

**POLISH ACADEMY OF SCIENCES
MEDICAL RESEARCH CENTRE**

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CONTENTS

Page

EXECUTIVE BOARD	4
MRC SCIENTIFIC COUNCIL	5
STAFF LIST	6
RESEARCH REPORT	33
Department of Neurophysiology	34
Department of Neurochemistry	37
Department of Neuropathology	42
Laboratory of Developmental Neuropathology	48
Laboratory of the Ultrastructure of the Nervous System	49
Department of Neurosurgery	51
Neuromuscular Unit	53
Department of Applied Physiology	58
Outpatient Cardiac Unit for Diagnosis and Therapy	62
Cardiovascular Laboratory	63
Department of Surgical Research and Transplantation	65
Department of Endocrinology	68
Library	69
MEDIPAN - Scientific Instruments Department	70
INTERNATIONAL COOPERATION	71
Visiting Scientists	72
Visits abroad	74
Participation in international meetings	79
SCIENTIFIC DEGREES	83
SCIENTIFIC MEETINGS ORGANIZED BY MEDICAL RESEARCH CENTRE	85
LIST OF PUBLICATIONS	86
Original Works	87
Communications	104

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RESEARCH REPORT

	Page
Department of Neurophysiology	34
Department of Neurochemistry	37
Department of Neuropathology	42
Laboratory of Developmental Neuropathology	48
Laboratory of Ultrastructure of the Nervous System	49
Department of Neurosurgery	51
Neuromuscular Unit	53
Department of Applied Physiology	58
Outpatient Cardiac Unit for Diagnosis and Therapy	62
Cardiovascular Laboratory	63
Department of Surgical Research and Transplantation	65
Department of Endocrinology	68
Library	69
MEDIPAN - Scientific Instruments Department	70

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MECHANISMS AND RESPIRATORY EFFECTS OF SIGNAL TRANSDUCTION IN THE CHEMOSENSITIVE STRUCTURES

(Assoc. Professor Mieczyslaw Pokorski)

We have studied the mechanisms of dopamine transport and release in the cat carotid body, using a continuous superfusion technique *in vitro*. A hypothesis was tested that apart from the known exocytotic vesicular dopamine release, a nonvesicular transport carried-mediated efflux of dopamine could contribute to its release upon stimulation. The evidence was found for an exchange/diffusion system, in which the movement of externally present dopamine into a cell stimulates the release of preloaded [³H]dopamine from that cell. This homoexchange of dopamine was found to be independent of the extracellular Ca²⁺ concentration and dependent on the Na⁺ gradient, which is consistent with its nonvesicular mechanism. These results suggest the existence of a Na-dependent carried-mediated efflux of dopamine, originating from a nonvesicular pool, which is a new finding with respect to the carotid body. Interaction of released dopamine with its membrane receptor may, through G-proteins, activate phospholipase C which cleaves phosphoinositides leading to the formation of second messengers which initiate cellular responses.

The hypothesis was tested that the peripheral chemoreceptor stimuli to breathing could also affect the laryngeal receptors. It was found that almitrine given into the laryngeal artery does not increase the activity of laryngeal afferents. This activity is enhanced only during the augmented breaths induced by almitrine.

Mechanisms of inhibitory, in certain conditions, effects of chemical stimuli to breathing were studied in the guinea pig. The hypothesis was verified that the reversed respiratory responses are due to the level of anesthesia and that anesthesia acts through depression of synaptic transmission. The results supported both hypotheses. A stepwise deepening of anesthesia evoked a depression of breathing in response to stimulants. This effect was abolished or reversed by the blockade of GABA inhibitory transmission.

STUDIES ON THE ANATOMICAL AND FUNCTIONAL CONNECTIONS OF THE BRAIN STEM STRUCTURES AND CEREBRAL CORTEX WITH RESPIRATORY MOTONEURONES

(Assoc. Professor Krystyna Budzińska)

In the studies concerning neural pathways from supramedullary structures to phrenic motoneurons we have checked the hypothesis coming from the results of the previous work that the motor nucleus of the Vth nerve (n.V.mt.) may be the substrate for the pneumotaxic center that is involved in the regulation of inspiratory and expiratory timing. The comparison of the effects of electric stimulation of n.V.mt., structures within and outside of the pneumotaxic region have shown that excitation of n.V.mt. activates mechanisms of inspiratory off-switch. Short-latency phenomena as well as characteristics of phasic response in the phrenic nerve activity combined with time course of off-switch excitability during subsequent phases of inspiration correspond very closely to the inspiratory inhibitory part of pneumotaxic mechanism. Stimulation of pneumotaxic complex in the site of nucleus parabrachialis medialis evokes different effects that are adequated to activation of inspiratory facilitatory mechanism. The results support a hypothesis of importance of the motor nucleus of trigeminal nerve in the respiratory phase-switching.

Bulbar post-inspiratory neurons inhibit practically all subpopulations of bulbar inspiratory and expiratory neurons and play major role in switching inspiration to expiration. On the base of this findings it was hypothesized that also phrenic motoneurons are inhibited or dysfacilitated due to activation of post-inspiratory neurons. It was found that phrenic motor nucleus have been inhibited (dysfacilitated) in the time periods closely resembling the discharges of post-inspiratory neurons. The inhibition have been transmitted via bulbo-spinal pathways crossed at the C4-C6 level.

The multichannel analog biomedical data acquisition system (named JULADC) has been completed. The purposes of the system are: 1. analog data collection, 2. storing collected data in a large computer mass storage, 3. presentation of recorded data in an analog - graphical form on the computer screen during the data acquisition (on-line) and from the mass storage (off-line) and 4. retriving of signal measures (parameters) from selected regions of the data. The system integrates three basic functions: data presentation (oscilloscope like system), data collection (tape recorder) and the ability of data processing (computer system). The data processing consist of: data segmentation, retriving the parameters of the data segments in the form of a parameter vector that describes an individual data segment, grouping the data parameters in a form of a data-base to be analyzed by means of any statistical tool. This system was applied in the described above neurophysiological studies.

NEW METHODS TO PREVENT THE DEVELOPMENT OF "DELAYED VASOSPASM" SYNDROME AND NEUROLOGICAL DEFICITS FOLLOWING SUBARACHNOID HEMORRHAGE

(Assoc. Professor Paweł Grieb)

Immunosuppressive properties of 2-chloro-2'-deoxyadenosine (2-CdA) have been evaluated in a series of *in vitro* tests. The drug has been found to suppress the activation of T-lymphocytes, but its primary target seems to be the B-cell system. Low (nanomolar) concentrations of 2-CdA effectively prevent B-lymphocyte activation and IgG synthesis. It has also been shown that the drug prolongs the mouse skin to allograft survival. The data collected so far on 2-CdA seem to indicate that it is a very promising agent for further evaluation in clinical immunosuppression and transplantology, including xenografting.

In patients with subarachnoid hemorrhage from ruptured intracranial aneurysm the response of T lymphocytes was found to be depressed. Considering this and several previous observations, a hypothesis of autoimmune etiology of intracranial aneurysms was put forward.

In the rabbit model of subarachnoid hemorrhage 2-CdA has been shown to prevent the development of angiopathy of intracranial arteries.

Low doses of 2-CdA have then been used in a group of patients with subarachnoid hemorrhage from ruptured intracranial aneurysm, who underwent early surgery (within 72 h from the bleeding episode). The treatment resulted in apparent full protection against development of the "delayed vasospasm" syndrome and/or neurological deficits in these patients, with no side effects.

THE ASSESSMENT OF CYTOTOXICITY OF 2-CdA (2-CHLORO-2'-DEOXYADENOSINE) AND 2-BdA (2-BROMO-2'-DEOXYADENOSINE) TOWARDS ORGANOTYPIC CULTURES OF CHOSEN STRUCTURES OF THE CENTRAL NERVOUS SYSTEM AND GLIAL TUMORS

Supported by the State Committee for Scientific Research: grant # 443449203 (Professor Mirosław J. Mossakowski)

Phosphorylation of 2-CdA by homogenates of human glial tumor tissues has been assayed. Glial tumors were cultured *in vitro* for assessment of the influence of the drugs on cell cycle kinetics with the use of DNA flow cytometry.

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EXCITATORY AMINO ACID RECEPTOR - DEPENDENT CALCIUM FLUXES IN THE HIPPOCAMPUS

(Professor Jerzy W. Łazarewicz)

The aim of these studies was to identify and to reveal the involvement of different natural calcium ionophores in injurious decompartmentation of Ca^{2+} to hippocampal neurons. Studies were focused on the role of ionic channels coupled to excitatory amino acid (EAA) receptors and of the L subtype of voltage-sensitive calcium channels (VSCCs). Microdialysis of the rabbit hippocampus *in vivo* was used to estimate the activity of calcium ionophores involved in Ca^{2+} fluxes after pharmacological stimulation and/or application of selective inhibitors. Changes in extracellular calcium concentration (Ca^{2+}_e) were measured with an indirect method based on detection of $^{45}\text{Ca}^{2+}$ radioactivity in dialysate, [^{14}C] sucrose being used as a marker of changes in extracellular space volume in the hippocampus. It was shown that application of KCl to the hippocampus induces a decrease of Ca^{2+}_e (by 25% at 65 mM KCl). This effect is the resultant of several mechanisms. The decrease in Ca^{2+} results in 70% from activation of NMDA receptors by endogenous EAA which are released due to KCl depolarization, whereas the activity of L channels participate in this effect in approx. 25%. Maximal activation of the L channels with Bay K 8644 increased this value to 30%. Moreover, it was found that the decrease in Ca^{2+}_e is partially compensated by paradoxical increase of Ca^{2+}_e , resulting from KCl-induced cellular swelling and shrinkage of the extracellular compartment in the hippocampus. Local NMDA application to the hippocampus leads to a large (60% at 5 mM NMDA) decrease of Ca^{2+}_e . Pharmacological characteristics indicate that this effect is primarily mediated by NMDA receptors. The effect of NMDA is only slightly (by 30%) inhibited by nimodipine and amiloride derivatives, indicating a relatively small indirect stimulation of the channels and $\text{Na}^+/\text{Ca}^{2+}$ exchangers. Changes in extracellular space volume of the hippocampus after NMDA application were brief and negligible. Local application of kainate, the other agonist of EAA receptors, results in the decrease of Ca^{2+}_e in the hippocampus. This effect is inhibited by DNQX, an antagonist of non-NMDA receptors. It was shown that kainate-induced Ca^{2+} fluxes result from indirect activation of several mechanisms including nimodipine-sensitive L channels and $\text{Na}^+/\text{Ca}^{2+}$ exchangers, without significant participation of NMDA receptors. Only a small portion of kainate-induced Ca^{2+} influx to neurons may be attributed to a calcium-permeable subclass of kainate channels. Altogether, these data indicate that Ca^{2+} fluxes in

the rabbit hippocampus *in vivo* through maximally stimulated NMDA channels considerably exceed Ca^{2+} fluxes through the L subtype of VSCCs. Therefore, these results point to NMDA receptors as primary targets to therapeutic intervention aimed to protect hippocampal neurons submitted to excessive stimulation.

ROLE OF NMDA RECEPTORS IN MECHANISM OF ARACHIDONIC ACID RELEASE IN BRAIN

Supported by the State Committee for Scientific Research: grant # 403209101 (Professor Jerzy W. Łazarewicz)

Studies aiming to estimate a role of NMDA receptors in the ischemia-evoked arachidonic acid (AA) release in brain were performed in Mongolian gerbils submitted to 5 min bilateral ligation of common carotid arteries, following by 5 min recirculation in normothermic conditions. Ischemia-evoked AA release was evaluated by measurement of the brain content of AA metabolites: prostaglandins (PG) D2 and F1 α and thromboxane B2. The experiments confirmed occurrence of prostanoid accumulation in the postischemic brain. Accumulation of PG D2 was significantly (by 35%) inhibited in animals treated with noncompetitive NMDA receptor antagonist MK-801 (0.8 mg/kg *i.p.*, 30 min before ischemia), whereas a decrease of other prostanoids was not statistically significant. It was shown in parallel experiments that pretreatment of animals with MK-801 significantly protects CA1 neurons from delayed selective injury only in animals that developed a prolonged postischemic hypothermia. These data indicate that NMDA receptors do participate in the ischemia-evoked AA release in the brain. However, there is no correlation between this effect and selective neuronal injury after ischemia.

