultrastructural changes in the midgut epithelium of *Acheta*
domesticus L. (Orthoptera, Gryllidae) during degeneration and regeneration. Ann Entomol Soc Am 101,151-158.

IMMUNOHISTOCHEMICAL AND in situ HYBRIDISATION STUDY ON THE ROLE OF GHRELIN EXPRESSED IN RAT THYROID GLAND

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Some results of our previous studies indicate modulatory effects of ghrelin on secretory function of rat and porcine thyroids and evidence an uptake of ^{125}I-ghrelin from systemic circulation to the gland of male. *In vivo* oposite effects could be recorded in relation to serum concentration of ghrelin and T_3 and T_4 following short-term starvation in rat. In an experiment performed on the porcine thyroid slices *in vtro* the ghrelin, when added to incubation medium with somatostatin jointly, diminished the effect of somatostatin which alone upregulated the TSH stimulated T_3 secretion. Because of controversial opinions on the thyroid cells producing ghrelin and responding to this neuropeptide as the objectives we intend to

take part in the discussion with results of the presented study. Expression of ghrelin and its receptors in thyroids was demonstrated immunohistochemically in paraffin sections fixed in Bouin fluid or formaldehyde, using antibodies (anti-ghrelin and anti-GHSR1a *Phoenix-Pharmaceuticals*); peroxidase and alkaline phosphatase were used as markers of the immune reaction. For positive control an expression of the hormone in ghrelin cells of gastric mucosa and other organs of the same subjects was accepted and for the negative control IgG and complete rabbit serum were used instead antibodies. Expression of genes for ghrelin and their receptors in rat thyroids was demonstrated in formaldehyde fixed sections applying RT-PCR technique and also *in situ* hybridisation technique using digoxygenin-labelled oligoprobes (5'-TTA GCT GGC GCC TCT TTG ACC TCT TC-3') specific for rat ghrelin and (5'-ACA CCA CCA CAG CAA GCA TCT TCA C-3') for rat ghrelin receptor mRNA; ovine anti-digoxygenin monoclonal antibodies (Fab fragments) were used to visualize the reaction site immunohistochemically. The immunohistochemical expression of ghrelin in parafollicular and follicular thyroid cells of adolescent and adult rats of both sexes was documented, and also expression of gene encoding ghrelin (mRNA) was demonstrated by RT-PCR in thyroid and by hybridocytochemistry in parafollicular and follicular thyroid cells. With RT-PCR the gene expression for GHSR1a was identified and with *in situ* hybridisation technique the mRNA for GHSR1a was shown in follicular cells. In follicular cells expression of the type 1a ghrelin receptor could also be demonstrated immunohistochemically. A confrontation of the results and the effects of uptake of ^{125}I-ghrelin from systemic circulation allow to conclude that rat thyroid may respond to both the exogenous ghrelin and that produced locally, and that besides paracrine/autocrine an intracrine action of ghrelin in thyroid follicular cells can not be excluded.

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EVALUATION OF RESPIRATORY MUCOSA BIOPSIES IN PATIENTS WITH CLINICAL DIAGNOSIS CILIARY DYSKINESIA

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Primary ciliary dyskinesia is a rare syndrome with a genetic background. The illness is caused by abnormalities in the structure of cilia. Those irregularities lead to dysfunction of ciliary motility, which in turn causes impairment of ciliary-mucous transport in the airways.

The aim of the presented work was to define abnormalities in the structures of cilia as parallel well as lesions in the respiratory epithelium and within the lamina propria of the mucosa in the patients with a clinical suspicion of primary ciliary dyskinesia. The indications for the biopsy of respiratory mucosa, were recurrent or/ and chronic bronchitis being untreatable or having frequent regressions with short or very short periods of remission. The examined material consisted of biopsies of the bronchial and/or nasal mucosa. There were collected 114 biopsies in total. The age of the patients ranged from 2 months to 62 years. It has been reported that in all examined material there were abnormalities in the ciliary structure. The most common irregularity was lack of inner dynein arms (68% cases) or lack of both dynein arms (12%), or mixed forms (20%). Lack of dynein arms was accompanied by other changes in the ciliary ultrastructure such as: abnormalities in the arrangement of microtubules, both peripheral and central as well as occurrence under the cell membrane groups of microtubules typical for a few cilia. Furthermore, among cilia there were microvillus cytoplasmic outpouching – single, in groups or branching. In this study the decrease in number or complete absence of cilia on the free area of ciliated cells was observed. In cells with few cilia, rete ridges was also mis-shaped. Evaluation of the structure of the epithelium, the changed proportion between goblet cells and ciliated cells was reported. Goblet cells appeared in higher numbers and focally formed clusters. In the lamina propria of the bronchial mucosa features of fibrosis of the extracellular space were present. Fibrosis was expressed by the presence of numerous fibrocytes and fibroblasts, and the deposition of collagen fibres, which locally formed bundles. In the lamina propria of mucous membrane, in addition to broad fibrosis, there were changes in a number, placement and structure of vessels. In patients with clinically diagnosed exacerbation of inflammatory processes numerous inflammatory cells, between epithelial cells, also were found.

Prolonged inflammatory processes lead to considerable damage of respiratory cells of epithelium. Multiple regenerations of bronchial epithelium with improperly held process of differentiation of basal cells led to squamous epithelial metaplasia and/or dysplasia in a few cases.The results of the present work reflect cause effect relationship between primary ciliary dyskinesia and following it lesions. The observed broad fibrosis of the lamina propria of the mucosa can be treated as the effect of recurrent inflammatory events resulting from inappropriate in ciliary transport and mucus retention. Moreover, long-term inflammatory processes causing frequent regeneration of epithelium can lead to irregularities in the structure epithelium.

