

**POLISH ACADEMY OF SCIENCES  
MEDICAL RESEARCH CENTRE**

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# POLISH ACADEMY OF SCIENCES MEDICAL RESEARCH CENTRE

Editor  
W. Dziejczak

Scientific Consultant  
H. Kaciuba-Uściłko, Professor of Physiology

Polish Academy of Sciences  
Medical Research Centre  
3 Dworkowa Str., 00-784 Warsaw - Poland  
Telephones: 49 64 93, 49 69 73  
Fax: 48-22 49 69 73

<http://rcin.org.pl>

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# STAFF LIST

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Polish Physiological Society

**M. Głowska**, M.D., Ph.D.

Member of:

European Respiratory Society

Polish Physiological Society

**P. Grieb**, M.Biol., Ph.D., D.Sc., Assoc. Prof. of Natural Sciences

Member of:

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European Respiratory Society

Polish Physiological Society

**H. Gromysz**, M.Biol., Ph.D.

Member of:

European Respiratory Society

Polish Physiological Society

**W. Janczewski**, M.Sc. (eng.)

Member of:

European Respiratory Society

Polish Physiological Society

**W. A. Karczewski**, M.D., Ph.D., D.Sc., Prof. of Physiology

Member of:

Committee of Physiological Sciences of Polish Academy of Sciences

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Polish Physiological Society  
Purkyne Czechoslovak Medical Society (Honorary Member)

Member of Editorial Boards of:  
Acta Neurobiologiae Experimentalis  
Journal of Physiology and Pharmacology

**M. Pokorski**, M.D., Ph.D., D.Sc., Assoc. Prof. of Neurophysiology

Member of:  
International Society for Neurochemistry  
European Respiratory Society  
American Physiological Society  
New York Academy of Sciences  
German Physiological Society

**J.R. Romaniuk**, M.Phys., Ph.D., D.Sc., Assoc. Prof. of Natural Science

Member of:  
European Respiratory Society  
Polish Physiological Society

**M. Ryba**, M.D., Ph.D., D.Sc., Assoc. Prof. of Neurophysiology

Member of:  
European Respiratory Society  
Polish Physiological Society

**R. Strosznajder**, M.Sc.

**B. Szereda-Przestaszewska**, M.D., Ph.D., D.Sc., Assoc. Prof. of Neurophysiology

Member of:  
European Respiratory Society  
British Physiological Society  
Polish Physiological Society

**B. Wypych**, M. Biol.

### Technical Staff

**L. Czerwosz**, M.Phys. (Member of European Respiratory Society), **N. Dziadosz**,  
M.Biol., **M. Janisz**, **U. Jernajczyk**, M.Biol., **K. Sroczyńska**, **T. Warnawin**

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### Scientific Staff

**K. Białynicka-Birula, M.D.**

**M. Chalimoniuk, M.Chem.**

Member of:  
Polish Biochemical Society

**B. Dąbrowska-Bouta, M.Biol.**

**K. Domańska-Janik, M.D., Ph.D., D.Sc., Assoc. Prof. of Medical Sciences**

Member of:  
International Neuropathological Society  
European Society for Neurochemistry  
Polish Biochemical Society  
Polish Neuroscience Society  
Polish Association of Neuropathologists

**W. Gordon-Krajcer, M.Pharm., Ph.D.**

Member of:  
International Society for Neurochemistry  
American Academy of Neurology  
Polish Biochemical Society

**L. Jabłońska, M.Biol. (postgraduated student)**

**I. Koładkiewicz, M.Chem.**

**B. Kwiatkowska-Patzer, M.D., Ph.D.**

Member of:  
International Society for Heart Research  
Polish Cardiac Society  
Polish Pediatric Society  
Polish Pharmacological Society

**M. Łałowski, M.Biol.**



**J.W. Łazarewicz**, M.D., Ph.D., D.Sc., Prof. of Medical Sciences

Member of:

Committee of Neurological Sciences of Polish Academy of Sciences

International Brain Research Organisation (IBRO)

International Society for Neurochemistry

European Society for Neurochemistry

Polish Biochemical Society

Polish Neuroscience Society

Polish Association of Neuropathologists

Member of Editorial Boards of:

Molecular and Chemical Neuropathology

Acta Neurobiologiae Experimentalis

Folia Neuropathologica

Member of Scientific Council:

Nencki Institute of Experimental Biology, PASci

**M. Puka**, M.Phys., Ph.D.

Member of:

European Society for Neurochemistry

Polish Biochemical Society

Polish Neuroscience Society

**U. Rafałowska**, M.Biol., Ph.D., D.Sc., Prof. of Natural Sciences

Member of:

European Neurochemical Society

New York Academy of Sciences

Polish Biochemical Society

Polish Neuroscience Society

**W. Rybkowski**, M.D.

**E. Salińska**, M.Biol., Ph.D.

Member of:

European Society for Neurochemistry

Polish Neuroscience Society

**M. Samochocki**, M.Chem.

Member of:

International Brain Research Organisation (IBRO)

International Society for Neurochemistry

European Society for Neurochemistry

Polish Biochemical Society

Polish Neuroscience Society

**E. Speina**, M.Biol.

Member of:

Polish Biochemical Society

**A. Stafiej**, M.Chem.

**J. Strosznajder**, M.D., Ph.D., D.Sc., Prof. of Medical Sciences

Member of:

International Brain Research Organisation (IBRO)

International Society for Brain Edema

International Society for Neurochemistry

European Society for Neurochemistry

American Society for Neurochemistry

New York Academy of Sciences

Polish Association of Neuropathologists

Polish Biochemical Society

Polish Neurological Society

Polish Neuroscience Society

**J. Sypecka**, M.Biol. (postgraduated student)

Member of:

Polish Biochemical Society

Polish Neuroscience Society

**J. Waśkiewicz**, M.Biol., Ph.D.

Member of:

European Society for Neurochemistry

Polish Neuroscience Society

**H. Wikiel**, M.Chem., Ph.D.

**B. Zabłocka**, M.Biol.

Member of:

International Brain Research Organisation (IBRO)

Polish Neuroscience Society

**T. Zalewska**, M.Pharm., Ph.D.

Member of:

International Neuropathological Society

European Society for Neurochemistry

Polish Association of Neuropathologists

Polish Biochemical Society

## **Technical Staff**

**T. Czechmańska, D. Kacprzak, S. Kuciak, A. Lenkiewicz, H. Nowińska, D. Pazikowska, I. Sawicz, M. Skorupka, A. Sobczuk, H. Zając, A. Ziembowicz**

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**M. Izak, secretary**

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**Head: Prof. Andrzej Kapuściński**

### Scientific Staff

**J. Albrecht**, M.Biol., Ph.D., D.Sc., Prof. of Biomedical Sciences

Member of:

European Society for Neurochemistry (Member of the Council)

Mayo Alumni Association

Polish Association of Neuropathologists

Polish Biochemical Society

Polish Neurosciences Society

Member of Editorial Boards of:

Journal of Neuroscience Research

Metabolic Brain Disease

Molecular and Chemical Neuropathology

**M. Barcikowska-Litwin**, M.D., Ph.D.

Member of:

World Federation of Neurology

Polish Association of Neuropathologists

Polish Neurological Society

**H. Borkowska**, M.Agr.Sci.

**L. Faff-Michalak**, M.Pharm., Ph.D.

Member of:

European Society for Neurochemistry

Polish Neurosciences Society

**R. Gadamski**, M. Vet., Ph.D.

Member of:

International Society of Neuropathology

Polish Anatomical Society

**W. Hilgier**, M.Pharm., Ph.D.

Member of:

European Society for Neurochemistry

Polish Biochemical Society

- A. Kapuściński, M.D., Ph.D., D.Sc., Prof. of Nuclear Medicine**  
 Member of:  
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 Polish Radiological Society  
 Polish Society of Nuclear Medicine
- E. Kida, M.D., Ph.D.**  
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 World Federation of Neurology  
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- S. Krajewski, M.D., Ph.D., D.Sc., Assoc. Prof. of Neuropathology**  
 Member of:  
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 Deutsche Gesellschaft für Neuropathologie und Neuroanatomie  
 Polish Association of Neuropathologists
- H. Kroh, M.D., Ph.D., D.Sc., Prof. of Neuropathology**  
 Member of:  
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 Polish Association of Neuropathologists  
 Polish Neurosurgeons Society
- E. Matyja, M.D., Ph.D.**  
 Member of:  
 World Federation of Neurology  
 Polish Neurological Society
- M.J. Mossakowski, M.D., Ph.D., D.Sc., Prof. of Neuropathology,**  
 Dr. h.c. of Medical School of Lublin
- Member of:  
 Committee of Biocybernetics and Biochemical Engineering of Polish  
 Academy of Sciences  
 Committee of Neurological Sciences of Polish Academy of Sciences  
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 International Society of Neuropathology  
 World Federation of Neurology  
 World Confederation of Neurosciences (Phronesis)  
 European Federation of Neuropathological Societies  
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Sociedad Iberoamericana de Ciencias Neurológicas  
Polish Academy of Sciences  
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Polish Pathological Society (Honorary Member)  
Warsaw Scientific Society

Corresponding Member of the Mexican Academy of Culture

Member of Scientific Councils:  
Institute of Biocybernetics and Biomedical Engineering, PASci  
Institute of Pharmacology, PASci  
Medical Research Centre, PASci

Member of Editorial Boards of:  
Bulletin de l'Académie Polonaise des Sciences  
Clinical Neuropathology  
Journal of Physiology and Pharmacology  
Nauka Polska  
Neurologia i Neurochirurgia Polska  
Neuropatologia Polska

**A. Pfeffer-Baczuk, M.D.**

**R. Pluta, M.D., Ph.D., D.Sc.**

Member of:  
European Society for Clinical Respiratory Physiology  
Polish Association of Neuropathologists  
Polish Neurosciences Society

**M.Z. Ratajczak, M.D., Ph.D., D.Sc.**

**G. Szumańska, Ph.D.**

International Society of Neuropathology  
Polish Cyto- and Histochemical Society  
Polish Association of Neuropathologists

**M. Śmiałek, M.D., Ph.D., D.Sc., Assoc. Prof. of Neuropathology**

Member of:  
International Society of Neuropathology  
Polish Association of Neuropathologists

**A. Taraszewska, M.D., Ph.D.**

Member of:

International Society of Neuropathology

Polish Association of Neuropathologists

**H. Weinrauder-Semkow, M.Biol., Ph.D.**

Member of:

International Society of Neuropathology

Polish Association of Neuropathologists

**I.B. Zelman, M.D., Ph.D., D.Sc., Assoc. Prof. of Neuropathology**

Member of:

Committee of Neurological Sciences of Polish Academy of Sciences

International Society of Neuropathology

Polish Association of Neuropathologists

Member of Editorial Board of:

Neuropatologia Polska

### **Technical Staff**

**J. Baraniecka, M.Chem. (eng.), H. Chrzanowska, A. Dubiel, I. Dybkowska-Anc, T. Emilian, I. Fręsko, E. Grzywaczewska, S. Januszewski, J. Kędzierska, M. Knejszuk-Wesoła, M. Kobryś, Z. Kowalska, W. Ogonowska, T. Pańkowska, M. Poławska, I. Przekop, I. Przybysz, M.Biol.Sc., J. Sawicki, M.Vet., B. Śliwińska, U. Tkaczyk, K. Wierzbicka, R. Wojda, M. Wyszogrodzka, M. Zielińska**

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## LABORATORY OF THE DEVELOPMENTAL NEUROPATHOLOGY

**Head: Prof. Maria Dąbbska**

### Scientific Staff

**M. Dąbbska, M.D., Ph.D., D.Sc., Prof. of Neuropathology**

Member of:

International Society of Developmental Neurosciences

International Society of Neuropathology

Société Française de Neuropathologie

Polish Association of Pediatric Neurologist

Polish Association of Neuropathologists

Polish Neurological Society

Polish Neuroscience Society

Editor in Chief of *Folia Neuropathologica*

Member of Editorial Board of:

Clinical Neuropathology

**L. Iwanowski, M.D., Ph.D., D.Sc., Assoc. Prof. of Neuropathology**

Member of:

International Society of Neuropathology

Polish Neurological Society

Polish Association of Neuropathologists

**I. Kuchna, M.D.**

Member of:

Polish Neurological Society

Polish Association of Neuropathologists

**M. Laure-Kamionowska, M.D., Ph.D.**

Member of:

International Society of Neuropathology

Polish Neurological Society

Polish Association of Neuropathologists

**D. Maślińska, M.D., Ph.D., D.Sc., Assoc. Prof. of Medical Sciences**

Member of:

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Polish Cyto- and Histochemical Society



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Member of:

Commission of Electron Microscopy of Polish Academy of Sciences

European Cell Biology Association

European Society of Pathology

Polish Association of Neuropathologists

Society of Polish Pathologists

**M. Frontczak-Baniewicz**, M. Biol.

**B. Gajkowska**, M.Biol., Ph.D., D.Sc., Assoc. Prof. of Medical Sciences

**M. Walski**, M.D., Ph.D., D.Sc., Assoc. Prof. of Medical Sciences

Member of:

Commission of Electron Microscopy of Polish Academy of Sciences

Society of Polish Parasitologists

Society of Polish Pathologists

## **Technical Staff**

**H. Bilski, W. Ciesielska, T. Barszcz**

## DEPARTMENT OF NEUROSURGERY

**Head: Prof. Zbigniew Czernicki**

### Scientific Staff

**J. Berdyga**, M.Sc., eng. (until August 1993)

**Z. Czernicki**, M.D., Ph.D., D.Sc., Prof. of Neurosurgery

Member of:

Commission of Pathophysiology of Intracranial Pressure (ICP) of  
Polish Academy of Sciences

Committee of Neurological Sciences of Polish Academy of Sciences

European Association of Neurosurgical Societies

Polish Society of Neuroanaesthesiology

Polish Neurosurgical Society

Member of Editorial Board of:

Neurologia i Neurochirurgia Polska

**J. Dziduszko**, M.D., Ph.D.

Member of:

Polish Neurosurgical Society

**E. Fersten**, M.Psych., Ph.D.

Member of:

Polish Neuroscience Society

Polish Psychological Society

**W. Grochowski**, M.D., Ph.D.

Member of:

Polish Neurosurgical Society

**D. Horsztyński**, M.D.

**J. Jurkiewicz**, M.D., Ph.D., D.Sc., Assoc. Prof. of Neurosurgery

Member of:

Commission of Pathophysiology of Intracranial Pressure (ICP) of  
Polish Academy of Sciences

European Association of Neurological Societies

Polish Neurosurgical Society

Polish Society of Neuroanaesthesiology

**E. Łuczywek, M.Psych., Ph.D.**

Member of:

Alzheimer's Disease and Related Disorders Polish Association

Polish Neuroscience Society

Polish Psychological Society

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**E. Mempel, M.D., Ph.D., D.Sc., Prof. of Neurosurgery**

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European Society of Functional and Stereotaxic Neurosurgery

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**W. Sapieja, M.D.**

Member of:

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Member of:

Polish Society of Neuroanaesthesiology

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Member of:

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Aphasieforschung und -behandlung"

Polish Neurosurgical Society

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**J. Walecki, M.D., Ph.D., D.Sc., Prof. of Neuroradiology**

Member of:

Commission of Neurobiology of Polish Academy of Sciences

Commission of Pathophysiology of Intracranial Pressure (ICP) of

Polish Academy of Sciences

Committee of Medical Physics of Polish Academy of Sciences

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European Society of Neuroradiology

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Member of:

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**W. Zabolotny, M.Sc. eng.**

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### Scientific Staff

**B. Badurska**, M.D., Ph.D., D.Sc., Assoc. Prof. of Neuropediatric

Member of:

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World Federation of Neurology

Polish Neurological Society

Polish Society of Neuropediatric

**H. Drac**, M.D., Ph.D.

Member of:

Polish Neurological Society

**A. Fidzińska-Dolot**, M.D., Ph.D., D.Sc., Prof. of Neurology

Member of:

International Neuropathological Association

World Federation of Neurology

Polish Association of Neuropathologists

Polish Neurological Society

**I. Hausmanowa-Petrusewicz**, M.D., Ph.D., D.Sc. Prof. of Neurology

Corresponding Member of Polish Academy of Sciences

Member of:

Committee of Biocybernetics of Polish Academy of Sciences

Committee of Neurological Sciences of Polish Academy of Sciences

World Federation of Neurology

Polish Neurological Society

Polish Society of EEG and Clinical Neurophysiology

Honorary Member of:

European Neurological Society

American Neurological Association

American Association for Electrodiagnosis

Bulgarian Neurological Society

Czecho-Slovak Neurological Society

French Neurological Society

Gaetano Conte Academy

German EEG Society

German Neurological Society

Italian Neurological Society

Member of Editorial Boards of:

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EMG and Clinical Neurology Journal

Journal of Neurological Sciences

Neuromuscular Disorders

Neurologia i Neurochirurgia Polska

**A. Kamińska, M.D., Ph.D.**

Member of:

World Federation of Neurology

European Society of Clinical Investigation

Polish Neurological Society

**M. Kozłowska, M.D.**

Member of:

Polish Neurological Society

**E. Kowalska, M.D.**

**I. Niebrój-Dobosz, M.D., Ph.D., D.Sc., Assoc. Prof. of Medical Sciences**

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Polish Neurological Society

Member of Editorial Board:

Journal of Neurology

**J. Rafałowska, M.D., Ph.D., D.Sc., Prof. of Neurology**

Member of:

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Polish Neurological Society

**K. Rowińska, M.D., Ph.D., D.Sc., Assoc. Prof. of Neurology**

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Polish Neurological Society

**E. Sawicka, M.D., Ph.D.**

Member of:

World Federation of Neurology

Polish Neurological Society

**K. Sieradzan, M.D., Ph.D.**

Member of:  
Polish Neurological Society

**H.M. Strugalska, M.D., Ph.D.**

Member of:  
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Polish Neurological Society