MECHANISM OF ARACHIDONIC ACID RELEASE AND ITS ROLE IN MODIFICATION OF TRANSDUCTION PATHWAY IN ADULT AND AGED BRAIN

(Professor Joanna Strosznajder)

In our studies on the mechanism of arachidonic acid release the main attention was concentrated on cross-talk between different receptor systems and postreceptor processes of transduction pathway.

Recently we have investigated the relationships between serotonergic and cholinergic receptor system for arachidonic acid release in adult and aged brain. For comparison this receptor relationships were studied for inositol phospholipid degradation. The second aim of our studies was to determine the role of arachidonic acid in signal transmission in the brain. In this case the significance of arachidonic acid in the GABA_A receptor function was investigated.

It was found that serotonin inhibits cholinergic receptor dependent arachidonic acid release by phospholipase A₂ action. This negative coupling between these two receptors system was observed in adult brain. Our studies indicate that activation of acylation reaction coupled to serotonergic 5HT_{1a} receptor is involved in the mechanism of this negative coupling. It was found that this type of receptor relationship does not occur in the aged brain, because aging specifically eliminates the cholinergic receptor dependent arachidonic acid release and serotonergic receptor activated arachidonic acid incorporation.

Moreover, we have also observed that serotonin abolishes cholinergic receptor-dependent inositol phosphate liberation in aged brain. Serotonin and carbachol-agonist of the cholinergic system stimulate more actively degradation of phosphoinositides in aged brain comparing to adult brain. It is possible that serotonin 5HT₂ receptor dependent arachidonic acid and diacylglycerol release known to be involved in PKC activation may be responsible for this negative coupling between both receptors for inositol phosphate formations.

Arachidonic acid, liberated during receptor stimulation, may act as a second messenger in signal transduction pathway and also as a neuromodulator. In our studies we have observed that arachidonic acid, in the concentration dependent manner, enhances agonist binding into GABA_A receptor of synaptoplasmic membranes. Concomitantly arachidonic acid decreases the binding of antagonist ³⁵S-t-butylbicyclophosphorothionate (³⁵TBPS) into convulsant site of GABA_A receptor chloride channel. Analysis of binding kinetics of this antagonist was used for the evaluation of chloride channel function. In adult brain arachidonic acid enhances affinity of the agonist to synaptic plasma membrane receptor (the value of K_D is decreased by AA) for high and low affinity. In the synaptic plasma membrane from aged brain arachidonic acid increases low affinity agonist binding into GABA_A receptor, however decreases high affinity muscimol binding into receptor. Arachidonic acid significantly suppresses affinity and the amount of binding sites for TBPS. Moreover, this fatty acid decreases (by about 35%) GABA dependent Cl uptake into synaptoneuroosomes from adult and aged brain. Ageing itself affects agonist and antagonist binding into receptor, and remains without effect on GABA_A dependent Cl uptake into synaptoneuroosomes. However, an analysis of TBPS kinetic binding parameters indicates that ageing modifies chloride channel function.

The results of our studies have shown that arachidonic acid may be a very important endogenous regulator of the neurotransmission processes in GABAergic receptor system.

In pathological condition, as for example in brain ischemia, arachidonic acid

may be responsible for disturbances of GABA_A receptor function.

We have investigated also the action of GABA metabolite gammabutyrolactone (GBL) on arachidonic acid release and phosphoinositide metabolism in normoxic and ischemic brain. Gammabutyrolactone was applied intraperitoneally in a dose of 300 mg per kg b.w. 10 min before sham operation and global ischemia in gerbils. It was observed that GBL stimulates phosphoinositide degradation and arachidonic acid release in normoxic brain. However, in the brain submitted to ischemia it enhances the survival time for ischemic insult and decreases the accumulation of lipid derived second messenger formation as arachidonic acid, diacylglycerol and inositolphosphates. Additionally, GBL enhances the phosphoinositides kinase activity, particularly phosphophosphatidylinositol kinase. In this way GBL protects the brain against depletion of bisphosphosphatidylinositol, which may play an important role in the dynamics of cytoskeleton in the neuron cells. Moreover, GBL enhances the resistance of synaptosomal and subsynaptosomal membrane for lipolytic activity stimulated by ischemic insult and occurring after ischemia.

THE METABOLIC AND STRUCTURAL CHANGES IN ISOLATED NERVE ENDINGS CAUSED BY TOXICITY OF LEAD

(Assoc. Professor Urszula Rafałowska)

The effect of lead on the uptake of GABA, dopamine, histamine and histidine was studied in the presence of different concentrations of calcium in synaptosomes obtained from chronically lead-treated rats and in synaptosomes with *in vitro* added lead.

Lead decreased the uptake of GABA, increased the uptake of dopamine and did not change the uptake of histamine and histidine. These effects were independent of calcium concentration.

Lead administration to rats changed the morphology of the synaptosomes which was manifested in a decreased number of synaptic vesicles and disturbed mitochondrial structure. Also the Na⁺/K⁺ -ATPase activity was diminished. These results suggest existing of several different mechanisms of lead toxicity on uptake, related to individual neurotransmitters, which are not necessarily connected with Pb²⁺/Ca²⁺ interaction.

Also, the effects of lead on morphology of capillaries and on activity of γ -glutamyltranspeptidase were examined. The opening of tight junctions between endothelial cells and disturbances in γ -GTP activity can be responsible for dysfunction in the capillary permeability.

MODIFICATIONS OF SIGNAL TRANSDUCTION AND GENE EXPRESSION IN CNS PATHOLOGY SUCH AS ISCHEMIA OR HYPOMYELINATION

(Assoc. Professor Krystyna Domańska-Janik)

The studies on the involvement of protein kinase C (PKC) in various cellular systems transducing ischemia-evoked signals were continued. The secondary effect of the postischemic, short-lasting PKC translocation/activation tested in vitro and in vivo, consisted of:

- an activation of cAMP signaling;
- a transient activation of early-response protein - ornithine decarboxylase.

As it was previously reported, the postischemic increase of the ability of brain tissue to accumulate cAMP could involve a reversible impairment of the inhibitory A1-adenosine signal as a result of PKC-dependent phosphorylation. In the present investigation we examined additionally the effect of fatty acids (FA) and some other ischemia-induced bioactive lipids on A1-adenosine receptor (A1-AR). The specific ligand binding to A1-AR was competitively and reversibly inhibited by unsaturated FA. The effective FA concentrations were in molar ranges with the potency as following: oleic<arachidonic<decosaenoic<linoleic<linolenic acids.

Further characterization of the dysmyelinating mutant pt rabbit was focused on the myelin protein expression in the 1 to 120 day-old animals. The analyses were performed in total brain homogenates and purified myelin by radioimmunoassay, PAGE and immunoblotting. The proteolipid protein (PLP) was most drastically reduced, whereas the level of other proteins corresponded to the degree of hypomyelination. In the study on 4 week-old rabbits the myelin specific RNA-s were analysed by the Northern-blot technique. The PLP mRNA was of normal size in the pt rabbits but its level was reduced to a greater extent than messages for the other myelin proteins.

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DAMAGE OF THE VASCULAR BED AND THE VASCULAR-TISSUE JUNCTION AND THEIR SIGNIFICANCE IN PATHOGENESIS OF STRUCTURAL AND METABOLIC ABNORMALITIES IN CEREBRAL ISCHEMIC SYNDROMES

(Professor Mirosław J. Mossakowski)

The studies were conducted with use of two experimental models of CNS ischemia: global cerebral ischemia after cardiac arrest in rats, and short-lasting forebrain ischemia in Mongolian gerbils, caused by bilateral ligation of the common carotid arteries.

In the first model the condition of the blood-brain barrier (BBB) was evaluated in the early postischemic period - after 3.5-, 5- and 10-min of cardiac arrest. The disseminated alterations of the BBB were shown in the cerebral cortex, subcortical structures, brain stem and cerebellum. Changes of BBB permeability involved capillaries and arterioles as well as venules surrounded by perivascular space. The preferent sites of the BBB damage were bifurcations of venules. Changes of vascular permeability were connected with tight junction opening, creation of vesiculo-canalicular structures, increased micropinocytotic activity of endothelium, as well as disintegration of endothelium. Comparable to other types of ischemia biphasic character of the barrier mechanism damage was observed. In the same conditions formation of microthrombi and thrombocytic aggregates after global cerebral ischemia was characterized. Thrombocytic aggregates of different dimensions in cerebral arteries and veins have been found from several minutes to several months after the incident of ischemia. The predilective sites of thrombocytic aggregates were in the same microcirculation regions where the BBB damage was observed. The passage of thrombocytes outside the vascular bed to the brain tissue was a noteworthy phenomenon. Prolonged formation of the intravascular thrombocytic aggregates indicates persistent impairment of the thrombocytic-endothelium interaction as a result of ischemia, and may play an important role in pathogenesis of postischemic encephalopathy.

Changes of leukotriene C4 (LTC4) concentration in the rat brain cortex and hippocampus and brain water content have been evaluated during 5-min clinical death and up to 2hrs after resuscitation. A significant increase of LTC4 concentration was observed in the early postischemic period, which normalized 2 hrs after resuscitation. Water content did not show any significant changes. The results question the hypothesis that LTC4 plays an important role in pathogenesis of cerebral edema.

Studies performed on short-term forebrain ischemia in Mongolian gerbils concentrated on electron microscopic characteristics of two neurotransmitters: gamma-aminobutyric acid (GABA) and glutamate distribution in structural elements of CA1 sector of hippocampus. Occurrence of GABA was combined with symmetrical synapses located on perikarya of pyramidal neurons, while glutamate appeared mainly in asymmetrical synapses located on dendrite ramifications of these cells. Ischemia in the early period produced a decrease of GABA content and a slight increase of glutamate content in synapses of hippocampus. Later on, the immunohistochemical picture of synapses normalized. The redistribution of glutamate from neuronal elements to the astrocytic glia might indicate participation of glia in metabolism of this neurotransmitter.

In the same experimental model Concavalin A (Con A), binding to neurons of CA1 sector of Ammon's horn, was characterized during the evolution of their postischemic injury. Reduction of Con A binding to pyramidal cells was progressing proportionally to the degree of neuronal injury in the CA1 sector, however, the above changes concerned also the nerve cells without histological damage. It is suggested that this phenomenon might represent only an inhibition of carbohydrate metabolism with subthreshold cell damage.

Investigations *in vitro* on the organotypic culture of the rat hippocampus undergoing hypoxia or exposure to excitotoxic agents showed specific lamellar structures in neurons and seldom in glial cells. These cytoplasmic structures might represent alterations in the activated granular endoplasmic reticulum as a result of hypoxia or an excitotoxic effect of kainic or quinolinic acid. Some of the lamellar structures are comparable to alterations observed in the early phases of postischemic delayed neuronal death.

THE MORPHOLOGICAL MANIFESTATION OF AGING DEPENDING ON CEREBRAL STRUCTURES AND PARTICIPATION OF SELECTED EXOGENOUS CHEMICAL AGENTS IN DEVELOPMENT OF DEGENERATIVE PROCESSES

(Assoc. Professor Irmina B. Zelman)

The investigations were focused on immunocytochemical and ultrastructural characterization of amyloid plaque in transmissible (Creutzfeldt-Jakob disease - CJD and Gerstmann-Straussler-Scheinker syndrome) and non transmissible amyloidoses (Alzheimer disease - AD). Comparative studies have shown that besides immunohistochemically "clean cases" with typical amyloid deposits (PrP or beta-A4) in the group of PrP-amyloidoses, cases with additional presence of beta-A4 deposits, and cases with replacement of PrP with beta-A4 amyloid plaque occurred. This is comparable to AD, however, in contrary to plaques in this pathological process, they do not have immunoreactivity of tau and ubiquitin. At present the interpretation of this phenomenon can be only hypothetical.