THE ULTRASTRUCTURE OF SOMATIC TISSUE WITHIN THE SEMINAL VESICLES IN THE EARTHWORM DENDROBAENA VENETA (ANNELIDA, CLITELLATA)

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The structure of seminal vesicles in an earthworm *Dendrobaena veneta* was studied with the use of light and electron microscopes. *D. veneta* had three pairs of seminal vesicles lying in 9, 11 and 12 segments of the body. Within the seminal vesicles spermatogenesis continued and the subsequent spermatogenetic stages were observed - clusters of primary and secondary spermatocytes, spermatids and spermatozoa. The inside of seminal vesicles was formed by somatic tissue not by celomic fluid as in other Annelids. Different cell types could be identified in the somatic tissue. One of them was represented by elongated cells with processes that varied in length and surrounded the germ cells. The cytoplasm of those cells and their processes contained relatively few organelles: elongated mitochondria, swollen tubules of endoplasmic reticulum and Golgi complexes, few lysosomes and granules of glycogen. The cells proved relatively numerous. The other type of somatic cells identified within seminal vesicles included three different types of cells with irregular elongated, fusiform or oval shapes without processes or with short lamellar ones. Their

cytoplasm was rich in organelles such as mitochondria, large lysosomes, endoplasmic reticulum, well developed Golgi complexes, bundles of microfilaments, glicogen granules and numerous vacuoles of various electron density. Some of them contained "bacterioid" crystals in the cytoplasm. Those morphologically diverse cells resembled coelomocytes, eleocytes and granulocytes identified in the celomic fluid of members of the family Lumbricidae.

MORPHOLOGY END MORPHOMETRY OF THE INFECTIVE JUVENILES OF HETERORHABIDITIS BACTERIOPHORA AND STEINERNEMA CARPOCAPSAE (NEMATODA) WITH THE USE OF SCANNING ELECTRON MICROSCOPY

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Profound and multi-faceted studies on entomogenous nematodes provide abundant information on their biology, morphology, physiology, ecology and biological control of insect pests. In the last twenty-year period new species and their local populations have been discovered and described. Nematodologists attempt to designate some distinctive features of hitherto described species to facilitate identification. Due to natural changeability within the described species and a number of approaches of material preparation adopted by researchers, there has emerged an urgent need for possibly simple, universal and fast methods of species identification. The present study attempts to portray the morphological and morphometric characteristics of nematode infective larvae using scanning electron microscopy (SEM) that would be useful in taxonomy and species identification. The present study focused on the infective stage morphology and morphometry of nematodes while the infective stage is comparable in all species of entomogenous nematodes. Besides, the type material can be obtained directly from insects in huge quantities. The studies used the freshly collected infective larvae which were killed at 60^0C and were then preserved in glutaraldehyd. After preservation, infective larvae were dehydrated and dried at the critical point, then coated with a 30 nm layer of gold-pallad and observed under scanning electron microscope (LEO 1430VP) at the voltage of 15 kV. Under SEM (500x) the precise length of infective larvae are obtained whereas enlargements of 3000x allow precise measurements of particular body parts. Enlaragements of 15000x in SEM showed that infective larvae have a very characteristic head region. The triangular shape of the mouth opening, the shape and location of labial papillae and the location of amphidia on the head are prominent. The cuticle covering the head section has a characteristic ring-shaped sculpture. Further to the end of the body the cuticle forms tessellate pattern and ridges. The pattern is easily observed under SEM and the location of the excretory pore in relation to the "squared field" of the cuticle sculpture can easily be determined. The cuticle of the final section of the infective larva richly sculptured and the shape and number of the cuticular ridges in front of the excretory pore distinctive. Observation and analysis of the majority of morphological and biometric properties is not possible under light microscopes. SEM offers much greater ranges of enlargements and enormous resolution as compared to light microscopy. The use of computer software for the microscope allows prompt measurements and digital analysis of the picture.

THE CHANGES DEVELOPED IN THE SMALL INTESTINE MUCOSA IN THE PIG DURING THE POSTNATAL LIFE

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Modification manifested in the architecture of gastrointestinal mucosa during postnatal period is often used to evaluate gut function, for instance during the development. Scanning electron microscopy (SEM) gives an opportunity to observe and analyze the surface of gut epithelium in three dimensions, but this technique is rarely used due to technical difficulties. The aim of the present study was to evaluate the development of the small intestine mucosa architecture using SEM technique. The experiment were carried out on piglets from birth until 38^{th} day of life. Four piglets from each age group were sacrificed just after birth, and at day 3, 7, 14, 21 and 38 of age. The entire gastrointestinal tract was harvested and the tissue samples were taken from duodenum, jejunum and ileum. Small intestine segments were taken and prepared for SEM analysis. At birth the surface of duodenal mucosa was covered with finger-like villi of uniform size. The jejunum mucosa villi at birth were finger-like and densely packed. The villi covered ileum mucosa was uniform in shape and size. Characteristic deep incisions around the villi were present in duodenum and jejunum, no extrusion zones on the villi tip were observed. The transversal furrows (deep incisions) in the following postnatal days gradually disappeared as the length of villi increased. At the day 7, singular shedding cells occurred on the villi surface. The villi observed at 7 days of age were finger-like shaped. From the day 14 villi observed in all segments were leaf-like in shape. The active extrusion zones were observed in duodenum and jejunum at 7, 14 and 21 days of age. At the day 21, the mucosa surface was folded with tongue-like shaped villi. The extrusion zones were active and contained numerous shedding cells, the knife-like incisions in extrusion zones were present. At weaning, short, leaf-like shaped villi were dominant at 38 day of life. The development changes in small intestine mucosa during the first 3 weeks were manifested in shape, size and density of villi. In conclusion, the development of the villi according to the part of the small intestine with age and the entire structure of small intestine mucosa undergoes profound structural changes.

INTERMEDIATE CELLS IN CHRONIC PANCREATITIS IN ALCOHOL ABUSERS

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Since the role and origin of pancreatic intermediate cells, having two phenoytypes of exocrine and endocrine cells, have not been elucidated yet, the study objective was an attempt to find out whether intermediate cells occur in chronic pancreatitis in alcohol abusers, what their structure and immunohistochemical features are and if their origin and role in pathological processes in the pancreas can be shown based on the observations made. The study material included 31 cases of chronic pancreatitis (n=31), 10 women and 21 men aged 29-50 years, with a history of long-term alcohol abuse. Frozen sections of the pancreatic head mass, fixed in buffered formalin or glutaraldehyde, were used for a/ histological stain-

ing, b/.immunohistochemical staining for the presence of insulin, glucagon, α-amylase and nestin, c/ ultrastructural examinations, d/ immunohistochemical investigations with colloid gold in an electron microscope for the presence of insulin. Analysis of the material revealed numerous intermediate cells showing ultrastructural and immunohistochemical features of follicular and endocrine cells, with the predominance of β-follicular or ductuloendocrine and ductuloacinar cells. As shown by immunohistochemical and ultrastructural investigations, intermediate cells constitute a phase in the development of endocrine cells and originate via differentiation of acinar and ductular cells.The presence of fibrillar inclusion bodies in intermediate cells revealed by ultrastructural examinations may be closely related to long-term alcohol abuse by patients.

