1st International Symposium on Deuterium Depletion

13-14 May 2010
Budapest, Hungary
1st International Symposium on Deuterium Depletion

13 – 14 May 2010

Budapest
General information

1st International Symposium on Deuterium Depletion is an international conference, wishes to give the researchers and medical practitioners a unique opportunity to get acquainted with this expanding new expanding field of science and share their results and experience.

The conference primarily focus on the biological effects of naturally occurring deuterium and its importance in cancer, diabetes and aging, but the scientific committee welcomed any other papers originating from basic research which reveal the role of deuterium in the different regulatory systems of the cells.

The basic concept of the conference is to gather all the scientists from the world, who have carried out basic or applied research on deuterium depletion, in order to share these results, experience, scientific and clinical information with the participants of the symposium and by these means to summarize this knowledge for the other scientists and clinicians involved in this field and other professional participants and share the basic rules of the application of DDW in the clinical practice.

Main topics of the Symposium

• Deuterium Depletion in Cancer Research and Oncotherapy
• Deuterium Depletion and Diabetes Research
• Deuterium Depletion in Anti-Aging Research

Language

• All presentation will be in English
  English-Hungarian synchronous interpretation will be provided.

Scientific Committee

• Gábor Somlyai Ph.D
• Gábor Jancsó D.Sc.
• Walther Bild M.D., Ph.D.
• Michael Aschner M.D., Ph.D.
• Yury Sinyak D.Sc.

Organizing Committee

Miklós Molnár M.D., Ph.D.
Ildikó Somlyai M.Sc.
Gábor Somlyai Ph.D.
Gábor Laskay Ph.D.

The official congress organizer agent for the symposium

CONNECTIONS
Event and Incoming Agency

Connections2000 Event and Incoming Agency
H-1016 Budapest, Hegyalja út 18.
Tel: 36-1-209-0380
Fax: 36-1-209-9334
E-mail: conn2000@conn2000.hu
Web: www.conn2000.hu

The official sponsor of the symposium

HYD
HYD LLC. for Cancer Research and Drug Development
Address: H-1118 Budapest, Ménesi út 104. Hungary
Postal address: H-1539 Budapest, P.O.B. 695. Hungary
Phone: +36-1-381-0765, +36-1-365-1660
Fax: +36-1-365-1661
E-mail: info@hyd.hu
Web: www.hyd.hu, www.preventa.org
Venue of the symposium
- Ramada Plaza Budapest Hotel
  Árpád fejedelem útja 94.
  H-1036 Budapest
  Web: www.ramadaplazabudapest.hu

Directions

Budapest Ferihegy Airport (BUD): 22 kilometers (14 miles) from the destination which takes approximate 30 minutes by car.

With public transportation: take the bus nr93 or nr200E to Köbánlya-Kispest (metro line 3). Take the metro till Árpád Bridge station than take the tram nr1 direction to Buda till the other side of the bridge (Szt. Lélek tér).

Driving directions: From the airport head for Ulloi Street, go to Hungária Boulevard and then Károly Boulevard, then across the Árpád Bridge and the hotel is on the left hand side. Budapest has an inexpensive and efficient public transport system with easy links between the metro, HEV trains, buses, trams and trolleybuses. Tickets can be bought from metro stations, kiosks and newsstands.

Budapest in General

Facts and figures
- Area: 525 square kilometres
- Population: 1 815 000
- Population density: 3456 persons / kilometre
- Administrative structure: 23 districts
  - Buda: comprising one-third of the area of the city on the hilly, right bank of the Danube
  - Pest: comprising two-thirds of the area of the city on the flat, left bank of the Danube
  - Islands: Óbuda Island, Margaret Island, Csepel Island
  - Bridges: seven public ones and two railways

Useful information

Important numbers
- Ambulance: 104
- Police: 107
- Fire Service: 105
- Auto Club help number: 188
- Universal inquires: 197
- Inland inquires: 198
- International inquires: 199
- Speaking clock: 180

Credit cards
- The list of most commonly used types: AMEX, Diners Club, Euro/Mastercard, JCB, Visa.

Electricity
- Hungary’s electricity network operates at 220-240 Volts. Plugs are the regular continental types.

Public Transport
- Budapest has an efficient public transport network. In general the buses, trams and trolleybuses operate between 4.30 am and 11.30 pm.
- Tickets can be purchased in advance at metro stations, ticket machines, tobacconists, news-agents and also at our information desk in the registration area of the congress.
- You may be requested to show your validated ticket on all public transport or at exit points of the metro by ticket inspectors. For this reason it is better to keep your ticket until the very end of your journey or until you have left the metro station.
# Scientific programme overview

## 1st International Symposium on Deuterium Depletion

### 9.30-10.00
Registration, welcome coffee

### 10.00-10.05
Opening

**Section 1.** Chairmen: Prof. Dr. Yu. E. Sinyak, Prof. Dr. Gábor Jancsó

<table>
<thead>
<tr>
<th>Time</th>
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<tr>
<td>10.30-10.50</td>
<td>Prof. Dr. László Boros, Prof. Dr. Ebrahim Azizi, G. Jancsó: PHYSIOLOGICAL EFFECTS OF HEAVY WATER</td>
</tr>
<tr>
<td>10.50-11.15</td>
<td>Dr. Gábor Somlyai, Dr. Walther Bild, W. Bild, V. Bild, I. Haulica: ENVIRONMENTAL DEUTERIUM AND CELL PROLIFERATION: IMPLICATIONS IN RADIOBIOLOGY</td>
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<tr>
<td>11.15-11.40</td>
<td>Yu.E. Sinyak, A.I. Grigoriev: RECOVERY AND USE OF DEUTERIUM-FREE WATER IN EXTENDED SPACE EXPEDITIONS</td>
</tr>
<tr>
<td>11.40-12.00</td>
<td>D.S. de Ávila, M. Aschner: PROTECTIVE EFFECTS OF DDW IN CAENORHABDITIS ELEGANS MODEL</td>
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### 12.00-13.00
Lunch

**Section 2.** Chairmen: Prof. Dr. Laszlo Boros, Prof. Dr. Ebrahim Azizi

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<tr>
<td>13.00-13.35</td>
<td>L. Boros, G. Somlyai: INTERMEDIARY METABOLISM AND MACROMOLECULE SYNTHESIS IN RESPONSE TO DEUTERIUM DEPLETION IN PANCREATIC, BREAST AND LUNG CANCER CELL LINES</td>
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<td>13.35-13.55</td>
<td>G. Laskay: CELL PHYSIOLOGICAL EFFECTS OF REDUCED DEUTERIUM CONTENT IN THE LEAVES OF ELODEA CANADENSIS</td>
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### 14.55-15.40
Coffee break

**Section 3.** Chairmen: Dr. Walther Bild, Dr. Gábor Somlyai

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<tr>
<td>15.40-16.00</td>
<td>M. Molnár, K. Horváth, T. Dankó, G. Somlyai: EFFECT OF DEUTERIUM OXIDE (D₂O) CONTENT OF DRINKING WATER ON GLUCOSE METABOLISM IN STZ-INDUCED DIABETIC RATS</td>
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<td>16.00-16.20</td>
<td>G. Somlyai, I. Guller, K. Krempels, I. Somlyai, A. Kovács: DEUTERIUM DEPLETION AS AN EFFECTIVE TREATMENT OPTION FOR PROSTATE CANCER - STATISTICAL EVALUATION OF A DOUBLE BLIND, RANDOMIZED PHASE II CLINICAL TRIAL AND A RETROSPECTIVE STUDY ON PROSTATE CANCER</td>
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<tr>
<td>16.40-17.00</td>
<td>Z. Gyöngyi, F. Budán, I. Szabó: LUNG CANCER AND DEUTERIUM DEPLETION. CLINICAL AND EXPERIMENTAL DATA</td>
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<td>17.00-17.25</td>
<td>G. Somlyai, I. Somlyai, M. Huszár, K. Krempels: MAIN RESULTS AND BASIC RULES OF THE HUMAN APPLICATION OF DEUTERIUM DEPLETED WATER IN COMBINATION WITH CONVENTIONAL ONCOTHERAPIES</td>
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### 17.25-17.35
Closing

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<td>18.00-</td>
<td>Reception</td>
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Biographies

Gábor Somlyai, Ph.D.

