REPORTS OF NUCLEOTIDES AND RELATED CONTENTS

Clinical Studies
Hospital Studies
Government Reports
Doctors Reports
World Health Organization
Imuregen Final Report
Introduction

This book is an assembly of some of very important studies, reports and research papers that were funded and supported by various governments, the World Health Organization, leading scientists and doctors all interested in changing the lives and health of the general population. It is not intended to make any statements about cure or treatment but instead, just to give the reader an idea about some of the incredible natural healing opportunities that the body has as well as nutrients and what you can do to support the body function and structure.

Many Experts around the globe realize that the miracle of the body and its ability to heal itself might be the best way to regain or improve your health. Many of the illnesses of today and yesterday are a result of our lifestyle, environment, the food we eat, stress and what we drink.

We have learned that if we give the body the nutrients it needs, the body will have a stronger immune system and perhaps avoid sickness and disease. Also, if the body is weak and/or sick, the studies have shown that the body can regain health with proper supplementation of the nutrients it needs to build the immune system and fight the problem.

Feel free to study many of the additional reports and studies that can be found in PubMed and other European Journals. There are so many more that talk about Nucleotides and immune system. I am sure that there will be many more. Today, the value of nucleotides is such that it is being put into baby’s formulas.

All studies we have seen have shown significant results and improvements. The word “significant” is actually used in many of the studies in their conclusions.

We hope that the information provided is of value to you.
# Nucleotides and related reports and clinical studies

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What is IMUREGEN?

THE VALUE OF DNA/RNA NUCLEOTIDE SUPPLEMENTATION

The science which promotes healthy aging is designed to preserve the human body in a healthy state for as long a period as possible. Thus it seeks to maintain one’s mind and body mentally alert and physically active so that one can enjoy contentment throughout all of life, relatively free of stress.

One of the challenges to healthy aging is the ability for the body to defend itself against disease and in increasingly unfriendly environment. The present approach for dealing with health problems is to treat the symptoms rather than the cause of the disease or discomfort by drugs and/or surgery. Although this is an effective means for resolving a serious infection or a life-threatening circumstance it is not a panacea for resolving all medical issues.

It has been the vision of the scientific community to find a way to strengthen the immune system, similar to vaccination, so that it will be ready to destroy pathogens; Imuregen was created with this vision in mind. Based on long term experimental research and clinical studies in Europe, it has been confirmed that Imuregen has positive effects on the total regeneration of the body and immune system. It is especially suitable for persons who are under extreme physical or mental stress. It fosters the regeneration of liver tissue and mucous membranes of the digestive and respiratory systems. It also provides protection from viral and bacterial infections.

Imuregen offers a new means of strengthening the immune system and promoting cell regeneration. By using highly efficient natural substances, such as essential nucleotides, amino acids, oligopeptides and trace elements, Imuregen is able to increase the body’s immunity and effect cell regeneration. Imuregen is introduced into the digestive tract in active form thus allowing the body to absorb only the substances it needs for optimizing performance, avoiding stress and disease, and to improve metabolism during a disease. Imuregen regulates the body’s stamina by modulating the cellular and hormonal function of the immune system.

Imuregen supplementation is thus one of the most rejuvenating, immune enhancing, and tissue supporting regimens ever to be discovered.

Numerous published scientific studies indicate very significant health benefits from DNA and RNA component supplementation. Almost every system of the body has documentation of improved health, vitality, or function from providing supplements of these fundamentally
important cellular elements, from infancy to advanced age. The following is a brief summary from the vast literature supporting the many published benefits of nucleotide supplementation in the diet.

**Tissue Regeneration**

In order to sustain health, virtually every tissue in the body must regenerate itself regularly. Having adequate supplies of all the nucleic acid bases may be one of the most significant limiting factors on whether a tissue will be able to express its greatest capacity for regeneration and self-repair.

Any tissue, in order to regenerate, requires the ability to make DNA and RNA to support the process of making new cells. Providing readily absorbed and assimilated nucleotides can be one of the most powerful ways to assist any tissue to repair and renew itself.

**Wound Healing**

Several studies in wound healing have assessed the effects of supplemental nucleic acids on wound healing. Compared to the control group, those receiving the supplements showed more rapid healing, greater tensile strength of the skin, and significantly reduced scarring.

**Endocrine Gland Repair**

Some of the tiniest organs in the body have the most profound effects on our health and well being. These are the endocrine glands that secrete minute amounts of hormones into the blood without which every function of the body can suffer.

- The pituitary gland
- The adrenal glands
- The thymus gland
- The thyroid gland
- The salivary glands
Intestinal Integrity, Maturation, and Bowel Flora

The intestinal lining replaces all of its cells every seven days. Only a single layer thick, this lining is highly dependent on a sufficient supply of nucleic acids to completely regenerate itself every week. If nutritional support is inadequate, defective regeneration of the intestinal mucosal lining impairs the enzymatic stages of digestion, which can lead to a vicious cycle of deteriorating digestion and nutritional status.

Upon administration of supplemental nucleotides, the appearance of the intestinal lining greatly improved, with regeneration of the height of the absorptive intestinal villi. In addition, the enzyme content and function of the Intestinal lining also greatly improved, permitting the animals to recover and thrive robustly.

Regen - antimicrobial peptides and proteins (AMPP)

AMPP’s have enormous importance for human health, because they kill microorganisms (bacteria, viruses, fungi, protozoa) on the interfaces between the external and internal environment of the body (intestine, lung, skin, urinary tract) and protects the circulatory system from the penetration of microbes (Yu & comp. 2010). AMPP regulate immune response and slows dangerous inflammatory response which are dangerous to the body.
HDL Cholesterol Levels
An additional finding in individuals who received nucleotide supplementation was an improvement in their blood lipid profiles. In particular, the individuals receiving added nucleic acids were found to have higher HDL cholesterol levels, the cholesterol fraction that protects against cardiovascular disease the higher the level.

Growth and Development
Studies in young laboratory animals have assessed the effects of supplementing DNA and RNA elements. Compared to control animals, the supplemented animals grew, developed, and increased muscle mass at a greater rate. Other vital proteins were also built more readily in the treated animals. The intestinal lining in particular matured more robustly in the supplemented animals. Research thus far indicates that the tremendous need for nucleic acids in growth and development is strongly beneficially supported through supplementing these vital nutritional elements.

Cellular Immunity
Cellular immunity refers in particular to immune cells that have the role of identifying cells in the body that have become abnormal, so that the abnormal cells can be removed. The main cellular changes sought through the cellular immune system are the development of cancer cells or various types of intracellular infection. The goal of the cellular immune system is to eliminate cancer cells or infected cells before they can become established in the body to cause serious illness. The main effectors of cellular immunity are cells that arise in the thymus gland. These cells are often called T cells for their thymic derivation, of which there are several types with varying functions. A special type of T cell called a cytotoxic T cell has the role of finding and sticking to abnormal cells, then releasing substances that selectively digest and clear the renegade cells.
Memory Enhancement

Many studies in animals and humans have found a dramatic improvement in memory function with nucleic acid supplementation. Whether it is the ability to remember the right pathway to get through a maze for a prize of cheese, or to remember facts and figures, giving supplements of DNA and RNA elements has highly significantly increased performance. Perhaps most dramatically, one researcher has focused on giving nucleic acids to persons with dementia. Even with advanced cases, if he went to high enough delivery levels to his patients, in almost every case memory improvement was very significant. The doctor reported that even in advanced cases of dementia dramatic memory recovery occurred if high enough levels of nucleic acids were given.

Longevity

It is perhaps functional nucleic acid deficiency that limits our potential for healthy longevity more than any other single factor. Of all the interventions that have ever been attempted to increase the life span of mammals, no method ever studied has been more powerful for mammalian life extension than nucleic acid supplementation. Compared to other techniques that have increased longevity of experimental animals up to 50%, administering nucleic acids has doubled and even tripled the usual maximum life span.

Added ATP Benefits

ATP stands for adenosine triphosphate, perhaps the most important of all the nucleic acid derivatives in the body. Its effects are so powerful and essential to cellular function, a description of its unique properties warrants special attention. ATP is the fundamental currency of every cell in the body. Virtually every activity in the body that requires energy uses ATP as the source of power. Whether the function is building complex molecules from building blocks, maintaining the
electric potential of cell membranes, or allowing muscle fibers to contract for mobility, speed, and strength, it is ATP that provides the electrochemical fuel. The oxygenating effects of nucleotide supplementation combined with a supply of ATP is likely to be additive and even synergistic at helping cells throughout the body achieve higher energy potentials and more ideal energy balances.

**Cellular Energy**

There are two fundamental ways ATP is generated in the body, one very efficient and one very wasteful. Efficient ATP production occurs through aerobic metabolism in the mitochondria, tiny organs or organelles within the cell that burn fuels like fat and glucose to generate ATP. Aerobic means that oxygen is used to completely burn a fuel for maximum ATP production. For example, the complete combustion of a single glucose molecule to carbon dioxide and water yields a rich harvest of 36 molecules of ATP. As nucleotides boosts cellular oxygen delivery, already making ATP production more efficient, the ATP has an ideal environment for further boosting cellular energy conditions; thus all the desirable ATP effects are likely to be even more potent.

The oxygenating effects of nucleotide supplementation combined with a supply of ATP is likely to be additive and even synergistic at helping cells throughout the body achieve higher energy potentials and more ideal energy balances.

**Neurological Effects**

ATP is the primary fuel that drives learning, memory, and concentration functions.
Cardiac Strengthening
The cyclic contraction of cardiac muscle is highly ATP intensive and thrives on aerobic metabolism. The combined oxygenation and ATP delivery effects of DNA/RNA formulas provide the heart with an enhanced energy supply for efficient function.

Muscle Performance
Skeletal Muscle also requires abundant quantities of ATP for muscular contraction. Supplemental ATP has been described as an explosive performance enhancer. Especially, if given with two other nutrient supporters of muscle function, creatine monohydrate and creatine pyruvate, muscle endurance, performance, and recovery can be significantly boosted.

Lung Function
ATP administration has been shown to have numerous beneficial effects on lung function, particularly the delicate lining membranes of the airways and alveoli. In some conditions, the blood pressure in the vessels in the lungs can rise too high, a condition known as pulmonary hypertension. ATP binds to the lining of the pulmonary vessels and stimulates a cascade of events that cause the blood vessels to relax and lower the pressure.

Cystic fibrosis is one of the most common inherited genetic diseases. Impaired water and electrolyte secretion from the bronchial lining results in thick secretions that block the bronchial tubes and result in recurring infections. ATP has been found to increase electrolyte and water secretion with improved clearance of secretions, offering hope of a new and useful intervention in this often aggressively progressive condition.
Antitumor Effects
In test tube studies, adding ATP has shown the ability to inhibit the growth of several types of human cancer cell lines. The types of cancer cells inhibited include pancreatic cancer, colon cancer, melanoma, androgen-independent prostate cancer (i.e., not responsive to male hormone manipulation, the most aggressive variant), breast cancer, myeloid and monocytic leukemia (bone marrow derived tumors of blood forming cells), and multidrug resistant colon cancer. In contrast, normal cells from these tissues showed less inhibition of growth or no inhibition at all, suggesting that increasing ATP outside cells may have a selective inhibitory effect on several cancer cell lines.

Improved Stress Management
Under conditions of metabolic stress, such as depriving a tissue of oxygen through reduced blood supply, a rapid and massive depletion of ATP within cells occurs. REgen has been described as a ~natural defense system~ to protect the tissues from the effects of severe oxygen deprivation. These protective effects include improved function of energy generating mitochondria, better electrolyte transport, increased ATP within cells, reduced oxygen consumption, and improved function of messenger molecules within the cells.
Stress is a condition in which there is a generalized reduction of blood flow and oxygenation to tissues below that required for their function. If stress is sustained, the prolonged tension from internal or external stressors may cause various physical manifestations – e.g., asthma, back pain, arrhythmias, fatigue, headaches, irritable bowel syndrome, ulcers, and suppress the immune system".
It can raise blood pressure, increase the risk of heart attack and stroke, increase vulnerability to anxiety and depression, contribute to infertility, and hasten the aging process.
Sexual Function

In human tissue studies, the increase of ATP and adenosine has been found to induce the smooth muscle relaxation that is essential for erectile function. In diabetic men, erectile dysfunction is common through several mechanisms. The erectile tissue of diabetic men has been found to be especially sensitive to the smooth muscle relaxation effects of ATP, offering them a hopeful avenue of recovery of erectile function.

*Statements have not been evaluated by the FDA. No claims of cure, diagnosis, substitute for medicine, surgery or even prevention of diseases are implied. Always consult your physician for any medical condition.

NO APPROVED THERAPEUTIC CLAIMS
IMUREGEN SUMMARY OF CLINICAL STUDIES AND PRODUCT INFORMATION

Industrial revolution of 20th century and subsequent worsening of environment/living conditions triggered onset of civilization diseases and brought humankind to such situation, that those former natural abilities of human organism for self-regeneration and self-healing started to fade/diminish. Our immune system is daily bombarded with adverse effect of civilization and pollution. Our daily fight with these will use almost all our defensive potential, so when we are exposed to sudden onset of flu, cold or other disease, our organism can be paralyzed. That’s why it is necessary to use prevention as a strategy, for to prepare our organism for this fight. There are many ways to prevention. For example we can go to environment not effected by industrial pollution and consume uncontaminated food there. It would be ideal, however, only a few can afford it. Most of us have to live where we can exist economically even if it is in the places affected by civilization’s contamination. Therefore there is no other way then to systematically look for alternative ways on how to prepare our organism for the most important fight – the fight with diseases. Famous Czech pharmacist Dr. Rakus started as one of the first in 30’s and mainly in 40’s of 20 century with concept that we have to treat human organism natural way and it can be done only with the extracts from herbal and animal tissues. Not by the artificially created chemical substances.

If we can use the proper technology and extract the most effective substances, - i.e. amino acids, peptides, oligopeptides, nucleotides and suitable organic salts – than we can win our fight. The man, after all, is part of the nature, and much more beneficial for him are natural complexes. These essential building elements have a great advantage. If a suitable technology is used, they are absolutely non-toxic, non-addictive and they do not settle in the organism. In addition, they effectively support the immune system.

Company “Jednota” started in highly specialized laboratories to manufacture product IMUREGEN on the base of amino acids, peptides, oligopeptides, minerals, nucleotides and suitable organic salts and vitamins. These in natural way increase energetic potential of human cells and bring immune system to harmony and thus maximize immune protection of organism.

In the year 1998 manufacturing and patent was brought by company UNIREGEN which continues in development, research and improvement of this unique product.

IMUREGEN is made from natural sources by a modern biotechnology, based on auto enzymatic disintegration of the macromolecular protein component. The product of this biotechnology is subject to long-term non-destructive careful extraction with a follow-up natural separation of a set of effective low-molecular substances having a catalytic effect on specific internal secretion glands while influencing favorably the intermedial metabolism of cells. The effective complex of IMUREGEN is a quantitatively characteristic set of amino acids and essential amino acids which are partly loose and partly in the form of low-molecular peptides and oligopeptides, tied to the disintegrative products of haem, i.e. chained porphyrine nuclei with loosened Fe++ and naturally formed compounds of elements essential for the organism [ e.g. Se, Mn, Zn, Mg, K ].
IMUREGEN - is a substance intended for mass prevention of civilization diseases, (including cancer, cardiovascular, diabetes, influenza and asthmatic diseases) also against bacterial infections. The complexes of essential amino acids are showing favorable effects on internal secretion glands. They incite the function of the pituitary gland and are functioning as regulators of the energy system by means of the neurohormonal apparatus of the organism. The oligopeptidic complexes incite the function of the thymus and stimulate the function of the spleen, which increases the immune response of the organism relatively quickly. The peptidic complexes provoke an important increase in lysoxine content in the blood, so that the effects of IMUREGEN may be compared to the function of unspecified antibiotics, without, however, their negative impact on the organism. The organically tied bivalent iron penetrates the intestinal wall without oxidation, thus increasing the blood pool between the spleen and the bone marrow. Its effects include improved memory; improved mobility and psychic balance, reduced fatigue and better physical and mental working ability. Under its influence the course and the frequency of allergies and common ailments, such as influenza, tonsillitis and colds, are improved. Wounds and common injuries heal more quickly. The long-term testing of IMUREGEN has proved that it is absolutely non-toxic, non-addictive and that it does not settle in the organism.

Clinical tests proved a significant strengthening of cell immunity, mainly increase of T-lymphocytes and their activity, induction of mucous immunity response.

Application of the IMUREGEN has shown that there were significantly increased values of lysozyme and increased values of many proteins. The application of the IMUREGEN significantly reduced the increase of the C-reactive protein and the orosomucoide, which are the symptoms of inflammatory processes. The top athletes and sport teams have shown an improvement of power/strength parameters and training capacity and further more, there was also proved the protective influence on liver parenchyma from the effects of physical load. When IMUREGEN was applied to the patients with hyperlipidemia, there was noticed a significant decrease of the cholesterol level. There was proved a significant reduction of sickness in work collectives in the normal season and during seasonal viral diseases. The use of IMUREGEN positively effects the health conditions of children, mainly in regions with extremely damaged environment.

In the past IMUREGEN was successfully tested in following medical institutions:

- VŠCHT Prague – Research department of Experimental Oncology- MUDr.Karel Cerný.
- Thomayer Hospital - Institute of clinical and experimental medicine (IKEM) in Prague-Krči - oncology, Head of Department MUDr.J.Korbelář CSc.
- University hospital in Motole, II. Childrens Clinic prof.MUDr.O.Hrodek, DrSc.,
- Hospital FN Na Bulovce –children’s department,, head of Department Doc.MUDr.Václav Špicák CSc.,
- Radiotherapeutic Hospital SFN Olomouc, Head of Department Doc.Dr.Macháček CSc.
- Clinic of Nuclear Medicine SFN Olomouc, Head master Doc.V.Rýznar CSc. Palackého university Olomouc Kraj.
- ÚNZ Ústí nad Labem – Department of Immunology , Head of Department MUDr.Jos.Richtr CSc.
- Clinic of Neurology SFN Olomouc Doc.MUDr.Jos.Klapetek CSc.
• Teaching Hospital Hradec Králové, Doc.MUDr.K.Martiník CSc.

All results from all testing show very positive role of IMUREGEN as a support treatment of malignant – cancer diseases. Some of the results are shown below.

Faculty Hospital Clinic of Neuroradiotherapy – Faculty of Medical Faculty Palackého University in Olomouc - report on application of product IMUREGEN, Clinic FNAL F Olomouc – report on IMUREGEN, Institute of clinical and experimental medicine of Academy of Medical Sciences - Novosibirsk.

Faculty Hospital, Clinic of Neurology of Medical Faculty - Palackého University in Olomouc

Head of Department: Professor MUDr. Jiří Hartl, DrSc

Report on application of product IMUREGEN

In the span of the years 1955 - 1989 in the Clinic of Neurology of Faculty hospital in Olomouc, many patients were treated for malignant cancerous growth, most often already metastasized. Neurological therapy was in all cases symptomatic. The basic sickness with malignant tumors (and metastasis) was treated in all patients with the basic of oncologic therapy by actinotherapy, cytostatic a chemotherapy. In a patient E.R.(case # 5 in this report) radioiodine 131 I was applied, later when metastasis were defirened, radiation treatment was stopped and patient treated further only by analgesics.

In all observed patients beside the basic oncology therapy was also used support treatment with product IMUREGEN. We have observed very interesting results with usage of IMUREGEN. The first patient (A.K.) with histology diagnosis of stomach (penetration of tumor into colon transversum, many metastases in omentum) is surviving practically 34 years after palliative surgery. Another patient (E.R.) with metastasis of thyroid is living more than 30 month just with minimal algic problems.

We provide this informative report on just some patients from many.

Case #1 Patient A.K., born. 13.1.1895.

In May 1955 proven big tumor of stomach. With penetration of stomach wall from angulus till pylorus. At the surgery (prof. Dr. Rapant, DrSc.) 25.5.1955 was found tumor penetrating to mesocolon transversum, further many metastases to omentum, Done palliative resection. After operation healing complicated by abscess in the wound.

Histology of results: reothelsarcoma ventriculi.

Therapy:
Aktinotherapy of body cavity (4p. a 1250 r), during aktinotherapy decrease of leukocytes.
In July 1955 started with product IMUREGEN, 2x 0,50 mg weekly.
Rtg control of stomach: 9.9.1956 (MUDr. Kameniček):
State after resection showing smooth relief, walls are smooth, gastrolith regular, no pain, free passage. No sighs of recidivism.
Conclusion:
In this patient was found big tumor of stomach. Infiltrating from angulus till pylorus. After surgery was used IMUREGEN. Rtg control of stomach and lungs did not shown any further
recidivism of cancer disease. With 34 years from palliative surgery, patient who is now 94 years old was healed.

Case #2 Patient H.S., Born. 15.3.1933.
From 1953 swollen local lymph nodes on neck. Patient hospitalized Faculty Hospital in Olomouc, where repeatedly treated with aktinotherapy. When in July 1955 one lymph node started to growth rapidly, puncture was performed and histology done. Suspected malignant tumor. The whole lymph node was malignant tumor in homolateral lob of thyroid gland.
Surgery done 9.8.1955 (doc. MUDr. Jan Burian)
There was done total removal of right lob and isthmus and complete histology done.
Histology test:
Thyroid gland with large metastatic infiltration.

Therapy:
After healing of the wound, patient went for radiation therapy in Oncology department in Brno.
2 month after surgery started supportive therapy with IMURGEN: 2x 0,50 mg daily.
Patient got pregnant during therapy. She refused interruption. Pregnancy went without complications.
She gave birth to healthy baby. Her health conditions were gradually getting better. Today she is retired and without any problems.

Conclusion:
At 22 years old patient was done removal of right lob and isthmus of thyroid gland (malignant tumor). Metastases proved also in two local lymph nodes. Patient treated for 3 years in institute of oncology.
As a support treatment was used IMURGEN. Patient’s health conditions were rapidly improving. So later she was removed from permanent disability support. 34 years after the surgery and support treatment with IMURGEN, the patient is healthy and retired. She is considered fully recovered.

Case #3 Patient S.H., born. 2.5.1925.
In February probatory excise of bleeding mole on right side of chest. Histology proved melanoblastom. 1.4.1980 re-excise, proved malignant melanoma. Applicated 5 cures of DTIC, Vincristinu. Further treatment abolished for allergy to therapy.
In May 1980 started support therapy with product IMUREGEN (6 moth daily 0,50 mg, later till now 2x 0,50 mg a week.). Patient is feeling well. All clinical controls are negative. The patient is famous music composer. After overcoming initial depression he regained full physical and mental abilities, and as a musical composer has written his best – lifetime musical piece for which he got awarded special award from Ministry of Culture.

Conclusion:
55 years old patient with malignant melanoblasthom in chest area and proven metastases in axilar nod absolved 5 cures of cytostatic and chemotherapy treatment. Immediately after surgery started supportive treatment with product IMURGEN. Almost 9 years after operational follow up is showing very good physical and mental state. The patient is on the top of his artistic creative ability.

Problems started beginning of August 1986. In just one week, she felt stiffness of the right breast and “inner temperature”, breast grew larger, red color of the skin of the breast and swelling of the right arm. On the day 5.8. was done mammography, which shown significant size difference in the breasts size. Didn’t show object itself (tumor). Patient hospitalized in oncology clinic. Objective exam shown that right breast is bigger, and in upper quadrant was found tumor of size 12 x 7 cm. Movable against lower part and with signs of boundary. Found also 2 hard movable nodes of diameter 1, 5 and 2 cm. At puncture found conglomerates of malignant cells.

Diagnosis: carcinoma of right breast, inflammation form, T 3 A 1bMO II

Started massive aktinotherapy and chemotherapy (sec.Cooper), totally applied 17 series of chemotherapy CMP.

Together with chemotherapy was applied product IMUREGEN 0,50 mg daily. Patient is handling massive chemotherapy unusually well, objects in right breast and axilla are disappearing. After more than 2 ½ years of follow-up, the patient is showing practically normal clinical findings hematologically and immunologically.

Conclusion:

At 38 years old patient was proved (after very short analysis) carcinoma of right breast inflammation form. Also at least 2 nodes in close axilla. Started massive aktinotherapy and chemotherapy with use of supportive therapy of IMURGEN. Patient is handling massive chemotherapy unusually well.

After 2 years and 9 months since surgery is clinical, hematological and immunological finding absolutely normal.

Case # 5 Patient E.R. born. 13.1.1922.

13.9.1983 found nodous struma, histology test of puncture of thyroid gland and nodes shown strong suspicion of malignant tumor. That’s why on 4.11.1983 was done total strumectomy.

Therapy: Liothyronin, KC1, Pyridoxin.

During illness found spread of metastasis. Applied radioiodine 131.

21.11.1986 proved metastasis in neck. Also proved osteological changes of expansive character in left humerus. In the short span of time also were found metastasis in lower part of spine and left femur.

Another application of radioiodine was suspended. And used only palliative therapy. (Mainly analgesics).

Therapy of IMUREGEN was started 1.December 1986 in doses 0,50 mg every second day. These doses used until today. After use of IMUREGEN the pain rapidly subsiding, physical and mental condition of patient is improving. Gain in body weight, improved appetite. Almost cachectical patient is obviously getting better.

Repeated regular clinical, rengenologic, hormonal and biochemical test show that malignant process has stopped. Patient is now home for two and half years in good shape and needs only common ambulatory care. She almost doesn’t use analgesics.

Conclusion:

At 63 years old patient was found malignant tumor of thyroid gland with metastases. After 3 years proven spread of tumor to lungs and bones, which brings terrible pain. Use of radioiodine was suspended.

After finding of the spread of cancer was started with therapy of IMUREGEN. Very soon (in about two weeks) is pain subsiding and general physical and mental state of patient is improving.
Regular clinical, rengenologic, hormonal and biochemical test show that malignant process has stopped. The patient is able to exist at home and normal ambulatory care is all what is needed she is 67 years old.

Doc.MUDr. Josef Klapetek, Csc., Neurologic clinic FN, Pavlova ul. 13, 77500 Olomouc In Olomouc 3. May 89

**Clinic of Faculty Hospital and Medical Faculty of UP Olomouc:**

**Proclamation about IMUREGENU**

So-called stimulating therapy has very long tradition. It is focused on increasing of immunizing abilities of organism. On speedy healing of wounds and other defects. And on defects of integrity of skin and mucus membrane. In radiation oncology helps with speedy healing after radiation treatment and better tolerance of radiation. In chemotherapy helps to reduce harmful side effects. Many years ago was used for this therapy so called autohemo - therapy, (own blood removed and later injected to muscles). Later on was this method improved by using products prepared mainly from natural sources by a modern biotechnology.  
First products RETISIN (first working name for IMUREGEN) and LYASTIN. Both these products were tested many years in oncology hospitals. Most often as stimulators of immunity of organism at malignant melanoma. In last years were these products replaced by product IMUREGEN, made with the same technology. Very positive effect on immune system was documented when using this product. Especially increase in numbers of white cells, which have important role in cellular immunity. In many cases was achieved significantly better tolerance of cytostatic used at chemotherapy. Up to date experience is showing that IMUREGEN, like it’s predecessors is very useful support in total oncology treatment. Positive finding is that we have never found any side effects of IMUREGEN.

Doc. MUDr. Jindřich Macháček, Csc., Head of Oncology clinic FN and LF UP Olomouc In Olomouc 16. 8. 1999

**Institute of clinical and experimental medicine, Academy of medical science, Novosibirsk.**

Testing of the influence of IMUREGEN on cell culture and on irradiated cell culture is showing stimulating effects. The density of growth of the cell culture is 2-3 times higher then in control culture.

Control 3,99%, experimental sample 5,68%. Kidney tissue was used as cell culture. After irradiating the cell tissues by a subtle dose of ultraviolet radiation, more than 50% of the tissue showed necrotic signs. The tested preparation (IMUREGEN) was applied after 24 hours and the cells began to normalize. The nuclei with nucleoli could be seen distinctly and they showed a good reception of staining agents. There appeared mitoses, which is unequivocal evidence of secondary regeneration. Therefore, the product IMURGEN was recommended to the Institute of Clinical and Experimental Medicine for application to persons irradiated after the Chernobyl disaster.  
The character of the testing further shows, that if worked into suitable carrier, the IMURGEN preparation will provide protection against ultraviolet radiation and will have a regenerative effect on the skin affected by UV radiation.

Testing was carried under the supervision of Academician V.P. KAZNACHEYEYEV.
In the last years started to come on the market products, which can prepare organism to fight bacterial and flu infection in very short time. They are called immune modulating, because human body will receive certain preparation, which will create antibodies against certain infections. This is a long-term application. Disadvantage is they work only against certain specific strain and do not work against the others.

Finally there was found the most efficient way to fight viral diseases. It consist of application of very effective elements - selected amino acids, nucleotides and other essential elements, which will organism use to naturally build and increase immunity. This way will humans, by natural and easy way build-up protection against illness and complications.

The most effective product to fight flu (influenza) is IMUREGEN, which is natural “medicine” to increase immune capacity. It consist of nucleotides, selected proteins, amino acids, minerals, loosened Fe++ and naturally formed compounds of elements essential for the organism, in optimal ratio. It very effectively modifies defense of organism against viral infections. It has no side effects.

Professor .MUDr. Martiník, DrSc

The extract from special studies and articles.

“Antibacterial peptides of organic origin, originally discovered in product IMUREGEN are the latest discovery of last twenty years of 20’es century. We are witnessing “genesis” of new science stream, which brings almost daily new findings. If scientists will continue with the research with this speed, we will witness unexpected development in treatment of diseases. Not only those which we still cannot successfully cure, but also those so far unknown, which will certainly arise in the future. As it happened in history of humankind over and over again.

RNDr. Petr Šíma, MUDr. I. Trebíchavský

“The healing abilities of product IMUREGEN, are known since the year 1955, when testing started in various clinics and oncology departments in Czechoslovakia and abroad. During this time was proven, that product has healing effects on healing wounds and other defects, or defects of the integrity of skin or mucus membrane. In radiation oncology IMURGEN helps with speedy healing of reactions after irradiation, and better tolerance for irradiation. In chemotherapy helps to minimize it’s harmful side effects. Product has been proven to be very useful and effective support of the important and unreplaceable total oncological treatment. Very positive finding was that in any medical case was never found any side effect of IMURGEN.

FN and LP UP Olomouc, Oncology Clinic.

In the past was known that product IMUREGEN works excellently, but it was not known why. Scientific research of this product is ongoing and it is being tested on experimental animal models in vivo, ex vivo and in vitro. In tissue cultures and also on selected clinical settings. Scientific research specialized in the field of dietary nucleotides lately comes to the conclusion, that oligo and polynucleotides, which are present in product IMUREGEN, are basic for synthesis of nuclide-acids at regeneration of tissue and influence mainly cellular part of immune response.

AV CR, Medical Faculty, Institute of health.

“Product IMUREGEN has scientifically and clinically proven “normalizing” function in immune system.
The product activates macrophages, big defensive cells, which can engulf “foreign” cells. It also stimulates functions of lymphocytes T and B. IMUREGEN stimulates immune defense of organism such, that organism can handle disease on its own.

Academy of Sciences, Czech Republic, Medical Faculty, Health department.

**Nucleotides and their importance at nutrition, prevention and immunity.**

This completely natural, Czech made food supplement supports natural regenerative abilities of human organism. It supports human body during all metabolic processes such as metabolism of proteins, fats and carbohydrates. Basics of IMUREGEN are nucleotides, which are parts for creation of very important nucleo-acids.

IMUREGEN, with its nucleotides – is product intended for mass prevention of civilization diseases, including carcinogenic, cardiovascular, diabetic, asthmatic and allergic diseases. It is the source of very important minerals, mainly iron and zinc, which it contains in very suitable easily absorbent form and so it accelerates production of protective bodies. IMUREGEN is important source of all essential amino acids and the basic building blocks of protein, needed for protection and regeneration of tissue in human body. We know that nucleotides positively influence some metabolic functions-absorption of iron, lowering cholesterol level and so positively influence course of cardiovascular disease.

Absolutely exceptional importance has application of IMURGEN at forming fully functional mucus membrane of intestine and establishing of all its important functions - at underdeveloped newborn as well at ill adults. Also at regeneration of slowly deteriorating mental and physical abilities of seniors. It improves course and frequencies of allergies, common illnesses – flu, strepthroat, common cold etc. It helps with speedy recovery at accident or after surgery conditions, IMURGEN works especially well as a prevention against cancerous diseases. In radiation oncology IMURGEN helps with speedy healing of reactions after irradiation, and better tolerance for irradiation. In chemotherapy helps to minimize it’s harmful side effects.

This product was found to be very useful at subsequent-follow up treatment after cancerous conditions. At long term testing IMURGEN proved to be non-toxic, non-addictive and it does not reside in organism.

**MUDr.Jan- Ševčík**


**APPLICATION OF IMUREGEN IN MODULATION OF HEALTH**

The last decade is characterized by the discovery of a series of information on effects of various nutritional, supplementary and supporting preparations, which besides simple substitutions are applied mainly in regenerating and immuno-modulating processes. These preparations are made from natural materials containing amino acids, polypeptides, nucleotides and minerals, inclusive some vitamins and albumens.