STRUCTURE OF THE GERMARIUM IN OVARY OF AN EARWIG, OPISTHOCOSMIA SILVESTRIS

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The reproductive system of *Opisthocosmia silvestris,* as those of other studied forficuloid earwigs is consits of paired ovaries attached to elongated lateral oviducts. The ovaries are meroistic-polytrophic and are composed of about 30 short ovarioles that consist of a terminal filament, germarium and vitellarium. The germaria of adult females are relatively short and comprise individual germ cells (presumably the cystoblasts), germ cell clusters, as well as small somatic prefollicular cells. All germ cell clusters, even the youngest, consist of two cells only that are connected by a single intercellular bridge. Using the TEM techniques we classified the germ cell clusters into 2 developmental categories: clusters in an early stage of differentiation consisting of a prospective oocyte and a pro-trophocyte (1) and differentiated clusters which are composed of an oocyte and a trophocyte (2). In the early stage of differentiation the cells constituting the cluster are morphologically similar and differ only in the organization of their nuclei. The nucleus of the pro-trophocyte comprises irregular chromatin aggregations, while that of the prospective oocyte is almost transluscent and contains postmeiotic chromosomes only. The cells are connected *via* intercellular bridge which contains the fusomal material. In their cytoplasm ribosomes, elements of RER, multivesicular bodies and numerous mitochondria are present. The latter are not distributed in the cytoplasm uniformly, but they are concentrated in the certain region of the cell forming a prominent mitochondrial cloud or Balbiani body (Bb). In both cells, the Bb tightly adheres to the nucleus and remains in a direct contact with the fusomal material. Cells consituing the differentiated clusters are morphologically different. They remain connected by an intercellular bridge which does not contain the fusomal material. The oocyte nucleus is relatively large. It contains chromatin aggregations and a spherical nucleolus. In the ooplasm numerous ribosomes, Golgi complexes and RER elements are present. The trophocyte nucleus envelope is slightly folded and pierced by numerous pore complexes. The cytoplasm of the nurse cell comprises elements of RER, multivesicular bodies and ribosomes. In both cells of the cluster (oocyte and trophocyte) prominent Bb is still present.

ULTRASTRUCTURAL ALTERATIONS OF DIAPHRAGM AND LUNG PARENCHYMA IN TRANSGENIC RATS WITH FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a progressive, lethal neuromuscular disease that is associated with the degeneration of cortical and neuromotor neurons. Overexpression of the SOD1 gene mutation causes pathological characteristics of ALS including mitochondrial dysfunction with greater oxygen consumption, increase of ATP production and hypermetabolism. Patients with ALS invariably develop respiratory muscle weakness and most die from pulmonary complications.In this study transgenic rats overexpressing the SOD1 gene were used w okresie przedobjawowym. Material obtained from diaphragm and lung parenchyma was fixed in 2% paraformaldehyde and 2,5% glutaraldehyde and postfixed in OsO_4

Diaphragm consists of white and red muscular cells characterized by differentiated ultrastructural changes. In white muscular cells we observed edematous mitochondria with lipid drops in the cytoplasm. The red muscular cells were rich in mitochondria with morphological features of division. Sometimes we observed muscular cells with significant destruction of contractile apparatus. Extracellular matrix in the analyzed material was rich in collagen fibrils. Our ultrastructural observations indicate on destruction and rebuilding of lung parenchyma.

In the area between the capillaries and alveolar epithelium there were often fibroblasts, miofibroblasts, and bundles of collagen and elastin fibrils. Ultrastructural alterations were also seen in the walls of capillaries. Endothelial cells were hypertrophic and rich in phagoloysosomes. Epithelial cells type II possessed lamellar bodies with beaten phospholipids lamellae. The alveolar vesicles were filled up by extracellular membrane of pulmonary alveolar lining layer. Our observations are confirmed by physiological studies of patients with ALS that develop pulmonary complications.

REBUILDING OF THE LUNG PARENCHYMA IN EXPERIMENTAL DIABETES MELLITUS

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The several studies of lung function in patients with insulin-dependent and non-insulin-dependent diabetes mellitus have found reduced values of forced vital capacity and forced expiratory volume compared to normal individuals. Earlier studies have revealed that hyperglycemia impairs NO production and diabetes causes endothelial dysfunction in humans and in experimental animals. Abnormalities in endothelial nitric oxide production contribute to reduction of endothelial contraction, increase of von Willebrand factor, increase of vascular permeability and increase of adhesion molecules. This study was designed to test the effect of altered concentrations of glucose in streptozotocin-induced diabetic rats on morphological elements of lung air barrier. Lung parenchyma was obtained 8 weeks after hyperglycemia. The material was processed for ultrastructural studies. Endothelium of capillaries in lung barrier was rich in pinocytic vesicles and Weibel-Palade bodies. In the lumen of the vessels there are platelets, granulocytes and monocytes connected with endothelial cells. Screening the material we paid attention to the capillaries char-

acterized by features of newly formed blood vessels. In some parts of lung the extracellular matrix was multiplicated and rich in collagen fibrils, thickened basement membranes and fibroblasts. Epithelial cells type II possessed well signed endoplasmic reticulum. In the lamellar bodies phospholipids lamellas were disappeared. The luminal part of these cells was characterized by aggregation of cytoskeletal fibrils and altered microvillage. Extracellular lining layer was altered and its elements were surrounded by fluid transudate. Decrease in cellular sensitivity of insulin-like growth factor contributes to greater protein synthesis and activation of growth factors. Such reaction has effect on pathophysiological changes in lung parenchyma.