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**M. Szymańska, secretary**



## DEPARTMENT OF APPLIED PHYSIOLOGY

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### Scientific Staff

**B. Bączyńska**, M.Biol., Ph.D.

**B. Bicz**, M.Biol., Ph.D.

Member of:

Polish Biochemical Society

**Z. Brzezińska**, M.Pharm., Ph.D.

Member of:

Polish Biochemical Society

Polish Physiological Society

**L. Budohoski**, Ph.D., D.Sc., Assoc. Prof. of Physiology

Member of:

Polish Biochemical Society

Polish Physiological Society

**J. Chwalbińska-Moneta**, M.D., Ph.D., D.Sc.

Member of:

Polish Physiological Society

Polish Society of Sports Medicine

**G. Cybulski**, M.Sc.(eng.), Ph.D.

**L. Dobrowolski**, M.Biol., Ph.D.

**A. Dubaniewicz**, M.Biol.

**I. Fałęcka-Wieczorek**, M. Biol., Ph.D.

**M. Górecka**, M.Biol. (postgraduate student)

**R. Grucza**, M.Sc.(eng.), Ph.D., D.Sc., Assoc. Prof. of Physiology

- H. Kaciuba-Uściłko**, Ph.D., D.Sc., Prof. of Physiology  
Member of:  
Committee of Physiological Sciences of Polish Academy of Sciences  
Thermal Commission of I.U.P.S.  
Polish Physiological Society  
Polish Society of Sports Medicine  
Member of Editorial Board of:  
Annals of Sports Medicine
- E. Kompanowska-Jeziarska**, M.Biol., Ph.D.
- B. Kruk**, M.Biol., Ph.D., D.Sc.  
Member of:  
Polish Physiological Society
- K. Krzemiński**, M.D., Ph.D.
- J. Langfort**, M.Biol., Ph.D.  
Member of:  
Polish Physiological Society  
Polish Society of Sports Medicine
- K. Nazar**, M.D., Ph.D., D.Sc., Prof. of Medical Sciences  
Member of:  
Committee of Physiological Sciences of Polish Academy of Sciences  
Research Group on Biochemistry of Exercise International Council  
of Sport Science and Physical Education  
Polish Physiological Society  
Polish Society of Sports Medicine  
Member of Editorial Boards of:  
Biology of Sports  
International Journal of Sports Medicine  
Journal of Physiology and Pharmacology  
Medycyna Sportowa
- W. Niewiadomski**, M.Sc. (eng).
- J. Sadowski**, M.D., Ph.D., D.Sc., Prof. of Medical Sciences  
Member of:  
Hungarian Physiological Society (Honorary Member)  
Polish Physiological Society

**E. Titow-Stupnicka**, M.Biol., Ph.D.

**E. Turlejska**, M.Biol., Ph.D.

Member of:

Polish Physiological Society

**A.W. Ziemba**, M.Biol., Ph.D.

Member of:

Polish Physiological Society

**E. Żernicka**, M.Biol.

#### **Technical Staff**

**B. Kurek, W. Radziszewska, J. Zwolińska**

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### **Scientific Staff:**

**W. Pawłowska-Jenerowicz, M.D.**

**M. Płachcińska-Bijak, M.D.**

**C. Romiszowska, M.D.**

**E. Wójcik-Ziółkowska, M.D., Ph.D.**

Member of:

Polish Cardiac Society

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### **Scientific Staff**

**Andrzej Ceremużyńska, M.D., Ph.D., D.Sc., Prof. of Medical Sciences**

Member of:

International Society for Heart Research

European Society for Clinical Investigations

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Łukasz

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Polish Immunological Society

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Member of:  
Polish Immunological Society

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Polish Immunological Society

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The Transplantation Society

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Member of:  
Polish Immunological Society

**U. Kubicka, M.Biol., Ph.D.**

Member of:  
Polish Immunological Society  
Secretary of Warsaw Branch of PIS

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Member of:

Polish Immunological Society

Poland-Japan Society for Exchange in Surgery

Society of Natural Immunity

The Transplantation Society

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**A. Namysłowski, M.D.**

**W.L. Olszewski, M.D., Ph.D., D.Sc., Prof. of Surgery**

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Visiting Professor Norwegian Radium Hospital, Oslo

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International Lymphological Society (Board Member)

Italian Lymphological Society

Korean Medical Association (Honorary Member)

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Immunology Section)

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Lymphology

Polish Journal of Immunology

Transplant International

Zeitschrift für Experimentelle Chirurgie

**E. Orlewska, M.D., Ph.D.**

Member of:

Polish Immunological Society

**D. Sadowska-Szablisty, M.Biol.**

Member of:

Polish Immunological Society

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American Chemical Society

American Peptide Society

Polish Chemical Society

Polish Biochemical Society

Polish Pharmacological Society

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Klinika

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**A. Misicka, Ph.D.**

Member of:

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American Peptide Society

**J. Nauman, M.D., Ph.D., D.Sc., Prof. of Medical Sciences**

Member of:

European Society for Clinical Investigation

European Thyroid Association

**M. Wroclawska, M.D.**

# RESEARCH REPORT

## DEPARTMENT OF NEUROPHYSIOLOGY

3 Dworkowa Str., 00-784 Warsaw

Telephone: 49 58 29

**Head: Assoc. Prof. Mieczysław Pokorski**

## NEUROTRANSMITTERS IN CHEMICAL REGULATION OF RESPIRATION

(Assoc. Prof. Mieczysław Pokorski)

Molecular phosphoinositide-related aspects of the transduction of chemical signals were studied in the cat carotid body *in vitro*. One project was concerned with the effect of ATP on the activity of phospholipase C acting against phosphatidylinositol-4,5-bisphosphate in the carotid bodies preexposed *in vitro* to normoxia or hypoxia. The results showed that ATP stimulated phospholipase C activity of the carotid bodies exposed to either gas condition, the stimulation being markedly augmented in hypoxia. These results indicate a modulatory role of ATP in transduction of the hypoxic signal. The effect of ATP is specific for the carotid body since the opposite, i.e., an inhibition of phospholipase C was found in the neural tissue of the brain stem. The importance of the molecular mechanisms of chemotransduction studied for the *in vivo* regulation of respiration was then tested in anesthetized, paralyzed ventilated cats. The effects of the specific phospholipase C inhibitor (PMSF) and an D2 receptor antagonist - spiperone, on respiration assessed from the integrated phrenic nerve activity were studied. It was found that spiperone but not PMSF depressed respiration and also the respiratory response to hypoxia. These results suggest the stimulatory influence on respiration of the D2 receptor present in the carotid body. The studies deepened our insight in to the still controversial role of dopamine in regulation of respiration. The importance of the phosphoinositide pathway for the *in vivo* respiratory responses remains to be further explored and established.

The effect of two neurotransmitters - serotonin and dopamine on the breathing pattern was studied in anaesthetized cats, in two different experimental series.

Generation and duration of an expiratory apnoea of the pulmonary chemoreflex induced by serotonin depended on preserved vagal pathways. The clear-cut apneogenic contribution of laryngeal afferentation was observed with serotonin challenge to the laryngeal vascular bed.

The ventilatory responses to serotonin administered via pulmonary circulation occurred likewise after vagotomy with limited effect on the respiratory timing.

Ventilatory depression induced by an intravenous injection of dopamine, affected to a larger extent the tidal component of the breathing pattern. The degree of this respiratory inhibition was enhanced by the consecutive exclusion of the sympathetic and vagal inputs. Bilateral section of the carotid sinus nerves abolished this chemoreflex.

## **STUDIES ON MECHANISMS SHAPING CENTRAL RESPIRATORY ACTIVITIES, PHRENIC MOTONEURON ACTIVITIES, AND THE ACTIVITIES OF THE UPPER AIRWAY MUSCLES**

(Assoc. Prof. Krystyna Budzińska)

The influence of hypoxia on the spontaneous activities of the phrenic and hypoglossal nerves was a part of the investigation on the factors leading to disproportion between the activities of these nerves - a phenomenon demonstrated in the obstructive sleep apnea syndrome. It was found that hypoxia caused an almost complete reduction of hypoglossal nerve activity, while the phrenic nerve activity diminished only slightly as compared to normal ventilation. Hypoxia, therefore, augmented the disproportion between the activities of the two nerves. It was also shown that vagotomy caused a manyfold increase of the hypoglossal but not phrenic nerve activity, which suggests a specific inhibitory influence of the vagus nerves towards the hypoglossal nerve.

In the next series of experiments, we have demonstrated that the rise in arterial pressure induced either mechanically or pharmacologically, suppressed the hypoglossal nerve activity (by  $27.6 \pm 12\%$ ) more than that of the phrenic nerve (by  $7.2 \pm 6\%$ ). Inspiratory upper airway resistance increased two-fold. A decrease of arterial pressure almost simultaneously activated hypoglossal nerve and phrenic nerve by  $42.3 \pm 9\%$  versus  $6.3 \pm 4.2\%$ , respectively. Upper airway resistance (cranial to the larynx) decreased. We believe that changes in the systemic pressure may disturb the balance between upper airway muscles and diaphragm activity promoting upper airway obstruction in humans.

Experiments carried out on monkeys were concerned with the conveyance of information from the cerebral cortex to the respiratory muscle motoneurons. It was found that magnetic stimulation of the cortex during resting or depressed breathing, or during reflex apnea does not influence appreciably the latency and

amplitude of the short-term responses of the respiratory muscles examined. The stimulation made faster, however, the switching of inspiration on expiration during depressed breathing. These results indicate that reflex modulations of the central generator of the breathing pattern do not interact with the transmission of information from the cortex to the respiratory motoneurons over oligosynaptic pathways. On the other side, respiratory drive seems important for the component of the cortex information that runs over polysynaptic pathways.

Another project performed was concerned with the role of the motor nucleus of the trigeminal nerve in the respiratory pattern generation. These studies were carried out on anesthetized cats, employing a method of a pharmacologic block of the nucleus. The results showed that this motor nucleus could be an anatomic substrate for the pneumotaxic center of the pons, i.e., the neuronal complex that is responsible for the inhibition of inspiration and switching over to expiration. It was also shown that respiratory modulation, due to peripheral chemoreceptor stimulation, is engendered via the motor nucleus of the trigeminal nerve aside of the previously known medullary structures.

Finally, other studies done suggest that expiratory neurons of the motor nucleus of the trigeminal nerve inhibit inspiration when stimulated by lung stretch. However, expiratory activities alone neither of the medulla nor of the pons are capable to inhibit inspiration when disconnected from the pulmonary stretch receptors after vagotomy.

## **SIGNAL TRANSDUCTION IN CAROTID BODY CHEMORECEPTORS**

(Assoc. Prof. Mieczysław Pokorski)

Supported by the State Committee for Scientific Research: grant # 402959101

We have previously reported that phospholipase C (PLC) plays a part in transduction of the chemical signals in the carotid body chemoreceptors in the cat. We have now attempted to work out the basic mechanisms regulating the PLC activity in the carotid body. We found that hypoxia enhances the PLC activity in both cytosolic and membrane fractions of the carotid body homogenate. Further, a role of G proteins in PLC activation was demonstrated in the studies employing the *pertusis* and *cholera* toxins. *Pertusis* toxin inhibited the PLC activation of hypoxia which points to an action mediated by a  $G_i$  protein. In another series of biochemical studies it was found that  $Mg^{2+}$  markedly enhances the PLC activation of hypoxia. In conclusion, these results lend support for the underlying role of phosphoinositide turnover in chemical signal transduction in the carotid body.

## **ROLE OF THE ENDOGENOUS BENZODIAZEPINE SYSTEM IN RESPIRATION**

(Assoc. Prof. Mieczysław Pokorski)

Supported by the State Committee for Scientific Research: grant # 402109101

The study was concerned with the physiological role of the benzodiazepine (BZ) receptor. The hypothesis was tested according to which endogenous BZ activity has a tonic inhibitory influence on respiration. Such an influence could be augmented by hypoxia which, on one side, has a central depressant effect on respiration and, on the other, modulates the BZ receptor. The problem was addressed using the specific BZ receptor antagonist, Anexate (Roche) which according to the hypothesis was expected to increase respiration. The study was carried out on 23 anesthetized cats. Pulmonary ventilation and the inhibitory Hering-Breuer reflex were measured in the contrasting conditions of hypoxia and hyperoxia before and after Anexate. Blockade of the BZ receptors, opposite to the conceptual premise, inhibited ventilation. The inhibition was independent of the chemical stimulus due to the gas condition. Anexate also potentiated the inhibitory Hering-Breuer reflex. The effects observed were mediated through mechanisms other than the GABA-BZ receptor complex, since the blockade of this complex with picrotoxin or bicuculline did not influence the results. In conclusion, the role of the BZ receptor in the central inhibition of respiration by hypoxia seems unlikely.

## **LARYNGEAL CONTRIBUTION TO THE RESPIRATORY RESPONSE TO CO<sub>2</sub>**

(Assoc. Prof. Małgorzata Szereda-Przestaszewska)

Supported by the State Committee for Scientific Research: grant # 663619203

It was shown that instantaneous flow of warm, humidified air enriched with 6% CO<sub>2</sub> through the anatomically isolated larynx induces a decrease in ventilation within the first minute.

It was due to the change in both components of the respiratory pattern: the fall in tidal volume and prolongation of the respiratory cycle. The decrease in ventilation appeared to be more pronounced following midcervical vagotomy and it was effectively abolished by the neurotomy of the superior laryngeal nerve.

## **ARACHIDONIC ACID IN SIGNAL TRANSDUCTION IN CAT CAROTID BODIES**

(M. Sc. Robert Strosznajder)

Supported by the State Committee for Scientific Research: grant # 6P20702905

Experiments were carried out to establish the optimal condition for arachidonic acid (AA) incorporation into membrane lipid.

The activity of arachidonyl-CoA synthase and acylation of lysophosphatidylinositol was investigated. It was observed that AA is actively incorporated into phosphatidylinositol (PI) and that this process is stimulated by dopamine. Hypoxia decreased AA uptake into PI, and had no effect on AA-CoA synthase activity.

## **OBSTRUCTIVE SLEEP APNEA AS A CONSEQUENCE OF A POSITIVE FEEDBACK BETWEEN THE LEVEL OF THE PHASIC NEURAL ACTIVITY OF THE NERVES SUPPLYING UPPER AIRWAY MUSCLES AND THE INITIAL TONIC TENSION OF THESE MUSCLES**

(Dr. Wiktor A. Janczewski)

Supported by the State Committee for Scientific Research: grant # 6P20702805

The project started in November 1993. Nine experiments have been performed on anaesthetized, vagotomized rabbits. The sensory muscle output was reduced several times during each experiment by means of a short lasting neuromuscular block induced by depolarizing agent - suxamethonium chloride (0.2 mg kg<sup>-1</sup> i.v.) or nondepolarizing neuromuscular blockers (Gallamine, Pancuronium, d-Turbocurarine). Electrical activities of the hypoglossal facial and phrenic nerves were recorded simultaneously.

We have found that muscle relaxation significantly reduces activity of the hypoglossal nerve (by 27%). The amplitude of the facial nerve was reduced less (by 13%) and phrenic nerve activity remained unchanged. We believe that this positive feedback between the initial tension of the upper airway muscles and the level of their phasic activity may be a partial explanation of a decrease in the upper airway dilator muscle activity during sleep or after administration of ethanol, or sedative drugs.

**FREE RADICAL MECHANISM IN THE PATHOGENESIS  
OF SUBARACHNOID HEMORRHAGE;  
CHARACTERIZATION OF IMMUNOSUPPRESSIVE PROPERTIES  
OF 2-CDA**

(Assoc. Prof. Paweł Grieb)

It has been shown that a lipid-soluble free radical scavenger ubiquinone (coenzyme Q<sub>10</sub>) prevents the development of ischemic lesions in a rabbit model of subarachnoid hemorrhage.

Immunosuppressive properties of 2-chloro-2'-deoxyadenosine (2-CDA) were further characterized with the use of *in vitro* models of B- and T-dependent immune reactions. Two clinical trials evaluating 2-CDA as a treatment for remitting-relapsing multiple sclerosis were initiated: an open pilot trial with 10 patients, and a double-blind placebo-controlled trial with 84 patients. These trials are in progress.

**EVALUATION OF CYTOTOXICITY OF 2-CDA AND 2-BDA  
ON ORGANOTYPIC CULTURES OF CHOSEN CENTRAL  
NERVOUS SYSTEM STRUCTURES AND ON GLIAL TUMORS**

(Prof. Mirosław J. Mossakowski)

Supported by the State Committee for Scientific Research: grant # 443449203

Organotypic cultures of neonatal rat hippocampus and cerebellum, incubated for up to 10 days with 2-CDA (2-chloro-2'-deoxyadenosine) and 2-BDA (2-bromo-2'-deoxyadenosine), 0.3-10  $\mu\text{m}$ , were evaluated with electron microscopy. No cytotoxic effects of the drugs were found. Maturation and differentiation of both neural and glial cells was normal. The same technique was used to assess effects of the drugs on organotypic cultures of human malignant gliomas initiated from surgical biopsy material. Here, signs of mitochondrial destruction (swelling and disintegration cristae) were observed following incubation with not less than 3  $\mu\text{m}$  of 2-CDA or 2-BDA.

Dissociated cultures of human gliomas were also initiated. Effects of the drugs on cell cycle of gliomas were evaluated with two-parameter DNA-protein flow cytometry. Preliminary data seem to indicate that 2-CDA in concentrations up to 1  $\mu\text{m}$  produces a dose-dependent disturbance of the cell cycle characteristic of the block of the S phase.