Gábor Somlyai graduated as a biologist at the University of Szeged in 1982. Between 1982 és 1990 he worked for the Plant Protection Institute of the Hungarian Academy of Sciences, Department of Plant Pathology, within this time period from 1983 to 1986 he was a scholarship-holder of the Hungarian Academy of Sciences as a postgraduate student for obtaining PhD. In 1988 he defended his thesis in molecular biology. In the same year he was a DFG-scholarship holder at Georg August University in Göttingen for 6 months, from the end of 1988 Dr. Somlyai held a postdoctoral fellowship at the University of Missouri (Columbia, Missouri, USA), where he worked in the field of genetic engineering and gene mapping. In the wake of Hungarian Nobel-prize winning researcher Albert Szent-Györgyi, who said that the true cause of cancer should be looked for at sub-molecular level, Somlyai began his investigations in 1990 as a senior research fellow at the Hungarian Institute of Oncology with the examination of whether the naturally occurring deuterium – the heavy isotope of hydrogen – has any role in the regulation of biological, i.e. intracellular molecular processes. In 1993 he established HYD Ltd. with his partners to carry out anticancer research based on his invention, drug development based on the proprietary procedure of deuterium depletion and to conduct drug registration. Between 1993 and 1997 he was the scientific director of HYD Ltd., from 1997 he became the CEO of the company. In 2000 his book entitled „Defeating cancer!” appeared in Hungary, from that time it has been published in Romania, Japan, the USA, South Korea and in China. Gábor Somlyai is the author of numerous scientific publications in Hungarian, English and German languages and recognized speaker of domestic and international conferences.

Gábor Jancsó, D.Sc.

Gábor Jancsó was born in 1941 in Budapest, Hungary. He graduated with a degree in chemistry from Eötvös Loránd University in 1964 and immediately joined the Central Research Institute for Physics of the Hungarian Academy of Sciences. He is currently a Scientific Adviser at the KFKI Atomic Energy Research Institute and holds a honorary professorship at the Eötvös Loránd University. In 1969-1970 and 1976-1977 he spent one-year study leaves at the Chemistry Department of The University of Tennessee; from 1982 to 1984 he was a visiting scientist at the Max Planck Institut für Chemie in Mainz. His research interests include the investigation of the effects of isotopic substitution on the properties of liquids and mixtures, molecular dynamics simulation of liquid water and aqueous solutions, and studies of the effects of intermolecular interaction on the vibrational properties of molecules in the condensed phase. More recently he has become involved with studies of the structural and dynamic properties of aqueous solutions using neutron diffraction. He is coauthor of more than one hundred twenty papers.

Walther Bild, Ph.D.

Graduate of the Faculty of Medicine, University of Medicine and Pharmacy “Gr T. Popa”, Iasi, Romania, in 1991. Scientific research begins in the Romanian Academy, as an employee of the Experimental and Applied Physiology Laboratory, led by the renowned scientist I Haulica (member of the Romanian Academy). Since 1998 became also part of the teaching staff at the University of Medicine and Pharmacy. From 1996 he begins doctoral studies in the biophysics of radiobiology. In 2005 became a specialist in nuclear medicine, took charge in 2009 of the Experimental and Applied Physiology Laboratory and holds the position of associate professor in the Department of Fundamental Sciences, University of Medicine and Pharmacy “Gr T. Popa”, Iasi, Romania. Since 1998 begins the collaboration with the first provider of DDW, the Institute of Cryogenics and Isotopic Research for expanding research in several directions, including the study of immunological effects, investigation or histological changes or of vascular reactivity induced by prolonged administration of DDW. In the period 1999-2000 is beneficiary of a scholarship from the Pharmacological Association Berlin-Brandenburg which allows the investigation of the cellular effects of various concentrations of DDW within the Department of Immunology Center for Molecular Medicine “Max Delbrück” in Berlin. Since 2000 the research took also a contractual form, the Laboratory of the Academy became a subcontractor in a national grant to study the biological effects of DDW within several research units. By 2004 he published 6 papers in extenso and presented 14 domestic and international communications regarding the biological effects of DDW. In 2002 he was part of the gold medal winning teams at the International Exhibition of Inventions, Geneva for “Biological Radioprotection” and also the gold medal at the Salon of Inventions “Eureka”, Brussels, for “Immune Activation”. Also has filed two patents for these innovations. His studies focused on the radioprotective effects and the investigation of the mechanisms by which they can be achieved. During recent years it has been demonstrated the hiperproliferative effect on cells in culture, the immunostimulating effect in vivo and in vitro and the stimulating effect of membrane proton pumps and intracellular alkalization as a mitosis triggering factor. Currently is still studying the biological effects of isotopic ratios alteration (using radioactive and non-radioactive isotopes) employing methods of electrophysiology and cell biology.
Yury Emelyanovich Sinyak, D.Sc.

Yury Sinyak was born 30 March 1932. Graduated in 1955 from the chemical faculty of Gorky State University on speciality radiochemistry. In 1955 - 1958 he postgraduate in Institute of Physical Chemistry of Academy of Sciences USSR and graduated with a Candidate of Chemistry Sciences degree. In 1961-1963 Yu. Sinyak - senior research assistant of Institute of Space Medicine, in 1963-2010 head of laboratory, head of Department of Life Support Systems in Institute for Biomedical Problems. In 1972 Yu. Sinyak defended the dissertation on degree of Doctor of Technical Sciences, in 1977 he was given rank Professor on speciality Physical Chemistry. From 1961 Yu. Sinyak have been developed the technologies for regenerative physical-chemical life support systems for crews of space ships and orbital space stations. Some technologies of Yu. Sinyak was used to creating of regenerative water supply systems for orbital space stations “Salut”, “MIR” and “ISS” (such systems as “SRW-C”, “SRW-U” etc.). Yu. Sinyak have been developed the structure of ecological life support systems based on biological-physical-chemical methods. In during last years Yu. Sinyak have been developed a new scientific direction: life support systems for creating and supporting of environment with optimal isotope composition of biogenical chemical elements (H2, O2, Ca, Mg etc.), including plants, animal and man. The main goal of such life support systems consist of removal of the heavy stable isotope (such as deuterium, 18O etc.) from water, atmosphere, plants, animals and man. He have developed the technology for recovery of the water with the lowered concentration of deuterium, based on the electrolysis of distilled water. Deuterium-free water has been used for: cultivation of the higher plants, breeding of birds – quails, research of anti-tumor properties, research of radiation shielding properties. It was shown, that deuterium-free water (and water with decreasing concentration of 18O2) provide positive medical-biological activity. Yu. Sinyak is author of more 300 sciences works, including manuscripts, monographs and patents. Yu. Sinyak is awarded with the order “Znak Pocheta”, he is Gagarin and Korolev Medalist of the Russian Cosmonauts Agency. He is academician of International Academy of Astronautics, Russian Academy of Cosmonautics named after K.E. Tsiolkovsky and Academy of Technological Sciences of Russion Federation, Honoured Inventor of Russian Federation, Honoured Scientist of Russian Federation and Russian Government Prize Laureate.

Michael Aschner, Ph.D.