PNEUMOCYSTIS CARINII- ULTRASTRUCTURAL FEATURES OF DIFFERENT TYPES AND EFFECT ON ALVEOLAR EPITHELIUM

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Pneumocystis carinii is a common cause of the pneumonia seen in immunocomprised hosts and particularly those diagnosed with AIDS. However, despite its clinical significance, progress in elucidating the basic biology of *P. carinii* has been slow. Because it is a very small organism that is difficult to grow in vitro and its taxonomic position as a fungus or protozoan have not been fully established. Ultrastructural analysis of *P. carinii* reveals a unique microorganism with morphological features hared by both fungi and protozoa. In our study *P. carinii* was produced in rats by the administration of corticosteroids and low protein diet. The rats were sacrificed at weekly intervals, and their lungs were observed by electron microscopy. On the alveolar epithelium surface, the first change wad a bleb formation beneath the type I pneumocyte which was covered by trophozoites. The contact of trophozoites was on the smooth cell surface, and we did not observe any instance in which the filopodia of the organism were tightly attached to the type I pneumocyte. On the attached surface the type I pneumocyte cytoplasm interdigitated with the membrane of the organism and extended between the organisms. Sometimes the alveolar surface was partially denuded of epithelium and the trophozoites were directly attached to the alveolar basement membrane. The alveolar interstitium showed proliferation of fibroblasts and increased collagen fibers. A few trophozoites were observed in interstitium. Type II pneumocyte showed a steady increase in size of the lamellar bodies and cellular volume. Our observations showed different types of P. carinii in alveolar space. In any times we did not observe types of *P. carinii* in type I or II pneumocytes.

THE CRUCIAL ROLE OF ELECTRON MICROSCOPY IN THE DIAGNOSIS OF FABRY'S DISEASE

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Fabry's disease is an X-linked inherited disorder caused by a deficiency of the lysosomal hydrolase, alpha-galactosidase A, which leads to the intracellular accumulation of glycosphingolipids in a variety of organs, including skin, kidney, heart, central nervous system, and cornea. One of the most severely affected organs in Fabry's disease is the kidney. The majority of patients show progressive renal failure and develop end-stage renal disease. The clinical diagnosis of Fabry's disease may be difficult when a family history is lacking, and the diagnosis may be missed if only routine light microscopy is done. We present a case of Fabry's disease in 24-year old man in whom diagnosis was done by ultrastructural examination of the renal biopsy that documented typical inclusion bodies in the cytoplasm with concentric lamellation and zebra or onionskin appearance. These osmophilic ovoid bodies were abundant in podycytes. The cellular inclusions were seen within lysosomes, surrounded by a single membrane, or were find free in the cytoplasm. In this case the family history was negative for renal diseases. A dominant clinical signs. in the onset of the disease were hypertension, acroparesthesiae and nycturia. Echocardiography examination revealed concentric left ventricular hypertrophy. Urine analysis demonstrated mild proteinuria. Light microscopy evaluation of the renal biopsy showed swelling and vacuolization of visceral podocytes and distal tubular cells, mesangial widening, and segmental glomerular sclerosis, tubular atrophy and interstitial fibrosis. On the basis of electron microscopy it was possible to establish the diagnosis of Fabry's disease, which was confirmed by a biochemical enzymatic assay, and demonstrated a decreased activity of alpha-galactosidase A. Diagnosis of Fabry's disease presents some difficulties because of the large variety of signs and symptoms, and it is thought that the disease may be more common than believed in the past. Therefore, the special attention must be done in electron microscopy evaluation of renal biopsy specimen to avoid an erroneous diagnosis. Early diagnosis of the disease is pivotal, because therapeutic intervention with enzyme replacement is the most effective way to treat Fabry's disease. In summary, we postulate that electron microscopy examination of kidney biopsy specimen is essential for investigation of storage diseases.

MORPHOLOGY AND ULTRASTRUCTURE OF VIRULENT AND TEMPERATE ENTEROCOCCUS FAECALIS BACTERIOPHAGES

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Infections caused by multidrug-resistant bacterial strains have become a serious medical challenge. Gram-positive cocci such as methicillin-resistant *Staphylococcus aureus* as well as vancomycin- and teikoplanin-resistant *Enterococcus faecalis* are the major causative agents of dangerous infections. The aim of the study was to designate the morphology and ultrastructure of seven *Enterococcus faecalis* bacteriophages from the Institute's phage collection. Four virulent *Enterococcus faecalis* phages were isolated from 46 environmental samples (city sewage, soil, natural water reservoirs).Three temperate bacteriophages were isolated from *Enterococcus faecalis* strains obtained from human biological material (urine, sperm, pus). The phages and host bacterial strains were propagated at 37°C on solid agar or broth medium (Difco) according to Adam's technique. Lysates were clarified by centrifugation (3000 x g for 20 min) and filtered through membrane filters of 0.22 µm pore size (Millipore). Pure phage lines were prepared using a five-step plague re-isolation technique. The

isolated phage were used to type two hundred *Enterococcus faecalis* strains. Positive lytic reaction in 67% of the tested strains was observed in contrast no positive lytic reactions were noticed in any of twenty-five *Enterococcus faecium* strains.The phage preparations for the electron microscopic study were made according to Ackermann's technique.The phage lysates were centrifuged at 25,000 x g for 60 min. and washed twice in ammonium acetate (0.1 M, pH 7.0). Sediments were deposited on carbon-coated Formvar films, stained with uranyl acetate, and examined in an electron microscope (TEM) Tesla BS540 magnification 60,000 x. Initial testing showed that all the phages belongs to family Siphoviridae with long, noncontractile tail sheath (B3 morphological type) according to Ackermann's classification schime.

ULTRASTRUCTURAL FEATURES OF THE BRAIN IN CASE OF THE ADULT POLYGLUCOSAN BODY DISEASE (IV TYPE GLYCOGENOSIS)

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Adult Polyglucosan Body Disease, APBD (IV type glycogenosis) is characterized by the deficiency of a branching enzyme - α-1,4glucan 6-transglucosylase.The deficiency is caused by mutations in the GBE1 gene located on human chromosome 3p12. In case of the APBD the storage of glycogen is present mainly in the central and peripheral nervous system.

We analyzed a case of a 45- year old unconscious woman who died on the third day after admittance to the hospital. She had suffered from headache, nausea and vomiting in the preceding month. She lost her job one year before. Cerebrospinal fluid pressure was increased with 350 mg% of protein and 105 mg % of glucose. Neuropathological examination revealed massive accumulation of polyglucosan bodies (PB) in the cortex and white matter of the whole brain. These bodies were found mostly in the white matter, around vessels and beneath the pia. The PBs were located in the proceses of neurons, astrocytes and microglial cells and generally resembled round or elongated spheres. The storage material in the cytoplasm of neurons and glial cells was visible as fine granules. Ultrastructurally, spherical PBs consisted of non-membrane-bound deposits of branching and densely packed filaments of about 8 nm in diameter. The filaments were mixed with fine granular and amorphous material and small vesicles. Big PBs located in distended axons caused disruption of their sheaths.

