



NATIONAL ACADEMY OF SCIENCES REPUBLIC OF ARMENIA

H. BUNIATIAN INSTITUTE OF BIOCHEMISTRY

THE 50-YEAR SCIENTIFIC PATHWAY

*“Unique paradise in the Universe, are gardens of memory,
whence nobody can expel you, even the God”*



PREFACE

The Institute of Biochemistry of the NAS RA after Academician Hrachya Buniatian is 50 years old. Since the origin of establishment of the Institute, biochemistry of brain – neurochemistry, has been identified as the main scientific theme, to which we remain true up to date. The scientific works of the Institute have always stood out with their original and actual character, have always been and remain in the center of attention of a large segment of scientists around the world. Presently, former members of the Institute's staff work in many scientific centers, of which more than 50 scientists in the United States. The efficient network of development of young scientists within our Institute allows designing outgoing plans for development of neurochemistry for many years in advance.

In 1980 the Academy of Sciences of USSR has made a decision to establish the journal “Neurochemistry” on the basis of our Institute. Today, the translated version of the journal is being published and disseminated around the world. The primary weakness of the Institute is, unfortunately, the outdated equipment requiring modernization. Laboratories serving the scientific research of the Academy of Sciences system and universities, with modern equipment and highly qualified specialists, such as the Spectral, Centrifugal, Electron-microscopic and, the only Radioisotope one in Armenia, worked in the Institute. Having a modern base for implementation of scientific works, the staff of the Institute presented reports at all prestigious scientific forums and was involved in many famous scientific communities. The Institute often has and continues to host Nobel Prize winners and other prominent specialists from all over the world.

Casting a glance at the past and looking with optimism outward into the future, we can boldly assume that the Institute of Biochemistry, proudly bearing the name of its founder Academician H. Buniatian, will carry on the traditions of our masters and continue to enrich the science with new achievements for many years to come.

Prof. Guevork A. Kevorkian, Ph.D, Member of Russian Medical-Technic Academy European Academy of Sciences and Art

THE 50-YEAR SCIENTIFIC PATHWAY OF H. BUNIANIAN INSTITUTE OF BIOCHEMISTRY OF NAS RA

The first biochemical studies in Armenia go back as far as to 1923-1924 initiated by Prof. H.Hovhannisyan in the Medical Department of the newly established State University. In 1935 a Department of Biochemistry was established adherent to the Institute of Chemistry within the Armenian branch of the USSR Academy of Sciences. There studies related to preservation of vitamins, as well as the effect of unsaturated phosphatides on lipids peroxidation were carried out. H.Buniatian studied the problem of neuro-humoral regulation of ascorbic acid metabolism in the organism, which made a great contribution to the development of metabolism and brain biochemistry in the organism. In 1943 the Institute of Physiology of AS was founded and the Department of Biochemistry was included in it. Later it became a separate scientific institution, on the basis of which the Institute of Biochemistry was established in 1961, organized and led by Academician H.Buniatian. Simultaneously with the set up of the Institute of Biochemistry, the main directions in biochemistry were formed, which rapidly developed in Armenia and found their worthy positions in different sections of biochemistry. In a short period the Institute of

Biochemistry developed into an important center for research of the problems of brain biochemistry, studies of which became widely recognized in Armenia and abroad.

Wide-scale studies were carried out by H.Buniatian and his students on clarification of the role of gamma-aminobutyric acid in metabolic processes. It was shown that this compound affects the increase of cell membrane permeability towards certain ions, glucose, amino acids and some other substances. Based on the obtained data it was confirmed that gamma-aminobutyric acid possesses an important function in the homeostasis of brain and the whole organism.

The modern high level studies carried out in the Institute of Biochemistry have become famous in our country and abroad. Noteworthy success has been achieved in revealing a number of delicate biochemical mechanisms of nervous tissue and the application of these results within medicine. Valuable data has been obtained concerning the effect of proline-rich polypeptides and their synthetic analogues on hematopoietic, antibacterial and antiradical properties. It should be noted that the above mentioned polypeptides possess curative and preventive (immunogenic) properties at

anthrax and the cattle tuberculosis treatment.

On the basis of the experimental model of maniac depression the contents of biogenic amines and the effect of Li-containing neuroactive peptides and lactic acid probionts on their level was studied. Using maniac depression model formed by pharmacological factors, the curative effect of Li-containing peptides was assessed. At diagnosis of a long-term crush syndrome at brain neurodegenerative injury a new way for the penetration of products of myoglobin proteolysis to brain was discovered.

The extracts obtained from endemic plants of the Armenian uplands, which significantly subdue the adenosine deaminase activity were discovered.

Interesting results were received on hemorphines effect, which increase β -endorphins activity in plasma of rats with diabetes.

The model mimicking clinical state of rat blood and bone marrow anaemia has been developed. The modern and quantity-dependent stimulating effect of cyclophosphamide on formation of nitrogen active compounds in brain was discovered.

Particular studies have been carried out to obtain NADPH oxidases from the cells of malignant tumor tissues, to form their NADPH-dependent superoxide and to assess ferri-haemoglobin restoring activities, combining them with

the proline-rich polypeptides as a possible anti-radical factor.

Results of studies obtained in the aging biochemistry have demonstrated that due to the stimulation of urea synthesis in liver under the effect of specially fused various compounds a significant decrease of ammonia contents and inhibition of the enzyme gamma-glutamyltransferase activity is induced.

Interesting data are received from experimental studies related to revelation of bioenergetic mechanisms in respect of ontofilogenetic relationships.

Acidic and basic phosphatases and glycogen phosphorylase activities were studied at rats with pharmacological desympathization. At stimulation of paraventricular nuclei in the cannon bone marrow a sound increase of glycogen phosphorylase activity is observed. It is supposed that the hypothalamus realizes its regulating activity via releasing hormones produced by neuroendocrine cells.

Important results are obtained on the effect of neurohormone "G" complex on blood clotting cascade mechanism.

New approaches are worked out based on the sensibilized silver nanoparticles which allow assessing antigens in concentration density from 20 to 30ng/ml.

A delicate distribution image for preserving RdCVF and the effects induced by light damage on eye retina of mice deprived of RdCVF-1 and RdCVF-2 genes because of bright light, as well as of taurine for eye retina defense system were studied.

These results provide evidence in favor of GABA-amide utilization in brain. It is indicated that ammonia derived from GABA-amide is more obvious in rats mitochondria subjected to aluminum intoxication. Data on the capability of the glutamine synthesis from asparagines has been received.

The Institute of Biochemistry has established close cooperation with scientific institutions of both the Republic of Armenia and abroad.

The Institute of Biochemistry actively participates in the works of “Neyrokhimiya”, a joint scientific publication of RAS and NAS RA, where the results of scientific research of the Institute are published periodically.

Significant works are carried out within the Institute for development of young scientists. For almost five decades a Specialized Council acts in the Institute, where hundreds of young specialists (from Armenia and other countries) have defended their dissertations in the field of biochemistry and molecular biology.

THE INTERNATIONAL ACTIVITY OF THE INSTITUTE

In spite of transition period difficulties the Institute has succeeded in preserving the existing huge scientific potential, restoring the old ties and establishing new ones with international and foreign scientific organizations, and integrating to the international scientific community. Extension and intensification of studies in H.Buniatian Institute of Biochemistry served the basis for establishing a wide network of international co-operation. Traditionally close ties with Russian Academy of Sciences escalated onto a new mutually beneficial level.

Since 1973 our Institute has established close scientific connections with the Institute for Neurochemistry and Drug Addiction in New York (later on named Center for Neurochemistry) headed by Prof.Abel Lajtha. A number of scientists visited this Center, made profound investigations on proteolytic enzymes of the brain, biosynthesis of catecholamines in vivo, neurohormonal regulation of proteolytic enzymes, etc. Prof.A.Lajtha and Prof.A.Galoyan have organized several joint symposia. As a result of many years' co-operation Prof.A.Lajtha has been elected a member of NAS RA. It is noteworthy that Prof.A.Galoyan is in the editorial board of Neurochemical Research published in New York.

Prof. A.Lajtha is a co-editor of *Neyrokhimiya* (issue of Armenian and Russian Academies). Prof.G.Hashim and Prof.B.Agranoff are in the editorial board of the same journal. On the other hand during past years numerous famous investigators of USA have visited Buniatian Institute of Biochemistry, such as Prof. P.Greengard and R.Guillemin (Noble Prize winners), A.Lajtha, A.Meister, V.Vernadakis, M.Saffran, L.Sokoloff, B.Agranoff, B.Lowenstein, A.Chobanian, S.Simonian, E.Noble, etc. Numerous scientific institutions and leading specialists of USA have established connections with the Institute of Biochemistry, such as: The Salk Institute for Biological Studies, Beckman Research Institute of the City-of-Hope, Boston University School of Medicine, Washington University, Miami University, Cancer Center in Frederick, Toledo University, etc.

Further is a short list of world scientific institutions with which we have established cooperation: UK Royal Society; Institute of Physiological Chemistry of Tübingen University, Free University in Berlin; Max Planck Institute of Neurobiology (Germany); A.N.Bach Institute of Biochemistry; Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of RAS (Moscow, RF);

Dalhousie University (Canada), Louisiana State University Medical Center, University of Pennsylvania, Medical Institute of Seattle (USA); Pasteur Institute (France); The University of Camerino (Italy); Coimbra University (Portugal); Korean Ginseng and Tobacco Research Institute (Korea).

The history of collaboration with Hungarian Academy of Sciences goes back to the 1960s and is still continued successfully (Prof.S.Vizi, M.Palkovits, A.Cillag, etc.).

As a general recognition of the achievements in biochemistry, the Armenian Association of Biochemists has been involved in the European Federation of Biochemical Societies (FEBS), and later, in 2002, became associated member of FEBS. Since 2010 AAB is included in the International Union of Biochemistry and Molecular Biology (IUBMB).

Development of international collaboration skills allowed applying and receiving both local State grants and ones from various international foundations, such as: INTAS, ISTC, CRDF, NFSAT, NATO Science Programme, DAAD, ANSEF, FEBS, etc.

Molecular Radiology and Imaging Society is a non-governmental organization registered in our Institute, which actively participates in elaboration

of medical documentation for the Scientific Center of Radiology to be established in Yerevan. This Center aims to produce short-lived radionuclides (iodine, fluorine, etc.) for diagnostic and treatment purposes to serve patients suffering with tumor diseases. Vahan Barseghyan, a well-known radiologist from Germany, is elected as the scientific consultant on medicine of the Center.

It should be noted that scientific secretary on international cooperation of the Institute Karine Gevorgyan's merits are great in enhancing the international cooperation. Since 1975 she has participated in International symposia held by the Institute as secretary of the Organizing Committees and interpreter, as well as the member of the Editorial Boards for preparation, translation and publication of Proceedings (1977, 1980, 1985, 1997, 2001, 2004, 2007, and 2009). Along with translation of numerous papers and abstracts, she participated with English translation in compiling the Russian-English-Armenian Explanatory Dictionary, 7000 words, Publ. House "Gitutyun", NAS RA.

FOREIGN LINKS OF LABORATORY OF METABOLISM OF ADENYLINE COMPOUNDS

I. Department Of Biochemistry Of Medical Academy, Gdansk, Poland.

In the frame of Soviet Union Program for researchers exchange with East Europe countries, in 1988 Sona Mardanyan visited for 3 months the Department of Biochemistry of Medical Academy in Gdansk, Poland. As a return visit, the Head of this Department, Prof. Marius Zydowo and Prof. Krystian Kaletha arrived for a week to Yerevan in 1991. The contact with Polish

colleagues was continued by three-month visit to Gdansk of Dr. Yelizaveta Sargisova in 1994. This visit was interesting by creation of contacts with Italian researchers. In the frame of a joint program of Polish colleagues with Prof. Fabio Tanfani from Italy, Dr. Sargisova visited Italy and the joint three-side investigations resulted in two scientific publications.

II. Institute of Biochemistry, Medical School, University of Ancona, Italy.

The collaboration with the Italian colleagues was continued due to the support by NATO SCIENCE PROGRAMME of a joint Project with Prof. Fabio Tanfani: «Studies on protein com-

plexes. Electron carrier complex [Adrenodoxin Reductase x Adrenodoxin]» (CLG.977079). The investigations in the frame of this Project resulted in scientific publication in the Journal Proteins.

III. Institute of Biochemistry and Biophysics, University of Tehran, Iran

Along with these collaborations, Sona Mardanyan established relations with Prof. A.A. Moosavi-Movahedi (Iran) and Prof. G. Cristalli (Italy). At the proposal of Prof. A.A. Moosavi-Movahe-

di, in 2001 S. Mardanyan visited IBBI for a week for discussions on possible joint collaboration which resulted in a three-side Project Proposal and publication of seven scientific articles.

IV. Department of Chemical Sciences, University of Camerino, Italy

The collaboration with the Department of Chemical Sciences, University of Camerino, Italy, had been directed on the investigation of different aspects of interaction of Adenosine Deami-

nase with Complexing Protein, both purified from different mammalian tissues in our Laboratory, and the inhibitors and substrate analogs, synthesized by the Italian researchers. After sev-

eral years of successful collaboration and printing of two articles, a Project Proposal had been submitted and awarded by NATO sci.: «Optimal Conditions for Adenosine Deaminase Interaction with Complexing Protein» (CLG. 979813). Besides the Project, our colleagues from Camerino University funded the additional visit of Dr. Alvard Antonyan to Italy for continuing the research investigations. Unfortunately, our next two joint Projects: «Interaction of Dipeptidyl Peptidases IV and II with Flavonoids» and «Expression, biochemical characterization and inhibition of dipeptidyl prolyl peptidase IV novel candidate to reduce the virulence of pathogens» were not funded by the NATO Programme. The long-term collaboration with the Italian colleagues led to signing of a scientific and cultural “Cooperation Agreement Signed Between The University

Of Camerino (Italy) And The National Academy of Sciences of The Republic of Armenia (Institute of Biochemistry)” on 08/10/2008. The aim of our collaboration in 2011-2012 will be the joint implementation of project: “Modulation by dietary flavonoids of Dipeptidyl Peptidases as new tolerable approach for the treatment of some pathologies”.

The active negotiations of Dr. Alvard Antonyan with colleagues from Germany resulted in invitations from Prof. Hua Fan (Campus Benjamin Franklin Charite, Berlin) and Prof. W. Saenger (Freie Universität, Berlin) and in 2010 Dr. Antonyan was awarded a grant by DAAD for performing the investigation on the joint Project: “The crystal structure of dipeptidyl peptidase II as a key for elucidation its function and physiological role”.

Foreign Links Of Laboratory Of Pathological Biochemistry And Radioisotope Methods

Center for Vascular Research of Lowe Cancer Research Center of the University of New South Wales (Australia), Director Professor Levon Khachigian, Academician, Foreign member of NAS RA

Nantes University, France, UMR CNRS 6204, Prof. Vehary Sakanyan Laboratory of Biotechnology, Academician, Foreign member of NAS RA

Academician Hrachia Khachatur Buniatian (01.05.1907 - 19.03.1981)



Academician, Prof., Dr. Hrachia Khachatur Buniatian (born in Bayazet city (at present Gavar), Armenia) was the founder of Biochemistry and Neurochemistry in Armenia. Together with several outstanding Armenian scientists (Hambarzumian V.A., brothers Orbelli, Asratian E.A., Sisakian N.A., etc.) he founded the Armenian Academy of Sciences. He was the Rector of the University, the Vice President of Armenian Academy of Sciences, the member of German Academy of Natural Sciences Leopoldina and the Member of Presidium of Armenian Academy of Sciences, the Chairperson of Biochemical and Physiological Societies of Armenia, the member and Council member of International Society for Neurochemistry, the director of the Institute of Physiology, the founder of the Institute of Biochemistry (of about 200 people), which he led till the end of his life.

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He brought up a school of Armenian biochemists comprising 16 professors and 60 PhDs in Biochemistry. He was invited as a lecturer to scientific Centres in England, Germany, Japan, USA, India, Hungary, Sweden, Canada, Spain, France, etc. The initially small group of scientists led by him, grew into a large world-known Centre for Neurochemistry in the former Soviet Union.

He was the founder and the Editor-in-chief of 14 volumes of "Problems of the Brain Biochemistry". Those volumes were distributed throughout the world and decorated the libraries of large neurochemical centres. Academician Buniatian

is the author of more than 250 scientific articles published in local, All-Union and International professional journals. In 1981, few months before his death, due to high authority of Armenian school of Neurochemists, the All-Union and International journal "Neurochimiya" was trusted to be edited and published in the Institute of Biochemistry of Armenian Academy of Sciences.

The main topics of his interest were:

Urea synthesis in brain. Adaptive properties and characterization of molecular forms of rat and chick brain arginase. The participation of N-acetyl-L-aspartic acid in brain metabolism. The

deamination of adenosine triphosphate, nicotinamideadenine- dinucleotides and mechanism of stimulation of AMP-aminohydrolase activity by hexokinase in brain. The role of adenosine diphosphate in regulation of nitrogen metabolism of rat brain mitochondria in early postnatal development and maturation.

Characteristics of brain glutaminase and the role of gamma-aminobutyric acid in the metabolism of glutamic acid, glutamine, catecholamines in brain.

Changes in carbohydrate and amino acid metabolism in brain and liver tissues, during experimental alloxan diabetes, acute brain edema, as well as in the course of embryonic and postnatal development. Characterization and regulation of multiple forms of phosphoprotein phosphatase in brain tissue.

Copper-containing proteins systems in white and gray matter, as well as in dopaminergic areas

of the brain and in chromaffin granules. Discovery and characterization of neurocuprein, a low molecular weight, extremely acidic copper-containing protein and its interaction with catecholamines.

The important role of ammonia in life-essential functions of the brain has been predicted already in 1962, from the above mentioned fundamental neurochemical studies developed in the Institute of Biochemistry of Armenian Academy of Sciences by Prof. Buniatian and his students. Nowadays, this field of Neurochemistry became one of the most exiting and extensively investigated ones. Most of scientific exchange programs and collaborative works with world-known scientific Centres in Germany, USA, Sweden and Hungary established and supported by Academician Buniatian and his students continue up to date and are reflected in multiple publications of the Institute.

Scientific Activity of Academician Armen Galoyan



Armen Galoyan was born on 1 May, 1929 in Armenia. The secondary school was finished with honours (golden medal). He has graduated from Yerevan State Medical Institute with honours diploma. In 1956 he defended his thesis on brain biochemistry for the degree of candidate of sciences in A.N.Severtsev Institute for Biology of Development in Moscow (under supervision of Kh.Koshtoyants). Afterwards he returned to Armenia, and since that (for over 50 years) he has been working in H.Buniatian Institute of Biochemistry of National Academy of Sciences in the Republic of Armenia.

He began his scientific experience in the Institute as a scientific worker, head of the Laboratory of Neurohormones Biochemistry, and for over 25 years (1981-2006) he was the Director of H.Buniatian Institute of Biochemistry of NAS RA.

In 1971 he was elected a correspondent member of Academy of Sciences of Armenia with specialisation "Chemistry of physiologically active compounds".