Participation of microglia in organization of PrP plaque in GSS was characterized in comparison with beta-A4 plaque in AD. The similar immunohistochemical and ultrastructural picture of microglial cells in both types of amyloid plaques suggests that basic differences between them are related rather to the molecular structure of amyloid proteins than to the mechanism of development of amyloid plaque per se. The direct contact of amyloid fibrils with endoplasmic reticulum of microglial cells indicates their participation in amyloidogenesis rather than of fagocytic function.

In the breakdown and organization of ischemic infarction in senile brains with Alzheimer type pathology, beta-A4 amyloid is scavenged and retained long in microglia/macrophages and astrocytic cells. Immunoreactivity of neurofibrillary degeneration markers was not found, probably because of very quick degeneration of this abnormal cytoskeleton elements.

In a case of progressive supranuclear palsy, besides characteristic and typical alterations for this syndrome, beta-A4 amyloid plaques have been additionally found in cerebral cortex, however, in different localization than in AD. Dementia of subcortical type was intensified by coexisting extensive lacunar state and degenerative changes of nerve fibers in the white matter.

It was shown that the ground of profound dementia in Parkinson's disease might be coexisting with degenerative changes of Alzheimer type. The latter could be so extensive that diagnosis of both pathological syndromes would be justified.

Quantitative evaluation of GFAP-positive astrocytes in aged normal rabbits and pt mutants with CNS hypomyelination did not show essential differences between two groups of animals. In brains of senile animals the results were comparable with remarkable accumulation of glial fibrils in cytoplasm and astrocytic processes. In opposite to the period of myelination, in which astrocytic gliosis is transitory in nature, progressing accumulation of glial fibrils leading to alterations of cellular structure, might represent the senile changes of astroglial population. Acceleration and intensification of this process in brains of mutants suggest that biochemical changes in the myelin membranes could be important factors in development of involutive gliosis in the white matter.

In investigations on the effects of exogenic chemical agents on central nervous system, the late effects of quinolinic acid injected into the rat hippocampus resulted in local degeneration of pyramidal neurons and postsynaptic dendrites. Glutamate released from degenerated neurons is probably responsible for changes in astrocytic processes, which differ from chronic pathological alterations with phagocytic activity.

In experimental model of tellurium intoxication in mature and developing rats, the reversible changes in the white matter were found. They manifested in the form of loss of myelin staining and inhibition of myelination. These changes were dependent on the injected dose of the toxic substance and were not related to impairment of the liver and kidney function. The latter was confirmed by biochemical and histological studies.

PROGRESSIVE HIPPOCAMPAL ATROPHY AND ADVANCEMENT OF DEMENTIA IN POPULATION OVER 65 YEARS. NEUROLOGICAL, RADIOLOGICAL AND NEUROPSYCHOLOGICAL EXAMINATIONS

(Professor Mirosław J. Mossakowski)

The research had two objectives: establishing a comprehensive protocol for the diagnosis of the Alzheimer's disease (AD) and defining patterns and norms for computer tomography assessment of hippocampal atrophy.

The studied group consisted of patients of Outpatient Clinic for Alzheimer's subjects and residents of a nursing home for elderly.

Clinical examination (both neurological and neuropsychological), laboratory findings and neurophysiological diagnosis allowed to eliminate subjects with multiinfarct dementia (MID) from the study. Cases of dementia related to cobolamine and folate acid deficiency were to be singled out through laboratory findings. However, such dementia was noted in neither elderly subjects, nor MID subjects, nor suspected AD cases. In the 8% of AD patients in whom a decreased cobolamine level was noted, the dementia proved not to be deficiency dependent.

Under neuropsychological examination verbal memory showed no statistically significant differences in normal elderly subjects and patients with degenerative brain diseases (Parkinson's) while it appeared markedly impaired in cases of suspected Alzheimer's disease.

Neuroradiological examination was next performed on patients with suspected Alzheimer's disease. The relation between the degree of selective hippocampus atrophy and the degree of dementia was established as a high statistical probability ($p < 0.001$).

METABOLIC AND STRUCTURAL CHANGES IN THE CNS FOLLOWING INTOXICATION WITH INORGANIC MERCURY

(Professor Jan Albrecht)

Previous studies revealed that a single i.p. administration of a moderate dose of mercuric chloride (MC) durably inhibits the rat cerebral microvascular Na/K ATPase. To compare the mechanism of this inhibition to that produced by incubated isolated cerebral microvessels with MC in vitro, the effects of both treatments were compared with regard to a) K^+ -activation kinetics of the enzyme; and b) ouabain binding, reflecting the number of active enzyme molecules. Both treatments produced a 40% decrease of the V_{max} value for the enzyme and a 60% decrease of ouabain binding. The similarities of the effects in vivo and in vitro suggest that the inhibition of the cerebromicrovascular Na/K ATPase follo-

wing in vivo administration of MC results from the direct interaction of mercuric ions with the enzyme. A histochemical analysis revealed the enzyme inhibition by in vivo treatment to involve cerebral microvessels of all the cortical layers. Electron microscopy confirmed the enzyme reaction to be very weak or completely absent in both the luminal and abluminal endothelial cell membranes, and the luminal plasmalemma showed invaginations and pinocytic vesicles indicative of changes in its transport functions. The enzyme inhibition coincided with profound perivascular swelling involving mainly the astrocytic enfeet.

A study performed on a primary culture of the rat cerebral astrocytes confirmed the inhibition by MC of the astrocytic uptake of the excitotoxic neurotransmitter glutamate - an event postulated to contribute to MC neurotoxicity. An analysis of the ability of membrane-penetrating and -nonpenetrating SH-protecting agents to prevent the inhibition allowed to ascribe the inhibitory effect to the interaction of MC with vulnerable SH groups located within, but not on the surface of the cell membrane. An ultrastructural study carried out on organotypic rat cerebellum directly confirmed the hypothesis that low, nontoxic doses of MC lower the threshold for neurotoxicity of exogenously added GLU.

EFFECTS OF AMMONIA ON THE GLIAL TRANSPORT AND METABOLISM OF NEUROTRANSMITTERS AND THEIR PRECURSORS

(Professor Jan Albrecht)

The studies have concentrated on the excitatory, glutamatergic system. Attention was paid to the differences between the responses of various enzymes related to this system in the synaptic and nonsynaptic, mostly astroglia-derived mitochondria. Hyperammonemia in vivo selectively stimulated the synaptic mitochondrial activity of α -ketoglutarate decarboxylase - a catalytic component of the α -ketoglutarate dehydrogenase complex involved in glutamate outflow to the Krebs cycle. Hyperammonemia in vivo also stimulated the glutamate dehydrogenase activity specifically in the direction of glutamate oxidation. The results taken together are indicative of an increased flow of glutamate through the tricarboxylic acid cycle. This may be considered to reflect an adaptation of the nerve endings to the energy deficit accompanying hyperammonemia. However, this adaptation may adversely affect glutamatergic neurotransmission.

Hyperammonemia was found to alter the sedimentation properties of both mitochondrial classes, as manifested by their increased contamination with a cytoplasmic marker enzyme - lactate dehydrogenase and, in the case of nonsynaptic mitochondria, by an increased activity of a membrane marker - acetylcholinesterase. Hyperammonemia in two models was also found to induce cerebral edema which correlated with the decrease of the cerebral glutamate content.

This result was interpreted to indicate participation of glutamate in osmoregulation (regulatory volume decrease).

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NEUROPATHOLOGICAL AND MORPHOMETRIC ANALYSIS OF CHANGES OCCURRING IN THE CNS DURING MATURATION

(Professor Maria Dąmbska)

The morphometric evaluation of neurons in hippocampal region was performed on four control and eight pathologic cases. The results obtained demonstrated differences in the degree of lesions after acute, severe and prolonged asphyxia in newborns.

The neuropathologic evaluation of autopsy material confirmed the relations between the time of damage and developmental age of examined brains. It was particularly evident in cases of newborns with cardiac failures and secondary cerebral infarcts.

A case of congenital cerebro-oculo-dermal malformations was described presenting presumably a new syndrome. The migration abnormalities in periventricular and subarachnoidal regions found in this case are of very rare type.

ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDIES OF THE NERVOUS SYSTEM IN HUMAN SUBJECTS WITH PRENATAL PATHOLOGY AND IN ANIMALS TREATED WITH NEUROTOXIC DRUGS

(Assoc. Professor Danuta Maślińska)

Studies performed in human beings revealed that in patients with Down's syndrome, accelerating process of aging, is connected with defective formation of transcription factor AP-1.

The response of neurons in human myenteric plexus to the harmful agents causing accumulation of amyloid beta protein was the same as that of neurons in the brain.

In peripheral and brain amyloidoses the pathomechanism of myenteric plexus degeneration was different.

In rabbits with vincristine neurotoxic side-effects the biphasic reaction of brain vessels was observed. The initial vasoconstriction was followed by the intensive pinocytotic process in wall of brain capillaries. Numerous cells of brain parenchyma underwent apoptosis.

LABORATORY OF THE ULTRASTRUCTURE OF THE NERVOUS SYSTEM

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INVESTIGATION OF THE REPARATIVE REACTION IN SELECTED CEREBRAL STRUCTURES (HYPOTHALAMUS, MEDULLA, CORTICAL STRUCTURES) FOLLOWING AN ISCHEMIC INCIDENT - THE ROLE OF BRAIN MACROPHAGES

(Professor Jerzy Borowicz)

The aim of the immunocytochemical study was to evaluate the functional and molecular aspects of synaptophysine (SY-38) in the hypothalamus-hypophysis system (UPP) and the hippocampus of both the control rats and in those following 5 min of complete ischemia. In the membranes of the Golgi apparatus system cisterns of the hypothalamic neurosecretory nuclei and in the microvesicle membranes of the hypophysis a high concentration of SY-38 was found. The presence of SY-38 was also revealed in the neurotubular membranes of the whole UPP of the animals examined. In the hippocampus the presence of synaptophysine was revealed in the synaptic vesicle membranes and on the neurotubules. Compared to the normal state, immunoreactivity increased after a 5 min ischemia. The results obtained after the ischemic incidents support the hypothesis that the immunoreactive microvesicles are, similarly to the synaptic vesicles, organelles committed to the specific neuronal transmission.

The influence of exogenous γ -butyrolactone (GBL) on the hypothalamus-hypophysis system (UPP) of a Mongol hamster undergoing a 10 min period of ischemia was investigated. The results obtained from the ultrastructural analysis did not confirm the protective influence of GBL; whereas they revealed slight disturbances of the neurosecretory process resulting from the damage of some cellular elements of the hypothalamus and hypophysis.

A study of the toxic influence of HgCl₂ on the brain cortex of rats was also carried out. Eighteen hours after the induction of HgCl₂ an accumulation of dense deposits in the edematous elements of the nerve and glial cells and a multiplication of macrophages were discovered. Observations made after 5 days revealed these processes to be reversible.

The investigations were carried out concerning the changes occurring in the rat brain within the late period (i.e. 6 to 12 months) after an incident of clinical death caused by 5-10 min of cardiac arrest. The area adjacent to the capillary vessels of the hypothalamic neurosecretive nuclei and of the temporal cortex

was analysed. The most important observation was the presence of single fibrils, and sometimes bundles, of collagen in the compartments between the endothelium and the pericytes, the pericytes and the basal membrane and also inside the perivascular phagocytes. It was concluded that in the material examined reparative processes occur in the area between the capillary vessels and the cerebral cells surrounding them. The investigations revealed the presence of fibrosis similar to that found in parenchymal organs. It is impossible to say, however, which of the investigated cells, in face of the lack of fibroblasts, responsible for the synthesis of collagen protein.