Dr. Aschner earned his B.S. from the University of Rochester, Rochester, NY, in 1980, and his Ph.D. in Anatomy and Neurobiology from the University of Rochester School of Medicine and Dentistry, Rochester, NY, in 1985. After a brief postdoctoral fellowship in Toxicology (at the same Institution), he assumed his first faculty position (Assistant followed by Associate Professorship) in the Department of Pharmacology at Albany Medical College, Albany, NY (1988-1994). For the next decade he joined the Department of Physiology and Pharmacology at Wake Forest University School of Medicine, Winston-Salem, NC. As of 2004, Dr. Aschner serves as the Gray E. B. Stahlman Professor of Neuroscience in the Department of Pediatrics at the Vanderbilt University Medical Center in Nashville, TN. He also holds joint appointments at the Kennedy Center for Research on Human Development and in the Department of Pharmacology. Dr. Aschner has served on numerous national and international toxicology panels (Institute of Medicine, US Environmental Protection Agency, Center for Disease Control), served and chaired a National Institutes of Health study section, and has authored approximately 300 peer-reviewed manuscripts and chapters in the area of neurotoxicology. He serves as Associate Editor (Neurotoxicology; Toxicological Science) and on the Editorial Boards (Toxicology; Acta Neurobiologiae Experimentalis; Alcohol) of several journals. He is a member of the Society of Toxicology, Society for Neuroscience, a Fellow of the Academy of Toxicological Sciences and a past president of the International Neurotoxicology Association. Dr. Aschner’s research interests are in the neurobiology and physiology of astrocytes and the mechanisms of central nervous system injury. Dr. Aschner has been particularly interested in metal uptake and distribution in the brain, devoting the last 25 years of his research to the mechanisms of transport of methylmercury, manganese, and uranium across the capillaries composing the blood–brain barrier, as well as their cellular and molecular mechanisms of neurotoxicity. Studies in the lab address basic mechanisms in various experimental models (C. elegans, tissue cultures and rodents) as well as follow-up on the sequelae of manganese deposition in the brains of human neonates. Most recently studies with DDW in the C. elegans model established its protective role against Mn toxicity and aging, likely by attenuating Mn-induced reactive oxygen species generation. The effect of DDW appears DAF-16 dependent, increasing the expression of antioxidants proteins and lifespan in C. elegans.

Daiana Silva de Avila, Ph.D.

Dr. Avila earned her bachelor in Pharmacy and Biochemistry from the Federal University of Santa Maria (RS, Brazil) in 2005 and her Ph.D. from the Toxicological Biochemistry Program at the same university in 2009. Dr. Avila assumed a postdoctoral position in Dr. Aschner laboratory in 2009, where she is currently developing her projects. Dr. Avila has been interested in the toxicity of several toxicants and evaluating the effects of antioxidants in the central nervous system using the rodent and the C. elegans as experimental models. She is currently engaged in studying the role of heat shock proteins and Parkinson’s disease related genes in manganese (Mn)-induced neurotoxicity using C. elegans. Recently, she assisted the Hydra group to elucidate the anti-aging effects of the deuterium depleted water (DDW) using the C. elegans model. Thus far, the group has found that DDW can restore the Mn-induced decrease in lifespan in C. elegans. They also found that restoration in the DAF-16 levels, a protein associated with antioxidants production, prolonged the lifespan in C. elegans.
Laszlo Boros, M.D., Ph.D.

Dr. Boros holds a Doctor of Medicine (M.D.) degree from the Albert Szent-Györgyi School of Medicine from Szeged, Hungary. Dr. Boros currently is an Associate Professor of Pediatrics, Endocrinology and Metabolism at the UCLA School of Medicine and Chief Scientific Advisor of SiDMAP, LLC. Dr. Boros is the co-inventor of the stable isotope-based dynamic metabolic profiling (SiDMAP) technology and its applications for drug testing that involves library screening, lead optimization and in vitro and in vivo phenotype profiling of living cell and host systems via flux measurements using heavy isotope labeled substrates with 13C or 2H (deuterium). His primary interest is mechanisms of drug resistance in pre-clinical drug testing studies using detailed analysis of the metabolic network. He trained as a house staff in his medical school in gastroenterology after receiving a research and training fellowship from the Hungarian Academy of Sciences, after which he was a visiting Scholar at the Essen School of Medicine in Germany. Dr. Boros also worked as a Research Scientist at the Ohio State University Department of Surgery. Dr. Boros is the recipient of the C. Williams Hall Outstanding Publication Award from the Academy of Surgical Research of the USA (1997), the Richard E. Weitzman Memorial Research Award from the University of California (2001) and the Excellence in Clinical Research Award from General Clinical Research Center at the Harbor-UCLA Medical Center (2004). Dr. Boros is an active member of the American Association for Cancer Research, the American Pancreatic Association, the American Physiological Society and the American Gastroenterological Association. Dr. Boros is currently serving on the Editorial Boards of the journals Pancreas and Metabolism as well as on various peer review panels including Hormone & Metabolic Research, Engineering Research Council of Canada, Molecular & Cellular Biochemistry, Analytical Biochemistry, Dutch Cancer Society (Nederlandse Kankerbestrijding), Oncogene, Nutrition & Cancer as well as the Federation of European Biochemical Societies (FEBS) Letters.

Gábor Laskay, Ph.D.

Dr. Laskay graduated as a biologist from the University of Szeged in Hungary in 1979. He started work at the Department of Biophysics, University of Szeged in 1979 and received his Ph.D. degree in Biophysics from the University of Szeged in 1981. His Ph.D. thesis was about the effect of the lipid environment on the functions of the photosynthetic apparatus in plants. Dr. Laskay did a 4-year post-doc at the Paterson Institute for Cancer Research in Manchester, U.K., from 1988 to 1992, where he was involved in various fluorescence studies comparing normal and cancer cells. In the meantime he received his Candidate degree (then a go-in-between Ph.D. and D.Sc.) in 1988. Later on dr. Laskay worked at the Department of Medical Chemistry, Medical School, University of Szeged, where he was involved in fluorescence studies related to Alzheimer’s disease. He had the chance to work at the Physiology Research Group, Limburg University in Belgium from 1996 to 1999 where he studied Ca2+-homeostasis in neural cells using fluorescent dyes. Since 1996 he has been an Assistant Professor at the Department of Botany (since 2007 Department of Plant Biology) where he teach the courses of Cell Biology, Plant Cell Biology, Fluorescence in Cell Biology, Cell Death Mechanisms. Dr. Laskay has published 35 listed scientific publications with a cumulative impact factor of 46.345, and have written a university lecture note (“Plant Cell Biology”), which has been published in 3 consecutive editions and has sold in more than 3,000 copies.

Ebrahim Azizi, Pharm.D., Ph.D.

Dr. Azizi got his Doctoral degree in Pharmacy in 1988 from Faculty of Pharmacy, Tehran University of Medical Sciences which is the first rank in the country. Then he was employed as instructor of Pharmacology and Toxicology in the same faculty shortly after graduation in 1988. Dr. Azizi started his PhD in the field of cancer genetics and chemotherapy in 1992 and finished with honor degree in 1997. Then he became Assistant Professor and Director of Molecular Research Lab of same faculty. In 2006, he was promoted to Associate Professor and in early 2010 to Full Professor. In addition to teaching Pharmacy and PhD students the theory and practical aspects of cancer genetics and chemotherapy, he is supervising and coordinating research projects, thesis and dissertations of qualified students. His research interests are as followings:
1) Genetics of tumor cells with respect to Drug Resistance, Apoptosis and Cell Cycle
2) Pharmacogenetics/genomics related to Population Variation in Drug Responses
3) Evaluating of New Synthetic or Plant derived compounds for Cancer Therapy at Cellular and Molecular level

His experiences in the field of DDW started 2 years ago when he received an invitation from R&D office of Food and Drug Administration of the Ministry of Health and Medical Education to study the anticancer properties of DDW. The project involved both cellular and molecular experiments to elucidate more on potentials of DDW in cancer therapy as reported by Prof. Somlyai and colleagues. The results that will partly be presented in the DDW Symposium were promising and therefore, they decided to continue this project at different aspects. Currently, they are doing experiments to further evaluate the Antioxidant properties of DDW in cancer cells in vitro as well as in Rats in vivo. As an academic member in the Medical Sciences, dr. Azizi sincerely does hope with close international collaborations and efforts, a new therapeutic gift will be presented to cancer patients around the world. He also highly appreciates valuable scientific contributions of Prof. Somlyai and colleagues related to DDW potential medical applications.
Radu Tamaian, B.Sc.