## DEPARTMENT OF NEUROCHEMISTRY

3 Dworkowa Str., 00-784 Warsaw

Telephone: 49 58 97

**Head: Prof. Jerzy W. Łazarewicz**

### **NMDA RECEPTOR-MEDIATED DESTABILIZATION OF CALCIUM HOMEOSTASIS IN CEREBRAL NEURONS: MECHANISMS AND PHARMACOLOGICAL PREVENTION**

(Prof. Jerzy W. Łazarewicz)

Changes in calcium homeostasis in the striatum of adult vs. immature rats at postnatal day (PND) 8-10, and in the hippocampus of adult rabbits, were evaluated by measuring of  $\text{Ca}^{2+}$  concentrations and  $^{45}\text{Ca}$  efflux in microdialysates. The efflux of [ $^{14}\text{C}$ ]sucrose was employed as a measure of changes in extracellular space volume. Studies focused on developmental aspects of destabilization of calcium homeostasis by NMDA in the rat striatum demonstrated that application of 5 mM NMDA for 20 min in the dialysis medium to the striatum of PND 8-10 rats induced 80% decrease in  $\text{Ca}^{2+}$  concentration and 46% decrease in  $^{45}\text{Ca}$  efflux. The above findings indicate a massive, long-lasting translocation of extracellular calcium to neurons. In the striatum of adult rats NMDA induced an increase in  $^{45}\text{Ca}$  efflux without significant changes in extracellular  $\text{Ca}^{2+}$  concentration, presumably reflecting balanced fluxes of calcium into and out from the neurons, without net accumulation of  $\text{Ca}^{2+}$  intracellularly. These results, demonstrating increased susceptibility of immature neurons to the NMDA-evoked destabilization of calcium homeostasis, may explain their increased sensitivity to NMDA toxicity (collaboration with Dr. H. Hagberg, Göteborg, Sweden).

Microdialysis of the rabbit hippocampus was utilised to study effects of the redox modulation and gangliosides on the NMDA-induced destabilization of calcium homeostasis. Sulfhydryl reagents and ascorbate were applied locally in the dialysis medium. It was shown that high, at least 50 mM, concentration of DTT is needed to induce any perceptible changes in  $^{45}\text{Ca}$  efflux. Two opposing components of DTT-induced calcium transient were separated: MK-801-sensitive decrease in  $^{45}\text{Ca}$  efflux which reflects calcium influx to neurons through NMDA channels, and an increase of  $^{45}\text{Ca}$  efflux as a result of DTT-evoked cellular swelling and condensation of extracellular components. DTNB and ascorbate in 10 mM concentrations did not interfere with changes in calcium homeostasis in the hippocampus evoked by 1 mM NMDA. These results point to the role of endogenous compensatory mechanisms preventing excessive redox modulation of the NMDA receptors *in vivo* by exogenous thiol reagents.



In the same experimental model effects of ganglioside GM1 (30 mg/kg b.w. administered i.p. or i.m. for two days, twice a day) on NMDA-induced disturbances in calcium homeostasis in the rabbit hippocampus were studied. Local application of 1 mM NMDA for 20 min induced a prolonged decrease in the extracellular  $\text{Ca}^{2+}$  concentration and in  $^{45}\text{Ca}$  efflux, resulting in the damage of CA1 neurons detectable after 24 h. In the contrary GM1-treated rabbits exhibited complete protection of CA1 neurons and NMDA-induced stimulation of  $^{45}\text{Ca}$  efflux. These results confirm neuroprotective activity of the ganglioside and suggest that stabilization of calcium homeostasis by GM1 may participate in the mechanism of neuroprotection.

Effects of acidifying the dialysis medium on extracellular concentrations of neuroactive amino acids were studied. It was shown that reduction of pH to 6.4 leads to a significant decrease in extracellular concentrations of glutamate and taurine. This effect is sensitive to DIDS, a blocker of  $\text{Cl}^-/\text{HCO}_3^-$  exchanger. This finding may suggest that  $\text{H}^+$  ions accumulating in the brain during ischemia may play a compensatory role decreasing extracellular concentrations of excitatory amino acid, glutamate (collaboration with Dr. A. Lehmann, Göteborg, Sweden)

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

(Prof. Jerzy W. Łazarewicz)

Supported by the State Committee for Scientific Research: grant # 403209101

Effect of NMDA receptor stimulation in the hippocampal slices of adult and developing rats at postnatal day (PND) 7 on tritiated arachidonic acid ( $[^3\text{H}]\text{AA}$ ) release was tested using superfusion with albumin-containing medium. In contrast to only slight stimulation of the release in adult rat hippocampus, in PND 7 rats a massive  $[^3\text{H}]\text{AA}$  release, dependent on the concentration of NMDA, sensitive to NMDA receptor antagonists, quinacrine and calcium-free medium was found. This effect was only partially inhibited by magnesium, whereas 7-Cl-ky-nurenic acid and ifenprodil inhibited this effect. Reduction of sulphhydryl groups with dithiothreitol (DDT) activated  $[^3\text{H}]\text{AA}$  release, that was inhibited by NMDA receptor antagonists. Thus, NMDA receptors in the immature rat hippocampus, that induce a highly pronounced  $\text{Ca}^{2+}$  and phospholipase  $\text{A}_2$ -mediated arachidonic acid release, are subjects to regulation by glycine, polyamines and redox modulatory sites, whereas their magnesium regulation is weak. These phenomena may participate in the mechanism of enhanced NMDA toxicity in PND 7 rats.

A similar protocol of superfusion of immature PND 7 rat hippocampal slices was adapted to study the release of selected metabolites of arachidonic acid,

prostanoids thromboxane B2 and 6-keto prostaglandin F<sub>1α</sub> under *in vitro* model ischemia-like metabolic conditions. In these experiments application of anoxic/ /aglycemic medium was accompanied by a temporary arrest of superfusion, then normoxic and normoglycemic superfusion was reestablished. The release of prostanoids after *in vitro* "ischemia" was observed. MK-801 and quinacrine reduced prostanoid release. These results suggest a role of glutamate release, NMDA receptor stimulation and phospholipase A<sub>2</sub> activation in the release of prostanoids in the hippocampal neurons under ischemic conditions.

## MODIFICATION OF INTRACELLULAR SIGNAL TRANSDUCTION IN BRAIN PATHOLOGY

(Assoc. Prof. Krystyna Domańska-Janik)

Delayed damage of hippocampal neurones following a short time, global cerebral ischemia, results from initiation of the autodestructive neurochemical cascade. The identification of these events as well as their timing would help to elucidate the mechanism and suggest the therapeutic strategies to alleviate effects of ischemic brain injury. The early postischemic changes, peaking at 3 hours after the insult, include the transient translocation (activation) of protein kinase C (PKC), increased enzymatic activity of ornithine decarboxylase (ODC) and elevated DNA binding activity of activator protein-1 (AP-1). The effectiveness of the known modulators of postischemic morphological outcome (MK-801, L-NAME, and ginkgolides BN 52020, BN 52021) was tested. The PKC and ODC activation, measured at 3 h after ischemia, can be effectively blocked by the drugs inhibiting directly NMDA-receptor gated calcium entry as well as these reducing the secondary impacts of high intracellular calcium concentration. In contrast, postischemic AP1 enhancement was only partially inhibited, suggesting involvement of an additional to calcium mobilization, mechanism(s) of its activation.

Further attention was focused on the role of platelet-activating-factor (PAF) in transduction of calcium signalling *in vitro* and *in vivo*. PAF antagonists: BN 52020 and BN 52021, were proved to exert neuroprotective effects after ischemia. Locally applied 0.5 μM PAF triggered calcium decompartmentation in microdialysed rabbit hippocampus *in vivo* and markedly potentiated this effect induced by NMDA. *In vitro*, PAF in pathophysiological concentration (10 nM) triggered Ca<sup>2+</sup> influx and activated PKC in the rat synaptosomal fraction. The data indicate that PAF can aggravate deleterious effect of elevated intracellular calcium on neuronal degeneration.

In cooperation with University of Arizona (Dr. D.E. Goll) the involvement of calcium and phosphoinositides in molecular mechanism of calpain activation was studied. Moreover, the pharmacokinetic study of a new, type-selective opi-

ate receptor ligands was performed in collaboration with Dr. H.I. Yamamura.

Besides, we have continued our study on molecular basis of the pt-rabbit mutation. The genetic defect was located on PLP gene, which confirms that pt-rabbit is an excellent model of the human Pelizaeus-Marzbacher disease. The developmental studies of major myelin specific proteins (PLP, MBP, CNP, MAG and MOG) in the brain homogenates and myelin of pt mutants and age matched control rabbits revealed reduction of the content of all determined proteins, whereas their incorporation into myelin (except PLP), remained close to normal. This indicates that the mutation, in spite of drastic PLP deficiency, does not affect myelin assembly process during development.

In the model of rat cardiac hypertrophy the mechanism of action of adenosine was studied in the respect of its role in cardiomyocyte relaxation.

The study concerning involvement of platelet-activating-factor (PAF) in ischemia-evoked signal transduction and aggravation of delayed neuronal injury was supported by the State Committee for Scientific Research: grant # 403199101.

## **REGULATION AND LOCALIZATION OF PHOSPHOLIPASES AND THEIR ROLE IN SIGNAL TRANSDUCTION IN THE BRAIN**

(Prof. Joanna Strosznajder)

Transduction of extracellular signals across the plasma membrane involves activation of several phospholipases (PLs) that generate multiple, sometimes inconvertible, lipid-derived messengers. Our studies refer to the intracellular distribution and to the regulation of the novel PLC, degrading phosphatidylethanolamine (PE) and to comparison with the other PLs in a signal dependent manner. Moreover, our investigations concerned the action of 5HT on glutamatergic receptor (GluR) dependent arachidonic acid (AA) release by PLA<sub>2</sub> and on this receptor mediated inhibition of AA uptake. In our study we have also aimed to evaluate the consequences of PLs activation by determining the lipid mediators influence upon Cl<sup>-</sup> channel properties. The novel enzyme we have found in the brain - Ca-independent PLC acting on PE has similar subcellular localization as the other PLs. The higher specific activity was observed in cytosol and SPM and these fractions next to synaptoneurosomes were used in further studies. It was observed that Ca<sup>2+</sup> in concentration dependent manner decreases PE-PLC. Concomitantly the higher AA liberation by DAG lipase cooperating with PE-PLC was found. Nonhydrozylable analog of guanine nucleotide, GTPS activates AA release in the presence of Ca<sup>2+</sup> ions, but remains without any effect on PE-PLC. Serotonin (10-100 μM) activates AA release in the presence of Ca<sup>2+</sup> by the action of PE-PLC/DAG-lipase. We have found that 5HT is a very potent regulator of several PLs. It acts directly through 5HT

receptors or indirectly through the action on other receptors. Serotonin by 5HT<sub>2</sub> receptor activates phosphoinositides (PtdIns) degradation by PLC and simultaneously stimulates AA release by PLA. Serotonin abolishes muscarinic cholinergic receptor (mAChR) dependent AA release and decreases inositol phosphate formation by PLC. Our findings indicate that protein kinase C is involved in regulation of mAChR dependent PLC. The activation of protein kinase C does not participate in the interaction between 5HT and mAChR. We have found that 5HT<sub>2</sub> and 5HT<sub>1A</sub> receptors are involved in AA turnover in the brain. Serotonin abolishes GluR stimulated AA release in the brain cortex and hippocampus and also decreases glutamatergic (GluR) dependent inhibition of AA uptake. Thus, an evidence was provided, that 5HT significantly modulates the toxicity of excitatory amino acids.

These results demonstrate the negative coupling between serotonergic and glutamatergic receptor system. The action of 5HT<sub>2</sub> receptor antagonist and 5HT<sub>1A</sub> receptor agonist on the liberation of second messengers ought to be taken into account considering the potential therapeutic implication of these compounds particularly 5HT<sub>2</sub> receptor antagonist in ischemic pathology. Our further studies demonstrated that products of phospholipases action, AA and DAG, which are known to be accumulated in aged brain, may be responsible for the modification of GABA<sub>A</sub>/Cl<sup>-</sup> channel properties during aging. The action of these lipid mediators on Cl<sup>-</sup> channel properties, evaluated by determination of kinetic values of picrotoxin induced dissociation of Cl<sup>-</sup> channel ligand (TBPS) in adult brain, correlates well with the effect of aging.

Summarizing our results demonstrated that 5HT is a very potent modulator of phospholipases including the new enzyme PLC/DAG lipase acting on PE. The inhibitory effect of 5HT<sub>1A</sub> agonist on GluR activated AA release, together with the ameliorating action of 5HT<sub>2</sub> receptor antagonist and 5HT<sub>1A</sub> receptor agonist on glutamatergic receptor-induced inhibition of AA incorporation, offers a potential therapeutic implication.

## **NITRIC OXIDE A POTENT MEDIATOR OF GLUTAMATE NEUROTOXICITY IN BRAIN ISCHEMIA**

(Prof. Joanna Strosznajder)

Supported by the State Committee for Scientific Research: grant # 6P20702704

The effect of a nitric oxide (NO) synthase inhibitor L-N<sup>o</sup>-nitroarginine (L-NOArg) on the neuronal survival in the CA1 region of the hippocampus of the brain submitted for 5 min to global cerebral ischemia was investigated. Concomitantly, the action of L-NOArg on glutamate-induced changes in turnover of arachidonic acid was studied. Complete forebrain ischemia in gerbils over a period of 5 min resulted in a consistent total neuronal degeneration of

the hippocampus CA1 area. L-NOArg was applied intraperitoneally in a doses of 1 and 30 mg/kg body weight 5 min before ischemia and 1, 3, 24 and 48 hours after ischemia. The gerbils were allowed to survive for 7 days. L-NOArg in a lower dose of 1 mg/kg b.w. sporadically preserved the neurons around the arteries in CA1 region. However, this inhibitor applied in a higher dose of 30 mg/kg b.w. had a protective action in 75% of the treated animals. L-NOArg exerted a significant neuroprotective effect on pyramidal neurons around the arteries and along the whole CA1 sector. In our studies on the mechanism of L-NOArg neuroprotection we have found that this compound eliminates glutamate and NMDA induced NO production and an inhibitory effect of this agonist on arachidonic acid uptake into membrane lipids, protecting in this way the brain against accumulation and destructive action of free arachidonic acid.

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

(Prof. Urszula Rafałowska)

The effect of lead on the release of GABA, dopamine and histidine (as precursor of histamine) was studied in synaptosomes obtained from chronically lead-treated rats and in synaptosomes with *in vitro* lead added. Lead administration to the rats decreased KCl dependent release of GABA, dopamine and histidine. Lead itself (independently from depolarizing condition) stimulated the release of previously accumulated neurotransmitters in synaptosomes (GABA and dopamine).

This effect depends on lead acetate concentration. On the other hand lead in different concentrations did not cause changes in the histidine release. This result shows that lead can attack the synaptic neurotransmission in the two ways: by depressing the Ca-KCl-evoked release of GABA, dopamine and histidine, and by selective stimulation of spontaneous release (independent from depolarization conditions) of GABA, DA but not of histidine. Simultaneously we have looked for mechanisms, which can be responsible for Pb-toxicity effect on neurotransmissions. We demonstrated that administration of  $\text{Pb}(\text{CH}_3\text{COO})_2$  *in vivo* and *in vitro* did not affect phospholipid composition and content in synaptosomes and the lipid peroxidation, but inhibits  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity. The decrease of  $\text{Na}^+\text{-K}^+\text{-ATPase}$  activity can change the gradients of sodium and potassium across the cell membrane and can be the reason of the disturbances in neurotransmission.

## ENERGY METABOLISM IN NERVE ENDINGS OF BRAIN UNDER LEAD TOXICITY CONDITIONS

(Prof. Urszula Rafałowska)

Supported by the State Committee for Scientific Research: grant # 6P20709304

The lead level in synaptosomes of brain, brain homogenates and also in the bone, hair, liver, kidney and blood from control and chronically poisoned rats was determined using the X-ray fluorescence with energy dispersion and spectrophotometer atomic absorption methods.  $Pb^{2+}$  level in the poisoned brain and in the brains synaptosomes was low comparing to bone, hair, liver or kidney. The  $Pb^{2+}$  level in the synaptosome fraction obtained from the lead-treated rats was two times higher than in the same fraction of control rats (1.1 ppm for control; 3.2 ppm poisoned rats). Such an increase of  $Pb^{2+}$  was high enough to decrease the  $Na^+-K^+-ATPase$  activity by about 25%. An inhibition of  $ATPase$  activity may change the gradients of sodium and potassium across the cell membrane and therefore disturb action of nerve endings.

Additionally we have introduced into experiments measurements of adenylnucleotides levels and oxygen consumption in control and  $Pb^{2+}$  poisoned synaptosomes.

## DEPARTMENT OF NEUROPATHOLOGY

3 Dworkowa Str., 00-784 Warsaw

Telephone: 49 54 10

**Head: Prof. Andrzej Kapuściński**

### VASCULAR AND METABOLIC PATHOGENETIC FACTORS OF THE LATE ISCHEMIC DAMAGE OF THE NERVOUS TISSUE

(Prof. Mirosław J. Mossakowski)

The studies were carried out on 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 experimental model the state and function of the blood-brain barrier (BBB) was evaluated in the different periods after 10-min brain ischemia. By means of radioisotope methods dynamics of BBB injuries was quantitatively characterized, revealing that they developed in 45% of animals after ischemic episode with a relatively mild, although statistically significant intensity. The BBB injuries were shown to have a biphasic nature. In the first phase developing immediately after ischemia, the most intensive alterations were observed in 120 min and 24 hr after resuscitation. The advanced late changes were found one week after ischemic episode. It was worth emphasizing that late BBB abnormalities found in the quantitative isotope studies were not observed in the previous ultrastructural studies with use of horseradish peroxidase. The early phase injuries were combined with the postischemic hemodynamic disturbances. The late phase injuries were assumed as secondary to the metabolic and structural tissue alterations. The quantitative radioisotope investigations supplemented previous ultrastructural studies with the use of the horseradish peroxidase in which topography of the BBB injuries was characterized and the route of tracer transvascular transport throughout damaged microvessels. In the other studies performed on this experimental model alterations of endothelium in cerebral arterioles, venules and capillaries were characterized. Advanced changes of the luminal endothelial surfaces were demonstrated in the early postischemic phase. They consisted in an appearance of numerous microvilli, formation of deep endothelial surface invaginations and foldings causing decrease of blood flow, production of thrombocytic aggregates and leukocytic conglomerates. On the other hand, progressing changes in the endothelial structure leading to their complete disintegration caused dysfunction of the BBB mechanisms.