In 1986 he was elected a full member with specialisation "Biochemistry". Since 1977 Prof. A.A.Galoyan has been leading Moscow subdivision of the Laboratory of Neurohormones Biochemistry of the Institute of Biochemistry, NAS RA. In 1995 it was reorganized into the Joint Laboratory of the Neurohormones Biochemistry (H.Buniatian Institute of Biochemistry of NAS RA, and A.N.Bach Institute of Biochemistry of

RAS). Prof.A.Galoyan is an honoured foreign member of the scientific council of A.N.Bach Institute of Biochemistry of RAS, honorary professor. He carried out joint studies with the scientists of the Institute of Organic Chemistry and Biochemistry in Prague, the Institute of Neurochemistry and Drug Addiction in New York, the Salk Institute in San Diego, the Rockefeller University in New York, Beckman Research Institute of the City of Hope, etc.

He participated in numerous scientific forums (held from North to South and from West to East) as a speaker, delegate of the country, he was

also elected the chairman of various symposia and conferences on molecular biochemistry, neuroendocrinology, and neurochemistry.

Prof. Abel Lajtha, the Editor-in-Chief of the journal "Neurochemical Research", Director of New York Neurochemical Center, prepared the third edition of the Handbook for Neurochemistry and Molecular Neurobiology, where A.Galoyan was invited to become the editor of the Neuroimmunology volume. This fact indicates Galoyan's general recognition in the field of neuroimmunology as well.

A.Galoyan made a valuable contribution to the development of priority areas in neurobiology being a Chairman of the Medical Biological Scientific Council in the Department of Life Sciences of NAS RA, Director of the Institute of Biochemistry, President of the Association of Armenian Biochemists. At the same time he pays great attention to the training of young scientists and co-ordination of different trends in biochemistry. At present, having a goal to clarify the mechanism of action for a number of hormones and biologically active compounds discovered by Galoyan and co-workers, basic scientific studies are carried out in numerous scientific centres of the world, such as Institute of Physiology of NAS RA, M.Heratsi State Medical University in Yerevan, Agricultural University in Armenia,

Department of Biochemistry and Molecular Biology in the School of Medicine and Health Sciences of Washington University, Miami University, Center for Neurochemistry in New York, Medical University in Vancouver, A.N.Bach Institute of Biochemistry of Russian Academy of Sciences, Institute of Physiological Chemistry in Tübingen, etc.

Great experimental data are accumulated on study of cardiotropic and immunomodulating properties by Galoyan and coworkers. This resulted in Galoyan's establishment of new concept on neuroendocrine cardiology and neuroendocrine immunology, which is acknowledged by the world scientific community. All studies carried out by Galoyan and coworkers are well described in his monographs: Biochemistry of Novel Cardioactive Hormones and Immunomodulators of the Functional System Neurosecretory Hypothalamus – Endocrine Heart. Nauka Publ., Moscow, 1997, 240 P. and Brain Neurosecretory Cytokines: Immune Response and Neuronal Survival. Kluwer Academic/Plenum Publishers, New York, 2004, 188p. Another article The Brain Immune System: Chemistry And Biology of the Signal Molecules published in 2008 by Springer in Handbook of Neurochemistry and Molecular Neurobiology, 3rd Edition, Neuroimmunology,

was a reflection of his many years scientific activity (eds. A. Galoyan, and H. Besedovsky), pp.155-195. Now Springer is going to publish Galoyan's new book with recent data: Brain Immune System Signal Molecules against Aerobic and An-

aerobic Infections (300 pages).

At present Academician Armen Galoyan successfully continues his studies and serves in the Institute of Biochemistry as a councilor.



Guevork A. Kevorkian, Ph.D., Doctor of Biol Sci., Professor



Since 2006 - Director of the H.Buniatian Institute of Biochemistry of the National Academy of Sciences

1972 - Biochemist, Yerevan State University, Biological Faculty, Dept. of Biochemistry

1977 - Radiochemist, Moscow State University, Chemical Faculty, Dept. of Radiochemistry

1979 - Ph.D., Biochemistry

1998 - Doctor of Sci., Biochemistry

2000 - Professor

2010 - Member of Academy of Medical Technical Sciences (Moscow, Russia)

2011 - Active Member of the European Academy of Sciences and Arts (Salzburg, Austria)

1967 - Laboratory Assistant, Lab. of Amino Acids Metabolism, Institute of Biochemistry

1979 - Senior Researcher, Lab. of Embriochemistry, Head of the Lab. of Radioisotopes Methods, Institute of Biochemistry

1993 - Research Deputy-Director, H.Buniatian Institute of Biochemistry

1994 - Head of the Lab. of Pathological Biochemistry and Radioisotopes Methods

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International Societies Memberships

Authority Person of European Atomic Energy Agency (IAEA)

Vice- President of Armenian of Association for Biochemists (AAB)

President of Armenian Association of Molecular Radiology and Imaging (AAMRI)

International Union of Biochemistry and Molecular Biology (IUBMB)
Federation of European Biochemical Societies (FEBS)
International Society for Neurochemistry (ISN)
European Society for Neurochemistry (ESN)
American Society for Neurochemistry (ASN)
International Brain Research Organization (IBRO)
Society for Neuroscience (USA)

Member of Editorial Boards

Vice-Editor-of-Chief Armenian Medical Journal
Neurochemistry (Joint Russian-Armenian (Institute of Biochemistry) Journal
Armenian Biological Journal
Neurochemical Journal
Science Advisory Board (USA)
Turkiye Klinikleri Journal of Medical Sciences
Turkish J. of Clinical Radiology

Grants and Honors

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1993, 1995, 1998, 2002, 2008, 2009 – Ministry of Science and Education
1994 – Open Society Institute (by George Soros Foundation)
1996 – American University in Armenia
1997, 1999, 2000 – International Society for Neurochemistry
2004, 2008, 2010, 2011 – IPP (USA)
2007 – Silver medal of Academician Norair Sisakyan (RAN, Moscow)
2010 – Gold Medal (ABI, USA)

Department of Neurohormone Biochemistry

Head: *Academician A.A.Galoyan*

Many years of investigation by Professor Armen Galoyan and colleagues studying the hypothalamic neurosecretion led to discovery of new groups of immunomodulators and cardioactive hormones produced by NSO and NPV of hypothalamus, which became the basis of a concept neuroendocrine cardiology and neuroendocrine immunology, which has tremendous potential significance for the theory and medical practice.

From the neurosecretory granules of hypothalamus and neurohypophysis the interleukines (I1-1a, I1-1b, I1-2, TNF-a, IL-6, etc) were isolated. Of particular interest for the theory of neuroendocrine immunology is phenomenon of interleukin neurosecretion from hypothalamus into general circulation discovered by Prof.A.Galoyan and coworkers. Accurate evidence on the biosynthesis and secretion of interleukins into circulation was obtained.

In 1967 Prof. A.Galoyan discovered the phenomenon of endocrine heart and demonstrated that ganglionic nervous cells of atria possessed endocrine function, and released peptide hormones that participate in the regulation of cardiac circulation. These studies demonstrate that the heart is not only a mechanical organ, and the atria are a hormone formation organ. Discovery of a neuroendocrine heart became a basis for development of a new scientific trend such as Neuroendocrine Cardiology. A.Galoyan and co-workers discovered a new target, heart, for cardioactive hormones, and established it to be under regulatory hypothalamic effect of specific proteins and peptides, which reached heart as by humoral way, as well as via nerve fibres. These investigations revealed new principles for two organs interaction: heart and brain which became the basis for studies in the Department.

Laboratory of Neurochemistry and Neuroimmunology of Very Dangerous Infectious Diseases

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A new group of cytokines, i.e. proline-rich polypeptides, was discovered in neurosecretory granules of hypothalamus-neurohypophyseal system. The discovery of a new type of cytokines of the neurosecretory hypothalamus, the proline rich peptides, brought changes in our understanding of the regulation of immune system as a whole. With the use of mono- and polyclonal antibodies, one of these peptides, PRP-1 (15 amino acid residues) named Galarmin, was found in immunocompetent blood cells, in neurosecretory cells of hypothalamus, in bone marrow granulocytes, and in brain and bone marrow PRP-1-ergic fibers. It was shown that PRP-1 peptide possesses the cytokine activity and stimulates the production of TNF α by macrophages and production of IL-1 and IL-6 by astrocytes. It also stimulates the antigen-presenting function of macrophages, expression and release of human growth factor by transformed BALB/c mice fibroblasts, etc. In other words, the PRP-1 is a classical cytokine. The discovered proline-rich peptides represent a new family of hypothalamic neuropeptides, which are synthesized in the form of a common precursor protein, NVAG (neurophysin- vasopressin-associated glycoprotein) by genetically determined mechanisms and released from the precursor by

proteolysis during axonal transport. PRP-1 is the regulator of both humoral and cellular immunity, differentiation of thymocytes, and myelopoiesis. It possesses strong antiviral (in vitro) and antibacterial activity (in vivo against *Salmonella typhimurium*, *S. cholerae suis*, *S. typhi*, *E. coli*, *Pseudomonas aeruginosa*, *Shigella Flexneri*, *Shigella Sonnei*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Bacillus anthracis* etc.). PRP-1 can be recommended in future for treatment of immunodeficiencies and different infectious diseases. It protects against aluminum neurotoxicity and abolishes the accumulation of this element in brain microstructures and visceral organs as shown by electron microscopic studies. PRP-1 expresses antineurodegenerative activity in the cases of spinal cord hemisection, trauma and crush syndrome, as well as in aluminum neurotoxicosis. PRP-1 also possesses antitumor activity in vitro against Jurkat cells, L929 fibroblasts, neuroinoma Hasser's ganglion cells; and in vivo against sarcoma 45 and human chondrosarcoma.

The role of hypothalamic neuropeptides in neuroimmune interactions is largely investigated. Armen Galoyan's scientific achievements are accumulated in data obtained for about 45 years by him and his coworkers, which resulted in estab-

lishment of two new pathways in neurobiology, neurochemistry and neuroendocrinology, namely neuroendocrine immunology and neuroendocrine cardiology. Discovery of brain new neurohormones and cytokines, deciphering of their complex chemical structures and elaboration of the methods for the chemical synthesis played a great role in clarification of the biochemical mechanisms, as well as treatment of immune, in-

fectious, neurodegenerative and tumor diseases. Discovery of interrelation between brain immune neurosecretion system and neurohumoral bone marrow belongs to a number of great achievements, which creates perspectives for hematology development. At present Prof.A.Galoyan works on introduction of the new medication “Galarm-in” to medical practice.

Laboratory For Neuroimmunology

Head: *Vardan S. Aprikyan, PhD, Dr. Sci.*



In our laboratory we investigate the action of the new brain proline-rich polypeptides (PRPs: PRP-1, PRP-2, PRP-3 and GXNH2), described by Prof.A.Galoyan and at first isolated from cattle and then human hypothalamus on the immune system including hematopoiesis.

We used the immunological methods in different test-systems. Our results indicated that PRP possess a pronounced immunoregulatory activity in humoral and cell – mediated immune response and modulate the functional activity of macrophages.

At first we carried out a study of the ability of PRPs to enhance in mice immune response to T-dependent and T-independent antigens (i.e. action as immune adjuvant).

Female pathogen-free BALB/c and C57B1/6 strains of mice were used for the experiments. Our results indicated the ability of PRP to stimulate recovery of myelopoiesis and enhance mature neutrophil function.

During comparison of treated effects at using GMCSF and PRPs we found and the analysis proved that PRP-1, GXNH2 are more efficient than GMCSF. However, the effect of PRP-2 in comparison with GMCSF is significantly weak and the influence of PRP-3 is almost the same as GMCSF. It is necessary to note that after GMCSF injection the mice have flabbiness,

oedema of extremities, loss of their activity and appetite. And after HP administration normalization of mice appearance, enhancing of their activity and appetite took place. PRPs comparison is done in the Tests 90-95 and the order of their effectiveness is determined as following: PRP-1, GXNH2, PRP-3, PRP-2.

PRPs have ability to induce the expression of colony-stimulating factors (CSFs). PRPs induced the mobilization of colony-forming units (CFU) from bone-marrow to blood in higher level, than CSFs. Under the joint action of PRPs with CSFs the number of CFU was higher. PRPs' activity

is similar to GM-CSF and G-CSF, but is more expressed. Trafficking of neutrophils and hematopoietic stem cells from bone marrow to blood was more intensively attached to PRPs than to CSFs. PRP is a powerful antibacterial agent. It was established that PRP does not possess an etiotropic effect. PRP does not change the quantitative and qualitative indicators of the growth and viability of microorganisms. It increases the survival ability of the experimental animals infected with lethal doses of different highly pathogen strains of Gram-negative and Gram-positive microorganisms such as: *Salmonella* and *Shigella* species, *Escherichia coli*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Mycobacterium tuberculosis*. PRP has the ability in mouse models to ameliorate or prevent development of *Salmonella* and *Shigella* species, *Escherichia coli*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Mycobacterium tuberculosis* infections. PRPs had a pronounced protective effect during the period of development of the infections. It was established, that PRP reduced the growth of bacteria in the internal organs of infected mice and the duration of the persistence

of the micro-organisms in the body was reduced after the use of PRPs and the elimination of bacteria from the body was enhanced. It stimulates formation of specific antibacterial antibodies, increases viability, accumulation and adhesion of phagocytes, increases their bactericidal properties, ingestion and killing of bacteria, antigen-presenting function and IL-1 synthesis.

It was established that PRP-1 has prophylactic action at *M. tuberculosis* infection. All the control mice died. And all the PRP-treated mice 24h before infection in high lethal doses remained alive (survival was 100%). It was established that PRP has therapeutic action at *M. tuberculosis* infection. All the control mice died. And the 91% of PRP-treated mice in period of development of infection in high lethal doses remained alive.

In other study PRP-1 was injected before BCG or simultaneously with BCG or after BCG. It was established that PRP enhances the effectiveness of prophylactic and therapeutic vaccinations with BCG up to 3,3-fold. PRP-1 may be used as an antibacterial immunocorrector and for creation of new generation vaccines against pulmonary tuberculosis.

Laboratory of Immunology

Head: *Tigran K. Davtyan, PhD, Dr. Sci.*



Dr. Tigran K. Davtyan, Senior Research Scientist in H. Buniatian Institute of Biochemistry, Scientific Centre of Drug and Medical Technology Expertise JSC, Armenia. He is a well known scientist, with great experience, in spite of him being young.

Dr. Davtyan approved to be very active man with personal initiatives, with creativity and eagerness to explore new ideas.

His research encompassed in over 70 journal articles, books and book chapters, has greatly increased our understanding of the basic molecular mechanisms of immunity, viral infection, and neuro-immune humoral regulation.

Dr. Davtyan together with Professor A. Galoyan studied the fundamental neurochemical, biochemical, immunological and cellular aspects of proline-rich peptide family action on hematopoietic progenitor cells proliferation, survival and differentiation, on innate and acquired immune system cells function and behavior, respiratory burst and phagocytosis induction, expression of surface receptors and activation markers. It has also led to his generation of novel immunomodulators – potential anti-HIV drugs (Iodine-lithium- α -Dextrine, ArmenicunTM). The main results of his research have been reported on numerous international conferences, meetings, symposia and. His international activity is

of success: Charity Missions to treat HIV/AIDS patients in Kinshasa, Congo Democratic Republic and in New Delhi, India, numerous reports were delivered by him as an invited lecturer. Dr. Davtyan won many highly-competitive international awards and grants. More than 20 years Dr Davtyan together with Dr. Gagik S. Hakobyan, PhD (Department of Internal Medicine, Yerevan State Medical University), and Dr Samvel A. Avetisyan, PhD (Department of Pathophysiology, Yerevan State Medical University), and Dr Nana R. Mkrtchyan, MD (Department of Rheumatology, National Institute of Health, Republic of Armenia) involved in the project of Familial Mediterranean Fever (FMF) study. They research

encompassed in over 25 journal articles on FMF patients, have greatly increased our understanding of the basic molecular mechanisms of the mechanisms of innate immunity, endotoxin tolerance and pharmacological regulation during FMF. The main results from this research group have been published in numerous peer-reviewed and prestigious international journals.



Laboratory of Chromatographic Methods for Peptides and Proteins Study

Head: *Dr. S. Chailian*



Researchers use RP-HPLC and PAAG-Electrophoresis techniques for isolation and purification of biologically active peptides from bovine and human hypothalamus. Particular emphasis is made on clarifying of the functional activity of the peptides in reference to molecular composition. At present they implement laboratory synthesis of Galarmin and its analogues in pharmacological industry.



Immunohistochemistry Group

Head: Silva Abrahamyan, Leading Researcher, Doctor of Sciences

Narine Tumasyan, Junior Researcher, PhD

Inesa Sahakyan, Junior Researcher



The Immunohistochemistry group has earlier studied the distribution of the protein-carrier of one of the hypothalamic coronary dilatatory glycopeptides, neurohormone “G” (PCG) in rat brain. PCG has been localized by immunohistochemical method in various brain regions, including the hypothalamus, amygdale, and the cerebral cortex. It was suggested that the coronary dilatatory compounds originating in the nervous system may be released into the circulating blood, bound to blood serum albumins and g-globulins, transported to the heart, and connected with the cardiac proteins. In addition to the humoral pathways, the neuronal pathway of PCG from the brain to the heart has been hypothesized. PCG localization in rat heart was later examined by immunohistochemistry. PCG-immunoreactive nerve fibers and varicosities were found around cardiac ganglionic cells and in close topographical contact with coronary vessels and capillaries.

Vagotomy induced no considerable changes in the distribution of PCG in cardiac neurons and nerve fibers in a different series of experiments. The detection of PCG in the superior cervical ganglion supported the hypothesis that PCG is of sympathetic origin. The presence of PCG in cardiac neuronal elements suggested a possible role of this peptide in cardiovascular regulations.

The group is currently studying specific (spinal cord hemisection, beta-amyloid peptide-induced AD) and non-specific (immobilization stress, stimulation of hypothalamic PVN, labyrinthectomy, and vibration of the vestibular apparatus) neuronal injury in rats. The aim is to identify the underlying mechanisms of the initiation and promotion of the neuronal recovery processes

proceeding from the treatment with proline-rich-peptide (PRP-1, named Galarmin), a natural cytokine of a common precursor neurophysin vasopressin associated glycoprotein).

Histological, histochemical (method on detection of Ca^{2+} -dependent acid phosphatase activity), and immunohistochemical (ABC) methods have been applied for the morpho-functional study. Data accumulated by the Group confirm the anti-neurodegenerative and protective actions of PRP-1 in the animal models mentioned above.

The morpho-functional investigations with use of the immunohistochemical techniques were focused on the interaction between the several neuropeptides (neuropeptide Y, hypothalamic immunophilin fragment, and PRP-1) and some biomodulators such as snake venom *Naja Naja Oxiana* (NOX) in normal and pathophysiological conditions.

Immunohistochemically, we have previously found that treatment with PRP-1 resulted in the recovery and growth of the nerve fibers, glia proliferation, and motoneuron survival in the trauma-injured rats [Abrahamyan et al. 2004]. In the experiments on the spinal cord (SC)-hemisectioned rats treated with NOX, immunohistochemically, using PRP-1-antiserum, NOX was suggested to exert the neuroprotective effect, in-

volving the PRP-1 in the underlying mechanism of neuronal recovery [Abrahamyan S.S. et al., 2007], based on the regeneration of nerve fibers growing through the trauma region, survival of the PRP-1-Ir neurons, and increase of the PRP-1-Ir nerve fibers and astrocytes observed in the SC lesion region.