MEMBRANE BLEBBING OF RL-34 AND OSTEOSARCOMA 143B CELLS UNDER OXIDATIVE STRESS

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Membrane blebbing is an early sign of toxicity. Blebbing can be observed in cells undergoing both apoptotic and necrotic cell death and can be caused by several forms of cell injury including oxidative stress. Hydrophilic *tert*-butyl hydroperoxide (*t*BH) in the presence of hemoprotein (cytochrom P-450) is converted to its free radical form and generates oxidative damage of membranes (swelling of mitochondria and endoplasmic reticulum) and intensive cytoplasmic membrane blebbing. It has been also shown that blebbing of the cell membrane is triggered by a major reorganization of the actin cytoskeleton, especially by an excessive polymerization of actin filaments under the surface of a cytoplasmic membrane.The aim of our study was to indicate whether *t*BH-induced membrane blebbing is an effect of oxidative stress or an early sign of apoptosis. Investigations were carried out on rat liver RL-34 and human osteosarcoma143B cell lines. Cells were treated with various concentrations of *t*BH (from 0,05mM to 1,5mM), time of exposure varied from 30 minutes to 4h, and examined in scanning and transmission electron microscope JEM 1200EX II (Jeol, Japan). Blebbing of the cell membrane induced by *tert*-butyl hydroperoxide was visualized by scanning electron microscope (SEM). Changes in the cytoplasmic organelles: nuclear condensation, endoplasmic reticulum dilatation, mitochondria swelling, and vacuolization of cytoplasm under higher doses of *t*BH were visualized by transmission electron microscope (TEM). Moreover, the test for the presence of phosphatidylserine or fragmentation of DNA by flow cytometry showed that morphological changes induced by *t*BH in both cell lines was not due to apoptosis. Whereas, high doses of *t*BH lead to nonapoptotic cell death. In conclusion, *tert*-butyl hydroperoxide-induced bleb formation in osteosarcoma 143B and rat liver RL-34 cell lines is not an initial sign of apoptosis phenomenon but results from an oxidative stress.

EXPRESSION OF CYCLIN A IN INTESTINAL BIOPSIES FROM CHILDREN WITH CELIAC DISEASE

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The celiac disease, is a permanent intolerance to ingested gluten that results in immunologically mediated inflammatory damage to the small-intestinal mucosa. Early diagnosis and management are important for the patients with this disease. In particular that patients with non-treated celiac disease have increased risk of malignancy, especially lymphomas. The aim of this study was to estimate the expression of cyclin A and describe its distribution in the intestinal biopsy specimens from children with suspected and confirmed celiac disease as well as to control. Investigated material consisted of 37 intestinal biopsies: 19 taken from patients with confirmed celiac disease, 9 from patients with its suspicion and 9 from healthy patients, who served as control. Immunohistochemical and immunogold methods were used to estimate cyclin A expression. In celiac disease samples morphological changes in epithelial cells, typical for disease, were shown. We observed weaker cyclin A expression, however there were also some cells with strong labeling in cytoplasm, near the nucleus. We assume that these areas could be transformed Golgi apparatus, vacuole-like structures or abnormal lysosomes. However they might also be droplets of fat, present there as a result of inability to transport resynthesized triglyceride into intracellular spaces. In control and suspected celiac disease

groups cyclin A was present in the brush border, nucleus and whole cytoplasm, especially in proximity to the nucleus. In conclusion, these studies enabled us visualized pattern of distribution of cyclin A but let us also to presume that observed decrease of expression and its distribution might function as additional factor which could be taken under consideration to establish terminal diagnosis. We are aware of the fact that these are very first observations and that this subject needs to be further investigated with the use of additional methods and samples.

THE HEPATOPANCREAS EPITHELIUM OF *PORCELLIO SCABER* LATREILLE, 1804 (CRUSTACEA, ISOPODA) – FINE STRUCTURE, FUNCTION AND REGENERATION

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The digestive tract of *Porcellio scaber* consists of a foregut and a hindgut, which are ectodermal in origin and both lined with cuticule. Hepatopancreas (midgut glands) is the only endodermal part of the digestive system of *Porcellio scaber*, which consists of two pairs of blind-ended tubules, not lined with cuticule. This structure functions as midgut in other groups of animals. Each tubule is surrounded by a network of striated muscles and composed of monolayer epithelium which is made up of two kinds of cells. The larger ones, usually binucleate and poliploid cells are called B and the smaller S cells. No regenerative cells were observed among differentiated cells in any of preparations examined. The B-cells are characterized by the presence of well developed microvilli, active Golgi complexes, extensive arrays of rough endoplasmic reticulum, numerous mitochondria and a lot of lipid droplets. This ultrastructural features indicate that the major role of the B-cells is secretion of digestive enzymes and absorption of nutrients. The main characteristic of the S-cells is a paucity of Golgi complexes and an extensive endoplasmic reticulum, as well as the presence of electron dense granules containing mainly heavy metals. We suggest that the major role of the small cells is to store the absorbed materials. The cells, which made up the epithelium of midgut glands differ in size. At the tip of this organ, which is covered by a cap of muscle cells, there are undifferentiated, small, mononucleate cells that lack microvilli. It is supposed that they serve as a source of regenerative cells that replace used up cells of the main epithelium. As the cells pass to the further part of the epithelium they enlarge, become binucelate, poliploidal and their adluminal surfaces become covered by microvilli. Similar sequences of changes can be observed in differentiating hepatopancreas epithelium in juvenile specimens.