These preparations can be used separately as a regenerative and stimulating diet. But the latest findings of research institutes (mainly Japanese and American) show that their significance is far wider, mainly because of effect of individual components (mentioned above) on many
immuno-regulative mechanisms. This is caused by the influence of preparations on regeneration of cell substrate and immunity directly through individual components of the preparation. We will inform you about these functions below:

When analyzing preparation IMUREGEN made by UNIREGEN Co. (approved by the Ministry of Health CR /CZ Rep., HEM – 350 – 29.10.1998 – 36469), we found that besides components mentioned by the producer, i.e. minerals, oligopeptides, free amino acids, vitamins, complex bound iron, there is also comparatively high share of dietary nucleotides (3-5 mg/ml).

Dietary nucleotides have recently been related to construction of normal immunity response. But it appears that they can take part in a number of biological functions and that they are basis of genetic codes (DNA, RNA). They also serve as a reserve of energy and mediate hormonal effect (cANP). Effects of dietary nucleotides were followed in detail in literature (mainly by Japanese and American research institutes) and they are given in brief in table 1. It is evident from the data in the table that dietary nucleotides do not only take part in increasing of cellular but also humoral mechanisms and secretion immunity. Of great significance is thought to be the effect on repair of intestine tenue (thin intestine) and thus not only mechanisms of cellular immunity are influenced, but there was definitely proved their effect on regeneration of microbial flora of intestinal tract, especially after an intensive and long lasting treatment by antibiotics. This concerns also repeated treatment.

The effect of individual amino acids on immunitary mechanisms has been known and described for a longer time. In those works it show a significant effect on repair of antitoxin and also cellular immunity response.
For example methionin increases antitoxin response and decreases risk of bacterial infections. Fenylalanin shows practically the same function.
Lower levels of arginine, lysine, hystidine are related to substantial decrease of cellular functions and also antitoxin response.
Polypeptides are known to be able to modulate ability of antitoxin immunity.
Iron effects immune response by direct mechanism. It is known that transferin saturated by iron has significant antivirus effect. Further significant effect is considered to be the influence of iron on utilization of iodine, which means significant contribution in solution of inadequate iodine level problem in population.
The groups of vitamins B can also be applied in regulation of immunity response, mainly in influencing of antitoxin response (thiamin, riboflavin). Both these vitamins have been proved to be linked with prevention of a number of infectious diseases.

Functional abilities of product Imuregen (already published some time ago) showed significant contribution of dietary nucleotides in nutrition of newborn babies and children. The content of nucleotides in mother’s milk is very high, while the share of nucleotides in cow milk is very low. This fact has led a number of manufacturers to supplement those kinds of milk, which are used for nutrition of babies and children with mixtures of nucleotides.
Some authors speak about humanization of formulas.
There are appearing scientific works, which write about significant contribution of application of nucleotides to adults, which leads to combination of diets containing nucleotides (Impact).
Examples of this nutrition policy in the world and also in this country lead us to consider possibility of supplementation of baby (children) food with application of nucleotides, and if needed with further micronutrients whose chronical absence in our population is known (ascorbic acid, zinc, iron, iodine).

It has been shown that is advisable to recommend use of product IMUREGEN in treatment of a number of clinical diseases – and namely in cases in which we intend to influence fast regeneration of cellular substrate, fast regeneration (repair) of physical and immunity functions.

Our report in detail on function of nucleotides is published in 'CZ hyg. 41, 1996, p.319 – 323.'

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Director MUDr. A. Vorderwinkler (M.D.) 2

SZÚ (State Health Institute), Prague
Senior Lecturer MUDr. (M.D.), Csc. 3

Evaluation of product IMUREGEN, company: UNIREGEN

We have found very significant amount of nucleotides in product IMUREGEN, beside the other important components. Those (nucleotides) were in the span of 12 - 21% of total volume. For this reason we consider this product to be significant regenerative product for immunity functions of organism, as it was documented in conference on nucleotides in San Diego, as well as in our article in magazine Czech Hygienic (1997). At present time are nucleotides used in many products intended for peroral supplementation of patients, mainly in after accident/surgery state (see World Congress in Munich, March 2000) and at patients in pre-surgery state. Numerous publications are showing reduction of 40-80% in post surgery complications and after accident infectious complication. From our own experience we can state that supplementation with IMUREGEN has great influence on regeneration of intestinal cells and the height of villi. It suppresses pathological changes of mucus membrane in intestines. Supplementation influences positively function of liver and add at regeneration and proliferation of immuno-competent cells. Product also functions as a regulating factor in hemopoiesis. Adding of nucleotides increases total immune capacity at cellular level and in humoral immunity. It increases activity of NK-cells. Supplementation with this product in higher doses works very well in regeneration of function of secretion immunity. We have proved increased resistance against infections of respiratory tract in children’s population. We are testing product IMUREGEN at present time in cooperation with The Czech Academy of Sciences and we applicate it in the complex of minerals.
and milk formula in children collectives. This pilot study is preparation for “grant” research project in cooperation with professor

MUDr. I. Hánou, DrSc- and RNDr. P. Šímov, CSc.
MUDr. Josef Richter, CSc.,OHS Ústí nad Labem

ANTIDOPPING COMMITTEE OF THE CZECH REPUBLIC
170 00 Praha 7, U Sparty 10
tel./fax 33 37 01 49, E-mail : antidopingvcr@mbox.vol.cz

Distributor - applicant : Uniregen spol. s r.o., Malé Poříčí
Na Brzdách 72, 547 03 Náchod
Name of product : Imuregen®
Producer :
Uniregen spol. s r.o., Malé Poříčí
Na Brzdách 72, 547 03 Náchod

Antidopping Committee of the Czech Republic on the basis of the expertise, according to which the product does not contain forbidden substances,

A G R E E

To the following marking of the product : “Suitable for sportsmen“ without any limitations in the market.

In Prague 1999-10-4

Seal : Antidopping Committee of the Czech Republic

Signature : PhDr. Jaroslav Nekola
Chairman of the Antidopping Committee
Of the Czech Republic
Czech Pediatric Society

Czech Medical Assoc. J.E.Purkyně

President : Prof. MUDr. Jiří Šolc, Csc., dětská klinika, FN, E.Beneše 13, 305 99 Plzeň
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Secretary : Doc. MUDr. Jozef Hoza, CSc.

UNIREGEN s r.o.
Malé Poříčí
Na Brzdách 72
547 03 Náchod

Prague 1998-11-25

Uniregen Co. put forward preparation Imuregen (previously approved Juvenil) to the Working Group for children’s gastroenterology and nutrition for approval.
An expert opinion is required in connection with the change of producer and new law on foods, particularly on foods intended for special nutrition.
Imuregen is intended for stimulation of immunity systém. It is produced of beef blood and contains amino acids, peptides, organic salts, bivalent iron. It has already been given to children before under the name Juvenil – see previous expert opinions.
The Working Group have no objections to giving the preparation to children over 1 year of age in doses of 10 to 30 drops daily.

MUDr. P. Frühauf , Csc
Doc.MUDr. O.Pozler , Csc
Doc. MUDr. J.Neoral , CSc

Working Group for children’s gastroenterology and nutrition at the Czech Pediatric Society

Signature
Czech Medical Association
PEDIATRIC SOCIETY

Working Group for
children’s gastroenterology
and nutrition
TESTING

THE INSTITUTE OF HYGIENY AND EPIDEMIOLOGY, CZECH MINISTRY OF HEALTH, DEPARTMENT OF NUTRITION

The Institute carried out the testing of the preparation as required by the Chief Hygienist of the Czech Ministry of Health before approving the standard, and made the following conclusion:

a) The preparation was tested for safety by microbiological and toxicological methods and was found suitable for human consumption without restriction.
b) The biochemical indicators of nitrogen metabolism are within standard, with a 10% reduction of the uric acid content.
c) Functional tests of the cardiovascular system showed a reduction of blood pressure and an improvement of the heart load resistance index.
d) After fourteen days of application of the preparation, the tested persons showed a 19.8% recent memory improvement.
e) The analytical values show an extremely high proportion of free amino acids (51.4%) with a well-balanced spectrum of 11%. Above limit values were found in tryptophan, but only in bonded form, found in free form were leucine, alanine, valine, serine and methionine.

Testing was carried out under the supervision of Prof. Augustin WOLF, M.D., D.Sc.

ENDOCRINIOLOGICAL RESEARCH INSTITUTE, CZECH MINISTRY OF HEALTH, DEPARTMENT OF EXPERIMENTAL ONCOLOGY AND RESEARCH LABORATORY FOR PSYCHOTRONICS AND JUVENOLOGY OF THE INSTITUTE OF CHEMICAL TECHNOLOGY, PRAGUE.

The purpose of the testing was to prove an increased biological activity of the organism. The preparation was administered to 156 malignant tumour patients, after their causal treatment, as supportive
therapy, and to another 12 patients suffering from repeated virus diseases and partial loss of immunity.

The effect of the preparation was evaluated in 15 different tumourous sites according to complete or partial remission and the period of its duration according to the Karnoff Index.

In about 90% of the tested persons there was a complete or partial remission in tumours of the mammary gland, the ovaries, the digestive tract and the rectum, and the malignity index became normalised.

In infaust cases a longer survival was recorded, and there was a strong mitigation, and even complete disappearance of pain, and the administration of generally toxic palliatives could be substantially reduced.

The eight-year clinical study showed that the preparation proved useful for the long-term therapeutical coverage of patients after the completion of protocolar therapy, when the malignant process is usually regarded as being eradicated, without the removal of the causes of the previous illness.

Patients suffering from repeated virus diseases showed a substantial reduction in the recurrence of their disease, by having the immune response of their organismus increased, as was verified by laboratory testing. On the basis of these long-term preclinical applications it can be said that the administration of this preparation has proved an improvement of the biological activity of the organism.

Testing was carried out under the supervision of Karel ČERNÝ, M.Sc. and Zdeněk REJDÁK, Ph.D.

SEARCH LABORATORY, TĚCHONÍN, J.E. PURKYNĚ, MILITARY MEDICAL FACULTY, CZECH ARMY.

Th purpose of the testing was to find whether it would be possible to use the preparation to influence immunocomplete cell systems and to gain basic information about the immunomodulating potential of the preparation. Its costimulative activity was monitored, and it was found that in concentrations from $1.5 \times 10^{-3}$ to $1.5 \times 10^{-9}$ g/ml the preparation inhibits significantly ($p < 0.05$) the lymphoproliferative response of splenic cells to Con A, PHA and LPS added to the cultures in optimum concentrations.

Other tests were carried out to find the proliferative response of splenic cells to mitogens after the administration of the preparation in vivo. The preparation was administration of the preparation in vivo. The preparation was administered to animals orally during seven days. In this
case, to the proliferative response of splenic cells to Con A (5 mg/ml) increased significantly (p 0.05) by from 0.015 mg/kg to 15 mg/kg of the daily dose. The response of splenic cells to PHA is significant in the full range of the doses used.

The administration of 15 to 150 mg/kg of the preparation in vivo increases the nonspecific cytotoxic activity of adhering peritoneal cells against the targets K 562 and P 815. The same result was achieved in studying the activity of natural killers in the spleens of mice.

SUMMARY: The testings show that the tested preparation influences the function of the immune system either directly or indirectly by neurohormonal regulation. According to findings on the radiation immunodeficient model, the influence of the preparation on the redistribution of blood-forming cells between the medulla, the spleen and the circulation field may be expected. Thus, the preparation may be described as a substance influencing favourably the organism's immune response.

Testing was carried out under the supervision of Col. Petr PROPPER, M.D., C.Sc.

REGIONAL HYGIENIC STATION, IMMUNOLOGICAL DEPARTMENT, ÚSTÍ NAD LABEM

The Station studied the immunological findings and the dynamics of proteins after the application of the preparation.

The testing involved 12 tumorous women patients aged from 38 to 58, to whom 2 mg doses of the preparation were administered during 25 days. Blood samples were taken before and after administration, and indicators shown in table 1 were determined. The findings were evaluated by the usual statistical methods.

The findings indicated in table 1 show that the preparation influences mainly the nutrition condition markers. There has been a statistically important increase in prealbumin values, which rose from the lower level of 0.219 g/l to 0.267 g/l. Also, a statistically important normalization of the transferin value occurred. The C-reactive protein alpha 1 – antitrypsine figures also show an important change.

The test has revealed an increase in the leucocyte count, a changed distribution of the lymphocytar population and the normalization of the proportion of terminal lymphocytes. A clinical as well as subjective improvement of the patients was noted while the preparation was administered.
Although the set of the tested patients was not fully representative, the unequivocal dynamics of the changes of certain indicators seems to be trustworthy. The dynamics of the changes in those proteins which are dependent on the nutrition condition testifies to the high probability of the favourable effect of the preparation. It seems that like many immunomodulators, the preparation, too, may influence immunemechanisms by the operation of free amino acids or oligopeptides. For example, the primary influencing of prealbumine is probably later projected into a favourable influencing of the lymphocyte population by „thymosin-like“ activity. The reduction of inflammation proteins and trace element carrier proteins will have an equally favourable impact. The presence of trace elements in the preparation is also expected to have a favourable effect. The findings reveal that the studied indicators influencing the immune response mechanisms are showing favourable results after the application of the preparation.

Testing was carried out under the supervision of Prof. J. RICHTER, M. D., C. Sc.

INSTITUTE OF CLINICAL AND EXPERIMENTAL MEDICINE,
ACADEMY OF MEDICAL SCIENCE, NOVOSIBIRSK.

The Institute tested the influence of the preparation on cell cultures and on irradiated cell cultures. The preparation showed a stimulating effect on the cell cultures. The density of the growth of the cell culture is twice to three times higher after adding the preparation than in control cultures. The mitotic activity is much higher than in the control culture: control 3.99%, experimental sample 5.68%. Kidney tissue was used as cell culture.

After irradiating the cell tissues by a subtle dose of ultraviolet radiation, more than 50% of the tissue showed nectoric signs. The tested preparation was applied after 24 hours and the cells began to normalize, the nuclei with nucleoli could be seen distinctly and they showed a good reception of staining agents. There appeared mitoses, which is unequivocal evidence of secondary regeneration. Therefore, the preparation was recommended to the Institute of Clinical and Experimental Medicine for application to persons irradiated after the Chernobyl disaster.

The character of the testing further shows that if worked into a suitable carrier, the preparation will provide protection against ultraviolet radiation and will have a regenerative effect on the skin affected by UV radiation.
Testing was carried out under the supervision of Academicien V. P. KAZNACHEYEV.

INSTITUTE OF PARASITOLOGY, POLISH ACADEMY OF SCIENCES, WARSAW.

The purpose of the testing was to establish the biological activity of the preparation. The Institute of Parasitology developed a method of measuring biological activity by means of studying photon emissions with the use of a photomultiplier.

The conclusions made on the basis of the model testing of the preparation is that the preparation influences oxidoreduction and oxidoperoxide conditions, mitigates or eliminates excited energy processes. It behaves like a low-molecular substance with strong penetrating ability. It rapidly increases the energy level of the cell, after which it shows a slow decline in its energy level. After repeated application the preparation improves the general energy balance of the cell.

Testing was carried out under the supervision of Prof. Stefan GRABIEC, D. Sc.

ACADEMY OF SCIENCES OF THE RUSSIA FAR EAST DEPARTMENT, COMPUTER CENTRE, KHABAROVSK.

The purpose of the testing was a comparison of the biological activity of the tested preparation with an etalon – the common preparation 'Salkoseyl', produced in the Switzerland. The biotest system chosen was one based on the reaction of erythrocyte suspension. The assumption was that the biological activity is the greater the larger the proportion of the difference of the selected indicators between the high and low concentration of the etalon in identical conditions. By repeated experiments with the use of the blood of white mice it was found that the studied preparation was 130% more effective than the etalon.

The purpose of further testing was to find a suitable method for measuring the preparation's standard and to make a comprehensive comparison with the above – mentioned etalon. Eight criteria were used.
for this purpose. The results, in comparison with the etalon, are shown in the diagrams.

The optimum criteria of measuring the standard of the preparation are the induction of erythrocyte aggregation (FGA) and the speed of erythrocyte setting. These criteria may be automated and the measuring may be carried out according to a special analyser.

Testing was carried out under the supervision of Georgie CHUICH, M. D., D. Sc.

INSTITUTE FOR THE MOTHER AND CHILD, RUSSIAN REPUBLIC, MOSCOW.

The testing was carried out on pregnant rats. The experiment had two variants.

a/ The first variant studied the indicator of biological usefulness of the preparation on equal groups of pregnant and non-pregnant rats. Both groups were offered a free choice of pure water and water with a 1% solution of IMUREGEN. The quantity of the liquid consumed was measured daily. An analysis of the measuring has revealed that pregnant rats from the 11th day of gravidity (the whole period of gravidity is 22 days) were showing strong preference for the water with a 1% solution of the preparation (35-40% more in comparison with non-pregnant rats). This shows that in conditions of a free choice, pregnant rats from the second half of the gravidity period prefer to drink a solution containing IMUREGEN.

b/ The second variant studied the influence of the preparation on the weight of pregnant rats and the increase in the level of haemoglobin in the blood of pregnant rats in the newly born rats.

The experimental group was receiving a 1% solution of the tested preparation, and the control group was getting pure water to drink. During the period of gravidity, the animals’ weight and their haemoglobin concentration were studied over 5, 10, 15 and 20 days. It was found that the weight increase was 25% higher in the tested group than in the control group. As from the 15th day, the experimental group showed a 10-15% increase in the haemoglobin content, as compared with the control.
group. The weight of the newly born rats the experimental group was 18% higher than in the newly born animals in the control group.

Testing was carried out under the supervision of prof. Svetlana ANIKIEVA, M.D., D.Sc.

APPLICATION OF IMUREGEN IN MODULATION OF HEALTH

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Senior Lecturer MUDr. (M.D.), Csc.3

The last decade is characterized by the discovery of a series of information on effects of various nutritional, supplementary and supporting preparations which besides simple substitutions are applied mainly in regenerating and immunomodulating processes. They are preparations made from natural materials containing amino acids, polypeptides, nucleotides and minerals inclusive some vitamins and albumens.

These preparations can be used separately as a regenerative and stimulating diet. But the latest findings of research institutes (mainly Japanese and American) show that their significance is far wider, mainly because of effect of individual components mentioned above on many immunoregulative mechanisms. This is caused by the influence of preparations on regeneration of cell substrate and immunity directly through individual components of the preparation.
We will inform you about these functions below.

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complex bound iron, there is also comparatively high share of dietary nucleotides (3-5 mg/ml).

Dietary nucleotides have recently been related to construction of normal immunity response. But it appears that they can take part in a number of biological functions and that they are basis of genetic codes (DNA, RNA). They also serve as a reserve of energy and mediate hormonal effect (cANP). Effects of dietary nucleotides were followed in detail in literature (mainly by Japanese and American research institutes) and they are given in brief in table 1. It is evident from the data in the table that dietary nucleotides do not only take part in increasing of cellular but also humoral mechanisms and secretion immunity. Of great significance is thought to be the effect on repair of intestinum tenue (thin intestine) and thus not only mechanisms of cellular immunity are influenced but it has definitely been proved their effect on regeneration of microbial flora of intestinal tract, especially after an intensive and long lasting treatment by antibiotics. This concerns also repeated treatment.

The effect of individual amino acids on immunitary mechanisms has been known and described for a longer time. In those works a significant effect is given on repair of antitoxin and also cellular immunity response. For example methionin increases antitoxin response and decreases risk of bacterial infections. Fenylalanin shows practically the same function. Lower levels of arginine, lysine, hystidine are related to substantial decrease of cellular functions and also antitoxin response. Polypeptides are known to be able to modulate ability of antitoxin immunity. Iron effects on immunity response by direct mechanism. It is known that transferin saturated by iron has significant antivirus effect. Further significant effect is considered to be the influence of iron on utilization of iodine which means, when supplementation of population is used, significant contribution even in solution of inadequate iodine level problem. Here we wish to stress that nucleotides also accelerate utilization of in human organism. The groups of vitamins B can also be applied in regulation of immunity response, mainly in influencing of antitoxin response (thiamin, riboflavin). Both these vitamins have been proved to be linked up with prevention of a number of infectious diseases.

Functional abilities of preparation Imuregen already published some time ago showed significant contribution of dietary nucleotides in nutrition of new-born babies and children. The content of nucleotides in mother milk is very high, while the share of nucleotides in cow milk is very low. This fact has lead a number of
manufacturers to supplement those kinds of milk which are used for nutrition of babies and children with mixtures of nucleotides. Some authors speak about humanization of formulas. There also appear works writing about significant contribution of nucleotides application to adults which leads to combination of diets containing nucleotides (Impact).

Examples of this nutrition policy in the world and also in this country lead to considerations on possibility of supplementation of baby (children) food with application of nucleotides and if need be further micronutrients the chronic and general absence of which in our population is known (ascorbic acid, zinc, iron, iodin).

It has been shown that besides indications given for preparation Imuregen because of newly found presence of nucleotides it is possible to recommend the preparation in a number of clinical diseases – and namely in ceses in which we intend to influence fast regeneration of cellular substrate, fast regeneration (repair) of psychical and immunity functions.

Our report in detail on function of nucleotides is published in „Čs. hyg., 41, 1996, p.319 – 323.“

Czech Pediatric Society
Czech Medical Assoc. J.E.Purkyně
President : Prof. MUDr. Jiří Šolc, Csc., dětská klinika, FN, E.Beneš 13, 305 99 Plzeň
Tel./Fax : 420 (0) 19 27 25 70
Phone/Fax : 420 (0) 19 74 20 745
Secretary : Doc. MUDr. Jozef Hoza, Csc.

UNIREGEN s r.o.
Malé Poříčí
Na Brzdách 72
547 03 Náchod

Uniregen Co. Put forward preparation Imuregen (previously approved Juvenil) to the Workong Group for children`s gastroenterology and nutrition for approval. An expert opinion is required in connection with the change of producer and new law on foods, particularly on foods intended for special nutrition.
Imuregen is intended for stimulation of immunity system. It is produced of beef blood and contains amino acids, peptides, organic salts, bivalent iron. It has already been given to children before under the name Juvenil – see previous expert opinions. The Working Group have no objections to giving the preparation to children over 1 year of in doses of 10 to 30 drops daily.

MUDr. P. Frühauf, Csc  
Doc. MUDr. O. Pozler, Csc  
Doc. MUDr. J. Nevoral, CSc  

Working Group for children’s gastroenterology and nutrition at the Czech Pediatric Society  

Signature  
Czech Medical Association  
PEDIATRIC SOCIETY  

Working Group for children’s gastroenterology and nutrition  

CHARLES UNIVERSITY IN PRAGUE  
PHARMACEUTICAL FACULTY IN HRADEC KRÁLOVÉ  
500 05 Hradec Králové, Heyrovského 1203, Czech Republic  

Date : 28th September 1999  
File No.: 33/ Nutr/ 437/ 99  
pages : 2  
Enclosure - sheets :  

PROTOCOL  

on expertise of preparation intended for sports nutrition  

Applicant : Uniregen spol. s r.o., Malé Poříčí, Na Brzdách 72, 547 03 Náchod  
Name of preparation : Imuregen ® (solution 50 ml)  
Kind of preparation : Food for special nutrition  
Producer : Uniregen spol. s r.o., Malé Poříčí, Na Brzdách 72, 547 03 Náchod  
Submitted documentation : Application to Antidopping Committee of the Czech Republic for expertise (1 sheet)
Abstract of Register of Commerce (1 sheet)

Decision of the Ministry of Health of the Czech Republic,
HEM-350-29.10.98-36469 (1 sheet)
Protocol – Analysis of amino acids etc (2 sheets)
Protocol – Analysis of Pb, Cd, Hg (1 sheet)
Protocol – Basic food characteristics (2 sheets)
Company standard – Imuregen – PN UN-01-98 (5 sheets)

Preparation description: Pellucid, slightly yellowish liquid, soup spices smell, beef bouillon taste.

Decision*

The present preparation does not create after taking any metabolic or psychic addictive response that could be classified as drug addiction and for this reason it cannot be considered as a doping narcotic substance.

The expertise only concerns the presented sample and the conclusions deduced from it can be applied on other products only if their composition and properties are completely the same as those of the presented product sample.

This expert opinion is a professional pharmaceutical expertise meant for binding decision of the Ministry of Health Authorities of the Czech Republic.

Signature:

Doc. RNDr. Lubomír Opletal, CSc.
Pharmaceutical Faculty Hradec Králové
Department of pharmaceutical botany and ecology

DEPARTMENT OF MIKROBIOLOGY ASCR (Academy of Sciences of Czech Republic)

Department of Imunology and Gnotobiology
Institute of Mikrobiology,
Academy of Sciences of Czech Republic

Vídešská 1083
142 20 Praha 4

Characteristic of regenerating abilities of Czech patented product Imuregen®

Czech patented food concentrate is showing significant abilities to increase immunity, in humans and animals, which was tested on experimental animals in vivo, ex vivo and in vitro on tissue cultures, and on selected clinical trials. Based on results from these testing, Imuregen® was approved by Chief Hygienist of Czech Republic as a supplement for food and beverages, and also for drinks, intended for special use. Especially for children and seniors in city
agglomerations, with increased levels of pollution, people recovering after surgeries etc., workers in heavy industry, sportsmen and for prevention of seasonal repeated inflectional epidemic diseases of upper respiratory tract.

Testing of Imuregen® has shown, that is contains protective factors* increasing readiness of anti-infective defense of organism, and components** necessary for growth and regeneration of tissue There have never been noted any signs of immunosuppression after using of Imuregen® in any experimental administration.

Verification tests, which were done in Sector of Immunology a Gnotobiology of Department of Microbiology of Academy of Sciences of Czech Republic have proven, that this product (Imuregen® ) has significant immuno regulating properties with positive influence, especially on manifestation of natural and cell immunity. In cooperation with Medical Faculty of Charles University in Prague, research has proved positive effects of dietary and drinking program supplemented with Imuregen®, on regeneration of mucous membranes in intestines. Which in final consequences means improved sorption function of intestine and increased use of nutrients, and at the same time optimization of function of mucous membrane immunity. That is why it is possible to apply Imuregen® as a supporting preparation on it’s own, or as an ideal supplement for beverages, milk, dairy products and other foods in the framework of preventative programs, where the target is to increase immunity of general population, especially children, youth and people recovering from surgeries, illness, infections, fatigue syndrome or after injuries and complicated surgeries (post-traumatic syndrome). Imuregen® can be especially recommended as a preventative-protective preparation, which increases resistance to infections in population living in areas afflicted by nature disasters or other catastrophes, and for personnel under stress in extreme conditions as in rescue and salvage operations, decontamination work, army combat, etc.

* Cationic peptides with pronounced anti-microbial abilities, some vitamins.
** oligo-and polynukleotides, essential for cell multiplication, amino acids, peptides, and some trace elements

RNDr. Petr Šíma, PhD

In Prague, 10. 1. 2003

Application of products Imuregen/Regen

In relation to health and disease prevention it is recommended to use only 1 capsule of Imuregen per day, in the weight category up to 50 kg of weight – children’s dose. Prophylactic dose for adult person is 2 capsules. For overweight and obese individuals the dose is doubled, the dose for individuals with critical BMI values of 32.5 and above - the recommended dose is increased to triple. Due to the large loss of zinc in sweat (loses up to 80% zinc), we recommend administration of one tablet of Imuregen Chelated Minerals. The dosage is adjusted according
to climatic conditions - at elevated temperatures, additional tablet of Imuregen Chelated Minerals should be taken.

For healthy individuals with increased physical activity (sports activity, wellness gym) we are adding one tablet of **Imuregen Eleutherooccus** regularly. During expected increased physical activity, such as sport competition, athletic competition and other sport activities, we recommend 2-3 capsules of **Imuregen Eleutherooccus**. It is indeed proven that this natural food supplement significantly reduced levels of lactic acid, thereby reducing the feeling of fatigue. In accordance with Australian literature studying various athletic and sport activities (swimming, cycling, rugby, gymnastics), this regime is very efficient and reduces the risk of infections in athletes with high training loads.

Administration of product **Imuregen** in chronic fatigue syndrome should be differentiated: we distinguish two units of chronic fatigue syndrome. We choose different treatment strategy in CFS induced by EB virus infection and completely different strategy for fatigue syndrome with clear clinical picture, where we first have to eliminate fatigue causes such as: pulmonary diseases, hematologic diseases, metabolic diseases, malignancies, infections of the urinary tract and over-training syndrome. **With CFS EBV infection** (definite diagnosis confirmed by PCR technique with the findings of more than 400 particles of EB virus in saliva) we recommend: two **capsules of Imuregen** or two teaspoons of **Imuregen liquid**, and two tablets of **Imuregen with Beta Glucans** preparation and one tablet of **Imuregen with Chelated Minerals**. The minimum treatment period is six months, then we check the value of the EBV virus in saliva by PCR technique. In the case of negative value of the virus we change the treatment scheme to the normal maintenance dose, as it is used in the healthy population, with paying attention to the weight of the individual and other factors. **In CFS not induced by EBV infection**, and excluding the above clinical manifestations, we recommend administration of **Imuregen capsules** at a dose of at least four capsules a day and two capsules of **Imuregen Eleutherooccus** and one capsule of **Imuregen with Chelated Minerals**. For most cases of CFS, especially where we measure mental stress score (particularly in patients with long-term unclear diagnoses stigmatized often by inadequate treatment), we also give one capsule of **Neurofit**.

In case of cancer diseases, nucleotides are used as adjunctive treatment which keeps the immune system in best performance. During radiation treatment we take advantage of the ability of pectin fibers to reduce radiation impact and we give **Imuregen with fiber – Imuregen V 270**. During radiation treatment we recommend to administer at least six capsules a day, then according to the clinical course of the patient's condition we serve the daily maintenance dose of four capsules of Imuregen V 270.

The initiation phase before the start of chemotherapy, respectively of radiotherapy, is supported by administration of **Imuregen with Vit.C+E**. We recommend two tablespoons a day for at least fourteen days before beginning of treatment. Increased intake of antioxidants reduces oxidative stress and reduces the risk of side-effects during treatment. We use the same mode in the preoperative phase of all cancer diseases, and the same applications regime at least fourteen days prior to surgery. During recovery time we reduce the dose and serve **Imuregen with Chelated minerals** in doses that we adjust accordingly as per observed plasma concentration values of zinc and foremost the values of iron (we monitor the risk of anemia by monitoring...
index of the ratio of transferrin receptors to ferritin). After all cancer treatment and in the recovery period we follow patients for the period of at least 4 years (monitoring of tumor markers, inflammatory proteins - CRP, SAA, IL6, orosomucoid, prealbumin and calculated ratio between orosomucoid and prealbumin - CSI Cancer Serum Index). Recovery period requires the same fundamental mode of the administration of Imuregen as in healthy individuals, but always keeping an eye on the current state of the individual, and in the case of finding of inflammatory symptoms, the incidence of viral infections, herpetic infections we apply “fighting ” dose of Imuregen with Beta Glucans with a minimum dose of two tablets twice daily.

The administration of Imuregen in patients with Type -2 diabetes requires the same treatment as the prevention in a healthy population, but it is necessary to monitor number of factors. Particular attention should be paid to diabetics with increased weight, where increased loss of minerals occurs, and also changes in microbial flora of intestinal tract (GALT microbiome ) and the resulting risks of frequent infections of mucosal surfaces. The impact of the above mentioned factors is high demand for reparations/regeneration of mucosal surface. These demands are covered by increased administration of Imuregen. Here we prefer to use Imuregen liquid at a dose of at least two teaspoons a day. As with other infectious states, in acute infections in Diabetics we recommend administration of increase dose of Imuregen liquid at a dose of up to one tablespoon three times daily with a support of one tablespoon of Imuregen with Vit.C+E. The rationale for this procedure is that, the preparation Imuregen not only contains nucleotides, but many other components with anti-bacterial properties, for example: antimicrobial peptides and proteins, defensins, hepcidins, cathelicidins, lysozymes, calprotectins, etc. The importance of these components of Imuregen culminates in recent years, when there is a continual growth of antibiotics resistant microorganisms and antibiotic treatment of many infections often fails.

Another positive effect of the administration of Imuregen is the influence on growth of mucosal GALT, and positive influencing of bacterial components with an effect, that may affect the metabolism of energy storage, regulation of values of Leptin and its effect on immunoregulatory mechanisms. At this point it is necessary to emphasize the general positive effect, which the long term administration of these Imuregen products have on preventing mild inflammatory reaction. It is known that subclinical and persistent inflammatory stimulation with the presence of relatively low levels of CRP are an inducer of atherosclerotic plaques. With Type -2 diabetics, according to our experience, this risk is many times higher, mainly due to the frequently increased weight, recurrent infections of the respiratory tract and especially infections of urinary tract and in the case of our population is graded by relatively high prevalence of smoking, where smokers are at a high risk of damage to the immune mechanisms due to oxidative stress and exposure to toxics in tobacco smoke.