Purpose of the current study is to examine the brain, SC, and bone marrow plasticity under the normal and a number of pathological conditions: immobilization (IMO) stress, electrical stimulation of the hypothalamic PVN, PRP-1 administration. By use of the radial astrocytes marker GFAP, neuroepithelial stem cell marker gene nestin, marker for synapses-synaptophysin, stem-cells marker and the polyclonal antibody raised by us against the synthetic PRP-1, adult brain cell regeneration was demonstrated in the newborn rats exposed to the prenatal IMO stress allowing us to support the hypothesis of PRP-1 involvement in this neurogenesis mechanism.

We also developed an enzyme linked immunosorbent assay (ELISA) for PRP-1 characterization, detection and quantification in human and animals biological fluids, including serum and plasma. This method gives a good detection range and sensitivity and is easy to apply. In addition, large number of samples can be analyzed rapidly and simultaneously.

Fellowships

Pavlov Physiology Institute, St. Petersburg, RF; Semmelweis University Medical School, Institute of Anatomy, Budapest, Hungary (Exchange Program between Armenian and Hungarian Academies of Sciences); Department of Histochemistry, Royal Postgraduate Medical School Hammersmith Hospital, London, UK (Royal Society Exchange Programme)

Memberships in professional, public organizations

Armenian Morphologists Association; Armenian Electron Microscopy Association; Armenian Association of Biochemists, European Association for Clinical Nanomedicine.

Travel Grant

IBRO Congress, Montreal; (Canada), 1st European Conference for Clinical Nanomedicine, Basel (Switzerland)

Publications

23 international publications, 3 publications in Armenian journals. Scientific data were extensively presented at international meetings.

Another group (A.Kirakosova, Cand.Biol.Sci.) investigates protein tyrosine kinase and biologically active modulators, such as Galarmin and its analogue d-15, and their regulation of activity, which represent a new way to examine the regulation of cell metabolism. Galarmin and d-15 analogue appeared to be natural inhibitors of PTK. We believe that Galarmin and d-15 can be used for the treatment of CPA intoxication.

The influence of Galarmin on rats bone marrow and spleen homogenate alkaline and acid phosphatase, glycogen phosphorylase activity (L.Ter-Tatevosyan, L.Arakelyan, Candidates of Biol. Sci.).

All the obtained data were statistically analyzed by the statistic packages GraphPad Prizm 3.03 and SPSS11.

Laboratory of pathological biochemistry and radioisotope methods

Head: *Guevork A. Kevorkian, Ph.D., Doctor of Biol Sci., Professor*



Laboratory Ph. D. Members:

Hripsime L. Hayrapetyan, Artashes G. Guevorgyan, Hranush F. Khachatryan, Grigor V. Gyulkhandanyan, Gurgen G. Batikyan, Barsегyan H. Vahag, Gohar R. Karapetyan, Krystine E. Danielyan, Aram G. Gyulkhandanyan, Lusine Gyulkhandanyan, Luiza H. Voskanian

Recent Publications: 275

In 1974 the Director of the Institute of Biochemistry Academician G. Buniatian organized the opening of the laboratory of radioisotope methods.

The employee of this laboratory G. Kevorkian was sent to Moscow State University, faculty of Radiochemistry for training on “Research methods using radio-active compounds” by mutual agreement between Academician G. Buniantian and Academician R. Khokhlov, the rector of Moscow State University.

After 2,5 years' study at the faculty of radio-chemistry in Moscow State University G. Kevorkian returned to Yerevan, and the laboratory of radio isotope methods began to function fruitfully, using methods with radio nuclides with low energy of radio activity, i.e. ^3H , ^{14}C , ^{32}P , ^{45}Ca etc. Laboratory of liquid scintillation methods was the only one in Armenia and received the status of inter-academic and inter-institutional laboratory. Since 1974 the Laboratory staff studied exchange of proteins, neurotransmitters, lipids, neu-

rohormones and carbohydrates, i.e. all the fields of Metabolism in Biochemistry. A special place in this research had the qualitative and quantitative determination of new neurohormones of hypothalamic part of the brain synthesized in the Institute of Biochemistry. During this period 26 doctoral and 75 PhD dissertations were done in this laboratory from different scientific centers of Armenia, including new methods of research using radioactive compounds. Thus, first time in the practice of measuring radioactivity by

scintillation method, the method of simultaneous measurement of three different radionuclides (by that time only two different isotopes simultaneously were measured worldwide) was developed and approbated. Due to this method, for example, it became possible to study simultaneously in one probe the speed of synthesis, degradation and phosphorylation of protein molecules. This method was adopted by several companies, which produce scintillation spectrometers. The principle of second method, which was worked out in our laboratory, is quantitative and qualitative definition of hormones, and especially, neurohormones by iodation with the help of radioactive ^{131}I . And the third express method is estimating the process of bio-damage of a certain microorganism related to a certain substrate. This method was worked out when the laboratory under my supervision studied the processes of bio-damage after the return of spaceships. A collection of microorganisms (approx. 450 strains) given to us by the Soviet Space Agency and NASA was tested.

Since 1993 the laboratory was renamed to laboratory of Pathological biochemistry and Radioisotope methods. The scientific profile of our laboratory was: consulting and practical works in the field of utilization of radioactive compounds in medical and biological experiments, study of myocardium metabolism during pathologies of

different etiology, as well as the impact of natural biological active compounds discovered and isolated from hypothalamus by Academician A. Galoyan.

The metabolic disorder of myocardium was studied on the models of isoproterenol necrosis, necrosis of myocardium with acute pancreatitis and crush syndrome or Bywaters' syndrome. While studying the processes of protein synthesis and degradation in subcellular structures of cardiomyocytes (mitochondria-M, sarcoplasmic reticulum-Sr and cytosol -C), translocation of Calcium ions, Ca^{2+} -binding properties and Ca^{2+} -affinity of membrane proteins M, Sr and C, enzymes systems of myocardium, electrolytic concentrations etc., we have discovered the resemblance of these parameters at all the three above-mentioned pathologies of myocardium. Thus, membrane proteins of Sr lose their affinity to Ca ions, and this function is very often performed by the protein that was discovered by us with molecular weight 32 kDa, which in normal state does not have affinity to Ca ions. We have secreted this protein and studied. It turned out, that normally this protein has alkaline reaction, and at pathologies, when incorporating acidic (aspartic and glutamic) amino-acids into its molecules, changes its physical and chemical parameters, and thus, begins to show affinity to Ca ions.

Another important phenomenon, registered by us, is the nature of necrotic damage of myocardium by toxic peptides, generated by the organism itself at pathological states. Thereby, myocardium infarction at pancreatonecrosis is induced under the effect of octapeptide, which is secreted by ischemic pancreas. At pathogenesis of crush syndrome after decompression of soft tissues the protein cleavage of myoglobin takes place, which results in discharge of myoglobin proteolysis products into the bloodstream. These products, formed at reperfusion of compressed muscle, interacting with superoxide radicals, change into toxins and spread all over the organism, causing infarction of myocardium, kidneys, neurodegenerative damage of brain, etc. The study of these four peptides, fragments of myoglobin, showed that the main toxin is nonapeptide, which corresponds to octapeptide at pancreatonecrosis, with a difference in thermal amino acid – arginine, which is absent in the structure of octapeptide.

Thereby, the results we received confirmed our hypothesis, that there is only one way of myocardium damage.

Another field of research in our laboratory covers biochemical disorders at bipolar disorders (BD). The experimental model of BD was worked out together with the Neurological Center of the University of Michigan (under the supervision

of Prof. B. Agranoff). The model, developed by them and based on creation of stress conditions for test animals during 14 days was completed by us on creation of these conditions with a glance at circadian rhythms, i. e. considering the main sensitive to stress biorhythms. The condition of animals was tested on psycho-emotional characteristics. The treatment of animals with BD was done applying synthetic lithium-containing neuroactive peptides and using probiotics (mixture of lacto bacteria compounds). The obtained results are very perspective.

The laboratory received the following grants during its years of activity:

1993, 1995, 1998, 2002, 2008, 2009 – Ministry of Science and Education

1994 – Open Society Institute

1996 – American University in Armenia

1997, 1999, 2000 – International Society for Neurochemistry

2004, 2008, 2010, 2011 – IPP (USA)

For the last 10 years the laboratory staff published 74 articles, mostly in international journals. 14 dissertation works were defended in PhD degree. Four of the laboratory staff now live and work by their specialization in USA, two in Sweden, one in Canada.

Laboratory Of Pathological Biochemistry, Group Of Bioengineering

Head: *Dr. G.V. Gyulkbandanyan, PhD*



A group consisting of four researchers (3 PhD) and post-graduate student conducting research in biochemistry, biophysics and biotechnology in the following areas:

1. Bioengineering (synthesis and testing of biologically active substances in vitro and in vivo)
2. Biotechnology (medical, microbiological, agricultural, nanobiotechnology)
3. The study of the molecular interaction of protein-ligand
4. Computer simulation of macromolecule-ligand interactions

I. BIOENGINEERING

The group conducts research on the physico-chemical, spectral and photophysical properties of proteins, peptides, water- and fat-soluble porphyrin synthetic or natural origin (from nettle and chlorophyll synthesizing microorganisms). Currently the group investigates new photosen-

sitizers of binary action (biochemical and photodynamic) by complexation of porphyrins and peptides for use in photodynamic therapy of tumors (PDT) and photodynamic inactivation (FDI) of hazardous micro-organisms in vitro and in vivo.

II. BIOTECHNOLOGY

a) *Medical Biotechnology*

A wide range of investigations on the efficacy of porphyrins and metalloporphyrins for use in

photodynamic therapy of tumors in vitro and in vivo was carried out. The research group with a

group on the synthesis of porphyrins (Yerevan State Medical University, head of group Dr. R.K.Ghazaryan) showed good anticancer activity of new photosensitizers (porphyrins and their water-soluble metal complexes) in vitro compared to known photosensitizers (chlorin e6, phthalocyanine, etc.). The destruction of tumor via necrotic pathway, as well as via induction

of apoptosis was shown. These porphyrins have been successfully used for photodynamic therapy of tumors in mice with implanted tumor sarcoma S-180 (Crokers sarcoma) in vivo (Fig. 1).

Currently investigations and testing of new water and fat-soluble porphyrins and their complexes with peptides for use in photodynamic therapy of tumors are carried out.



Fig. 1. A mouse with a tumor before and after photodynamic action of porphyrin Zn-TOEt4PyP (72 hours).

b) Microbial Biotechnology

Currently, photodynamic inactivation of microorganisms via photosensitizers (porphyrins) is one of the most promising areas for destruction of antibiotic resistant organisms. For cationic porphyrins and metalloporphyrins with different

positive charge (+2 to +4) and peripheral groups we showed high photodynamic efficacy against both Gram (+) (*Staphylococcus aureus*, *Staphylococcus epidermis*), as well as against Gram (-) (*E. coli*, *Salmonella sp.*) microorganisms (com-

plete destruction of the bacteria at concentrations ranging from 0.5 to 10 μM). High efficacy of photosensitizers against especially dangerous microorganisms has also been shown (Fig. 2).

Currently investigations and testing of new water and fat-soluble porphyrins and their complexes with peptides for use in photodynamic inactivation of bacteria are carried out.

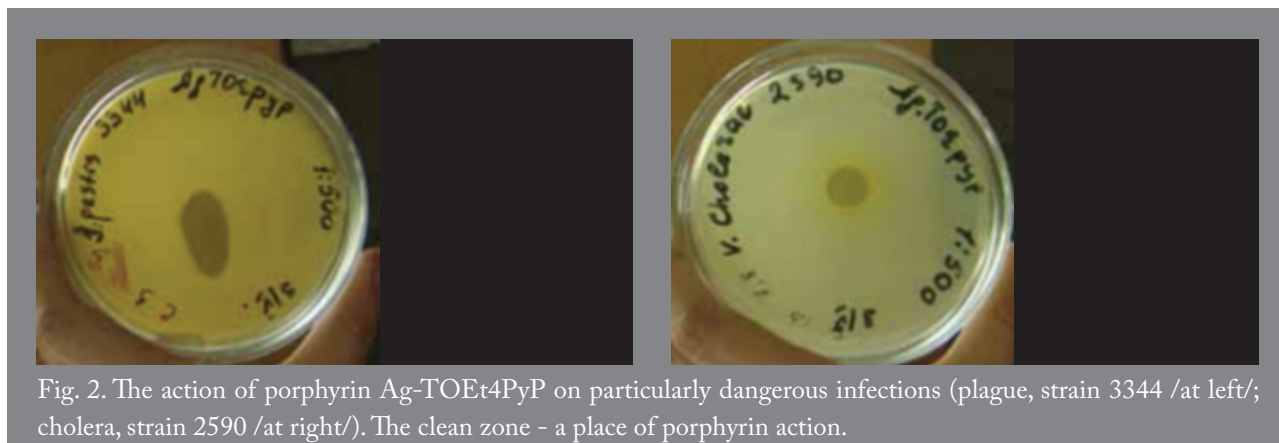


Fig. 2. The action of porphyrin Ag-TOEt4PyP on particularly dangerous infections (plague, strain 3344 /at left/; cholera, strain 2590 /at right/). The clean zone – a place of porphyrin action.

c) *Agricultural biotechnology*

Fungicides, which are used at present against root rot of plants in agriculture are insufficiently effective. They are relatively toxic and are used in high amounts/concentrations. We have developed new approaches and methods of struggling against root rot of plants by means of new synthetic porphyrins (analogs of natural porphyrins).

The results of studies have shown high efficiency of synthetic porphyrins against root rot of plants both in greenhouses and fields (Fig. 3, 4). Increase of harvest for cereal crops (winter wheat) and vegetables (cucumbers, tomatoes and pepper) on the infected soils was about 12-20%.

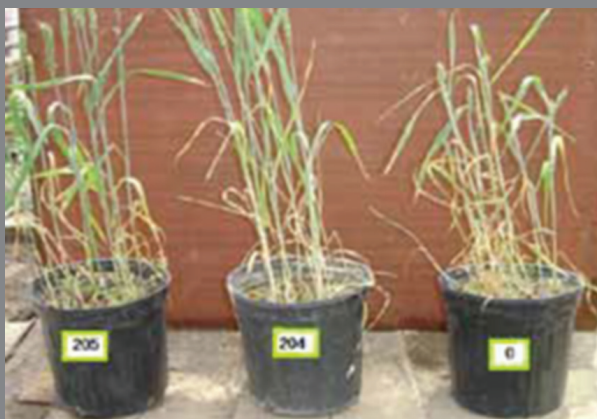


Fig. 3 (on the left). Action of porphyrins on growth of wheat in greenhouse in pots with root rot. 0: control, plants were not treated; 204 and 205: plants were treated by various porphyrins.

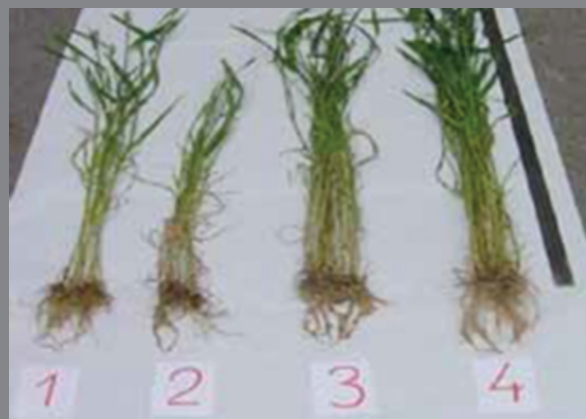


Fig. 4 (on the right). Action of porphyrins on growth of ears and corns of wheat. 1 – plants from healthy field, plants were not treated by porphyrin; 2 – plants from root rot contaminated fields, plants were not treated by porphyrin; 3 and 4 – plants from root rot contaminated fields, plants were treated by porphyrins.

III. STUDY OF MOLECULAR INTERACTION OF PROTEIN-LIGAND

The high affinity of porphyrins to the serum albumin, serum lipoproteins and hemoglobin indicates a role of these proteins as the main endogenous carriers of porphyrins in the realization of photodynamic therapy of tumors. It is obvious that by means of “usually” applied research methods (fluorescence, UV-, VIS-, NMR-spectroscopy, circular dichroism etc.) a large num-

ber of experiments with different proteins and porphyrins at the same time with preserving the identical experimental conditions cannot be carried out. For a large set of synthesized porphyrins and several probable carrier-proteins tasks of complexes formation are divided into multiple tasks to optimize the conditions for binding of each pair of protein–porphyrin and the com-

petitive role of some most important long-chain fatty acids. One of the new effective methods of solving such problems of multidimensional biology is the method of small molecules microarray (SMM). The microarray technology is based on the binding or immobilization of small molecules or proteins to the surface and this allows successfully identifying the interactions of small molecule–protein. For the first time we describe the development and application of the SMM method for studying the interaction of cationic porphyrins with some carrier-proteins of blood, as well as the influence on this process of fatty acids. To study this process in cooperation with

IV. COMPUTER SIMULATION

The method of computer simulation significantly complements experimental methods to predict the possibility of protein–ligand interactions (molecular docking), as well as screening sites of ligand binding. The molecules HSA (www.pdb.org, file ID: 1o9x.pdb) and hemoglobin (www.pdb.org, file ID: 1KOY) were chosen as models for studying the docking of the protein with ligands. As ligands porphyrins with different hydrophobicity and the content of hydroxyl groups, as well as the most important fatty acids of blood (stearic and palmitic acids) were selected. The docking procedures and visualization was per-

the company ProtNeteomix Co. (Nantes, France, Prof. Sakanyan V.A.) we designed and created the first porphyrin microchip (Fig. 5).

Via the method of SMM it was shown that long-chain fatty acids (palmitate, stearate) can compete with porphyrins for binding sites on molecules of serum albumin and hemoglobin, greatly reducing the degree of binding of protein–porphyrin pair. This fact should be considered in photodynamic therapy of tumors and in the case of calculation the dose of photosensitizer, apparently, it is necessary to take into account the level of fatty acids in the blood of a patient.

formed by means of the Graphical User Interface AutodockTools1.4.5. Program (ADT) (<http://mgltools.scripps.edu/>), using in it the functions AutoGrid and AutoDock. Molecular docking of protein–ligand allowed to get much new information on the binding of porphyrin, fatty acids and their complexes with proteins, as well as to identify possible sites for binding of ligands to albumin (Fig. 6), and hemoglobin.



Fig. 5. The microchip with 30 immobilized porphyrins. Each porphyrin immobilized in spots 4-fold.

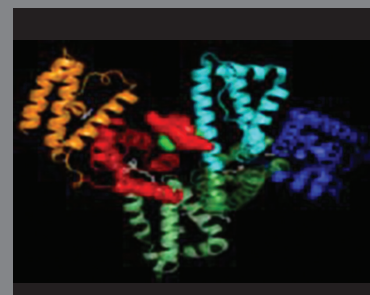


Fig. 6. Docking of the complex [porphyrin+palmitic acid] with a molecule of SA.