OVARY ORGANIZATION IN TUBIFICIDAE (ANNELIDA, OLIGOCHAETA)

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Among Annelida ovary organization and oogenesis are well-known in Polychaeta, where a lot of various types of ovary organization and different patterns of oogenesis have been described. However, in Oligochaeta our knowledge about ovaries architecture and oogenesis is insufficient. Our preliminary studies include two genus: *Limnodrilus* sp. and *Tubifex* sp., both belonging to Tubificidae. Generally, we have found that ovary in Tubificidae, are small and irregular structures composed of interconnected germ cells forming cysts (clones) enveloped by flattened somatic cells. In a cyst each germ cell is connected via its own intercellular bridge to a common cytoplasmic mass, a cytophore. The cytophore occupies the central position in a cyst. In studied tubificids, germ cells within cysts differentiate into oocytes and numerous trophocytes, what is unusual feature among Oligochaeta *sensu stricto* (except *Dendrobaena veneta*) and it is characteristic rather for leeches. Next feature, which also is similar to ovary cords found in Erpobdellidae and Hirudiniformes, is polarization of the Tubificidae ovary. We distinguished three zones: 1st – on the top of ovary (the top part), where oogonia and forming clones of undifferentiated germ cells occur; 2nd – middle part of the ovary (the middle part), where germ cells differentiate into oocytes and trophocytes, and 3rd – basal part, where growing oocytes undergo previtellogenesis/early vitellogenesis, and gradually protrude into coelom, while vitellogenic and ripe oocytes float freely in the coelom. Numerous trophocytes, relatively few oocytes and extensive cytophore are features, which distinguish ovaries in studied tubificids from other Oligochaeta *sensu stricto*. It is worth mention here about follicular cells in Tubificidae ovary. This flattened cells surround external surface of the ovary, as well as enter inside it, where they accompany the germ cells and play supportive role in oogenesis. The plasma membrane of follicular cells surrounding growing oocytes folds deeply, forming invaginations which eventually seem to form channels throughout their cytoplasm. Similar organization of follicular cells has been described in many leeches. In summary, our studies shown, that ovary architecture in representatives of Tubificidae is different than described for others Oligochaeta *sensu stricto*, but resembles the ovary cords found in leeches.

FOLLICULAR CELL DIFFERENTIATION IN POLYTROPHIC OVARIES OF NEUROPTERA

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Clusters of germ cells (cystocytes) that fill the proliferative zone (germarium) of the neuropteran ovariole are accompanied by two types of somatic cells. Externally located prefollicular cells (pFCs) that border on directly the ovariole basal lamina, form processes penetrating among the germ cells. Internal somatic interstitial cells are located among cystocytes with no contact with the basal lamina. pFCs transform into epithelial follicular cells (FCs) that invest the germ cell cluster. Cluster of cystocytes together with their covering follicular epithelium forms the egg chamber. Initially FCs are few. With the progress of previtellogenic and vitellogenic growth of the egg chamber their number increases. Somatic cells of the previtellogenic egg chamber fall into two distinct categories (subpopulations). More numerous FCs are externally supported by the ovariole basal lamina, while a small number of interstitial cells remains located among germ cells inside the ovariole. The FCs either stretch over the nurse cells or form a cuboidal epithelial cover on the lateral aspects of the oocyte. Interstitial cells occupy different positions within the egg chamber. They can be found among the nurse cells, at the nurse cell/oocyte interface or between neighboring egg chambers. In the advanced stages of vitellogenesis cuboidal FCs at both poles of the oocyte migrate centripetally. In consequence, vitellogenic oocyte becomes gradually encapsulated by the epithelial layer. Converging cuboidal FCs at both extremities

of the oocyte drag behind the basal lamina and retain their epithelial character. At late vitellogenesis, FCs at the anterior pole of the oocyte become columnar and form a characteristic epithelial fold. The latter comprises FCs that are engaged in the formation of the aeromicropyle, and those that mould the micropylar canals. The latter form processes that penetrate inside the forming aeromicropyle. These processes are supported by an elaborate cytoskeleton. All the remaining FCs are engaged in the synthesis of the main body chorion. The interstitial cells do not seem to contribute to the formation of the eggshell.

OOCYTE ULTRASTRUCTURE OF THE AGELENID SPIDER TEGENARIA ATRICA (KOCH)

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The oocytes of some spiders show asymmetrical distribution of organelles in the form of a prominent cytoplasmic accumulation termed the Balbiani body. Balbiani bodies comprise mitochondria, endoplasmic reticulum, Golgi elements, *nuage* material and lipid droplets. The oocytes of *Tegenaria atrica* (Koch) do not seem to contain any Balbiani body equivalent. Instead they house numerous rod-shaped bacteria. In early previtellogenic oocyte microorganisms form a cap-shaped accumulation in close vicinity of the centrally located oocyte nucleus. In the advanced stages of previtellogenesis this accumulation rounds up and is observed in a distance from the eccentrically situated oocyte nucleus. The ultimate fate of bacteria as well as their functional significance during oogenesis remain unclear. Ultrastructural analysis of late previtellogenic oocytes revealed that besides bacteria the ooplasm contains also dictyosomes, heterogenous vesicular structures and numerous mitochondria with characteristically swollen cristae.

ELECTRON MICROSCOPY IN POLAND

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The first electron microscopy laboratory for medical use was founded by prof. Janusz Groniowski in 1956 rok at the Departament of pathologic Anatomy in Poznań. During next two years two members of this staff (F. Kaczmarek and W. Djaczenko) constructed a prototypic ultramicrotome with thermic movement. First scientific paper with electron microscope use was presented by prof. J. Groniowski during 4th International Electron Microscopic Conference in Berlin. In Poland the first scientific meeting on the use of el-mi in biology in medicine was held in Poznań in 1959 during the Conference of Polish Anatomopathologic Society. The Electron Microscopy Commission (EMC) was founded as a Part of Polish Academty of Sciences in 1961 by prof. Janina Kowalczykowa (Committee of Pathomorphology), prof. Zygmunt Grodziński (Committee of Zoology) and prof. Janusz Groniowski (Committee of Pathomorphology). Then in the following years the activity of the EMC was based on specialists from histology, embryology, cytology, pathomorphology, oncology, veterinary, botany, paleontology, and agricultural sciences. The Presidents of EMC were as follows: prof. J. Groniowski (in years 1960-1968), prof. Andrzej Vorbrodt (1968-1975), prof. Wincenty Kilarski (1975 -1984), prof. Wenancjusz Domagała (1984-1987), prof. Leszek Cieciura (1987-1996), and prof. Wiesława Biczysko (since 1996-present). Polish Commission on Electron Microscopy became a member of international societies such as International Federation of Societies of Electron Micorscopy (since 1972) and Committee of European Societies for Electron Microscopy (established in 1976). Under auspicies of Commission there organized regular conferences and symposiums. First one took place in Warszawa (in 1963). The following conferences were organized annually or biannually in several places such as Kraków, Lublin, Szczecin, Gliwice, Uniejów, Poznań, Gdańsk, Kazimierz nad Wisłą, Międzyzdroje, Oleśnica, Jabłonna, Rydzyna, Bydgoszcz, Porąbka-Kozubnik, Kiekrz, Wrocław, Białystok.