In inflammations of the joints we always define the origin of the disease and distinguish the administering of Imuregen products: in autoimmune joint disease and in post infectious joint disease (Chronic Borreliosis). In both cases we follow the basic regime for healthy individuals and it is also recommended to use Imuregen with Beta Glucans in the maintenance dose of one tablet daily, and in the event of flare up of the disease- two tablets a day. Other Imuregen products are chosen more or less according to the recommended treatment
of the rheumatologist, in which case we follow balancing of oxidative stress with the application of **Imuregen with Vit.C+E** at a dose of one **teaspoon** twice a day.

In diseases resulting from impaired intestinal mucosa, administration of **Imuregen** is chosen according to the clinical diagnosis. Here are some selected examples of where we use the effects of the Imuregen products: For HP disease after re-treatment of the disease (according to the Maastricht Consensus triple) we try to repair the affected mucous membrane by application of **Imuregen liquid** one **teaspoon** twice a day. Continuously monitor for the incidence of HP in the organism (HP ELISA test in the stool). After a period of convalescence we pay attention to maintaining good condition of the immune status by continuous use of the Imuregen preparation as in healthy individuals. Virtually the same process is kept with patients treated by a gastroenterologist for inflammatory disease of GI (ulcerative colitis, Crohn's disease) with the fact, that with these individuals we recommend intermittent application of **Imuregen with Vit.C+E**.

Dysmicrobia manifestations (including those after long term antibiotherapy) we solve by application of **Imuregen with Beta Glucans** at a dose of one tablet twice a day for at least two months and simultaneously we give quality probiotics (multi strain probiotics). With these diseases we base dosage formulations by clinical manifestations of the disease and the patient's symptoms and, of course, we follow the influence of supplementation quality by testing of values of faecal **calprotectin**. At this point, we note that all manifestations of inflammatory gastrointestinal tract can be very well monitored by monitoring values of salivary albumin and salivary CRP. ([C-ReactiveProtein](#))

Diseases resulting from stress stimuli are handled individually, the optimum is a basic examination of salivary cortisol values for the definition of stress load. It is known that psycho-immunomodulation can control a number of different clinical manifestations. With these patients we recommend Neurofit in higher and long-term doses at least two to four capsules a day for one month, then two tablets daily. We do not forget the possibility of **seasonal affective disorder**, so we regularly monitor the patients and adjust dose accordingly.

In our practice we very often meet with the disabilities of function of liver parenchyma, these are usually post-infectious inflections (hepatitis A, B, C, E, infectious mononucleosis, and others). For these individuals, we recommend use of **Imuregen DNA** at an initiating dose of three capsules a day for a period of one month and then maintenance dose of one capsule twice a day. We realize that the composition of **Imuregen** is a natural food supplement with hepatoprotective effect. In the toxic liver damage (acute ethanol disability) we have used repeatedly mega-doses of Imuregen product with excellent effect and clear impact on the repair of entire spectrum of liver tests, after a six-week Imuregen administration. In these patients, we have used a dose of two **tablespoons** of Imuregen liquid per day throughout the monitored period until normalization of both: the laboratory and the clinical findings. Due to the psychological impacts we have continued in these patients with administration of **Neurofit** at a dose of one capsule twice daily.

Towards the note on the possible effects of the **Imuregen** in terms of increased blood pressure, we state that we cannot confirm your findings. We believe, however, that it would be
appropriate to verify this claim, not only with the administration of the preparation, but also with the placebo effect, of course, with corresponding standard measurement technique (repeated BP measurements in thirty minutes intervals).

An interesting chapter is the administration of Imuregen in individuals with allergies. Given that a large proportion of allergic individuals whom we monitor are undergoing oral vaccination regimen using appropriate allergens (allergy monitoring measuring the specific IgE method CAP phadiatop) we have positive experiences with these individuals, with adding of any of available Imuregen product in dosage, which corresponds to dose of healthy population, or adding concentrated agent directly into the oral vaccine. In these patients findings of improvement are in both laboratory and clinical.

Fat metabolism (cholesterol, HDL, cholesterol index) can be affected in our opinion, only partially and we do not find even after long-term administration of the preparation significant changes in values. More or less the effect could be achieved by long-term administration of Imuregen V 270, however for a really good answer we would have to perform necessary studies with a placebo.

Applications of nucleotides and related products is widely monitored and studied and at present we have at least sixty papers dealing with this issue. I refer to the available database, or we can provide a list of citations of works that deal with this issue. In any case, there are very interesting studies of Australian origin, following application of nucleotides to top athletes, and polar explorers. These studies are supported by high-quality laboratory testing, monitoring especially the parameters of mucosal immunity, which comports with us- we also advocate for non-invasive methods of samples of biological material.

Ústí nad Labem, Czech Republic MUDr. Josef Richter, CSc.

**Health Institute at Ústí nad Labem**

400 01 ÚSTÍ NAD LABEM, MOSKEVSKÁ 15

Re: The expert opinion on preventative function of product IMUREGEN (IMUN Plus) on viral infections including Bird Flu virus.

Centrum of Immunology and Microbiology, department: Research and International cooperation.

Influenza (flu) is illness affecting mainly people with weakened immune system. Most often the high risk groups (seniors and people with chronic illness). World Health Organization recommends vaccination against flu as an important part of prevention against this illness. But vaccination effectiveness is not higher than 70%, mainly because of weakened immune system of many people, which is not able to completely ensure immunity against flu. It is known that it is partially caused by deficiency of various micro-nutrients (minerals, vitamins, amino-acids) and other substances able to repair weakened immune system and mucus membrane which ensure our resistance to illness.
That is why to strengthen immune system should be our choice not only before epidemic, but also during flu epidemic and when being ill with flu or other illness of upper respiratory tract. IMUREGEN is excellent for this purpose. The product IMUREGEN (IMUN Plus) can be used on its own or added to drinks. It contains mix of nucleotides, polypeptides, amino-acids, ascorbic acid (vitamin C) (also zinc-gluconate, and herbal extracts).

IMUREGEN increases absorption of micro-nutrients including iron, which increases human anti-flu immunity, and have direct effect on some viruses, mainly flu virus. The herbal additions contain substances, whose anti-oxygenous effect is together with zinc, guarantee of minimizing risk of infections and influencing their course.

Product IMURGEN:
- Increases immunity
- Improves quality of mucus membrane
- Improves function of central nervous system
- Enhances power of organism as a whole
- Works as a prevention
- Improves resistance against viral infections including flu viruses and bird flu viruses
- Increased immunity during preventative use leads to increased immune answer after vaccination against any flu.

Ústí nad Labem, Czech Republic 27.10.2005 MUDr. Josef Richter, CSc.

**Imuregen - antimicrobial peptides.**

All living organisms are protected from parasitic microorganisms by immune system. One of the important factors of the immune system immediate protection are its own substances that have an antimicrobial effect. They either stop the proliferation of microbes or kill them directly. Such antimicrobial substances were first discovered in the human body, by the discoverer of penicillin Sir Alexander Fleming in 1922. His discovery, unfortunately, did not have a followers. And other similar substances were discovered 40 years later. Today (fifty years later) we know that these substances are found in all living organisms and represent a fundamental pillar of innate immunity.

Throughout the animal kingdom can be several million different substances with antimicrobial effect. They are species-specific and, moreover, for each species there are dozens of different substances. Many hundreds of them have been discovered in mammals, and dozens in people. They occur in all tissues, but their number is highest in white blood cells, in the glands and secretions, mucous membranes and other barriers of organism (see recent review of Dr.Sima & comp. 2003). Most of them are peptides, but since these also include small proteins, thus they are properly called AMPP - antimicrobial peptides and proteins.

**AMPP have enormous importance for human health,** because they kill particularly transit microorganisms (bacteria, viruses, fungi, protozoa) on the interfaces between the external and internal environment of the body (intestine, lung, skin, genitor-urinary tract) and protects the circulatory system from the penetration of microbes (Yu & comp. 2010). Residential harmless commensal microflora of the skin and mucous membranes is living in an environment containing
AMPP.

Antimicrobial effect of AMPP is caused by:

1.) Binding to the parasite membrane and subsequent penetration into the cell interior, where they can bind to DNA directly, or directly by penetration of parasitic cell membrane and killing them by osmosis (these are called cationic peptides - defensins, katelicidins, BPI)
2.) Enzymatic lysis of important microbial structures (lysozyme, elastase, phospholipase A2)
3.) Linkage of vital substances (lactoferrin - bound iron and calprotectin - zinc binding)

In addition to the above effect, AMPP regulate immune response and slows dangerous inflammatory response which are dangerous to the body. Beneficial effects on human health is caused at many probiotics mainly by induction and secretion of AMPP in the intestinal lining and intestinal immune cells (Trebičavský I. and I. Splichal, 2006). Their content in the blood varies depending on activity of white blood cells. These cells are a storage depots of tens of AMPP.

Substrate from which Imuregen is prepared, contains dozens of antimicrobial peptides. From cathelicidins - there are are bacteneics Bac-5 and Bac-7 (which at a concentration of 2-50 μg/ml, kill: dangerous gram-negative enterobacterias of salmonella, klebsiella and E. coli and common triggers of frequent nosocomial “hospital” infection), myeloid antimicrobial peptides BMAP 27, 28 and 34 and two very small peptides indolicidin and dodekapeptid. Indolicidin, which contains the most amino acid tryptophan (39%) of all known protein, binds to the membrane and the parasite and penetrates into the cells where binding to DNA to inhibit its synthesis. Thus kills gram-negative and gram-positive bacteria, fungi and parasitic protozoa.

Of the thirteen beta-defensins BNDB that occur in the granules of white blood cells, there were ten of them Isolated from the substrate Imuregen (BNDB-1, 2,3,6,7,8,9,10,11 and 13). All of these defensins have antimicrobial effect on the golden staphylococcus and E. coli. Were also found lysozyme, lactoferrin, and BPI (bactericidal permeability-Increasing Protein) calprotectin (synonym calgranulin or MRP 8 / 14), which constitutes 30% of all proteins of the cytoplasm of neutrophils (these cells represent half of the white blood cells) and is highly effective against yeasts, staphylococci and salmonellae (Stříž Trebičavský and 2004) and numerous antimicrobial enzymes such as elastases, cathepsin G, azurocidin, and phospholipase A2.

Lactoferrin of the substrate Imuregen, breaks by proteolysis (for example, pepsin in gastric juice) into a very effective lactofericin. This substance has a strong antimicrobial effect, and has a positive anti tumor effect and effect against high pressure.

Literature

Ilya Trebičavský
UNIREGEN

We, signed below, recommend the product Imuregen containing important bio-active substances. Based on long-term experimental and clinical tests, it was confirmed that the product Imuregen has positive effects on the total regeneration of the organism and the immune system. It is especially suitable for people under extreme physical and/or mental stress. It fosters restoration of liver tissue and mucous membranes of digestive and respiratory system and it also provides protection from viral and bacterial infections (Hepatitis, leptospirosis, Weil’s disease, salmonellae, typhoid diseases).

For the above stated reasons the product Imuregen is especially suitable for personnel under stress in extreme conditions, as in rescue and salvage operations and decontamination work in flood-affected regions.

MUDr. Josef Richter, PhD.
Department of Science and Research
KHS Ústí nad Labem

RNDr. Petr Šíma, PhD.
Sector of Immunology and Gnotobiology
Institute of MicroBiology, Academy of Šience CR

Ústí nad Labem, 28 August 2002
(This above statement was released during summer 2002 as a respond to the extreme flooding in Europe)

UNIREGEN S.R.O. (A Limited Liability Company)

Hitherto Conclusions from Repeated Testing of the Preparation IMUN +

Supplementation of human nutrition with immunity-enhancing substances can be very beneficial, because our human bodies have become imbalanced. The overwhelming cause of this imbalance is poor lifestyle and environment, pollution, stress, nutritional deficiencies, toxicity and infections, and they all erode our health and lead to a decrease in our immune system effectiveness. The reduction of immunity causes various diseases of viral, bacterial and metabolic character. The only solution is strengthening – balancing – of the organism stamina – its immunity. Optimal solution can be offered by the product IMUN+, made by company Uniregen.
**IMUN +**
Food concentrate with efficient component of **nucleotides**

**IMUN +** is a natural complex of essential amino acids, nucleotides, oligo-peptides and trace elements. These substances in their complex are able to activate, and positively increase the immune system of a human. There are able to harmonise the organism stamina, from the point of view of immuno-modulation of cell and hormonal part of the immunity.

Bio-complex of active substances creating efficient regeneration and stimulation dietetics.

The food supplement **IMUN +** ranks among modern preparations produced on the purely natural basis. It is designed for adults and children from 1 year of age and it does not contain alcohol or any inorganic chemical substances. It is particularly suitable for diabetics and for athletes supporting the muscle mass growth and improving regeneration of strength/endurance, during exercise or training.

**IMUN +** is one of the most efficient preparations fighting the flu.

**IMUN +** offers new approach to solving the fight against viral diseases by using the application of highly efficient substances – selected amino acids, nucleotides and other essential substances used by the organism to increase it’s immunity.

Using of **IMUN +** will boost natural resistance to illness and disease and will naturally improve immune system capacity. People will avoid light flu as well as possible negative effects on vital organs – heart, lungs or kidneys – and consequent danger for the whole organism.

**UNIREGEN S.R.O. (A Limited Liability Company)**

**Composition**
This purely natural product was developed at the beginning of the 50’s under management of a popular pharmacist Dr. Rakús in Czech laboratories. It contains a balanced complex of the most efficient plant and animal substances. Essential amino acids, nucleotides, proteins, peptides, oligo peptides, organic salts and vitamins in natural relations increase the power potential of human cells, which can stimulate the immune system.

**Use**
**IMUN +** is absorbed in the digestive system in an active form – the organism absorbs only the substances it needs for managing the performance, to avoid stress and civilisation diseases and
for improvement of metabolic process during a disease. The product IMUN + does not contain sugar and it is suitable even for diabetics. It can be used for an unlimited time period without any risk of addiction, or negative side effects.

**Effects**

**IMUN +** reduces chronic fatigue, eliminates feelings of weakness and depressions, speeds up healing and regeneration and has positive effects on the digestive system, kidney and liver function.

**IMUN +** is suitable for healthy people as well as for people under mental or health stress, in any age groups.

Healthy individuals can use **IMUN +** in case of fatigue and as prevention of stress situations. It reduces the risk of the organism damage by civilisation diseases.

When used by athletes, it increases the muscle growth and positively effects the power metabolism by arranging several ways of power supply to cells. It improves regeneration of strength/stamina.

When used by ill people, it creates a base for speeding up the process of healing, reduces the time of regeneration and thus improves the efficiency of treatment. It is suitable as a prevention of arteriosclerosis and it is one of the basic substances in the methodology of JUVEENOLOGY (postponement of ageing and keeping and improvement of active life from the mental, physical and sexual point of view). It is suitable for both acute and chronic illness. Application of **IMUN +** is beneficial as prevention of the illness as it eliminates excessive deviations in the organism homeostasis and even long-term chronic diseases can be significantly improved or even cured.

**IMUN +** does not have any harmful side effects.

**UNIREGEN S.R.O. (A Limited Liability Company)**

The basic biologically active substances of **IMUN +** are:

**Nucleotides**

- the synthesis of nucleotides in liver is very demanding from the point of view of energy and that is why the intake of exogenous nucleotides in the periods of increased demand (after surgery, traumas, burns or system diseases) is necessary.
- Period of growth of organism up to the adult age, significantly increase the demands of the organism towards the consumption of nucleotides. They are applied also in induction of anti-substance response and they non-specifically increase the production of immunoglobulins, mainly of the IgG and IgM class.
- experiments proved, that nucleotides increase resistance to infection with Staphylococcus pyogenea and Candida albicans, they also increase production of interleucine IL2 and increase functions of microfages.
• they significantly stimulate the creation of lymphocytes, which are a base of immunity.
• they improve the utilisation of vitamins and trace elements.

**Amino acids**

• some of them directly effect immunity
• arginine and glutamine have immuno-regulation functions and they cause an increase of proliferation of lymphocytes and an increase of some sub-populations of lymphocytes.

**Iron**

• necessary for arrangement of cells proliferation and bio-synthesis of ribo-nucleotides to deoxiribonucleotides, the basic elements of DNA.
• deficit of iron leads to reduction of dermal cell response, reduction of lymphocytes proliferation, reduction of bactericide capacity, it also reduces the anti-substance response, it increases the sensitivity towards infections.

**Proved facts related to the preparation IMUN +**

Clinical tests proved a significant strengthening of cell immunity, mainly increase of T-lymphocytes and their activity, induction of mucous immunity response. Application of the IMUN+ has shown that there were significantly increased values of lysozyne and increased values of many proteins.

The application of the IMUN+ significantly reduced the increase of the C-reactive protein and the orosomucoide, which are the symptoms of inflammatory processes.

The top athletes and sport teams have shown an improvement of power/strength parameters and training capacity and further more, there was also proved the protective influence on liver parenchym from the effects of physical load.

When IMUN+ was applied to the patients with hyperlipidemia, there was noticed a significant decrease of the cholesterol level.

There was proved a significant reduction of sickness in work collectives in the normal season and during seasonal viral diseases.

The use of IMUN+ positively effects the health conditions of children, mainly in regions with extremely damaged environment.

UNIREGEN S.R.O. (A Limited Liability Company)

**Evaluation of the IMUN + product in relation to cellular part of immunity**

Prof. MUDr. J. Kocian, DrSc., 1st Clinic of Internal Diseases IPZV – FNT Prague 4

In our clinic we tested products with high contents of polynucleotides – i.e. substances contained in cytoplasm of all and any cells. As soon as the 3rd day from the start of the application of products, there was an increase in the numbers of white blood cells responsible for protection of the body against infections – the increase fluently continues up to the 28th days when the numbers of cells reach as much as 200% of the original values.

It was mainly the **Imun +** preparation that proved well.
Evaluation of the IMUN + product in relation to infections of the upper and lower respiratory system, tonsillitis and flu.
ZORA Olomouc, MUDr. H. Černochová

The product Imuregen was given to a selected group of employees in the ZORA Olomouc plant during the period of increase of viral diseases. The employees were in the age of 19 – 64 years. The sickness leave representing a comparative group was significantly lower in employees using Imuregen. Serving the preparation – mainly in large work teams with high physical or mental load – is efficient and economical in the periods of an increased occurrence of respiration viral and bacterial diseases.

TT Viadrus ŽDB a.s., MUDr. Masařek

The product Imuregen was daily given to a selected group of employees of the foundry in the TT Viadrus ŽDB, a.s. plant.
For evaluation of efficiency of the Imuregen: comparison has shown decrease in the illness rate of the upper respiratory tract by 47%.

MUDr. Štaket from the department of work and medical care:

He recommends IMUN + product to be used mainly for professions:
• under mental stress (managers, drivers)
• working in the environment with exposure to carcinogens chemical
• working under the risk of ionising radiation
• working with organic solvents
• working in physically demanding jobs
• working in shifts work

Immunological Findings in Groups of Children after Compensation Measures

National Institute of Health – department of Immunology in Ústí nad Labem
Doc. MUDr. J. Richter, CSc., MUDr. Ladislav Pelech
200 children permanently living in a region with high degree of environmental pollution spent 14 days in a region with healthy environment and they were given product Imuregen. As a result, their parameters of secretion immunity have significantly improved. Decrease of albumin values signalises a significant reduction of the inflammable irritation of the respiration system. There were also significantly reduced the specific antibodies IgE of the respiration type of allergy. On the basis of hitherto experience, just the stay in the region with high-quality air positively influences 80 – 90% of people for a period lasting for 2 – 3 months. This period can be significantly prolonged by using the product Imuregen.
Findings from children supplemented with the IMUN + product.

KHS Ústí nad Labem, OHS Ústí nad Labem, OHS Litoměřice
Doc. MUDr. J. Richter, Dr. S. Richterová, Dr. T. Kolinová

Group of 35 children in the age of 11 – 15 years, who were given product Imuregen for the period of 1 month, was observed. Statistically was proved a significant reduction of inflammable reactions, there was increase of the values of secretion immunity and there was normalisation of many values of biologically important proteins, mainly the transferine.

Monitoring the Effects of the IMUN + product in a group of 53 persons in VÚ Kbely
Clinical Laboratory ÚVN Prague – Strešovice, Dr. M. Švec

The product Imuregen was continually given to the group of 53 people aged 20 – 50 years, for a period of 2 months. Comparison of laboratory tests was done for the evaluation of cellular immunity before application of Imuregen to group, and after termination of Imuregen product use. The Rosette test proved a significant increase of T – lymphocytes and the test of lymphoblast transformation proved a significant increase of their activity.

Preliminary Findings in Population of Seniors supplemented with product IMUN+.
KHS Ústí nad labem, OHS Litoměřice, OHS Chomutov
Doc. MUDr. J. Richter, D. Jílek, V..král, A. Vorderwinker, Dr. T. Kolinová, M. Hanusová

Total of 151 seniors from nursing homes were monitored – seniors received a dose of Imuregen for a period of 3 months. The values of IgG and IgE globins which prove optimisation of the immunity system regulation, were normalised. Significant decrease of the C-reactive protein proves the significant anti-inflammation effects of the product Imuregen. Normalisation of the transferine values confirms the positive adjustment of the iron metabolism. There was also a significant decrease of the SP3 protein values, which are indicators of hormonal dysfunction but also possibly of tumours activity or inflammations. The dynamics of changes of the pre-albumin values confirms significant improvement of nutrition status and an improvement of detoxification processes.

Verification of Effects of IMUN + in the Sphere of Top-Performance Sports
Administration of Top-Performance Sports – Prague, RNDr. P. Fořt, CSc.

The extensive study of power demanding sports and endurance types sports confirmed highly positive effects of the product Imuregen mainly in the following spheres:

- significant improvement of power/strength/stamina parameters, improvement of training capacities
- significant reduction of sickness rates
- improvement of the regeneration course
- protective influence on liver parenchyma from the effects of physical load
• stimulation of proteo-synthesis

Use of IMUN + in Patients with Elective Surgery
Department of Surgery of the Hospital Na Homolce, OKBHI Hospital Na Homolce, Institute of Experimental Medicine, Academy of Science Czech Republic.

MUDr. M. Hladký, MUDr. V. Táborský, MUDr. J. Červinka, MUDr. M. Průcha, MUDr. M. Dostál

The product Imuregen was given to 24 patients in the dose of 2x1 tablet/day 7 days before, and 30 days after surgery. The patients showed a statistically significant decrease of activity values of liver amino-transfers that were significantly pathological at some patients before starting the application of Imuregen. Furthermore, there was statistically significant decrease of the transferine values, which proves positive restoration effects of Imuregen during inflammation. There was also proven a modulation effect of Imuregen in the sphere of cellular immunity due to statistic increase of the amount of CD 3+ lymphocytes in comparison with the control group. The results confirm suitability of Imuregen use as a non-specific supporting product for patients, where it can be possible to expect immunodeficiency in the sphere of cell-mediated immunity.

Clinical Testing of the IMUN + Product at the Surgery Department
Hospital in Mladá Boleslav
MUDr. A. Skřivánek

Patients with so-called unhealable defects were selected for clinical tests – i.e. patients with poorly healing fractures, decubites and patients with varicose ulcers or with burns of 3rd degree. After 3 months of serving Imuregen, all the patients showed a significant improvement. While the healing time – i.e. the reduction of the term of treatment – reached 7 to 10 days.

Immunological Findings and dynamics of Proteins after Application of IMUN +
Regional Hygienic Station in Ústí nad Labem
DOC. MUDr. J. Richter, CSc.

Patients aged 39 – 58 years with diagnoses of non-plasmatic diseases were given Imuregen in the dose of 2 mg/day for the period of 25 days. Chemical examinations before and after the therapy proved statistically significant increase of pre-albumine values, normalisation of transferine values and values of C – reactive protein of alpha I – antitripsyne. There was also proved an increase in the number of leucocytes, adjustment of distribution of lymphocyte population and normalisation of the share of terminal lymphocytes.
**Nucleotides. A review and current advances**

The endogenous supply of nucleotides is maintained de novo synthesis and the salvage pathway. Because there are metabolically costly process, it is more efficient to use already formed nucleotides. This is particularly true in rapidly dividing tissues, such as intestinal and lymphoid tissues, which require nucleotides for the synthesis of nucleic acids. One DNA replication requires at least $10^9$ nucleotide molecules. An exogenous source of nucleotides, such as dietary supplement, could optimize the tissue function by sparing the cost of de novo synthesis or salvage. This may be especially important during periods of rapid grows.

Dietary nucleotides can improve the grows and differentiation of the intestinal cell lines, the response to injury of the intestinal track and positively influence the bacterial flora in infants. In the intestine of breast fed infants, bifidobacteria were predominant, while gram-negative bacteria predominate in those of infants fed cow’s milk based formula. Breast milk contains about hundred times the level of nucleotides present in cow’s milk. Nucleotides supplemented milk increased HDL and decreased VLDL in infants, suggesting that nucleotides enhance lipoprotein synthesis particularly in the intestine.

Feeding nucleotides to infants improve hepatic function and restoration and has been associated with increased polyunsaturated fatty acids in erythrocytes, suggest in that nucleotides play a role in the conversion of 18C essential fatty acids to 20C-22C very long chain polyunsaturated fatty acids.

In humans, rapidly proliferating lymphocytes require an exogenous supply of nucleotide for optimal function even if de novo synthesis and salvage pathways have been demonstrated. – lymphocytes seem to require dietary nucleotides for normal maturation and function. In mice and humans, a nucleotide free diet significantly depressed IgM and IgG antibody production from spleen cells. Host resistance to bacterial and fungal infection decreases on nucleotide free diets. In human, parenteral and/or peroral solutions with nucleotides given to postoperative cancer patients improve immune function and infectious complications and length of hospital stay was reduced compared to a control group.

A large number of clinical studies on human infants has been conducted to investigate in particular the gastrointestinal and immunological effects of dietary nucleotides. Beneficial effect
of dietary nucleotides on mucosal regeneration, immune function have been demonstrated. 'Immunonutrition' is a term which has been adopted to describe diets that contain several additives. Some in the form of glutamine. Arginine, fish oils and nucleotides alone or in combination.

Results of an intervention study-Iodine supplementation of Gipsy children for a period of three month with humanized milk ( nucleotides and polypeptide) has regulating effect in the iodine metabolism as well as in secretory immunity. In supplemented children a significantly lower frequency of diseases and shorter periods of morbidity were recorded.

MUDr. Josef Richter, PhD.
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Ústí nad Labem 15.11.2002

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In Ústí nad Labem 25th January 2000

Risk of contamination through infectious spongiform encephalopathy

According to the report and findings of the WHO consultative commission, whose session was held from 9th to 11th February 1998 and solved problems of viral particles and prions infection risk from initial material (beef blood corpuscles), the risk is zero.

This risk as mentioned in the material above is also reduced by method of preparation. Its final product is a mixture of amino acids, polypeptides and nukleotides under the trade name IMUREGEN.

Possible more detailed information in case of doubts can be get at the WHO consultants´ addresses (prof. Herbert Budka, Institute of Neurology, Vienna, Austria and prof. Henry Barona, WHO, Geneva).

Signature
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SUMMARY OF CLINICAL RESEARCH FOR 
IMUREGEN

MUDr. Josef Richter, CSc.
Summarization of the properties and application of the product

IMUREGEN

Fully-fledged nutrition adequate to age is one of the basic conditions for the harmonic body development. It provides for all physiological functions of human organism, including immunity. That’s why it must incorporate balanced content of low-molecular substances (trace elements, minerals, vitamins, etc.) and macromolecular ones (saccharides, fats, and proteins). Some of these ingredients need higher intake in some life periods, primarily for growth of body, ageing, recovery, increased physical load or in the case of infectious diseases. This demand is caused by higher utilization of the proteins, trace elements, vitamins, and antioxidants.

To provide high-quality development of immune system, which is able to react to infectious load, it is necessary to assure higher supply of some substances, which are normally available. We have to point out dietary nucleotides, their consumption in conditions of higher load and during convalescence is increased to such a level, when they become essential. Based on recent discoveries, the dietary nucleotides in combinations with other substances, like amino acids, N-3 fat acids, beta glucans, and vitamins are considered to be the basic compositional units of immunonutrition.

High efficiency of IMUREGEN is just based on high contents of nucleotides. Nucleotides (further NT) comprise nitrogenic base, pentose sugar and group of phosphates. Nitrogenic base consists of purine and pyrimidine. Cells of mammals, bacteria and plants contain various nucleotides. Cellular NT contained in IMUREGEN is possible to find in milimolar concentrations and they may have a lot of functions:

1. They secure energetic metabolism.
2. Precursors of nucleic acids (DNA and RNA) consist of nucleotide units.
3. They serve as physiological mediators of many metabolic processes.
4. They are constituents of coenzymes.
5. They are carriers of activated messengers for a lot of reactions (e.g., glycogenic a glycoprotein synthesis, metabolism of phospholipids).
6. They work as cellular agonists.
7. They can work as effective agonists providing impulses to intracellular transduction cascade including cAMP.
Thirty years ago, there were discussions at symposium of the American Society of Nutrition, whether there exist biochemical or physiological arguments to consider dietary NT essential from the nutritional point of view, in spite of their insignificant role in nitrogen metabolism.

Then IMUREGEN was already successfully tested. It was supposed, that main source of NT in organism is endogenic synthesis of purines and pyrimidines, which is more effective than use of dietary NT arising from degradation of nucleic acids in food.

NT can be synthesized in endogenic way, this was used as a reason to deny their essential role in nutrition. New research in 90’s of previous century gave clear evidence that dietary NT are important and from many points of view indispensable nutritional component. An organism is able use them directly from food avoiding their highly energetic demanding de novo synthesis.

Insufficient supply of NT via food may not express only as an imbalance of immune functions, it may reflect disorder of physiological functions of the gut and its microbiome, liver, heart, developmental retardation of CNS, immune system, and general growth. Just at correction of lack in NT supply, the IMUREGEN provides excellent results. Supplementation is highly effective even in relative low dosage.

**Importance of dietary nucleotides (dNT) contained in the IMUREGEN during postnatal period**

An adult gets daily 1.5 to 2.5 g of dNT on an average, it is much more than the whole intake of vitamins. For sucklings and infants in the period of accelerated growth is necessary to provide sufficient supply of nucleotides, which are necessary especially for maturation of GI tract and its enzymatic equipment, and for metabolism of lipoproteins and linoleic acid. NT take part in the growth of microbiome and selection of microorganisms positive for physiological functions and suppression of pathogenic microorganisms. Maternal milk is indispensable source of NT.

It contains 20 to 25 % of non-protein nitrogen on an average whilst cow milk only 2 to 5 %. From the 5th to 8th month of lactation, the maternal milk contains 10 to 120 mg/L DNA and 100 to 600 mL RNA. Human maternal milk is generally considered to be "gold"
standard” for suckling nutrition. Now available infant nutrition is prepared in industrial way to approximate to maternal milk. Cow milk is supplemented by nucleotides.

Such enriched formulas were available in Japan in 60’s of previous century. In 1991, Scientific Committee for Food recommended supplementation by 10 mg dNT/L, later it was increased to 70 mg/L. There are used dNT, which add a flavor to infant formulas. Pleasant, so called „fifth flavor” umami contains glutamic acid and IMP. There were messages, that such supplemented formulas were consumed by sucklings in increased amounts due to adaptation of taste. Optimal intake of dNT for sucklings is 160 mg/kg per day and it corresponds to average weight increase 10 g/kg per day.

New findings about significance of NT show that their relevance in adult nutrition is remarkable as well. Supplementation by the IMUREGEN is being linked with restoration of lost immune functions. Administration of dNT contained in the IMUREGEN results in fast and vigorous improvement of disordered human physiological functions.

**IMUREGEN and metabolic functions**

Nucleotides in the IMUREGEN are building blocks of nucleic acids and they as mono-, di-, and triphosphates are involved in practically all metabolic functions. They represent integral part of protein, saccharide, and lipid metabolism as well as metabolism of nucleic acids. In form of nucleosides, they take part in metabolism of active substances, especially co-enzymes. They work in energetic metabolism as donators of phosphate, their mediator function is necessary for aggregation of blood platelets and vasodilatation.

It is estimated that during 24 hours is destructed and renewed about 300 g of enterocytes in an adult. Further load and high demand is increasing during induction of immune response due to clonal expansion and merging of lymphoid cells into effector ones. Another amount of nucleotides is necessary for regeneration of tissues, renewal of mucous epithelia, induction of haemopoiesis and renewal of skin cells as well. Supplementation by the IMUREGEN successfully prevents complications arising from the lack of nucleotides.

**IMUREGEN and gastrointestinal effects**

Dietary NT contained in the IMUREGEN play significant role in the development of GI. It increases production of mucosal protein, DNA, height of villi and other activities of...
intestinal mucosa. Repeatedly was demonstrated positive influence of the IMUREGEN on reparation of intestinal damage both in models and in children and adult population. Our tested IMUREGEN proved significant anti-inflammatory effect of administered NT in children allergic population. It resulted in decrease of salivary albumin and at the same time there were higher levels of secretory IgA and salivary lysozyme. These significant changes came before improvement of health, including higher resistance and diminished morbidity of respiratory tract. In literature there are published reduction of mortality and decrease of inflammatory symptoms in model experiments.