Radu Tamaian graduated as a bachelor in biology and geology at the Babes-Bolyai University in Cluj-Napoca (Romania) in 2000. From 2003 he is a PhD Student in Science (Biology) at the Faculty of Biology, University of Bucharest, Romania. Radu Tamaian has been employed at National Research and Development Institute for Cryogenics and Isotopic Technologies – ICIT Rm.Vâlcea, Romania since October 2000. Between 2000 and 2007 he was responsible for the monitoring and microbiological control of the production of deuterium depleted water (light water). Starting with 2002 he has been involved in research as following: Investigation of hydrogen’s transport in atmosphere-soil-plants structures using natural isotopic tracers; Studies of hydrogen’s isotopes’ translocation and accumulation in plants; Experimental researches in the field of deuterium depleted water (DDW) effects over laboratory animals’ health (toxicology, immunology and oncology); Determination of deuterium’s distributions by mass-spectrometry in laboratory and farm animals under effect of DDW; Experimental researches in the field of natural drugs and synthetic therapeutics (antitumoral compounds derived from naphthaquinone ligands using DDW as solvent). National projects in the field of hydrogen’s isotopes and deuterium depletion: The determination and use of biological effects of deuterium depleted waters on some animal organisms in normal and pathological conditions (scientific responsible); Methods of prevention and treatment for cancer at humans and big animals (project responsible); The investigation of hydrogen’s transport in atmosphere-soil-plants structures using natural isotopic tracers (project responsible);The study of hydrogen’s isotopes’ translocation and accumulation in plants (project responsible); Hydrological studies using isotopic tracers (team member); Transfer and technology development to obtain deuterium depleted water and its derivatives (team member). His undergoing project: Obtaining and characterization of new targeted-nanodrugs with naphthaquinoninc active substance (project director).

Miklós Molnár, M.D., Ph.D.

Miklós Molnár graduated as a bioengineer at the Budapest University of Technology and Economics in 1979 and earned his M.Sc. degree as chemical engineer at the same university in 1981. He graduated as a medical doctor at the Semmelweis University Faculty of Medicine in 1988. In 2001 he received Ph.D. degree in Pathophysiology at Semmelweis University Faculty of Medicine. Between 1985 and 1986 he was a Research Assistant between 1989 and 1991 a Postdoctoral Fellow at the Dept. of Obstetrics and Gynecology at St. Louis University, St.Louis, USA. Between 1988 and 1992 dr. Molnár was an intern at the Institute of Pathophysiology at Semmelweis University Faculty of Medicine. In 1992 and in 1996 he held a Visiting Professor position at the Dept. of Obstetrics and Gynecology at St. Louis University, St.Louis, USA for 2 months. Between 1992 and 1993 he was an assistant lecturer at the Institute of Pathophysiology at Semmelweis University Faculty of Medicine. In 1998 he was a visiting professor at the Dept. of Physio-Pharmacology at St. Louis University, St.Louis, USA for 2 months. Between 1993 and 2001 he was an Assistant Professor at the Institute of Pathophysiology at Semmelweis University Faculty of Medicine. Since 2001 he has been an Associate Professor at the Institute of Pathophysiology at Semmelweis University Faculty of Medicine. Dr. Molnár is the recipient of numerous awards and honors. He has been a council member of the Hungarian Physiologic Society since 1999. Dr. Molnár is the author of 60 original articles, the total number of his publications is 126.

Kristina Krempels, M.D.

Kristina Krempels graduated as a medical doctor in 1991 from the Semmelweis University Medical School in Budapest, Hungary. She joined as a medical student in the neuroendocrine research team at the 2nd Department of Anatomy of the Semmelweis University in 1987; and participated in the research of cellular and molecular aspects of the regulation of pituitary function and the hypothalamic regulation also after her graduation as an assistant professor. She gained teaching experience in anatomy, histology and embryology both at the Hungarian and German language course of medical students at the gross anatomy and histology laboratory classes, and lectures. Getting a postdoctoral fellowship at the National Institute of Neurological Disorders and Stroke in Bethesda, Maryland U. S. A. between 1994 and 1996 she investigated the distribution of somatostatin receptor mRNAs using in situ hybridization and immunohistochemical techniques. At the Human Serum Production & Medicine Manufacturing Co. Ltd., (Gödöllő, Hungary) she took part in planning of bioequivalence studies from 1997. Since 2004 she has been working for the HYD LLC for Cancer Research and Drug Development and gained experience using the novel proprietary procedure of deuterium depletion for the treatment and prevention in various types of cancer.

Zoltán Gyöngyi, Ph.D.

Zoltán Gyöngyi graduated at Kossuth Lajos University as a biologist in Debrecen in 1996. From 1996 he was a Ph.D. student at Szent-Györgyi Albert Medical University, Szeged, Hungary and at the University of Pécs from 1998. He earned his Ph.D. degree from the University of Pécs in 2003. Between 2003 and 2006 he was a postdoctoral fellow at CNRS UMR (Reserch Training Network) in Nice, France. From 2001 he was an assistant lecturer, from 2004 a lecturer at the Public Health and Preventive Medicine, Medical School, Pécs University, Hungary. His fields of interests are the Molecular mechanisms of life, Carcinogenesis and Cancer prevention. He is the author of 15 scientific papers (impact factor 30, citations 53). His papers in the field of deuterium depletion: Virág V, Varjas T, Gyöngyi Z, Somlai G, Ember I, Nádasi E: The possible role of natural products in the dietotherapy of cancer-related weight loss: an animal model. ACTA ALIMENTARIA 36(2): 249-256, 2007., Gyöngyi Z, Somlai G: Deuterium depletion can decrease the expression of c-myc Ha-ras and p53 gene in carcinogen-treated mice. IN VIVO 14(3): 437-439, 2000. Their main results of deuterium depletion research: Regulation of genes, playing a key role in the cell cycle regulation and tumour development, is sensitive to deuterium depletion according to animal experiments.
Abstacts

Naturally occurring deuterium is essential for the normal growth rate of cells


The role of naturally occurring D in living organisms has been examined by using deuterium-depleted water (DDW) (30-100 ppm) instead of water containing the natural abundance of D (150 ppm). DDW significantly decreased the growth rate of L929 fibroblast, HT-29 colon, A4, MDA and MCF-7 breast, PC-3 prostate, M19 melanoma cell lines. The inhibitory effect was more significant when the D-concentration of the culture medium was gradually decreased in 3-5 steps. To investigate the anticancer effect of DDW in vivo, human breast adenocarcinomas; MDA and MCF-7 were transplanted into CBA/Ca mice. The drinking water of the animals in the treated group (17) was replaced with DDW (30 ppm) one day after transplantation. Eighty days after the transplantation all mice in the control group perished (10) except one, meanwhile 70% of the animals (12) were still alive in the treated group. In an other experiment, PC-3 tumorous prostatic cells were transplanted into CBA/Ca mice. The DDW treatment started on the 18th day. Twelve days later the tumors were removed from mice and histologically examined. Cells being in mitosis and apoptosis were counted. It was found that in the control group, that received water with normal (150 ppm) D-content, 3.6% of the cells were in mitosis and only 1% in apoptosis. The ratio was almost the opposite in the treated group, where only 1.5% of the cells were in mitosis, while 3% of the cells were in apoptosis. In order to reveal the molecular background of the inhibitory effect of DDW COX-2 gene expression was investigated in healthy myometrial and HT-29 colon tumorous cell line in medium with 20-80-150-200-500-1000 ppm D. It was found that deuterium depletion inhibited COX-2 expression and the inhibition correlated with the D-concentration. At the same time there was a strong correlation between the COX-2 expression and the intracellular prostaglandin concentration. When the prostaglandin was added back to the culture medium it diminished the inhibitory effect of DDW. The application of DDW also proved to influence the expression of genes encoding different kinases using nanocapillary quantitative real-time PCR analysis technique and to modify the activity of amilorid-sensitive Na+/H+ antiport system.