In the same experimental model changes of the GABA-ergic neurons in the nucleus reticularis thalami were characterized. This GABA-ergic structure is

selectively sensitive to ischemia being exposed to the excitotoxic effect of glutamate of cortical afferent innervation. By means of the immunocytochemical technique with use of colloidal gold, a remarkable decrease of GABA content in the neuronal perikarya in the nucleus reticularis as well as in the symmetric synapses was demonstrated in the early period after ischemia. Changes in neurons were more advanced and appeared earlier. Immunocytochemical alterations normalized 24 hr after ischemia. The experimental data indicated the temporary reduction of the GABA-ergic activity increasing damaging effect of the excitatory amino acid neurotransmitters, active in this thalamic structure.

The immunocytochemical analysis of the blood serum proteins content in the brain tissue taken post mortem in patients after cardiac arrest was complementary to the studies on the BBB disturbances after experimental cardiac arrest. It was shown that serum proteins (albumin and fibrinogen) accumulated in the brain with different intensity and in different periods after ischemic episode. They accumulate by diffusion in the subependymal and submeningeal regions, in astrocytes and neurons. The hypothesis was proposed that diffusion reaction observed both in subpial and subependymal regions resulted from dysfunction of the cerebrospinal fluid-tissue barrier mechanisms. Intracellular accumulation of proteins in astrocytes represented removal of proteins which were extravasated to the brain tissue during and immediately after the acute ischemic phase, whilst the neuronal deposits seem to reflect injury of cellular membranes of the surviving although damaged neurons (penumbra phenomenon).

In the second experimental model investigations were focused on morphological analysis of terminal vascularization (vessels in diameter less than 12.5  $\mu$ ) in the CA<sub>1</sub> and CA<sub>3</sub> hippocampal sectors characterized by a different vulnerability to ischemia. It was shown that terminal vascularization of the ischemia-sensitive CA<sub>1</sub> sector was poorer than that in ischemia-resistant CA<sub>3</sub> sector when calculated per surface unit as well as per number of neurons. Moreover, the average dimensions of vessels, their exchange and flow surfaces in CA<sub>1</sub> sector were also lower. The obtained data indicate lower microcirculation efficiency in the CA<sub>1</sub> area than in the CA<sub>3</sub>. These differences can aggravate neuronal injuries in the CA<sub>1</sub> sector induced by the cytotoxic effects of glutamate.

Histochemical studies of protein-bound glycoconjugates participating in the molecular structure of the cellular membranes of morphological elements of the CNS and its vessels were also performed after short-lasting cerebral ischemia. The immunohistochemical analysis concerned these structures of the CNS which did not show any evident morphological alterations. Significant changes in glycoconjugate distribution in the vessel endothelial cells, neurons and glial cells were observed after ischemia. The above findings indicate that in cellular CNS components without any visible histological abnormalities, changes in the chemical structure of their cellular membranes appear, which can significantly affect their functional state and metabolism. Changes in the vascular endothelium, leading to dysfunction of its transport function seem to be of particular importance.



The comparative morphometric evaluation of the state of cerebral microcirculation was investigated in two ischemic experimental models: the short-lasting ligation of carotid arteries in Mongolian gerbils and cardiac arrest in rats. In both groups of animals statistically significant abnormalities of microcirculation were observed during the first days after ischemia. In Mongolian gerbils in all studied periods after ischemia the content of blood vessels decreased, however, it was different in various cerebral structures. The blood reflow to the vascular network of CA<sub>1</sub> sector of hippocampus was delayed. In rats after cardiac arrest the significant increase of blood content in the vascular bed was observed in all studied periods after resuscitation except 1 h, when distinct hypoperfusion dominated. It was suggested that nature and intensity of microcirculation disorders in different types of brain ischemia depend on experimental model applied.

## **STRUCTURAL MANIFESTATIONS OF AGING IN BRAIN REGIONS WITH DIFFERENT NEUROTRANSMITTER CHARACTERISTICS**

(Assoc. Prof. Irmina B. Zelman)

The comparative evaluation of morphological changes was performed in four subcortical nuclei representing different neurotransmitter systems (basal nucleus of Meynert-NBM, nucleus locus ceruleus - LC, dorsal raphae nucleus - DRN and substantia nigra - SN), in brains of normal elderly subjects at the age ranging from 71 to 90 years (group I) and in brains of persons aged 59-85 years with neuropathologically proven Alzheimer's disease (AD) - group II. In the first group the most extensive neuronal loss and neurofibrillary degenerations were found in LC, whereas in the second group marked neuronal atrophy and numerous neurofibrillary tangles were demonstrated in all investigated nuclei, except SN. In comparison with the first group, in AD brains neuronal loss was most pronounced in NBM. The earlier development of neurofibrillary tangles in the group II can be explained by generalized unspecific acceleration of cytoskeleton pathology in AD brain. In both investigated groups neurofibrillary tangles appeared mostly in LC and DRN and they were rare in SN. Decreased ubiquitin immunoreactivity of neurofibrillary tangles in LC and SN may indicate the delayed degradation of abnormal tau-protein in these nuclei. The differences in nature and intensity of glial reactions in particular nuclear structures suggested different dynamics of neuronal loss in the nuclei examined. The obtained results point out the lack of quantitative differences in morphological alterations among NBM, LC, DRN and SN, though differences in intensity of neuronal degenerations and concomitant glial reactions suggest participation of various pathogenetic mechanisms in their development.

In collaboration with the Department of Anatomy, School of Medicine, Gdańsk, morphometric estimation of neuronal loss, the number of neurofibril-

lary tangles and amyloid plaques (revealed by immunocytochemical stainings) were performed in hippocampal formation including hippocampus, entorhinal cortex and subicular complex, in senile cases without cognitive impairment, in cases with vasogenic dementia and in AD cases. The pattern of pathological changes was characteristic and similar in all three groups with their greatest intensity in the second layer of entorhinal cortex, molecular layer of the dentate gyrus and CA<sub>1</sub> sector of Ammon's horn. In non-demented group the changes in neuronal density were not found, but the presence of neurofibrillary tangles and amyloid deposits in hippocampal formation may be a cause of slight memory disturbances, often found in senile persons. In vascular dementia alterations in hippocampal formation were not marked, so impairment of intellectual functions in this group does not seem to be directly linked with its damage. The relatively small damage of hippocampal formation in vascular dementia and severe damage of this structure in Alzheimer dementia gives rise to the assumption about different pathogenetic mechanisms in both of them.

The studies aimed in ultrastructural characteristics of the neurotoxic effects of sodium tellurite intoxication on myelin in adult and developing rats revealed dose- and age-dependent, mostly reversible myelin structure abnormalities in brain hemispheres and optic nerves. They consisted in multiple focal swellings and condensation of myelin lamellae and myelin sheath thinning. Myelin changes were accompanied by abnormalities in axonal fibers, synaptic endings and alterations of some neurons and glial cells, more pronounced in developing animals. The tellurium-dependent myelin alterations may be connected with disturbances of the cholesterol biosynthesis at the level of conversion of squalene to the epoxysqualene.

## **FOCAL BRAIN ATROPHY IN COMPUTED TOMOGRAPHY (CT) ASSESSED IN COMPARISON WITH CLINICAL SYMPTOMS OF PROGRESSING DEMENTIA. PROSPECTIVE STUDIES**

(Prof. Mirosław J. Mossakowski)

Neurological, neuropsychological and radiological examinations of 112 patients from outpatient clinic for Alzheimer's disease (AD) and aged persons from the nursing home were performed. Diagnosis of probable AD was based on informations from anamnesis, laboratory data and neurological and neuropsychological screening examinations. Detailed neurological examination was compared with focal brain atrophy visualized on CT pictures. Similarly to our previous data a striking heterogeneity of AD cases was observed. This inclined us to separate a group of AD patients with an early onset of the disease process (>65 years) and to analyzed it in order to characterize a typical clinical picture of this particular group. Only one statistically significant element was specific

for "young" AD patients. It consisted in that during the first clinical examination the hippocampal atrophy was less pronounced than in the group of "late" (<65 years) onset AD cases, although the clinical state assessed by neuropsychological examination was the same. Another group of AD cases included patients in which parkinsonian syndrome was accompanying progressive dementia. In AD patients with secondary extrapyramidal features the clinical symptomatology was significantly more severe. In 1993 the first data from prospective CT observations in AD patients were collected. Assessment of hippocampal fissure (according to de Leon projection) as a symptom of limbic atrophy was used as a reliable tool in clinical prognostic diagnosis of dementia progress.

## **INTERACTIONS OF THE GLUTAMATERGIC SYSTEM WITH OTHER NEUROTRANSMITTER SYSTEMS IN INORGANIC MERCURY NEUROTOXICITY AND HEPATIC ENCEPHALOPATHY**

(Prof. Jan Albrecht)

### **Inorganic mercury neurotoxicity**

A histochemical analysis of the activity of alkaline phosphatase (AP) - a cerebral capillary marker enzyme normally located on the luminal endothelial cell membranes, was performed in rats injected with 6 mg/kg of mercuric chloride (MC), i.p. The study revealed an almost instant enzyme inhibition, involving cerebral microvessels of all the cortical layers. Electron microscopy confirmed the enzyme reaction to be very weak or completely absent, which was followed by translocation of the activity to the abluminal side of the endothelial cell membranes. The translocation and coexistent ultrastructural changes in the luminal membrane (invaginations, pinocytic vesicles) were indicative of increased leakage of the cerebral vessels, and indirectly, of their increased permeability to mercuric chloride.

A study performed on a primary culture of the rat cerebral astrocytes revealed a marked stimulation by MC of the astrocytic release of the excitotoxic neurotransmitter glutamate (GLU) and localized the stimulatory effect on vulnerable SAH groups located within, but not on the surface of the cell membranes. This result added credence to the hypothesis that MC at low doses exerts neurotoxicity indirectly, by potentiating the excitotoxic effects of GLU. In consistence with this hypothesis, an earlier ultrastructural study carried out on an organotypic culture of the rat cerebellum demonstrated that low doses of MC lower the threshold for neurotoxicity of exogenous added GLU. A follow-up study showed that neurotoxic effects of a combined administration of MC plus GLU can be prevented by dithiothreitol - an agent protecting the intramembranous SH groups.

### **Hepatic encephalopathy (HE)**

The studies have concentrated on elucidating the mechanism by which HE promotes GLU excitotoxicity and, subsequently, leads to downregulation of different GLU receptor classes. HE in the thioacetamide model was found to induce a many-fold increase of the brain uptake index for ornithine (ORN), which in the brain serves as an alternative GLU precursor. Enhanced ORN transport to the brain, in conjunction with its increased metabolism towards GLU, may increase the amount of GLU available for release. Another study revealed that HE in the same model leads to a 70% loss of cerebral cortical GABA<sub>B</sub> receptors that are involved in the negative control of GLU release.

### **Taurine - a potential neuromodulator and osmoregulator in hyperammonemic conditions**

The effect of pathophysiologically relevant (1-5 mM) concentrations of ammonia on the release of taurine (TAU) was measured in cultures of retinal (Müller) glia and cerebellar astrocytes. Short-term treatment with ammonia induced a sodium- and chloride-dependent release of TAU from Müller glia, which may reflect a "programmed" neuroprotective response, preventing hyperexcitation by ammonia of adjacent neurons. By contrast, a 24-h treatment of cerebellar astrocytes with 1 mM ammonia led to an uncontrolled increase of basal TAU release (TAU "leakage") and at the same time, to the disappearance of the osmoregulatory TAU release in response to elevated concentrations of potassium ions in the medium.

## **TRANSPORT MECHANISMS OF NEUROACTIVE AMINO ACIDS IN RABBIT MÜLLER GLIA IN CULTURE**

(Prof. Jan Albrecht)

Supported by the State Committee for Scientific Research: grant # 6920705905

The studies were initiated in November 1993. In cooperation with Prof. Andreas Reichenbach (Leipzig University) preliminary experiments were performed on the effects of different modulators of ion channels and intra- and extracellular pH on the potassium- and ammonium-dependent release of the inhibitory amino acid taurine from cultures of Müller glia.

## DYSEMBRYOPLASIA AS A MORPHOLOGICAL BASIS FOR TEMPORAL EPILEPSY

(Prof. Halina Kroh)

The idea of dysembryoplastic neuroepithelial tumors (DNT) was suggested by C. Daumas-Duport in 1988. In 1993 the abnormality was introduced to the WHO classification as a new entity observed in simple and complex forms. In the world literature there are only a few papers dealing with this topic (Mayo Clinic).

Our preliminary investigation concerned temporal gliomas in young patients affected by medically intractable seizures. The study included also 5 patients with similar symptomatology in brain neoplasia localized in other brain regions. Among the patients operated in years 1988-1993 at the Neurosurgery Department of Warsaw Medical School, particularly involved in surgical treatment of medically intractable epilepsy were 22 patients aged from 7 to 39 years; majority of them were 12-20 years old. Chosen patients suffered of partial complex type of fits (psychomotor and temporal seizures), the disease history lasting from 2 to 18 years. Most often the fits appeared around 10th year of life.

Biopsy material was examined using histo- and immunohistochemical techniques. In 17 patients, temporal lobe gliomas presented various degree of anaplasia. In each case the tumor involved temporal cortex, in some also subcortical white matter and in 2 patients invaded neighbouring lobes. In biopsies we could not find either pathological foci limited to the cortex, or additional organized heterotopic structures (complex form). Majority of cases showed neuronal heterotopias in the white matter, evidenced by neuron specific enolase reaction. Material taken from 2 patients contained typical structures indicating DNT origin of tumors (simplex form confirmed by immunocytochemical staining).

## **LABORATORY OF DEVELOPMENTAL NEUROPATHOLOGY**

3 Ludwika Pasteura Str., 02-093 Warsaw

Telephone: 22 96 27

**Head: Prof. Maria Dąmbaska**

### **NEUROPATHOLOGICAL AND QUANTITATIVE EVALUATION OF CHANGES OCCURRING IN THE MATURING NERVOUS SYSTEM**

(Prof. Maria Dąmbaska)

Neuropathological evaluation included six cases of cystic encephalopathy in newborns, in two of them involving only periventricular white matter, and therefore named cystic leucoencephalopathy. An extensive cystic lesion in the brain hemispheres could be considered as a special type of lesions occurring in the maturing brain.

The morphometric evaluation of neuronal population in the hippocampal region demonstrated differences in density and maturity of nerve cells in hippocampal sectors in the brain of newborns. The picture of hypoxic lesions in this region examined in perinatal period, and evaluated with morphometric methods during the last two years were summarized and presented in a doctoral thesis. A preliminary morphometric analysis of neuronal maturation in dorsal vagal nucleus of humans was performed, constituting the first part of a study concerning maturation of this structure.

### **ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL EXAMINATION OF THE NERVOUS SYSTEM IN HUMAN PERINATAL PATHOLOGY, INHERITED DISEASES AND ANIMAL EXPERIMENTAL MODELS**

(Assoc. Prof. Danuta Maślińska)

Studies performed on human autopsy brains revealed that in accidentally intoxicated, normal children strong immunoreactivity of neurons with the monoclonal amyloid beta antibodies is similar to that observed earlier in children with neuronal ceroid lipofuscinoses (NCL). Since, extracellular immunoreactivity deposits (preamyloid) in those brains have not been found it is concluded that overexpression of amyloid beta precursor protein (APP) observed in neuronal stress of children is not concomitant with amyloidogenic processing of this protein.

In studies performed with adults affected with NCL (Kufs disease) extracellular deposits were found and they were indentified as typical nonfibrillar amyloid beta immunoreactive material.

It may be concluded that beyond overexpression of APP some additional events (eg. age-dependent) are required for increasing amyloidogenic alternative processing of the protein in brain leading to deposition of extracellular preamyloid.

Ultrastructure of the sciatic nerve was studied in rabbits treated with vincristine. The results provided an evidence, that the pathogenesis of vincristine neuropathy is more complex than it was previously described. Two alternative ways of the nerve fibre destruction: Wallerian degeneration and axonal atrophy were observed but never both of them affected the same fibre. In some fibres a down regulation of perikarial neurofilament formation attenuated an axon caliber following by the formation of myelin sheath. The infolded myelin compressed the axons resulting in desruption of them into pieces. In other nerve fibres the profound destructive effect of vincristine upon microtubule formation affected axoplasmic transport and caused accumulation of organelles in Ranvier's nodes. Arrested organelles underwent degeneration followed by disconnection of the nerve fibre. Then, Ranvier's nodes become *locus minoris resistantiae* in vincristine intoxication. Additionally, in the vincristine affected sciatic nerve a temporal sequence of pathological events, including the construction of perineurial and endoneurial vessels, was observed. The changes were followed by edema of perivascular and endoneurial connective tissue and Schwann cell cytoplasm.

## **LABORATORY OF THE ULTRASTRUCTURE OF THE NERVOUS SYSTEM**

3 Dworkowa Str., 00-724 Warsaw

Telephone: 49 54 20

**Head: Prof. Jerzy Borowicz**

### **THE ASSESSMENT OF THE INFLUENCE OF GLOBAL BRAIN ISCHAEMIA ON THE ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL PICTURE OF THE HYPOTHALAMO-HYPOPHYSIAL SYSTEM AND ON THE REPARATIVE PROCESSES IN CHOSEN PARTS OF THE BRAIN (HYPOTHALAMUS, BRAIN STEM, CEREBRAL CORTEX)**

(Prof. Jerzy Borowicz)

Immunocytochemical electron-microscope studies of the hypothalamo-hypophysial system and the thalamus of the rat were carried out. The main concern of the study was the localisation and physiological effects of neurotransmitters (GABA and glutamine) in the postischemic period. Preliminary analysis of the results showed that neurohypophysial microvesicles are morphologically and biochemically identical with synaptic vesicles. They contain classical neurotransmitters and take part in specific neurotransmission (e.g. they control the exocytosis of neurosecretory granules).