Substantial changes are elicited by given dNT through influencing the intestinal microflora. It results in significant growth of Bifidobacterium in intestinal tract with consequent decrease of pH in intestinal contents. Low pH is able to prevent proliferation and growth of pathogens, especially Bacteroides and Clostridium. Effects of IMUREGEN on Bifidobacterium are reported in children fed industrially produced children nutrition supplemented by dNT.

IMUREGEN as an exogenous source of dNT is able to optimize function of intestinal epithelia especially in the period of accelerated growth and during convalescence after damage of mucosa. Administration of IMUREGEN has positive effect on a lot of inflammatory diseases of GI, e.g., ulcerous colitis, Crohn’s disease, and irritable bowel syndrome. In the same way was repeatedly proved positive influence on reparation of intestinal mucosa after bacterial infections of intestinal tract. IMUREGEN possesses significant influence on healing of diarrhoeic disorders caused by lactose diet.

**IMUREGEN and its effects on the liver**

The liver plays important role in provision of dNT for organism, mainly through active synthesis and release of dNT for other tissues. Extracellular nucleotides influence growth of hepatocytes and their regeneration as well. Disorder of hepatic tissue leads to increased consumption of nucleotides where regeneration of tissue is replenished by increased synthesis of DNA and RNA. IMUREGEN improves function of hepatic parenchyma and its reparation, is involved in detoxification of mycotoxins and significantly ameliorates post-stress disorders. We proved that administration of the IMUREGEN in conditions with significant damage of hepatic tissue results in rapid decrease of aspartate aminotransferase (AST), alanin aminotransferase (ALT) and glutamic acid-pyruvate transaminase (GPT).
The same positive effect of IMUREGEN was repeatedly proved in models on experimental animals.

It was revealed that levels of mycotoxins in the liver of pork and poultry supplemented by IMUREGEN were significantly lower than in animals with a standard diet. Higher levels of mycotoxins in feces of experimental animals were statistically significant. IMUREGEN modulates growth of hepatocytes and their regeneration including synthesis of glycogen. Positive effect of IMUREGEN was proved in cases of toxin-induced fibrosis of hepatic parenchyma. Hepatoprotective effect of IMUREGEN was proved in cases of EBV infection with damage of hepatic tissue.

Our experience shows significant preventive influence of IMUREGEN given prophylactically in frame of fluid intake as well as in workers with high exposition to chemical substances (negation of hepatotoxic effects). This experience led us to administration of high doses of IMUREGEN to intoxicated individuals. Fourteen days of daily administration of IMUREGEN resulted in normalization of all indicators of hepatic damage (liver function tests, tissue polypeptidic antigen, orosomucoid). In longitudinally observed groups of individuals with the risk of exposition to hepatotoxic substances, we also found significant decrease of cholesterol levels with increase of HDL and normalization of the index.

**Influence of IMUREGEN on lipid metabolism**

In children with diet supplemented with dNT was found increase of levels of HDL already in the first month after birth. It seems that IMUREGEN increases synthesis of lipoproteins especially in intestinal tract. Administration of the IMUREGEN leads to increase of omega 3 and omega 6 PUFA in plasma and in RBC membrane in experimental animals.

**IMUREGEN and immunity**

It was repeatedly proved that IMUREGEN increases specific and non-specific immune functions, proliferation activity of lymphoid cells with the consequent corresponding reaction of both cell and humoral immune response. Administration increases not only numbers but first of all the activity of natural killers, production of some cytokines. This may be used for reparation of tumorous conditions during and after conventional therapy.
Significant effect of IMUREGEN is proved towards the parameters of humoral immunity, including B lymphocytes-regulated production of antibodies together with increased resistance to bacterial and viral infections. Endogenous nucleotides positively influence antibody response and keep its optimal function. Significant influence on levels of antibodies against tetanus in vaccinated children population was proved. In comparison with control group there were statistically significant differences in antibody responses. Repeatedly was proved protective effect of IMUREGEN in therapy of mycotic infections. Nucleotides influence mechanisms of cellular immunity. They speed up lymphoproliferation induced by alloantigen, correct immunosuppression induced by malnutrition, increase number of NK cells and their activity. They increase production of IL-2 and take part in increase of number of macrophages and at the same time they increase their fagocytary activity. It is proved that IMUREGEN increases proliferation of bone marrow and proliferation of peripheral neutrophiles.

Administration of IMUREGEN increases resistance against infections both in animal models and humans. In an experimental model was proved significant resistance to infections caused by Candida albicans and Staphylococcus aureus. Survival of exposed animals was statistically significant in comparison with control group.

IMUREGEN adjusts the ratio of cellular subpopulations Th1 and Th2 and in such way significantly decreases risk of sensibilisation and induction of IgE response. Already 0.5 \% of dNT contained in IMUREGEN proved reduction of induction risk of allergic symptoms in experimental model of inhalation allergy. It was proved that administration of IMUREGEN to premature children was connected with significant increase of CD4+ lymphocytes.

**IMUREGEN and secretory immunity**

Long term administration of IMUREGEN results in positive influence on factors of mucosal immunity. It was revealed, that there is increase of secretory IgA, significant decrease of albumin and C-reactive protein together with overall recovery. In groups with physical load, IMUREGEN induced increase of secretory IgA, IgG and IgM in saliva. In comparison with placebo group, there was increase of mucosal lysozyme levels and so provision of higher resistance to infectious load.
Dietary nucleotides in IMUREGEN influence generally production of antibodies via T helpers and the earliest phase of antigen presentation. Immune response is regulated by interaction with T regulation cells. In repeated studies, following health conditions of children hospitalized in sanatorium for pulmonary and allergic diseases we revealed that after 1 month of IMUREGEN administration there was not only significant improvement of secretory immune response represented by increase of SIgA, salivary IgG and lysozyme together with decrease of salivary C-reactive protein. Moreover, there was significant decrease of cortisol in saliva representing normalization of stress response.

There was not only significant improvement of respiratory functions of followed children with decrease of levels eNO by 15%, besides that there was improvement of physical condition represented by increased endurance. Measured entrance values of 6-minute walk test were increased after stay in sanatorium by 17%. Control testing showed persistence of positive findings during 6 months, slow worsening reflected mostly staying in environment contaminated with exhaust gases, chemicals, products of local stoves and in more than 50% of cases there was exposition to indoor contaminants (smoking parents).

**IMUREGEN and CNS**

Appropriate intake of dNT contained in IMUREGEN in early development results in positive influence on growth of CNS and improves memory. There in literature is possible to find reports on significant improvement of health conditions in individuals with destruction of brain functions, e.g., in patients with dementia. It was proved that ATP is indispensable not only for learning, but also for memory and ability to concentrate. It is important for mediation of signals from CNS to PNS.

**Cardiovascular system and IMUREGEN**

Heart contractions are ATP-dependent and supplementation of nucleotides assures physiological function of cardiovascular system.

**Tumors and IMUREGEN**

Administration of the preparation may increase efficacy of antitumor chemotherapy and reduce the collateral symptoms. IMUREGEN is able to decrease toxicity of some antitumor
substances. Co-administration with doxorubicin revealed increase of level by 50% in tumor cells in comparison with patients without IMUREGEN. The same positive effects of given dNT were described in therapy of pulmonary tumors. There are discussed two possible effects of IMUREGEN in tumorous conditions. Firstly it is an increased effect of IMUREGEN, on the other hand it is increased function of immune mechanisms. Our experience points to ability of IMUREGEN to keep constant number of white blood cells or to prevent their decrease much more than in patients without IMUREGEN.

**Increased physical load and IMUREGEN**

IMUREGEN has its use in individuals with increased physical load. Regular administration in professions with increased physical demands decreases morbidity and inability to work. In model study of more than 2,000 individuals exposed to high physical load and to high temperatures, administration of IMUREGEN in drinking fluids led to decrease of inability to work by 50%. The same application of IMUREGEN with the same effectiveness is useful for some professions (firefighters, policemen, drivers, etc.).

Administration of IMUREGEN alone or in mixtures with other substances is now in focus and it is used by a lot of top level athletes. On this spot, it is necessary to distinguish administration of substances and their doses during increased training load, in period of competitions, and in period of convalescence. Our experience shows that administration of IMUREGEN led not only to increase in performance, but at first to reduction of morbidity during period of competitions. Positive effect of IMUREGEN may be mediated via increased resistance to stress. We revealed that in children administered IMUREGEN was swiftly reduced level of salivary cortisol.

MUDr. Josef Richter, CSc.
Expert of the World Health Organization
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THE FINAL REPORT

About the solution of the program project with grant from Internal Grant Agency of The Ministry of Health of Czech Republic

Name of program: Protective and imunomodulative influence of supplementing RNA, comparing of clinical testing and experimental model.

Registry number: NJ 6888 - 3
Date of research of project: 2001 - 2003

In Ústí nad Labem 29. 1. 2004

Health Institute, City: Ústí nad Labem
Moskevská 15, 400 01, Ústí nad Labem
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INTRODUCTION

Inadequate nutrition has its big share on health deterioration of general population, not only by poor eating habits, but also by unbalance of basic nutrition components in food. It is generally calculated fact, that 15-25% of all death is related to improper nutrition.

The optimal presence of vitamins and trace elements in nutrition/food, is constantly repeated basic demand, influencing quality of health of the population. Inadequate composition of nutrition/food influences our immune system analogically just like food deficiency, especially at both sides of age span of humans, in the period of growth (childhood) and at the period of ageing. The present styles/conceptions of the world health policy are based on these ideas/conclusions. These policies concentrate on preventative programs with effort to reach possibly the widest parts of population. For these reasons our health policies should include research and clinical verification of different food supplements, in which their positive influence on metabolic, physiologic a immune functions has been proven. These aspects are substantially recognised in developed countries, where increased attention is paid to the questions of food supplementation with various micronutrients. Japan, USA, and some European countries have been developing programs for so called functional food. Lots of above mentioned aspects are being closely looked at WHO materials „The Health for all in 21st century“.

The goal of our project was verification of the possibility to influence the health and immune system by nutrition and supplementing by nutritional products with high content of DNA. We have explored the effects of highly purified DNA of yeast origin on selected immune parameters, in the experiments and in clinical and preventative studies. We have found that the some result can be achieved by product Imuregen which has high content of amino acids, polypeptides, oligo a
polynucleotide. Advantage of this product is very good financial accessibility (the
cost for one day application is 20x lower than German product “Torula”). We have
stated/determined the optimal dose of the supplement in relation to dosage for
preventative treatment and dosage for medical treatment. In the aim of the
project we have started from assumption that immunoregulating effects of the
exogenous DNA are very useful for synthesis of nucleo acids during cell division.
Dietary nucleotides are, from the view of energetic coverage in this application
much more suitable also because their partial fission in GI tract leads to their
fast absorption and thus to the improved cellular reparation of different tissues
of the organism, which ere dependent on supply/feeding of exogenous
nucleotides. In many cases it can be stated that in the importance of nutrition,
dietary nucleotides are from this view essential. Absence of dietary nucleotides
in human organism can lead even to the symptoms of malnutrition, which is
described under the name NAIDS (Nutritionally Acquire Immune Dysfunction
 Syndromes). We have tried in our study not only to verify the functionality of
supplementation with dietetary nucleotides, but also their positive effect in
preventative and clinical application. In the layout of individual clinical studies we
have surpassed the scope of initial goals, due to the fact, that we have achieved
important results with application of dietetary nucleotides in many
diseases/illnesses. For this reason we continue studying the clinical indications/
applications further. We recommend the possibility of wide spread use of
product Imuregen in the form of beverages (which is at present time produced
by company “Dobra Voda”) as substantially beneficial to public.

Due to the fact that the scope of the study was so broad, many other
articles/sub-studies will be published during year 2004. Publications in Czech
Republic and abroad in English language will be gradually published, some after
processing of finished stages, others after finishing of studies on sufficient
number of followed individuals.
EXPERIMENTAL PART

1. **Goal of the experimental part of project**

It was necessary in the scope of the project, (which is the verification of possibility of the positive influence on immune system by nutrition, by the way of supplementing with food products with high content of dietary nucleotides (dNT) and thus positively influence the general health of population), to supplement clinical studies with the results from experimental animal models, which cannot be obtained in clinical studies on humans, for practical or ethical reasons. And which are important for understanding of mechanisms of immunoregulating effects/influence of dNT.

1.1. **Starting premises:**

The exact mechanism of influencing of immune processes dNT is not entirely understood. It is believed, that dNT in organism works on many levels. Especially by increasing supply of endogenous nucleotides, which are used by proliferating and differentiating immuno-competent cells. Intensive onset of creation of nucleotides after activation of immuno-competent cells by antigen, is explained by high demands on preparation of energetic metabolism, needed for synthesis of nuclei acids, and also that lymphoid cells have limited
ability of synthesis and endogenic reutilization of especially pyrimidine nucleotides.

It has been proven, that reducing of dNT in food will be reflected by lowering of immunologic readiness of organism, especially by reaction of cellular (T-component) immunity. (lowering proliferation of lymphoid cells after alogenic induction, lowering of production of interleukin-2 and lower expression of receptor for IL-2) and humoral (B-component), which is effective in anti infection immunity.

On contrary, supplementation of dNT supports not only normal physiologic functions, but it becomes necessary for regeneration processes. Lots of indication shows that immunocyts stimulated by antigen and the cells metabolically actively participating on immune functions (macrophages, granulocyts etc.) have increased demand on supply of dNT.

Proliferation differentiating processes of antigen activated lymphocytes demand additional increased supply of nucleotides (ensured by endogen synthesis, or by reutilization from exogenic source - nutrition), which are used in energetic metabolism and later in synthesis of nuclei acids at clone expansion during immune response.

2. Experimental model

For setting of basic/starting parameters of antiinfection and antitumor immunity were, as the experimental animals used mice of defined inbreded group Balb/c (MBÚ AV ČR, Praha) and mice of defined inbreded group C57Bl/6 (AnLab, Praha). 3 month old, and average weight 20g, kept in standard conditions (temperature, illumination, unified basic diet ad libitum); control and testing groups were 16 mice each. C57Bl/6 mice were s.c. inoculated by melanoma cells
B16F10 (Weizmann Inst., Rehovot, Israel). All experiments were done in two parallel tests and repeated three times.

As a source of dNT were used standard highly purified yeast RNA (Ribonucleic acid-core Torula sp. Type II-C, Sigma-Aldrich Chemie, Gmbh, Steinheim Germany) and product Imuregen® (Uniregen s.r.o., Náchod) in preventative time protocol (4 weeks) drinking(feeding) program *ad libitum* and dosage (100 mg/l (kg) dNT) set by programs recommended by speciality literature data.

Using/coming from above shown assumptions, we have set basic/starting parameters of antiinfection and antitumor immunity after application of dNT by set harmonogram of the project on mice experimental model in comparison with control (dNT unefected) animals.

3. Results

3.1. Influence of production of IL-2, IL-4, IL-12, TNF-α and IFN-γ

3.1.1. Criteria of selection of cytokines:

**IL-2** is the marker of activation of Th1 lymphocytes by antigen. It is the growth factor for T and B cellular population and also for Tc and NK cells. In “Th” cells it stimulates production of IL-4 and IFN-α. It stimulates proliferation and differentiation of CTL and NK cells and increases their cytotoxicit activity. It also increases proliferation and differentiation of B cells. Increase of production of IL-2 means increased T component immunity and IL-2 is therapeutically used in oncology.
**IL-4** is produced by Th2 lymphocytes and it stimulates differentiation of B cells for production of IgM a IgG antibodies. It also supports differentiation of CTL and granulocytes, decreases production proinflammation cytosines (example. TNF-α).

**IL-12** is produced by cells of monocytare-makrofage system and supports activation and cytotoxiciting of NK cells and their transformation in to more effective LAK cells similarly like IL-2. Further more, it is the key stimulator of production of IFN-γ in Th1 cells. It is applicated/used in treatment of tumorous and parasitic diseases.

**TNF-α** (cachectin) has cytostatic and cytotoxic effect on tumorous cells. As a proinflammation factor (endogen pyrogen) induces inflammation (through stimulation of production mainly of IL-1 and GM-CFS) and it supports the toxic effects of endotoxin G- bacteria's. It produces TNF-α and is inhibited by other cytosines and also by IL-4.

**IFN-γ** has regulating pleiotrop effects in immune system. Among others, it supports differentiation of B and T cells and stimulates expressing of HLA-1, by which it makes easier/assist in antitumores hit/effect by CTL. IFN is activator of macrophages and endothelial cells. Recombinated IFN-γ is used in prevention and therapy of viral diseases (Actimun).

3.1.2. Arangement of experiment:
Preventative application of dNT (Torula) for 30 days to the mice, group C57Bl/6, inoculated by tumorous melanoma cells. Concentration of cytosines was identified by method ELISA (see Picture 1).

**Picture 1. Comparing concentrations of researched cytosins against control group.**

<table>
<thead>
<tr>
<th>Cytosines</th>
<th>kontrola</th>
<th>Torula</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-gama</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.1.3. Conclusion:

Preventative 30 days application of dNT on tumorous mice (mice with tumor) shown substantial increase of IL-2, IL-4, IL-12 and the most of IFN-γ, compared to control group. While the production of TNF-α was not effected and it's concentration remained on the level of control group.

It can be stated, that preventative application of dNT supports production of regulatory important cytosines, especially those, which are important for development of anti tumour composition of immunity. Which is: directly stimulating differentiation and activity of CTL and the cells NK (IL-2, IL-4, IL-12, IFN-γ), some from these are already used therapeutically (IL-2, IL-12, IFN-γ), or are important for development of complex immunity. Which means also of
B-component, on which resides antiinfection immunity (IL-2, IL-4) and the cells of monocytarly-makrofag system, important at processing and presentation of antigen, at T and B cellular co-operation (IFN-γ). On contrary, preventative application of dNT did not result in increase of TNF-α, which is an important pro-inflammatory factor, and it can be indirectly assumed, that it could be result of increased production of IL-4, which TNF-α inhibits. (see above).

We can conclude, that effects of dNT applied by the form of preventative long term drinking/beverage regime(Torula and Imuregen), in the environment of experimentally induced tumours process (mice-melanoma), have clearly supportive influence on production of cytosines, positively influencing preparation and development of antitumoral component of immunity. On contrary, it does not effect amount of production of TNF-α, which, even for it’s cytostaticly-cytotoxic effects, is mainly strong proinflamating factor.

3.2. Quantification of cells making haemolytic antibodies IgM a IgG

3.2.1. Criteria of stating of amounts of IgM a IgG:

IgM presents main group of antibodies at onset of primary immune reaction. Effective humoral immunity in later phases of immune response, rests mainly in antibodies class IgG. Quantification of cells, making haemolytic antibodies IgM and IgG can give information, to what level are, by preventative application of dNT influenced not only differentiation of precursor immunocompetent cells for both classes of immunoglobulins, but also the dynamic of molecular shift of IgM in IgG during primary immune reaction.
3.2.2. Arrangement of experiment:

Absolute amount of spleen lymphoid cells creating haemolytic antibodies class IgM and IgG after i.p. immunization by T-dependant antigen (0,5 ml 4% sheep's erythrocytes, 5th. day of primary immune response) were detected by modified Jerneho “plak” method, where the cells secerning antibodies are identified by binding guinea pig complement and creation haemolytic zone.

Mice group Balb/c were compared after 30 days of application of preventative drinking program/regime dNT (Torula and Imuregen). Values are presented as the averages of absolute amounts of cells creating haemolytic antibodies. Determined/detected in individual tested animals and calculated to $10^8$ lymphoid cells. The average values of parallel tests, which were repeated three times, are shown in Table 1. and Picture 2.

Tab. 1. Average absolute numbers of cells producing haemolytic antibodies IgM and IgG in conversion on $10^8$ spleen cells.
<table>
<thead>
<tr>
<th></th>
<th>IgM</th>
<th>SD</th>
<th>IgG</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5837</td>
<td>236</td>
<td>33281</td>
<td>3563</td>
</tr>
<tr>
<td>Torula</td>
<td>15889</td>
<td>770</td>
<td>115945</td>
<td>1599</td>
</tr>
<tr>
<td>Imuregen</td>
<td>14674</td>
<td>269</td>
<td>105170</td>
<td>3344</td>
</tr>
</tbody>
</table>

Obr. 2. Average absolute numbers of cells producing haemolytic antibodies IgM and IgG in conversion on $10^8$ spleen cells.

3.2.3. Conclusion:

After 30 day preventative application dNT in the form of drinking/beverage regime at both tested products, Torula and Imuregen substantially increased absolute amounts of cells creating/producing haemolytic antibodies IgM and IgG in spleen of immunized animals in comparison with control animals, not
supplemented with dNT. Differences between both tested products (Torula and Imuregen) were not substantial. However, increase of absolute number of cells is not exhaustive (exceeds only 1st order).

It can be expected, that after long term supply of dNT in the form of preventative drinking/beverage regime with tested samples of Torula and Imuregen is created the tendency for non exhaustive stimulation of differentiation of immunocompetent cells towards production of humoral protective antibodies and thus to higher readiness of B cellular component of immune system, on which cellular capacity directly depends the effectiveness of antiinfection immunity.

3.3. Influencing of cytotoxicity of effector cells (Natural Killers NK)

3.3.1. Criteria determination of cytotoxic capacity of NK cells:

Determination of cytotoxic activity of lymphoid cells enables to obtain information on effect of dNT on differentiation of NK cells.

3.3.2. Arrangement of experiment:

Cellular cytotoxicity is quantified by measuring of amount of $^{51}\text{Cr}$, which is released from marked target (tumorous) cells (T), which are killed by effector
NK, from cells (E) of tested animal. As a cytotoxic testing system were used target cells of mice tumorous line YAC-1 (American Type Culture Collection).

Compared were mice Balb/c, after 30 day application of preventative drinking regime dNT, products Torula and Imuregen. The values are shown as an average percent of cytotoxic activity of splenocytes, after 3,5 and 18 hours incubation of effector cells with target cells YAC1, marked $^{51}Cr$, in ratios E : T: 200 : 1; 100 : 1; 50 : 1; 25 : 1. The values of cytotoxic activity were calculated by equation:

$$
\% \text{ cytotoxicity} = \frac{(cpm_{exp} - cpm_{spont} : cpm_{max} - cpm_{spont})}{100}
$$

From the maximal yields of cytotoxicity and are expressed as averages of percent of cytotoxicic activities, established in individual tested animals and 3 x repeated tests. (Picture. 3).

Picture. 3. Mean cytotoxic activity of lymphoid effector cells of splean (NK), after preventative application of drinking regime dNT: comparison of products Torula and Imuregen.
3.3.3. Conclusion:

30 days preventative application of dNT in the form of drinking regime with both tested products Torula and Imuregen unsubstantially increased cytotoxic activity of effector cytotoxic splenocytes (NK cells) oriented on target tumours cells YAC1 in *in vitro* model cellular system.

It can be judged, that long term supply of dNT presented in products Torula and Imuregen, by the form preventative drinking regime will have tendency positively stimulate some part of antitumor component of immunity. These results are corresponding with findings at testing of proliferative activity co-stimulated by mitogen ConA, which stimulates mainly T-cellular sub-population (see part bellow. 3.4.3).
3.4. Determination of proliferative activities of lymphoid cells

3.4.1. Criteria of determining proliferative activities:

Immunocompetent cells after encounter with antigen, go through proliferating processes, in which course are differentiated in terminal stage of effector cells. Therefore the determining of proliferative activity of lymphoid cells is the substantial information on the state of immune potential of organism. Costimulation by T-dependent mitogen ConA determines influence of tested matter on T cellular component, costimulation LPS gives a picture on influence on B cellular component.

3.4.2. Arrangement of experiment:

Cellular proliferation in proliferative test ex vivo is quantified by measuring of amount $^3$H-thymidin, which is in S phase of cellular cycle incorporated in nuclear/nucleus DNA.

Mice group Balb/c were compared after 30 days of application of preventative drinking regime dNT products Torula and Imuregen. Measured was the complete basal ("tranquil") proliferation of splenocytes uninfluenced by mitogens and compared with proliferation after co-stimulation ConA and LPS. Average values of proliferative activities are presented as proliferous indexes, stating the intensity of cellular fission (see Picture 4).
3.4.3. Conclusion:

Total basal proliferation of lymphoid spleen cells is not substantially influenced after 30-day preventative application dNT in the form of drinking regime at both tested products (Torula and Imuregen). There are substantially increased proliferative activities of co-stimulated lymphoid cells dNT on tested/influenced mice (using Torula and Imuregen), namely ConA induced T cellular subpopulation. Compared to proliferative activities of control group (uninfluenced dNT).

(Comp. above part: 3.3.3.)

Application of dNT administered via products Torula and Imuregen in the form of long-term preventative drinking regime significantly stimulates proliferating lymphoid cells induced by T and B mitogen. It can be expected, that supply of dNT will more positively influence T cellular component of immunity.
3.5. Flow cytoometry

By the flow cytoometry were identified specific surface markers IgM and IgG, CD4 and CD8, CD11b, CD14, CD45 and Dx5 on splenocytes of experimental mice in both groups, which were supplied with dNT (Torula and Imuregen) and control mice (without supply of dNT). Application of dNT in preventative 30 day drinking regime did not influence expression of any followed markers compared to control group. For this reason we do not show resulting values.

3.5.1. Conclusion

On experimental model (mice Balb/c; tumor carrying mice C57Bl/6) preventative application of dNT (source Torula and Imuregen) given for 30 days in the form of drinking regime did not have toxic effects on organism.

Immunologic tests, focused on basic manifestation of natural and specific humoral and antitumoral immunity, didn't show inhibiting effects in any experimental set up/arrangement.

Preventative application of dNT conclusively supports cellular and humoral component of immunity and thus entire immune readiness of organism.
Experimental testing indicated, that dNT might become suitable supplement of nutrition, recommended in preventive health programs.

3.6. Regeneration of soft tissue of ilea by diet enriched with nucleotides.

Epithelium of digest tract has very important role not only in digesting and absorption of nutrition, but also in transfer of antigen and pathogen signals for intestines lymphatic tissue. Different stages and forms of malnutrition are considered as the most frequent cause of dysfunction of the Immune system.

Current Scientific research on dietary nucleotides comes to the conclusion, that oligo and poly-nucleotides, which are present in the food (nutrition) present the most important parts of nutrition due to their importance for all kinds of metabolic and energetic process in human organism. Nucleotides are the base for synthesis of nucleo acids at regeneration of tissue. Exogen nucleotides are necessary building stone for some cellular systems, which are unable to use endogen sources, for example cells of red blood line, white blood line, central nervous system and partially cellular substract of liver tissue.

Most of the up to date medical publications, show positive influence of dietary nucleotides on growth and maturing of intestines epithelium. We have previously published the results of testing on animal model, with application of extracts of animal origin, with substantial impact on increase of immune response.

3.6.1 Material and Methodic
In this study were 3 month old female mice group: Balb/c, fed by: 1. standard diet, 2. diet enriched by Imuregen supplement 3. standard diet and water enriched by Imuregen supplement for the time of four weeks. In all cases was used product Imuregen.
Supplement dose of 100mg per 1kg of food. Or 100mg per 1L of water contained Imuregen, product of company: Uniregen, s.r.o., Nachod Czech Republic.
Samples of terminal Ilea from all three groups were taken for histology test/examination and for observation in raster electronic microscope. The tissue for histology testing was fixed, coated in paraffin, dyed with hematoxyline eosin and by method on proving acid mukopolysacharids by Hale-Muller and studied in electronic microscope.
The length of villi was evaluated morphometrically. The samples for raster electronic microscope were dried by hexamethyldisylazan, gold coated and looked at/examined under raster electronic microscope Tesla BS 301.

3.6.2 Results
There were found no pathological changes of intestines villi in experimental groups. The highest growth of intestines villi were observed in group #3, which was fed with standard diet and water supplemented with Imuregen. Evaluation of morphometric data have proven statistically significant difference in the length of villi of experimental groups. (graph 1)
Morphology of individual selected founds from groups 1 – 3 is shown in pictures 1,2 and 3.
Morfometric evaluation of the height of villi (lining) of terminal ilea.

Graphic presentation of test results

2. Diet enriched by Imuregen – dose 100mg/1kg
3. Supplementation of drinking regime with Imuregen – (standard diet + Imuregen in drink – dose 100mg/1L) (Animals had free access to drink and food, experimental nutrition was served for duration of four weeks.)

3.6.3 Statistical evaluation and conclusion:

Differences of measured values of the heights of villi in control group and both experimental groups were evaluated by Snedecorov F-test. To evaluate value of zero hypothesis about difference of averaging mean measured values, the Student’s t-test was used. The differences in averaging mean values of the height of villi were considered to be statistically significant, if value of testing characteristic was bigger than relevant kvantil of Student’s discrimination to 5% level significance (p < 0.05).
3.6.4 Conclusion.
In this experiment was found statistically significant growth of the mean length of villi of terminal ilea of mice group Blab/c after four weeks of serving of the mix of oligo-polynukleotides in dietetic product IMUREGEN, in comparison with control group. The most significant growth was proven in the case of serving Imuregen in drink. (p < 0.005) than it was in food with the same product (Imuregen) (p < 0.025). There were found no pathological changes of intestines villi in experimental groups. This test/experiment has proved positive influence of mix of nucleotides in dietetic immune stimulating product Imuregen on regeneration and growth of epithel of terminal ilea.

4. Preventative programs

There is more and more evidence about the fact, that even in the society with high standard of living, unbalanced supply of nutrition components exists, which demands solution by compensation nutritional means. There have been efforts in the last years to increase quality of supplementation. Together with vitamins and minerals are being introduced/recognised another micronutrients, like for example flavonoids, DNA and nucleotides. In this section of work/research, the effort was to verify the effect of the products with high contents of oligo and polynucleotides on growing children population.
4.1.1. Summary and method

Number of children from preschool settings was divided in two groups. The first group was given Imuregen supplemented food, the second group was given food with placebo. The course of supplementation is shown on picture 1.

<table>
<thead>
<tr>
<th>Suplementing</th>
<th>March</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>October</th>
<th>November</th>
<th>December</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>14</td>
<td></td>
<td>20</td>
<td></td>
<td></td>
<td>4</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Supplementation was done during the scope of 3 month in the spring, and again in the fall.
During the study, samples of saliva were taken always before supplementation and after supplementation, in both springtime and the fall.

The concentration of lyozym, SIgA and albumin in the saliva, was followed by method of simple radial immunodiffuse. Secretion index was calculated from values SigA and albumin.

Sickness/sicktime of children was observed/followed during supplementation, from the view of frequency of sickness and from the view of length/duration of sickness.

The results were statistically evaluated with the use of Microsoft Excel and with the use of programs accessible in InStat 3. Two samples from individual children were evaluated with the use of the two sided pair T-test.

4.1.2. Results

When comparing values of the supplemented and the control groups, it is obvious from the results, that the biggest changes that we have found were at values of albumin. While in the supplemented group, decrease of values of albumin (decreased inflammatory reaction) always occurred after the course of supplementing with Imuregen, in the control group value increase occurred in both testing seasons (spring and fall) (pic.2).
Less pronounced are changes of values of lysozyme, where in the controlled and in the supplemented, changes were more noticeably effected by seasonal and circadian rytmicity (pic. 3). Only after spring supplementation there was noticed more pronounced increase of values of lysozyme in the group of supplemented.

Obr. 2: Values of salivary albumin at supplemented and control

Obr. 3.: Values of lysozyme at control and supplemented children
We have found similar findings in values SIgA, where significant change was detected in supplemented group, only in the spring sample.

Values SIgA in control and supplemented children (log. averages)

As it is clear from graph 4, the findings from secretion index (ratio albumin / SIgA) are statistically highly important, which is influenced mainly by decrease of inflamative irritation in the group of supplemented.

Ratio SIgA / Albumin in supplemented and control (log. averages)
The significant reduction of sickness in followed group of supplemented children is a substantial benefit of supplementation with Imuregen. In the "influenced" group the sickness was decreased by 50% and statistically significantly shorten the amount of sick days in supplemented children. We consider these findings as beneficial also from the point of view, that they verify the importance of rational supplementing and document our opinion, that recessive trend on supplemental policy of children population is not a good solution. We realise this particularly in the context with joining European Union, where supplementing mainly by milk products is common norm.

5. Supplementation of school age children

5.1. - Rom (Gypsy) population

We have done complex observation of children’s population of elementary schools in city Ústí nad Labem, as an entry study for project SZO-RHN. In the scope of this study were observed not only economical and social criteria, but also health aspects. As a beginning, we have evaluated literature information, showing predisposition for a number of illnesses, for example increased prevalence of malfunction of thyroid gland. We have adopted (started with) model of supplementation, in which we used humanised milk (addition of nucleotides-Imuregen) with daily addition of 150ug iodide potassium. (Prepared by company Nutricia Opočno.) Supplement was served for duration of 2 month.