We suggest that cells are able to regulate the D/H ratio and its changes can trigger certain molecular processes. One possibility to modify the D/H ratio is the activation of the H+-transport system which prefers to eliminate H+ resulting in a higher D/H ratio within the cell. We suggest that the changing D/H ratio can simultaneously regulate the expression of certain genes and the activity of enzymes having key role in cell cycle regulation and in other molecular mechanisms. We suppose that the naturally occurring D is the key element of a hitherto unknown sub-molecular regulatory system (SMRS).

Physiological Effects of Heavy Water

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Since the discovery of deuterium on Thanksgiving Day, 1931, the effect of heavy water (D,O) on various living organisms has been widely investigated. The availability of heavy water in the mid-1950's in quantities of hundred tons at a reasonable price greatly facilitated the research on the effects of deuterium in biological systems. The physiological effects of heavy water will be demonstrated on some selected examples.

There are some striking effects of heavy water on simple organisms. While early experiments indicated that the incorporation of deuterium into algae was incompatible with life, it turned out later that by gradually changing the deuterium concentration of water in media used for growing algae can be fully adapted to growth in D2O. Another example is the mold Aspergillus niger which grows well in media containing D2O, but loses its ability to synthesize its characteristic black pigment and becomes alabaster-white.

Whereas simple organisms can be adapted to grow in D2O, higher (vascular) plants as well as animals resist full deuteration. Experiments carried out with mice showed that animals drinking 30% D2O and deuterated to about 25% in the body fluids, live normal life span, however their reproductive capacity becomes severely impaired. Deuterium content of 15-20% in the body fluids was found to be the threshold for toxicity in animals. Since deuterium substitution results in a decrease of the rates of reactions the question arose already at an early stage after the discovery of deuterium whether the growth of tumors could be inhibited by the administration of heavy water. Experiments carried out on mice with lymphosarcoma and mammary cancer showed that although the administration of 40% D2O decreased the growth rate of the tumors, the mice treated died more rapidly due to the toxic effects of deuterium.

It is an interesting question how toxic deuterium or heavy water is to humans. If it is accepted on the basis of animal experiments that the “critical” deuterium concentration (the double of which would cause severe physiological effects) is about 10%, then one can easily estimate that a person of 70 kg may drink 4.8 liter heavy water without serious consequences. Thus heavy water cannot be considered toxic to humans. This is an important conclusion since heavy water is widely used for the estimation of total body water.

It has been shown that the effect of heavy water, and thus of increased deuterium concentration, on various physiological processes has been extensively investigated. However, until 1993, when it was demonstrated by Somlyai and his co-workers that sub-normal deuterium concentration leads to the inhibition of cell division in cultured proliferating animal cells, very little was known about the sensitivity of biological processes to deuterium depletion.
Environmental deuterium and cell proliferation: implications in radiobiology

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Knowing the radio-mimetic effects of deuterated water (heavy water) and having a tool for achieving the partial depletion of deuterium (deuterium-depleted water), the effects of chronic administration of this water were tested in animals, which were then subjected to irradiation with gamma radiation in various doses.

Deuterium-depleted water (DDW) demonstrated to be an effective way to significantly reduce the deuterium concentration in animals subjected to prolonged treatment. Deuterium concentration decreased to about 90 ppm, compared to a normal concentration of about 140-145 ppm deuterium/protium.

Prolonged treatment with DDW significantly protects animals from irradiation with $\gamma$ radiation at LD$_{50}$ dose. The dose modifying factor (DMF) could be calculated at 1.41. The association with the strongest current chemical radioprotective (amifostine) did not increase significantly the effects of treatment with DDW. Protective effects were present also while administering LD$_{50}$ of a chemical radiomimetic preparation derived from nitrogen-yperite (hydrochloric embihine).

The antioxidant action is present but not strong enough to explain the radioprotective effects reported. The strongest action was unspecific stimulation of immunologic parameters.

The investigation of the mechanisms by which the stimulatory action could take place in the rapidly proliferating cellular compartments (hematopoetic and gastrointestinal mucosa) using DDW as medium for normal and neoplastic cells in culture showed significant effects of cell proliferation stimulation. The study of the mechanisms that stimulate proliferation demonstrated the possible involvement of $K^+$/H$^+$ ATP-ase in the mechanisms that regulate cell division.

In conclusion, DDW can be regarded as a radiobiologically active substance.

Recovery and use of deuterium-free water in extended space expeditions

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On long space flights (for example, the Lunar or Martian program) substance circulation based life-support systems will be used. Such systems will include the higher plants, single-celled seaweed and other heterotrophs. This creates need for the development of non-standard technologies for the cultivation and gathering of food products with improved medicinal and biological values.

Long space expeditions will be subject to high levels of radiation, which creates the need to develop new methods of radiation shielding.

Besides, cosmonauts, being only human, can be exposed to diseases. The search for methods that increase the efficiency of the immune systems of cosmonauts is one of the major factors in lengthy space expeditions.

In the State Scientific Center of Russian Federation-Institute for bio-medical problems of the Russian Academy of Sciences long term research has shown that in the solution of these problems deuterium-free water shows promising results and has positive medicinal and biological qualities. With this goal in sight we have developed the technology for its obtainment, based on the electrolysis of distilled water with the subsequent transformation of received gases (hydrogen and oxygen) in water with the lowered concentration of deuterium. In the first stages of the decomposition of water on the cathode, a light isotope of hydrogen is formed: protium, as a result of this oxidation, deuterium-free water is produced.

Deuterium-free water has been used by us for:

1) Cultivation of the higher plants,
2) Breeding of birds – quails,
3) Research of anti-tumoral properties,
4) Research of radiation shielding properties.

Cultivation of the higher plants shows that seed production in higher plants such as the thale cress (Arabidopsis thaliana, Dijon) and field mustard (Brassica rapa) increases by 150-200% after being watered with «deuterium-free» water.

In quails (the Japanese quail: Coturnix coturnix japonica) an increase in growth rate and organ weight of the birds, in particular the reproductive organs, is seen.

Research of deuterium-free water as an anti-tumoral substance showed decrease in growth rates of some tumors, and decreases in metastasis speed have been revealed, as well as an increase in the life duration of animals.

Research of radio-protective properties of «deuterium-free» water has shown that its consumption by animals, irradiated with gamma rays from Cobalt-60, led to increased life duration, and a decrease in speed and count of cataract formations in the eyes.

In long space flights deuterium-free water can be received with the use of regeneration systems from a condensate of atmospheric moisture, urine and other sources.