Grants

| | |
|-----------|--|
| 2003-2004 | Grant of Armenian National Science and Education Fund in USA (ANSEF), Grant ANSEF No. NS97, Principal Investigator G.V. Gyulhandanyan) |
| 2004-2005 | Grant ANSEF No. 04-NS-biotech-81 5-92, Principal Investigator G.V. Gyulhandanyan |
| 2005-2006 | Grant Du Pont de Nemours International S.A. (USA-Switzerland), Principal Investigator G.V. Gyulhandanyan |
| 2006-2007 | Grant ANSEF No. 839-NS-biotech, Principal Investigator Investigator G.V.Gyulhandanyan |
| 2010-2011 | Grant USA-Armenia (CRDF, BPG STEP), Principal Investigator G.V.Gyulhandanyan Grant Belorussia-Armenia 11PB-016, Principal Investigator .V.Gyulhandanyan |

Gyulhandanyan Aram: FEBS Collaborative Experimental Scholarship 2008 by theme “Study of porphyrins binding to transporter proteins” (Head: Academician of NAS RA, Prof. Vehary Sakanyan, Laboratory of Biotechnology, UMR CNRS 6204, Nantes University, France).

PhD thesis defence

1. A.G.Gyulhandanyan, “Complexation of porphyrins with serum albumin” (specialty “Bioinformatics”), 2010
2. L.Zh.Gyulhandanyan, “Complexation of porphyrins with hemoglobin and cytochrome c” (specialty “Biochemistry”), 2010

PhD students

1. A.H. Muradyan (2005-2008, defended in Germany in 2010)
2. S.O. Gasparyan (2010)

Patents and Publications

1. Over the past 5 years in the field of study the effectiveness of new porphyrins for photodynamic therapy of tumors, inactivation of bacteria and elimination of root rot of plants we obtained 3 Patents in RA.
2. Over the last 5 years we published more than 30 scientific papers, as well as more than 10 presentations at international symposia in Europe, USA and Armenia.

Laboratory Of Pathological Biochemistry, Group Of Myocardium Metabolism

Head: *Dr. A.G.Guevorkian, PhD*

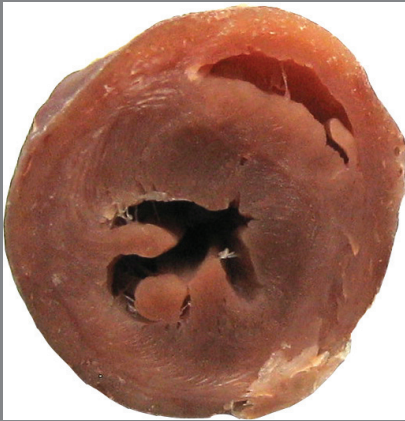


In 2010 Senior Researcher of H.Buniatian Institute of Biochemistry Dr. Artashes Gevorgyan received an invitation from the Director of Center for Vascular Research of Lowe Cancer Research Center of the University of New South Wales (Australia) Professor Levon Khachigian to investigate PRP anti-inflammatory properties during myocardium ischemia reperfusion injury in their center.

For these investigations a model of rat heart's left ventricle vessel ligation was chosen. The animals were divided into three groups: first group had undergone i.p. injection of vehicle (saline). The other two groups were injected two different doses of PRP. Each rat passed an ultrasound heart test to measure left ventricle ejection fraction. After that a ligature was placed on the left ven-

tricle of the rat for 40 minutes. In two minutes after ligature placement each animal had undergone i.p. injection of PRP. In two hours after the microsurgery the ultrasound tests were repeated. The next day, after 24 hours again the ultrasound test was repeated to get data for left ventricle ejection fraction. After that the animal underwent euthanasia, after which its heart was extracted for further histochemical analysis.

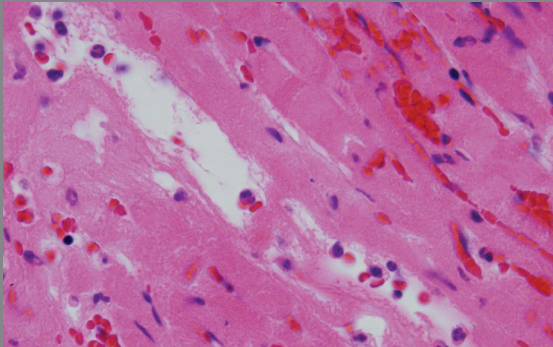
All the data we received was very promising and received a positive feedback from the leading cardiologists, and the further investigation was awarded with a grant from the Government of Armenia to carry out research on pigs.



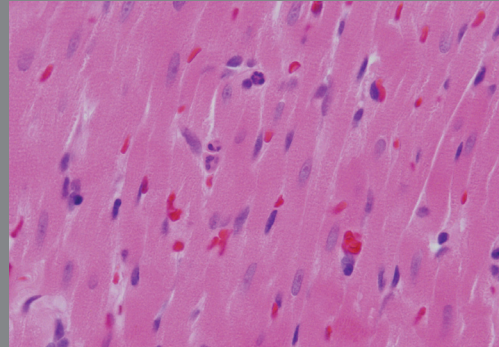
Saline intraperitoneal injection 200 μ



200 μ 4 μ g PRP intraperitoneal injection



Saline intraperitoneal injection – Neutrophils number in the sample is 40 (in average).



4 μ g PRP intraperitoneal injection – Neutrophils number in the sample is 7 (in average).

Laboratory of Lipids

Head: *Konstantin G. Karageuzyan, Academician*



Since January 1, 1962 K.G. Karageuzyan was appointed Deputy Director on science of the Institute and served until 1986. Simultaneously, since May 1965 he was Head of Laboratory on Functional Biochemistry of Nervous System, later on since December of the same year he became Head of Laboratory of Lipids. Under his leadership the laboratory has united over 30 scientists, including: Kazaryan B.A., Hovakimyan S.S., Hovsepyan L.M., Amirkhanyan H.M., Poghosbekova S.D., Amirkhanyan L.T., Aghababova A.A., Babok Yu.V., Alexandryan D.V., Vartanyan G.S., Adonts K.G., Martikyan A.R., Dadayan M.A., Poghosyan A.Yu., and many others.

The scientific area of the laboratory was mainly focused on the problems of neurohumoral regulation of lipids metabolism in norm and pathology (infarction of myocardium, tuberculosis, diabetes, intoxications and stressory situations of different origin, radiation injuries, damages of CNS sympatho-parasympathetic reactions, edema and other pathologic states of the organism).

We have established for the first time the fact of conditioned-reflectory regulation of blood coagulation process, which brought about an essential change in scientific understanding of the discovery. It obtained a general recognition among scientific authoritative staff in the neighboring and other countries, and attained high appreciation by Levon Abgar Orbeli, leader of the neurophysiology, who represented my authorship on that fact in the Proceedings of the

USSR Academy of Sciences, 1958, v. 118, N1, p. 142-145, which became an issue for numerous discussions on international level. Study of biochemical mechanisms for regulatory basis of the hemocoagulation processes revealed a number of new phenomena manifesting an important participation of phospholipids different categories of pro- and antioxidant effect in it. Particular attention was paid in the activity of the Laboratory of Lipids to the study of structural-functional pe-

cularities of choline-containing phospholipids. As it has been first established in joint studies with the Institute of Physiological Chemistry of Cologne University, the GABA introduced intra-carotidally, nitrogen labeled, participates in choline formation involved in the biosynthesis of phosphatidylcholines in CNS, intensely released via blood outflowing from brain and participating in the activation of hemocoagulation processes in it. Based on the indicated changes we have estab-

lished a fact of adrenaline-like effect of GABA introduced into carotid artery with developed arteriovenous difference in the rate of blood coagulation dominating in blood outflowing from brain (v. jugularis interna) in comparison with the one flowing into brain via arteria carotis. The above mentioned achievements were qualified by the Presidium of AS of ArmSSR as outstanding and marked with praiseworthy thanks.

Drug resistant tuberculosis and therapeutic efficacy of newly synthesized compounds with antioxidant activity

The main accent of our investigation was made on the method of combined antioxidant therapy in the clinics and experiments for complicated cavernous forms of lung tuberculosis. It was proposed to arrange study of patients treated with efficient antibacterial preparations known, such as the use of chemopreparations (combination of streptomycin with isoniazid, in some cases with rifampicin, prothionamide, ethambutol, as well as the above indicated combinations of antioxidant effect, sodium thiosulfate known as a-tocopherol synergist (vitamin E), calcium precipitate of ds-RNA obtained by us, etc. Being one of the main components of the endogenous system of the cell antiradical defense, and being damaged by lung

tuberculosis, the a-tocopherol manifests its high activity in the presence of sodium thiosulfate. Besides, the sodium thiosulfate being a supplier of free sulfur in the tissues of the organism, simultaneously plays a role of a reliable stimulator for the activity of other components of the indicated system of cell antiradical defense (glutathione synthase, glutathione reductase, glutathione peroxidase, and reduced glutathione).

We have used some preparations that improve repair processes in lungs and increase immunity, as the recovery of lungs cavity decay takes place not at all patients. One of the substances of that type is Larifan (ds-RNA) and its derivatives as a calcium precipitate of ds-RNA (Ca²⁺-ds-RNA).

That preparation named “Zetapol”, which was synthesized by a group of scientists from the Armenian National Academy of Sciences, appeared to be a very good remedy of antimicrobial and antiviral effect at very different states of the organism. Its main peculiarity is to increase the non-specific resistance of the organism being able to induce the activity of tissue interferon on the level of molecular mechanism. The results of basic researches carried out earlier on studying the peculiarities of combined effect of Larifan and Vitamin E indicated a high therapeutic effect of the method applied for the treatment of the patients as the unique remedy against the foot and mouth disease and extremely effective for tuberculosis (copyrights of the USSR State Registration N 1374494, dated 15.10.1987; N 161738, dated 01.09.1990 on “Method of differential diagnosis of tuberculosis and acute pneumonia”; N 1713585, dated 22.10.1991 on “Method of pulmonary tuberculosis treatment”). It is necessary to mention that the studied compound has a property to stimulate the whole immune system, which is of extra value for providing preventive measurements against viral and infection diseases in military subdivisions.

At present it is supposed to enlarge and perfect the above-mentioned studies for more de-

tailed investigation of phospholipids metabolism in blood certain elements and tissues damaged with tuberculosis in the experimental and clinical issues. The indicated studies were fulfilled simultaneously with the study of the dynamics of processes taken place at lipids free radical oxidation. It is of a special importance to make studies taking into account the known opinion about the role of lipids peroxidation products in the molecular-biological mechanisms of pathogenesis at any diseased state of the organism, especially at drug resistant tuberculosis of lungs. A new search will be realized on revelation and clarification of the specific antioxidant properties of Ca^{2+} -ds-RNA in the etiopathogenesis of tuberculosis process, which will give new information to elucidate delicate biochemical and molecular-biological changes in initiation, development and generalization of pathologic processes. The experiments will be carried out in vivo and in vitro on guinea pigs and rabbits with modeled lung tuberculosis.

So, understanding the situation and urgent need we should like to demonstrate clearly that any new method for prevention and treatment of the disease should be supported both by the local government and international foundations contribution. This phenomenon becomes stronger if to take into consideration the accepted interna-

tional indicators: recent disease trends, quantity of population at risk, international migration, extent of cross-boarder, etc.

The experimental part has been carried out on guinea-pigs, and the immune system response

was studied at the treatment of lungs tuberculosis too (some data were reported at New York Academy of Sciences forum held in 2000, and represented also on the 4th World Congress on Tuberculosis held in Washington in 2002).



Laboratory of Neuropeptides Biochemistry

Head: *Dr. Nina Barkhudaryan, Ph. D., Dr. Sci.*



Laboratory Members:

Sarukhanyan Flora, scientific researcher, assistant to secretary of AAB

Zakaryan Naira, senior scientific researcher

Zakaryan Hermine, junior scientific researcher

Hunanyan Ovsanna, junior scientific researcher

Gevorgyan Hasmik, scientific researcher

In early 1990s several laboratories from different countries have reported about existence of hemorphins, a new family of endogenous non-classical opioid peptides derived from haemoglobin (Hb), in human and animal organisms.

Among those laboratories was the group of Neuropeptides Biochemistry, a research unit of the Institute of Biochemistry, where 5 hemorphins with the following structure were isolated from bovine hypothalamus: VVYPW, VVYPWT, LVVYPWT, VVYPWTQR, LVVYPWTQRF, which represent the fragments 32-36, 32-37, 31-37, 32-39, 31-40 of the b-chain of bovine Hb. The primary structure of mentioned peptides was determined due to collaboration with Dr. Friedrich Lottspeich (Max-Planck Institute of Biochemistry, Martinsried, Germany).

At present a growing body of data demonstrates that hemorphins exhibit a wide spectrum of biological activity by affecting different recep-

tors' function.

The contribution of Laboratory of Neuropeptides Biochemistry to the field of hemorphins research is presented below.

It has been established that hemorphins modulate in vitro the activity of Ca^{2+} /calmodulin(CaM)-dependent enzymes (such as myosin light chain kinase, 3'5'cAMP phosphodiesterase, and Ca^{2+} /CaM- dependent protein phosphatase calcineurin) by binding to CaM (Kd 2-10 nM), demonstrating biphasic response on enzyme activity. Hemorphins are able to induce Ca^{2+} -independent regulation of mentioned enzymes as well, by binding to Ca^{2+} free CaM. It has been shown that hemorphins may affect

the basal activity of calcineurin by interaction with the regulatory sub-unit B of enzyme, which shows 35% structural homology with CaM. Recently, it has been shown that hemorphins regulate calcineurin activity in vivo in pathophysiology of stress, cancer and diabetes.

In 1993 it was revealed that brain high molecular weight aspartic proteinase participate in the generation of LVV-hemorphin-7 from b-chain of bovine Hb by cleavage of Leu30-Leu31 and Phe40-Phe41 bonds in vitro (collaborative work with Dr. F. Lottspeich, DAAD 1992 Research grant). Later, in 2001 it was shown that brain cathepsin B in vitro participates in the generation of hemorphins-7, LVV-hemorphin-5 and hemorphins-5 from LVV-hemorphin-7, acting both as dipeptidyl carboxypeptidase and endopeptidase. It is suggested that the same cascade mechanisms of hemorphins generation take place in vivo as well.

It has been demonstrated that hemorphins are regulators of hypothalamo-pituitary-adrenocortical (HPA) axis by interaction with cytokines (tumor necrosis factor- α (TNF α), interleukin(IL) -2, neuropeptide/ hormone (corticotrophin-releasing factor, CRF), corticosterone and neurotransmitters (serotonin, dopamine). Hemorphins were shown to recover HPA axis activity in pathophysiology of stress and diabetes.

Hemorphins were found to change the sensitivity of calmodulin to its antagonists trifluoperazine, chlorpromazine, vinblastine and vincristine and others. All of these compounds are drugs that are implicated in the treatment of different diseases, including cancer. Therefore, in 1992 hemorphins were predicted to be potential drugs for the treatment of severe diseases without side effect. Indeed later (2006-2011) it has been demonstrated that hemorphins act as homeostatic agents in response to stress, diabetes and cancer (sarcoma-45). Moreover, plasma glucose-lowering effects of LVV-hemorphin-3 and hemorphin-7 were demonstrated. Hemorphins (LVV-hemorphin-3, hemorphin-7 and LVV-hemorphin-7), depending on their structure, differently inhibit tumor growth in sarcoma-45 bearing rats (collaborative work with Dr. H. Stepanyan, Institute of Fine Organic Chemistry NAS RA). Maximal activity in inhibition of tumor growth demonstrates hemorphin-7, which is the most potent among the hemorphins in μ -opioid receptors (MORs) binding. It has been determined that hemorphin-7 regulates DNA binding activity of transcription factors NFAT, AP-1 and NF κ B. It has been proposed that molecular mechanisms of hemorphins action in physiology and pathophysiology involve integration of Ca²⁺/CaM/calcineurin/NFAT pathway

with MORs function. It has been shown that hemorphins (LVV-hemorphin-3, hemorphin-7 and LVV-hemorphin-7) may affect DNA structure by inducing the conformational changes of its molecule (collaborative work with Prof. Y. Dalyan, Yerevan State University, Faculty of Physics). By using modern proteomic methods and approaches it has been demonstrated in collaboration with Dr. Lottspeich (DAAD 2005 Research grant) that intraperitoneal injection of hemorphin changes the expression levels of some proteins (ferritin heavy chain, cyclophilin A and others) in mouse brain (C57BL/6 strain, age 14 weeks, 25-30 g). Importantly, these 2 proteins are known to be involved in pathophysiology of cancer and diabetes. Very recently (collaborative work with Dr. F. Lottspeich, DAAD 2010 Research grant) it has been demonstrated that treatment of streptozotocin-induced diabetic rats with LVV-hemorphin-3 induces the changes in the expression levels of some Ca^{2+} -binding proteins (calbindin 28 kDa, neurocalcins (a and d), complexins I and II) in diabetic rats (Wistar line, 200-220g) brain. It should be noted that

these Ca^{2+} -binding proteins have a functional link with diabetes. In 1997 we proposed that one of the main functions of hemorphins in the cell is the regulation of cytosolic free concentration of Ca^{2+} by binding with CaM. Because all of mentioned Ca^{2+} -binding proteins participated in the regulation of intracellular calcium concentrations, so that finding indicating on regulatory influence of hemorphin on the expression levels of mentioned Ca^{2+} -binding proteins in diabetic rats brain confirm our previous proposition about participation of hemorphins in the regulation of calcium homeostasis.

At present, epidemiologic evidence suggests that cancer incidence is associated with diabetes, as well as certain diabetes risk factors and treatment. It is suggested that diabetes treatments influence the risk of cancer or cancer prognosis. Because hemorphins exhibit both anti-cancer and anti-diabetic effect, it is suggested that they have good prospects for applied medicine for creation of new effective complex drugs without side effects.

Grant Support

1. VolkswagenStiftung Research grant for 2 years (2006 April-2008 March) common work with Department of Molecular Pathology, Institute of Pathology, University Würzburg, Germany on project: Cytosolic partner of calcineurin – regulators of activation, differentiation and carcinogenesis of lymphocytes
2. Research work in the Department of Pharmacology and Psychiatry of the University of Pennsylvania (Philadelphia, PA, U.S.A.), supported by Fulbright Research Program (2002-2003).
3. Max-Planck Society 1993 Research grant, DAAD Research Grants 1992, 2002, 2005, 2010.

Recent Publications

1. Barkhudaryan N., Dosch D., Gabrielyan A., Kellermann J., Lottspeich F. 2008. Study of the effect of hemorphins on mouse brain proteome by using different proteome analysis approaches. Proc. Int. Conf.” Biotechnology and health” & DAAD Alumni seminar, Yerevan, 2008, pp.36-41.
2. Barkhudaryan N.H., Sarukhanyan F.P., Garybyan D.V., Stepanyan H.M., Grigoryan I.E., Dalyan Y.B. 2009. LVVYPW demonstrates the ability to affect DNA structure. Proc. Int. Conf.” Biotechnology and health” & DAAD Alumni seminar, Yerevan, pp. 21-29.
3. Barkhudaryan N., Zakaryan H., Sarukhanyan F., Gabrielyan A., Dosch D., Kellermann J, Lottspeich F. 2010. Hemorphins act as homeostatic agents in response to endotoxin-induced stress. Neurochem. Res. 33:925-933.
4. Barkhudaryan N. H., Hunanyan O.V., Gabrielyan A.V., Serfling E. 2010. Hemorphin-7 regulates DNA-binding activity of NF-AT, AP-1 and NF-κB transcription factors in stimulated Jurkat T cells. Blood (Ministry Health, Republic Armenia), 10:70-74.
5. Sarukhanyan F., Barkhudaryan N. 2011. LVVYPW regulates immune-neuro-endocrine changes in pathophysiology of streptozotocin-induced diabetes. Medical Science Armenia, LI (N2): 63-71.
6. Sarukhanyan F, P. 2011. Serotonin 5HT_{2A} receptors and NMDA glutamate receptors participate in blood glucose lowering effect of LVVYPW in streptozotocin-induced diabetic rats. Blood (Ministry Health, Republic Armenia), 11: 27-31.