5.1.1. Summary and method
In the group of 120 Rom (Gypsy) children we took samples of saliva before and after nutrition intervention. From the complete number of 120 children, we have got pairs of saliva samples from 77 children, which is 64.2%. 33 children were given milk with supplement, 34 children were given milk without supplement. Children were given milk daily, in amount 200 ml of milk.

Obtained samples of saliva were evaluated/examined by method of simple radial immunodiffuse, and values of SIgA, of albumine and lysozyme were followed/examined. We have used again ratio of SIgA and albumine to calculate secretion index. Statistical evaluation was done with use of program: Microsoft Excel and InSTAT 3.

Graph 1.: Salivar albumine before and after suplementingi (log values)
Graph 2.: Albumine in saliva of control group

Graph 3.: Albumine in saliva of suplemented children
5.1.2. Results and discussion (green: before, blue: after)

In graph#1 are shown values of salivary albumine in supplemented and unsupplemented children before and after nutritional influence. It is obvious, that in supplemented children occurred substantial decrease in values of albumine from mean value 117 mg/l saliva to value 79 mg/l saliva. While in unsupplemented were values insignificantly increased from value 95 mg/l saliva to 105 mg/l saliva. Higher values in control group show chronic inflaming irritation, which can also be related to the state of oral hygiene.

Surprising are findings of single doubles/couples of albumine before and after supplementation, at control group and at supplemented group. While in control group occurred in some cases increase and decrease of values (graph 2.). In graph#3 is quite obvious very significant decrease in values of albumine in supplemented children, mainly in samples with extremely high level of albumine. This decrease is so significant, that in the values signalling inflammation (over 100 mg/l saliva) were found only 7 individuals from original 14 of extremely increased findings, after supplementing with Imuregen. These finding documents significant anti-inflammatory effects of nucleotides, probably on the base of
activation of immune functions of compete immune system GALT. As we have proved on animal models, substantial growth and reparation of intestines villi leads probably not only towards increase of immune functions, but also by activation of alpha and beta defenzines to comprehensive improvement of homeostasis of immune functions. In the graph 4. Is shown, that local production of IgA is in supplemented children much more pronounced, than in children not supplemented. It is necessary to mention here, that selected group of followed/tested population was often in nutritional deficit and, as we have found from survey, our supplied snack was for most children the second meal of the all day.

5.1.3. Conclusion

Nutritional supplementation by milk enriched by nucleotides (Imuregen) and polypeptides substantially influenced parameter of secretional immunity. The result is more evident/pronounced in the group of Rom (Gypsy) children mainly for the reason of covering necessary basic energetic needs. We have proved in context with opinion of the other authors, the importance of ecoimmune nutritional interference in regulation and keeping/maintaining of homeostasis of immune mechanisms of mucus membrane.

6. New ways of supplementation

6.1. Senior population

There are many components of food/nutrition, which in current epidemiological studies show important benefit in prevention of tumours,
cardiovascular and autoimmune diseases. Even when these nutrients have much higher importance mainly in early age, we cannot underestimate their effect on health in mature and elderly population.

After experiences in our workplaces with application of nucleotides, (where the world’s literature presented much evidence on their importance in reparation of many physiological functions), we have initiated study on effects of nucleotides in the preparation complex, consisting of:

- 1 mg Imuregen
- 5 mg dihydroquercetin
- 50 mg vitamin C
- 20 mg Zn lactate

Preparation was given to the groups of senior population in various institutions Ústeckého region.

6.1.1. Summary and method

3-month of serving product Imuregen to 30 seniors, was controlled/compared with the group of 15 individuals, to whom was provided placebo product. To follow up the effects, we have used samples/collections/takings done in the frame of regular complete health check-up. Beside the series of 17 parameters of humoral immunity, we have also followed quality of psychological state of individual and his/her wellness. Evaluation was done from the initial check up, and the check up after termination of program.

6.1.2. Results and discussion
Evaluation of the results in observed individuals with supplement and without supplement was very difficult, mainly because of age heterogenesis of the group and often there were pronounced changes at entry check-up, which often signal serious illness. Inspite of this, we have noticed significant influence in the markers of inflammation, where we have proved decrease of values C-reactive protein from 75 to 43 mg/l (P=0,01). In connection with this we have found improvement of values of complement’s components - mainly C3, where was increase from 96 to 123 mg (p=0,001). Important is also increase of value of prealbumin from 0,221 mg/l to 233 mg/l, primarily for the reason of it’s decrease in the control from 0,219 to 0,174 mg/l (p=0,047). It shows significant improvement of nutritional state. Very surprising for us are findings of changes in values of rheumatic factor, where we have found after 3 month of supplementation significant decrease (p=0,0039) compared to control group (graph 1.). It corresponds with significant decrease in values of circulating immune complexes, while in control group, increase in values occurred. Improvement in quality of immune response even after short term supplementation is documented by increase in mean values of natural antibodies (xenoaglutinin), which at control group didn’t show any change.

Graph 1.: Rheumatic factor before and after supplementing of seniors
6.1.3. Summary and conclusion

We have repeatedly proved the importance of supplementation in senior population with the use of supplement containing nucleotides. We also take into account obvious additive effect of positive psychological alteration of this population group/sample. We believe that nutritional influence has importance even in senior population, mainly for the reason to positively influence health and psychological well being. In accordance with literature we consider necessary to pay attention to this issue, as senior population will show continuous increase in coming years.
CLINICAL PART

1. Benefits of speleotherapy with nutritional regime for treatment of allergic diseases

Significant growth of allergic diseases in children's population leads towards the quest for another possible medical treatments, which would enhance improvement of health in affected children. One of the possible ways is to remove child away from harmful exposure in domestic environment (living space) as well as from exterior environment. For treatment of allergic children in our republic are already used capacities of various establishments with suitable climate for additional treatment of allergies. Some of these are (for example) therapeutic stays with speleological therapy regime, where additional therapy/treatments
including nutritional treatment are used. In the scope of research we have observed/explored the influence of speleological therapy on health state of allergic persons.

1.1. Summary and method

In the first stage we have compared healthy individuals in situ (were not sent to treatment facility) with allergic individuals as well without sending them to treatment facility, and allergic individuals, who underwent speleotherapy treatment/regime. In the first group of healthy individuals we observed 23 children in the age of 8-12 years. In the second groups of allergics (without sending them to treatment facility) we observed 21 children of the same age average. And in the third group we observed 31 children, who underwent 3 weeks treatment in facility "Zlatých horách" with speleotherapy treatment, (without nutritional addition). In all observed individuals were taken samples before commencing study, next one after undergoing of 3 weeks treatment/regime and the last sample was taken three month later. In saliva were evaluated/examined values IgG, SIgA, IgM, lysozyme and albumin. The results were statistically evaluated with the use of program Microsoft Excel and InSTAT 3.

1.2. Results and discussion

It is obvious from graph 1, that values of albumin at healthy individuals were statistically significantly lower, compared to allergic children who were sent to treatment facility and to those who were not sent to facility. These values were practically not changed during the whole course of observation of the control group of healthy children. Also the values of salivary albumin in allergic children who were not sent to facility did not show statistically
substantial changes during the all course of observation. However, in children with speleotherapy regime we have found significant decrease of values of albumin, after going through speleotherapy regime. But after three month period values of albumin were gradually increasing. Inspite of this, these values were substantially lower then those of allergic children who were not sent to treatment facility and there was no difference from findings in healthy children.

Graph 1: Salivary albumin in allergic children and control (average values and SD)

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>zdraví</td>
<td>54.8</td>
<td>52.2</td>
<td>58.8</td>
</tr>
<tr>
<td>allergici bez výjezdu</td>
<td>88.2</td>
<td>86.3</td>
<td>91.2</td>
</tr>
<tr>
<td>speleoterapie</td>
<td>95.1</td>
<td>26.8</td>
<td>65.5</td>
</tr>
</tbody>
</table>


Graph 2 shows, that values SIgA are statistically substantially higher in allergic children, then it is in control group of children. This is also partially caused by contamination of saliva by serum IgA. In allergic children who underwent speleotherapy treatment was found substantial decrease in values SIgA, which lasted even in last taken sample.
Dynamic of changes IgA and albumin was shown in values of secretion index (graph 3), where we have found important increase in the second taken sample followed by decrease to the same values as in individuals without speleological treatment.
1.3. Conclusion

In this first part we have proved beneficial influence of therapeutic stay with treatment/regime of speleotherapy in children with allergic disease/illness. We consider important to follow this effect repeatedly with the use of longer stay/regime of treatment and with the back-up of another treatments, which would positively influence health/well being of allergic population. For this reason we have initiated treatment/regime of speleotherapy in combination with nutritionally influencing children population.
B: dtto without supplement
C: chronic infection of upper respiratory tract + supplement Imuregen
D: dtto without supplement

In the continuation of this study we have tested total of 45 children with bronchial asthma and 43 children with chronic infections of upper respiratory tract. As is obvious from graph 1, pronounced improvement in findings occurred, mainly in children with nutritional regime (supplement with poly and oligonucleotides - Imuregen), group A and C. Values of albumin in individuals with bronchial asthma were statistically significantly decreased, as well as in children with chronic infections of upper respiratory tract. Improvement in children without supplement was less pronounced, but provable. Overall findings are in accordance with literature sources, which presents, that even short supplementation with corresponding dose of nutritional supplement can lead to fast modification of immune parameters.

We continue in this study with evaluation of samples, which are done 6 month after undergoing through treatment regime.

2. Influence on health conditions in individuals with clinical manifestation of 
Chronic fatigue syndrome with definition of EBV infection

Clinical manifestation of fatigue is accompanying occurrence of many chronic infections, nutritional insufficiency (deficit of iron) and others, up to day,
unspecified diseases. It is obvious, that diagnosis of chronic fatigue syndrome is expression of inability of medicine to closer define and specify whole group of diseases. Aspects of chronic EBV infection, respectively polyherpetic syndrome, (study by Japanese authors) are constantly followed subject in world literature. All persons affected by this clinical syndrome are showing together with clinical manifestation of tiredness/fatigue also high score of Holmes’s criteria. In the scope of complex observation and treatment of this syndrome, we have used beside treatment by transmissible factor also possibilities of nutritional supplementation in observed individuals by use of minerals and for this study also product Imuregen, with high content of polypeptides, oligo a polynucleotides.

2.1. Summary and method

We have included total of 26 patients in the group, one half with placebo preparation, second half supplemented with product Imuregen in dosage 2x2 tablets daily for duration of 3 month. Before and after nutrition supplementation was done complex/complete immunologic check up, observing parameters of humoral immunity, antibody response against heretic viruses (HSV1, HSV2, HSV6) and against CMV and EBV. Cellular immunity was examined with immunofenotypicality by method of flow cytometry. Based on findings on animal model, we have paid attention mainly to observation of spontaneous cytotoxicity cellular populatin (NK).

2.2. Results

Results are shown in graph 1. While in the group with placebo were not found important changes in this lymphocyte subpopulation, in the group
supplemented with nucleotides was increase in the number of cells from average value 257 to 354 cells in microliter of serum (p=0.000913).

NK lymphocytes in EBV infection after nutritional intervention.
(N = supplemented, P = placebo)

Together with improved findings in cellular lymphocytic subpopulations occurred also the improvement in parameters of Holmes's criteria, important improvement of clinical symptoms and in improvement functional liver function tests.

2.3. Conclusion

By nutritional supplementation we have achieved important adjustment of laboratory parameters and clinical indications of symptoms, in the patients with chronic fatigue syndrome on base of activated EBV infection.

3. Supplementation of patients with inhalation allergy, caused by mites in domestic dust
Continuous increase in indications of inhalation allergies in the world, as well as in our republic is serious health problem. In the present time we are finding in the epidemiological studies, done in "Ústecko" regions/counties already more then 50% of respondents with positivity of complete IgE, or specific antibodies IgE against inhalation allergens, food allergens and latex allergens. In this population's sample with positive responsiveness we are finding more than 30% of individuals with clear clinical presentations of inhalation allergy. Beside the classical treatment of allergic diseases, we see more and more of nutritional treatments used around the world, not only with preventative aim (pregnant women), but also with the effort to accelerate corresponding basic treatment.

3.1. Summary and method

We gradually include in the group persons with inhalation allergy, defined by clinical presentations of symptoms together with activity of specific antibodies IgE against allergen d1 (Dermatophagoides pteronyssin). In all individuals was examined/checked complete basic spectrum of inhalation allergens with the use of diagnostic sets made by company Phadiatop. Further more were observed the values of complete IgE, values ECP, values of basic proteins and values of eosinophils. In this spot we refer to the first results of the study, where we served/added with the basic treatment also the supplement of nucleotides in the dose of two tablets a day and individual vaccine against mites in domestic dust. Totally was supplemented 12 individuals, placebo was given to 8 individuals.

3.2. Results and discussion
The results in supplemented patients with respiratory allergy treated by standard treatment, treated by standard treatment, vaccination with supplementation and without supplementation (with placebo), are shown in graph 1.

It is obvious from the graph, that course of de-sensibility since the entry exam, is during one year of application of nucleotides together with vaccine substantially more effective. Statistically important decrease in antibodies against d1 occurred, in comparison with the group-using placebo, however even in this group is noticeable important effect of vaccination. This improvement is probably caused by the reparation processes, renewing intestinal mucus membrane, with the induction of other mediators, which influence recognition of given medicine and it’s effect. We also calculate with the possibility of induction another regulating mechanisms, on cellular level and also the level of immune processes in intestinal mucus membrane (betalyzins, mucins etc.). We intend to continue to study this problematic issue. Quick reparation of clinical symptoms in
supplemented individuals, with respiratory allergy, leads us to the efforts for the same treatment in allergic manifestations in food allergies and atopy.

4. Supplementing with nucleotides in inflaming diseases of gastrointestinal tract

Chronic unspecified inflammations of gastrointestinal tract, are very often accompanied by extreme loses, not only energetic, but also loses of micronutrients. Changes in the specific immune response and in unspecific response are generaly studied in the patients with this clinical condition, and there is continuos effort for detailed definition of pathogenesis of sickness. The attention to this problem was given by the medical team in “Usti Hospital” under the leadership of Professor Bitter MD, in the beginning of the seventies. We cooperate with this hospital in regular time frames. In accordance with our previous findings, we are trying to equalize effects of chronical inflammation on parameters of immune response in the patients with ulcerative colitis and with Crohn’s disease. In this study we have paid attentions to the questions of energetic supplementation with the use of DNA and nucleotides on the group of 30 patient with ulcerative colitis.

4.1. Summary and method

We have chosen for this study 30 patients diseased with ulcerative colitis, with defined clinical course, histologically verified. We are observing total of 17 women of average age 63,5 years (in the span of 44 - 90 years) and 13 man of average age 65,7 let (in the span of 38 - 79 years).
All observed patients were supplemented with product Imuregen with high content of oligo and polynucleotides and they were immunologically observed for duration of 2 month of supplementation with the dose: 2 - 6 tablets a day (by the physical weight of the patients). The control samples were done/taken after 6 months since the beginning of supplementation. In this part of the study we have focused on following of parameters of humoral immunity and mainly of the indicators of inflammation and indicator of nutritional status.

4.2. Results and discussion

In graph 1 are shown values of albumin at entry examination/testing, after the application, and in the third sample are values from 6 month after the application of product Imuregen. We have found statistically important the increase in values of prealbumin between first and second sample, and between first and third sample, which documents not only improvement in nutritional status, but also improvement of immune potency in observed individuals. The higher value of prealbumin documents higher level of immunoregulating functions of organism. It is well known that prealbumin has the structure very close to thymus elements (Thymosin), acting as a modulator of T-cellular immune answer. We have also found important reaction in the observed component parametres of the complement C3 (graph 2).
We have found statistically important decrease of values C3 practically in all observed persons, which documents on one hand decrease in reaction of chronic inflammation, and on the other hand substantially decreased consumption of component C# (graph 2). It corresponds with the dynamic of changes in values of immunoglobulins class M. Where after the first increase in values in the sample after supplementing, comes to the normalizing of values, This normalizing documents the substantially lower infectious alteration and probably increased ability of secretionary immunity to equalize antigen impulses via GI tract (graph 3). It indicates, that nucleotides contribute (as well as in model tests) to accelerate repair of mucous membrane of GI tract, increase production of mucin and other components of unspecified immunity like for example lysozyme and betadefenzine. Together with this adjustment we can also proclaim important feeling of satisfaction/contentment of the patients with this treatment. Due to the fact that the base group of registered patients of Masaryk Hospital in the city Ústí nad Labem, is comprehensive/wide enough and well clinically defined, we consider as a beneficial to follow up with these individuals. Not only from the view of nutritional influence, but also from the view of their more detailed...
immunogenetic classification. In the frame of this study we have found, that sickness with ulcerative colitis effects more often patients with dysfunction of trypsin (finding of ZZ deficit alpha 1 antitrypsin), which happens in these afflicted 40x more often then in normal population.

Graph 3.: The effect of supplementation with Imuregen on component parametres of complement C3

Values IgM after supplementation with Imuregen in the sick with ulcerative colitis
4.3. Conclusion

Our results prove, that supplementation with dietary nucleotides contributes substantially to reparation of immune functions of the sick persons affected by ulcerative colitis disease.

5. Final conclusion

In the scope of this project we were considering the possibility of using product made in Czech Republic, suitable for application in nutrition supplementation, in preventative and clinical medicine. In comparison with other products DNA, we have found that products of domestic production on base of oligo and polynucleotides have practically the same effects as economically expensive products DNA, prepared from yeast, (company SIGMA Germany). We are documenting its use in preventative applications on children at preschool and school age and in elderly population. We also document the effects in several clinical studies on particular diseases, as demonstrated in experimental studies done in the scope of this project. Good experience with this product (Imuregen) including its financial affordability leads to suggestion about the possibility of wide use of this product (Imuregen) in the drinking program application. Especially for population which is substantially affected
by higher risk of their profession and exposition of harmful chemical and physical emissions.
Based on our findings, we have recommended using formula of products: oligo and polynucleotides (Imuregen) together with mix of flavonoids (Grape seed oil) and vitamin C, and low amount of Zinc.
In the pilot study we have tested the effects of this formula/mix in small short test series on workers at company GLAVERBEL (manufacturer of industrial glass). We had the possibility to define consumptions of drinks with the above mentioned formula and the resulting effect of this experiment, (by the use of computer systems). We have found that application of this formula in the critical flue epidemic 2003/2004 led to substantially reduced sickness among the workers.
Based on these results/data, we have recommended, together with The Institute of Work Medicine/SZU (Professor Cikrt), the wide spread of this application to the other industrial productions – workplaces with higher health risks.
At present time, the drink with above-mentioned formula (oligo and polynucleotides (Imuregen) together with mix of flavonoids (Grape seed oil) and vitamin C, and low amount of Zinc) is programmed and approved by The Chief Hygienic of the Ministry of Health of Czech Republic for distribution and sale to the public. Company Good Water prepares the drink product in Czech Republic.

**Literature of grant:**


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23) Richter, J.: Naše praktické zkušenosti s IMUNOREM® (v tisku)

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In this era of an increasingly enlightened public about the health benefits of nutritional supplements, there is a new area perhaps more overlooked than any other. This central area of health and nutrition is the ingestion of dietary nucleic acid bases, NUCLEOTIDES, which are the essential building blocks of DNA and RNA.

Numerous studies in animals and humans show dramatic benefits in health, function, and survival with the supplementation of nucleic acid elements. These effects are so powerful that survival in life threatening assaults ranging from radiation to infection to shock has been markedly increased. From the standpoint of longevity studies, no single method has increased longevity more than supplementing DNA and RNA elements.

Nucleotide supplementation is thus one of the most rejuvenating, immune enhancing, and tissue supporting regimens ever to be discovered.

Recent evidence indicates that the body is often not able to make enough DNA and RNA to protect, repair, and regenerate cells to their optimum function. This is especially true for cells that have high turnover rates such as the intestinal lining that may fully replace itself every week. The demand for production may particularly exceed synthetic capacity under conditions of stress in which the demand for greater cell activity and function becomes acute, particularly for the dynamic populations of cells in the immune system.

When demand exceeds production capacity, DNA and RNA base components become essential nutrients for protecting and preserving health. Numerous lines of evidence will be presented to show the far reaching health benefits of supplementing DNA and RNA during health stresses and even for general well being and longevity.

Numerous published scientific studies indicate very significant health benefits from DNA and RNA component supplementation. Almost every system of the body has documentation of improved health, vitality, or function from providing supplements of these fundamentally important cellular elements, from infancy to advanced age. The following is a brief summary from the vast literature supporting the many published benefits of nucleotide supplementation in the diet.
Thousands of published scientific studies in the metabolic pathways related to the formula components also suggest the following potential benefits of these nutrients:

1. Cell membrane repair
2. Increased SAMe and serotonin levels
3. Balance of neurotransmitter chemistry in the brain
4. Increased melatonin with immunologic, antiaging, and antioxidant effects
5. Regeneration and repair of insulating myelin sheaths of nerves
6. Rejuvenating degenerating proteins into functional proteins again
7. Required for full antioxidant potency of antioxidant proteins in every cell and tissue
8. Repair of joints and cartilage
9. Reduced risk of colon and other cancers
10. May help reverse precancerous lesions to normal cells
11. Improved histamine clearance
12. Supports detoxification pathways in the liver

When DNA and RNA are ingested intact, they are intensely metabolized by intestinal bacteria and the intestinal lining.

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Tissue Regeneration

In order to sustain health, virtually every tissue in the body must regenerate itself regularly. It is now known for example that even neurons in the brain have the capacity to regenerate. Having adequate supplies of all the nucleic acid bases may be one of the most significant limiting factors on whether a tissue will be able to express its greatest capacity for regeneration and self repair.

A study in rats looked at the ability of the liver to regenerate depending on whether or not injections of nucleic acid bases were given. In this study, the rats had 70% of their livers surgically removed. The animals that received IV nucleic acids showed liver regeneration rates that were significantly greater than the untreated control animals.
Any tissue, in order to regenerate, requires the ability to make DNA and RNA to support the process of making new cells. Providing readily absorbed and assimilated DNA and RNA bases can be one of the most powerful ways to assist any tissue to repair and renew itself.

Wound Healing

A wound, surgical or otherwise, results in severing the usual integrity of tissue organization. It is a special case of tissue regeneration in which cells migrate into the area of the wound to either regenerate new tissue or to fill the defect with scar tissue. The type of healing depends on the tissue ~ the liver will tend to restore normal liver cells in the wound, whereas the skin will tend to fill the breach with scar to heal the opening and restore strength.

Several studies in wound healing have assessed the effects of supplemental nucleic acids on wound healing, especially of surgical wounds. Compared to the control group, those receiving the supplements showed more rapid healing, greater tensile strength of the skin, and significantly reduced scarring.

Endocrine Gland Repair

Some of the tiniest organs in the body have the most profound effects on our health and well being. These are the endocrine glands that secrete minute amounts of hormones into the blood without which every function of the body can suffer.

The pituitary gland located at the base of the skull has been called the ~master gland~ because it makes hormones that control the functions of other endocrine glands. It secretes hormones that regulate the thyroid and adrenal glands, the ovaries and testes, and the production of breast milk. The posterior region of the gland exerts control over the kidneys to adjust fluid balances throughout the body. Perhaps most important for longevity, the pituitary also makes growth hormone, that has been shown to have some of the most powerful age-reversing effects of any hormone ever studied. Inadequate nutritional support to this gland can have devastating and far-reaching effects throughout the body.

The adrenal glands, situated atop the kidneys, secrete adrenaline and noradrenaline, the fight or flight hormones. These powerful hormones increase heart rate and blood flow to muscle so that the body is immediately prepared for vigorous physical activity. In our ancestral past, this rapid preparation was a key to surviving in a hostile environment. However, modern living often puts a chronic stress on the adrenal glands, the myriad stimuli that surround us tending to keep the fight or flight mechanism constantly activated. The result is often varying degrees of adrenal burnout, exhausting the reserves of the gland to make the fight or flight hormones when really needed. Burned out adrenals give rise to a chronic low energy state, fatigue, and poor stress tolerance, like depleted batteries that fail to get recharged. These glands are especially prone to nucleotide deficiency under chronic stress, a condition that supplementation can help to restore, much as giving a long needed recharge to a nearly totally drained battery.

The thymus gland, residing behind the breastbone, is often considered the organ of rejuvenation and longevity. It is the gland in which the T cells of the immune system are formed and given identity. Upon release it is the T cells in particular that help find and destroy cancer cells and cells that have become afflicted with viruses. The thymus gland tends to shrink with time, yet specific supplementation has been found to bring this vital organ back to more youthful function. In particular, providing nucleic acid bases for this gland with very dynamic cell turnover can significantly rejuvenate this gland and its life preserving activities.
The thyroid gland, at the base of the neck in front of the windpipe, produces thyroid hormones, the main control mechanism for setting basal metabolic rate. In some circles it is believed that we are in the midst of an epidemic of undetected deficiency of thyroid function. Tests of thyroid function may not show overt clinical disease, but low-level deficiency can significantly reduce quality of life. Effects are subtle and can include generally low energy, sluggish bowel function, lack of initiative, tendency to depressed mood, and weight gain with great difficulty losing the added pounds. Dietary iodine and the amino acid tyrosine are important building blocks to make thyroid hormone naturally from the gland. In addition, correcting insufficient nucleic acid production under stress will also support recovery of a sluggish gland.

The salivary glands reside in several pockets in the mouth. Although not as essential as the other glands to sustain life, they provide a vital role in the first stages of preparing food for complete digestion. These metabolically active glands also require a rich supply of nucleic acids to maintain adequate salivary flow.

Nucleotide supplementation can thus be a very powerful tonic to sustain and boost the functions of the most vital glands in the body. These glands set our level of energy, our ability to respond to stress, our capacity to maintain strong immune defenses, the hydration of our bodies, and a wide range of hormone balances essential to a high quality of life.

Intestinal Integrity, Maturation, and Bowel Flora

The intestinal lining replaces all of its cells every seven days. Only a single layer thick, this lining is highly dependent on a sufficient supply of nucleic acids to completely regenerate itself every week. If nutritional support is inadequate, defective regeneration of the intestinal mucosal lining impairs the enzymatic stages of digestion, which can lead to a vicious cycle of deteriorating digestion and nutritional status.

Cellular Immunity

Numerous studies in animals and humans have shown that supplementing nucleic acid elements has profoundly beneficial effects on boosting the function of lymphoid tissue. In part, the reason for this is that lymphoid tissue is highly dynamic such that cells that have become sensitized to microbial invaders or cancer cells need to divide rapidly to make an army of specifically targeted cells to eliminate the invader.

A rich supply of nucleic acids, often beyond that the body can readily make, may be required for all the activities required for expanding the cells that prevent a minor invasion from becoming an overwhelming infection or uncontrolled malignancy.

Published studies have particularly demonstrated that cellular immunity is significantly strengthened with nucleic acid supplementation. Research that has examined natural killer cell function has shown especially dramatic effects on increasing the activity and function of these tumor surveillance and elimination cells. Improved health of body tissues in general and enhanced cellular immunity in particular, likely accounts for the vastly improved outcomes observed in the face of a wide range of minor to life threatening insults.

Memory Enhancement

An adequate pool of RNA is needed to manufacture new proteins that are essential to memory function. Although other support nutrients are an important factor, optimum memory function is not possible without a rich supply of nucleic acids.
Many studies in animals and humans have found a dramatic improvement in memory function with nucleic acid supplementation. Whether it is the ability to remember the right pathway to get through a maze for a prize of cheese, or to remember facts and figures, giving supplements of DNA and RNA elements has highly significantly increased performance.

Perhaps most dramatically, one researcher has focused on giving nucleic acids to persons with dementia. Even with advanced cases, if he went to high enough delivery levels to his patients, in almost every case memory improvement was very significant. The doctor reported that even in advanced cases of dementia dramatic memory recovery occurred if high enough levels of nucleic acids were given.

**Longevity**

It is perhaps functional nucleic acid deficiency that limits our potential for healthy longevity more than any other single factor. Of all the interventions that have ever been attempted to increase the life span of mammals, no method ever studied has been more powerful for mammalian life extension than nucleic acid supplementation. Compared to other techniques that have increased longevity of experimental animals up to 50%, administering nucleic acids has doubled and even tripled the usual maximum life span.

In a landmark study, a strain of rats was used that had a usual life span of 800-900 days. The study began with all of the animals at day 750, rather advanced in age at the entry of the test protocol. Half of the animals were used as controls and received their standard diet, housing, and care. The treatment group animals were given identical conditions with the exception of receiving weekly injections of DNA and RNA. After eight weeks the control rats looked much worse than at the start of the study, losing fur and muscle mass, and showing reduced physical activity. In sharp contrast, at this time in the study, the treated animals actually looked and behaved like younger animals. They regrew fur and increased their muscle mass, had renewed libido, and were significantly more active.

By day 150 of the study, all of the untreated control animals had died. In dramatic contrast, the minimum additional life span in the treated animals was 850 days, minimally doubling the usual life span of the animals. Perhaps most noteworthy, the longest lived animal in the treatment group survived 1500 days from the start of the study.

This is the greatest life extension ever reported for a mammal; nearly triple the usual maximum life span. It is especially remarkable because the animals were of advanced age at the start of the study. Weekly injections of DNA effectively increased the remaining life spans of the animals by 500-900%.

It is as yet unknown whether even greater degrees of life extension could be achieved by beginning nucleic acid supplementation at an even earlier age, before any organ deterioration had occurred. It is likely that the longevity achieved would be at least as great or greater.

Any intervention that slows, stops, and reverses the loss of methyl groups from DNA is slowing, stopping, and even reversing aging at the DNA level. Supplemented nucleotides provide the most powerful nutrient factors for improving DNA methylation ever studied.

The most sensitive blood chemistry test for determining the rate of methyl group loss from DNA is called homocysteine. The higher the homocysteine level above 4-6, the greater the rate of methyl group loss from DNA. The teenage level of homocysteine is 4-6, which tends to rise.
1-2 points per decade, such that at 60-70, the level is typically 12-15 or higher. Elevated homocysteine has in parallel with aging effects also been found to be a cardiac risk factor. Above a level of 6.3, cardiac risk rises exponentially, with a level of 15 carrying a risk four times, and a level of 20 carrying a risk that is nine times greater for a major cardiovascular incident than that of the general population.

In a major double blind randomized placebo controlled clinical study the effects of laser-enhanced nutrients on homocysteine reduction and other important metabolic factors and clinical symptoms. This study was reviewed and approved by the Western Institutional Review Board as meeting the international standards for the design and safety of human clinical research.

Perhaps the single most powerful homocysteine-lowering nutrient is trimethylglycine, or TMG. This molecule has three methyl groups to donate, hence the prefix trimethyl. It is also known as betaine because it is a natural substance derived from sugar beets.

Previous research has shown that high dose betaine can reduce the risk of mortality the first year after a heart attack from 25% to 0%. In addition, high dose betaine in conjunction with vitamin cofactors has been the only intervention that has permitted women with a genetic disease of high homocysteine levels (homocystinuria) to conceive and have normal gestations and deliveries. In other persons with homocystinuria, adding betaine has reversed neurologic defects and caused gray hair to darken and lost hair to regrow.

The study formula for boosting methyl group transfers and reducing homocysteine contained betaine as the main component. In addition vitamin cofactors that are known to support these pathways were also included, particularly vitamins B6, B12, and folic acid. Niacin was also included to assist with fat metabolism in addition to homocysteine reduction, for further cardiovascular support.

The treatment group received increasing doses of the methylation formula over a 3-month period of time, whereas the placebo group only received sugar pills. Subjects had blood drawn at baseline and every month for homocysteine levels, blood counts, and other metabolic tests. Subjects completed daily written reports and each week completed an extensive standardized symptom survey.

By the completion of the study, the treatment group showed very dramatic metabolic improvements not seen in the placebo group. Homocysteine levels for the treatment group started at values that on average carry about double the cardiac risk that by the end of the study reduced to values at less than the risk of the general population. The reduction at every dosage level was highly statistically significant, the homocysteine reduction for the group suggestive of a 20-30 year physiologic reduction in this aging measure.

The placebo control group showed no significant change in homocysteine level. The study compellingly demonstrated that the laser enhanced methylation formula was the essential factor in significantly lowering homocysteine and thereby improving methyl group transfer chemistry. In addition to reduced homocysteine, the treated subjects also showed highly statistically significant improvement of several clinical symptoms that included the following:

1. Reduced anxiety
2. Decreased body aches and pains
3. Elevation of mood
4. Decreased paranoia and obsessive-compulsive scales
5. Reduced hostility
6. Much decreased global symptom profile (all symptoms taken together in one comprehensive score)

Reducing homocysteine and improving methyl group transfers does more than protect and repair DNA and lower cardiac risks. Thousands of published scientific studies in the metabolic pathways related to the formula components also suggest the following potential benefits of these nutrients:
1. Cell membrane repair
2. Increased SAMe and serotonin levels
3. Balance of neurotransmitter chemistry in the brain
4. Increased melatonin with immunologic, antiaging, and antioxidant effects
5. Regeneration and repair of insulating myelin sheaths of nerves
6. Rejuvenating degenerating proteins into functional proteins again
7. Required for full antioxidant potency of antioxidant proteins in every cell and tissue
8. Repair of joints and cartilage
9. Reduced risk of colon and other cancers
10. May help reverse precancerous lesions to normal cells
11. Improved histamine clearance
12. Supports detoxification pathways in the liver

ATP Benefits

ATP stands for adenosine triphosphate, perhaps the most important of all the nucleic acid derivatives in the body. Its effects are so powerful and essential to cellular function, a description of its unique properties warrants special attention. ATP is the fundamental currency of every cell in the body. Virtually every activity in the body that requires energy uses ATP as the source of power. Whether the function is building complex molecules from building blocks, maintaining the electric potential of cell membranes, or allowing muscle fibers to contract for mobility, speed, and strength, it is ATP that provides the electrochemical fuel.