DEPARTMENT OF NEUROSURGERY

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BRAIN ELECTRICAL ACTIVITY MAPPING (BEAM) IN PRIMARY GENERALIZED EPILEPTIC SEIZURES

SOMATOSENSORY EVOKED POTENTIAL (SSEP) STUDY IN DIAGNOSIS OF THE LEVEL OF SPINAL CORD DAMAGE

(Professor Eugeniusz Mempel)

The computer analysis of paroxysmal and interparoxysmal generalized epileptic discharges (the BEAM method) allowed to localise epileptic foci and to determine directions of the propagation of discharges in several cases. These discharges spread within one and both hemispheres, also spread into the cerebral midline structures. The frontal paramedial foci with discharges into the midline structures were mostly the cause of seizures characterized by unconsciousness. Other studies allowed to discover so called "the mirror-like foci" in homological areas of the contralateral hemisphere within cerebral frontal lobes. BEAM as a new noninvasive method allows to reveal epileptic localization in the cortex of the brain in primary generalized epileptic seizures more often, in spite of the opinion of their centrencephalic origin.

SSEP studies in the cervical discopathies showed conductivity reduction within peripheral part as well as in the part of C2-C6 spine. These studies have practical importance in diagnosis of damages in cervical part of vertebral column.

EARLY SYMPTOMS OF DISTURBANCES OF INTRACRANIAL VOLUME PRESSURE RELATIONS

(Assoc. Professor Zbigniew Czernicki)

One of the important problems studied in our Department was an early diagnosis of disturbances in the intracranial volume-pressure relations. In the experiments carried out on cats, the usefulness of harmonic alternations of ICP pulse wave in the estimation of intracranial volume reserve was evaluated. It was confirmed, that the ICP pulse wave analysis could inform earlier than other parameters about the intracranial system changes. So, it seems to be very informative in the intensive care of neurosurgical patients.

Studies with different rates of lumbar infusions allowed to elaborate the new model of infusion test. The test was checked under experimental conditions. It was proved, that application of three succeeding infusions (first - low rate - 0.06 ml per min, second - high rate - 0.3 ml per min, the third - again low rate - 0.06 ml per min) allowed to investigate the dynamics of CSF out-flow resistance changes depending on the intracranial pressure. The new method should be useful and important in clinical diagnosis of low-pressure hydrocephalus. The transcranial Doppler studies (TCD) showed the usefulness of the method in examination of focal brain vessel CO₂ reactivity. The measurements were done in patients after brain injuries and the correlation of vessel reactivity disturbances with localisation of brain lesions in CT was found. The Acetazolamid application allowed to evaluate the cerebral vascular reserve. This is a reason why the obtained information is very important in taking decision on surgical treatment of cerebral circulation insufficiency.

The transcranial Doppler (TD) was found also to be a very useful method in mental activity investigations. The estimation of blood flow changes during neuropsychological studies (considering hemispheric asymmetry and brain atrophy) was elaborated. The method should be very useful e.g. in the clinical diagnosis of low-pressure hydrocephalus.

LEARNING ABILITY IN ELDERLY PERSONS

(Professor Jadwiga Szumska)

The studies showed that in the degenerative diseases (Alzheimer and Parkinson diseases) besides the general deterioration of cognitive processes the verbal memory is very much disturbed. This dysfunction is caused by the linguistic function disorders and/or memory disturbances. Therefore, in the present studies both - linguistic and memory functions were evaluated in processes of learning.

In the clinical experiment learning ability was studied using verbal and nonverbal material with successive and simultaneous strategy applied. The study was carried out on 42 subjects, 20 young (control group) and 22 elderly persons. Statistical analysis confirmed the learning ability deterioration concerning both material types and both strategies. The most disturbances were observed in verbal-simultaneous learning procedure.

The obtained results suggest hemispheric interaction disorders as a cause of the disturbances observed. Significantly worse results obtained in the group of persons from Nursing Home in comparison with those in independently living persons could be due to the wrong organization of this institution or some nondiagnosed brain pathology.

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MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF IMMATURE AND AGING MUSCLE

(Professor Anna Fidziańska)

In the last few years the most attention has been paid to neuromuscular diseases in which the concomitance of architectural myofibril abnormality and desmin filament storage occur. Desmin has a special significance for the maintenance of the regular structural organization of sarcomere-associated cytoskeleton. Disorganization or remodeling of the cytoskeleton, as observed in pathological reactions, may change muscle protein expression.

For this reason we started the study on the distribution of desmin in the muscle specimens taken from patients with congenital myopathies by using antibodies against the intermediate filaments - vimentin, desmin.

As a first report we have presented a slowly progressive myopathy with focal myofibrillar disorganization of targetoid or core-like nature. Aberrant accumulation of 8-10 μm filaments seen at the ultrastructural level as a main content of core-like structures corresponds to the presence of desmin positive inclusions in light microscopy. This may represent a new disease entity due to an unknown myofibrillar protein defect that produces abnormalities in the structure of the muscle contractile elements. Presented myopathy indicates also that core-like changes, contrary to structured core devoid of desmin found in the central core disease, are a different entity and require further morphological as well as biochemical investigations.

Age-related changes in neuro-muscular system have been recently extensively studied both in humans and experimental animals. It is well known that the muscle strength and muscle mass decline with age. The morphological changes described by several investigators in the senile muscle are consistent with those found in neurogenic atrophy. Indeed, a progressive loss of motor neurons and terminal axons seems to be the main characteristic of the aging muscle.

Recently, in a group of very old rats, we observed severe motor deficit with marked decrease of muscle force and mass accompanied by muscle stiffness.

In electromyographic examination spontaneous tonic activity was present in all examined muscles.

Diverse morphological changes found in muscle in light and electron microscopy correspond to chronic denervation atrophy. A number of the already

known abnormalities could be demonstrated. However, sarcoplasmic reticulum (SR) tubular formations, which have been observed so far, only after a long standing experimental denervation of the muscle, were seen for the first time in unlesioned rats. This fact could be explained by more advanced age of our experimental animals and, in consequence, more advanced denervation atrophy. The other interesting finding, not yet observed in senile muscle, is the extensive splitting of muscle fibers with very well preserved morphology of splitted fragments.

Therefore, it seems that no single characteristic abnormality or set of morphological changes occur, which could identify markedly functionally altered senile muscle.

To our knowledge, in such very old rats no complex functional or ultrastructural analysis of the mechanism responsible for the impairment of neuromuscular function has been done so far.

ABNORMALITIES IN X-LINKED AND LIMB-GIRDLE DYSTROPHIES

(Professor Irena Hausmanowa-Petrusewicz)

About 15% of patients previously diagnosed as limb-girdle dystrophy after DNA testing and staining for dystrophin turned out to be X-linked type of dystrophy (mostly Becker type).

Western blotting technique was applied to detect parvalbumin and S-100 protein in muscles from Duchenne's dystrophy patients and patients with other muscle diseases and 5 age-matched normals. Duchenne's dystrophy muscles were found to contain significantly decreased amounts of parvalbumin and S-100 protein. The parvalbumin level in Duchenne's dystrophy was not related to the age of patients and the stage of their disease. The S-100 protein was decreasing progressively with age of the patients. In advanced stage of dystrophy S-100 protein was present only in trace amounts. In other primary myopathies and neurogenic atrophy cases both parvalbumin, as well as S-100 protein levels were similar to those observed in healthy subjects.

The results suggest that a decrease in the content of parvalbumin and S-100 protein in dystrophic muscles may contribute to the elevation of Ca^{2+} level in the sarcoplasm with subsequent activation of the Ca^{2+} -dependent proteolysis and ion transport disturbances.

The muscle biopsies performed in chronic progressive neuromyopathy, involving proximal as well as distal muscle, display a numerous unusual sarcoplasmic inclusions. These filamentous inclusions found in subsarcolemmal areas were closely related to Z-band. Immunocytochemical study, using polyclonal and monoclonal antibodies, revealed that the inclusions consisted of dystrophin positive material. Overproduction of dystrophin-related protein and/or dystrophin mutation is discussed.

MYASTHENIA - IMMUNOLOGICAL CHARACTERISTIC IN MYASTHENIA

(Assoc. Professor Barbara Badurska)

The question is how to treat myasthenia in elderly patients - the answer has been for a long time a matter of disagreement between clinicians, immunologists and surgeons.

The course of the disease and validity of treatment have been retrospectively reviewed in 82 patients with myasthenia gravis with the onset of first symptoms after 65 to 81 yrs of age.

Associated autoimmune diseases were found in 14% of patients: in 4% rheumatoid arthritis, in 3% diabetes mellitus, in 1% systemic lupus erythematosus and in 6% thyroid disease. Ocular signs alone at the beginning of disease were more common in older group. A significantly greater percentage of older patients progressed to severe disease with bulbar signs.

Forty patients were treated surgically and 60% of them received steroids both before and after operation.

In 45% of surgically treated patients thymoma was found (in 20% encapsulated and in 25% infiltrating). All the patients with infiltrating tumors went through radiotherapy, steroid and/or cyclophosphamide treatment.

Duration of the follow-up in the surgically treated group was about 8 yrs after operation, the longest being 20 yrs.

The results in the patients with thymoma, who underwent thymectomy, did not differ significantly from those without thymoma, 70% of these patients were asymptomatic on medication (steroids, azathioprine or cyclophosphamide).

ACQUIRED AND HEREDITARY NEUROGENIC LESIONS

(Assoc. Professor Katarzyna Rowińska)

The main problems were: the new concept of Guillain-Barré syndrome and remodelling of denervated motor unit.

A classical form of Guillain-Barré (G-B) syndrome is considered to be the acute inflammatory demyelinating polyradiculoneuropathy, in which morphological examination of the nerves reveals first of all foci of primary demyelination associated with infiltrates of lymphocytes and macrophages. Recently, some morphological descriptions indicating acute axonal changes have appeared. In our material examined electrophysiologically and morphologically different possibilities of axonal lesion in G-B have been presented and discussed. The block of conduction was found only in some cases of acquired neuropathies - mostly in pure motor neuropathies. The diagnostic yield of the sensory threshold level

changes in the peripheral nerve lesion was demonstrated in G-B and other neuropathic syndromes.

The motor unit desintegration and reintegration, is a process which occurs as a result of different kind of lesions (neurogenic, miogenic) and of the compensation of the damage. The above mentioned processes pass consecutive steps and each step of reintegration is characterized on the model of the motor neuron disease. One of the characteristic feature of the motor unit reintegration is the complexity of motor unit potentials (MUAP).

The complexity of each component - a component is more complex when it has more peaks and turns of different amplitudes and different frequency. The similarity of components - the signal is more complex when components differ among themselves.

The filling of signal - this factor depends on the number of components and relative duration of components and breaks.

The analysis of complex MUAP with satellite components based on the 231 electromyograms (EMG) was performed in 84 patients and 20 healthy volunteers.

The patients' group comprised cases of quickly progressing Amyotrophic Lateral Sclerosis (ALS) and the cases of slowly progressing benign and intermediate form of Spinal Muscular Atrophy (SMA).

Cases of Duchenne Muscular Dystrophy (DMD) were considered as s.c. diseases control.

The complex MUAPs appeared significantly more frequently in the progressing muscle diseases than in the control group and more frequently in the slowly progressing SMA than in quickly progressing ALS group.