Laboratory of Developmental Biochemistry

Head: *Dr. Aprikyan G.V., Dr. Sci.*



The Laboratory of Developmental Neurochemistry was established in 1973 by Doctor Professor Aprikyan G.V.

The first studies of experimental gerontology in Armenia were conducted in this laboratory.

The Laboratory's areas of research include molecular and biochemical mechanisms of action of plant roots of the *Bryonia alba* ("P") and Korean red Ginseng ("G"), their comparative effect on life span prolongation, the regulation of amino acid metabolism, the role of N-acetyl-L-aspartic acid (NAA) in brain function, mechanisms of ammonia formation and neutralization, age-related regulation of the functions of neurotransmitter amino acids, norepinephrine and serotonin.

It was shown that NAA plays an important role in different functional state of CNS and stimulates release of neurotransmitter amino acids from synaptosomes.

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It appeared that the reason of inefficiency of the glutamate-dehydrogenase pathway in ammonia formation in in vitro condition is a result of over reduction of pyridine-nucleotide co-enzymes. Feature of the uptake, release and regulation of neurotransmitter amino acids in nerve endings, in the enriched fractions of neurons and glial cells during aging are established. Data obtained indicated that glial cells, unlike neurons, while retaining the potency of reproduction in aging, partially assume the function of neurons, in particular, the function of neutralization of neurotransmitters after their release into

the synaptic cleft.

The data obtained indicated that although aging reduces the intensity of metabolic processes, the possibility for their regulation is preserved. Studies have shown that during early post-natal development the content of glutamine in the brain is significantly higher than the content of ammonia, however, subsequently it gradually decreasing in old age, becoming less than the amount of ammonia, which is explained by reduced synthesis of glutamine and neutralization of ammonia.

For many years the laboratory conducted diverse studies to elucidate and understand the effect of *Bryonia alba* roots on life span of albino rats in comparison with the effect of Korean red G". Long-term studies have shown that P has the property of prolonging life expectancy. Originally, it was shown that at 50% mortality (LD50) in albino rats at the age of 13 and 18 months roots of "P" prolong survival of the animals by 30-40%. In animals aged 14, 17, 21 and 24 months, P" increases life span from 32 to 41%, with the greatest effect obtained in older (24 month) animals.

Subsequent studies have shown that "P" is rich in free amino acids; including glutamic acid, threonine, tyrosine, arginine, and γ -aminobutyric acid. Published data show that "P" contains many biologically active compounds: cucurbitacins, tetracyclic triterpens and their glycosides, polysaccharides, prostaglandin like compounds, carbohydrates, proteins, polysaccharides, unsaturated fatty acids, etc.

Experiments in the laboratory showed that P" stimulates synthesis of DNA, proteins and cAMP, inhibits lipid peroxidation, increases activity of superoxide dismutase, and activates synaptic transmission with participation of the neurotransmitter amino acids, norepinephrine and serotonin by stimulating their release and in-

hibiting their uptake, especially during aging. P", as well as "G" significantly stimulate oxygen consumption by mitochondria of various organs from young and old animals. Therefore, similar to Korean "G", "P" is a reliable source of compounds that are involved in prolonging the life span. The Laboratory obtained patent of the Russian Federation on the effects and usefulness of "P".

Research conducted jointly with the Institute of Cosmetology in Moscow, showed that P" used as an additive to a cream applied topically for 20-day significantly increases the amount of soluble proteins and total lipids in skin. Data suggested use of P in creams for dry and aging skin.

In recent years laboratory studies are focused on the mechanisms and treatment of the syndrome hyperammonemic liver diseases and encephalopathic complications. A special amino acid mixture was created, which effectively reduces the concentration of ammonia and stimulates synthesis of urea in animals with hyperammonemia syndrome. This effect is significantly enhanced by stimulating the synthesis of ATP. The Laboratory actively continues research in this direction. The results of work performed in the laboratory presented at international conferences by professor Aprikyan GV and colleges in many countries, including Britain, Italy, Czechoslova-

kia, Japan, China, Sweden, USA, Greece and the former Soviet republics. Head of the Laboratory professor Aprikyan was elected as a member of the International Society of Gerontology, European Society of Biochemistry, a member of the International Association of Natural Medicine (Japan), Russian Academy of Natural Sciences (Armenian Branch), a member of the Editorial

Board of journals: “Aging and Longevity” (Kiev) and “Neurochemistry” (Moscow) as well as a member of the Presidium of the SU Society of the Board of Gerontology and Geriatrics (Kiev), scientific council of gerontology of SU Academy of Medical Sciences and section of Neurochemistry of SU Academy of Sciences.

Laboratory of Neurospecific Proteins Biochemistry

Head: *Rima Srapiionian, PhD, Dr. Sci., Professor*



Prof. Srapiionian is an expert in neurochemistry, neuroendocrinology and bioorganic chemistry for over 35 years. She was the first in discovery of the novel family of neurospecific cardioactive protein-hormonal complexes (PHC) in the mammalian hypothalamus. The results of multiyear research work allowed identifying PHC as a biochemical system through which the chemical regulation of metabolism and function of the brain and visceral organs, especially of the heart. Her laboratory has studied various aspects of PHC function in cardiovascular system, in modulation of neurons within the intrinsic cardiac nervous system and participation in adaptation of the organism in a whole. Among the regulatory systems of the heart function are the hypothalamic cardiotropic neurohormones delivered to the heart by humoral mode and via nervous fibers, as well as atrium glycopeptides liberins of hypothalamic neurohormones, taking part in feedback between hypothalamus and heart.

The principal focus of their studies has been the transporting function and preprohormonal role of PHC, because it has been established in our laboratory that the synthesis of highly active neurohormones (NK, NC, NG) occur in the form of precursors without considerable activity, but playing the role of protoactive molecules. Study of some physical, chemical and biochemical properties suggested that neurohormones (NK, NC, NG) apply to a group of multiple forms. Twelve coronary dilator neuropeptides by four in

each group were separated.

On the other hand, the localization of PHC in synaptic fraction and, particularly in neurosecretory granules, might suppose their participation in synaptic activity. We have demonstrated the influence of PHC on hippocampal glycoprotein fucosilation and their involvement in some processes specific for nervous tissues and in mechanism underlying long-term memory formation.

In the past several studies of this laboratory were aimed at classifying the role of PHC's regu-

lation functional state of blood coagulating system, particularly, the effect on hemocoagulation some indexes and FXa step of coagulation cascade. These results imply the intriguing connection between hormonal, endocrine and cardiovascular functions with regulation of hemostasis.

Prof. Srapionian has more than 140 scientific publications, supervised 7 PhDs and possesses

valuable experience of international collaboration; she worked in Germany, Hungary, USA, Russia.

The studies were supported by some grants: RFFR, the International Science Foundation M7B000, ISTC, № A1124, 2004; ISTC, № A1701, 2009.

Laboratory of Embryochemistry

Head: *Prof. Armen A. Simonyan*



Ontophylogenetic relationships of the cell bioenergetic mechanisms

One of the important functions of each cell is the energy generation from the external environment resources, which occurs in the cycle of the oxidation and reduction reactions by synthesis of ATP from ADP and inorganic phosphate.

As a result of years of research in our laboratory important data have been obtained on the bioenergetic mechanisms of different stages of the birds and mammalian ontogenesis and determination of the ontogenetic and phylogenetic evolutionary relationships of these mechanisms. The findings have both fundamental and applied importance, and served as a base for arising of evolutionary bioenergetics in Armenia.

The biochemical features of energy metabolism, respiration and oxidative phosphorylation and related enzymatic systems activity formation and development in embryonic and postembryonic ontogenesis of poultry were shown, in different biological systems directly associated with the formation and development of physiological functions.

The revealing of the relationships of free and phosphorylation linked oxidation in the brain by

Prof. A. Simonyan, appears as one of the natural bioenergetic mechanisms, that provides formed cells with sufficient supply of energy.

A compound of the lipoprotein nature unknown before has been isolated from the bird embryonic liver in certain period of development. It has an important biological role and appears a regulatory factor in developing embryo tissues to maintain the homeostasis of free and bound oxidation reactions.

The important data were obtained regarding the changes of bioenergetic processes in the birds developing embryo cells under the influence of cold stress. It has been shown especially, that moderate cold stress significantly induces the activity of enzymes participating directly in the processes of energy formation and uptake. These data have a fundamental and applied role. On the basis of that theoretical data the new scientifically-based technology of the breeding of poultry chicks was developed. The use of this technology raises the birth-rate of chicks by 8-12 % and increases significantly the viability of offsprings.

The important research was done regarding the ontogenetic changes of humoral regulation of the energy metabolism processes, the relationship of thyroid hormones and mitochondrial function and the biochemical and morphological changes of these processes.

Results obtained in the Laboratory of Embryo-chemistry allowed making an important conclusion for the first time, which has general biological interest. The Haeckel-Muller's biogenetic law, which reflects the evolutionary relationship of ontogenesis and phylogenesis, is completely usable also for the biochemical and, especially, bioenergetic reactions in the cells and subcellular structures at molecular level. This conclusion

explains anew and expands our understanding of the existing morphological, anatomical, embryonic classical basing of evolutionary relationship of ontogenesis and phylogenesis, adding to them the basing related to the biochemical and bioenergetic metabolism. Such a formulation is an important biological discovery.

In recent years, the scope of scientific research of the laboratory was expanded and deepened. It is known that epilepsy and liver cirrhosis are common today in various countries, including Armenia. Investigation of the changes of biochemical and bioenergetic processes in different biological structures of the organism in these pathologies may have not only the fundamental, but also practical importance. The data obtained in our laboratory showed that the attacks of experimental epilepsy bring to alterations of phylogenetically stable condition of phospholipid-phospholipid interactions in membrane structures of brain tissue. The processes of peroxidation in lipid enzymatic and nonenzymatic systems are intensified. The trend of intensification of the reactions of free radical oxidation of lipids is observed. Under the same experimental conditions suddenly increases the activity of enzymes involved in energetic reactions. It is important to note that in case of this pathology background

the administration of the minimum amount of α -tocopherol and its synergist sodium thiosulfate to experimental animals significantly regulates observed metabolic alterations.

It is proposed to use these materials in pre-clinical trial for studied pathology as endogenous therapeutic drugs with antioxidant and antitoxic properties.

The important data regarding the alterations of phospholipid-phospholipid interactions, as well as the changes of enzymatic systems activity involved in bioenergetic metabolism have been obtained also in pathology of experimental liver cirrhosis.

During the past decades the lowering level of Lake Sevan, the abrupt changes of hydrobiological conditions, the intensifying of eutrofication and its consequences has brought to the significant changes of lake fauna and flora. The different anthropogenic stress factors directly affect especially the endemic fish species, survival of which

is exposed to danger. Studying of the influence of changed ecological conditions of the lake on the alterations of biochemical and bioenergetic mechanisms of sustainable systems, which has been formed phylogenetically in endemic fish tissues and subcellular structures, is one of the main research areas of our laboratory. It will allow considering the reasons of the loss of Lake Sevan's endemic fish species from the molecular-biochemical point of view. In conjunction with the other works it will be used for saving these fish species and increasing their reserves.

In parallel with research works Prof. Simonyan has performed significant work also in the area of biological vocabulary composing in Armenian. He has published the "Russian-Armenian biochemical defining dictionary" (over 1500 terms), "Russian-English-Armenian biological defining dictionary" (over 7000 terms), as well as "English-Russian-Latin-Armenian biological dictionary" (over 80 000 terms).

Laboratory of “Metabolism of Active Oxygen”

Head: *Prof. M.A. Simonyan*



The laboratory is founded on the basis of scientific-productive group “Biooxidant” of the Institute of Biochemistry of NAS RA in 1995. By the methods developed by us we have obtained and sent to users 250 g of electrophoretic homogenic antioxidant enzymes and other metalloproteins for the total sum of USD 600 000. We developed new methods of obtaining well-known metalloproteins of antioxidant activity (MAA) (CuZn-SOD, Mn-SOD, catalase, ceruloplasmin and transferrin) and new types of metalloproteins of pro-oxidative activity – MPA (isoforms of NADPH oxidase, super-oxid- producing lipoprotein of blood serum suprol of mammals, cytochrome b5) for analytic and preparative needs.

For the first time electrophoretic homogenic NADPH oxidase (cytochrome b558) were obtained from erythrocyte membranes, nuclei and mitochondria of immune system organs cells (spleen, bone marrow, etc.) as well as other cells. Using these methods molecular-biochemical mechanisms of blood oxidative damages and tissue cells of different pathologies (malignant tumors, cardiovascular, kidney diseases, diabet, intoxications with heavy metals, carbon dioxide, radiation damages of different origin etc.) were determined. These mechanisms associated with peculiar quantitative and qualitative changes of MAA and MPA, which can be used as new

and sensitive diagnostic tests at diseases and as markers of drug validity. For the first time the existence of extracellular NADPH oxidase was shown, formed at different diseases with decreasing the erythrocyte stability. NADPH oxidases are extracted both from normal cells, as well as from atypical cells (from malignant tumor cells: lymphosarcoma, stomach, small intestine cancer, lung and Ehrlich ascites tumor, sarcoma-45, sarcoma-37, etc.). For the first time ferryHb-reducing properties of NDPH oxidase are shown. That indicates the importance of NADPH oxidase as for immune system, as well as for oxidative homeostasis regulation, mitochondria and genome

redox processes. The releasing phenomenon of NADPH oxidase from membrane, nuclei and mitochondria of immune system organs cells and other cell types (liver, heart, kidneys) close to physiological conditions is detected. It serves as the basis for revising the concept, that NADPH oxidases are not “aborigines”, but they are “new-comers”. At the weakness of erythrocyte stability the unstable complex formation of NADPH oxidase with Hb is observed in damaged parts of erythrocytes (a result of unsaturated fatty acid peroxidation). That type of effect is observed particularly at malignant tumors. The activation and inactivation mechanisms of NADPH oxidases are determined. Inactivation factors (gamma irradiation, ultrasound, CO₂ intoxications) induce irreversible aggregation of NADPH oxidase. NADPH oxidase activators (laser irradiation, some cytokines, including proline rich peptide – PRP) in many cases have the antioxidant effect via catching HO radicals, like PRP. In this case NADPH oxidase is a receptor for PRP, which has the immune-modulating effect and stimulates oxidative homeostasis by increasing the NADPH depending O₂-producing and ferr-iHb-reducing activity of NADPH oxidase. According to the obtained results the new concept

of malignant tumor autotherapy in post-operational period against the metastases is put forth: 1) After the removal of the tumor tissue extract suprol from the blood (80-100 ml) of the patient, activate in vitro with transmitting metal tracks for O₂ producing and inject it intravenously (produced O₂ will cause lipid peroxidation of metastases membranes, promoting their apoptosis. 2) After the removal of the tumor tissue induce the antibody formation against intravenously injected isoform of NADPH oxidase (or cyt b558), obtained from the cells of the removed tumor tissue of the patient. In the future we plan to approve and apply these conceptions in the experiment and clinics. The molecular-biochemical mechanisms of NADPH oxidase extracellular formation, as a response of blood adaptation system at pathology, will be determined. The production of NADPH oxidases by developed methods will be set for commercial purposes (1g of a peptide is USD 100 000). We have participated in 3 ISTC granted projects. Seven candidate dissertations were defended referring the problem, 2 more will come soon. 52 scientific papers have been published in local and foreign journals during the period from 2005 to 2011.

Laboratory of adenyline compounds metabolism

Head: *Mardanyan Sona*



List of researchers:

Sargisova Yelizaveta, Ph.D; leading researcher
Sharoyan Svetlana, Ph.D; senior researcher
Hayrapetyan Hripsime, Ph.D.; senior researcher
Antonyan Alvard, Ph.D; leading researcher
Andreasyan Nune, Ph.D; senior researcher
Harutyunyan Hayk, Ph.D; senior researcher
Movsisyan Naira, MS student; senior laboratory assistant
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6. Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran.

intra-republic

1. L. Mnjoyan Institute of Fine Organic Chemistry, Armenian NAS
2. Faculty of Chemistry, Department of Physical Chemistry, Yerevan State University
3. Institute of Molecular Biology, Armenian NAS.

Grants

1. 1997- CAEN Grant of the International Union of Neurochemistry
2. 1999- NFSAT Award N ISIB 03-02
3. 2000- ANSEF Grant No 04-NS-biochem-812-38
4. 2001- NATO SCIENCE Collaborative Linkage Grant No. 977079
5. 2004- NATO SCIENCE Collaborative Linkage Grant No. 979813
6. 2009- CAEN Grant of the International Union of Neurochemistry
7. 2010- DAAD award
8. 2011-1f105 Grant of the Ministry of Education and Science of Republic of Armenia.

Publications

2006-2011: 11 articles in peer review Journals and 11 abstracts in international conferences.

The main achievements

Two Adenosine Deaminase isoenzymes and related to them Dipeptidyl Peptidases II and IV, identical with the antigen CD26 on activated T-cells, are the subjects of researches at the Laboratory for several years. We are working on elucidation of the molecular and catalytic properties of the enzymes, their physiological role, and probability of their use in medicine.

Relation to pathologies: a) The monitoring of ADA activity in the pleural fluids of Armenian tuberculosis Hospital manifested the usefulness of application of this index in differentials diagnosis of tuberculosis. b) At in vivo experimental chronic and acute aluminum toxicosis in rats, the ADA1 activity in brain decreased, the change

of DPP activity was negligible, but these activities in the blood plasma increased significantly at acute, and moderately – at chronic toxicosis. c) The involvement of ADA1 and ADA2 in the post-ischemic systemic inflammatory response and the association of functional ADA1 nt22 G>A polymorphism were studied. The significant elevation of ADA2 and non-significant of ADA1 levels in the blood of stroke patients was shown. In the studied groups, the genotype frequencies for nt22 G>A did not differ. The data indicated that ADA2 might be involved in the pathogenesis of ischemic stroke.

Interaction with substrates a) A proline-rich cytokine from neurosecretory granules of bovine

neurohypophysis, PRP-1, was shown as a new natural substrate for DPPIV, but not for DPPII. The catalytic parameters of catalyzed by DPPIV enzymatic breakdown of PRP-1 were evaluated. b) The kinetic parameters of ADA1 free and in ADA1/DPPIV-CD26 complex showed higher affinity of substrates to the complex. Together with a stronger activity of some inhibitors towards the complex, these data proved that the complex formation enhances the neutralization of toxic nucleosides in the extracellular medium. c) Conformation of ADA1 free and in the presence of inhibitors was studied by the method of selective quenching of fluorescence emission. The manifestation of substantial distortion in local environment of Trp residues in ADA1/inhibitor complexes relative to free ADA1 was received. d) The influence of reactive oxygen producers on the activity of ADA2 in vitro showed the increase of substrate affinity to the enzyme. The participation of SH-groups of ADA2 in this effect was excluded. The components of extracellular matrix strengthened this activation in vitro, proposing a probable role of protein environment in oxidative stress in vivo.