The results of the research received while developing space life-support systems can be used in medicine, fundamental biology, agriculture and other areas of science.
The nematode Caenorhabditis elegans is a unique model organism for studies on longevity and the aging process. The worm is an attractive system because of its size (1mm in length), short life span (~ 20 days) and its homology to the human genome (60-80%). The knowledgebase obtained through genetic analyses strongly suggests that endocrine signalling plays a key role in many of the pathways that alter the aging process in C. elegans, including insulin/IGF-1-like signalling. In order to study the putative anti-aging effects of depleted deuterium water (DDW), we used a well-standardized manganese (Mn) model of toxicity in C.elegans, in which it has been demonstrated that Mn causes lethal osmoregulation defects, developmental delay, dopaminergic neurodegeneration and decrease in lifespan. In the present study we analyzed the ability of DDW to attenuate the Mn-induced effects. A synchronous population of N2 (wild type) worms was treated with 35 mM MnCl2 (4000 worms per tube) for 30 minutes and then washed 3 times to remove residual Mn. Next, worms were treated for 48 hrs with a D-concentration of 150 ppm (M9 buffer in regular water), 120 ppm (M9 buffer prepared with DDW in a ratio 25:75 of M9 in regular water) or 90 ppm DDW (M9 buffer prepared with DDW in a ratio 50:50 of M9 in regular water). After the treatment, worms were washed and placed on plates for life span assay or prepared for protein extraction and immunoblotting. We observed that Mn caused a shortened lifespan in worms, which was reversed by DDW (120 and 90 ppm). As lifespan is a direct measurement of aging and as DDW showed this anti-aging property, we decided to study the DAF-16 pathway in Mn/DDW treated animals. Interestingly, we observed that DDW was able to restore the DAF-16 expression that had been reduced by Mn exposure. In addition, superoxide dismutase (SOD) and AKT, downstream and upstream proteins in the DAF-16 pathway respectively, were altered by Mn exposure; however, their expression was also restored by DDW treatment. Taken together, these results demonstrate that DDW may play a protective role against Mn toxicity and aging, likely by attenuating Mn-induced reactive oxygen species generation. The effect of DDW is likely mediated by the DAF-16 pathway, as a transcriptional factor that increases the expression of antioxidants proteins and increase the lifespan in C.elegans. Further studies are necessary in order to clarify the exact molecular mechanisms of the anti-aging activity of DDW, especially whether it is only related to the DAF-16 pathway.

Keywords: DDW, manganese, C.elegans, aging.

Intermediary Metabolism and Macromolecule Synthesis in Response to Deuterium Depletion in Pancreatic, Breast and Lung Cancer Cell Lines

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Hydrogen atoms of water participate in virtually all ion exchange and substrate – product transport reactions through the cell membrane and hydrogen also acts as the reducing equivalent in energy producing as well as reductive macromolecule synthesis reactions in all living cells. Deuterium depletion of water in cell culture media or body fluids temporarily decelerates cell growth in vitro and induces tumor regression in vivo. The exact mechanism and the effects of deuterium depletion on mammalian cell intermediary metabolism are not fully known. Potential mechanisms of carcinogenesis include the following: 1) Deuterium incorporation from common water into DNA increases its fragility thus accelerates mutations, aging and cancer; 2) Deuterium affects the kinetics of reductive synthesis and the generation of NADP+ thus altering membrane fatty acid and cholesterol synthesis; 3) Deuterium alters tricarboxylic acid cycle and intermediary metabolism by altering carbon flow and the rate of product synthesis and energy production. Stable isotope-based metabolic profiling studies were performed to determine metabolic flux-modifying effects of deuterium depleted water (DDW: 100, 50 and 25 ppm) as compared to normal deuterium-containing water (150 ppm) on [1,2-13C2]-D-glucose metabolism in cultured pancreatic (MIA-PaCa), lung (H-441) and breast (MCF-7) ductal carcinoma cells. Deuterium depleted water (DDW) did not significantly alter glucose uptake, oxidation and glycogen synthesis in any of the cell lines. Pentose cycle flux relative to glycolysis decreased in MIA-PaCa cells. RNA ribose synthesis and turnover also decreased in MIA-PaCa cells after 25 ppm treatment. TCA cycle substrate flux decreased in MCF-7 breast tumor cells. Lignocerate (C:24) and palmitate syntheses were decreased in MIA-PaCa cells and cholesterol synthesis was decreased in MCF-7 breast tumor cells. Based on these observations it is evident that decreased deuterium to hydrogen ratios regulate sterol and fatty acid precursor synthesis, which likely affects the rate of divisions and cellular proliferation via limited reductive synthesis and new membrane formation.
Cell physiological effects of reduced deuterium content in the leaves of Elodea canadensis

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The effect of reduced deuterium (D) concentration of water was studied on several important cell physiological parameters in intact isolated leaves of the aquatic macrophyte Elodea canadensis by incubating the leaves in nutrient solutions prepared with distilled water having natural (150 ppm) or low (87 ppm) concentration of D in the presence of light. In normal water Elodea leaves induced a gradual alkalinization of the external medium, whereas in low-D water an external acidification was detectable in the first 30 minutes, the rate of which decreased gradually, and after about 2 hours the external alkalinization recovered to a rate similar to that of the control. Associated fluorimetric studies using the potential-sensitive fluorescent probe 3,3′-dipropyl-thiacarbocyanine (di-S-C3-(3)) revealed an increase in the dye fluorescence, indicative of a higher resting membrane potential (hyperpolarization) of the cells. Since the time-course of the hyperpolarization and that of the external acidification was comparable, they both could be associated with an increased activity of the H+-ATP-ase present in the plasma membrane of the cells. These observations demonstrate the ability of Elodea cells to detect and respond to a reduction in the D-concentration of water. It is concluded that a sudden perturbation of the pre-existing intracellular D/H ratios might be responsible for the observed cellular responses. Importantly, these responses are of transient nature, and the physiological processes of the plant cells adapt to the altered conditions within hours.

Antiproliferative effects, cell cycle alterations and apoptosis induction of DDW on T47D and HT-29 human carcinoma cell lines.

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Cancer therapy is still a major obstacle among medical oncologists around the world. Failure in chemotherapy of tumor cells is mainly related to occurrence of drug resistance. Cell cycle alterations and or decreased apoptosis induction following chemotherapy are primarily due to molecular changes of cancer cells. Studying new compounds alone or in combination with potent chemotherapeutic drugs such as Doxorubicin can open new strategic approach in cancer therapy. Previous reports indicated the potential anticancer effects of Deuterium Depleted Water (DDW). Therefore, we studied the antiproliferative effects of 2 types of DDWs (A and B) produced by AEO on human breast T47D and colorectal HT-29 carcinoma cells in comparison to Doxorubicin (Dox) using MTT assay. Cell cycle alterations and apoptosis induction following exposure of cancer cells to DDWs (A and B) were also evaluated by flowcytometry using DAPI and Annexin V-FITC/PI reagents, respectively. Both types of DDWs showed no apparent effects on proliferation of T47D and HT-29 cell lines. But the antiproliferative effect of Dox was slightly increased when prepared in DDWs in comparison to Dox alone. The S phase in cell cycle pattern of T47D cells was significantly increased only in Dox prepared in type A of DDW (Dox A). In the HT-29 cells, the G2/M phase of cell cycle was significantly increased in both Dox A and Dox B in comparison to Dox alone. The T47D cells exposed to Dox A and Dox B in comparison to Dox alone showed increased apoptosis and necrosis, respectively. Significant increase in apoptosis induction of HT-29 cells was observed following exposure to Dox A and Dox B in comparison to Dox. In conclusion, these data supports the previously reported findings on significant potential of DDWs on cancer cells to enhance the effectiveness of chemotherapy.