The electron-microscopic studies were also carried out in order to evaluate the toxicity of sodium tellurine in the hypothalamo-hypophysial system, the white matter and the ophthalmic nerve and to follow up reparative processes in the precapillary vessel zones of animals that survived 10 to 12 months after transitory clinical death of 10 minute duration. It was demonstrated that myocytes, macrophages and pericytes take part in the production of collagen. Morphological analysis of the participation of these cells in the processes of perivascular fibrosis was carried out. The obtained results suggest that the collagen fibrills, observed in the analysed material, play an important part in reparative processes taking place between the capillary and precapillary vessels and the brain.



## DEPARTMENT OF NEUROSURGERY

16 Barska Str., 02-315 Warsaw

Telephone: 22 36 43

**Head: Prof. Zbigniew Czernicki**

### COMPENSATION AND AUTOREGULATION DISTURBANCES IN SUPRATENTORIAL BRAIN LESION

(Prof. Zbigniew Czernicki)

In 1993 the Neurosurgical Clinic Department was opened after a long period of renovation. Thus, the whole activity became concentrated on clinical work.

The studies on the adaptatory and compensatory mechanisms and autoregulation in patients with focal brain damage were connected with the studies of regional cerebrovascular reactivity, disturbances of evoked potentials and memory disturbances after focal stereotactic brain damage. These investigations proved clinical usefulness of the cerebrovascular reactivity examination and a possibility of estimation of preoperative situation and the post-surgery changes. The electrophysiological examination of the "fair field" and cranial evoked potentials is a noninvasive method which allows to reveal brain cortex damage and conductivity disturbances in thalamocortical distance.

The investigations on memory disturbances in patients with focal damage of medial temporal structures showed hemispheric interaction disorders and right hemisphere domination in sensoric-visual memory.

The experimental studies were carried out on the CSF out-flow disturbances caused by a local brain damage and on the mechanisms of propagation of vasogenic edema after cold injury.

The studies on CNS out-flow disturbances showed that a local administration of definite volume of blood (model of intracerebral haematoma) causes less disturbances in the intracranial volume-pressure relations than the same blood volumes administrated to CSF space (model of subarachnoid haemorrhage). In the latter case CSF out-flow mechanisms are disturbed.

Examination of tissue with vasogenic edema showed a marked increase in ouabaino-like substance concentration which may cause an inhibition of sodium-potassium pump, and in consequence, play at least partly a role in propagation of brain edema.

As the most important achievements we consider application of somatosensory evoked potentials for estimation of nerve root disorder level in patients with cervical discopathy and an analysis of neuropsychological changes in the dementia syndrome.

## **THE FREQUENCY CHARACTERISTICS OF FOCAL EPILEPSY DISCHARGE BRAIN MAPPING**

(Prof. Eugeniusz Mempel)

Studies on the clinical usefulness of automatic frequency characteristics analysis in the brain mapping were performed, because of divergences in literature about validity of this method in localization of epileptic foci. Localization of these foci plays the main role in surgical treatment of the epilepsy.

It was found that 1) frequency analysis may confirm epileptic process in nontypical course of the disease without clinical symptoms of epilepsy; 2) frequency spectrum analysis allows to reveal and localize precisely the epileptic foci; 3) this analysis permits to disclose epileptic foci in an early stage of disease when the other diagnostic examinations fail; 4) this analysis allows also to reveal a predominant focus among 2 or 3 existing ones, while the other diagnostic methods bring insufficient or even wrong information. It was confirmed by surgical verification with corticography. Surgical treatment improved patient's condition very much.

In some other investigations relationships between brain mapping and others noninvasive diagnostic methods (EEG, CT, MRI) in revealing and localization of epileptic foci in hemispheres were sought for.

## **NEUROMUSCULAR UNIT**

1a Banacha Str., 02-097 Warsaw

Telephone: 659 75 06

**Head: Prof. Irena Hausmanowa-Petrusewicz**

The main aim of our research in 1993 was to introduce modern methods into myology. Therefore, the work was concentrated on molecular genetics, immunology and immunocytochemistry.

### **MORPHOLOGICAL CHARACTERISTICS OF IMMATURE AND AGING MUSCLE**

(Prof. Anna Fidzińska)

1. The distribution of N-CAM (neural cell adhesion molecule) in muscle specimens taken from neonates with X-linked recessive centronuclear myopathy (XLRCNM) was investigated. It was shown that all small muscle cells with centrally located nuclei were decorated by embryonic isoform of N-CAM suggesting a failure in muscle maturation. To explain this finding ultrastructure of motor end-plates was studied. The results of our studies have shown that aberrant undeveloped motor end-plates, found in neonates with XLRCNM, may be responsible for muscle immaturity.

2. Another study was devoted to the muscle cytoskeleton organization. Investigations with anti-desmin antibodies allowed us to separate from congenital myopathies a new disease entity - hereditary desminopathy in children. Abnormally accumulated desmin deposits were observed in muscle fibres of 5 cases representing 3 families. This finding requires further morphological as well as immunocytochemical investigations.

3. Effect of aging on skeletal muscle regeneration has been studied in senile (3 years old) rats. Most research on muscle regeneration has been performed - so far - using young animals. Experimental muscle regeneration in senescence has received little attention. We are not aware of any ultrastructural studies of regenerating old muscle. The results of our preliminary electron microscopic studies showed that after Bupivacaine injection the senile muscle is able to regenerate. The stages of muscle fiber regeneration are similar to those observed in young muscle. Muscle fiber reconstitution, however, is slower and less active than in young animals.

## SPINAL MUSCULAR ATROPHY - CLINICAL, MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS

(Dr. Katarzyna Rowińska)

The clinical differential diagnosis and laboratory findings concerning the Spinal Muscular Atrophy (SMA) were summarized and revised (Hausmanowa-Pertusewicz). The diagnostic procedure for SMA, including new laboratory method was elaborated. The utility of modern electrophysiological, ultrasonographic and imaging methods beside genetic ones in the SMA diagnosis were discussed.

Additionally the study was undertaken with regard to a concept that the Guillaine-Barre syndrome may involve more than one type of pathological processes. The examined material - sural nerve biopsy taken from subjects who fulfilled clinical criteria for acute G-B revealed as follows:

- 1 - Morphologically G-B is not uniform polyneuropathy,
- 2 - Demyelination/remyelination as well as coexistence of segmental demyelination/remyelination and axonal degeneration can be found in the nerves,
- 3 - Presence of pure axonal changes does not exclude diagnosis of G-B but interpretation of such changes must be treated with caution (H. Drac, Z. Jamrozik).

Other investigations concerned motor unit changes in the neurogenic lesion. The prevalence of motor unit double discharges was analyzed in the electromyogram of the patients with neuromuscular disorders and in control subjects. The diagnostic yield of double discharges in early detection of neurogenic lesion was discussed.

In the successive paper the computer methods for MUP complexity evaluation with developed system of data presentation were described.

## ELECTROPHYSIOLOGICAL, IMMUNOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

(Prof. Irena Niebrój-Dobosz)

**Electrophysiological characteristics.** No conduction block has been found in cases tested since 1989. The conduction block was present, however, in 14 cases diagnosed as demyelinating neuropathy (two cases were congenital), or pure motor neuropathy with high titer of anti-GM<sub>1</sub> antibodies.

**Immunological studies.** Fifteen cases of ALS were examined. The titer of GM<sub>1</sub> antibodies, the presence of paraproteins, levels of IgG, IgA and IgM in blood serum and cerebro-spinal fluid were examined. The titer of GM<sub>1</sub> antibody-

ies over 1:400 in serum, usually present in controls, was increased in four ALS cases. In cerebrospinal fluid the anti-GM<sub>1</sub> titer over 1:4 was found in six cases. No monoclonal protein bands were present. The level of IgG was over the normal range only in one case. In cerebro-spinal fluid of all ALS patients the IgG level was normal. There was no IgA and IgM in CSF of ALS patients or control subjects.

**Morphological examination.** The studies of astrocytes of brain stem and spinal cord specimens of 15 ALS cases were performed with the use GFAP and S-100 staining. Concomitant vascular permeability was also examined, but the results obtained are still not conclusive.

**APPLICATION OF NEW MARKERS IN DIAGNOSIS  
OF POLY- AND DERMATOMYOSITIS;  
AN ATTEMPT TO APPLY A SELECTIVE THERAPY**  
(Prof. Irena Hausmanowa-Petrusewicz)

Clinical, electromyographic and immunological studies of inflammatory myopathies associated with various connective tissue diseases or isolated inflammatory myopathies were performed using indirect immunofluorescence and double immunodiffusion. Antibodies against Mi2, RNP, PMScl and anti t-RNA synthetase antibodies were determined. For the first time in the Polish population antibodies Mi2 were found in dermatomyositis. Antibody Ku which is frequent in Asiatic population, was also detected in Polish cases. We have established neurological and neuromuscular manifestations in the overlap syndrome - scleromyositis associated with PMScl antibody. The main clinical characteristics of this syndrome are some features of dermatomyositis combined with scleroderma-like indurations of the hands and face.

Among 49 cases of scleromyositis, one case was associated with myasthenia gravis, a coexistence not described previously.

**MORPHOLOGIC, ELECTROPHYSIOLOGIC AND GENETIC  
FINDINGS IN DYSTROPHIES (EXCLUDING X-LINKED) i.e.  
FACIO-SCAPULO-HUMERAL, EMERY-DREIFUSS, LIMB-GIRDLE**  
(Prof. Irena Hausmanowa-Petrusewicz)

The aim of the studies was to check whether using DNA and dystrophin tests some cases of x-linked dystrophy can be identified among the patients classified previously as limb-girdle dystrophy. Fourteen cases from 45 families turned out

to be dystrophinopathies or manifesting carriers (13 boys and 1 girl). These investigations are extremely important for establishing correct diagnosis, particularly in sporadic cases, both in males and in females.

The subjects with Emery-Dreifuss dystrophy the peculiar predominance of 2 type muscle fibers might explain the characteristic EMG changes. However, these results require further confirmation.

The recruitment pattern of dystrophic muscle was studied in a large group of boys affected by dystrophy and in age-matched control group. The wire electrodes were used and isometric contraction of biceps br. muscle was recorded at different percentage of maximal voluntary effort. The findings indicate a higher rate of firing of dystrophic muscle which increases proportionally to the severity of dystrophic process.

### **ANALYSIS OF DNA IN DUCHENNE AND BECKER DYSTROPHY AND IN CHILDHOOD SPINAL ATROPHY**

(Prof. Irena Hausmanowa-Petrusewicz)

Supported by the State Committee for Scientific Research: grant # 407799101

The purpose of the study was to analyse relationships between gene deletion, dystrophin amount and phenotype in Duchenne and Becker's dystrophy. The series consisted of 193 patients with Becker and Duchenne dystrophy (BMD and DMD). The applied methods for DNA analysis were Southern-blotting and PCR.

Proportion and distribution of deletions seem to be in Poland similar to other European countries, however, there were also a few very rare deletions (e.g. selective deletion of exon 43). Phenotype in BMD turned out to be variable, abortive cases were quite frequent. We studied also the dystrophy in sporadic and familiar female cases.

The collaboration with the Genetic Laboratory of Columbia University was continued - recently 35 families with spinal atrophies (SMA) were collected, and previously described linkage to chromosome 5 was confirmed. An important finding was linkage disequilibrium in the Polish patients with SMA indicative of founder effect.

In collaboration with the Institute of Genetics in Bonn the data base for SMA cases was initiated.

## THE ROLE OF IMMUNOCYTOCHEMICAL METHODS IN DIAGNOSIS OF NEUROMUSCULAR DISORDERS

(Dr. Anna Kamińska)

Supported by the State Committee for Scientific Research: grant # 408799101

During the last year our work was focused on immunocytochemical studies of intermediate filaments (IF) using specific antibodies against desmin and vimentin. Increased fluorescence of desmin has been shown in regenerating fibers in various neuromuscular disorders as well as in case of central core myopathy.

Morphological changes similar to central core myopathy were produced experimentally in tenotomized muscle.

Our results indicate that tenotomy influence significantly expression of desmin in the soleus muscle fibers. Vimentin, which occurs in high concentration only in fetal and regenerating muscle cells did not reappear in tenotomized muscle fibers. It seems that the changes in intracellular organization of desmin parallel the morphological alterations in myofibrillar structure. The similar pattern of accumulation of desmin and acridine orange (AO)-RNA fluorescence in the area of intensive myofibril formation and hypertrophy indicates that desmin plays some role in the organization of newly formed myofibrils.

## DEPARTMENT OF APPLIED PHYSIOLOGY

17 Jazgarzewska Str., 00-730 Warsaw

Telephone: 40 40 47

**Head: Prof. Hanna Kaciuba-Uściłko**

### HEMODYNAMIC, METABOLIC AND NEURO-HORMONAL RESPONSES TO PHYSICAL EXERCISE IN MEN

(Prof. Krystyna Nazar)

1. Changes in blood growth hormone (hGH), catecholamines (CA) and lactate (LA) were determined during graded exercise of increasing intensity up to volitional exhaustion in 22 endurance athletes at the age from 17 to 56 years. It was found that in younger subjects (17-35 yrs,  $n=12$ ), plasma concentration of hGH increased in exponential manner with a rapid rise after reaching exercise load of approx. 60-70% of maximal oxygen uptake ( $VO_{2max}$ ). The calculated "hGH threshold" occurred in the similar range of exercise loads as the threshold of plasma CA and anaerobic threshold detected on the basis of blood LA concentration. Moreover, close correlations were found between plasma hGH and adrenaline ( $r=0.71$ ,  $p<0.001$ ), noradrenaline ( $r=0.81$ ,  $p<0.001$ ) or blood LA ( $r=0.64$ ,  $p<0.001$ ) concentrations. In the elder group (36-57 yrs,  $n=10$ ) resting plasma hGH level was significantly decreased in comparison with the younger group and it did not show typical increases during exercise whereas the thresholds of plasma CA and LA were similar in the two groups. The data indicate a decrease in the reactivity of the hypothalamo-pituitary system to exercise with ageing even in well fit subjects involved in regular training.

2. Previous investigation from this Laboratory demonstrated that during graded exercise, choice reaction time (RT) decreases with work load increment up to approx. 75% of maximal oxygen uptake ( $VO_{2max}$ ), and then it rapidly increases. The work associated with the best psychomotor performance exceeded anaerobic (lactate) threshold by approx. 25%. The curvilinear U-shape relationships were found between RT and plasma catecholamine (CA) concentrations. The aim of the present study was to follow up changes in RT during continuous constant-rate exercise at work loads above and below anaerobic threshold. The changes in RT were then related to blood lactate (LA) and CA responses to the effort. Sixteen endurance trained male subjects (aged  $21.3\pm 1.1$  yrs) participated in the study. It was found that during 20-min exercise above anaerobic threshold (AT), heart rate (HR), blood LA, and plasma CA levels were progressively increasing with time, whilst RT was decreasing until the end of the test, reaching 88% of the pre-exercise value. During 60-min exercise



below AT, blood LA and HR steady state was maintained, with only small increases in plasma CA. Choice RT reached its minimum after 40 min of exercise, and then stabilized at the level lower by 17.5% from that measured before exercise. Linear inverse correlations were ascertained between RT and plasma adrenaline ( $r=-0.65$ ), or noradrenaline ( $r=-0.68$ ) during exercise above AT. However, no such relationships were found in case of 60-min exercise, suggesting that an association between psychomotor performance during exercise, and circulating CA exists only when the latter show fast and relatively large changes.

3. Work tolerance of occupationally active, hypertensive patients was studied under laboratory conditions. For this purpose electrocardiogram (ECG) was recorded, systolic (SBP) and diastolic (DBP) blood pressure as well as heart rate (HR) were measured at various work loads during bicycle ergometer exercise, and during static handgrip at 30% of maximal voluntary contraction (MVC). Thirty four untreated male patients with essential hypertension aged 20 to 40 years, and 94 (61 males and 33 females) patients being on their routine medication, aged 20 to 65 years participated in the investigation. The control group consisted of 69 healthy men aged 20 to 60 years. The patients treated with beta-adrenolytics were excluded from the study. The results indicate that there are no significant differences in the responses of SBP, DBP and HR between untreated and treated groups of patients of similar age and both of them showed an exaggerated SBP increases during dynamic exercise. The slope of the regression between exercise HR and SBP was significantly greater in hypertensives than in controls. In 14% patients from the elder groups (40-65 yrs) inadequate HR increases in relation to work load were demonstrated and in 11 persons abnormalities in exercise ECG (mostly ST-segment depression) were noted. The increases in SBP and DBP during handgrip were greater in patients than in control subjects and did not depend on the patients' age. It is concluded that the physical load in occupational work for hypertensive patients below 40 yrs of age should not exceed 30% of maximal oxygen uptake (HR 100-120 beats/min). In elder patients individual evaluation of work tolerance is necessary. Static effort as a constant element of occupational tasks of hypertensive patients ought to be eliminated.

4. In cooperation with the Department of Rehabilitation, Sahlgren's Hospital, University of Gothenburg (Sweden) changes in malonyldialdehyde (MDA) in blood and muscles were studied during exhaustive exercise in young healthy volunteers. It was demonstrated that MDA formation occurs mostly in muscle fibers of high oxidative potential, rich in capillaries (type I). In another series of experiments the subjects were pretreated for 2 weeks with free radical scavengers (superoxide dismutase). This treatment reduced post-exercise muscle pain and oedema, and decreased both plasma and muscle MDA, and lactate concentrations. The findings indicate that free radical formation plays an important role in development of muscle fatigue.

5. In cooperation with the Institute of Sport (Warsaw), and the Department of Sports Medicine, Military School of Medicine (Łódź) the interrelationships between indices of aerobic and anaerobic capacity were analyzed in trained boys and girls (aged 13-17 years). The study showed that in trained adolescents there is a close association between aerobic and anaerobic performance measures, and between the laboratory and field fitness test items.