Interaction with inhibitors: a) The influence of Armenian highland plants used in folk medicine and/or as a food on the activities of DPPIV and ADA1 was studied. The values of IC₅₀ were evaluated. The obtained results allow recommending some selected plants for use at treatment of

diabetes mellitus in combination with the anti-diabetic drugs. b) The influence on the activity of ADA1 of more than 30 compounds, synthesized at the Institute of Fine Organic Compounds of Armenian NAS, was studied. The values of IC₅₀ were determined. Some structure-functional relationships were observed. Unusual difference in inhibition of catalytically identical high- and low-molecular ADA1 was observed. c) The inhibition of DPPII and DPPIV with several flavonoids was studied, Shtern-Volmer constants for quenching of Trp fluorescence at titration with flavonoids were determined.

Relation to immune system: a) The similarity of the dynamics of IgG to that of DPPIV-CD26, and of the dynamics of IgM to that of ADA1 at immunization of rats with human erythrocytes, suggested the probability of ADA1 participation in Th2, and of DPPIV – in both Th2 and Th1 immunity. b) The responsibility of human blood monocytes for activation of ADA2 at inflammation, and importance of monocytes interaction with other cells for this activation was shown. Correlation of ADA2 activity dynamics with the dynamics of IgG at human measles vaccination was shown.

Interaction between components: Using a resonant mirror biosensor, fluorescence polarization, and differential spectroscopy techniques, the ability of DPPII to bind ADA1, similar to DPPIV, was shown for the first time.

Laboratory of enzyme activity regulation

Head: *Kamalyan R.G., Prof.*



List of researchers:

Vardanyan Anahit, Ph.D; researcher

Khachatryan Narine, junior researcher

Publications

2006-2011: 10 articles in scientific journals and 7 abstracts in conferences.

The main direction of investigation is the metabolism of neurotransmitter amino acids, its regulation and the role in the brain and the immune system function.

In the model experiments with mitochondrial and synaptosomal fractions of rat brain cortex we could demonstrate the evidence of alternative way of GABA formation from glutamine.

In our experiments the usage of the inhibition of phosphate activated glutaminase (PAG) by 6-diazo-5-oxonorleucine (DON) did not influence the output of GABA from glutamine. The data obtained were support by the investigations of other authors, which have shown the absence of phosphate and hydrogen ions action on the ^{15}N -GABA formation from ^{15}N -glutamine, 5 times higher output (issue) of $^{14}\text{CO}_2$ from ^{14}C -glutamine compare ^{14}C -glutamate in hippocampus slices.

It has been established that inhibitor of GABA-T ethanolamine-O-sulfate (EOS) injection leads to elevation of GABA level in the brain mito-

chondrial and particularly synaptosomal fraction. It is important to note that we did not observe any statistically significant decrease in glutamine concentration in those experiments. In the same time incubation of synaptosomes in the presence of PAG activator phosphate stimulates glutamine utilization without GABA generation. Pyridoxalphosphate (PLP) also promotes glutamine utilization, but with elevation of GABA level, especially of rats received in vivo EOS.

GABA generation from glutamine was more pronounced in EOS treated rats in the presence of both phosphate and PLP.

Our data represent evidence for GABA genera-

tion from glutamine without PAG participation.

The higher production of $^{14}\text{CO}_2$ from glutamine in comparison of glutamate supports existence of direct way of glutamine decarboxylation. The evidence of that way suggests GABA-amid formation and its presence in the brain. We have shown the presence of GABA-amid in the brain with using the methods of high voltage paper electrophoresis and HPLC. As a standard GABA-amid, synthesized by direct ammonolysis of GABA, was used. GABA-amid was poured by ion-exchange chromatography and identified by elementary analysis, mass-spectroscopy and NMR-spectroscopy methods. GABA-amid and related compounds (glutamine, GABA, EOS) were used in electrophysiological experiments. It was shown that GABA-amid leads to inhibition the neuronal activity after some latent period. These experiments testified that GABA-amid is an inactive form of GABA in brain. The GABA-amid utilization in brain cortex mitochondria was studied. The ammonia generation from GABA-amid was shown. This process is more pronounced in aluminium intoxication rats. Reaction inhibits by GABA. The last was utilized in mitochondria in presence of ammonia and ATP. The data obtained testify GABA-amid formation from GABA and deamidation of last in the brain mitochondria.

The alternative way of GABA formation from glutamine more pronounced in the aluminum neurointoxication and possibly in other brain disorders.

It was shown that glutamine family amino acids involve in the pathogenesis of aluminum intoxication. The aluminum administration to rat is accompanied with essential changes of amino acids content and activities of its metabolism enzymes, glutamate decarboxylase and PAG. The general change is GABA content increase mainly by GABA-amid. It is accompanied by decrease in PAG activity and increase in glutamate decarboxylase activity. The experiments with transgenic mouse with preference synthesis of amyloid precursor protein (APP) were shown more vulnerability of glutamateergic mechanisms in comparison with GABA-ergic.

Thus, the generally accepted way of GABA formation from glutamine is glutamine[®]glutamate[®]GABA. Alternative pathway is glutamine[®]GABA-amid[®]GABA. The latter way escapes intermediate formation of glutamate and protects GABA-ergic neurons from excitotoxic effects of glutamate and ammonia.

The evidence of this metabolic pathway of glutamine suggested the study of its regulation. Regulation of this pathway may have an important role in the GABA-glutamate ratio in different psychotic and neurodegenerative disorders.

In other series of experiments the reciprocal regulation by glutamate and α -ketoglutarate the succinate dependent Ca^{2+} uptake in animal heart, liver and brain homogenates and mitochondria was exposed. This phenomenon is based on the initiation of the influx and efflux of the oxaloac-

etate in the aspartatetransaminase reaction. This is supported by sensibility of the Ca^{2+} uptake to transaminase inhibitor aminooxiacetate. The participation of transamination in the mechanism of regulation and support of Ca^{2+} homeostasis in mitochondria was examined.

Eye Research Laboratory

Head: *Petrosian Andranik Ph.D.*



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Later on they discovered that tauret is a natural component of the eye retina and patents are received in USA, Canada and in some countries of European Union and also they received a patent in Armenia. In successful development, due to these studies, it will be possible to create a medicine for human vision recovery.

A.A.Petrossian managed many projects in mentioned direction; he had a numbers of international grants, as 2002-2004 – budget grant of Rep.of Armenia 0757; 2005 – 2007 budget grant

Since 1978, after returning from Saint-Petersburg Dr.Andranik Petrossian, has lead the investigations in the group of Neurochemistry of the retina dedicated to the neuroactive amino acids. Later on the base of this group the Eye Research laboratory was created.

In 1986 Dr. Andranik Petrossian and Dr. Jasmine Haroutounian discovered tauret and by their suggestion tauret was synthetized. It is a substance, where taurine is connected via Schiff bound with the derivative of vitamine A- retinaldehyde. Then it was shown that tauret – retinilidentaurine allows to accelerate vitamine A turnover in the visual cells of the eye and and can regenerate the visual cells after light induced damage.

of Rep. of Armenia 0629; 1994 - Fellowship Grant of American Association for Advancement of Science (AAAS); 1997 - Research Grant of Norwegian Foundation of Science, Oslo; 2003 - ANSEF Grant No. NS 32 & 2005 - Grant 05-NS Biochem 821-60; 2003 - FEI – Philips Company Technical Grant - Transmission electron microscope Philips CM-10 Crio; 2007 Grant of Eco-Net, EGIDE joint with Dr. Thierry Leveillard INSERM, France;

A.M.Petrossian supervised the Ph.D thesis

“The comparative study of the role of taurine and its content in different structures of the eye”. He participated in many conferences devoted to taurine and primary mechanisms of vision- in Russia, Japan, and USA. Norway, Germany, Italy, Mexico and others.

During last years A.Petrosian initiated cooperation with Armenian, Russian, American and French scientists to jointly use taurine and its derivatives in ophthalmology.

A.M.Petrosian made systematic efforts to renew instrumentary in his lab. In 2008 jointly with Thierry Leveillard (France), Professor Armen Vartanian (Armenia), he organized an International Symposium devoted to the Defense mechanisms of retina. Leading specialists from Armenia, Russia, USA, France and Great Britain were participants of the symposium.

Dr.Petrosian has many patents in different countries concerning taurine and tauret application in ophthalmology.

Patents & Applications:

Taurine derivatives usable in the treatment of ophthalmic disorders.

| | |
|------|--|
| 1999 | Canada Patent. - CA2312842 A1 - 1999-06-17 |
| 2001 | Japan Patent Appl - JP2001525362T T - 2001-12-11 |
| 2002 | European Patent EP1037622 2002-11-06 |
| 2003 | China Patent Appl. CN 1290162T 2001-04-04 |
| 2002 | USA Patent No: 6,391.924 B1 Issue - 2002 -05- 21 |
| 2007 | France, Germany, Great Britain, Italy Patent 1037622 |
| 2010 | Armenia Patent 2379A |

Group of biochemistry of membrane proteins

Head: *Manukian Karine H., MD, leading scientist*



Study Of Distribution, Some Properties And Specific Features Of Protein And Lipid Moieties Of Proteolipids From Cellular Membranes Of Nervous And Other Tissues

Members of group

Kazarian Tatiana I., PhD, senior scientist

Kirakosian Laura G., PhD, senior scientist

Levonian Karine L., senior scientist

Stepanian Hasmik A., senior scientist

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The main problem of long-term investigations carried out by the group of biochemistry of membrane proteins was the study of widely distributed hydrophobic integral membrane proteins – proteolipids (PLs) and lipids bound with them in cellular membranes of nervous tissue (especially of myelin, where PLs are most abundant) and other tissue in normal and pathological states, as well as during ontogeny.

The data were obtained about localization, some physico-chemical properties, protein composition, molecular weight, amino acid composition, terminal amino acids, as well as lipid and especially phospholipid composition of PLs, isolated

from different parts of nervous system, various subcellular particles of brain (two fractions of myelin, mitochondria, microsomes, synaptosomes, synaptic membranes, synaptic vesicles, nuclei) and from other organs (heart, liver, kidney, lymphoid organs etc.) and their subcellular particles. It was shown that parallel with some similarities there are definite differences in protein, as well as in lipid, especially phospholipid, moieties of PLs from various cellular membranes depending on their localization and possible function. On ground of results obtained the peculiarities of the lipid components of PLs from different cellular membranes were characterized for the first time.

It was shown that the lipid moiety of all PLs studied is composed mainly from phospholipids, it contained also glycolipids and cholesterol. Loosely bound with PL proteins (PLPs) are cholesterol, cerebroside and neutral phospholipids (phosphatidylcholine, sphingomyelin, phosphatidylethanolamine), more tightly bound-acidic phospholipids and sulfatides. Characteristic features of lipid composition of PLs from different cellular membranes depend mainly on content and correlation of tightly bound with PLPs acidic phospholipids, especially, phosphatidylserine, diphosphatidylglycerol, phosphatidylinositol and inositol 4,5-bisphosphate.

It must be noted that lipids bound with PLPs probably play an important role in preserving the conformational integrity of these membrane proteins and their functions.

Definite differences between the rate of in vivo incorporation of Pi^{32} into the phospholipids of total lipid extracts and phospholipids, loosely and especially more tightly bound (phosphatidylserine, diphosphatidylglycerol, phosphatidylinositol) with PLPs of whole brain were obtained.

Our recent investigations revealed peculiarities of protein and lipid (phospholipid) moieties of PLs from endoplasmic reticulum, as well as from mitochondria of various functionally differing

organs (brain, heart, liver, kidney). They showed that the prevalence in the lipid fractions more tightly bound with PLPs of one or both of the two acidic phospholipids, phosphatidylserine and phosphatidylinositol in the case of microsomes, or of diphosphatidylglycerol and phosphatidylinositol in the case of mitochondria is the characteristic feature of PLs from these subcellular particles of organs studied. In the case of endoplasmic reticulum the most differences were observed between PLs of brain microsomes and heart sarcoplasmic reticulum. PLs of brain microsomes (where myelin PLs are synthesized) by their protein and lipid composition have many similarities with myelin PLs and like to them were characterized by prevalence of only one tightly bound phospholipid-phosphatidylserine, while in heart sarcoplasmic reticulum – phosphatidylinositol predominated.

During last years peculiarities of PLs from the primary (thymus) and secondary (spleen, lymphatic node) lymphoid organs of some mammals were also studied. Now in definite calls of these three organs expression of major myelin PLP and its isoform, protein DM-20, was revealed. These proteins parallel with myelin basic protein are antigens of autoimmune diseases including multiple sclerosis in humans. Our investigations at first showed that PLs are present in quite defi-

nite amounts, comparable with other organs, in all of lymphoid organs of some mammals studied. Like other organs loosely bound with PLPs are neutral phospholipids, more tightly – acidic, especially two of them, phosphatidylserine and phosphatidylinositol. These two phospholipids prevailed in more tightly bound with PLPs lipid fractions of PLs from thymus and spleen, while in lymphatic node, like to PLs from brain and its myelin, phosphatidylserine predominated. Conclusion was made that in tissues of immune system besides other cellular membranes of PLs, myelin PLs are also present.

The data obtained in the course of investigations of specific features of PLs from different cellular membranes bring us to conclusion about the existence of several types of PLs in functionally different cellular membranes of nervous and other tissues, which differ in their properties and specific features of protein and especially lipid (phospholipid) components. Three of them are most pronounced: one – in myelin, the second – in mitochondria, the third – in heart sarcoplasmic reticulum. The first is characterized by predominance of phosphatidylserine in more tightly bound with PLPs phospholipid fraction, the second – of diphosphatidylglycerol, the third – of phosphatidylinositol. One more new PL was isolated by us from brain cell nuclei.

In the course of investigations, concerning the study of brain PLs during development of some nervous system pathologies, the data were obtained about considerable decrease of quantity of PLPs and phospholipids loosely and more tightly bound with them in neocortex and some more in hippocampus during development of such a heavy affection of brain as aluminum neurotoxicosis, which is recognized as one of the possible models of Alzheimer disease.

Another important plane of several years' investigations concerns the study of PLs from brain and other organs during postnatal development of rat. Some regular features of changes of concentration, as well as of composition of protein and lipid moieties of PLs were elucidated. Special attention was paid to study of formation of protein and mainly of phospholipid moieties of PLs from myelin, microsomes (where myelin PLs are synthesized) and premyelin fractions (SN-4) during myelinogenesis and effects of inhibitors of protein synthesis (cycloheximide) on these processes.

In order to elucidate the possible role of PLs in structure and functions of cellular membranes (especially of myelin) stable lipid – PL artificial flat membranes were reconstructed from lipid – PL mixtures of cattle brain white matter. Contact interaction of two membranes was studied. It was

shown that PLs together with the bound lipids may form the zones of high conductivity for cations, especially for K^+ .

The goal of investigations planned to be fulfilled in future is the further study of specific features of PLs from different cellular membranes of

brain and other organs in normal and pathological states for future characterization and classification of these unique proteins, as well as investigation of possible functions of PLs and lipids bound with them in cellular membranes.



Group of Physical Chemistry of Proteins

Head: *Gasparyan V. K., Ph.D.*



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It means that processes that will take place on the surface of these nanoparticles will bring to changes in bands of SPR. Moreover, these nanoparticles have affinity for SH groups and as a result the protein molecules that usually contain such groups can be easily immobilized on their surface. As a rule immobilization on gold nanoparticles does not influence the properties of bounded biomolecules.

We have applied these properties of gold and silver nanoparticles for medical diagnosis for determination of various diagnostic relevant an-

Nanotechnology is a revolutionary direction in modern science that opens new horizons in biology and medicine. This direction involves research and development of materials, sizes of which are extended from 1 to 100 nm. These sizes are comparable with sizes of molecules and these materials bound with appropriate molecules can be applied for investigation of molecular processes in vitro, as well as of processes that take place in live cells without interference of their functions.

Moreover, in such nanoscale materials optical properties are determined by boundary processes so-called surface plasmon resonance (SPR).

In this aspect gold and silver nanoparticles have particular interest for scientists. SPR bands of these nanoparticles lie in visible region.

tigens. At first stage we have prepared gold or silver nanoparticles by reduction of appropriate metal salts with some reducers. As a result stable nanoparticles were obtained, which were applied further for immobilization of antibodies. In particular for immobilization we have used antibodies to C-reactive protein, myoglobin and h-IgG. Addition of biological fluid containing antigens to noted antibodies brought to interaction of antigen-antibody and, as a result, to drastic changes in optical spectra of metal nanoparticles. These changes correlated with concentration of these

antigens. A diagnostic test system was developed for specific and sensitive detection of noted antigens. Then the investigations were conducted with the aim to improve this assay. The developed approaches permit oriented immobilization of affinity purified antibodies on the surface of these nanoparticles. As a result all antibodies immobilized on the surface of metal nanoparticles retained their antigen binding properties and sensitivity of assay was significantly increased.

Further immobilization of DNA and DNP on these nanoparticles was conducted and this system was applied for diagnosis of some autoimmune diseases.

At this time new nanoparticles of these metals

are prepared (nanorods, nanodisks), which have more interesting optical properties that extend their application as for drug delivery, as well as for treatment of some diseases.

Moreover, we are involved in investigation of processes of apoptosis and factors that have apoptotic and anti-apoptotic action. A technology was developed for detection of early stages of apoptosis based on annexin V. The principle of detection is based on the fact that the first stages of apoptosis are characterized by translocation of phosphatidylserine from inner part of plasma membrane to outer part and annexin V is able to bind this phospholipids. Now effects of some apoptosis inducing factors are studied.

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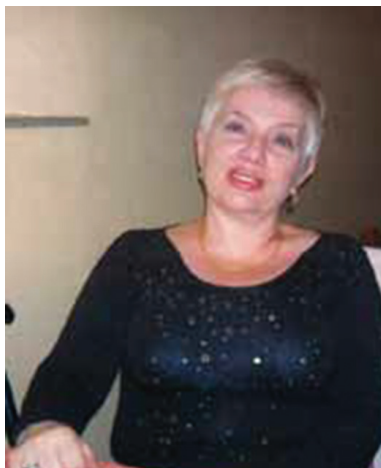
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Microbiology Research Group

Head: *Agnessa A. Agababova, PhD.*



Publications (2001-2011): 30

Bacterial translocation and its role in formation of symbiotic relations with microorganisms

E.coli is one of the most studied types of the microorganisms, but its role in the development of several inflammatory diseases remains to be elucidated. According to our findings the negative consequences of microorganism translocation comes only when bacteremia develops and specific clones of E.coli are involved in the processes of bacterial migration with both protective and negative effects: elevated sulfur-resistance, factor of bacterial persistence, toxins e.t.c.

Thus, E.coli facilitates intestinal and abenteric escherichioses differing from each other by feature of symbiotic relations with macroorganism. As a rule, the first one forms parasite – hostal relations, whereas the second one comes into commensal relations with the host.

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Principles of E. coli interaction with erythrocytes in acute leucosis

We have obtained the data concerning the function of system “bacterium-erythrocyte” taking into account the level of expression of the microorganism’s properties and their effects on the erythrocyte from patients with leucosis and observed anizocitosa and pokilocitosa. During

the investigation of the interaction of E. coli and leukemic erythrocytes we have found the phenomenon of intraerythrocyte location of bacteria and destroying of the membranes of erythrocytes and changes in their pathomorphology.