Cellular Energy

There are two fundamental ways ATP is generated in the body, one very efficient and one very wasteful. Efficient ATP production occurs through aerobic metabolism in the mitochondria, tiny organs or organelles within the cell that burn fuels like fat and glucose to generate ATP. Aerobic means that oxygen is used to completely ~burn~ a fuel for maximum ATP production. As nucleotides boosts cellular oxygen delivery, already making ATP production more efficient, the ATP has an ideal environment for further boosting cellular energy conditions; thus all the desirable ATP effects are likely to be even more potent.

Inefficient ATP production occurs through anaerobic metabolism. Anaerobic means without oxygen, so very little energy and ATP are extracted from fuels. When glucose is broken down through anaerobic metabolism, each molecule of glucose only gives rise to 2 molecules of ATP, wasting 95% of the potential glucose energy. Further, the byproduct of this reaction is two molecules of lactic acid, which makes the cells more acidic and less functional. In athletes, lactic acid accumulation causes muscle fatigue and the ~burn~, whereas in cancer cells lactic acidosis is a long recognized metabolic disturbance that can promote a dwindling spiral of progressive malignancy.
The direct suppression of tumor cell lines by ATP is likely related to increased cellular energy efficiency. The oxygenating effects of nucleotide supplementation combined with a supply of ATP is likely to be additive and even synergistic at helping cells throughout the body achieve higher energy potentials and more ideal energy balances.

Neurologic Effects

ATP is the primary fuel that drives learning, memory, and concentration functions. ATP is essential to maintain the membrane potentials that permit nerves to integrate and transmit signals throughout the central and peripheral nervous system.

In addition, giving ATP or its breakdown product adenosine intravenously has shown pain relief comparable to injected morphine for pain due to ischemia (impaired blood flow). Two surgical studies have shown a 25% reduction in the need for postoperative narcotic pain relievers when adenosine was given IV.

Perhaps most remarkable, peripheral neuropathic pain is one of the most difficult pain syndromes to manage. Excruciating constant pain may resist all but the most drastic measures. IV adenosine for 45-60 minutes reduced neuropathic pain for 6 hours to 4 days in 86% of persons tested.

Cardiac Strengthening

The cyclic contraction of cardiac muscle is highly ATP intensive and thrives on aerobic metabolism. The combined oxygenation and ATP delivery effects of DNA/RNA formulas provide the heart with an enhanced energy supply for efficient function.

Providing intravenous ATP has been shown to slow conduction through the AV node, which has been used to slow down certain excessively fast heart rates called tachycardias. Occasionally chest symptoms can occur with rapid intravenous infusions of ATP that resolve within seconds after stopping the infusion. ATP is not known to cause excessively slow heart rates in persons whose heart rates are normal.

Muscle Performance

Skeletal Muscle also requires abundant quantities of ATP for muscular contraction. Supplemental ATP has been described as an explosive performance enhancer. Especially, if given with two other nutrient supporters of muscle function, creatine monohydrate and creatine pyruvate, muscle endurance, performance, and recovery can be significantly boosted.

Lung Function

ATP administration has been shown to have numerous beneficial effects on lung function, particularly the delicate lining membranes of the airways and alveoli. In the lung, branching tubes called bronchi and then bronchioles deliver air to and from the tiny air sacs called alveoli. The alveoli form a large membrane only a single cell in thickness through which capillary blood can pick up a new supply of oxygen and unload carbon dioxide with every breath.

In vitro, or test tube level research, has shown that ATP increases secretion of surfactant in the alveoli. Surfactant is an essential substance that keeps the alveoli from collapsing when the breath is exhaled, preserving integrity of functional gas exchange. The bronchial tubes are lined with tiny brush like structures called cilia that are constantly
sweeping particulates that get into the lung upward and outward. ATP not only increases the ciliary beat frequency, it also increases the secretion of mucus and water from the bronchial lining, to help keep the lungs clear at all times.

In some conditions, the blood pressure in the vessels in the lungs can rise too high, a condition known as pulmonary hypertension. When given intravenously, ATP binds to the lining of the pulmonary vessels and stimulates a cascade of events that cause the blood vessels to relax and lower the pressure.

Cystic fibrosis is one of the most common inherited genetic diseases. Impaired water and electrolyte secretion from the bronchial lining results in thick secretions that block the bronchial tubes and result in recurring infections. ATP has been found to increase electrolyte and water secretion with improved clearance of secretions, offering hope of a new and useful intervention in this often aggressively progressive condition.

**Cellular Immune Enhancement**

Natural killer cells and cytotoxic T cells as reviewed are subtypes of effector lymphocytes that have a vital role in immune defense against tumors and virus-infected cells. Recent research suggests that ATP may play an important role in the mechanism through which these effector cells eliminate the target abnormal cells. In test tube studies, ATP has been shown to enhance the ability of cytotoxic lymphocytes to rupture the membranes of tumor cells.

**Antitumor Effects**

In test tube studies, adding ATP has shown the ability to inhibit the growth of several types of human cancer cell lines. The types of cancer cells inhibited include pancreatic cancer, colon cancer, melanoma, androgen-independent prostate cancer (i.e., not responsive to male hormone manipulation, the most aggressive variant), breast cancer, myeloid and monocytic leukemia (bone marrow derived tumors of blood forming cells), and multidrug resistant colon cancer. In contrast, normal cells from these tissues showed less inhibition of growth or no inhibition at all, suggesting that increasing ATP outside cells may have a selective inhibitory effect on several cancer cell lines.

Administering ATP may also enhance the effectiveness of cancer chemotherapeutic agents, increasing the antitumor effect of a given dose, or greatly reducing the dose required for a therapeutic effect. In particular, decreasing the dose of the treatment agents can dramatically reduce the toxicity of these antitumor drugs.

For example adding ATP to the drug doxorubicin to cultures of human ovarian cancer cells doubled the tumor cells eliminated compared to using doxorubicin alone. When ATP was given, 30-50% more doxorubicin accumulated in the cancer cells, whereas giving ATP to healthy human cells did not increase the accumulation of the drug.

In a randomized human clinical study, intravenous ATP was given to patients with advanced lung cancer at 2-4 week intervals. Whereas the control patients lost 2 pounds per month, the treated patients had stable to slightly increased weight. Over the six months of the study, the control patients lost one third of their muscular strength, while the ATP treated patients lost no strength. Although some medications may maintain weight in cancer patients, this is usually due to fat gain while muscle is lost. Intravenous ATP is the first intervention ever studied that appears to be able to maintain muscle mass, body weight, and muscle function in advanced cancer patients.
Thus ATP may be broadly beneficial in supporting antitumor cell biology. ATP enhances cellular immune function, inhibits the growth of several types of tumors, and in some cases may be able to cause direct elimination of tumor cells. In addition, ATP protects from radiation injury and may preserve weight and muscle strength. Further study will be needed to assess the full range of benefits it may provide. Given its high safety profile, ATP use may be one of the most beneficial adjuncts developed for supportive care, enhancing the results of conventional treatments.

Improved Human Survival of Shock

Under conditions of metabolic stress, such as depriving a tissue of oxygen through reduced blood supply, a rapid and massive depletion of ATP within cells occurs. Giving ATP or its metabolite adenosine has been described as a "natural defense system" to protect the tissues from the effects of severe oxygen deprivation. These protective effects include improved function of energy generating mitochondria, better electrolyte transport, increased ATP within cells, reduced oxygen consumption, and improved function of messenger molecules within the cells.

Shock is a condition in which there is a generalized reduction of blood flow and oxygenation to tissues below that required for their function. If shock is sustained, organ failure or death may occur. Once shock is reversed, supportive measures to assist tissue recovery can significantly affect quality of outcome.

In a study of 32 patients with acute kidney failure or multiple organ failure due to shock, highly beneficial effects of intravenous ATP were observed. The patients were randomly divided into the treatment group that received intravenous ATP or the control group that did not. The survival rate of 73% in the control group was increased to 100% survival in the ATP treatment group, showing the powerful tissue restorative effect of this intervention.

Sexual Function

In human tissue studies, the administration of ATP and adenosine has been found to induce the smooth muscle relaxation that is essential for erectile function. In diabetic men, erectile dysfunction is common through several mechanisms. The erectile tissue of diabetic men has been found to be especially sensitive to the smooth muscle relaxation effects of ATP, offering them a hopeful avenue of recovery of erectile function.

The one precaution is that the purine nucleic acids adenine and guanine are metabolized to uric acid in the body. Persons with elevated uric acid or a history of gout may have a very slightly increased risk of an episode of gout while taking nucleic acid supplements. Because of the very high potency and bioavailability of the nucleic acid elements in known nucleotide formulas, the specific quantities of purine bases are well below that usually associated with an increased risk of elevating uric acid.


Gur S, Ozturk B. Altered relaxant response to adenosine and adenosine 5~'-triphosphate in the corpus cavernosum from men and rats with diabetes. Pharmacology 2000; 60: 105-112.


Frankel P. The methylation miracle: unleash your body~s natural source of SAMe. St. Martin~s Mass Market Paper 1999


Czech patented food concentrate is showing significant abilities to increase immunity, in humans and animals, which was tested on experimental animals in vivo, ex vivo and in vitro on tissue cultures, and on selected clinical trials. Based on results from these testing, Imuregen® was approved by Chief Hygienist of Czech Republic as a supplement for food and beverages, and also for drinks, intended for special use. Especially for children and seniors in city agglomerations, with increased levels of pollution, people recovering after surgeries etc., workers in heavy industry, sportsmen and for prevention of seasonal repeated inflectional epidemic diseases of upper respiratory tract.

Testing of Imuregen® has shown, that is contains protective factors* increasing readiness of anti-infective defense of organism, and components** necessary for growth and regeneration of tissue. There have never been noted any signs of immunosuppression after using of Imuregen® in any experimental administration.

Verification tests, which were done in Sector of Immunology and Gnotobiology of Department of Microbiology of Academy of Sciences of Czech Republic have proven, that this product (Imuregen®) has significant immunoregulating properties with positive influence, especially on manifestation of natural and cell immunity. In cooperation with Medical Faculty of Charles University in Prague, research has proved positive effects of dietary and drinking program supplemented with Imuregen®, on regeneration of mucous membranes in intestines. Which in final consequences means improved sorbtion function of intestine and increased use of nutrients, and at the same time optimization of function of mucous membrane immunity. That is why it is possible to apply Imuregen® as a supporting preparation on it’s own, or as an ideal supplement for beverages, milk, dairy products and other foods in the framework of preventative programs, where the target is to increase immunity of general population, especially children, youth and people recovering from surgeries, illness, infections, fatigue syndrome or after injuries and complicated surgeries (posttraumatic syndrome). Imuregen® can be especially recommended as a preventative-protective preparation, which increases resistance to infections in population living in areas afflicted by nature disasters or other catastrophes, and for personnel under stress in extreme conditions as in rescue and salvage operations, decontamination work, army combat, etc.
* Cationic peptides with pronounced anti-microbial abilities, some vitamins.

** oligo- and polynucleotides, essential for cell multiplication, amino acids, peptides, and some trace elements

RNDr. Petr Šíma, PhD

In Prague, 10. 1. 2003
IMUREGEN® is a product with significant bioactive effects. It is non-toxic, without mutagenic activity and exhibits immunoregulatory properties. It has been approved as a Czech product by the Ministry of Health of the Czech Republic (HEM-350-29.10.1998-36469) and is patented and trademarked.

a) National Institute of Public Health, Prague
b) Institute of Microbiology of the Academy of Sciences of the Czech Republic, Prague, Jan Evangelista Purkyně Military Medical Academy, Hradec Králové, Regional Hygienic Station, Ústí nad Labem

Imuregen® is prepared by auto-enzymatic digestion of animal tissues using a precisely controlled technological process. It contains a mixture of free essential amino acids, low-molecular peptides and cationic proteins, nucleotides, and organically bound iron and zinc in optimal ratios.

1) Amino acids are the building blocks of proteins. Amino acids that must be supplied to the body in the form of food are referred to as essential.
2) Proteins are composed of several polypeptide chains, which consist of peptides (short chains of amino acids).
3) Cationic proteins are low molecular weight proteins characterised by antimicrobial and antibiotic properties. They are part of the natural immunity of the body.
4) Nucleotides are the building blocks of nucleic acids. Deoxyribonucleic acid (DNA) is contained in the core (nucleus), and in the cytoplasm of animal and plant cells, carrying the encoded characteristics of the organism in the form of genes, and ribonucleic acid (RNA), important for transcription and the ultimate manifestation of these properties.
5) Iron (Fe) is an important trace element necessary for proper growth, development and functional maturation of cells and tissues.
6) Zinc (Zn) is an essential trace element, ensuring the functionality of the immune system.

Bioactive properties of Imuregen®
The ingredients contained in Imuregen® are important to the growth and division of cells, and tissue construction. It plays a supporting role in liver disease, healing of wounds and fractures and restoration of the function of the digestive system after intestinal infection.

Immunoregulatory properties of Imuregen®
Imuregen® supports the overall anti-infective immunity of the body. It increases the readiness of the immune system by giving the body components, which are necessary for the development of protective and anti-inflammatory factors and antibodies, and which also inhibit the accompanying symptoms of allergic diseases.
Thanks to these immunoregulatory properties, Imuregen® is recommended as a preventive product before the onset of seasonal infectious diseases as well as a supportive product in the treatment of conditions where the body’s immunity is reduced for whatever reason.
Therefore, its regular use is suitable for children who do not yet have fully developed immune function as well as for the elderly with reduced efficiency of the immune system, and also during any states reduced efficiency of the immune system in adults (polluted environment, chemical plants, hard labour, challenging sports training, recovery from surgery, injuries and burns, starvation, or infectious and other diseases).

Use of Imuregen®
The balanced bioactive and immunoregulatory properties of Imuregen® make it an ideal supportive dietary supplement that may be used alone in the form of drops, and capsules and tablets, or as an ingredient in food products. It does not affect the taste of food or aroma of beverages.

Conclusion
Preventive application of IMUREGEN® did not exhibit generally toxic effects on the body. Immunological tests focusing on the core symptoms of innate immunity (distribution of NK cells), specific immunity T and B component (relative proportions of cell subpopulations CD4, CD8 and IgM and IgG) and the formation of humoral IgM and IgG showed no inhibitory effects.
The product does not exhibit properties that would lead to excessive exhaustive stimulation of observed immunological parameters and unproductive immunostimulation, resulting in the exhaustion of the immune capacity of the body.
Tests demonstrate that the product has desirable sparing immunostimulatory properties and optimally selected time and preventive dosage application will increase the readiness of both natural and specific cellular and humoral immunity

RNDr. Petr Šima, CSc.
IMUREGEN

JUVENIL

Regenerative and mass prevention preparation
Food concentrate

Characteristics and concept

Producer and distributor:

UNIREGEN, spol. s r.o.
Malé Poříčí, Na Brzdách 72
547 03 Náchod
tel./fax.:+420(441)201 24
www.imuregen.cz

JUVENOLOGY AND MASS PREVENTION

Ten years ago, the UNIREGEN spol. s r.o., Male Porici was the first in the former Czech Republic to start a program, which not only propagated the juvenological concept and mass prevention, but also began to implement it with a concrete production program.

What is the JUVENOLOGICAL CONCEPT? The juvenological concept is striving to preserve the condition characteristic of a healthy and young organism as long as possible and
consequently to prepare conditions for active psychic and physical longevity. In other words, to prolong the time of active life and to prepare conditions for a contented life in old age relatively free of stress.

What is MASS PREVENTION? The civilization diseases and the deteriorated environment have brought about a situation in which we must give equal care to the healthy as to the sick. The outbreak of a disease always signifies an upsetting of the balance of the organism. It is therefore our duty to delay the moment of its outbreak as much as possible or to prepare conditions in the organism that will ensure a short and unproblematic course of the disease. Therein lies the sense of mass prevention: to see to it that the energy balance in the organism may support its natural self-regeneration and self-recuperation abilities as long as possible.

There are different approaches to the juvenological concept and mass prevention. For example we may go to live in an environment relatively unaffected by excessive industrialization, and eat uncontaminated food. This would be ideal. But we know that only few people can do this today. So there is not much left for us but to begin supporting our organism daily, while staying where this is required by our professional duties. It was no coincidence that so much importance was attached to vitamins, trace elements and minerals in our organism. While helping us, these substances, however, could not solve all problems. For decades researchers have concerned themselves with the importance of amino acids and especially essential amino acids, both in loose form and in
the form of peptides and oligopeptides. They knew that these were yet other invaluable building elements for the organism. It is true that the organism produces them itself, but in a worsened environment it is unable to produce a sufficient quantity of these elements and, in addition, if the organism gets into trouble, it produces even less of them. So there is nothing left for us but to add them to the organism to make it prepared for the moment when the disease strikes, so that it can strike back!

It was good luck for us in Bohemia that as far back as in the late 1930's, and specially in the 1940's, the well-known Czech pharmacist Dr. Rakús developed a concept according to which all problems of the organism must be tackled in a natural way, with the help of vegetable and animal tissues. If we can get the most effective substances, i.e. amino acids, peptides, oligopeptides and suitable organic salts, from them by a suitable technology, we shall have won. This concept of Dr. Rakús was brought to its climax in the early 1950's. Unfortunately, this was a time when another concept prevailed — namely the idea that chemotherapy can solve everything. Its advantage was that it was using sufficiently well defined chemical substances. What it did not take into consideration, however, was that man, after all, is part of the nature, and that much more beneficial for him are natural complexes provided by nature, in suitable combinations of amino acids, essential amino acids, peptides and oligopeptides. We already know quite a few things about the mechanisms of their effects, but must learn still more about them. These essential building elements
have a great advantage. If a suitable technology is used, they are absolutely non-toxic, nonaddictive and they do not settle in the organism. In addition, they effectively support the immune system.

The UNIREGEN comp.ltd. at Malé Poříčí began to manufacture a tissue preparation, first in pilot plant conditions, and later in normal production conditions, based on amino acids, essential amino acids, peptides, oligopeptides, organic salts, bivalent iron and haemoglobin. Its working name JUVENIL and its trade name IMUREGEN. The first preparations produced at UNIREGEN were subject to different kinds of testing. We would like to inform you about the results of the testing of JUVENIL – IMUREGEN, the product we are manufacturing at Malé Porici.

**NAME OF PREPARATION : IMUREGEN**

**DECLARATION :** FOOD CONCENTRATE for separate use or as a supplement in combination with other natural stimulants, such as vitamins, trace elements, minerals, roughage and as a component of suitable victuals.

**BRIEF CHARACTERISTICS:** IMUREGEN is made from natural sources by a modern biotechnology, based on autoenzymatic disintegration of the macromolecular protein component. The product of this biotechnology is subject to long-term non-destructive careful extraction with a follow/up natural
separation of a set of effective low-molecular substances 
having a catalytic effect on specific internal secretion glands 
while influencing favourably the intermedial metabolism of 
cells. The effective complex of IMUREGEN is a quantitatively 
characteristic set of amino acids and essential amino acids 
which are partly loose and partly in the form of low-molecular 
peptides and oligopeptides, tied to the disintegrative products 
of haem, i.e. chained porphyrine nuclei with loosened Fe++ and 
naturally formed compounds of elements essential for the 
organism [ e.g. Se, Mn, Zn, Mg, K ].

COMPOSITION:
1. amino acids
a/ essential amino acids loose
   leucine
   valine
   histidine
   lysine
   fenylalanine
   threonine
   methionine
   isoleucine
   arginine
other loose amino acids
    alanine
    serine
    glycocol
    proline
    thyrosine
    glucamin acid
    apar.acid

b/ in the form of oligopeptides and peptides

2. lipides
3. proteins
4. F++ in natural form
5. mineral salts
6. nucleoproteins

PROPERTIS

IMUREGEN - a food concentrate - is a substance intended for mass prevention of civilization diseases, including carcinogenous, cardiovascular, diabetic and asthmatic diseases. Its effects may be specified according to its structure. The complexes of essential amino acids are showing favourable effects on internal secretion glands. For example, they incite the function of the pituitary gland, but only up to the physiological norm, and are functioning as regulators of the energy system by means of the neurohormonal apparatus of the organism. The oligopeptic complexes incite the function of the
thymus and stimulate the function of the spleen, which increases the immune response of the organism relatively quickly. The peptidic complexes provoke an important increase in lysoxine content in the blood, so that the effects of IMUREGEN may be compared to the function of unspecified antibiotics, without, however, their negative impact on the organism. The organically tied bivalent iron penetrates the intestinal wall without oxidation, thus increasing the blood pool between the spleen and the bone marrow. The above-mentioned components contained in IMUREGEN have a polyvalent effect and influence the organism in a non-specific way: they strengthen the organism nonspecifically in directions, in which its own regulation system does not ensure a correct energy balance. Its effects include improved memory; improved mobility and psychic balance, reduced fatigue and better physical and mental working ability. Under its influence the course and the frequency of allergies and common ailments, such as influenza, tonsillitis and colds, are improved. Wounds and common injuries heal more quickly. It mitigates pain, including pain in the terminal states of cancer, so that the administration of ordinary analgesics is sufficient, without the need to use strong painkillers [narcotics]. IMUREGEN has oxidoreduction properties, mitigates or eliminates excited conditions, and slows down conditions with strongly excited energy processes. The long-term testing of IMUREGEN has proved that it is absolutely non-toxic, non-addictive and that it does not settle in the organism.
INDICATION

Due to the polyvalent effects of essential amino acids and low-molecular peptides and oligopeptides, IMUREGEN is suitable for use in prevention and as a supportive means for carciogenous, cardiovascular and diabetic diseases and for the reduction of the incidence of influenza, tonsillitis and colds and for mitigating their course, as well as for prevention, to protect and maintain a good energy balance of the organism in all age groups.

For middle-aged and older people it is suitable as a means to improve their physical condition and to ease their mental fatigue and exhaustion, and in cachexic conditions. It can be used with advantage in the terminal states of cancer. For younger people it is suitable for prevention and as a supportive means in allergies.

COUNTERINDICATION

Has not been proved

DOSAGE

For direct dosage the preparation is mostly used in liquid form. The dry matter is usually dissolved in distilled water according to dosage requirements. If the application is
intended for mass prevention, in conditions of increased fatigue and exhaustion, a double to triple dose is recommended. There is no danger of overdose. Application is best on a lump of sugar or a piece of bread, to be absorbed slowly in the mouth. Long-term application is recommended.

APPLICATION of IMUREGEN: IMUREGEN - drops or capsules.
The Role of Nucleotides in Adult Nutrition\textsuperscript{1,2}

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\textbf{ABSTRACT} Dietary nucleotides, found in normal diets, have been recently determined to be required for normal immune defenses. Rejection of cardiac transplants, graft-vs.-host disease, and delayed cutaneous hypersensitivity in animal models are all suppressed by a diet deficient in nucleotides. T lymphocytes seem to require dietary nucleotides for normal maturation and function. Host resistance to bacterial and fungal infections is decreased in mice on nucleotide free diets; addition of RNA or uracil prevents this vulnerability to infection. Dietary RNA is required to restore lost immune function after protein deprivation. Adequate calories and protein alone do not return immune function to normal. Dietary nucleotides can restore lost immune function even during protein starvation and weight loss. Because all parenteral and most enteral nutrient solutions are nucleotide free, clinical studies were undertaken comparing a new nucleotide containing diet (Impact) to a standard high protein enteral feeding. In two separate double blind clinical studies the patients fed the enteral diet containing nucleotides had improved immune function compared with patients receiving a nucleotide free diet. In addition, infectious complications and length of hospital stay were reduced in postoperative cancer patients fed Impact compared with a control group. J. Nutr. 124: 160S-164S, 1994.

\textbf{INDEXING KEY WORDS:}
- dietary
- nucleotides
- lymphocyte
- immunity

Conditional requirements for dietary substrates have become important considerations in human nutrition. The best defined of these substrates are amino acids, such as arginine, which are required for growth in infants and for maintaining host immune responses in animals. Another amino acid, histidine, is required by persons with chronic renal failure. Nucleotides are ubiquitous in cells, in either monomeric or polymeric form, and are vital for the function of the organism. The metabolism and importance of these substrates have been reviewed in this supplement [Bustamante et al. 1994, Carver 1994, Jyonouchi 1994, Rudolph 1994, Sanderson and He 1994, Uauy 1994] and elsewhere [Rudolph et al. 1990]. Recent literature in experimental transplantation has demonstrated that exogenous nucleotides are important for maintaining host immunity to allogeneic tissues; restoring specific immune responses to foreign antigens requires providing exogenous nucleotides. Thus, exogenous nucleotides appear to be required for maintaining specific host immunity.

\textbf{INFLUENCE OF NUCLEOTIDES ON LYMPHOCYTE FUNCTION AND CELLULAR IMMUNITY}

These observations, while significant, have been relevant primarily to clinicians in suppressing the immune responses for successful organ transplantation. To test the hypothesis that dietary nucleotides might influence nonspecific host responses to infective organisms, the contralateral footpads of BALB/c mice fed a protein-free (PF) diet were injected with BALB/c spleen cells (Kulkarni et al. 1989). Syngeneic inoculation was chosen for controlling for nonspecific immune responses...
immune stimulation. After inoculation, the mice were either continued on the PF diet or switched to a nucleotide-free INFI, nucleotide-free plus 0.25% RNA (NFR), nucleotide-free plus 0.06% uracil (NFU), nucleotide-free plus 0.06% adenine INFAJ or commercial diet (containing -0.25% RNA by weight based on analysis in our laboratories). The mice were weighed daily, and were killed 7 d after inoculation. The popliteal lymph nodes from both hind limbs were harvested, and a stimulation index of immune responsiveness was calculated by dividing the weight of the allogeneically stimulated nodes by the weight of the contralateral popliteal nodes.

All mice that were switched from a PF diet regained the weight that they had lost. No difference was noted in the weight restoration between these groups. In contrast, the mice that were maintained on the PF diet continued to lose weight, and their weight loss was 30% when they were killed.

The restoration of immunity depended on the presence of either RNA or uracil in the diet. In the mice maintained on the NF diet, even though they regained the lost weight, the immune response was equivalent to that in the mice continued on the PF diet!Table 1). Thus, providing calories and protein alone is insufficient to reverse the immunosuppression induced by protein starvation. Dietary nucleotides or dietary pyrimidines were necessary to restore the lost immune function [Pizzini et al. 1990]. These findings, if extended to humans, may help explain the lack of association in previous clinical studies of an improved nitrogen balance after nutritional support with any change in the infection or mortality rates.

### INFLUENCE OF NUCLEOTIDES ON HOST RESPONSE TO INFECTION

The studies discussed above focused on the influence of dietary nucleotides on lymphocyte function and cellular immunity. To test the hypothesis that these findings altered the host response to infective organisms, we intravenously inoculated BALB/c mice that were fed an NF, NFR, NFU, NFA or commercial diet with either fungal (Candida albicans) or bacterial (Staphylococcus aureus) pathogens. The amount of RNA, pyrimidine [uracil] or purine [adenine] was calculated on the basis of the amounts of the nucleotides or nucleobases present in a normal commercial diet. All mice were maintained on the assigned diets for 3 wk before inoculation. In the mice inoculated with C. albicans, survival was significantly greater (\( P < 0.02 \)) in those fed the NFR and NFU diets than in those fed the NF and NFA diets [Fanslow et al. 1988]. Decreased resistance to fungal infection was reflected in the ability to culture more viable organisms from the spleens of the animals fed the NF and NFA diets, a finding that, because of the importance of cellular immunity in resistance to fungal infections, might be expected.

In a study of the influence of the diet on resistance to bacterial infection, a dose of S. aureus that was lethal in 50% of mice fed a commercial diet resulted in 100% mortality when it was given to mice fed an NF diet. Supplementation with RNA or uracil resulted in increased survival after inoculation with the bacteria, but supplementation with adenine had no beneficial effect [Kulkarni et al. 1986]. Because cellular immunity is not known to have an important role in host defenses against gram-positive bacterial infections, the macrophage responses were examined. The phagocytic ability of the macrophages could not account for the differences in survival. Macrophages isolated from the NFR group had a greater ability to engulf radiolabeled bacteria and no difference was observed in the phagocytic ability of macrophages isolated from the NF and commercial groups, yet the mortality was higher in NF diet-fed mice. Instead, the bactericidal ability of splenic macrophages after phagocytosis appeared to correlate with in vivo resistance to S. aureus. The ability to kill engulfed bacteria was greater in the mice fed the NFR, NFU and commercial diets than in those fed the NF and NFA diets. Further studies showed that the production of superoxides in macrophages from animals fed the NF diet was less than that in animals fed the NFR and chow diets [Kulkarni et al. 19941. Thus, the requirement for dietary nucleotides appeared to be important for nonspecific as well as specific host defenses.

These studies are significant for two reasons. First, bacteria and fungi are important pathogens in critically ill hospitalized patients and are major contributors to morbidity and mortality. Second, all total parenteral nutrition solutions and most enteral feeding solutions are nucleotide-free. These studies suggest that depriving these critically ill patients of dietary nucleotides might adversely affect their ability to fight infection.

### INFLUENCE OF NUCLEOTIDES ON LYMPHOCYTE RESPONSIVENESS

The finding that macrophage as well as lymphocyte function appears to depend on exogenous nucleotides has suggested a more basic role for these substrates. In a mouse model of bone marrow transplantation to study acute graft-vs-host disease, the ability to cause graft-vs.-host disease in H2-incompatible irradiated recipients was less in donors maintained on an NF diet than in donors fed commercial diet [Kulkarni et al. 1984]. The influence is not absolute. The ability to cause graft-vs.-host disease was suppressed in radiation chimera donors fed the NF diet for 6-8 wk after
bone marrow infusion; normal alloreactive ability was regained in radiation chimera donors that were fed commercial diet for 15 wk. These findings are most consistent with a maturation arrest of T lymphocytes or their precursors. Supporting this hypothesis is the finding that the responsiveness to interleukin-S, an important cytokine that directs lymphocyte maturation, is decreased in bone marrow harvested from mice fed an NF diet and is normal in that harvested from mice fed an NFR diet (Kulkarni et al. 1992). The ability to generate splenic colonies of bone marrow cells that are transplanted into hosts fed an NF diet is less than that of bone marrow cells from donors fed a commercial diet or an NFR diet. Hosts fed an NFR diet support normal bone marrow engraftment, but engraftment is suppressed in hosts that are fed an NFA diet. Thus, the influence of dietary nucleotides on the immune system may be much broader, and they may affect a more primitive population of immunopotent cells than was initially believed.

Nonspecific host defense mechanisms appear to require dietary nucleotides for optimal function. The bactericidal activity of macrophages is suppressed in animals fed an NF diet, resulting in increased mortality from bacterial challenge. Supplementation with dietary RNA prevents the compromise of host defenses. In these studies and in evaluations of lymphoproliferative responses, pyrimidines mimic the action of RNA and purines are usually ineffective in maintaining immune responsiveness.

### CLINICAL EFFECTS OF NUCLEOTIDES

For clinical studies, dietary nucleotides have been incorporated into casein-based enteral feeding formula with two other immunopotent substrates, fish oil and arginine. Fish oil, which is rich in (n-3) fatty acids, has been shown to increase lymphoproliferative responses to lectins (Kinsella et al. 1990). The suggested mechanism for this effect is the increased production of prostaglandin E3 rather than E2, the latter of which is synthesized from arachidonic acid. Prostaglandin E2 appears to suppress lymphoproliferative responses (Goodwin and Ceuppens 1985). Arginine is a dibasic amino acid that promotes thymic development, increasing lymphocyte numbers and response. The mechanism by which it exerts these effects remains to be elucidated.

In an animal model that examined the effects of single substrates on the responses of popliteal lymph nodes to injected allogeneic spleen cells, fish oil and dietary RNA appeared equivalent in maintaining the lymphoproliferative responses and arginine appeared less effective (Kulkarni et al. 1989). Furthermore, when RNA and fish oil were combined with arginine, an additive effect on the immune function was seen. These effects were also seen in the previously described model of challenge with *S. aureus* (Fig. 1). This research laid the foundation for the development of an enteral formula that combines nucleotides, fish oil and arginine [Impact, Sandoz Pharmaceuticals, East Hanover, NJ].