6. In cooperation with Department of Physiology, Medical School in Białystok the effect of low-carbohydrate diet on concentrations of ammonia in plasma and sweat during prolonged exercise was studied in young healthy volunteers. An evidence was provided that reduced carbohydrate availability enhances ammonia formation, and its excretion with sweat.

**RISK FACTORS OF PRIMARY HYPERTENSION:  
PROSPECTIVE STUDY**  
(Professor Krystyna Nazar)

Supported by the State Committee for Scientific Research: grant # 413109101

Exaggerated blood pressure response to exercise (BPRES) in normotensives is considered as an indicator of predisposition to hypertension. The aim of this study was to find out whether elevated BPRES is associated with any other hemodynamic, metabolic or hormonal abnormalities and to follow their evolution during three years. Out of 181 male students (aged 20 to 23 yrs) 39 showed elevated BPRES. As a criterion of abnormal BPRES systolic blood pressure >200 mmHg at 150W or lower exercise loads attained during an incremental bicycle ergometer test was used. Fifteen students with elevated (E) and 15 with normal BPRES (C) participated in further examinations. Seven subjects from group E had family history of hypertension. In the first year of the study in 8 students echocardiography revealed left ventricle hypertrophy. Body mass index, fat content, waist to height ratio, plasma lipid concentrations, as well as fasting blood glucose (BG) and plasma insulin (IRI) were similar in group E and C. The E subjects had also normal plasma catecholamines and renin activity at rest and during exercise, but their plasma cortisol and sodium levels in erythrocytes were higher than in group C. During oral glucose tolerance test (75 g), only in one subject from group E abnormal BG and plasma IRI responses were noted. In this group, however, significantly lower increases in cardiac output and higher total peripheral resistance were found than in group C. In the 2nd year of observation (1993) in three out of 15 subjects BPRES normalized but in two an elevation of resting BP was found. In the remaining subjects the majority of the examined indices were similar to those found in the previous year.

## FACTORS MODIFYING LIPID METABOLISM IN THE RAT SKELETAL MUSCLE

(Assoc. Prof. Leszek Budohoski)

1. The aims of this study were: 1) to establish the relationship between fatty acid (FA) metabolism in isolated skeletal muscle of the rat and FA availability, and 2) to investigate the effect of triiodothyronine ( $T_3$ ) administration *in vivo* on FA uptake and utilization by the muscle *in vitro*. Accumulation of free fatty acids (FFA), the rate of their oxidation to  $CO_2$  and synthesis of triacylglycerol (TG) were measured using  $^{14}C$ -labelled palmitate. The soleus muscle strips were incubated for 20 min in the medium enriched with albumin-bound palmitate at concentrations varying from 0.5 to 2.0 mmol/l. The rate of all three processes significantly correlated with the medium FA concentration, although only the relationship between TG synthesis and concentration of FA in the medium was linear ( $r=0.866$ ). The other two processes showed a tendency towards saturation at high FA concentrations following the logarithmic curves ( $r=0.762$  and  $0.694$  for  $CO_2$  production and FFA accumulation, respectively). Muscles taken from the rats treated for three days with  $T_3$  ( $75 \mu\text{g}/100 \text{ g}$ ) were incubated for 20 min at 1.0 mmol/l of palmitate. In comparison with euthyroid controls FA accumulation ( $p<0.05$ ) and oxidation ( $p<0.01$ ) were increased, whilst TG synthesis was similar. The initial muscle TG content in  $T_3$  treated rats was slightly greater than in the untreated animals ( $p<0.01$ ). In conclusion, the data confirmed the relationship between FFA availability and their utilization by skeletal muscle *in vitro*. They also indicate that  $T_3$  stimulates skeletal muscle FA uptake and oxidation.

2. The effect of chronic hindlimb suspension (head-down tilt) on lipoprotein lipase activity (LPL) in the rat myocardium was studied in cooperation with Laboratory of Physiology, Faculty of Medicine Grange Blanche, University of Lyon (France). The data showed that after 1 day of suspension the heparin releasable (active) form of LPL in the myocardium increased by approx. 60% in comparison with control animals ( $p<0.01$ ). This was accompanied by a decrease in triacylglycerol (TG) content. After 35 days of suspension LPL activity was still enhanced (by approx. 40%,  $p<0.01$ ), and TG content in the cardiac muscle showed further decrease. The data emphasize increased utilization of plasma and intramuscular lipids by chronically overloaded heart.

3. Studies on the effect of diets enriched with fat of different origin on lipid metabolism in the rat myocardium were continued. Male Wistar rats were fed for 4-weeks after weanling diets containing 30% of fat in the form of butter and lard, cod-liver oil, or sunflower oil. Activity of the heparin-releasable form of LPL in myocardium was reduced in rats fed all kinds of fat enriched diets in comparison with control animals fed standard diet. Activity of residual (intracellular) form of LPL remained unchanged in rats on diet with sunflower oil, while in the remaining groups it was decreased. The plasma and cardiac muscle TG

concentrations were increased in rats maintained on the fat enriched diets, whereas the total plasma cholesterol level was elevated only in the animals on butter/lard diet. The inverse correlation between activity of heparin releasable form of LPL and TG content in myocardium ( $r=0.51$ ,  $p<0.01$ ) supports the hypothesis that lipid content within the cell is at least one of the factor determining the muscle cell potential to hydrolyze the lipoprotein TG.

## **AN IMPORTANCE OF RELATIONSHIPS BETWEEN AGE, BODY MASS, AND PHYSICAL FITNESS IN MODIFYING METABOLIC EFFECT OF GLUCOSE IN SUBJECTS OF BOTH SEXES**

(Dr. Andrzej Ziemia)

Supported by the State Committee for Scientific Research: grant # 402199101

It has been postulated by some authors that decreased postprandial thermogenesis may be a factor promoting development of obesity. However, only few attempts were made to find a link between the magnitude of thermal effect of glucose (TEG) and body mass, or hormonal control of metabolism. In present study oxygen uptake ( $VO_2$ ) and  $CO_2$  production ( $VCO_2$ ), blood glucose (BG), plasma insulin (IRI), adrenaline (A) and noradrenaline (NA) concentrations were measured after ingestion of glucose (75 g) in 48 healthy men (aged 23-67 yrs) and in 57 healthy women (aged 21-57 yrs). Body mass index (BMI) in men ranged from 18.8 to 40.8 and in women from 18.9 to 40.1  $kg/m^2$ . TEG, as well as BG and IRI responses to glucose load were calculated as areas under the curves (auc) obtained from 2 h determinations following glucose ingestion. The ratio of  $BG_{auc}/IRI_{auc}$  was also calculated.

In men, significant correlations were found between TEG and BMI ( $r=-0.37$ ),  $IRI_{auc}$  ( $r=-0.65$ ),  $BG_{auc}/IRI_{auc}$  ratio ( $r=0.55$ ) and the maximal post-glucose NA level ( $r=0.79$ ). In women, significant relations were found between TEG and age ( $r=-0.30$ ), BMI ( $r=-0.31$ ),  $IRI_{auc}$  ( $r=-0.33$ ),  $BG_{auc}/IRI_{auc}$  ratio ( $r=0.43$ ) and the maximal post-glucose plasma NA level ( $r=0.65$ ). In conclusion, the present results demonstrated that the thermogenic effect of oral glucose in subjects of both sexes depends, to a large extent, on insulin response to glucose load and the glucose-induced activation of the sympathetic nervous system. The data suggest that overweight and aging leading to decreased insulin sensitivity may reduce thermogenic effect of food, which in turn promotes further body mass gain.

# PHYSIOLOGICAL REACTIONS TO EXERCISE AND HEAT LOADS IN WOMEN IN RELATION TO THE PHASE OF THE MENSTRUAL CYCLE

(Assoc. Prof. Ryszard Grucza)

Supported by the State Committee for Scientific Research: grant # 402169101

Thermoregulatory responses to exercise in relation to the phase of the menstrual cycle were studied in 10 women taking (P) and in 10 women not taking oral contraceptives (NP). Each subject was tested for maximum aerobic capacity ( $VO_2\max$ ) and for 50%  $VO_2\max$  exercise in follicular (F) and luteal (L) phase of the menstrual cycle. Since the oral contraceptives could prevent ovulation quasi-follicular (qF) and a quasi-luteal (qL) phases of the menstrual cycle were assumed for P subjects. Exercise was performed on a bicycle ergometer at ambient temperature of 24°C and relative air humidity of 50%. Rectal ( $T_{re}$ ), mean skin ( $T_{sk}$ ), mean body ( $T_b$ ) temperatures and heart rate (HR) were measured. Sweat rate was estimated by a continuous measurements of air humidity above the chest ( $Ph_{chest}$ ) by a ventilated capsule. Gain for sweating was calculated as a ratio of increase in  $Ph_{chest}$  to the appropriate increase in  $T_{re}$  for whole period of sweating (G) and for unsteady-state ( $G_u$ ) separately.  $VO_2\max$  did not differ significantly either between the groups of subjects or between the two phases of the menstrual cycle. In P, rectal temperature threshold for sweating ( $T_{re_{td}}$ ) was 37.85°C in qL and 37.60°C in qF ( $P < 0.01$ ) and corresponded to a significant difference in resting  $T_{re}$ . Rectal temperature,  $T_{sk}$ ,  $T_b$  and HR increased similarly during exercise in qF and qL. No menstrual phase related differences were observed in dynamics or in the gain of sweating.  $G_u$  was 13.5  $kPa \cdot ^\circ C^{-1}$  in qF and 13.0  $kPa \cdot ^\circ C^{-1}$  in qL (NS), while G attained respective values of 6.0 and 4.5  $kPa \cdot ^\circ C^{-1}$  (NS). In NP,  $T_{re_{td}}$  was greater in L than in F (37.70 vs 37.47°C,  $P < 0.02$ ) with significantly greater value of  $T_{re_{rest}}$ . Dynamics and gain for sweating were also greater in L than in F.  $G_u$  was 36.8 vs 16.6  $kPa \cdot ^\circ C^{-1}$  ( $P < 0.01$ ) while G was 6.4 vs 3.8  $kPa \cdot ^\circ C^{-1}$  ( $P < 0.05$ ), respectively.  $T_{re}$ ,  $T_{sk}$ ,  $T_b$  and HR increased significantly greater in phase F than in L. It is concluded that in women performing moderate exercise there is a greater temperature threshold and the gain for sweating in phase L than in F. Application of oral contraceptives reduces the differences in the gain for sweating making thermoregulatory responses to exercise more uniform.

## **MECHANISM CONTROLLING INTRAMUSCULAR LIPID METABOLISM**

(Professor Hanna Kaciuba-Uściłko)

Supported by the Polish-American Maria Skłodowska-Curie Joint Fund II:  
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In cooperation with the Department of Physiology, SUNY Health Science Center, University of Syracuse (U.S.A.) triacylglycerol (TG) synthesis was evaluated in different skeletal muscle fiber types of the rat. The incorporation of plasma-derived fatty acids (FA) into the intramuscular TG pool of red (FTR) and white (FTW) gastrocnemius and soleus muscle fiber sections was assessed using an isolated hind quarter preparation of adult rats (n=41). A high flow rate (50 ml/min) of 95%/5% O<sub>2</sub>/CO<sub>2</sub> - Krebs-Henseleit buffer (pH 7.4, 5mM glucose, 100 μU insulin, normal amino acids, 5 g/100 ml albumin) delivered 0.25 to 2.0 mM [9,10-<sup>3</sup>H]palmitate (0.1 μCi/ml). Uptake of FA into the TG pool was fairly linear with time within the range of FA concentration from 0.5 to 2.0 mM. Synthesis rate of TG was the least in low-oxidative FTW and high in the high-oxidative red sections (FTR and soleus). Since intramuscular TG content remained constant, TG turnover increased as a function of plasma FA concentration. In conclusion, the data showed that intramuscular TG metabolism markedly differs among fiber types.

## **FUNCTIONAL EVALUATION OF THE RAT KIDNEY CHRONICALLY EXPLANTED UNDER THE FLANK SKIN**

(Prof. Janusz Sadowski)

In order to create experimental conditions which would enable an easy access to the kidney without inducing deep anesthesia and performing extensive acute surgery, the left kidney of Wistar rats was chronically displaced ("explanted") out of the abdominal cavity and placed under the flank skin. In addition, the animals were surgically prepared for urine collection, separately from each kidney. Three or four days later clearance studies were performed under light plane ethanol + chloralose anesthesia. The explanted kidney was characterized by the glomerular filtration rate (GFR) similar to that of the contralateral organ. There were no significant differences between the two kidneys regarding their ability to produce concentrated or diluted urine or to generate the electrolyte or total solute hypertonicity of the medulla. The only detected difference between the two kidneys was that clearance of p-aminohippurate, a measure of total renal blood flow, was on the average 14% lower on the operate side

$p < 0.01$ ). Sodium and total osmole excretion were slightly higher in the intact kidney. The data indicate that the rats with the explanted kidney can be used in experimental studies requiring placement of electrodes, probes or other sensors in the kidney, especially those in which surgical level of anesthesia and the trauma of acute surgery should be avoided. An additional advantage of the model is a possibility of collecting urine from the exposed kidney, which enables simultaneous separate measurement of renal clearances in the explanted and intact kidney.

**THE CORTICO-MEDULLARY ELECTROLYTE GRADIENT  
IN RAT RENAL INTERSTITIUM:  
EFFECTS OF HORMONES CONTROLLING SALT TRANSPORT  
IN HENLE'S LOOP**  
(Prof. Janusz Sadowski)

Supported by the State Committee for Scientific Research: grant # 413129101

In 1993 the investigations were carried out to answer a question whether prostaglandins modulate the cortico-papillary electrolyte gradient in rat renal interstitium.

Modulation of the cortico-papillary electrolyte gradient by prostaglandins (PG) was studied in the kidney of anaesthetized rats. The intrarenal PG activity was altered by PG synthesis blockade with indomethacin (Ind) or meclophenamate (Me) and by intrarenal infusion of PGE<sub>2</sub>. The interstitial electrolyte concentration in the medulla was recorded in the kidney *in situ* as tissue electrical admittance (reciprocal impedance); the total renal blood flow (RBF), inulin clearance and renal excretion were measured simultaneously. Ind and Me (15 mg/kg h) increased tissue admittance by 15-20% in the inner and by 12-15% in the outer medulla whereas PGE<sub>2</sub> (300 ng/kg min) decreased admittance by 14 and 8%, respectively. Analysis of tissue slices confirmed an increase in electrolyte concentration after PG blockade; tissue non-electrolytes did not change. RBF and inulin clearance were not affected by intrarenal PG activity changes. Urine concentration increased after PG blockade and did not change during PGE<sub>2</sub> infusion which caused delayed changes in the concentration and excretion.

The data indicate that PG can modulate *in vivo* the intrarenal electrolyte gradient. A joint analysis of the dynamics of gradient and renal function changes suggests that PG modify the medullary ionic hypertonicity by affecting NaCl transport in the ascending limb of Henle's loop.

**CONSTRUCTION AND SOFTWARE DESIGN  
OF A DEVICE ENABLING 24-HOUR MONITORING  
OF HAEMODYNAMIC HEART ACTIVITY  
USING IMPEDANCE CARDIOGRAPHY - REOMONITOR**  
(Dr. Gerard Cybulski)

Supported by the State Committee for Scientific Research: grant # 8S50601305

The aim of the 3-year project (realization began in September 1993) is to construct the prototype of a device enabling continuous 24-monitoring of haemodynamic heart activity basing on impedance cardiography signals (ICG) simultaneously with ECG. In assumption the device would be characterized by the following features:

- simultaneous recording of ECG and ICG signals on PCMCIA v.2.01. FLASH CARDS,
- initial analysis of data performed in real time,
- report analysis prepared on PC and possibility to do partial report on palm-top portable computer.

By the end of 1993 the electrical scheme of the device was elaborated and partly tested. Besides, some important procedures of the program for data analysis were prepared. According to the plans, the model of the device should be build by the end of July 1994.



## **OUTPATIENT CARDIAC UNIT FOR DIAGNOSIS AND THERAPY**

5 Pawińskiego Str., 02-106 Warsaw

Telephone: 658 46 43

**Head: Dr Ewa Wójcik-Ziółkowska**

### **EVALUATION OF CORONARY BLOOD FLOW RESERVE IN PERIMENOPAUSAL WOMEN**

(Dr Ewa Wójcik-Ziółkowska)

In women the coronary heart disease (CHD) mortality increased in Poland in the last decade. Moreover, fatality of myocardial infarction (MI) in women is greater than in men. Therefore, this study was designed to establish a battery of noninvasive tests for early diagnosis of CHD in women with the special emphasis to the coronary perfusion assessment.

The total number of women examined was 65. They were divided into 4 groups according to the clinical criteria: group 1 - patients after MI (n=11, age  $54.5 \pm 6.9$  yrs), group 2 - patients with typical angina (n=21, age  $52.4 \pm 6.6$  yrs), group 3 - patients with angina-like chest pain (n=20, age  $49.5 \pm 7.4$  yrs), group 4 - subjects without CHD symptoms (n=13, age  $49.2 \pm 7.0$  yrs). In all women the following examinations were made: resting ECG, 24h ECG using Holter method, resting echocardiography, dynamic exercise test, scintigraphic perfusion by single photon emission tomography with Tc 99 MIBI-SPECT.

In group 1 all patients showed impairment in myocardial perfusion in ECG (resting, exercise and 24h). In 90% of the patients impairment in myocardial contractility was proved by echocardiography and that in coronary perfusion by the radioisotope examination.

In group 2 (women with angina) exercise test was positive in 90% cases and the Holter recording revealed myocardial ischemia in 76% of the patients, whilst impairment of myocardial perfusion was proved using radioisotope method in 38% of women.

In group 3 positive results of exercise test were obtained in 85%, and those of Holter method in 60%, but the impaired coronary perfusion was detected only in 20% of the patients.