In the SMA group a positive correlation between duration of the disease and complex MUAPs prevalence and a negative correlation between muscle force preservation and the number of the satellite components in one complex were found. In the DMD group there was no correlation between duration of the disease and the number of the complex MUAP. Statistical analysis revealed, however, a correlation between the muscle force and duration of the individual complexes. The results confirmed a hypothesis that there are different ways of the MU remodelling in the primary myopathic and neurogenic muscle lesions. In the primary muscular lesion the appearance of the satellite potentials reflects variability of the muscle fibers diameter. In the neurogenic lesion the satellites are the result of the increasing signal desynchronisation which takes place during the progressive de- and reinnervation. In both types of the muscle damage, however, the prevalence of the satellite components is the result of active remodelling of the motor unit.

A concept of the complex MUP potential classification is preserved on the basis of mathematical model. The complex internal structure of a potential reflects the pathological changes of the MU architecture and is a result of some remodelling and reorganization. The parameters describing the complexity of the MUAP are elaborated and complexity coefficient of a signal is defined.

DNA ANALYSIS IN DUCHENNE AND BECKER DYSTROPHY AND CHILDHOOD SPINAL MUSCULAR ATROPHY

Supported by the State Committee for Scientific Research: grant # 407799101 (Professor Irena Hausmanowa-Petrusewicz)

Dystrophy

DNA was isolated from 175 subjects of 41 families with Duchenne (Becker) dystrophy. DNA analysis was done using Southern blotting with application of 6 c DNA probes. For a rapid detection of deletion PCR was introduced.

The reanalysis of 30 families previously diagnosed as limb-girdle dystrophy was made and 9 of them turned out to be dystrophy of Becker's type. Dystrophin testing was performed in 65 male patients and in 12 females. One female patient with Duchenne-like clinical features turned out to have a translocation and had no dystrophin, the other were manifesting carriers with reduced amount of dystrophin.

Among examined families some unusual ones were found e.g.: the coexistence in the same family of Becker and Duchenne phenotypes or obvious X-linked inheritance of Duchenne-like phenotypes with normal dystrophin.

SMA

Thirty five SMA families were examined in collaboration with Laboratory of Genetic of Psychiatric Institute of Columbia University. All of them are mapped to chromosome 5, many of them express an intrafamilial clinical variability.

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PHYSIOLOGICAL EFFECTS OF INTERACTION BETWEEN PSYCHOLOGICAL AND PHYSICAL FACTORS

(Professor Krystyna Nazar)

In order to investigate relationships between psychomotor fitness, activity of the sympathoadrenal system and anaerobic threshold, in 22 young men blood lactate (LA), plasma catecholamines (CA) and multiple reaction time (RT) were determined during graded, incremental exercise. It was found that plasma CA concentrations increase exponentially during exercise with the thresholds at the work loads close to the blood LA threshold. It was also demonstrated that the subjects' psychomotor skill, estimated on the basis of RT measurements, improves with exercise intensity, reaches its optimum above LA and CA thresholds and then rapidly decreases.

To check the possibility that psychological stress impairs carbohydrate tolerance, oral glucose tolerance test (OGTT) was performed in 8 young healthy men under three conditions: (i) standard nonstressful, (ii) exposure to industrial noise (92 dBA) or (iii) performing the arithmetic Kracpelin's tasks. The stressors were applied for 20 min of each 30 min of OGTT. Venous blood glucose (BG) response to 75 g glucose ingestion was significantly lowered ($p < 0.001$) when the subjects were exposed to noise in comparison with two other conditions, but the glucose-induced increases in plasma insulin, noradrenaline levels and blood pressure were not affected by the stressors. In an additional experiment it was proved that the lowered BG response to glucose load during exposure to noise is caused by diminished arterialization of venous blood due to the peripheral vasoconstriction. It is concluded that psychological stressors of moderate severity do not impair carbohydrate tolerance in healthy persons.

Continuing the study on the effects of carbohydrate restricted, ketogenic diet on metabolic and hormonal responses to exercise, it was demonstrated that the 3-day ketogenic diet results in an increased maximal oxygen uptake and anaerobic threshold estimated during graded exercise. Besides, in the subjects on this diet an elevation of the plasma β -hydroxybutyrate, free fatty acid and plasma catecholamine levels was found both at rest and after exercise, whereas the plasma insulin concentration was decreased. The exercise-induced increases in blood lactate were lower and decreases in blood pH, base excess and standard bicarbonate levels more pronounced on ketogenic than on normal diet.

Effects of low energy diet (1000 Kcal/day for 4 weeks) and phosphate supplementation on metabolic and endocrine responses to physical exercise and oral glucose load were examined in 36 overweight women. The data confirmed our

previous study showing an increase in the resting metabolic rate in dieting subjects treated with phosphates. However, this treatment had no influence on mechanical efficiency during exercise, and glucose-induced thermogenesis. No differences were found between the subjects supplemented with phosphates and those on Placebo in resting and post-exercise blood glucose, lactate, plasma free fatty acid, adrenaline, cortisol, growth hormone, insulin, and testosterone levels, whilst the respiratory exchange ratio was slightly higher and the plasma β -hydroxybutyrate lower with than without phosphate treatment. Post-exercise plasma adrenaline was reduced after 4 weeks of energy restriction only in the group on Placebo. Blood glucose, insulin and noradrenaline responses to glucose load were not affected by phosphate supplementation.

A pattern of changes in blood lactate was investigated in rats during multistage, incremental treadmill exercise. It was demonstrated, for the first time, that in rats the lactate threshold can be detected. It occurs at blood lactate levels of 4 mmol/l, similarly as in man, and shows high reproducibility. This experimental model was used to find out whether prolonged hyperadrenalinemia, induced by sc implantation of adrenaline tablets, releasing the hormone at a constant rate (approx. 1.6 μ g/min) affects the anaerobic threshold. The results indicate that hyperadrenalinemia sustained for 12 h decreases exercise performance, and the intensity of exercise at which lactate threshold occurs. After more prolonged elevation of blood adrenaline (60 h) the anaerobic threshold returns to control values. The latter phenomenon may be, at least partly, due to the down regulation of adrenergic receptors.

To find out whether the impaired glucose utilisation by skeletal muscles, described previously in hypothyroid (THY) rats, could be reversed by prolonged activation of β -adrenergic receptors, both euthyroid and THY rats were implanted with slow releasing adrenaline tablets (see above) 12 h before sacrifice. In the soleus muscle the rates of 2 deoxyglucose (2DG) transport, lactate (LA) formation and glycogen synthesis were measured in vitro at various concentrations of insulin in the incubation medium. In THY rats hyperadrenalinemia increased the rate of 2DG transport to muscle cells and glycogen synthesis, but it did not influence lactate production. Responsiveness of all these processes to insulin was enhanced by adrenaline treatment and the insulin sensitivity of LA production and 2DG transport was improved. The data suggest that prolonged hyperadrenalinemia partly reverses the effect of thyroid hormone deficiency on muscle glucose utilization.

Continuing the study on an influence of skeletal muscle contractile activity on their sensitivity to insulin, it was shown that 15 min electrical stimulation of the rat sciatic nerve greatly increases the in vitro measured sensitivity of glucose transport, lactate production and glycogen synthesis to insulin of the soleus muscle impaired by previous (12 h before sacrifice) tenotomy. Extending the stimulation up to 30 min did not cause any further changes in insulin sensitivity either in tenotomized or in intact muscles.

RISK FACTORS OF PRIMARY HYPERTENSION: PROSPECTIVE STUDY

Supported by the State Committee for Scientific Research - grant # 413109101
(Professor Krystyna Nazar)

Twenty nine persons with exaggerated blood pressure (BP) response to physical exercise were identified among 145 male students aged 20-23 yrs with normal BP at rest. The aim of this study has been to find out whether elevated BP response to exercise is associated with any other hemodynamic, metabolic or hormonal abnormalities, and to follow up their evolution during at least three years. In the first year of the investigation 15 students with elevated (group E) and 10 students with normal (group N) BP response to exercise were examined. In 8 subjects from group E echocardiography revealed left ventricle hypertrophy but in none ECG abnormalities were found. Body mass index, fat content, waist to heap ratio, plasma lipid concentrations, as well as fasting blood glucose (BG), and plasma insulin (IRI) levels were similar in group E and N. Subjects from group E had also normal plasma catecholamine concentration and renin activity both at rest and during exercise, but their plasma levels of cortisol, and sodium concentration in erythrocytes were higher than in group N. During the oral glucose tolerance test (75 g) only in one subject from group E abnormal BG and plasma IRI responses to glucose load were noted, however, in this group significantly lower increases in cardiac output, and higher total peripheral resistance were found in comparison with group N.

INTRAMUSCULAR LIPID METABOLISM

(Assoc. Professor Leszek Budohoski)

A new method of measuring in vitro the labelled free fatty acid uptake and metabolism in the rat skeletal muscle was elaborated. This included determination of the optimal conditions of the soleus muscle incubation (time of incubation, concentrations of necessary substrates etc) as well as verification of the intramuscular free fatty acid (FFA) and triacylglycerol (TG) content estimation. Preliminary experiments were carried out to establish the relationship between the concentration of ^{14}C -palmitate in the incubation medium and the rate of labelled TG synthesis, CO_2 production and concentration of the labelled intramuscular FFA. Besides, the effects of glucose and insulin added to the the medium on the ^{14}C -palmitate uptake and metabolism were investigated. The results indicate that glucose, irrespectively of the insulin presence, enhances significantly the rate of intramuscular TG synthesis.

Changes in two forms of lipoprotein lipase (LPL) activity (heparin releasable and residual) and TG content in the rat soleus muscle were followed during 48h hyperadrenalinemia, produced by sc implantation of slow releasing adrenaline

tablets (1.6 μ /min). Both forms of LPL activity were significantly diminished after 6 and 12 hrs of hyperadrenalinemia, and then returned or even exceeded the control values. These changes can not be attributed either to plasma FFA, which were elevated throughout the whole 48 hrs or to muscle TG that were progressively increasing. The data suggest that adrenaline per se inhibits muscle LPL activity. The increase of the enzyme activity after 24 hrs of sustained hyperadrenalinemia may result from the down regulation of adrenergic receptors.

A MODEL OF CHRONICALLY EXPLANTED KIDNEY IN THE RAT (PRELIMINARY STUDIES)

(Professor Janusz Sadowski)

In previous studies we have successfully applied measurements of tissue electrical admittance (reciprocal impedance) for estimation of the electrolyte hypertonicity of the renal medulla in the rat. The studies were conducted in acute experiments performed in animals under barbiturate anesthesia, directly after extensive surgery necessary for exposure of the kidney. A serious limitation of such an approach has become apparent when we attempted to study hormonal control of the cortico-medullary electrolyte (mostly NaCl) gradient. It is known that both barbiturates and acute surgery are powerful stimulants of multiple hormonal systems, e.g. of the release of the antidiuretic hormone, a factor modifying the magnitude of the gradient.

In order to study physiological control of the medullary ionic hypertonicity in the kidney, an experimental model was developed enabling admittance measurements in the absence of acute surgery, under light anesthesia with alpha-glucosylchloralose, an agent devoided of vasopressin stimulating action.

In Wistar rats the left kidney was explanted out of the abdominal cavity under the flank skin. The renal artery and vein were left intact, passing across the flank muscles via an unsutured window within the original incision. The ureter was cut close to the bladder and exteriorized for urine collection.

The proper experiments were conducted 3 days later, under i.v. chloralose, 30-40 mg/kg body weight. Electrodes for admittance measurement were inserted into the kidney after cutting the sutures of the skin overlying the flank wound. Inspection of the exposed kidney confirmed its viability. The renal excretion data and the in vitro comparison of the medullary tissue composition indicated a moderate impairment of the explanted versus contralateral kidney function, by about 30%.

The model is of potential value for the study of the intrarenal salt gradient fluctuations, with but only minimal, anesthesia and in the absence of acute surgery. Its application can be extended to all experimental projects in which easy repeated access to kidney tissue (e.g. collecting biopsy material) is required. Before such attempts, more precise evaluation of the explanted kidney function, including measurements of the glomerular filtration rate, are necessary.