In two clinical studies, Impact was compared with an isocaloric casein-based high-nitrogen enteral formula (Osmolite, Ross Laboratories, Columbus, OHJ. In a double-blind study conducted at the University of Minnesota with septic or critically ill patients in the intensive care unit, 11 patients were randomly assigned to Impact and 9 to Osmolite (Cerra et al. 1990). Both patient groups were similar with respect to demographics, prestudy nutritional assessment and severity of illness. Nitrogen delivery was controlled for, and both groups received equivalent amounts of nitrogen. Because Impact contains nearly 12 g/L more amino acids and/or protein than Osmolite, the caloric intake in the Impact group

### TABLE 1

Effect of various diets on in vivo popliteal lymph node (PLN) response

<table>
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<tbody>
<tr>
<td>PF-PF</td>
<td>31 ± 0.6</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.6</td>
<td>29 ± 0.7</td>
</tr>
<tr>
<td>PF-NF</td>
<td>29 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>22 ± 0.2</td>
</tr>
<tr>
<td>PF-NFR (0.025%)</td>
<td>74 ± 0.6</td>
<td>1.7 ± 0.2</td>
<td>57 ± 0.5</td>
<td>43 ± 0.3</td>
</tr>
<tr>
<td>PF-NFR (2.5%)</td>
<td>70 ± 0.8</td>
<td>1.4 ± 0.2</td>
<td>5.6 ± 0.9</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td>PF-NFU (0.6%)</td>
<td>98 ± 1.0</td>
<td>1.7 ± 0.3</td>
<td>7.9 ± 1.0</td>
<td>58 ± 0.6</td>
</tr>
<tr>
<td>F-F</td>
<td>86 ± 1.2</td>
<td>1.5 ± 0.3</td>
<td>71 ± 1.2</td>
<td>67 ± 1.3</td>
</tr>
</tbody>
</table>

1Values are means ± SEM. *PF, NF vs. NFR (0.025%), NFR (2.5%), NFU (0.6%), F P < 0.05.  
2Diet abbreviations used: F, commercial rodent diet; NF, nucleotide-free diet; NFR, nucleotide-free diet + RNA; NFU, nucleotide-free diet + uracil; PF, protein-free diet.
was less. The patients were examined before enteral feeding and throughout the course of the study for lymphoproliferative responses to phytohemagglutinin, concanavalin A and tetanus toxoid protein. Both groups of patients remained on study for an average of 9 d. The average total duration of hospitalization was 37 d in the Impact group and 55 d in the Osmolite group. Because of the small number of patients and the wide standard error, this difference was not statistically significant. However, the trend is interesting in light of subsequent clinical studies.

The difference between the groups in the immune response to supplementation was statistically significant. There was no change in the immune response throughout the study in the Osmolite group, with the lymphoproliferative responses at the study’s end being practically identical to those before enteral feeding. In contrast, there was statistically significant and progressive improvement in the lymphoproliferative responses to phytohemagglutinin, concanavalin A and tetanus toxoid protein in the Impact group, with the greatest responses being observed at the study’s conclusion. Tube feeding with Osmolite failed to improve the initial immune suppression noted in the critically ill patients, while the Impact did improve it significantly. This improvement occurred despite there being no difference in nitrogen balance between the two groups.

In the second study, Daly et al. [1992] studied Osmolite and Impact in 85 postoperative gastrointestine cancer patients. The caloric intake was controlled for in this study, which resulted in the Impact group receiving significantly more nitrogen than the Osmolite group, and, as expected, the nitrogen balance was better in the Impact group. However, no matter how large the positive nitrogen balance in the Osmolite group, no improvement in the immune response and no association between the nitrogen balance and the immune function were noted in this group. In contrast, a statistically significant improvement in the immune function was demonstrated in the Impact group. The clinical outcome associated with this improved immune response was a 70% reduction in infectious and wound complications in the Impact group (11% incidence) from that in the Osmolite group (37% incidence). This reduced complication rate resulted in a 22% reduction in the length of hospitalization in the Impact group.

The results of these two comparative clinical studies suggest that specially formulated enteral solutions that contain nucleotides favorably influence the outcome in hospitalized patients. Enteral feeding solutions that have not been evaluated for their effects on the immune response may not be optimum for critically ill patients.

**Summary**

Exogenous nucleotides appear to support normal nonspecific and specific immune defenses. Investigations in animal models and recent clinical literature suggest that administering dietary nucleotides will help to minimize infectious complications, improve clinical outcomes and provide the most cost-effective nutritional support available.

**Literature Cited**


Effects of Dietary Nucleotides on Immune Mechanisms and Physical State in Children with Chronic Respiratory Problems

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Abstract: Heavy industrial pollution and/or passive smoking negatively affect the overall immune health of a population. We used a model of children with chronic respiratory problems and evaluated the effects of short term supplementation of their diet with nucleotides. We measured the level of sIgA, eNO levels and total physical activity. We found positive effects of oral supplementation with commercially available nucleotides. Our data demonstrate strong improvements in physical activity, strong decrease of eNO levels and maintaining of the levels of sIgA. We conclude that supplementation with nucleotides offers an easy way to improve health conditions in children.

Keywords: Nucleotides, sIgA, Immunity, Children, Physical Activity, eNO

Introduction

A long-term focus of our laboratory is the research of how the environmental conditions affect the health of population. We are particularly interested in the possible regeneration of physical status of children exposed to environmental pollution of both internal and external environment (Lee et al., 2013; Richter et al., 2014; Vaclav et al., 2013a; 2013b; 2013c). We are focused most of all on children with chronic respiratory problems caused by living in industrial regions of northern Bohemia and northern Moravia where the extremely high concentration of environmental contamination helped to gain the term Black Spot of Europe. The fact that this region is populated mostly by people with lower education, often disregarding the basic rules of healthy living, with a prevalence of smoking reaching 60-90%, makes the health situation even worse. The effects of passive smoking are further increased by the small area of apartments, often with less than 10 m² of space per person. In addition, this population traditionally consumes less fruit and vegetables than the rest of the Czech population. A high frequency of automobile traffic, with the average age of a car reaching almost 15 years, is responsible for extreme pollution from exhaust fumes. The prevalence of respiratory problems and allergies in this region is more than two times higher than in the rest of the Czech Republic.

These facts led us to this study to improve the health of the population at the biggest risk-the children. The first step is to guarantee a short-term stay in an environment with very low pollution (app. 20% of the original level). It was found that the climatotherapy treatment together with speleotherapy, higher physical activity and improved nutrition resulted in significant medical improvements (Vetvicka et al., 2013a; 2013b; 2013c; Ostojic et al., 2013). As confirmed by other authors (Schuh, 1993), we showed that this improved regime leads to significant improvements of immune functions, lowering of the risk of infections and improvements in the state of current diseases such as respiratory problems or asthma bronchiale (Buka et al., 2006; Davis et al., 2004). In addition, it is clear that a high prevalence of diseases and related deaths resulting in extreme pollution of the environment have high economic effects (Lee et al., 2013; Buka et al., 2006; Linares et al., 2010).

We decided to test the effects of improving the nutritional conditions by supplementing the food with samples with high level of nucleotides. Nucleotides in the form of Nucleic Acids (NA) affect several
biochemical processes necessary for adequate functions of the living organism. Nucleic acids are monomeric units with both RNA and DNA genetic code (Devresse, 1999; Lerner and Shamir, 2000). They are involved in biosynthesis of glycogen, such as UDPP galactose is involved in synthesis of lactose of UDP glucose. In addition, nucleotides are parts of some coenzymes NAD, FAD and A. In addition, they serve as a biological regulator-cAMP decreases cascade of second messenger, which has a key role in regulation of biological processes and simultaneously represents an extremely important source of energy (Lerner and Shamir, 2000).

An animal model indicated strong effects of 4 weeks supplementation with dietary Nucleotides (dNT) used in drinks on development and quality of terminal ileum (Slizova et al., 2004). In addition, positive effects of dNT on growth and quality of intestinal flora and iron absorption were also demonstrated (Lerner and Shamir, 2000). Human milk contains a higher level of dNT and higher biological availability of iron than bovine milk (Leach et al., 1995; ATSCPSCPFL, 2002; Thorell et al., 1996). In breast-fed infants dNT positively influenced NK cell activity and levels of IgM, IgA and slgA. The addition of dNT increased the effect of vaccination (Lerner and Shamir, 2000; Leach et al., 1995). dNT also affected liver function and liver regeneration and stimulated production of some components of the complement cascade. On the other hand, dNT deficit (in dNT-free diets) resulted in suppression of some immune reaction, mostly in decrease of IL-2 and IL-3 production and in decrease in numbers of pluripotent stem cells. It is not surprising, therefore, that this complex role of dNT is currently the focus of extensive interest of both theoretical scientists and clinicians. More and more groups study individual aspects of postnatal development and development of malnourished children living in areas with a contaminated environment. In addition, more and more attention is focused on how to improve immune functions before the start of the expected epidemic of infectious diseases. Supplementation with dNT might offer some help in regeneration processes, normalization of metabolic, physiologic and immune functions in a wide range of patients, from people with high stress to chronically ill patients or the elderly.

In this study, we report our results in supplementation of food with high content of dNT in children from a heavily contaminated environment.

Materials and Methods

Protocol

The same protocol that was previously described (Vaclav et al., 2013b) was used throughout this study. Briefly, a randomized trial compared one dose of Imuregen (Uniregen, Nachod, Czech Republic), 2 mg/day-4 weeks and a placebo in children. A total of 138 children from the sanatorium were enrolled in the 4-week trial testing. The trial was conducted at the Sanatorium EDEL (Zlate Hory, Czech Republic) and the study was approved by the Ethics committees of the Public Health Institute based in Usti nad Labem and Sanatorium EDEL, Zlate Hory, Czech Republic. As a part of the clinical evaluation at the starting point of the study, all children were tested for their height, weight and BMI (Body Mass Index) and its percentile was calculated in relation to age and sex (Table 1). Ninety three children (43 females and 50 males) formed the group supplemented with Imuregen, 45 children (24 females and 21 males) formed the control (placebo) group. This study was performed in agreement with Helsinki declaration (revised version 2000.09.01) and in full compliance with the rules for clinical testing in the Czech Republic. Parental consent was given in all cases. Subjects were routinely evaluated by the medical staff. Imuregen is a food supplement approved by the Chief hygienist of the Czech Ministry of Health.

Tests

In all subjects we obtained saliva at the beginning of the study and at the conclusion of their stay in Sanatorium. We used identical times (between 8 and 9 am) for sampling, so the possible influence of circadian rhythms could be eliminated.

Saliva was collected using a commercial Salivette device (Sarstead, Orsay, France). After two minutes of chewing, the cotton swab was added into a sterile container and centrifuged at 1,000 g for 15 min and stored at -15°C. We measured the levels of slgA using nephelometer Siemens BMII as suggested by the manufacturer.

Table 1. Body mass index and its percentile calculated in relation to age and sex

<table>
<thead>
<tr>
<th></th>
<th>Age- mean (SD)</th>
<th>BMI-mean (SD)</th>
<th>Percentiles mean (SD)</th>
<th>BMI &lt;90% percentiles</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>MEAN</td>
<td>BMI</td>
<td>Percentiles</td>
</tr>
<tr>
<td>Nucleotides</td>
<td></td>
<td>MEAN</td>
<td>BMI</td>
<td>Percentiles</td>
</tr>
<tr>
<td>Males</td>
<td>50</td>
<td>10,7 (3,01)</td>
<td>19,31 (3,89)</td>
<td>61,46 (31,33)</td>
</tr>
<tr>
<td>Females</td>
<td>43</td>
<td>9,55 (2,98)</td>
<td>18,03 (3,21)</td>
<td>58,86 (27,91)</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td>MEAN</td>
<td>BMI</td>
<td>Percentiles</td>
</tr>
<tr>
<td>Males</td>
<td>21</td>
<td>11,00(2,18)</td>
<td>18,77 (2,99)</td>
<td>58,52 (28,09)</td>
</tr>
<tr>
<td>Females</td>
<td>24</td>
<td>10,62 (2,58)</td>
<td>18,89 (4,79)</td>
<td>53,33 (35,62)</td>
</tr>
</tbody>
</table>
All participants absolved at the beginning and at the end of the 6 Min Walk Test (6MWT) based on suggested development, including evaluation of additional parameters such as frequency before and after physical stress, oxygen saturation, interruption of stress due to tiredness. These tests were done 2 h after the meal. We used recommendations of the American Thoracic Society (ATSCPSCPFL, 2002).

In addition, both weight and height were measured and BMI calculated. BMI levels were subsequently transferred into age percentiles (Body Mass Index for Age Percentiles) according to the CDC (2000). We found that CDC percentiles are identical to levels found in the population of Czech children of the same age population as determined by The National Institute of Public Health, Prague, Czech Republic. All tested children had minimal physical activity for 2 h prior to the test with guaranteed no special energetic income. The track used for all testing was long and wide enough to ensure no risk of physical constraints during the test.

At the beginning and the end of the study we also evaluated eNO levels using the American Thoracic Society recommendations (ATSCPSCPFL, 2002) as described in our previous studies (Vetvicka et al., 2013c).

During the whole stay at the Sanatorium EDEL, all children were subjected to the same dietary regime corresponding to their age category.

**Statistical Analysis**

Statistical significance was evaluated by a pair t-test and unpaired t-test using a GraphPad Prism 502 software (GraphPad Software, USA).

**Results**

Figure 1 summarizes our measurements of the 6MWT in children at the beginning and at the end of their stay in Sanatorium EDEL. We divided all children not only into supplemented and control (placebo) groups, but also based on sex. In the supplemented group, we found a significant increase in 6MWT levels in both the female and male groups—from 448 to 470 in males, from 403 to 427 in females. The results in control groups were minor -12 m increase in male group, 5 m increase in female group.

Figure 2 shows the levels of eNO at the beginning and the end of the experiment. The decrease of the level at the end of the stay is significant, particularly in the male group, where we found a decrease from 25.3 to 14.48 ppb. In females, the decrease from 15.04 to 11.39 ppb was less pronounced, but still significant. A decrease in the eNO levels was also observed in the control group-non-significant in the male group (from 19.08 to 11.57 ppb) and significant in females (from 14.88 to 11.79 ppb).

Our findings of sIgA levels were not significantly changed by diet supplementation with dNT (Fig. 3). In male group, the levels were not changed (ap. 185 mg L\(^{-1}\)). In females, we found a non-significant decrease from 208.9±34.6 to 153.8±36.6 mg L\(^{-1}\). However, in the control group we found a significant decrease in the sIgA levels in saliva of both groups. The decrease in the male group was from 309.7±33.4 (mg L\(^{-1}\)) to 169.0±32.9 and in female group from 217.0±20.9 to 102.1±18.7. Mean values and SD for all our data are summarized in Table 2.

![Fig. 1. Effect of supplementation with nucleotides on six minute walk test. A - day 1, B - day 30](image-url)
Fig. 2. Levels of eNO in children supplemented with nucleotides or placebo. A-day 1, B-day 30

Fig. 3. Effect of supplementation with nucleotides on sIgA levels in saliva. A-day 1, B-day 30
Table 2. Mean values and SD for all presented data

<table>
<thead>
<tr>
<th></th>
<th>Nucleotides mean (SD) A</th>
<th>Placebo mean (SD) B</th>
<th>mean (SD) A</th>
<th>mean (SD) B</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 MWT</td>
<td>448 (85)</td>
<td>469 (85)</td>
<td>465 (65)</td>
<td>478 (79)</td>
</tr>
<tr>
<td></td>
<td>403 (83)</td>
<td>427 (83)</td>
<td>461 (95)</td>
<td>466 (83)</td>
</tr>
<tr>
<td>SIgA</td>
<td>185,4 (22,7)</td>
<td>182,0 (32,2)</td>
<td>309,7 (33,4)</td>
<td>169,0 (32,9)</td>
</tr>
<tr>
<td></td>
<td>208,9 (34,6)</td>
<td>153,8 (36,6)</td>
<td>217,3 (20,9)</td>
<td>102,1 (18,7)</td>
</tr>
<tr>
<td>eNO</td>
<td>25,3 (27,2)</td>
<td>14,5 (10,6)</td>
<td>19,0 (21,1)</td>
<td>11,6 (9,7)</td>
</tr>
<tr>
<td></td>
<td>15,4 (17,8)</td>
<td>11,4 (8,5)</td>
<td>14,9 (13,4)</td>
<td>11,8 (10,8)</td>
</tr>
</tbody>
</table>

Discussion

Nutritional intervention in the form of food supplementation, with nucleotides, micronutrients, bovine colostrum or glucans, in groups with increased physical challenge have become the main research approach of numerous laboratories (Ostojic et al., 2013; Davis et al., 2004; Gunzer et al., 2012; Ostojic and Obrenovic, 2012; Riera et al., 2013; Stier et al., 2014; Wolvers et al., 2006). It is the results of our quest for an increase of immune reactions not only in athletes, but also in clinical medicine (Ciaccio et al., 2014) and in possible renovation of damaged mucosal immunity in children exposed to effects of environmental contaminants (Richter et al., 2014; Vetvicka et al., 2013a; 2013b; 2013c). Based on these trials, several commercial supplements appeared on the market, including Oral Impal from Nestle (Ciaccio et al., 2014; Sublingual nucleotides, extract from germinated barley (Ostojic and Obrenovic, 2012) or Immunoactive from Bioiberica Spain (Wolvers et al., 2006).

Our previous studies showed significant effects of dNT on repairs of mucosa in distal ileus (Slizova et al., 2004). Our data demonstrated that even short term supplementation with dNT affected physical stress related changes by holding the levels of salivary IgA steady, which was strongly suppressed in the control group of children. As high doses of dNT were shown in murine models to increase the allergic reactions (Lerner and Shamir, 2000), it is important to carefully find the optimal dose of dNT. In our current study, we found no dose-related problems, neither clinically or in the laboratory. The effects of dNT supplementation suggests their importance in keeping the optimal quality of physiological functions and supporting some mechanisms of both specific and nonspecific immune reactions. Physical conditions in children supplemented with dNT were significantly improved in the female group. The results of the 6MWT in the male group were at the limit of statistical importance, most probably due to the higher number of obese individuals (over 40%). A male group showed in relation between BMI percentile and 6MWT statistically significant linear regression at p<0.0001 level. We can presume that improvement in 6MWT levels might be in relation to strong improvements in eNO values, which were found in all groups. It is clear that the abandoning of the highly polluted conditions (both external and internal such as passive smoking) plays an important role in these findings. Our results confirmed the data achieved by other groups that the 6MWT is a simple, objective and reproducible comparison of the functional capacity both in clinical and rehabilitating programs (ATSCPSCPFL, 2002; Grinnel, 2010; Ulrich et al., 2013).

The salivary samples for evaluations of the level of mucosal immunity were used primary for the easy and non-invasive way to obtain samples. Saliva offers a possibility to test the whole range of important information about the state of mucosal immunity, which can be further used for determination of the general quality of immune reactions. Currently, medical practice offers a wide spectrum of these indicators (Gillum et al., 2013; Koh and Koh, 2007; Krasteva and Kiselova, 2011; Malamud and Rodriguez-Chavez, 2011). We used sIgA because it offers a good indication for testing of the effects of physical stress on mucosal immunity (Gillum et al., 2013; Yi and Moochhala, 2013).

Conclusion

From our results, we can conclude that supplementation with nucleotides caused significant improvements in all tested reactions.

Acknowledgement

This study was supported by the Technology Agency of the Czech Republic TACR TA 0202094.

Author’s Contributions

Josef Richter: Participated in all experiments, coordinated the data-analysis and contributed to the writing of teh manuscript.

Vladimir Svozil: Participated in expriments, evaluated all patients.

Vlastimil Kral: Designed the research plan and orgnized the study.

Lucie Rajnohova Dobiasova: Coordinated the patients groups, performed the data analysis.
Ivana Stiborova: Coordinated the patients groups, performed the data analysis.
Jitka Pohorska: Coordinated the patients groups, performed the data analysis.
Vaclav Vetvicka: Participated in all experiments, wrote and translated the manuscript.

Ethics

The study was approved by the Ethic committees of the Public Health Institutes of both Sanatorium Edel and ZU Usti nad Labem.

References

CDC, 2000. Body mass index-for-age percentiles. CDC.


Re: The expert opinion on preventative function of product IMUREGEN (IMUN Plus) on viral infections including Bird Flu virus.

Centrum of Immunology and Microbiology, department: Research and International cooperation.

Influenza (flu) is illness affecting mainly people with weakened immune system. Most often the high risk groups (seniors and people with chronic illness). World Health Organization recommends vaccination against flu as an important part of prevention against this illness. But vaccination effectiveness is not higher than 70%, mainly because of weakened immune system of many people, which is not able to completely ensure immunity against flu. It is known that it is partially caused by deficiency of various micro-nutrients (minerals, vitamins, amino-acids) and other substances able to repair weakened immune system and mucus membrane which ensure our resistance to illness.

That is why to strengthen immune system should be our choice not only before epidemic, but also during flu epidemic and when being ill with flu or other illness of upper respiratory tract. IMUREGEN is excellent for this purpose.

The product IMUREGEN (IMUN Plus) can be used on it's own or added to drinks. It contains mix of nucleotides, polypeptides, amino-acids, ascorbic acid (vitamin C) (also zinc-gluconate, and herbal extracts).

IMUREGEN increases absorption of micro-nutrients including iron, which increases human anti-flu immunity. The herbal additions contain substances, which increase immunity and have direct effect on some viruses, mainly flu virus. Their anti-oxygenous effect is together with zinc, guarantee of minimizing risk of infections and influencing their course.

Product IMUREGEN:

- Increases immunity
- Improves quality of mucus membrane
- Improves function of central nervous system
- Enhances power of organism as a whole
- Works as a prevention
- Improves resistance against viral infections including flu viruses and bird flu viruses
- Increased immunity during preventative use leads to increased immune answer after vaccination against any flu.

Ústí nad Labem, Czech Republic 27.10.2005 MUDr. Josef Richter, CSc.
Chřipka je onemocněním napadajícím zejména osoby s oslabeným imunitním systémem. Nejčastěji pak rizikové skupiny (seniory, jedince s chronickými nemocemi). Světová zdravotnická organizace doporučuje očkování proti chřipce jako důležitou část prevence tohoto onemocnění, ale jeho účinnost nepřesahuje 70%, především díky oslabenému imunitnímu systému řady osob neschopného zcela protichřipkovou imunitu zajistit. Je známo, že na tomto se podílí nedostatek různých mikronutrientů (minerálů, vitamínů, aminokyselin) a dalších látek schopných reparovat nejenom narušený imunitní systém, ale i povrchy sliznic zajišťujících naší odolnost.

Proto posilování imunitního systému je věcí volby a to nejenom v období před epidemií, ale i v průběhu epidemie a při onemocnění nejenom chřipkou ale i jinými chorobami horních cest dýchacích. Tomu slouží přípravek IMUREGEN (IMUN Plus), obsahující směs nukleotidů, polypeptidů a aminokyselin, kyselinu askorbovou (vitamín C), (případně i glukonát zinku a rostlinné extrakty).

Nápoj zaručuje nejenom podmínku kvalitního pitného režimu, ale i zvyšování příjmu mikronutrientů včetně železa, které v navázané formě na lidské bílkoviny zajišťuje protichřipkovou imunitu. Složky rostlinné obsahují řadu látek s působením na zvyšování imunity i s přímým ovlivněním různých virů a především chřipkového viru. Jejich antioxidační působení je spolu se získáním zárukou snižování rizika infekcí a ovlivněním jejich průběhu.

Preparát
- zvyšuje imunitu
- zvyšuje kvalitu sliznic
- zlepšuje funkce centrální nervové soustavy
- posiluje organizmus
- působí preventivně
- zvyšuje odolnost proti virovým infekcím včetně infekce chřipkovým virem a virem ptačí chřipky
- zvýšení imunity při preventivním podávání vede i ke zlepšení imunitní odpovědi po očkování proti jakékoliv chřipce

Ústí nad Labem 27.10.2005 MUDr. Josef Richter, CSc.
Extract from Technical Studies and Articles

"Antibacterial peptides of animal origin discovered in the IMUREGEN preparation are the news of the last 20 years of the passed 20th century. We are fellows of appearance of a new scientific branch, bringing – really day after day – new knowledge that can not be fully characterised, even on the basis of best efforts. In case of the scientists to pay the same efforts to the research as by now, we will witness unpredicted development of new attitudes towards treatment of human diseases. Not only those that we can not successfully treat regardless their knowledge, but also those still unidentified, that will undoubtedly appear in the future, as well as it used to be again and again in the course of human history."
RNDr. Petr Šíma, MUDr. I. Trebicchavský

"The healing effects of the IMUREGEN preparation are known as soon as from 1955, when tests started in various clinics and oncology departments in our country and abroad. In the course of the period it was proved that it has provable healing effects in acceleration of wound healing as well as other defects or failures of skin and mucosal surfaces integrity. In radiation oncology, it supports acceleration in healing of wounds caused by irradiation, its better tolerance, in case of chemotherapy there are reduced the negative adjacent and side effects. The preparation was proved to be a highly useful supporting amendment to the basic and non-replaceable general oncology care. There was also a positive knowledge that no negative side effects have ever been recorded in any case."
FN a LP UP Olomouc, Clinic of Oncology

Just in the past it was known that the preparation IMUREGEN works very well, but there was not known the method. But the research work with the preparation goes on and it has been tested on experimental animal models in vivo, ex vivo and in vitro, in tissue cultures, as well as in selected clinical work places. The scientific works engaged in recent years in problems of dietary nucleotides come to a conclusion that oligo- and polynucleotides, contained in the preparation IMUREGEN, are a base for synthesis of nuclear acids during regeneration of tissues and they affect mainly the cellular part of immunity responses."
Academy of Sciences of the CR, Faculty of Medicine, Health Institute

"The preparation IMUREGEN has scientifically and clinically proved "normalising" function of the immunity system. In fact, the fact activates macrophages, large protective cells, able to absorb foreign cells and simultaneously it supports the activities of lymphocytes of the type T and B. Its activities stimulate the immunity protection up to such level, that it finally manages any disease."
Academy of Sciences of the CR, Faculty of Medicine, Health Institute

"In fact that means that we have available an efficient and absolutely safe preparation against cancer, inflammation and for healing, which is absolutely non-toxic. IMUREGEN represents a method that reaches – during basic oncology treatment, chemotherapy or irradiation – much better results without health-damaging side effects."
Hippokrates: "Let medicaments are your food and food your medicaments."

Nucleotides and their meaning in nutrition, prevention and immunity
This fully natural, Czech food supplement contains a complex of the most efficient tissue substances supporting natural regeneration powers of human body. It mainly saves the powers of our body during all and any metabolic processes connected with conversion of proteins, but also fats and carbohydrates. The base of IMUREGEN contains nucleotides, being finished parts for creation of very important nucleic acids.

IMUREGEN and its nucleotides – that is a means designed for mass prevention from civilisation diseases, like cancerous, cardio vascular, diabetic, asthmatic and allergic diseases. It is a source of minerals that are of extreme importance for a man, like ferrum and zinc, containing them in highly favourable and easily usable forms and so it speeds up the development of protective substances. It is an important source of all and any needed amino acids, basic building elements of proteins needed for protection and renewal of human body tissues. We know that nucleotides positively affect some metabolic functions – adsorption of ferrum, production of muscular glycopene, they reduce the cholesterol level and so they positively affect the appearance and development of heart and vascular diseases.

There is also an extreme importance of IMUREGEN serving for development of fully functional mucous membrane of small intestine and development of all and any of its important functions at immature new born babies as well as at ill adults, respectively during renewal of gradually disappearing physical and mental skills of seniors. It improves the course and frequency of allergies, usual diseases, like flu, angina, cold, etc. There improves the healing of injuries and post-operation statuses and the preparation is an important means of prevention of tumour diseases. In radiation oncology it helps to heal reactions to irradiation, their better toleration, in case of chemotherapy it reduces negative side effects. The preparation was found to be very useful for consequent treatment of post-tumour conditions. Based on long-time testing, IMUREGEN proved its non-toxicity, non-addiction and no deposits forming in human organism.

MUDr. Jan Ševčík

Use of Imuregen for Health Modulation
The recent decade is characterised by discoveries of many information on effects of various nutritious, supplementary and supporting preparations, that are used – together with simple substitution - mainly for regeneration and imuno-modulation procedures. These are preparations made of natural materials with contents of amino acids, polypeptides, nucleotides and minerals, with contents of some minerals and proteins.
The preparations may be served separately as regeneration and stimulation dietetics, but new information (mainly those from Japanese and American scientists) show that their importance is much more extensive, mainly due to effects of the above stated individual substances to many imuno-regulation mechanisms. These are caused by the influence of preparations on renewal of cellular substrate and by affection of immunity directly by individual parts of the preparation. Information on such functions will be provided here below.

During an analysis of the IMUREGEN preparation produced by the company Uniregen (approved by the Ministry of Health of the CR, HEM -350 - 29.10.1998 - 36469) we found that – together with the substances, specified by the manufacturer, i.e. minerals, oligopeptides, free amino acids, vitamins, complex-bound ferrum, the preparation contains quite a high share of dietary nucleotides reaching the values of 3 - 5 mg/ml.

Dietary nucleotides were recently connected with development of standard immunity response. It has been proved, that they may participate in many biological functions, that they create a base of genetic information (DNA, RNA), that they serves as a power stock and they mediate hormonal influences (cANP). The effects of dietary nucleotides were monitored in detail in literature (mainly by Japanese and American scientists) and they are specified in detail in table no. 1. Based on the table it can be seen that they participate in an increase of mechanisms of cellular, as well as humoral and secretion immunity. The important factors include effects on reparation of small intestine cells, affecting mechanisms of cellular immunity as well as showing clear effects on renewal of microbial flora of the intestinal tract. That applies mainly after extensive long-lasting or repeated treatment with antibiotics.

The influence of the amino acids on immunity mechanisms is known and described for quite a long time. There is specified an important effect referring reparation of anti-body and cellular immunity response. E.g. methionine increases the anti-body response and reduces the risk of bacterial infections appearance, phenylalanine shows practically the same functions. Reduced levels of arginine, lysine, hystidine are specified in relation to significant decrease of cellular and anti-body response functions. Polypeptides are known thanks to their ability to modulate the ability of anti-body immunity. ferrum affects the immunity response by direct mechanism. It is known that ferrum-saturated transferine shows significant anti-virus effects. Another important effect is considered to be the influence of ferrum on absorption of iodine, which means in relation to supplementation of deficit population an important contribution even in solution of insufficient iodine level matter. We consider purposeful to put stress on the fact that nucleotides – inter alia – accelerate ferrum absorption in human organism. Even the group of B vitamins may apply in regulation of immunity response, mainly in affection of anti-body response (thiamine, riboflavin).In relation to both of the vitamins, there was proved their participation in protection against many infectious diseases.
The before-specified function abilities of the IMUREGEN preparation showed an important contribution of dietary nucleotides to nutrition of new born babies and children. Breast milk shows high values of nucleotides, while their share in cow milk is very low. That causes many manufacturers to supplement milk used in children nutrition with a mixture of nucleotides. Some authors speak of humanisation of formulas. But there also appear works stating significant contribution of nucleotides application at adults, which leads to setup of diets with nucleotides contents (Impact). Samples of the nutrition policy in the world lead in our country to consideration referring the possibility of children nutrition supplementation while using nucleotides and possibly other micro nutrients, with known chronic and general insufficiency in our population (ascorbic acid, zinc, ferrum, iodine).

It proves that – together with indications specified in the IMUREGEN prepartion – thanks to newly established presence of nucleotides contents it is possible to recommend the preparation in many clinical diseases. Mainly in cases when we intend to use nutrition for affecting fast regeneration of cellular substrate, reparation of psychic and immunity functions.

Detailed information on function of nucleotides can be found in our communication - Čs hyg, 41, 1996, p.319-323.

(Literature available at authors)

Richterová, S.1, Richter, J.2,Turek, B.3
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SZÚ Praha, Doc.MUDr.J.Kříž, Csc.

In Ústí nad Labem 27.3.1999
Assessment of the Imuregen preparation (company UNIREGEN, s.r.o.)

Except for the signed composition, i.e. mineral substances, proteins, natrium, free amino acids, fats, carbohydrates and cholesterol, we found in the preparation quite significant quantity of nucleotides. It reached the extent of 12 - 21% of the total charge. Due to these reasons we consider the preparation to be an important regeneration means for immunity functions as proved by the conference on nucleotides in San Diego as well as our clear article in Československá Hygiena (1997). At the present time, nucleotides are used in many dietary preparations for mouth supplementation of patients mainly with extensive post-traumatic conditions (see the World Congress in Munich, March 2000) as well as at patients in pre-operation stage. Numerous publications show reduction of post-operation complications and post-injury infections complications in as much as 40 - 80%. On the basis of our experience we can say that supplementation affects the renewal of intestinal cells, height of cilia, it inhibits the endotoxine – induced intestinal translation of bacteria and it inhibits pathological changes of intestinal mucosa. The supplementation affects the functions of liver, regeneration procedures and it supports renewal and proliferation of immunocompetent cells. The preparation acts as a regulation factor of haemopoiesis. Addition of nucleotides increases the general immunity capacity in cellular section as well as in humoral section of immunity. It increases the activity of NK cells. The preparation supplementation in a high dose supports renewal of secretion immunity functions, we proved an increased resistance mainly against infections of airways at children. At the present time, we are testing the preparation in cooperation with the Czech Academy of Sciences and we serve it in a complex with minerals in milk formula to children groups. This pilot study is a preparation for grant research in cooperation with professor MUDr. I. Hána, DrSc. and RNDr. P. Šíma, CSc.