In group 4 (controls) no changes in resting ECG were found and normal coronary perfusion was assessed by SPECT. However, in 46% subjects from this group symptoms of myocardial ischemia were detected in 24h ECG and in 61% in exercise ECG.

The results obtained in this study showed that the applied set of noninvasive methods of myocardial perfusion evaluation differentiates the groups of patients

assigned being on clinical criteria. High percentage of positive results of exercise tests in all groups suggests the possibility of many false-positive outcomes of the test and decreases its reliability in diagnosis of coronary perfusion impairment in women. Among the tests used in this study the radioisotope (SPECT) examination was proved to be of the highest diagnostic value. However, even using this method we were unable to clearly differentiate the groups of patients with typical angina and atypical chest pain. It seems likely that using adenosine or Dipyridamol would increase the specificity of the isotope examination of cardiac perfusion disturbances in women.

### **ASSESSMENT OF CORONARY BLOOD FLOW RESERVE IN SILENT MYOCARDIAL ISCHEMIA BY NONINVASIVE METHODS**

(Dr Ewa Wójcik-Ziołkowska)

Supported by the State Committee for Scientific Research: grant # 4S40202204

The investigation started in July 1993 in cooperation with the Laboratory of Nuclear Medicine of the National Institute of Cardiology. The group of 57 male patients participating in this study included 25 patients without angina after myocardial infarction (MI) and 32 patients with silent CHD without MI. The diagnosis of CHD in the latter group was established on the basis of positive results of exercise tests, occurrence of ischemic changes in 24h ECG and impairment of myocardial contractility in post-exercise echocardiography. In all patients coronary perfusion was assessed using radioisotope examination (SPECT Tc 99 MIBI). In a group of patients without MI, in 42% cases transient impairment of coronary perfusion was found during exercise whilst in 58% coronary blood flow was normal. Five patients from this group underwent coronary angiography and were qualified for surgical revascularization. In 73% of the post-MI patients stable or transient disturbances in coronary perfusion were detected by the isotope examination and only in 27% the results were negative. The study will be continued next year introducing pharmacological agents, such as adenosine to improve the diagnostic value of the SPECT test.

## **CARDIOVASCULAR LABORATORY**

3 Dworkowa Str., 00-784 Warsaw

Telephone: 49 73 55

**Head: Prof. Krystyna Cedro-Ceremużyńska**

### **INFLUENCE OF ANTIOXIDANT VITAMINS UPON THE INDICES OF TISSUE LIPID PEROXIDATION AND OXYGEN FREE RADICAL GENERATION BY LEUKOCYTES IN ACUTE MYOCARDIAL INFARCTION**

(Prof. Krystyna Cedro-Ceremużyńska)

Damaging effect of oxygen free radicals (OFR) upon cellular membranes and their influence on the extension of myocardial ischemic injury has been documented in experimental studies. Enhanced free radical generation is a likely consequence of hormonal and metabolic responses to acute myocardial infarction (AMI). Neutrophils, activated in response to myocardial injury are an important source of OFR. Enhanced free radical generation may lead to exhaustion of antioxidant defense mechanisms. Clinical studies suggest that neutrophil activation during AMI aggravates tissue injury.

In view of the above, an intervention capable to reduce neutrophil OFR production and to prevent depletion of antioxidants seems to be fully justified. We have previously shown in healthy subjects that ingestion of antioxidant vitamins C and E decreases OFR production by isolated neutrophils assayed by chemiluminescence (CL).

In the present study, vitamins C and E were administered to the patients with AMI randomized to receive either conventional treatment plus vitamin C and E in a dose of 600 mg/day for 14 days (VIT group, n = 23) or conventional treatment only (control group, n = 22). The aim was 1) to investigate the effect of vitamin supplementation upon leukocyte OFR generation and serum lipid peroxides, 2) to evaluate serum levels of vitamins C and E in the course of AMI. All measurements were performed on the 1st and the 14th day.

The results obtained have shown that vitamin supplementation decreases significantly leukocyte CL, suggesting decreased generation of OFR and/or enhanced scavenging capacity of neutrophils in VIT patients. An increase in serum lipid peroxides, known to occur in the course of AMI and confirmed in our control patients, was prevented in VIT suggesting attenuation of peroxidative injury in cells and tissues.

As expected, basal serum ascorbic acid and tocopherol levels were within the range associated with an increased risk of ischemic heart disease. Within 2

weeks from the onset of AMI serum ascorbic acid increased significantly even in the patients without vitamin supplementation suggesting that its previous fall was associated, at least partly, with the acute phase of the disease.

We conclude from this preliminary study that supplementation with antioxidant vitamins is advisable in AMI. Well controlled study on a larger number of patients is needed to evaluate clinical benefits of antioxidant intervention.

**DEPARTMENT OF SURGICAL RESEARCH  
AND TRANSPLANTATION**

5 Chałubińskiego Str., 02-004 Warsaw  
Telephone: 21 49 23

**Head: Prof. Waldemar L. Olszewski**

**NEURO-ENDOCRINE REGULATION OF LYMPHOCYTE  
EXTRAVASATION TO SKIN AND LYMPHOID TISSUE**

(Prof. Waldemar L. Olszewski)

To improve the local immune reactions in skin, methods influencing the process of protein and immune cells extravasation were examined. A lumbar sympathectomy was performed in dogs and, after 7 days, efferent lymph vessels of popliteal lymph nodes were cannulated and lymph was collected for 6 consecutive hours. In the desympathectomized lymph node, blood flow rate was higher than in controls. Thus, denervation enhanced blood flow, but did not affect capillary protein filtration and lymph formation. Interestingly, it diminished leukocyte extravasation in nodes. These results point to an evident role of sympathetic innervation in modulation of immune cell extravasation.

An analysis of immune cells, sequestered to human peritoneal cavity was performed. Cells were obtained from the intraoperative wash-out of peritoneal cavity of patients undergoing elective cholecystectomy. It was found that most of helper/inducer CD4<sup>+</sup> (92.4%) and suppressor/cytotoxic CD8<sup>+</sup> (73.1%) peritoneal cavity lymphocytes belonged to memory cell population (CD45RO<sup>+</sup>). Cells from peritoneal cavity displayed more intensive fluorescence of CD2, CD11a and CD11c molecules than peripheral blood lymphocytes. These observations and higher numbers of CD54<sup>+</sup> (ICAM1<sup>+</sup>) cells in peritoneal cavity suggest a constant antigenic stimulation occurring in peritoneal cavity.

**EXPERIMENTAL VASCULARIZED BONE MARROW  
TRANSPLANTATION**

(Assoc. Prof. Barbara Łukomska)

Supported by the State Committee for Scientific Research: grant # 446229203

Previous studies revealed that bone marrow transplanted in a vascularized graft of hind-limb to irradiated rats repopulates recipients bone marrow cavities

within 10 days. In the present study, an evaluation of the function of repopulated cells and the influence of humoral factors from sera and bone marrow cell culture supernatants on bone marrow cell proliferation was performed. It was observed, that ten days after hind limb transplantation the responsiveness of bone marrow cells to mitogens (PHA, ConA, PWM) was restored to control values. Addition of sera from hind-limb graft recipients to third party bone marrow cell cultures, supplemented with PHA, produced a slight but evident stimulatory effect. Supplementing bone marrow cells with 24 hr-bone marrow culture supernatants (50%) produced a 3-fold increase in 3HTdR incorporation in the presence of PHA. These findings point to an active hemopoietic process in recipients' bone marrow cavities following vascularized bone marrow grafting. The studies on the nature of humoral factors produced in the vascularized bone marrow transplant are in progress.

### **MECHANISM OF BONE MARROW CAVITY REPOPULATION AFTER VASCULARIZED BONE MARROW TRANSPLANTATION**

(Prof. Waldemar L. Olszewski)

Bone marrow cells, transplanted in a vascularized graft of hind limb, repopulate recipients bone marrow. The transplanted cells display a high predilection to lodge in this compartment. Although the numbers of bone marrow cells transplanted in a vascularized graft was much lower than those given intravenously, the repopulation process was completed already after 10 days. These results suggest that only few hemopoietic precursor cells, transplanted together with stromal cells in their spatial relationship, were necessary for rapid repopulation of bone marrow cavities in irradiated syngeneic recipients. Interestingly, an enhanced traffic of bone marrow cells from the transplanted hind limb to bone marrow cavities and lymphoid organs of irradiated recipients, compared with non-irradiated, was noted. This was most likely the effect of stimulation of hemopoietic and stromal cells by irradiation.

### **HUMORAL REGULATION OF THE IMMUNE RESPONSIVENESS TO TRAUMA**

(Prof. Waldemar L. Olszewski)

Mechanism of regulation of the immune responsiveness was studied in rats after transfusion of syngeneic venous blood to normovolemic recipients. No significant changes were found in WBC, while a decrease was noted in spleen cell

numbers/g tissue and bone marrow (BM) cell numbers/femur. Analysis of BM revealed a significant drop in the percentage of cells of erythroid lineage and an increase of cells of myeloid and lymphoid lineages on days 3, 7 and 14 after blood transfusions. Elevated numbers of OX7<sup>+</sup> stem cells in BM were noted. Blood, spleen and BM lymphocytes demonstrated a higher autotransformation rate and an increased responsiveness to mitogens. The results suggest that syngeneic blood transfusions cause a major adoptive changes in the bone marrow and affect immune responsiveness of blood spleen and BM lymphocytes.

An influence of human skin lymph on proliferation of tumor cells of dermal origin was investigated. It was found that human skin lymph inhibited growth of HT3-epitheloid carcinoma cells, HACAT-transformed keratinocyte cells, and HELA-epitheloid carcinoma cells, the latter also when tested in a serum-free system. No effect on A431-epidermoid carcinoma cell proliferation was noted. IL1, IL6 and IL8 did not influence growth of the examined cell lines, within concentrations seen in skin lymph. The results suggest that the effect of human skin lymph on proliferation of examined cell lines of dermal origin was mediated by other lymph growth factors than IL1, IL6 and IL8 cytokines.

## **MECHANISM OF CELLULAR REJECTION IN SKIN, LIVER AND VESSELS**

(Prof. Waldemar L. Olszewski)

The humoral mechanism of elimination of allogeneic lymphocytes, injected intravenously to rats treated with donor-specific transfusions, was studied. Using immunoblotting, it was found that antibodies present in sera of such rats bound to other antigens than MCHC class I and II. Elimination of allogeneic cells was not dependent on the presence of these molecules on cells used for immunization of rats. Administration of CsA, together with donor-specific transfusion, significantly decreased elimination of injected lymphocytes, suggesting an inhibition of humoral response by CsA.

Distribution of CsA-treated lymphocytes infused into untreated syngeneic recipients with syngeneic or allogeneic heart transplants, was examined. An evident decrease in traffic of CyA-treated lymphocytes to allogeneic grafts was noted, although accumulation still remained higher than in syngeneic grafts. Moreover, CyA caused an enhanced extravasation of lymphocytes to lymphoid organs.

Distribution of cellular alloantigen and alloserum given to recipients of allo-transplanted hearts and skin was investigated. It was found, that donor lymphocyte-alloglobulin complexes accumulate in spleen and liver. Alloglobulin against donor transplantation antigens did not accumulate in the heart and skin grafts, although the graft survival time was prolonged. These findings indicate

that antigen-alloserum acts upon the effector system of the recipient and not on the graft itself.

Further studies on the effect of Kupffer cells and opsonisation on the process of elimination of allogeneic cells in rat liver were carried out. To block the function of Kupffer cells Gadolinium chloride was injected i.v. Preliminary results suggest that blocking of hepatic macrophages does not affect trapping of allogeneic lymphocytes in liver.

## **IMMUNOREGULATORY ROLE OF LIVER**

(Prof. Waldemar L. Olszewski)

Supported by the State Committee for Scientific Research: grant # 404289101

The aim of this project was to examine adhesion molecules on liver sinusoidal cells and lymphocytes marginating in the sinusoids and to evaluate their role in the adherence process. A test for lymphocyte adherence to frozen liver sections was used. Population of mononuclear cells eluted from liver revealed more CD56<sup>+</sup>, CD8<sup>+</sup> and CD54<sup>+</sup> (ICAM1<sup>+</sup>) cells, and bright CD11<sup>+</sup> and CD18<sup>+</sup> cells. These populations displayed high adhering properties. On liver sinusoidal cells CD54 (ICAM1), ICAM2, ICAM3 (mostly Kupffer cells), VCAM1 and LFA3 (CD58) molecules were identified, but no ELAM1 was found. These observations document a high expression of adhesion molecules, both on blood cells marginating in liver, and sinusoidal lining cells. Basing on these findings, it is likely that pharmacological intervention decreasing or increasing expression of adhesion molecules may help to regulate immune processes in liver.

## **IMMUNE RESPONSIVENESS TO TRAUMA IN MAN - MOBILIZATION OF MONONUCLEAR CELLS AND CYTOKINE RELEASE**

(Dr Irena Grzelak)

Supported by the State Committee for Scientific Research: grant # 403699101

In patients undergoing selective cholecystectomy, peripheral blood mononuclear cell population was characterized using monoclonal antibodies. An increase of the percentage of CD1<sup>+</sup> (dendritic cells with thymocyte antigen) was observed on days 1 and 3 after operation. A significant rise of CD45R0<sup>+</sup> (memory cells), CD13<sup>+</sup>, CD14<sup>+</sup>, CD33<sup>+</sup> (cells of myeloid lineage), CD34<sup>+</sup> i CD38<sup>+</sup>



(stem cells, pre-B, myeloid), TdT<sup>+</sup> (pro-B, pro-T clls, thymocytes) was noted between days 1-7. There was also an elevation of percentage of HLA-DR<sup>+</sup> cells and a decrease of CD3<sup>+</sup> (T cells), CD4<sup>+</sup> (helper/inducer) and CD8<sup>+</sup> (suppressor/cytotoxic). No changes in frequency of CD22<sup>+</sup> (B cells) or CD11/CD18<sup>+</sup> (adhesion molecules) cells were recorded. Thus, moderate trauma evokes an influx of stem cells, immature myeloid lineage and pre-B cells. Mobilization of immature immune cell populations to blood circulation suggests an inadequacy of peripheral reserves of phagocytic and antigen presenting cells for scavenging and repair of the damage tissues.

## **SKIN GRAFTS - CELLS INITIATING REJECTION**

(Dr. Hanna Galkowska)

Supported by the State Committee for Scientific Research: grant # 411299101

In the study on the mechanism of spontaneous lymph dendritic cell-lymphocyte cluster formation, the involvement of adherence molecules - CD58 (LFA3) and CD54 (ICAM1) was observed. The monoclonal antibodies against these molecules inhibited cluster formation. Anti-fibronectin antibodies reduced lymph cell clustering. Other antibodies, such as anti-CD18, CD49d,e,f, had rather proaggregatory effect on cluster formation. A reduced cell clustering in the presence of lymph was observed *in vitro*. After supplementation with rIL1 $\beta$ , the percentage of cell clusters was significantly increased. IL1 $\alpha$  had no effect on this process. This last finding may suggest the involvement of IL1 receptor inhibitor in lymph cell clustering *in vivo*.

## **DEPARTMENT OF ENDOCRINOLOGY**

1a Banacha Str., 02-097 Warsaw

Telephone: 659 75 62

**Head: Prof. Janusz Nauman**

### **NEW DRUGS FOR NEW METHODS OF NEUROLOGICAL TREATMENT**

(Assoc. Prof. Andrzej W. Lipkowski)

Traditional search of drugs for neurological disfunction treatment is focusing 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 administredrugs 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) - 17495 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 1993 was prepared. It contains 513 citations.

## **MEDIPAN**

1/13 Flory Str., 02-586 Warsaw

Telephone: 48 22 62

Telephone/fax: 49 30 79

### **Head: Andrzej Lasek**

MEDIPAN is a manufacturer of a special equipment needed by various medical service units. In the past few years it has concentrated on construction and production of microprocessor infusion drip pumps and microprocessor infusion syringe pumps. In 1993 models manufactured in the previous years were considerably modified to comply with modern worldwide requirements.

The new 604 model of the microprocessor infusion drip pump can be easily and quickly operated, since setting of the required parameters is very simple. This model is provided with an alarm system (both acoustic and luminous) which ensures the patients' safety. High technical level of performance, and reliability of the pump make the device quality comparable to that produced by western companies.

The new 612 model of the microprocessor infusion syringe pump has been enriched with several fairly new technical possibilities. Due to them the pump calculates automatically the speed of drug dosage after introducing to the program such informations as e.g. the required total drug dose, patient's body mass etc. A possibility of using syringes of 6 different volumes (1, 5, 10, 20, 50 and 60 ml) widens also an opportunity of the programming system. Besides, the size as well as weight of the device have been markedly diminished. All the above characteristics make this pump easy and quick to use by the medical staff (doctors and nurses). Thus, the 612 model is considered as fairly innovatory, being comparable with the devices produced by a well known U.S. company BAXTER.

The new 611 model of the microprocessor infusion. Syringe pump has been improved in comparison with the previous type. Three kinds of syringes (10, 20 and 50 ml) can be used in this model, and it is possible to administer quite large amounts of fluid (or drugs) if necessary (bolus injection).