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RISK FACTORS OF CORONARY ARTERY DISEASE IN MIDDLE-AGED WOMEN

(Dr Ewa Wójcik-Ziółkowska)

Although the number of infarctions in female population increases the results of standard noninvasive tests conducted in the diagnosis of coronary artery disease (CAD) are often false positive (30%).

The study presented is a part of the project concerning the pathomechanism of coronary insufficiency in women. The aim of the investigation was to determine the risk factors of CAD in a group of 112 women. An attention has been focused on the following possible risk factors: obesity, smoking, glucose intolerance hormonal dysfunction, hypertension, family history of heart disease, hyperlipidemias.

The patients were divided into three groups: Group I - 70 women (mean age 51.7 ± 7.3 yrs) were included to the study because of the anginal-like chest pain and/or abnormal resting ECG. Group II - 18 women (mean age 60.8 ± 10.9 yrs) after myocardial infarction (MI). Group III - 24 women (mean age 44.7 ± 7.8 yrs) consisted a control group without CAD symptoms and with normal ECG.

In all groups body mass index (BMI) was similar, although in group I and II overweight was frequently observed. The frequency of smoking habit was significantly greater in group I and III in comparison with the group II after MI. Glucose intolerance was often found in group II, whilst hormonal dysfunction was demonstrated in groups I and II but not in the control group. Hypertension occurred in the women from groups I and II only. Patients from group I and II had family history of heart disease. Hyperlipidemia was observed most frequently in the post-MI group, however, in the patients from the group II - the concentrations of blood lipids were significantly higher than in the control group.

It should be pointed out that six of the analysed risk factors were absent in the control group, without any CAD symptoms. Therefore, we conclude that presence of those factors is crucial in the process of qualifying the patients for the group with high risk of CAD.

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HORMONAL AND METABOLIC REACTION TO ACUTE MYOCARDIAL INFARCTION

(Professor Krystyna Cedro-Ceremużyńska)

During the last year, Cardiovascular Laboratory has been involved in clinical investigation carried out in the Department of Cardiology, Postgraduate Medical School in Warsaw.

Research program included:

1) Studies on the mechanisms influencing survival and outcome in acute myocardial infarction (AMI).

This study aims to assess separate and combined effects on vascular mortality of 3 treatments: nitrates, angiotensin-converting enzyme inhibitor and magnesium, added to currently standard therapy in patients with AMI. Our particular aim was to investigate metabolic, humoral and hemodynamic mechanisms influencing the course and outcome of AMI and underlying the effectiveness of these interventions.

Previous investigation of our group and of other authors have shown that the magnitude of adrenergic response to AMI and activation of renin-angiotensin-aldosterone system influence severity of clinical course and adversely affect the outcome of the disease. Therefore, in the patients enrolled into ISIS-4, we measure blood catecholamines and their urinary excretion, plasma renin activity, angiotensin II and aldosterone, as well as indirect indices of enhanced free generation, such as serum lipid peroxides, erythrocyte SOD activity, chemiluminescent response of leukocytes. This is complemented by evaluation of the contractile function of the heart (Echo) and assessment of heart rhythm (24 h Holter monitoring). This study is blind, opening the code is planned for Autumn 93. Summing up of our results will then be possible.

2) Influence of antioxidant vitamins upon the indices of tissue lipid peroxidation and leukocyte free radical generation in acute myocardial infarction (AMI).

Damaging effect of oxygen free radicals upon integrity of cell membranes and extension of myocardial ischemic injury has been well documented in experimental studies. Enhanced free radical generation is a likely consequence of hormonal and metabolic response to AMI which may lead to exhaustion of endoge-

nous antioxidant defense mechanisms.

We have examined clinically available indices of enhanced free radical generation: chemiluminescent response of isolated leukocytes to an activating agent, serum lipid peroxides, erythrocyte SOD activity as well as serum endogenous antioxidants, Vit. C and α -tocopherol, in patients with AMI. Patients routinely treated with currently standard medication are randomly assigned to Vit. C+E supplemented (orally 600 mg daily for 14 days) or non-supplemented group. After completion of the study planned for May-June 1993 (30 patients in each group) the results will be analysed to assess:

- oxidant-antioxidant balance in acute phase of AMI;
- the effect of supplementation with antioxidants upon the indices of free radical generation in the course of the disease.

The results may provide biochemical basis for routine supplementation with exogenous antioxidants in the course of myocardial infarction.

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NEURO-ENDOCRINE REGULATION OF CAPILLARY TRANSPORT OF CELLS INTO THE TISSUE SPACE AND LYMPHATICS

(Professor Waldemar L. Olszewski)

Lymphocyte circulation and migration through tissues is of crucial importance for the immune surveillance. The influence of thermal stress on lymphocyte recirculation was investigated in a rat model. After 8-hours hyperthermia (42°C), distribution of lymphocytes to spleen and lymph nodes was lower than in normothermic controls. Pharmacological sympathectomy (6-OHDA) abrogated these differences. Blocking of α - and β -adrenergic receptors on lymphocytes (Labetalol) did not influence the migration index, suggesting that catecholaminergic innervation of secondary lymphatic organs may influence lymphocyte mobilization after stress. The distribution of lymphocytes to bone marrow (BM), following thermal stress was much higher than in controls. Adrenalectomy abrogated this effect, while dexametazone caused an increased distribution of lymphocytes to BM, both in normothermic and hyperthermic rats. In vitro dexametazone-treated lymphocytes also showed a higher migration rate to BM. The results suggest that distribution of lymphocytes to BM may be regulated by endogenous glucocorticoids.

For the elimination of foreign antigen and inhibition of tumor cell growth, transport of immune cells to tissue space remains the main problem. In the present study the effect of increased venous pressure and hyperthermia (42°C) on lymphocyte migration into the afferent and efferent lymph of dog hindlimb was investigated. Hyperthermia increased the leukocyte output into afferent lymph 6-times, whereas into the efferent - 85-times. Increased venous pressure enhanced leukocyte output into afferent lymph twice, whereas into efferent - decreased 3-times. Hyperthermia, therefore, stimulated leukocyte migration to skin and lymph nodes to the higher degree than did increased venous pressure.

TRANSPLANTATION OF ALLOGENEIC CELLS AND TISSUES

(Professor Waldemar L. Olszewski)

Rat allogeneic heart or kidney grafts are rejected within 6-8 days, while lymphoid cells, transplanted i.v. across the MHC barrier are eliminated within 6 hr.

In the present studies, the mechanism of accelerated elimination of allogeneic lymphocytes by DST-treated recipients was studied. It was shown that DST effect on i.v. transplanted lymphocytes may be mediated by non-cytotoxic opsonizing antibodies. They probably block the donor specific MHC antigen epitopes on organ graft endothelial cells, thereby preventing the attack of circulating donor specific cytotoxic lymphocytes. At the same time, however, they opsonize i.v. transplanted lymphocytes, facilitating their destruction in circulation and host lymphoid organs.

Another study was devoted to the bone marrow (BM) grafting. Repopulation of bone marrow cavities after i.v. transplantation of BM cells is a slow process, whereas engraftment of BM in hind limb graft leads to repopulation of hemopoietic system of irradiated recipient already in 10 days, due to preserving the functional relationship of hemopoietic and stromal cells in the bone. Since engraftment of vascularized BM in hind limb is a complicated procedure, it was studied whether the implantation of BM coagulum to liver and spleen will be sufficient for recipient repopulation. It was found, that after 10 and 30 days, histological structure of BM was preserved in 30% of experiments on the average.

REGULATORY FUNCTIONS OF LYMPHOID CELLS

(Professor Waldemar L. Olszewski)

In the first part of this project the mechanism of immune "enhancement" was studied. The question was asked, what is the distribution of donor antigen and alloserum in heart and skin graft. It appeared that alloserum against donor antigens did not accumulate in grafts, despite of its effect on prolongation of graft survival. The results suggest that alloserum acts on the effector arm of the recipient immune response.

Another problem studied was the effect of CyA on in vivo migration of i.v. injected lymphocytes. The recipient of lymphocytes was treated i.v. with CyA (10 mg/g b.w./day, during 7 days) before injection of syngeneic ⁵¹Cr labelled lymphocytes. It was found that thoracic duct lymphocytes are distributed in CyA-treated recipient similarly to saline and CyA dissolvent (Kremofor Elger)-treated recipient. Only in vivo CyA-treated lymphocytes (from treated donor) showed a diminished migration to blood, and increased accumulation in the lymphoid tissues.

An original test of lymphoid cell adherence to liver cells was also signed for the study of the mechanism of selective trapping of lymphocytes in liver sinusoids and, especially, for identification of adhesion molecules, responsible for the adherence process. Human blood lymphocytes, placed on frozen sections of hu-

man or rat liver, adhered to endothelial cells of sinusoids, Kupffer cells, while to a lesser degree, hepatocytes. Among lymphocytes, suppressor/cytotoxic cells (OX8⁺) displayed the highest adherence abilities. The number of cells which adhere to liver cells depends on their activation as well as on activation of endothelial and Kupffer cells.

A study was devoted to the problem of phenotypical characterization of human peritoneal cells. It was found that most peritoneal CD8⁺ (75%) and CD4⁺ (90%) T lymphocytes belong to the population of memory cells, (in blood 30% and 50%, respectively).

IMMUNE RESPONSE AFTER TRAUMA

(Professor Waldemar L. Olszewski)

Tumor cells, both primary and metastasizing, proliferate, at least at the initial stage, in the normal tissue fluid. Lymph is the tissue fluid drained via lymphatics. In the present studies, the effect of IL1 α , IL1 β and IL6, the cytokines present in human skin lymph, on tumor cell growth was investigated. The strongest antiproliferative activity was displayed by IL6. Since in human skin lymph these cytokines were present together, their effect in combination was studied. The results suggest that the effect of their action in combination, may differ from that observed when a single cytokine is present. To confirm that skin cytokines may be responsible for antiproliferative effect of lymph, blocking of their activity was performed, using anti-cytokine antibodies. Blocking of IL1 α , IL1 β and IL6 activity abrogated the inhibitory effect of lymph on tumor cell proliferation. These cytokines may be, therefore, responsible for regulating tumor cell proliferation in human skin.

In another study, the effect of syngeneic blood transfusion (SBT) on subpopulations and functions of mononuclear cells from blood (B), spleen (SPL) and bone marrow (BM) was examined in rats. The model corresponds to the clinical situation of massive blood transfusions, which affects the immune responsiveness. SBT brought about an increase in frequency of myeloid and lymphoid lineage cells and OX7⁺ stem cells in BM, as well as release of OX7⁺ cells in blood circulation. Decreased responsiveness of B, SPL and BM cells to mitogens may reflect a diminished frequency of immunocompetent cells due to dilution by less mature BM cells or inhibition of DNA synthesis capacity by putative factors produced in response to surplus of blood cells.

A study considered the problem of the potential source of humoral factors, following trauma, accelerating *in vivo* hemopoiesis. It was found, that the increase in BM volume was most efficient after a large tissue trauma, while infiltrating and proliferating cells in the anastomotic wound seem to be a potent source of cytokines for BM hemopoietic cells.

DEPARTMENT OF ENDOCRINOLOGY

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Head: Professor Janusz Nauman

NEW DRUGS FOR NEW METHODS OF NEUROLOGICAL TREATMENT

(Assoc. Professor Andrzej W. Lipkowski)

Traditional search of drugs for neurological disfunction treatment is focussing on compounds with high pharmacological permeability, and as high as possible selectivity. However, such approach has limitations, because the nervous receptor systems are not selective themselves - the selectivity being a result of space selective secretion. Thus, following nature, we are looking for neurological drugs which have low permeability and their action may be regulated at the place of distribution. The first targets of our research are new drugs for pain treatment. New technics of anesthesia allow to administre drugs directly to the place of their needed action. The neuropeptide analogues with low permeability may create a new generation of drugs useful for these anesthesia technics.