MECHANISMS OF LUNG MACROPHAGES ACTIVATION BY EXOGENOUS SURFACTANT

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From the time when exogenous surfactants were introduced and used for treatment of acute respiratory distress syndrome there some studies published on side effects of such therapy. There were already done several attempts for understanding of unwanted outcomes of a single dose of exogenous surfactant on the lung parenchyma, but the mechanisms of those changes remain not fully understood. The aim of the present study was to evaluate the different modes of activation of mononuclear cells after treatment with exogenous surfactant. In the present study we used peripheral blood mononuclear cells (PBMC) derived from healthy volunteer donor. For further studies we used PBMCs or peripheral blood lymphocytes (PBL) or monocytes alone. Freshly isolated PBMCs were magnetically labeled with CD14 MicroBeads according to standard protocol and then treated with increasing concentration (from 0.125 to 40.0 mg of lipids per 1 ml of medium) of semi-natural surfactant (Curosurf). In next study stage cell colonies were first pre-treated with 40mg/ml surfactant for 1 hour and then washed and finally treated with selected surfactant concentrations, eg. 4, 0.5 and 0.125 mg of surfactant mg per 1 ml. Cell cultures revealed dose-dependent activation of PBMC or PBL or monocytes alone. PBMC proliferation was augmented by the presence of lymphocytes pre-stimulated with surfactant (2500cpm vs. 27000 cpm, non-prestimulated and surfactant prestimulated cell colonies respectively). On the other hand, in our studies, results after very high surfactant concentration (100µl of surfactant for 100µl cell suspension) revealed decreased number of cells comparing groups treated with lower concentrations.

In conclusion, our results suggest that high concentrations of surfactant possibly destroy cells in culture. These studies revealed that the level of lymphocyte-macrophage axis activation depends on the surfactant concentration. Moreover the stimulation of cell proliferation depends on the mode of surfactant treatment. Low concentration surfactant pre-treatment causes augmented cell proliferation. The latter phenomenon should be studied with focus on cell-cell crosstalk.

ULTRASTRUCTURE, DISTRIBUTION AND TRANSOVARIAL TRANSMISSION OF ENDOSYMBIOTIC YEAST-LIKE MICROORGANISMS IN *CONOMELUS ANCEPS* (INSECTA, HEMIPTERA, FULGOROMORPHA: DELPHACIDAE) AND *METCALFA PRUINOSA* (INSECTA, HEMIPTERA, FULGOROMORPHA: FLATIDAE)

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In fulgoromorphans, like in other plant sap-sucking hemipterans, endosymbiotic microorganisms commonly occur. In contrast to remaining hemipterans harboring endosymbiotic bacteria, members of Delphacidae and Flatidae families harbor endosymbiotic yeast-like microorganisms. In the body cavity of *Conomelus anceps* and *Metcalfa pruinosa*, in close vicinity of the ovaries large syncytial organs of irregular shape termed mycetomes are present. The mycetomes are surrounded by a one-layered epithelium composed of small, flattened cells. The mycetome cytoplasm is tightly packed with endosymbiotic rod-shaped yeast-like microorganisms. The endosymbionts are surrounded by a thick cell wall composed of two distinct layers. In the central part of the cell a large, spherical nucleus with a single nucleolus is localized. The rest of cytoplasm is filled with ribosomes, mitochondria and cisternae of rough endoplasmic reticulum. The yeast-like microorganisms reproduce by budding. The yeast-like endosymbionts, like bacterial endosymbionts in remaining hemipterans, are maternally inherited by transovarial transmission. The yeasts become released from the mycetomes and migrate towards the ovarioles containing vitellogenic oocytes. The microorganisms transverse the cells of the ovariole stalk (pedicel) and enter the perivitelline space. Simultaneously, at the posterior pole of the oocyte a deep depression is formed. The yeasts accumulate in the oocyte depression and form a characteristic "symbiont ball".

ULTRASTRUCTURE OF EGG CAPSULES IN EUROPEAN AND TROPICAL STONEFLIES (PLECOPTERA: PERLIDAE)

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Stoneflies are amphibiotic insects. Embryonic and larval development of the representatives of the family Perlidae, takes place in well oxygenated water. A mating period occurs on land. Females lay packets of eggs into water. Each egg is covered by egg capsule which is equipped with a specialized attachment structure. Egg capsule is multilayered, radially and regionally differentiated. Egg capsule organization facilitates crucial physiological functions, in particular efficient gas exchange both in aquatic and land environments. Numerous (multiregional) studies carried in many stoneflies species show that egg capsules of all the investigated stoneflies are species specific (Fenoglio and Rościszewska, 2003). Therefore they can serve as important taxonomical criterion (Knispel *et al*., 2002). In this contribution the organization of the egg capsules in several tropical (Equadoran, Nicaraguan and Bolivian) species belonging to the family Perlidae is described. The results and the appropriate results which concern the European species of the mentioned family are compared. Adaptations of the egg capsules to the different environments are discussed.

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OVARY STRUCTURE AND OOGENESIS IN THE MOUSE FLEA CTENOPHTHALMUS AGYRTES (SIPHONAPTERA: CTENOPHTHALMIDAE). HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES

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Histological and ultrastructural studies have shown that ovaries of *Ctenophthalmus agyrtes* (Siphonaptera: Ctenophthalmidae) are composed of panoistic ovarioles. An individual ovariole consists of a terminal filament, germarium, vitellarium and ovariole pedicel. The germarium houses differentiating germ cells and somatic prefollicular cells. In the basal part of the germarium prefollicular cells surround oocytes, forming ovarian follicles. The vitellarium is composed of developing ovarian follicles in a linear arrangement. In the apical part of the vitellarium early previtellogenic oocytes are localized. They are relatively small and contain large, spherical germinal vesicles (oocyte nuclei), containing heteromorphic nucleolar masses (as a result of rDNA amplification). During subsequent stages of oogenesis nucleolar masses grow and disperse. From early previtellogenic stages oocytes possess two types of ooplasm: transparent and dense. The amount of the dense cytoplasm increases gradually towards the end of oogeness. Follicular cells surrounding the oocytes change their shape during oogenesis. In early previtellogenic stage follicular cells form squamous mono-layered epithelium. In advanced previtellogenesis follicular cells become cuboidal and contain large, spherical nuclei with prominent nucleoli. During this stage the number of follicular cells remarkably increases, as a result of high mitotic activity. During vitellogenesis follicular cells start to synthesize precursors of egg envelopes in the form of dense, prechorional granules that are secreted into the space between the follicular epithelium and the oocyte, where they accumulate and form subsequent layers of the egg shell. During this stage the cytoplasm of follicular cells contain sabundant rough endoplasmic reticulum, numerous mitochondria, ribosomes and dictyosomes. Between the neighbouring ovarian follicles groups of somatic cells (interfollicular cells) can be also observed. Morphology and ultrastructure of these cells are entirely different from follicular cells surrounding the oocytes.