# INTERNATIONAL COOPERATION

## VISITING SCIENTISTS

### Department of Neurophysiology

H. Gautier                      Atelier de Physiologie Respiratoire,  
Faculte de Medicine St. Antoine,  
Universite Pierre et Marie Curie, Paris, France

### Department of Neuropathology

A.S. Lossinsky                Institute for Basic Research in Developmental  
Disability, New York, USA

### Department of Neurosurgery

W. Amcheslavsky,  
A. Potapov                    Institute of Neurosurgery M. Burdenko,  
Moscow, Russia

N. Beritashvili                Institute of Physiologie, Georgian Academy of  
Sciences, Tbilisi, Georgia

### Neuromuscular Unit

A. Vincent                    Institute of Molecular Medicine,  
Oxford, Great Britain

### Department of Applied Physiology

O. Hanninen                 Department of Physiologie, University of Kuopio,  
Finland

B. Kapitaniak                Laboratory of Work Physiology, Medical Faculty  
Pitie-Salpetriere, CNRS, Paris, France

A.J. Sargeant                      Department of Muscle and Exercise Physiology,  
Faculty of Human Movement Sciences,  
Vrije Universiteit and Universiteit van Amsterdam,  
Amsterdam, The Netherlands

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

### **Department of Surgical Research and Transplantation**

Ch. Balabaud                      Laboratoire des Interactions Cellulaires, Université  
de Bordeaux II, Centre National de Recherche  
Scientific, Bordeaux, France

C. Hammer                        Institut für Chirurgische Forschung, Klinikum  
Grosshadern, Ludwig-Maximilians-Universität  
München, Munich, Germany

S. Jamal                            Madras Medical College, Indian National Science  
Academy, Madras and Thanjavur, India

N. Miller                          British Heart Foundation, St. Bartholomew's Medical  
School (Hospital), London, Great Britain

K. Ogawa                          Department of Surgery, Tokyo Women's Medical  
College, Tokyo, Japan

Y. Idezuki  
T. Kajiwara                        II Department of Surgery,  
University of Tokyo, Japan

### **Department of Endocrinology**

D.B. Carr,  
S.K. Szyfelbein                    Massachusetts General Hospital, Harvard Medical  
School, Boston, USA

M. Manning                        Department of Biochemistry, Medical College of  
Ohio, Toledo, USA

T. Suzuki,  
J. Kamei                            Department of Pharmacology, Hoshi University,  
Tokyo, Japan

H. Nagase                         Basic Research Laboratories, Toray Ind., Japan

## VISITS ABROAD

### Department of Neurophysiology

- K. Budzińska Instituto di Fisiologia Umana I, Universita di Milano, Milan, Italy
- M. Pokorski Medical Physiology, Sports/Cybernetics, University of Copenhagen, Denmark
- Pulmonary Function Laboratory of the First Department of Medicine, Tokyo Women's Medical College, Japan (long term visit)
- J. Romaniuk Pulmonary Division, Department of Medicine, Metro Health Medical Center, Cleveland, USA (long term visit)

### Department of Neurochemistry

- W. Gordon-Krajcer Institute for Basic Research in Developmental Disabilities, Staten Island, New York, USA (long term visit)
- M. Łałowski Department of Pathology, New York University Medical Center, New York, USA (long term visit)
- J. Łazarewicz Faculty of Medicine, Institute of Neurobiology, University of Göteborg, Sweden
- Research Unit for the Neurochemistry of Mental Diseases, South African Medical Research Council, Department of Chemical Pathology, University of Stellenbosch, Tygerberg, South Africa
- Neurology Unit, Johannesburg Hospital, Johannesburg, South Africa
- M. Puka Department of Histology, Institute of Neurobiology, University of Göteborg, Sweden (long term visit)

- J. Strosznajder      Institute of Biochemistry, University of Perugia, Italy  
 Institute of Biochemistry, University of Catania, Italy  
 Cancer Research Centre, Heidelberg, Germany  
 European Laboratory of Molecular Biology,  
 Heidelberg, Germany
- J. Waškiewicz      Department of Chemistry and Biochemistry,  
 The University of Oklahoma, Norman, USA  
 (long term visit)
- H. Wikieł      Roswell Park Cancer Institute, Buffalo, USA  
 (long term visit)
- B. Zabłocka      Rudolf Magnus Institute, Utrecht University,  
 Utrecht, The Netherlands (long term visit)
- T. Zalewska      Department of Animal Sciences, University of  
 Arizona, Tucson, USA (long term visit)

### **Department of Neuropathology**

- H. Borkowska      Department of Biomedical Sciences, University of  
 Tampere, Finland (long term visit)
- L. Faff-Michalak      Abteilung für Zelluläre Neurophysiologie,  
 Carl-Ludwig-Institut für Physiologie, Universität  
 Leipzig, Germany (long term visit)  
 Institute for Neurobiology, University of Heidelberg,  
 Germany
- E. Kida,  
 R. Pluta      Institute for Basic Research in Developmental  
 Disabilities, Staten Island, New York, USA  
 (long term visit)
- S. Krajewski      La Jolla Cancer Research Foundation, La Jolla, USA  
 (long term visit)
- M. Ratajczak      Department of Pathology and Laboratory Medicine,  
 The School of Medicine, University of Pennsylvania,  
 Philadelphia, USA (long term visit)



### **Laboratory of Developmental Neuropathology**

D. Maślińska                      Institut for Basic Research in Developmental  
Disabilities, Staten Island, New York, USA  
(long term visit)

### **Laboratory of Ultrastructure of the Nervous System**

B. Gajkowska                      Laboratoire de Biologie et Ultrastructure du Noyau  
de L'Institut de Recherches Scientifiques sur le  
Cancer, Villejuif, France

### **Neuromuscular Unit**

J. Borkowska,  
A. Karwańska                      Institute of Genetic, University of Bonn, Germany

I. Hausmanowa-  
-Petrusiewicz                      Department of Neurology, Columbia University,  
New York, USA

### **Department of Applied Physiology**

Z. Brzezińska                      Department of Rehabilitation Medicine, University of  
Göteborg, Sweden (long term visit)

L. Budohoski                      Department of Physiology, Health Science Center,  
State University of New York, Suracuse, USA  
(long term visit)

G. Cybulski                      Centre for Biological and Medical Systems, Imperial  
College of Science, Technology and Medicine,  
London, Great Britain (long term visit)

L. Dobrowolski                      Physiologisches Institut der Ruprecht-Karls-  
Universität Heidelberg, Germany  
(long term visit)

R. Grucza,  
B. Kruk                      Laboratory of Physiology, University of Kuopio,  
Finland

- H. Kaciuba-Uściłko,  
K. Nazar  
Institute of General and Experimental Pathology,  
University of Graz, Austria
- Department of Physiology, Health Science Centre,  
State University of New York, Syracuse, USA
- Department of Physiology, NASA, Moffett Field,  
USA
- H. Kaciuba-Uściłko  
Division of Pharmacology, University of Aberdeen  
Marischal College, Aberdeen, Scotland
- Department of Environmental and Occupational  
Medicine, University Medical School, Aberdeen,  
Scotland
- Department of Biochemistry, University of Oxford,  
Cellular Nutritional Research Group, Oxford,  
Great Britain
- 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
- Department of Pediatrics, Karolinska Institutet,  
Stockholm, Sweden
- R. Grucza,  
A. Ziemba  
Laboratory of Work Physiology, Medical Faculty  
Pitié-Salpêtrière, Pierre and Marie Curie University,  
CNRS, Paris, France
- J. Langfort  
Department of Physiology, URA D 1341 CNRS,  
Lyon, France
- E. Żernicka  
Laboratoire de Physiologie, Faculté de Médecine  
Grange Blanche, Université Claude Bernard (Lyon I),  
France

## Department of Surgical Research and Transplantation

- |   |  |
|---|--|
| W.L. Olszewski,<br>B. Łukomska,<br>U. Kubicka,<br>M. Zalewska | Indian National Science Academy, New Delhi,<br>Stanley Medical School, Madras,<br>VCRC, Pondicherry,<br>Medical College, Allepey, Kerala,<br>India |
| B. Łukomska   | Laboratoire des Interactions Cellulaires,<br>Universite Bordeaux II, CNRS, France  |
| W.L. Olszewski,<br>I. Grzelak                                 | Norwegian Radiumhospital,<br>Oslo, Norway  |
| W.L. Olszewski  | The Medical College of Saint Bartholomew's<br>Hospital, University of London, Great Britain  |
| W.L. Olszewski  | Ludwig-Maximilianas-Uniwersität München<br>Klinikum Grosshadern, München,<br><br>Universität Kassel, Humanbiologie, Germany                        |

## Department of Endocrinology

- |                               |   |
|-------------------------------|---|
| J. Kiljański                  | Allegheny-Singer Research Institute,<br>Pittsburgh, USA (long term visit)   |
| A.W. Lipkowski                | Massachusetts General Hospital,<br>Harvard Medical School, Boston, USA  |
| A.W. Lipkowski,<br>A. Misicka | Department of Chemistry, University of Arizona,<br>Tucson, USA  |
| I. Maszczyńska                | Department of Pharmacology, University of Arizona,<br>Tucson, USA   |
| R. Gniadecki                  | Department of Dermatological Research,<br>Leo Pharmaceutical Products Ltd. A/S,<br>University of Copenhagen, Ballerup, Denmark<br>(long term visit) |

## PARTICIPATION IN INTERNATIONAL MEETINGS

Central/Eastern Europe/US Regional Symposium on Drug Abuse Research, Budapest - Szeged, Hungary, February 28 - March 3, 1993: *A.W. Lipkowski*

25. Jahrestagung der Deutschen Gesellschaft für Neurotraumatologie und Klinische Neuropsychologie, Munich, Germany, March 18-20, 1993: *J. Jurkiewicz*

Lymphologica'93, Berlin, Germany, April 2-4, 1993: *W.L. Olszewski*

5th Interscience World Conference on Inflammation, Antirheumatics, Analgesics, Immunomodulators, Geneva, Switzerland, April 25-28, 1993: *J. Strosznajder*

45th Annual Meeting of American Academy of Neurology, New York, USA, April 25 - May 1, 1993: *I. Hausmanowa-Petrusewicz*

X European Meeting of Alzheimer Associations, Strasbourg, France, April 27-28, 1993: *M. Barcikowska*

The Ninth International Symposium "Brain Edema 1993", Yokohama, Japan, May 16-19, 1993: *Z. Czernicki, M.J. Mossakowski, R. Pluta*

Fifth International Conference on Computerized and Quantitative EMG, Hamilton, Canada, May 19-21, 1993: *I. Hausmanowa-Petrusewicz*

International Symposium on Microcirculation Stasis in the Brain, Tokyo, Japan, May 20-21, 1993: *M.J. Mossakowski*

XXVIII Congress of the European Society for Surgical Research, Turku, Finland, May 23-26, 1993: *I. Grzelak, U. Kubicka, P. Bryła, A. Namysłowski, W.L. Olszewski*

17th Meeting of the European Group of Lymphology, Milan, Italy, May 28-29, 1993: *W.L. Olszewski*

Symposium EAMDA - SMA Meeting, Boston, USA, May 30 - June 2, 1993:  
*I. Hausmanowa-Petrusewicz*

International Symposium: The role of glia in CNS pathology and repair: basic and clinical aspects. Warsaw, Poland, June 14-15, 1993: *J. Albrecht, M. Barcikowska, K. Domańska-Janik, L. Faff-Michalak, E. Matyja, M.J. Mossakowski, R. Pluta, A. Taraszewska, I. Zelman*

XIII International Symposium "Puijo Diet, Exercise and Women's Health", Kuopio, Finland, June 22-23, 1993: *E. Wójcik Ziółkowska*

22nd Meeting of the Federation of European Biochemical Societies, Stockholm, Sweden, July 4-9, 1993: *K. Domańska-Janik*

Joint Annual Meeting of the British Association of Dermatologists and Canadian Dermatology Association, Oxford, England, July 6-10, 1993: *W.L. Olszewski*

International Narcotics Research Conference, Skövde, Sweden, July 10-15, 1993: *A.W. Lipkowski*

International Conference: Cellular, Biochemical and Molecular Aspects of Reperfusion Injury. The New York Academy of Sciences, USA, July 11-14, 1993: *J. Strosznajder*

Satellite Symposium of the 11th International Biophysics Congress "Heavy Metal Induced Alterations in Excitable Membranes", Tihany, Hungary, July 22-23, 1993: *L. Jabłońska*

XXXII Congress of the International Union of Pharmacological Sciences, Glasgow, Scotland, August 1-6, 1993: *K. Budzińska, M. Szereda-Przestaszewska*

Symposium on Temperature Regulation, IUPS Thermal Physiology Commission. Satellite Symposium: Thermal Physiology 1993, Aberdeen, Scotland, August 9-13, 1993: *R. Grucza*

"Dolichol and Related Lipids" A satellite Meeting to the XIIth International Symposium on Glycoconjugates, Zakopane, Poland, August 11-14, 1993: *P. Grieb*

6th International Symposium "New Frontiers in the Biochemistry and Biophysics on Diagnosis and Treatment of Stroke, Neurotrauma and other Neurological Diseases", Martin, Slovak Republic, August 16-19, 1993: *M. Chalimoniuk, K. Domańska-Janik*

14th Biennial Meeting of the International Society for Neurochemistry, Montpellier, France, August 22-27, 1993: *J. Albrecht, L. Faff-Michalak, J. Jabłońska, J. Łazarewicz, E. Salińska, J. Strosznajder, R. Strosznajder, J. Sypecka, B. Zabłocka*

13th International Congress of EEG and Clinical Neurophysiology, Vancouver, Canada, August 29 - September 4, 1993: *I. Hausmanowa-Petrusewicz (Honorary President)*

Elba International Neuroscience Program, 1993 Workshop and Summer School "Developmental Neurobiology: Neuroimmunology, Neurogenetics and Neurotoxicology from the Fetus to the Adolescent", Marina di Campo, Isola d'Elba, Italy, September 1-12, 1993: *R. Strosznajder*

International Congress on Recent Developments in Immunology. Potential Clinical Impact on Surgery and the Treatment of Infections, Würzburg, Germany, September 3-4, 1993: *U. Kubicka*

German-Polish Symposium on Neuropharmacology, Zakopane, Poland, September 5-9, 1993: *M. Barcikowska*

XVth World Congress of Neurology, Vancouver, Canada, September 5-10, 1993: *I. Hausmanowa-Petrusewicz*

16th Annual Meeting of the European Neuroscience Association, Madrid, Spain, September 18-21, 1993: *P. Grieb*

14th International Congress of Lymphology, Washington, USA, September 20-26, 1993: *W.L. Olszewski*

WHO/TDR/ISL Joint Program on Lymphatic Filariasis, Bethesda, USA, September 22, 1993: *W.L. Olszewski*

ENP 1993 Autumn School "Neural Mechanisms Involved in Psychological and Biological Time Measurement", San Feliu de Guixols, Costa Brava, Spain, September 22-27, 1993: *B. Wypych*

Annual Congress of the European Respiratory Society, Firenze, Italy, September 25-29, 1993: *K. Budzińska, M. Głogowska, W. Janczewski, M. Szereda-Przestaszewska* (Dr. Maria Głogowska received a "Daily Poster" award, sponsored by ASTRA, for the best poster of the day)

26th Danube Symposium for Neurological Sciences, Innsbruck, Austria, September 30 - October 2, 1993: *M. Barcikowska, A. Taraszewska*

2nd International Meeting on the Therapy of Muscle Dystrophies and Related Disorders and the 1st Mediterranean Meeting of Myology, "Gaetano Conte" Academy, Ischia (Naples), Italy, September 29 - October 4, 1993: *I. Hausmanowa-Petrusewicz*

International Symposium on Cardiomyopathies: Pathogenic Mechanism and Clinical Aspects, Warsaw, Poland, September 30 - October 1, 1993: *B. Kwiatkowska-Patzer*

International Congress FIMS 7th European Sports Medicine Congress, Nikosia, Cypr, October 5-19, 1993: *R. Grucza*

38. Jahrestagung der Deutschen Gesellschaft für Neuropathologie und Neuroanatomie, Berlin, Germany, October 6-9, 1993: *Z. Czernicki, H. Kroh*

Meeting European School of Oncology "Growth factors (cytokines): from basic science to the clinic", San Servolo Island, Venice, Italy, October 19-21, 1993: *B. Lukomska*

6th Congress of the European Society for Organ Transplantation, Rodos, Greece, October 25-28, 1993: *M. Durlik, H. Gałkowska, W.L. Olszewski*

Arbeitskreis ICP - Hirnödeme - Hirndurchblutung in der Deutschen Gesellschaft für Neurochirurgie, Heidelberg, Germany, October 29-30, 1993: *Z. Czernicki*

International Symposium of Polish Immunological Society "The Role of Cell Adhesion Molecules in Immunopathology", Warsaw, Poland, November 14-16, 1993: *P. Bryła, S. Durowicz, H. Gałkowska, M. Jastowska-Englisz, U. Kubicka, B. Łukomska, M. Maksymowicz, W.L. Olszewski*

Meeting of the European Federation of Neurological Societies, Berlin, Germany, December 8-11, 1993: *M. Barcikowska*



# SCIENTIFIC DEGREES

## DOCTOR'S DEGREES

### **Lidia Faff-Michalak**

The activity of the enzymes which provide a link between energy metabolism and amino acid neurotransmitter synthesis in the rat brain during experimental hepatic encephalopathy

*(Department of Neuropathology)*

### **Iłona Fałęcka-Wieczorek**

Effect of fat enriched single meal or a long-term fat diet on physiological responses to prolonged exercise in dogs

*(Department of Applied Physiology)*

### **Wiktor Janczewski**

Respiratory activity after splitting the brainstem - the role of crossed neural connections

*(Department of Neurophysiology)*

### **Izabela Kuchna**

Evaluation of hippocampus in human newborns after perinatal hypoxia: A morphometric analysis of neuronal changes

*(Laboratory of Developmental Neuropathology)*

**Małgorzata Puka**

Taurine release in brain ischemia: role of N-methyl-D,aspartate receptors and pathogenic consequences

*(Department of Neurochemistry)*

# SCIENTIFIC MEETINGS

ORGANIZED BY THE MEDICAL RESEARCH CENTRE

Practical Course of Staining with Monoclonal Antibodies, February 10-11, 1993;  
sponsored by DAKOPATTS, Denmark

International Symposium: The Role of Glia in CNS Pathology and Repair:  
Basic and Clinical Aspects, June 14-15, 1993

XXV Course of Basic Microsurgical Techniques, June 22-24, 1993;  
sponsored by CYANAMID OVERSEAS Corp.

Practical Course of Staining with Monoclonal Antibodies, October 6-7, 1993;  
sponsored by DAKOPATTS, Denmark

Course of Cytometry Technique "Immunocount System", November 23-24, 1993;  
sponsored by ORTHO

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