THE LIBRARY

5 Pawińskiego Str., 02-106 Warsaw

Telephone: 658 46 77

Head: Krystyna Marczakowska

The library constitutes one Department of the Medical Research Centre and acts as an information source for scientists.

Scope and the subject profile: physiology, neurosciences and experimental surgery including transplantology.

Present holdings:

books - monographic and serial volumes (Polish and foreign) - 17412 periodicals, newspapers (number of titles) - 500

Reference aids:

catalogues - alphabetical: book, periodicals and microfiches

- subject: books

main card-files - bibliographical list of papers published by scientists of the Medical Research Centre Polish Academy of Sciences from 1967.

Users:

scientific workers of the Medical Research Centre, interlibrary loans available for all scientific Institutes in Poland and abroad.

Bibliography of library: a list of new books and current periodicals is prepared weekly. On the basis of the Scientific Citation Index a report of citations of papers published by MRC Scientists in 1991-1992 was prepared. It contains 1193 citations.

MEDIPAN

1/13 Flory Str., 02-586 Warsaw

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Telephone/fax: 49 30 79

Head: Andrzej Lasek

MEDIPAN is a manufacturer of a special equipment for medical service units' needs.

This equipment is represented by microprocessor infusion drip pumps and microprocessor infusion syringe pumps.

Once it had carried on a wide range of construction and production activities. To withstand competition with foreign products of this type and manufacture equipment of the same technical level as the equipment of well-known world companies MEDIPAN had to diminish the scope of its constructional works. In the result the last year's activity can be characterized by visible specialization in the field of construction and production of infusion drip pumps and infusion syringe pumps. Models manufactured in the previous years were considerably modernized according to the latest world construction and technical solutions and the new ones were produced in MEDIPAN.

Models 601 SP and 605 SP Microprocessor Infusion Drip Pumps represent the first group of equipment. The stress was put here on precision of operation and energy saving.

Employment of a new type of the instrument's enclosure resulted in softening the driving system operation and weight reduction, whereas modification of microprocessor control system increased the flow accuracy.

Model 611 Microprocessor Infusion Syringe Pump has been improved by adapting its program to cooperation with three sizes of syringes (10, 20, 50 ml) instead of one (50 ml). Construction works were completed and a set of a quite new type of instrument - Model 604 Microprocessor Infusion Drip Pump - was produced.

Techno-economic data and therapeutic-clinical directions were worked out for the Model 612 Microprocessor Infusion Syringe Pump, which represents completely new, more sophisticated type of infusion pumps, on the highest world technical level. It cooperates with 6 sizes of syringes and enables administration of a drug with the flow rate automatically calculated by the pump's memory on the basis of such parameters as: patient's body mass, a dose of a drug and its concentration.

Each instrument manufactured in MEDIPAN has the approval of the Polish Ministry of Health and Social Welfare as well as complies with the EEC standards.

Specialization and endeavours to remain competitive on the world markets have led to the situation that MEDIPAN's constructions are ahead of other Polish constructions on this field.

INTERNATIONAL COOPERATION

	Page
Visiting Scientists	72
Visits abroad	74
Participation in international meetings	79

VISITING SCIENTISTS

Department of Neurophysiology

- K. Murphy Department of Medicine, Charing Cross and Westminster Medical School, University of London, United Kingdom
- I. Poliaczek Department of Medicine, Komensky University, Martin, Czecho-Slovakia
- R. Torrance Laboratory of Physiology, University of Oxford, United Kingdom

Department of Neurochemistry

- S. Pfeiffer Department of Microbiology, University of Connecticut Health Center, USA

Department of Neuropathology

- A. Lossinsky Institute for Basic Research and Developmental Disabilities, New York, USA
- D.L. Meyer Laboratory of Neuroanatomy, Georg-August University, Götting, Germany

Laboratory of Developmental Neuropathology

- K. Shütt Institute of Neuropathology, University Clinic Steglitz, Berlin, Germany

Department of Neurosurgery

A.L. Krawczuk
G.A. Lichterman
Institute of Neurosurgery, Medical
Academy of Sciences, Moscow, Russia

Neuromuscular Unit

A. Eisen
University of Vancouver, Canada

B. Hecht
Institute of Pathophysiology, Academy of
Medical Sciences, Moscow, Russia

K. Zerres
S. Rudnik-Schönborn
D. Röhrig
Institute for Human Genetic, Bonn,
Germany

Department of Applied Physiology

D. Desplanches
Laboratory of Physiology, Department of
Medicine Lyon Grange Blanche, Lyon,
France

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Department of Physiology, University of
Kuopio, Finland

H.C.G. Kemper
Department of Health Science, Faculty of
Human Movement Sciences, University of
Amsterdam, Netherlands

S. Porta
Department of Functional Pathology,
University of Graz, Austria

R. Terjung
Department of Physiology, College of
Medicine, State University of New York,
USA

Department for Surgical Research and Transplantation

P.B. Sivaraman
Department of Urology, Chengalput Medical
College, Chengalput, Madras, India

VISITS ABROAD

Department of Neurophysiology

- K. Budzińska Institute of Physiology, University of Florence, Italy
- Gromysz H. Laboratory of Physiology, Department of Medicine, University of Nancy, France
- Pokorski M. Laboratory of Physiology, Department of Medicine, University of Nancy, France
Department of Biochemistry and Biology, University of Valladolid, Spain
Department of Pharmacology and Therapeutics, Faculty of Medicine, Porto, Portugal
Fakulty Detskeho Lekarstvi, University Karlovy, Praha, Czecho-Slovakia
- J. Romaniuk Department of Medicine, Metro Health Medical Center, Cleveland, Ohio, USA (long term visit)

Department of Neurochemistry

- K. Domańska-Janik Laboratory of Neurochemistry, University of Vadois, Lausanne, Switzerland
- W. Gordon-Majszak Institute for Basic Research and Developmental Disabilities, New York, USA (long term visit)
- M. Łalowski Department of Pathology, New York Medical, Research Centre of Mental Disabilities, New York, USA (long term visit)
- J. Łazarewicz Institute of Neurobiology, University of Goteborg, Sweden

M. Puka	Institute of Neurobiology, University of Goteborg, Sweden (long term visit)
M. Samochocki K. Domańska-Janik	Institute of Biochemistry, University of Perugia, Italy (long term visit)
J. Strosznajder	Baylor College of Medicine, Houston, Texas, USA (long term visit)
J. Waškiewicz	Department of Chemistry and Biochemistry, University of Oklahoma, Norman, USA (long term visit)
H. Wikieł	Roswell Park Cancer Institute, Buffalo, USA (long term visit)
T. Zalewska	Muscle Biology Group, College of Agriculture, University of Arizona, Tucson, USA (long term visit)

Department of Neuropathology

J. Albrecht	Department of Pharmacology and Toxicology, Albany Medical Center, Albany USA (long term visit)
L.Faff-Michalak	Carl-Ludwig Institut für Physiologie, Universität Leipzig, Germany
W. Hilgier	Department of Emergency Medicine, Wrigh State University, Dayton, Ohio, USA (long term visit)
S. Januszewski E. Kida	Institute for Basic Research and Developmental Disabilities, New York, USA (long term visit)
S. Krajewski	La Jolla Cancer Research Foundation, California, USA (long term visit)
M. Ratajczak	Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, USA (long term visit)

Laboratory of Developmental Neuropathology

M. Dąmbska
D. Maślińska
Institute for Basic Research and Developmental
Disabilities, New York, USA
(long term visit)

Laboratory of Ultrastructural Nervous System

B. Gajkowska
Cancer Research Center, CNRS,
Villejuif, France (long term visit)

Department of Neurosurgery

Z. Czernicki
Department of Neurosurgery,
Tokyo Medical and Dental University,
Japan (long term visit)

Z. Czernicki
J. Jurkiewicz
Department of Neurosurgery,
St. Jacob Hospital,
Vilna, Lithuania

Z. Czernicki
J. Jurkiewicz
Burdenko Neurosurgery Institute,
Moscow, Russia

Neuromuscular Unit

I. Hausmanowa-Petrusewicz
Department Pediatrics,
Hadassah Hospital,
Jerusalem, Israel

K. Rowińska-Marcińska
Department of Neurology,
University of Helsinki, Finland

K. Sieradzan
Department of Anatomy and
Developmental Biology,
University College London,
United Kingdom
(long term visit)

Department of Applied Physiology

- Z. Brzezińska Department of Rehabilitation Medicine,
University of Goteborg, Sweden
(long term visit)
- G. Cybulski Centre for Biological and Medical Systems,
Imperial College of Science, Technology and
Medicine, London, United Kingdom
(long term visit)
- L. Dobrowolski Institute of Physiology,
University of Heidelberg, Germany
(long term visit)
- R. Grucza Department of Physiology,
University of Kuopio, Finland
- H. Kaciuba-Uściłko Department of Pathophysiology,
K. Nazar University of Graz, Austria
- E. Kompanowska-Jezierska Institute of Physiology,
University of Copenhagen,
Denmark (long term visit)
- B. Kruk Laboratory of Work Physiology,
Medical Faculty Pitie-Salpetriere,
CNRS, Paris, France
- K. Krzemiński Department of Health Sciences,
Sargent College of Allied Health Professions,
Boston University, USA
(long term visit)
- J. Sadowski Department of Physiology and Biophysics,
University of Lund, Sweden

Panum Institute, Copenhagen, Denmark

Cardiovascular Laboratory

- K. Cedro-Ceremużynska Wellcome Research Laboratories,
London, United Kingdom

Department of Surgical Research Transplantation

- P. Bryła
Thanjavur Medical College, Thanjavur, India
Tuberculosis Research Centre, Madras, India
Vector Control Research Centre, Pindocherry, India
- H. Galkowska
Department of Surgery, University of Turku, Finland
- I. Grzelak
Norway Radium Hospital, Oslo, Norway
(long term visit)
- U. Kubicka
Tuberculosis Research Centre, Madras, India
- B. Łukomska
Laboratoire des Interactions Cellulaires,
University of Bordeaux, France
- W. Olszewski
Institute for Surgical Research,
University of Munich, Germany
Department of Human Biology,
University of Kassel, Germany
Norwegian Radium Hospital, Helsinki, Norway
Thanjavur Medical College, Thanjavur, India
Vector Control Research Centre, Pindocherry, India
Tuberculosis Research Centre, Madras, India
Hopital Cognac-Jay, Paris, France
British Heart Foundation, London,
United Kingdom

Department of Endocrinology

- A. Lipkowski
Department of Chemistry,
University of Arizona, Tucson, USA
(long term visit)

PARTICIPATION IN INTERNATIONAL MEETINGS

Workshop on "Therapeutic Principles in Spinal Muscular Atrophy", Karlsruhe, Germany, January 10-12.

I. Hausmanowa-Petrusewicz

12th ENMC International Workshop "The Limb Girdle" Muscular Dystrophies, Baarn, Netherlands, February 28 - March 1.

I. Hausmanowa-Petrusewicz

Congress of Swiss Society of Experimental Biology, Basel, Switzerland, March 9-10.

K. Domańska-Janik

3rd International Symposium of Lymphology, Lisbon, Portugal, March 19-21.

W.L. Olszewski

92th Congress of Japan Surgical Association and 2nd Symposium of the Japan-Poland Society for Exchange in Surgery: "Surgery Toward 21st Century", Tokyo, Japan, March 25-28.

B. Łukomska, W.L. Olszewski

Conference for Liver Research, Ashikawa, Japan, March 29.

B. Łukomska, W.L. Olszewski

2nd International Symposium on Endothelium-Derived Vasoactive Factors, Basel, Switzerland, April 22-25.

G. Adamczyk

First International Congress of the Cell Transplant Society, Pittsburgh, USA, May 31 - June 4.