Effect of low-level laser irradiation on rat gut microflora

We have demonstrated that a single irradiation by SCL provokes time-dependent changes in microflora of healthy rats, completely suppresses the growth of normally occurring resident strain of *E. coli* and stimulates that of lactose-negative forms that indicate to a mild stressor effect of LLLT, which may influence the microbial spe-

cies, particularly in gut micro-flora there through interference with host homeostasis and the right balance of immune system kept by microbes, which might be involved in the development of infectious processes under altered physiological circumstances.

Morpho-hystochemical pattern in mice ascite carcinoma

In 2011 a new study in this direction has been initiated.

Research Group Of Nad(P)/Nad(P)H-Dependent Systems

Head : *Nina H. Movsesyan Ph.D.*



45 publications (2000-2011)

Nitric oxide (NO) is an important messenger molecule generated from L-arginine by calcium-dependent constitutive forms of NO synthase (cNOS), neuronal (nNOS) involved in neurotransmission and endothelial (eNOS) regulated vascular tone and tissue perfusion in nervous system and calcium-independent inducible form (iNOS) contributed to the innate immune response following a variety of infectious or inflammatory insults.

The iNOS appears to be the only form that can synthesize high levels of NO for sustained periods in the immune cells that could be pathogenic in states of systemic inflammation. Recent studies highlight the importance of differing pathological effects driven by the different NOS isoforms, when assessing their roles in neuroinflammation/degeneration, and immunosuppression, as well.

Compression-Injury (CI) syndrome is characterized by persistent pain, cardiovascular, neuroendocrine, and immunological systemic responses, the outcome of which depends on a number of factors including the health of the individual and therapeutic approaches used to

overcome this injury. A crucially important participatory component of these responses involves the characterization of L-arginine-dependent synthesis of NO.

L-arginine-dependent NO synthesis and D-glucose Uptake in Rat Immune Cells Following Muscle Compression Injury and Treatment with Newly Synthesized N α -para-butoxybenzoyl L-Arginine Lithium and Sodium Salts

Newly synthesized inhibitors of nitric oxide synthase (NOS) activity sodium N α -para-butoxybenzoyl-L-argininate (NaNPBA) and lithium N α -para-butoxybenzoyl-L-argininate (LiNPBA) were examined following treatment of rats with regard to their ability to modify activity the calcium-calmodulin-independent “inducible” NOS (iNOS) and D-glucose uptake in rat leukocytes: tissue-derived thymocytes and splenocytes, as well as blood-derived lymphocytes, monocytes, and neutrophils prior to compression injury (CI) and 2 and 24 h following CI. These examinations were performed using the Griess-Ilosvay reaction to measure the level of nitrogene oxides (NO x) and related nitrosocompounds (NC), as well as L-arginine-dependent production of NO x and NC during long-term incubation of mentioned cells isolated from rats treated with 0,9% NaCL

(saline) (control rats), NaNPBA, or LiNPBA. Both NaNPBA and LiNPBA modified iNOS produced NO x and related NC, however the pattern of these changes varied based upon cell type, when compared to cells obtained from saline-treated control rats either prior to or following CI. D-glucose uptake by these cells was also affected by NaNPBA and LiNPBA treatments and the pattern of changes in D-glucose uptake also varied based upon cell type, when compared to cells obtained from saline-treated control rats prior to and following CI. It is concluded that the iNOS activity, level of NO x and related NC and D-glucose absorbance of rat immune cells are linked and contributed to pathophysiological changes in CI and could be modulated by novel N α -substituted arginine derivative salts, NaNPBA and LiNPBA in early stages of CI.

D-Glucose Uptake and Nitrergic Response in Brain, Blood and Bone Marrow following *Clostridium perfringens* Infection. Effects of Red Light Irradiation

A single intra-peritoneal injection of gram-positive pathogen Cp (LD50) causes a complete suppression of the cNOS activity in the striatum and the hippocampus and its remarkable decrease

in the cortex and the hypothalamus with a simultaneous significant upregulation the iNOS in all the brain regions at 48 h after Cp administration. At the same time, an elevated level of reactive ni-

trogen species (RNS) and a diminished content of L-arginine were observed in the brain regions of Cp-infected rats. The cNOS activity dropped in bone marrow, but activated in blood neutrophil and mononuclears, whereas the iNOS was significantly upregulated in marrow and neutrophils and inhibited slightly in mononuclears of Cp-infected rats. *C. perfringens*-induced innate nitrergic response was accompanied with an elevated level of reactive nitrogen species of above mentioned brain regions, blood leucocyte subpopulations and bone marrow. Neurological deficit, hemorrhage and inhibition of hematopoiesis, possibly, associated with expansive iNOS stimulation and a drop of constitutive NOSs in brain and bone marrow. Both iNOS and cNOS of neutrophil might be contributed to NO overproduction in blood and therethrough hypotension and hemorrhage occurred in clostridial infection. An elevated D-glucose uptake observed in hypothalamus, hippocampus and neutrophils of *C. perfringens*-infected rats was correlated with the iNOS activity. Contrary, diminished D-glucose uptake was observed in cortex, striatum, blood mononuclears and bone marrow cells, and appeared to be regulated by L-arginine in NO-independent pathway.

In our preliminary studies we have investigated

in vivo and in vitro the effects of low-level laser irradiation using helium-neon (He-Ne) laser ($\lambda=632,8$ nm) on rat brain-blood-marrow nitrergic system depending of wave mode, beam diameter (0,03; 1,3 cm), dose and time of intravenous, contact and non-contact treatment under physiological circumstances. The most pronounced NO modulating activity associated with a down-regulation of iNOS was seen at 900 mJ energy of He-Ne laser radiation and appropriate treatment parameters (vide infra) which were used to treat *Clostridium perfringens* (Cp) infection. A single non-contact (10-mm distance from the crown) radiation using a semiconductor laser (SCL) and /or light-emitting diode (LED) with similar parameters (continuous wave, $\lambda=654$ nm, fluence=1.27 J/cm², time exposure=600 s) was applied to rats at 24 h after Cp administration. Both SCL and LED irradiation reduced the RNS level and NO production by the iNOS and showed a tendency to recover the L-arginine content in the brain in 24 h post-treatment. A treatment with SCL caused a 9.0, 5.3, 4.3 and 3.9-fold decrease in the iNOS activity of hypothalamus, cortex, hippocampus and striatum, respectively, as compared to non-treated Cp-infected rats, and restored to normal the cNOS activity of all the brain regions. SCL upregulated the cNOS of marrow and iNOS of mononu-

clears, and modulated the latter in marrow and neutrophils. A treatment with LED negligibly attenuated a Cp-induced inhibition of cNOS and reduced the iNOS activity of 2.3, 1.9, 1.9 and 1.2 times in striatum, cortex, hippocampus and hypothalamus, respectively. LED downregulated the iNOS in marrow and blood immunocompetent cells, but could not restore the cNOS activity in marrow. In addition, Semi-conductor laser irradiation exerted the pronounced modulation effect on D-glucose uptake in brain-blood-bone marrow system and hydroxyl radical-scavenging ability of plasma dropped thrice in clostridial infection.

The results show that a treatment with SCL in the red region of spectrum modulates a nitrenergic response to Cp-infection more efficiently than that of LED with similar wavelength, energy density and time exposure through a differential regulation of the catalytic activity of distinct NOS isoforms and associated D-glucose supply in the brain regions, bone marrow and blood immunocompetent cells. Thus, SCL may be used, perhaps as an adjunctive therapy to prevent a nitrosative stress-mediated injury and energetic processes in brain, blood and bone marrow during Cp-infection.

Brain, Blood and Bone Marrow Nitrenergic Response following Cyclophosphamide Treatment

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An immunosuppressive drug cyclophosphamide (CPA) widely used in chemotherapy mediates immunosuppressive and tumoricidal effects via nitric oxide (NO) and its reactive intermediates. Therapy with CPA has a direct myelosuppressive effect and is related to a risk of developing cytopenia, leucopenia etc, associated with immune systems damage, particularly with innate immune nitrenergic response. In this context, this study was performed to ascertain the CPA effect on the pharmacologically and functionally distinguished NOS isoforms of brain, blood

and bone marrow in the rat immunosuppression model of CPA-induced inhibition of hematopoiesis accompanied with granulopenia, thrombocytopenia and aplasia, as well.

Daily intraperitoneal injection of cyclophosphamide (CPA) (50 mg·kg⁻¹ of body weight) for 5 days resulted in reduced levels of marrow and blood cellularity, which was most pronounced in 18 days post-treatment (pt). On day 9 after CPA treatment in vivo and in vitro testing suggested a down-regulation of Ca(II)/calmodulin(CaM)-independent “inducible” NO synthase (iNOS)

and Ca(II)/calmodulin(CaM)-dependent “constitutive” NOS isoform (cNOS) in marrow indicating that signals in addition to NO appear to be involved in the inhibitory effect of CPA on hematopoiesis. The most pronounced granulopenia, thrombocytopenia, aplasia, inhibition of hematopoiesis, as well as hemorrhage observed on day 18 pt are accompanied with the elevated levels of the NOS substrate and metabolites (L-arginine, reactive nitrogen species (RNS) and L-citrulline) resulted from up-regulation with the iNOS, with a lesser contribution of cNOS to systemic NO in blood and bone marrow. CPA increases thoroughly the cNOS activity of all the leucocyte subpopulations indicating a possible contribution of the cNOS to the development of CPA’s immunosuppressive effects in blood. Biphasic response to CPA of nitrergic system suggests that signals in addition to NO might be involved in CPA-induced inhibition of hematopoiesis, while a gradual increase of neutrophil and platelet NOS activity appeared to be contributed to a CPA-induced development of granulopenia, thrombocytopenia and hemorrhage, that should be taken into account in the design of therapeutic agents.

We have found that on day 18 of CPA-treatment the cNOS is inhibited completely in the cortex, striatum and hippocampus, partially in hypothalamus, with simultaneous activation of the iNOS of the mentioned regions to different extent. Of interest, the cNOS activity of cerebellum was subsequently increased after CPA-treatment and strongly depended on the presence of L-arginine and cofactors in the assay system. L-arginine and NOS cofactors (flavine cofactors, BH4 and NADPH) together affect differently the activity of distinct NOSs in the main brain regions, blood platelet and leucocyte subpopulations and bone marrow of CPA- treated rats, indicating an opportunity of their exogenous use to modulate the systemic nitrergic response. Thus, a CPA-induced prolonged cytopenia is accompanied with a time-dependent and complicated nitrergic response in the brain-blood-marrow axis, suggests a complex regulatory network involved in the pathogenesis of conditions associated with CPA treatment, particularly an overproduction of RNS in the brain could be involved in the neurodegeneration that should be taken into account in the design of chemoimmunotherapy sequencing.

Subcellular Nitrergic Mechanisms of Limbic Regulation and Early Immune Response at Chronic Stress-induced Depression-like Behavior of Rats. Effects of GABA Lithium Salt.

A few clinical and several pre-clinical studies strongly suggest involvement of the nitric oxide (NO) signaling pathway, as well as mitochondrial dysfunction in these disorders. The NO system is a potential target for antidepressant and anxiolytic drug action in acute therapy, as well as in prophylaxis. However, the roles of distinct NOS isoforms, especially recently discovered mitochondrial NOS, remain unknown.

We have shown that immediately after chronic circadian stress (CCS) (a rat model established in our laboratory) and four days later, CCS-induced depression-like behavior of rats is accompanied by a substantial persistent elevation of the L-arginine, L-citrulline and reactive nitrogen species (RNS) levels (in vivo), associated with a simultaneous up-regulation of the inducible nitric oxide synthase (iNOS) (in vitro) in both cytosolic and mitochondrial compartments and a down-regulation of the cytosolic constitutive NOS isoforms (cNOS) (in vitro) in the rat major regions involved in the anticipatory chronic stress responses prefrontal cortex, striatum, hippocampus and hypothalamus. Mitochondrial cNOS was dropped twice in the hypothalamus, while remained unchanged in the rest of the brain regions. A single

intraperitoneal injection of GABA lithium salt (GABALi) at the subsufficient mild dose of 0.9 mg/kg modulated the CCS-induced changes in the L-arginine, L-citrulline and RNS levels and the iNOS, whereas the cNOS activity was not restored. Modulatory effect of the GABALi on the mitochondrial iNOS/NO persistent activation in all the brain regions studied might be useful for prevention of stress-induced nitrosative stress leading to mitochondrial disfunction and energy impairment possibly involved in the pathogenesis of depression.

Experimental data from both animal and human studies have confirmed the immunosuppressive effect of stress. It is well known that iNOS/NO is involved in the immunosuppression and antiproliferative tone in bone marrow. Decreased immune function has been reported in depressed patients, once more suggesting the interactions between the nervous and immune systems and the relationships between mental processes. Now we have been studying the above mentioned biochemical pattern in platelet, immunocompetent cells and bone marrow following chronic stress-induced depression-like behavior of rats.

We have also studied (2000-2006):

Effect of Hypothalamic Peptides on the Endogenous ADP-ribosylation in Synaptic Membranes of Rat Cerebral Cortex.

Effect of Novel Neurospecific Protein-Hormonal Complexes on Nitric Oxide Synthesis in Rat Brain and Whole Blood.

Nitric Oxide Synthase Isoforms Activity in Whole Peripheral Blood of Healthy Humans and Patients with Posttraumatic Stress Disorders: affective disorders (aggression, anxiety, depression).

Nitric Oxide Synthase Isoforms Activity in Plasma and Immunocompetent cells of Blood at familial Mediterranean fever.

Study of the different isoforms of lactate dehydrogenase using NAD(P)/NAD(P)H as cofactors in various organs of mammals in norm and pathology.

Study of NAD binding to nuclear, mitochondrial membranes of brain and liver, and synaptic membranes of brain as well.

Laboratory of metabolism of nucleotides and nucleosides

Head of the Laboratory: *Dr., Prof. Alexander V. Arutjunyan*



The Laboratory of metabolism of nucleotides and nucleosides was created on the base of the identical research group in 1979.

The investigations carried out in the Laboratory headed by Prof. Arutjunyan were dedicated to the study of metabolism of adenylate compounds in nervous tissues and in muscle, regulation of the activity of a key enzyme of purine-nucleotide cycle, AMP-deaminase, and the mechanism of ADP-ribosylation of synaptic membranes proteins as well as the ammonia formation from amino-acids in brain.

Prof. Arutjunyan performed joint investigations with colleagues in Hungary, USA and Japan.

Since 1990 Prof. Arutjunyan is a Head of the Laboratory of biochemistry and clinical-diagnostics of the D.O. Ott Institute of Obstetrics and Gynecology of Russian Academy of Medical Sciences



Laboratory of Amino acid metabolism

Head: *Prof. Kazaryan Boris*



The laboratory staff worked on study of metabolism GABA, metabolism of bound and free amino acids, tyrosine-hydroxylase activity in brain. They also indicated the forming of ninhydrin-positive compound in the brain, after injection of GABA in carotid artery. The common carotid artery has a paired structure, meaning that there are two in the body, one for each half. The left and right common carotid arteries follow the same course with the exception of their origin. The right common carotid originates in the neck from the brachiocephalic trunk. The left arises from the aortic arch in the thoracic region.

The left common carotid artery can be thought of as having two parts: a thoracic (chest) part and a cervical (neck) part. The right common carotid originates in or close to the neck, so it lacks a thoracic portion.

Only the left common carotid artery has a substantial presence in the thoracic region. It originates along the aortic arch, and travels upward through the superior mediastinum to the level of the left sternoclavicular joint, where it is continuous with the cervical portion.

After injection in carotid artery the gamma-aminobutyric acid, in the flow-off blood from brain there was a ninhydrin-positive compound, consisting of seven amino acids, which had apparent vasoconstrictor agent properties, raising the blood pressure 5-7 times.

B.Kazaryan passed away in USA in 2008.

Laboratory Of Nucleotides And Amino Acids

Head: *Prof. Sedrak G. Movsesyan (1962-1979) , Karabashyan V. Levon (1979-1980)*



The general direction of investigations was the metabolism of energy and amino acids in particular in ammonia formation and neutralization in the brain. The ways and mechanisms of ammonia metabolism have a special significance for brain in connection with its toxic action on brain functions and the absence of urea cycle in the brain.

The general achievements of laboratory were:

1. The revealing of the reaction of NAD deamination in the brain, i.e. the recovery of new source of ammonia in the brain and product of that reaction deamino-NAD
2. The participation of deamino-NAD in the release of α -amino nitrogen of amino acids with formation NAD-succinate by analogy with adenosuccinate
3. The advancement of working hypothesis for NAD-deamino-NAD cycle as an important mechanism of the release of α -amino nitrogen from amino acids in brain
4. The study of the role of nucleotides (ADP, ATP, NAD, deamino-NAD) pyridoxalphosphate (PLP) and Mg^{++} -ions in the amino acids and energy metabolism in the brain and liver mitochondria
5. The significance of NAD-deamino-NAD cycle in the mitochondrial reactions has been studied
6. It was shown that deamino-NAD increase energy potential of heart muscle and may be used as a therapeutic means in cardiac pathology,
7. The glutamate dehydrogenase, its separation, purification and study of physicochemical, kinetic and regulatory properties,
8. The role of aspartate transaminase and glutamate dehydrogenase in the integration of mitochondrial reactions,
9. The significance of NAD-deamino-NAD transition in regulation of oxidative phosphorylation,
10. The study of mitochondria permeability for NAD and deamino-NAD.

The Laboratory of Physical Chemistry of Proteins

Head: *Prof. Robert Nalbandyan*



Robert Nalbandyan (1937-2002) was an Armenian chemist, the co-discoverer of photosynthetic protein plantacyanin, a pioneer in the field of free radicals, and a noted and prolific writer on various subjects in the field of chemistry.

Born in Yerevan, Armenia and educated at Moscow State University in Moscow, Russia, Nalbandyan lived and worked in Yerevan for most of his life, where he also headed a laboratory and lectured. He was recognized as one of Soviet Union's most prominent chemists, and in his research collaborated with fellow chemists in the USSR, US, Europe and Australia. When the Armenian Soviet Socialist Republic (SSR) struck for independence in 1989, Nalbandyan became a prominent critic of the nationalist movement, which he felt was foolhardy and was merely agitating the people for political gain.

The energy shortage, economic woes, and virtual blockade experienced by the tiny republic after independence seemed to justify his concerns. In 1996 he left the country and emigrated to the United States.

Primarily known in the scientific community for his research work with proteins, Nalbandyan was also recognized among his fellow scientists as a progressive thinker in other fields of chemistry, including neurochemistry.

This Laboratory began active researches in 1970, just after Robert Nalbandyan's completion

of PhD courses and returning from Moscow. The first group of researchers included Vardan Haykazyan and Sona Mardanyan – Nalbandyan's friends from PhD courses in Moscow, and Maxim Symonyan. Kira Markosyan and Nvard Grygoryan joined them shortly afterwards. Later, the Laboratory staff was completed with Svetlana Sharoyan, Harutyun Shaljyan, and many others, often those who participated in the Laboratory works while still studying.