Keywords: Cancer therapy, DDW, T47D, HT-29, Cytotoxicity, Cell Cycle, Apoptosis

Studies concerning DDW use for deuterium's depletion in animal organism and for synthesis of new naphthaquinonic antitumoral compounds

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Until the 90’s, it didn’t exist the possibility to reduce the quantity of deuterium in external or internal medium of living organisms. With the discovery of DDW (deuterium depleted water or light water), it was obtained a rapid and easy way to reduce deuterium in biological systems by the simple installation for obtaining the deuterium depleted water. In our studies we used DDW produced at our institute (patent WO/2006/028400; PCT/RO2005/000011 – Process and installation for obtaining the deuterium depleted water), which is the main ingredient for the commercial product QLARIVIA™. The experiments followed two main directions: the first one was about monitoring deuterium’s concentration decrease in animal’s body using DDW as depleting agent; the second one consisted in the synthesis of new antitumoral compounds derivated from

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naphthoquinone ligands using DDW as solvent. Literature mentioned the antiproliferative and/or antitumoral effects [1,2,3,4] of DDW, but without showing the comparative deuterium ratio in normal condition and after administration of DDW. In this respect, our deuterium depletion studies were carried out on laboratory and farm animals, nourished with standard food and drinking water (witness lot) and DDW (experimental lots). Biological samples (liquid and solid samples) were analyzed by mass-spectrometry. These new synthesized antitumoral compounds consist in metal complexes with naphthoquinone derivatives. In the process of synthesis, DDW was the solvent for transition metals (Ni, Cu, Co) salts in order to decrease the deuterium content of our new compounds. The result of first experimental series shows that in natural conditions, the organism has the tendency to accumulate deuterium through bioaccumulation process. On the other hand, the grade of deuterium depletion seems to be under influence both of specimen genotype and behavior, and also of deuterium’s depleting medium concentration – moreover, this effect is time dependent. The result of second experimental series was the obtaining new types of anticancer compounds with low deuterium content for in vitro and in vivo future experiments. As a conclusion, DDW can be used as a synergic agent for deuterium depletion in both biological assays and chemical processes for a possible combined anticancer therapy.

The effects of administration and local application of deuterium-depleted water on dogs and cats suffering from spontaneous malignancies

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Dogs and cats bearing malignant tumors were given Vetera-DDW-25® A.U.V. deuterium-depleted water (DDW) containing deuterium (D) at a concentration of 2.8 mmol/L (25 ppm) instead of normal tap water, which contains 16.8 mmol/L D (150 ppm). The DDW therapy was combined with surgery when conditions were sufficient for the excision of the tumor or DDW was applied as a single treatment in numerous cases. Mammary tumors in 81 dogs and 14 cats showed a response rate higher than 70%; more than 50% of the animals achieved complete recovery. Similar effectiveness was observed in 43 dogs and 3 cats bearing rectal tumors. More than 70% of cats with lymphoid leucosis achieved complete response. Sarcomatoid tumors in dogs showed a short-term response or no response at all. As a result of DDW consumption, a gradual decrease of the serum D-concentration was detected in dogs and cats during the 12-week DDW treatment, which was restored when DDW intake was replaced again with ordinary tap water. The gradual decrease of serum D-concentration raised the question whether it is possible to increase the effectiveness of DDW if we can reach a more rapid decrease in D-concentration especially in certain types of tumors, such as ulcerative, extensive, relapsed, metastasizing tumors, malignant melanoma, and sarcomatoid tumors which were fairly resistant to per os (PO) DDW treatment. In order to get a sharp decrease in D-concentration a new injectable formulation of DDW was developed. Dogs affected with malignant tumors of poor prognosis were submitted to treatment with DDW injections. Local treatment induced regression of tumors that were resistant to previous PO DDW administration and produced advantageous conditions for subsequent surgical interventions. Microscopic evaluations of the tumors verified that DDW injection induced disappearance of tumor cell infiltration, demarcation and consequent rejection of invasive tumors, nevertheless, DDW injections were harmless to healthy tissues in the environment of the tumor. Simultaneous PO and local treatment was initiated in some animals to enhance therapeutic effectiveness and to prevent relapses. As a result of the combined treatment, dogs became tumor-free and asymptomatic and 3 to 3.5 years after therapy they were still alive. DDW injection alone or in combination with surgery offers an effective cancer treatment of aggressive tumors with poor prognosis.

Effect of deuterium oxide (D₂O) content of drinking water on glucose metabolism in STZ-induced diabetic rats

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Deuterium, a stable isotope of hydrogen, binds to oxygen to form D₂O. D₂O exist in the environment at 1/6700 of H₂O (150 ppm) and is expected to have some biological effects. Several lines of evidences suggest that D₂O inhibits insulin release from pancreatic islets. Very little or no data is available on the action of lowering D₂O content of the cellular environment. Some experimental and clinical observations suggest that depletion of D₂O has anti-mitotic effect in various tumor cells. Some clinical observations also suggest that depletion D₂O interfere with glucose metabolism in diabetic patients. In our experiments we wanted to test the effect of removal of D₂O on the glucose metabolism in streptozotocin (STZ)-induced diabetic rat model. Diabetes was induced by a single ip. injection of 60mg/kg body weight of STZ. After 2 weeks, animals were randomly distributed into several groups to test the effect of D₂O (25-150 ppm) on glucose metabolism in diabetic animals with or without 2x1 U/day insulin treatment. The following parameters were tested: serum glucose, fructose amine, HbA₁c, -creatinine, -TBARS and -insulin; urine glucose, -creatinine and -protein. At the end of the experiments, 8 weeks of treatment, membrane associated GLUT-4 content was estimated by western-blot technique from m. soleus. Our results indicate that STZ treatment significantly increased serum glucose, fructose amine, HbA₁c and TBARS concentration. Depletion of D₂O did not influence any of the measured parameters in animals.
not received insulin. However the measured parameters were significantly lower in those animals received lower D₂O containing drinking water and insulin treatment. The membrane associated GLUT-4 was significantly higher in these animals also. These data suggest that D₂O depletion enhance insulin effect on GLUT-4 translocation and potentiate glucose uptake in diabetic animals. The mode of action of D₂O depletion is not fully understood and needs further experiments to elucidate this question.

**Deuterium depletion as an effective treatment option for prostate cancer – Statistical evaluation of a double blind, randomized phase II clinical trial and a retrospective study on prostate cancer**

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In order to investigate whether DDW might exert an anticancer effect in humans and improve the results of conventional treatments, a four-month long double blind, phase II, placebo controlled clinical trial was conducted on prostate cancer. In addition, beside the 44 evaluated patients in the phase II clinical trial, the course of the disease was also retrospectively evaluated in 91 patients consuming DDW parallel with the conventional forms of treatments. Summarizing the changes in prostate volume during the 4 months' period of the phase II clinical trial in the treated group a net decrease of 160.3 cm³ was achieved, on the contrary, the result was 54 cm³ in the control group. Furthermore, in those 7 patients, who achieved PR (p=0.046), the prostate volume became smaller by 125.2 cm³ in total, and the patients reached 32%, 64%, 18%, 70%, 47%, 20%, and 52.6% decrease respectively in comparison to the size detected at entering the trial. One patient showing PR in the control group achieved 13.4 cm³ (59%) decrease. Urination complaints ceased in 8 patients of the treated group, but none of the patients experienced changes in their complaints in the placebo group (p=0.0041). During the extended follow-up of the 44 patients, in the first year (from the date of entering the trial), 2 patients (9.1%) died in the treated group and 9 patients (40.9%) in the placebo group (significantly lower mortality in the treated group; Fisher’s Exact Test, p=0.034).