W.L. Olszewski

43 Jahrestagung der Deutschen Gesellschaft für Neurochirurgie, Frankfurt am Main, Germany, May 10-13.

J. Jurkiewicz

2nd International Symposium of Neuroanesthesia, Łódź, Poland, May 15-16.

J. Berdyga, Z. Czernicki, J. Jurkiewicz, G. Stępińska

27th Congress of the European Society for Surgical Research, Zaragoza, Spain, May 20-23.

P. Bryła, M. Jaskłowska-Englitz, W.L. Olszewski, D. Sadowska-Szablisty

International Congress of Electromyography and Clinical Neurophysiology, Jerusalem, Israel, June 8-12.

I. Hausmanowa-Petrusewicz

7th European Colloquium of Renal Physiology, Napoli, Italy, June 12-26.

L. Dobrowolski, E. Kompanowska, J. Sadowski

3rd International Symposium of Lymphology, Buenos Aires, Argentina, June 17-19.

B. Łukomska, W.L. Olszewski

2nd International Symposium on Dendritic Cells in Fundamental and Clinical Immunology, Amsterdam, Netherlands, June 21-25.

H. Galkowska

7th International Catecholamine Symposium, Amsterdam, Netherlands, June 22-26.

H. Kaciuba-Uściłko, K. Nazar

Meeting of International SMA Consortium, Bonn, Germany, June 26-28.

I. Hausmanowa-Petrusewicz

4th European Meeting of Neuropathology, Berlin, Germany, July 14-19.

M. Barcikowska, M. Dąbbska, H. Drac, A. Fidziańska, B. Gajkowska, A. Kapuściński, H. Kroh, I. Kuchna, M.J. Mossakowski, M. Samochocki, J. Strosznajder, G. Szumańska, M. Walski

First International Symposium on Neurobiology and Neuroendocrinology of Aging, Bregenz, Austria, July, 19-23.

M. Barcikowska

21st Annual Meeting of the Federation of European Biochemical Societies (FEBS'92), Dublin, Ireland, August 9-14.

M. Samochocki

9th General Meeting of the European Society for Neurochemistry, Dublin, Ireland, August 16-21.

K. Domańska-Janik, L. Faff-Michalak, L. Jabłońska, M. Łatowski, M. Puka, M. Samochocki, J. Strosznajder, B. Zabłocka

14th International Congress of the Transplantation Society, Paris, France, August 16-21.

M. Jaskłowska-Englisz, B. Łukomska, M. Maksymowicz, W.L. Olszewski

6th International Symposium on Cells of the Hepatic Sinusoid, Antwerp, Belgium, August 23-30.

W.L. Olszewski

8th International Congress of Immunology, Budapest, Hungary, August 23-28.
P. Bryła, M. Jaskłowska-Englisz, U. Kubička, A. Namysłowski

2nd Annual Congress of European Respiratory Society, Vienna, Austria, August 29 - September 3.
W. Janczewski, M. Pokorski, M. Szereda-Przestaszewska

11th International Meeting on Neuromuscular Diseases, Marseille, France, September 10-12.
I. Hausmanowa-Petrusewicz

First International Congress of the Polish Neuroscience Society, Warsaw, Poland, September 20-23.

J. Albrecht, M. Barcikowska, E. Kida, M. Dąbwska, K. Domańska-Janik, H. Drac, L. Faff-Michalak, I. Hausmanowa-Petrusewicz, L. Jabłońska, W. Karczewski, M. Krynicki, I. Kuchna, J.W. Łazarewicz, E. Łuczywek, D. Maślińska, M.J. Mossakowski, M. Muzylak, R. Pluta, M. Puka, K. Rowińska-Marcińska, E. Salińska, M. Samochocki, J. Sypecka, B. Zabłocka

International Obesity Conference: "Ephedrine, Xanthines and Other Thermogenic Drugs to Assist the Dietary Management of Obesity", Geneva, Switzerland, September 24-26.
A. Ziemba

1st Meeting of the European Federation of Microsurgical Societies, Roma, Italy, September 26-29.
W.L. Olszewski

8th International Symposium of the European Alzheimer's Disease Association, Brussels, Belgium, September 23-27.
M. Barcikowska

National Institutes of Health Experimental Immunology Study Section Workshop: "Molecular Immunology and Prospects for Cancer Immunotherapy", St. Petersburg Beach, Florida, USA, October 3-4.
B. Łukomska

8th International Natural Killer Cell Workshop and First Meeting of the Society for Natural Immunity: "Molecular and Cellular Aspects of Natural Killer Cell Triggering and Signalling", St. Petersburg Beach, Florida, USA, October 4-6.
B. Łukomska

Symposium "Intrakranieller Druck, Hirnodem und Hirndurchblutung", Lubeck, Germany, October, 29-31.
Z. Czernicki

25th Danube Symposium for Neurological Sciences, Budapest, Hungary, November 4-7.

M. Barcikowska, J. Mossakowski

2nd International Conference on Advances in Pulmonary Rehabilitation and Management of Chronic Respiratory Failure; Prevention of hypoxaemia in COPD, Venice, Italy, November 4-7.

M. Pokorski

2nd Japan Symposium on Peptide Chemistry, Shizuoka, Japan, November 9-13.

A. Lipkowski

65th Annual Scientific Sessions of the American Heart Association, New Orleans, USA, November 16-19.

K. Cedro-Ceremużyńska

1st International Seminar on Biological Signals Processing, Gliwice, Poland, November 16-20.

W. Niewiadomski

SCIENTIFIC DEGREES

DOCTOR'S DEGREES

Leszek Dobrowolski

Role of prostaglandins in modulation of the cortico-papillary electrolyte gradient in rat kidney

(Department of Applied Physiology)

Elżbieta Kompanowska-Jeziarska

Vasopressin control of the cortico-papillary electrolyte gradient in rat kidney

(Department of Applied Physiology)

Maciej Krynicki

The mechanisms of influence of hyperthermia on whole body distribution of recirculating lymphocytes

(Department for Surgical Research and Transplantation)

Elżbieta Salińska

Mechanisms of ischemia-evoked changes in extracellular calcium concentration in rabbit hippocampus in vivo

(Department of Neurochemistry)

HABILITATIONS

Barbara Kruk

Interrelationship between metabolism and body temperature during exercise

(Department of Applied Physiology)

Ryszard Pluta

Attempt to prevent the consequences of complete cerebral ischemia in rabbit by use of prostacyclin

(Department of Neuropathology)

Katarzyna Rowińska-Marcińska

The analysis of the activity of compound potentials motor unit

(Neuromuscular Unit)

SCIENTIFIC MEETINGS ORGANIZED BY THE MEDICAL RESEARCH CENTRE

Practical Course of Staining with Monoclonal Antibodies, February 5-7,
sponsored by DAKOPATTS, Denmark

Symposium "Postthrombophlebitic Syndrome, leg ulcers", March 12,
sponsored by Lek Lubljana, Yugoslavia

Experimental and Clinical Studies of Brain Ischemia. April 25

Symposium "Immunological Problems in Surgery, Transplantology and Oncology - Diagnostic and Treatment, Clinical and Experimental Studies", October 8,
sponsored by Ortho Diagnostik System

Practical Course of Staining with Monoclonal Antibodies, October 14-15,
sponsored by DAKOPATTS, Denmark

24th Course of the Basis Microsurgical Techniques, October 25-27,
sponsored by Cyanamid, USA

LIST OF PUBLICATIONS

	Page
Original works	87
Communications	99

Original works

1. Adamczyk G., Lembowicz K., Walski M., Olszewski W.L.: Vasa vasorum and endothelial cell function in veno-venous grafts. *Journal of Vascular Research* 1992, 29, 75-76.
2. Albrecht J.: Combination of diclofenac and codeine in the treatment of postoperative pain. *MED-WELT*. 1992, 43, 6, 515-519.
3. Badurska B., Ryniewicz B.: Immunosuppressive treatment for juvenile myasthenia gravis. *Europ. J. Pediat.* 1992, 151, 215-217.
4. Barcikowska M., Kida E., Joachimowicz E., Siekierzyńska A.: Cerebellar granular layer degeneration in small cell lung cancer: paraneoplastic cerebellopathy or artifact? *Neuropatol. Pol.* 1992, 30, 2, 155-163.
5. Barcikowska M., Kujawa M., Wiśniewski H.M.: Beta-amyloid deposits with- in the cerebellum of persons older than 80 years of age. *Neuropatol. Pol.* 1992, 30, 3-4, 283-291.
6. Barcikowska M., Mirecka B., Papierz W., Bogucki M., Niewodniczy A., Liberski P.P.: Alzheimer's disease case with clinical picture mimic Creutzfeldt-Jacob disease. *Neurol. Neurochir. Pol.* 1992, 43, 703-710.
7. Berger J.R., Scott G., Albrecht J., Belman A.L., Tornatore C., Major E.D.: Progressive multifocal leukoencephalopathy in HIV-1-infected children. *AIDS* 1992, 6, 8, 837-841.
8. Bergan T., Jorgensen N., Olszewski W.L., Zhang Y.: Azithromycin pharmacokinetics and penetration to lymph. *Scand. J. Infect. Dis.* 1992, Suppl. 83, 15-21.
9. Bojarski P., Marchel A., Kroh H.: Intramedullary metastatic tumour. *Neurol. Neurochir. Pol.* 1992, 26, 3, 393-398 (in Polish).
10. Bryła P., Olszewski W.L.: Transcapillary transport of immune cells from blood to afferent and efferent lymph. *Immunologia Polska* 1992, 17, 176-177 (in Polish).
11. Budohoski L., Opas M.: Effects of insulin and tumor promoting, TPA, on glucose transport and metabolism in retinal pigmental epithelium in vitro. *Biochem. Int.* 1992, 27, 777-782.

12. Carr D.B., Lipkowski A.W.: Mechanism of opiate analgesic agents. In: Principles and practice of anesthesiology. Eds.: B. Covino, J. Tinker, M. Rodgers. Mosby, St. Louis, 1992, chapter 52.
13. Ceremużyński L., Barcikowski B., Lewicki Z., Wutzen J., Gordon-Majszak W., Famulski K.S., Kłoś J., Herbaczyńska-Cedro K.: Stress-induced injury of pig myocardium is accompanied by increased lipid peroxidation and depletion of mitochondrial ATP. *Exp. Pathol.* 1991, 43, 213-220.
14. Challiss R.A.J., Richards S.J., Budohoski L.: Characterization of the adenosine receptor modulating insulin action in rat skeletal muscle. *Eur. J. Pharmacol.* 1992, 226, 121-128.
15. Chrapusta S., Grieb P.: Evidence for sex steroid receptors in feline brainstem. *Neuroscience Letters* 1992, 142, 167-170.
16. Czernicki Z.: Brain injury and edema. In: *Microcirculation of the Brain*. Eds. G. Mchedlishvili, M. Tomita, R. Tuma. Nova Science Publishers Inc., New York, 1992, pp. 252-254.
17. Czernicki Z.: Evaluation of intracranial pressure changes using transcranial Doppler. *Neurol. Neurochir. Pol.* 1992, 3, 375-382 (in Polish).
18. Czernicki Z., Berdyga J., Stępińska G., Jurkiewicz J.: Application of physiological loadings in evaluation of intracranial volume-pressure relations. An experimental study. *Neurol. Neurochir. Pol.* 1992, 5, 677-684 (in Polish).
19. Czernicki Z., Uchman G.: Rheological changes in neurological patients. *Anest. iInten. Ter.* 1992, 5-6, 566 (in Polish).
20. Czernicki Z., Walecki J., Jurkiewicz J., Grochowski W., Tychmanowicz K.: Intracranial volume reserve determination using CT images, numerical analysis and lumbar infusion tests. An experimental study. *Acta Neurochir.* 1992, 115, 43-46.
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