THE CHANGES OF THE CELL'S ULTRASTRUCTURE IN THE BODY COVER DURING THE EMBRYONIC DEVELOPMENT IN THE GRASS SNAKE NATRIX NATRIX L

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The scaled reptilian epidermis changes throughout life and periodically regenerates and is shed. The shedding mechanism involves morphologically different keratinous materials that are present in the adult, regenerating and embryonic epidermises of snakes and other lepidosaurians. The small pieces of skin were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde 1:1 in 0,1 M phosphate buffer, pH 7, 4 in 4ºC, dehydrated and embedded in LR White embedding medium. We analyzed cell's ultrastructure of the body cover in the grass snake (*Natrix natrix* L.) over the full length of the embryo's body at each developmental stage. The semi- and ultra thin sections were immunostained with use of specific anti-α antibody. Our findings indicated that: the stratum germinativum contains columnar cells, which are attached to basal membrane by hemidesmosomes. The cytoplasm of the columnar cells is transparent for electrons, contains bundles of intermediate filaments and glycogen accumulations. Among columnar cells, there are isolated pigment cells. The cytoplasm of pigment cells contains many vesicles filled with granules of pigment, mitochondria and smooth endoplasmic reticulum. The layers of cells lying above the stratum germinativum contain mitochondria and rough endoplasmic reticulum. These cells are joined to the columnar layer by desmosomes. The desmosomes are also visible between lateral surfaces of the flat and columnar cells. At the end of the integument differentiation one can distinguish respective epidermal layers. The innermost layer of epidermis is the stratum germinativum, which contains many branched pigment cells. Above this single layer are the single mesos layer and then thick β layer (or α and β layers), thin Oberhäuten layer, and the periderm. These layers correspond to the distribution of two different types of keratin. On the inner scale surface and hinge regions the epidermis is thinner than on the outer surface. On the outer surface of scales and shields the β layer is very much ticker than the α layer, but on the inner surface of scale and in the hinge region the α layer is predominant. Before the hatching the epidermis forms the first embryonic shedding complex and at the end of the developmental stage, XII periderm layer begins to detach.

A RAPID FLOW OF SPERMATOZOA THROUGH THE VECTOR TISSUE IN PISCICOLA RESPIRANS (ANNELIDA, HIRUDINIDA)

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In fish leeches (Piscicolidae) indirect (hypodermic) insemination has evolved. These leeches have no penis, during copulation the spermatophores are released on the specialised region of the body wall known as copulatory area or copulatory region. Spermatozoa leave the spermatophore and have to reach the ovaries somehow. The way in which they do this is not fully understood. In piscicolids beneath the copulatory area a specialized connective tissue, the vector tissue occurs which is thought to guide the spermatozoa toward the ovaries. The vector tissue is supposed to be an outgrowth of the ovary wall. To date the structure of the vector tissue has not been observed during massive sperm transfer through it. Here we present first ultrastructural observation of massive sperm flow from the spermatophore throughout the vector tissue to ovaries. Additionally, we show the structure of the spermatophore and a connection between spermatophore and body wall. The results that we obtained show that the sperm transfer is massive and rapid, the migrating spermatozoa form huge aggregations which push aside the vector tissue cells in such a way that between these cells voluminous gaps are formed. Unexpectedly to our previous suggestions the ultrastructural pictures show that the long cytoplasmic processes of plasmatic and granular cells forming the main mass of the tissue are not engaged in sperm transport. However, the wall of vector tissue built mainly from the vesicular cells and thick basal laminae remains intact and prevent the sperm flowing out of the tissue. We suggest that the sperm is pumped from the spermatophore into vector tissue with a high pressure, and as a result the vector tissue cells are pushed aside and spermatozoa can freely pass through it.

STRUCTURE OF THE GERMARIUM IN OVARY OF AN EARWIG, *OPISTHOCOSMIA SILVESTRIS*

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The reproductive system of *Opisthocosmia silvestris,* as in other studied forficuloid earwigs consits of paired ovaries attached to elongated lateral oviducts. The ovaries are meroistic-polytrophic and are composed of about 30 short ovarioles that consist of a terminal filament, germarium and vitellarium. The germaria of adult females are relatively short and comprise individual germ cells (presumably the germ line stem cells or the cystoblasts), germ cell clusters, as well as small somatic prefollicular cells. All germ cell clusters, even the youngest, consist of two cells only that are connected by a single intercellular bridge. Using the TEM techniques we classified the germ cell clusters into 2 developmental categories: clusters in an early stage of differentiation consisting of a prospective oocyte and a pro-trophocyte (1) and differentiated clusters which are composed of an oocyte and a trophocyte (2).

In the early stage of differentiation the cells constituting the cluster are morphologically similar and differ only in the organization of their nuclei. The nucleus of the pro-trophocyte comprises irregular chromatin aggregations, while that of the prospective oocyte is almost transluscent and contains postmeiotic chromosomes only. The cells are connected *via* intercellular bridge which contains the fusomal material. In their cytoplasm ribosomes, elements of RER, multivesicular bodies and numerous mitochondria are present. The latter are not distributed in the cytoplasm uniformly, but they are concentrated in the certain region of the cell forming a prominent mitochondrial cloud or Balbiani body (Bb). In both cells, the Bb tightly adheres to the nucleus and remains in a direct contact with the fusomal material. Cells consituing the differentiated clusters are morphologically different. They remain connected by an intercellular bridge which does not contain the fusomal material. The oocyte nucleus is relatively large. It contains chromatin aggregations and a spherical nucleolus. In the ooplasm numerous ribosomes, Golgi complexes and RER elements are present. The trophocyte nucleus envelope is slightly folded and pierced by numerous pore complexes. The cytoplasm of the nurse cell comprises elements of RER, multivesicular bodies and ribosomes. In both cells of the cluster (oocyte and trophocyte) prominent Bb is still present.