The cumulative time of DDW consumption of the retrospectively evaluated 91 patients was 139.2 years. The time period from the initial diagnosis to the end of the follow-up was 350.1 years. The median survival time (MST) of the 91 patients was 11.02 years although 46 of them (50.5%) had distant metastasis either before the start of the DDW treatment or after that. Due to the extremely low death rate (4 patients, 8.8%) in spite of the 157 years cumulative follow-up period in the population without distant metastasis, we could not calculate the MST. Investigating the patients with distant metastasis developed within one year after the diagnosis, the MST was 5.4 year (64.8 months) suggesting that the administration of DDW may had an effect resulting in longer MST comparing to other studies with progressive metastatic prostate cancer showing 15-20 months long MST. The results suggest that DDW might reduce the mortality of prostate cancer, since it was able to delay progression as well as to prolong MST in patients with histologically confirmed prostate cancer.

**A Retrospective Study to Evaluate the Effect of Deuterium Depletion on the Survival of Metastatic Breast Cancer Patients**

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Breast cancer is one among the most prevalent forms of malignant tumors worldwide. Development of locally advanced, recurrent and/or metastasizing disease is the primary determinant of the outcome and represents a high risk for breast cancer patients. The anticancer effect of deuterium depleted water (DDW) has been revealed in the treatment of spontaneous malignancies in dogs and cats. The therapeutic efficacy of per os (PO) DDW treatment was also confirmed in a human, phase II double blind clinical trial on prostate cancer. The aim of the present study was to investigate the impact of DDW consumption in addition to conventional forms of treatments on the survival of metastatic breast cancer (MBC) patients. The records of 74 women suffering from MBC were retrospectively evaluated for the period between January 1993 and May 2005 and all but 6 of the 74 patients had previously undergone intensive and repeated prior conventional therapy and had limited expectations on survival time. In the 74 patients 135 distant metastases were diagnosed before DDW treatment commenced. Conventional cancer therapy was supplemented with PO DDW treatment; i.e. daily water intake of the patients was replaced by DDW. DDW consumption and simultaneous conventional treatment elicited response or halted tumor growth in 74.3% of the 74 MBC patients. Median survival time (MST) from the diagnosis of the distant metastasis was 47.7 months. Probability for 1-, 2-, 5-, and 8-year-long survivals were 93.7%, 77.8%, 33.4%, and 9.1%, respectively. The subgroup of patients achieving complete (CR), or partial response (PR) had MST of 63 and 50 months, respectively, while the subgroups showing no change (NC) or progression of disease (PD) reached a MST of 35 and 31 months respectively. Taking the amount and the D-concentration of the DDW consumed, the lengths of the treatment and body weight into consideration, a new parameter called deuterium depletion unit (DdU) has been introduced. Frequency of CR and PR was significantly higher (p=0.0028) in patients consuming DDW at a dose higher than 1 DdU and borderline significant results (p=0.046) were achieved when deuterium depletion reached 0.6 DdU. The great majority of the patients whose disease progressed or showed no change consumed DDW at a low dose and/or at irregular intervals. Application of DDW as an oral anticancer agent simultaneously with the conventional forms of treatment remarkably prolonged MST; it produced twice to three times longer MST in patients achieving CR or PR. We suggest that DDW can be integrated in the conventional treatment regimens of MBC patients.
Lung cancer and deuterium depletion. Clinical and experimental data.

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In spite of the development of the therapies, the shortness of the survival of lung cancer patients is still remained disappointing. In this manner finding new adjuvant strategies is in the focus of cancer cure. Deuterium depletion represses cell division in plants and suppresses some genes, which are involved in tumour formation. In this experiment we compared expressions of genes, which are involved in lung cancer development to clinical outcomes of lung cancer patients consuming deuterium depleted water. In animal experiment, carcinogenic DMBA was applied to induce over-expression of p53, bcl-2 and Kras in lung tissue of mice, while animals drank deuterium depleted water. In clinical study, 129 patients received diverse chemotherapy and radiotherapy and drank deuterium depleted water as an adjuvant, non-toxic therapy. Deuterium depleted water could significantly diminish DMBA-induced expression of p53, bcl-2 and Kras in the lungs of mice. Within patients, median survival was 25.7 months, CI [22.3; 29.1] in men and 73.6 months, CI [50.6; 96.7] in women with statistically significant difference. Median survival of subjects with brain metastasis was 26.9 months, CI [20.9; 32.9]. Cumulative 5 year survival probabilities were 15 %, 60 % and 26 % in male, female and patients with brain metastasis respectively. Deuterium depletion could prevent the DMBA-induced over-expression of p53, bcl-2 and Kras genes as an anti-carcinogenic agent in animals and could extend survival of lung cancer patients as an anticancer adjuvant therapy especially within women maybe because of suppression of cancer-related genes.

Main results and basic rules of the human application of deuterium depleted water in combination with conventional therapies

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Beside the phase II clinical trial, that had been conducted with 44 prostate cancer patients, a data base has been created from 1992 involving all those patients who had been subjected to the administration of deuterium depleted water (DDW) for at least one day. This data base refers to 1,969 patients (949 male and 1020 female patients), who fulfilled this main criterium between October 1992 and April 2010. The cumulative time from diagnosis to the end of the follow-up period was 5,136 years, from the start of DDW consumption to the end of the follow-up, this value was 2,692 years. The cumulative time of DDW administration was 1,892 years. The median age of the investigated population was 55 years. The evaluated 1,969 patients represented 59 different localizations of the tumor. The incidence of the main tumor types among the examined patients was almost identical with the same data of the National Cancer Registry. The median survival time (MST) of the whole test population was 7.7 years. Investigating the length of MST and the duration of DDW consumption, a strong correlation was found. MST was calculated for the sub-groups of patients consuming DDW for 0-3, 3-6, 6-12, 12-24 and longer than 24 months and an MST of 0.7, 4.5, 5.8, 9.9 and 17.8 years was found, retrospectively. 583 patients died (303 males and 280 females) out of 1,969 during the follow-up period. 59.3% of the patients died within 6 months, 36.1% within 3 months after the start of DDW consumption. Investigating the possible causes of unsuccess in these cases we found that the median time of DDW administration was only 37 days in patients consuming DDW for not longer than 3 months, while it was 288 days in those sub-group where patients consumed DDW for longer than 3 months, suggesting that the length of DDW administration is a determinative factor influencing the outcome of the disease. There was a sub-group of patients (99), who, in most cases, interrupted the use of DDW after its long-term consumption, but later on they started again and repeated the cures in spite of the fact, that they were in remission. The median time from the diagnosis to the start of DDW was 174 days, the length of DDW consumption was 1,015 days. Since only 8 patients died in this sub-group during the 585 years' cumulative survival time of the follow-up period from the diagnosis, it was not possible to calculate the MST because of the extremely low death rate.

We conclude that the integration of DDW treatment into the conventional oncotherapies may increase the MST of cancer patients. The efficacy of DDW depends on the length of DDW-treatment. Based on the statistical analysis of homogenous populations of metastatic breast-, lung-, and prostate cancer patients has been carried out earlier, and the present evaluation of the data of 1,969 patients using DDW, we conclude, that through the integration of DDW into the widely accepted and applied oncotherapies a 2-3 fold increase may be achieved in the MST of cancer patients suffering from the most common tumor types.
The main activities of our Connections2000 Event and Incoming Agency, which has been working for 10 years, are comprehensive preparation, organization and arrangement of conferences, international congresses, doctor-patient appointments, press conferences, trainings, banquets, social events and incoming programs into Hungary.

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- EULAR Autumn Conference for PARE - 2008
- Hungarian Cancer Society - Nursing Session I, Roving Conference and Exhibition - 2008
- International World Day of Nurses-Record Day of Oncological Nurses - 2008
- XXVII. Congress of Hungarian Cancer Society - 2007
- “Kékgyöző” Days 2007 National Conference and professionals Exhibition for Oncological Nurses - 2007
- 19th Meeting of the European Association for Cancer Research -2006
- NEW HORIZONS in Treating Cancer 4th International Conference for Organizations Representing People with CML or GIST -2006
- 8th Annual Congress of European Society of Oncology -2006

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