The main efforts of the Laboratory researches, managed by R. Nalbandyan, were directed

towards purification of proteins and enzymes. Actually, Robert Nalbandyan created at the Institute of Biochemistry of Armenian NAS a unique school of experts in isolation and purification of biologically active compounds from the natural sources: fungus, plants and mammalian tissues. In the Laboratory methods were developed for preparation of highly purified proteins and enzymes and it became the sole supplier of such preparations to numerous research and medical organizations of the former USSR.

As subjects of investigation, mainly metal-containing proteins and enzymes were chosen. Usually, the components of the same enzymatic system were purified for study their interaction, the influence of one to another, the optimal conditions for complex formation and development of physiological activity, etc. Numerous copper and ferrum containing proteins and enzymes were purified and studied: laccase, hemocyanin, ceruloplasmin, plastocyanin, superoxide dismutase, catalase, cytochromes c, cytochrome-c-oxidase, cytochrome P-450 and the components of electron transferring chain from NADPH to P450, monoamine oxidase, ferredoxins, etc. Preparation of these proteins of the highest available purity allowed studying their detailed physical and chemical properties, the interaction with dif-

ferent biological active compounds, elucidation of function and special role of each metal atom, their ligand environment, etc.

The investigations were conducted on elucidation of the role of reactive oxygen species in various physiological processes. It was shown that some copper-containing proteins prevent peroxidation of lipids. The metallothioneins of animal and plant origin were purified and used in clarification of pathways for removal of toxic metals (Cd, Hg, Pb) from organism, the protein synthesis in response of action of toxic metals was studied.

Further extending of scientific interests of Laboratory demanded development and application of immunological methods, by which the intracellular localization of some proteins under study was elucidated; the homology of some copper-containing proteins was demonstrated.

One of the main interests of the Laboratory was associated with investigation of copper metabolism. During these studies, two new, earlier unknown, copper-containing proteins were discovered. One of them – an extremely acid low-molecular protein from brain and adrenal medulla, named neurocuprein. The physical and chemical characteristics of neurocuprein, confirming its uniqueness, were described: amino

acid composition, optical and ESR spectra, etc. The investigations directed to understand the possible physiological role of neurocuprein have shown that it can donate or accept copper from other copper-containing proteins. Based on these data, a concept was developed about copper pathways in organism.

Another new copper-containing protein, named plantacyanin, was purified from various plants. It was shown that this protein plays a key role in processes of photosynthesis: its antibodies inhibited the process of oxygen evolution.

In the early 70-th of the last century in the Institute of Biochemistry of Armenian NAS, a Central Laboratory of Spectral Methods of Armenian NAS had been created with Academician H.Buniatian efforts.

The unique scientific results obtained in the Laboratory of Physical Chemistry of Proteins headed by Prof. R. Nalbandyan, which demanded modern high-level technology, served as prereq-

uisites for creation of this Laboratory.

For the Spectral Laboratory, the following equipment was purchased:

Spectrofluorimeters Perkin-Elmer MPF-44A

Dual-wavelength, Dual-beam, Chance Spectrophotometer

Circular Dichroic Spectrometer Jobin-Yvon Mark M3

Spectrophotometer Specord M-40

Atomic Absorption Spectrometers, AAS-1 and AAS-30

Besides the researchers of the Laboratory of Physical Chemistry of Proteins and of the whole Institute of Biochemistry, this equipment served to the benefit of the scientists from many other Scientific Centers of Armenia, as, for example, the Yerevan Physics Institute, Institute of Applied Problems of Physics, etc.

Prof. R.Nalbandyan passed away in USA in 2002.

Laboratory Of Enzymes



Head (1962-1987): *G.T. Aduntz (1915-1997), Dr. biol. Sci., Prof.*

Head (1987-1991): *G.K. Parsadanjan (1938-1998), Dr. biol. Sci.*

The laboratory was founded in 1962. The staff consists of 11 coworkers, including two Doctors and 5 Candidates of Sciences (PhD).

Theoretical investigations

In the Laboratory investigations on the enzymes participating in the processes of carbohydrate phosphoric metabolism on the level of the cell, tissue and the whole organism are made.

Main Achievements

Metabolism of phosphoric compounds in the organism is one of the key problems of the enzymatic metabolism. In the laboratory phosphatase enzymes are studied. For the first time in 1962 an idea was given about isoenzyme character of the alkaline phosphatase. The type and cell peculiarities of phosphatases, their spreading and subcellular distribution, changes in the enzyme activity at a definite functional and pathological states possible ways of hormonal regulation of phosphatases are revealed. A scheme of isolation of separate molecular forms of phosphoprotein phosphatases is worked out. With the increase of antropogenic pressure on the environment at present, there appeared a necessity of studying the pollutants' influence on human and animal activity. Vanadium, which is close to phosphates by its physico-chemical properties and which induces numerous disorders in the organ-



ism, can be referred to them. The investigation made allows supposing that vanadium compounds possess an inhibiting effect on the above-mentioned enzymes.

Problem-oriented investigations

The regulation of molecular forms of phosphatases by the influence of a number of biologically active substances and vanadium compounds is planned to be studied. There is also an aim to search for mainly such natural compounds, which will defend the organism from negative influence of pollutants.



Laboratory of Neurotransmitters

Head of Laboratory: *N.Yesayan, Prof.*



Ph.D members of the Laboratory

E.K. Kazarova, A.R. Armenyan, L.N. Arakelyan, M.D. Chiflikyan, A.M. Petrosyan, P.V. Tozalakyan, Zh.E. Harutyunyan.

The Head of the laboratory, Nvard A.Yesayan, has been living in the United States since 1979. In the above-mentioned period the laboratory has studied the mechanisms of interaction between the endogenous biogenic amines (noradrenalin and serotonin) and neuroactive amino acids in the processes of neurotransmitive regulation of the nervous activity. Among the neuroactive substances great attention was drawn at gamma-aminobutyric acid (GABA) for its definite role in the processes of CNS suppression in mammals. The quantitative shifts, exchange, capture and release processes, as well as

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subcellular distribution of monoamines during in vivo and in vitro experiments under the influence of GABA and ganglioblocking medication gangleron and quateron, which increase the release of biologically active amines, have been studied. Some studies have been devoted to finding out the activity of separate non-organic ions, stipulating certain changes of monoamines. Electro-physiological studies of GABA affect on the neuronal membrane have also been carried out and it was shown that the direction of its affect depends on the functional state of nerve endings, where non-organic ions (K^+ , Na^+ , Ca^{2+} and Cl^-) may play quite a significant role. Some studies were devoted to the interaction of GABA with NA receptors and it was revealed that this activity is suppressed by bicuculline and picrotoxine.

Since 1972 studies were carried out in cooperation with radioisotopic laboratory (Head of the laboratory Prof. G.A.Kevorkian) with application of labeled neurotransmitters.

042 Professional Council

Scientific Secretary: *H.Hayrapetyan, PhD, docent*



Ph.D. and Doctor of Sciences degree 042 Professional Council, within H. Buniatian Institute of Biochemistry of the Academy of Science of RA, functions since 1976. The first founding president of the Council was the Head of the Institute of Biochemistry of the Academy of Science of RA Academician Hrachya Buniatian. The Scientific Secretary of the Council from the day of its foundation till 2009 has been Doctor of Biological sciences, Professor Armen Simonyan. From 1981 the Head of the Council has been Academician of the Academy of Science of RA Professor Armen Galoyan. Starting from 2009 the President of the Council is the Head of H. Bunyatyan Institute of Biochemistry, Doctor of Biological Sciences, Professor Guevorg Kevorkian. The Scientific Secretary is Doctor of Biological Sciences, associate professor Hripsime Hayrapetyan. The Council is an inter-institutional scientific body, which, since the day of its foundation, has carried out valuable scientific and organizational work in training and preparation of human resources with high scientific qualification in the field of biochemistry, molecular and cellular biology and genetics. More than 500 doctoral and candidate dissertations have been discussed and approved here.

In 2011 the Council has been renamed to 042 Professional Council on Biochemistry and Molecular Biology within H. Buniatian Institute of Biochemistry of the Academy of Sciences of RA.

Based on the archive data, personal conversations with Prof. H.Bunitian, PhD fellows of Prof. N.Sisakyan, letters of Prof. M.Sevak's son living in the USA, and other documents, I would like to introduce you to the immense merit of three great scientist-biochemists and patriots of Armenia in protecting the Armenian apostolic church from the intentions of the communist party of the USSR, and particularly, from the plans of demolition of churches brought forward by N. Khrushchev, the General Secretary of Communist party of the USSR.

Immediately after his election Vazgen requested Soviet citizenship, something 'that has been my dream for years'. He then set out, despite the problems within the church in Armenia, on a three-month tour of Middle Eastern diaspora churches. The main aim was to secure the election of a pro-Soviet Catholicos in the rival Catholicosate of Cilicia, based in Beirut. He was unsuccessful, but from Cairo immediately denounced the election of the new Catholicos Zareh as 'irregular'. While in the city he met President Gamal Abdel Nasser, then being wooed by the Soviet Union. On a later leg of the journey to Western Europe he visited the Archbishop of Canterbury, Dr Geoffrey Fisher.

The Soviets were impressed with his diplomatic skills and, at a key meeting with the Soviet prime minister, Marshal Nikolai Bulganin, in Moscow on 12 May 1956, Vazgen won concessions for his church. These included the reopening of churches, the enlarging of the Echmiadzin seminary and the ability to accept money for church work from Armenians abroad.

Vazgen saw good relations between local and emigre Armenians as an important part of his programme, and in 1960 made a further visit to emigre communities in Europe and the Americas. He also made efforts to heal the rift he had exacerbated in 1955-56 between the Echmiadzin and Cilicia jurisdictions. He took the Armenian Church into the World Council of Churches in 1962 at the same time as other Soviet churches joined.

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With the aim to avoid undesirable consequences from the USSR government, the Catholicos of All-Armenian Vazgen –I turned to the intelligence of Armenia, who had world recognition and often visited Armenian Diaspora abroad. Among them was Academician H. Bunitian,

who immediately also included Academician N. Sisakian in the list of Armenians with remarkable reputation in other countries. In the same period Prof. Manaseh Sevak from the USA, already started to take active participation in the Armenian Diaspora of USA. It was decided to hold a collective discussion on this important issue and find ways to consolidate the nation regardless of where they lived. A neutral territory, where M. Sevak could enter freely (until the 60s he was simple afraid to come to USSR), was selected as a meeting point. This place was Venice, more specifically the San Lazzaro island, where the Armenian Congregation of Mkhitarians was situated. The photo depicts three prominent sons of Armenia, biochemists Academician N.Sisakyan, Prof. M.Sevak and Academician H. Bunitian (from left to right). Their active joint efforts resulted in organization of the Catholicos's visit to the America (north and south), his warm welcome, discussion of national problems and issues, future plans, actions, etc.

Unfortunately the archive materials are not sufficient for complete description of the occurrences of those years, search is still carried on, nevertheless, based on the factual data, it may be concluded that such robust wave of activism of the Armenian diaspora was one of the reasons for the Communist party leadership to dismiss

its intention of infringement of the Armenian apostolic church, and to understand that though Armenians are scattered around the world, this does not imply absence of unity, unified idea of protection of the Armenian church.

The Armenian people will not forget the self-sacrifice and courage shown by three world-famous biochemists H. Bunitian, N. Sisakyan and M. Sevak.



Three Armenian Prominent Biochemists Saved Armenian Apostolic Church

His Holiness Vazgen I (also Vasken I, Armenian: Վազգեն Ա, born Levon Garabed Baljian; September 20, 1908—August 18, 1994) was the Catholicos of the Armenian Apostolic Church between 1955 and 1994, in one of the longest reigns of the Armenian Catholicos. A native of Romania, he began his career as a philosopher, before becoming a Doctor of Theology and a

Biography

Vazgen was born in Bucharest to a family belonging to the Armenian-Romanian community. His father was a shoemaker and his mother was a schoolteacher. Young Levon Baljian did not initially pursue the Church as a profession, instead graduating from the University of Bucharest's Faculty of Philosophy and Letters. After graduation, he became a philosopher and published series of scholarly articles.

As his interests began to shift from philosophy to theology, Baljian studied Armenian Apostolic Theology and Divinity in Athens, Greece. He eventually gained the title of vardapet, an ecclesiastical rank for learned preachers and teachers in the Armenian Apostolic Church roughly equivalent to receiving a doctorate in theology. In the 1940s, he became a bishop, and then the arajnord (leader) of the Armenian Apostolic Church in Romania.

member of the local Armenian clergy. The leader of the Armenian Apostolic Church hierarchy in Romania, he became Catholicos during the 1950s, moving to the Soviet Union and residing in the Armenian SSR. Vazgen I led the Armenian Church during the dissolution of the Soviet Union, and was the first Catholicos in newly-independent Armenia.

His rise through the hierarchy of the Church culminated in 1955 when he was elected Catholicos, becoming one of the youngest Catholicos in the history of the Armenian Apostolic Church. He would reign until his death in 1994. During his long time as Catholicos, he managed to assert some independence for his church in face of the totalitarian Soviet rule in the Armenian SSR, and lived to see religious freedom restored under Armenia's national government in 1991.

From then on, he was very busy renewing ancient Armenian churches and reviving institutions of the church. He saved a number of church treasures by establishing the Alex Manoogian Museum of the Mother Church. Vazgen intensified contacts with the Armenian Catholic Church, with the aim of reuniting both wings of Armenian Christianity.

San Lazzaro Island

The oldest information about Armenians living in Italy goes back to the 6th-8th centuries. Later, in the 9th-10th centuries, a great number of Armenians moved to Italy from Thrace and Macedonia. They were the descendants of Paulicians chased from Armenia by emperor Constantin.

As to Armenian communities, they were formed in Italy in the 12th-13th centuries, when active trade was going on between Cilician Armenia and Italian big city-republics as Genoa, Venice and Pisa. Under Cilician Armenian king Levon II (1187-1219) (also known as King Leo II of Armenia), treaties were signed between the two parties, according to which Italian merchants had the right to open factories and to develop industrial activities in the Armenian Kingdom of Cilicia and Armenian merchants could do the same in Italian towns. These treaties were periodically renewed, as long as the Cilician Armenian Kingdom existed. In the 13th century the number of Armenians in Italy increased because of the new wave of emigrants after the invasion of Tatars and Mongols.

Beginning with the 15th-16th centuries the process of catholicizing Armenians was strengthened in Italy which greatly contributed to their assimilation with Italian people. Nevertheless, some Armenian organizations continued to func-

tion with the aim to preserve national identity. As a result first Armenian books were printed in Venice.

Besides, in the beginning of the 18th century the Armenian Congregation of the Mechitarists (Armenian: ՄԻԻԹԱՐԵԱՆ, also spelled Mekhitarists), was founded in Venice, on the St. Lazzaro Island (San Lazzaro degli Armeni). It exists up till now with its monastery, library, manuscripts depository and publishing house, and is considered as a centre of Armenian culture in Italy.

There is also the reputable Moorat-Raphael College in Venice for general education with student body from Armenians from many countries and Collegio Armeno (The Pontifical Armenian College) in Rome for preparation of clergy in the Armenian Catholic Church.

The cloister of the monastery on the island of San Lazzaro (Saint Lazarus) near Venice, Italy, headquarters of the Mechitarists.

The Monastic Headquarters of the Mekhitarist Order is on the island of St. Lazarus in Venice (San Lazzaro Monastero Armeno in Italian). It is located on San Lazzaro degli Armeni, (Armenian: Սուրբ Ղազարոս Կղզի, English: Saint Lazarus Island), a small island in the Venetian Lagoon, lying immediately west of the Lido; completely occupied by an Armenian Catholic

monastery that is the mother-house of the Mekhitarist Order. It is considered as one of the world's foremost centers of Armenian culture.

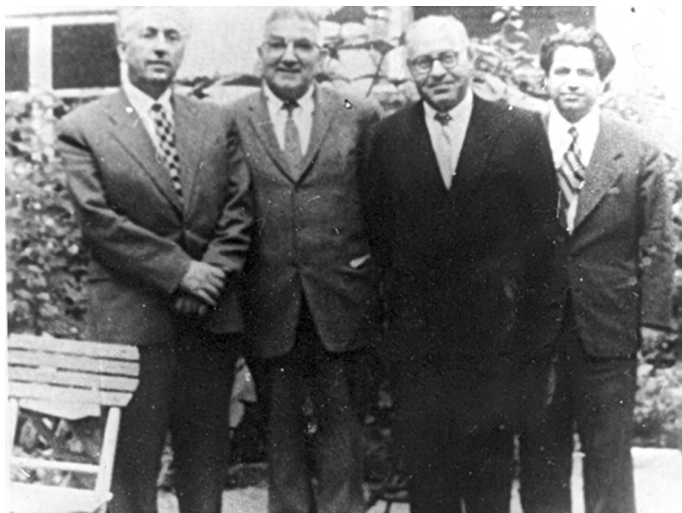
The beginnings of the island's Armenian history started when Mekhitar da Pietro and his seventeen monks built a monastery, restored the old church, and enlarged the island to its present 30,000 square metres, about four times its original area.

Its founder's temperament and natural gifts for scholarly pursuits immediately set the Mekhitarist Order in the forefront of Oriental studies: the monastery published Armenian historical, philo-

logical and literary works and related material, renowned for their scholarship and accuracy as well as for the beauty of the editions, on its own multilingual presses.

The island also houses a 150,000-volume library, as well as a museum with over 4,000 Armenian manuscripts and many Arab, Indian and Egyptian artifacts collected by the monks or received as gifts.

The Mekhitarist Order also publishes the longest-running Armenian periodical, the academic "Pazmaveb".



Norair Martiros Sisakian



Norair Sisakian (1907-1966), Soviet-Armenian biochemist. Sisakian was one of the founders of space biology and is known for proposing chloroplasts as poly-functional cell structures. He also contributed greatly to the Soviet space program. Here, in his role as Academic Secretary of the USSR Academy of Sciences' Biological Sciences Department, Secretary of UNESCO he is answering journalists' questions at a news conference on studying preliminary data obtained during Sputnik 9 and Sputnik 10 space flights involving animals. These animals were two dogs, some mice and a guinea pig. Photographed in the Academy's conference hall in Moscow, Russia, on 28 March 1961.

Manase Kirakos Sevag (Karageuzyan)



Manaseh Sevak was born in Sis, on December 23, 1897. Since 1920 he had lived in the United States, where in 1921 he graduated from Yale University. Afterwards, he entered the Columbia University of New York and in 1929 successfully graduated having defended a thesis in the field of biochemistry. In 1926, together with associates, he established the American-Armenian scientific society. His scientific skills were formed in 1931-1932 in the University of Munich, and in 1932-1934 in the University of Berlin. Since 1935 to 1966, being associate professor, he worked in the department of microbiology of medical school of Pennsylvania University, Philadelphia, where in 1946 he became the holder of the scientific title of a "professor". The scientist's main studies are devoted to matters of microbiology and biochemistry, exchange of nucleic acids and protein compounds, development of methods for receipt and purification of the mentioned compounds with the use of chloroform.

In the period of 1963-1967 Manaseh Sevak was the first Chairman of the Armenian Historical Research Society in Philadelphia. Since 1952 he was a member of the New York Academy of Sciences and a number of other scientific organizations. In 1960 he became a foreign member of the Academy of Sciences of Armenian SSR. Five years later, in 1965, he was a laureate of Tigran Kabakchyan prize of the American-Armenian Student Union.

Manaseh Sevak died on November 23, 1966 in Philadelphia.



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