MUDr. Josef Richter, CSc., OHS Ústí nad Labem
The expert opinion on preventive function of product IMUREGEN on viral infections.

Do you know that one flu epidemic in recent history killed more people than first world war? Do you also know that in the flu epidemic not only people die, but also they can they develop chronic damage of lungs, heart and brain which can result is many disabled people. Also people who are being treated for more serious illnesses can die very quickly for failure of life important functions of organism. Not only during epidemic, but also as a result of undergone virosis, many years after the epidemic. Flu epidemic occurs as a disease of the upper and lower respiratory tract. It can be fairly mild illness, however in small children, health compromised individuals or in elderly it can cause such a serious illness, that basic physiological functions are affected very fast and result is many short term or chronic complications.

If a person is treated for some serious illness and viral sickness complicates it, the death can be the result. Because patient did not take important preventative steps.

Research has shown that serious damage to the organism may not occur, if endangered population is prepared for incoming epidemic. Flu (influenza) viruses are changing constantly and mild virus causing running nose and fever can become killing virus even for healthy individual. Just remember „Spanish“ flu, killing people all over the world.

Treatment of complicated viral diseases is very difficult and expensive and often useless. If organism cannot defend itself, then the course of disease can be very dramatic. Only effective protection is to prevent contact with sick people. Not to go to public places where more people are together, but it is difficult way of protection/prevention, because infected person is able to infect hundreds of other people before the symptoms are showing.

Pharmaceutical industry came with many preparations to fight viruses, but their effectiveness is judged skeptically. What is mostly recommended are vitamin preparations and local desinfection. Results of studies did not uniformly confirm their importance. We all know uselessness of mass vaccination, because vaccine is not good at all for changing structure of flu virus, which is coming.

In the last years started to come on the market products, which can prepare organism to fight bacterial and flu infection in very short time. They are called immune modulating, because human body will receive certain preparation, which will create antibodies against certain infections. This is a long-term application. Disadvantage is they work only against certain specific strain and do not work against the others. Finally there was found the most efficient way to fight viral diseases. It consist of application of very effective elements – selected amino acids, nucleotides and other essential elements, which will organism use to naturally build and increase immunity. This way will humans, by natural and easy way build-up protection against illness and complications.

The most effective product to fight flu (influenza) is IMUREGEN, which is natural „medicine“ to increase immune capacity. It consist of nucleotides, selected proteins, amino acids, minerals, loosened Fe++ and naturally formed compounds of elements essential for the organism, in optimal ratio. It very effectively modifies defense of organism against viral infections. It has no side effects.

Conclusion:
If you want effective prevention against viral illness and prevent complicated course of flu, use product IMUREGEN. You will prevent not only onset of mild course of viral illness and running nose, but mainly prevent complications like inflammation of sinuses, lungs and other damage to organism. In children, elderly and weekend individuals will prevent death. Use the possibility to use products, which do not have side effects and help to fight viral infection. They create needed immunity, which will protect you against flu.

Profesor MUDr. Martiník, DrSc
Imuregen - a antimicrobial peptids.

All living organisms are protected from parasitic microorganisms by immune system. One of the important factors of the immune system immediate protection are its own substances that have an antimicrobial effect. They either stop the proliferation of microbes or kill them directly. Such antimicrobial substances were first discovered in the human body, by the discoverer of penicillin Sir Alexander Fleming in 1922. His discovery, unfortunately, did not have a followers. And other similar substances were discovered 40 years later. Today (fifty years later) we know that these substances are found in all living organisms and represent a fundamental pillar of innate immunity.

Throughout the animal kingdom can be several million different substances with antimicrobial effect. They are species-specific and, moreover, for each species there are dozens of different substances. Many hundreds of them have been discovered in mammals, and dozens in people. They occur in all tissues, but their number is highest in white blood cells, in the glands and secretions, mucous membranes and other barriers of organism (see recent review of Dr. Sima & comp. 2003). Most of them are peptides, but since these also include small proteins, thus they are properly called AMPP - antimicrobial peptides and proteins.

AMPP have enormous importance for human health, because they kill particularly transit microorganisms (bacteria, viruses, fungi, protozoa) on the interfaces between the external and internal environment of the body (intestine, lung, skin, genitor-urinary tract) and protects the circulatory system from the penetration of microbes (Yu & comp. 2010). Residential harmless commensal microflora of the skin and mucous membranes is living in an environment containing AMPP.

Antimicrobial effect of AMPP is caused by:

1.) Binding to the parasite membrane and subsequent penetration into the cell interior, where they can bind to DNA directly, or directly by penetration of parasitic cell membrane and killing them by osmosis (these are called cationic peptides - defensins, kathelicidins, BPI)
2.) Enzymatic lysis of important microbial structures (lysozyme, elastase, phospholipase A2)
3.) Linkage of vital substances (lactoferrin - bound iron and calprotectin - zinc binding)

In addition to the above effect, AMPP regulate immune response and slows dangerous inflammatory response which are dangerous to the body. Beneficial effects on human health is caused at many probiotics mainly by induction and secretion of AMPP in the intestinal lining and intestinal immune cells (Trebichavsky I. and I. Splichal, 2006). Their content in the blood varies depending on activity of white blood cells. These cells are a storage depot of tens of AMPP.

Substrate from which Imuregen is prepared, contains dozens of antimicrobial peptides. From cathelicidins - there are are bactenecins Bac-5 and Bac-7 (which at a concen-
The administration of 2-50 µg/ml kills dangerous gram-negative enterobacteria of salmonella, klebsiella and E. coli and common triggers of frequent nosocomial "hospital" infection. Myeloid antimicrobial peptides BMAP 27, 28 and 34 and two very small peptides indolicidin and dodekapeptid. Indolicidin, which contains the most amino acid tryptophan (39%) of all known protein, binds to the membrane and the parasite and penetrates into the cells where binding to DNA to inhibit its synthesis. Thus kills gram-negative and gram-positive bacteria, fungi and parasitic protozoa.

Of the thirteen beta-defensins BNDB that occur in the granules of white blood cells, there were ten of them isolated from the substrate Imuregen (BNDB-1, 2, 3, 6, 7, 8, 9, 10, 11 and 13). All of these defensins have antimicrobial effect on the golden staphylococcus and E. coli.

Were also found lysozyme, lactoferrin, and BPI (bactericidal permeability-Increasing Protein) calprotectin (synonym calgranulin or MRP 8 / 14), which constitutes 30% of all proteins of the cytoplasm of neutrophils (these cells represent half of the white blood cells) and is highly effective against yeasts, staphylococci and salmonellae (Striz Trebichavsky and 2004) and numerous antimicrobial enzymes such as elastases, cathepsin G, azurocidin, and phospholipase A2.

Lactoferrin of the substrate Imuregen, breaks by proteolysis (for example, pepsin in gastric juice) into a very effective lactofericin. This substance has a strong antimicrobial effect, and has a positive antitumor effect and effect against high pressure.

Literature

Ilya Trebichavsky
Malnutrition is an accompanying effect of HIV infection due to a lot of reasons. At first, the infested people are not provided with sufficient amount of food, they have decreased appetite, further there are altered metabolism and decreased absorption of nutrients. Both the insufficient intake of energetic sources of food and micronutrients deepen cachexia and lead to higher risk of some other infectious diseases which then frequently have fatal outcome. In era of high-quality treatment of retroviral infection, the adequate and reasonable nutrition becomes a base for long-term survival of infested individuals. Professionally managed nutrition regimen is a basic prerequisite for the infested individuals. Imuregen preparation, due its composition - high ratio of amino acids and nucleotides, grants provision not only of energy but of building elements as well. The preparation enables fast reparation of immune system, it secures physiological functions of the gastrointestinal tract and in such way it renews and makes stronger both immune system and enteric mucosa (GALT). It enhances detoxication via repcrqtion of hepatic cells, increases quality of body functions, diminishes exhaustion and influences functions of central nervous system as well. The preparation meets almost all criteria published in a guide of ANSA (Association of Nutrition Services Agencies), in a material called Guidelines for agencies providing food to people living with HIV disease. It contains a lot of micronutrients, from those recommended mostly vitamins of B series (812). It contains microelements, high dose of iron, amino acids and many antioxidants.
In our studies, we have proved a significant effect on restoration of cellular immune response, especially via activation of NK-cells. We have documented significantly positive effect on repair of enteric mucosa with activation of parameters of secretion immunity together with a significant improvement of specific antimicrobial immunity. Thus we confirmed the results of J.P. Schaller (2004) who used dietary nucleotides for the same purposes. We can state that the preparation meets all criteria for its incorporation into the category of functional food and that it is suitable for wide use.

MUDr. Josef RICHTER, CSc.
Zdravotná ústava se sídlem v Ústí nad Labem
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Nutritional effects on chronic active EBV infection with a preparation containing amino acids, nucleotides, apple pectin and fibre

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Summary

Nutritional supplementation using Imuregen, containing dietary nucleotides, amino acids and other components with the addition of apple pectin and fibre, was used in 36 patients suffering from chronic fatigue syndrome. Half of the monitored patients was administered a preparation not containing any active components. Before and after supplementation, the natural killer population (NK cells) was monitored, and statistically relevant increases in NK cells were found established in supplemented patients after a three-month nutritional intervention. The laboratory findings revealed established a significant change. The issue of supportive nutritional intervention in the clinical disorder of EBV-induced chronic fatigue syndrome is being discussed.

Key words: nucleotides; apple pectin; chronic fatigue syndrome; NK cells

Chronic fatigue syndrome is characterized by serious health disorders accompanied by symptoms of extensive fatigue and a number of other clinical symptoms. There have been many attempts to define chronic fatigue syndrome, of which two criteria have prevailed in the last two years; i.e. the British criterion (Oxford), taking into account particularly mental symptoms, and the American criterion (CDC), emphasizing particularly physical symptoms...
the illness, corresponding with the hypothesis of infection or immunological etiology of the illness (Reid et al., 2000; Straus et al., 1993).

Clinical symptoms of the fatigue syndrome can also be affected by nutrition, for example, such as an insufficient supply of iron or great losses of it. An iron deficiency is often a secondary symptom of chronic inflammatory alteration based on persisting microbial infection. In the group of patients showing clinical symptoms of chronic fatigue syndrome examined at our centre, an iron deficiency in more than half of the monitored patients was established.

The second group of individuals often linked with the clinical symptoms of iron deficiency are patients showing chronic active EB virus infection (Kimura et al., 2001; Wallace et al., 1999).

Based on the favourable background materials achieved from pilot clinical studies and model experiments (Slizova et al., 2004), we have decided to carry out this study, mollitoril Ig supplementation by a preparation containing nucleotides and amino acids supplemented with apple components (apple pulp, pectin and fibre). The preparation is produced by Uniregen, Czech Republic and approved by the Ministry of Health to be applied as a nutritional supplement.

Group allld methodology

A total of 36 patients showing symptoms of chronic active Epstein Barr virus infection as well as the diagnosis of chronic fatigue syndrome were placed in the this group. The followillg diagnostic criteria were established in all the patients:
- length of the disorder 'ilnd problems exceeding 6 months
- il lreased temperature
- myalgia, or arthralgia
- increased EBY antibody levels (VCA, IgG, EA, AB and EBNA)
- others

All the monitored patients were examined for cell-mediated and humoral immunity both before the nutritional intervention and 2 weeks after supplementation. Half of the patients was administered the preparation containing active components (Table I), the other half was administered the preparation without the active component (placebo), both for a period of 3 months. The natural killer population (NK cells) was established by immunophenotypisation using the flow cytometry method, and the results were statistically evaluated using MS Excel and Instat 3A programmes.

Results

The group of supplemented individuals showed an increase in NK cells from 247.3 to 354.7 cells. The control group showed 292.9 cells before supplementation, there being no statistically significant change after the supplementation, and the number of cells established amounted to 287.1 (Table 2). The findings of the supplemented group of patients are significant from the statistical point of view - p < 0.001 (Tuckey-Kramer, Multiple comparison test).

Discussion

This study is based on the experimental findings that prove that supplementation with nucleotides affects immunity, but also other physiological mechanisms. Apart from others, in the course of our experimental work we have monitored the influence of dietary nucleotides on the regulation
stimulation of ideal epithelium in experimental animals, and we have established essential morphological changes in Balb/c mice (Sřizova et al., 2004). We have also established an increase in number of NK cells in supplemented patients showing clinical symptoms of fatigue syndrome already after a relatively short period of supplementation. The favourable findings in the experimental part of the study were an impulse for us to use the Imuregen preparation as a nutritional supplement in a number of other patients showing deficiency in cell immunity response. In this part of the study, the findings achieved in patients suffering from chronic active EBY infection and chronic fatigue syndrome are presented. The reason why the preparation was administered was the favourable effect of dietary nucleotides on better functioning of fast dividing cells as well as hepatocytes (Carver, 1994), established both in experimental and clinical testing, Moreover, one of the EBY infection symptoms is a slight alteration of liver functions. At the same time, a diet with increased number of dietary nucleotides improves a number of cell immunity functions (Jyonouchi et al., 1996). In addition, we should not forget the positive effects of other preparation components, particularly pectin and fibre (Aprikian et al., 2003; Gonzales et al., 1998). Favourable effects on the lipid level and the anti-oxidation effects of the components is secondarily reflected in improvement of immunity functions, particularly in the regulation of NK cells, which might be a symptom of pectin effects on fat metabolism. At the same time, pectin affects cholesterol metabolism and it significantly reduces 137 Cs level (Nesterenko et al., 2004).

The most important findings, in our opinion, are not only the changes in the NK cells, but particularly subjective and objective manifestation of favourable changes in the clinical condition of patients. The clinical symptom rate has been reduced and the feeling of "well-being" has been increased.
Based on both experimental and clinical administration of Imuregen, the preparation appears to be an effective supplementary nutritional reparation to improve the health of patients showing chronic fatigue syndrome.

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References


COMMENTARY

Dietary nucleotides and preterm infant nutrition

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Nucleotides (NT) have been added to formulas for term infants in the US since the mid-1980s, and to formulas for preterm infants since 2002. The primary justification for these additions was evidence in animals and humans that dietary NT enhance development of the gastrointestinal and immune systems.1,2 A lower incidence of diarrhea,3–5 increased response to vaccination,6–9 and effects on natural killer cell activity10 and immune cell subset distribution11 have been reported in term infants. We are aware of only one published study reporting dietary NT effects in preterm infants: serum concentrations of immunoglobulin G to β-lactoglobulin were significantly greater in preterm infants fed NT-supplemented formula for 30 days.9

In the early 1990’s, we participated in a multi-center, randomized clinical trial to investigate dietary NT effects in very low birth weight preterm infants. There was no significant effect of NT supplementation on indices of immune function — the primary outcome measure. At one of the three sites, there was a greater incidence of necrotizing enterocolitis (NEC) among infants fed the NT-supplemented formula (five of 40 infants (12.5%) in the supplemented group versus 0 of 44 infants in the unsupplemented group). A direct causal relationship between the NT-supplemented study formula and the development of NEC could not be established, since the incidence of NEC in the study group was equivalent to the incidence among similar non-study infants admitted to our neonatal intensive care units at that time. The results of this study were not submitted for publication due to several significant methodological issues related to the study design.

Other reports published since the completion of the study have renewed our interest in the role of dietary NT in gastrointestinal function. We and others have reported that dietary NT-supplemented formula was associated with sustained postprandial increases in superior mesenteric artery blood flow velocity in preterm10 and term infants.11,12 The clinical significance of dietary NT effects on splanchnic blood flow is unclear, since postprandial blood flow velocities were more similar between infants fed human milk and infants fed formula with no added NT, than they were between infants fed human milk and infants fed formula with added NT. However, we speculate that NT effects on the gastrointestinal and immune systems may relate to NT effects on splanchnic blood flow, perhaps via effects on gut-associated lymphoid tissues. Additional studies are needed to clarify the relationship between NT-related effects on intestinal blood flow and gastrointestinal function. Studies in animals provide interesting yet conflicting observations. In rodents, dietary NT reportedly protect against and accelerate healing following bowel injury,1,13–17 and accelerate changes in intestinal lymphocyte maturation.18 However, Matheson et al.19 reported that an immune-enhancing diet containing fish oil, arginine and NT increased ileal blood flow and proinflammatory cytokines in rats, and Caplan et al.20 reported that dietary NT abrogated the beneficial effects of long-chain polyunsaturated fatty acids on intestinal necrosis in rat pups.

Despite uncertainties regarding the role of dietary NT in nutrition for preterm infants, several published clinical trials that have enrolled large numbers of infants provide presumptive term evidence of the safety and efficacy of NT supplementation for infants. Three randomized, well-controlled, multi-center studies in term infants enrolled a total of more than 300 infants into each of the control and study groups.5,6,21 Two of the studies reported that dietary NT supplementation enhanced infants’ responses to vaccination,4,5 while one reported that immune cell profiles of infants fed NT-supplemented formula tended to be more like those fed human milk.8 A more recent study reported that infants fed NT-supplemented formula had significantly higher responses to tetanus toxoid.22

Unfortunately, there are no published studies in which large numbers of preterm infants were fed formulas that differed only in NT content. O’Connor et al.23 conducted a multi-center, randomized trial to investigate the effects of long-chain polyunsaturated fatty acid supplementation on very low birth weight preterm infants; more than 100 infants were enrolled into each group. Both the study and the control formulas contained added NT. A reference group of human milk-fed infants was also included. The incidence of adverse events did not differ among the study groups. Three to 4% of infants fed the NT-supplemented
formulas developed confirmed NEC, while no infants in the human milk-fed group developed NEC. Since the study and control formulas both contained added NT, these data do not address efficacy issues related to dietary NT. However, the similar incidence of adverse events between the human milk-fed and the formula-fed groups provides presumptive evidence of the safety of NT-supplemented formula for preterm infants.

The existing evidence suggests that dietary NT-supplemented formula may enhance maturation of the gastrointestinal and immune systems in infants. Since many of the morbidities in preterm infants relate to immaturity of these systems, NT-immune systems in infants. Since many of the morbidities in preterm infants relate to immaturity of these systems, NT-supplemented formula may benefit this population. Studies in which NT are added as single ingredients to preterm formula are needed to clarify any safety issues, and to further establish the role of NT in nutrition for preterm infants.

References

Diet enriched by Nucleotides supports growth and regeneration soft membrane of intestines (Ilea – small intestine).

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**Introduction**

Epithelium of digest tract has very important role not only in digesting and absorption of nutrition, but also in transfer of antigen and pathogen signals for intestines lymphatic tissue. Different stages and forms of malnutrition are considered as the most frequent cause of dysfunction of the Immune system. Scientific research in last years on problematic of dietary nucleotides comes to the conclusion, that oligo and poly-nucleotides, which are present in the food (nutrition) present the most important parts of nutrition due to their importance for all kinds of metabolic and energetic process in human organism. Besides that, Nucleotides are the base for synthesis of nucleic acids at regeneration of tissue.

Most of up to date results show, that dietary nucleotides are necessity for growth maturing of intestines epithelium. We have documented in the previous experiments on animal model the immune-regulatory abilities of product: Imuregen, which is the mix of dietary nucleotides and has influence mainly on cellular part of immune response.

**Material and Methodic**

In this study were 3 month old female mice group: Balb/c, fed by: 1. standard diet, 2. diet enriched by Imuregen supplement 3. standard diet and water enriched by Imuregen supplement for the time of four weeks. Supplement dose of 100mg per 1kg of food. Or 100mg per 1L of water contained Imuregen, product of company: Uniregen, s.r.o., Nachod Czech Republic.

Samples of terminal Ilea from all three groups were taken for histology test/examination and for observation in raster electronic microscope. The
tissue for histology testing was fixed, coated in paraffin, dyed with hematoxyline eosin and by method on proving acid mukopolysacharids by Hale-Muller and studied in electronic microscope. The length of villi was evaluated morfometricly. The samples for raster electronic microscope were dried by hexamethyldisylazan, gold coated and looked at/examined under raster electronic microscope Tesla BS 301.

Results
There were found no pathological changes of intestines villi in experimental groups. The highest growth of intestines villi were observed in group #3, which was fed with standard diet and water supplemented with Imuregen. Evaluation of morfometric data have proven statistically significant difference in the length of villi of experimental groups.
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<th>Control group.</th>
<th>Experimental group #1</th>
<th>Experimental group #2</th>
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<tr>
<td>Standard diet and drinking regime</td>
<td>Diet enriched by Imuregen dose 100mg/1Kg</td>
<td>Diet (normal) and drinking regime enriched by Imuregen liquid. Dose 100mg/1L</td>
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**Control group.**

- Standard diet and drinking regime

**Experimental group #1**

- Diet enriched by Imuregen dose 100mg/1Kg

**Experimental group #2**

- Diet (normal) and drinking regime enriched by Imuregen liquid. Dose 100mg/1L
Statistical evaluation and conclusion:

Methodic of statistical evaluation. Differences of measured values of the heights of villi in control group and both experimental groups were evaluated by Snedecorov F-test. To evaluate value of zero hypothesis about difference of averaging mean measured values, the Student’s t-test was used. The differences in averaging mean values of the height of villi were considered to be statistically significant, if value of testing characteristic was bigger than relevant kvantil of Student’s discrimination to 5% level significance (p < 0.05).

Morfometric evaluation of the height of villus of terminal ilea.

Graphic presentation of test results

1. Standard diet. (Yellow bar)
2. Diet enriched by Imuregen – dose 100mg/1kg (Blue bar)
3. Supplemantation of drinking regime with Imuregen – (standard diet + Imuregen in drink – dose 100mg/1L) (Animals had free access to drink and food, experimental nutrition was served for duration of four weeks.) (Red bar)
Conclusion.
In this experiment was found statistically significant growth of the mean length of villi of terminal ilea of mice group Blab/c after four weeks of serving of the mix of oligo-polynukleotides in dietetic product IMUREGEN, in comparison with control group.

The most significant growth was proven in the case of serving Imuregen in drink. (p < 0.005) than it was in food with the same product (Imuregen) (p < 0.025). There were found no pathological changes of intestines villi in experimental groups.

This test/experiment has proved positive influence of mix of nucleotides in dietetic immune stimulating product Imuregen on regeneration and growth of epithelium of terminal ilea.
*Villi = fine soft processes of living cells, in the small intestines each contain a central vessel or lacteal, surrounded by a plexus of capillaries. Their function is to increase the surface of the small intestine thereby aiding absorption.
UNIREGEN

We, signed below, recommend the product Imuregen containing important bio-active substances. Based on long-term experimental and clinical tests, it was confirmed that the product Imuregen has positive effects on the total regeneration of the organism and the immune system. It is especially suitable for people under extreme physical and/or mental stress. It fosters restoration of liver tissue and mucous membranes of digestive and respiratory system and it also provides protection from viral and bacterial infections (Hepatitis, leptospirosis, Weil’s disease, salmonellae, typhoid diseases).

For the above stated reasons the product Imuregen is especially suitable for personnel under stress in extreme conditions, as in rescue and salvage operations and decontamination work in flood-affected regions.

MUDr. Josef Richter, PhD.
Department of Science and Research
KHS Ústí nad Labem

RNDr. Petr Šíma, PhD.
Sector of Immunology and Gnotobiology
Institute of MicroBiology, Academy of Science CR

Ústí nad Labem, 28 August 2002

(This above statement was released during summer 2002 as a respond to the extreme flooding in Europe)

UNIREGEN S.R.O. (A Limited Liability Company)

Hitherto Conclusions from Repeated Testing of the Preparation IMUN +

Supplementation of human nutrition with immunity-enhancing substances can be very beneficial, because our human bodies have become imbalanced. The overwhelming cause of this imbalance is poor lifestyle and environment, pollution, stress, nutritional deficiencies, toxicity and infections, and they all erode our health and lead to a decrease in our immune system effectiveness. The reduction of immunity causes various diseases of viral, bacterial and metabolic character. The only solution is strengthening – balancing – of the organism stamina – its immunity. Optimal solution can be offered by the product IMUN+, made by company Uniregen.
**IMUN +**

Food concentrate with efficient component of **nucleotides**

**IMUN +** is a natural complex of essential amino acids, nucleotides, oligo-peptides and trace elements. These substances in their complex are able to activate, and positively increase the immune system of a human. There are able to harmonise the organism stamina, from the point of view of immuno-modulation of cell and hormonal part of the immunity.

**Bio-complex of active substances creating efficient regeneration and stimulation dietetics.**

The food supplement **IMUN +** ranks among modern preparations produced on the purely natural basis. It is designed for adults and children from 1 year of age and it does not contain alcohol or any inorganic chemical substances. It is particularly suitable for diabetics and for athletes supporting the muscle mass growth and improving regeneration of strength/endurance, during exercise or training.

**IMUN +** is one of the most efficient preparations fighting the flu.

**IMUN +** offers new approach to solving the fight against viral diseases by using the application of highly efficient substances – selected amino acids, nucleotides and other essential substances used by the organism to increase it’s immunity.

Using of **IMUN +** will boost natural resistance to illness and disease and will naturally improve immune system capacity. People will avoid light flu as well as possible negative effects on vital organs – heart, lungs or kidneys – and consequent danger for the whole organism.
UNIREGEN S.R.O. (A Limited Liability Company)

- **Composition**

This purely natural product was developed at the beginning of the 50’s under management of a popular pharmacist Dr. Rakús in Czech laboratories. It contains a balanced complex of the most efficient plant and animal substances. Essential amino acids, nucleotides, proteins, peptides, oligo peptides, organic salts and vitamins in natural relations increase the power potential of human cells, which can stimulate the immune system.

- **Use**

IMUN + is absorbed in the digestive system in an active form – the organism absorbs only the substances it needs for managing the performance, to avoid stress and civilisation diseases and for improvement of metabolic process during a disease. The product IMUN + does not contain sugar and it is suitable even for diabetics. It can be used for an unlimited time period without any risk of addiction, or negative side effects.

- **Effects**

IMUN + reduces chronic fatigue, eliminates feelings of weakness and depressions, speeds up healing and regeneration and has positive effects on the digestive system, kidney and liver function.

IMUN + is suitable for healthy people as well as for people under mental or health stress, in any age groups.

Healthy individuals can use IMUN + in case of fatigue and as prevention of stress situations. It reduces the risk of the organism damage by civilisation diseases.

When used by athletes, it increases the muscle growth and positively effects the power metabolism by arranging several ways of power supply to cells. It improves regeneration of strength/stamina.

When used by ill people, it creates a base for speeding up the process of healing, reduces the time of regeneration and thus improves the efficiency of treatment. It is suitable as a prevention of arteriosclerosis and it is one of the basic substances in the methodology of JUVENOLOGY (postponement of ageing and keeping and improvement of active life from the mental, physical and sexual point of view). It is suitable for both acute and chronic illness. Application of IMUN + is beneficial as prevention of the illness as it eliminates excessive deviations in the organism homeostasis and even long-term chronic diseases can be significantly improved or even cured.

IMUN + does not have any harmful side effects.
The basic biologically active substances of **IMUN +** are:

**Nucleotides**
- The synthesis of nucleotides in liver is very demanding from the point of view of energy and that is why the intake of exogenous nucleotides in the periods of increased demand (after surgery, traumas, burns or system diseases) is necessary.
- Period of growth of organism up to the adult age, significantly increase the demands of the organism towards the consumption of nucleotides. They are applied also in induction of anti-substance response and they non-specifically increase the production of immuno-globulins, mainly of the IgG and IgM class.
- Experiments proved, that nucleotides increase resistance to infection with *Staphylococcus pyogenea* and *Candida albicans*, they also increase production of interleucine IL2 and increase functions of microfages.
- They significantly stimulate the creation of lymphocytes, which are a base of immunity.
- They improve the utilisation of vitamins and trace elements.

**Amino acids**
- Some of them directly effect immunity
- Arginine and glutamine have immuno-regulation functions and they cause an increase of proliferation of lymphocytes and an increase of some sub-populations of lymphocytes.

**Iron**
- Necessary for arrangement of cells proliferation and bio-synthesis of ribonucleotides to deoxiribonucleotides, the basic elements of DNA.
- Deficit of iron leads to reduction of dermal cell response, reduction of lymphocytes proliferation, reduction of bactericide capacity, it also reduces the anti-substance response, it increases the sensitivity towards infections.

**Proved facts related to the preparation IMUN +**

Clinical tests proved a significant strengthening of cell immunity, mainly increase of T-lymphocytes and their activity, induction of mucous immunity response. Application of the IMUN+ has shown that there were significantly increased values of lysozyne and increased values of many proteins. The application of the IMUN+ significantly reduced the increase of the C-reactive protein and the orosomucoide, which are the symptoms of inflammatory processes. The top athletes and sport teams have shown an improvement of power/strength parameters and training capacity and further more, there was also proved the protective influence on liver parenchen from the effects of physical load. When IMUN+ was applied to the patients with hyperlipidemia, there was noticed a significant decrease of the cholesterol level. There was proved a significant reduction of sickness in work collectives in the normal season and during seasonal viral diseases.
The use of IMUN+ positively effects the health conditions of children, mainly in regions with extremely damaged environment.
UNIREGEN S.R.O. (A Limited Liability Company)

Evaluation of the IMUN + product in relation to cellular part of immunity
Prof. MUDr. J. Kocián, DrSc., 1st Clinic of Internal Diseases IPZV – FNT Prague 4
In our clinic we tested products with high contents of polynucleotides – i.e. substances contained in cytoblasts of all and any cells. As soon as the 3rd day from the start of the application of products, there was an increase in the numbers of white blood cells responsible for protection of the body against infections – the increase fluently continues up to the 28th days when the numbers of cells reach as much as 200% of the original values. It was mainly the Imun + preparation that proved well.

Evaluation of the IMUN + product in relation to infections of the upper and lower respiratory system, tonsillitis and flu.
ZORA Olomouc, MUDr. H. Černochová
The product Imuregen was given to a selected group of employees in the ZORA Olomouc plant during the period of increase of viral diseases. The employees were in the age of 19 – 64 years. The sickness leave representing a comparative group was significantly lower in employees using Imuregen. Serving the preparation – mainly in large work teams with high physical or mental load – is efficient and economical in the periods of an increased occurrence of respiration viral and bacterial diseases.

TT Viadrus ŽDB a.s., MUDr. Masařek
The product Imuregen was daily given to a selected group of employees of the foundry in the TT Viadrus ŽDB, a.s. plant. For evaluation of efficiency of the Imuregen: comparison has shown decrease in the illness rate of the upper respiratory tract by 47%.

MUDr. Štaket from the department of work and medical care:
He recommends IMUN + product to be used mainly for professions:
- under mental stress (managers, drivers)
- working in the environment with exposure to carcinogens chemical
- working under the risk of ionising radiation
- working with organic solvents
- working in physically demanding jobs
- working in shifts work

Immunological Findings in Groups of Children after Compensation Measures
National Institute of Health – department of Immunology in Ústí nad Labem
Doc. MUDr. J. Richter, CSc., MUDr. Ladislav Pelech
200 children permanently living in a region with high degree of environmental pollution spent 14 days in a region with healthy environment and they were given product Imuregen. As a result, their parameters of secretion immunity have significantly improved. Decrease of albumin values signalises a significant reduction of the inflammable irritation of the respiration system. There were also significantly reduced the specific antibodies IgE of the respiration type of allergy. On the basis of hitherto experience, just the stay in the region with high-quality air positively influences 80 – 90% of people for a period lasting for 2 – 3 months. This period can be significantly prolonged by using the product Imuregen.

**Findings from children supplemented with the IMUN + product.**

KHS Ústí nad Labem, OHS Ústí nad Labem, OHS Litoměřice
Doc. MUDr. J. Richter, Dr. S. Richterová, Dr. T. Kolinová

Group of 35 children in the age of 11 – 15 years, who were given product Imuregen for the period of 1 month, was observed. Statistically was proved a significant reduction of inflammable reactions, there was increase of the values of secretion immunity and there was normalisation of many values of biologically important proteins, mainly the transferine.

**Monitoring the Effects of the IMUN + product in a group of 53 persons in VÚ Kbely**

Clinical Laboratory ÚVN Prague – Strešovice, Dr. M. Švec

The product Imuregen was continually given to the group of 53 people aged 20 – 50 years, for a period of 2 months. Comparison of laboratory tests was done for the evaluation of cellular immunity before application of Imuregen to group, and after termination of Imuregen product use. The Rosette test proved a significant increase of T – lymphocytes and the test of lymphoblast transformation proved a significant increase of their activity.

**Preliminary Findings in Population of Seniors supplemented with product IMUN+.**

KHS Ústí nad labem, OHS Litoměřice, OHS Chomutov
Doc. MUDr. J. Richter, D. Jílek, V. král, A. Vorderwinker, Dr. T. Kolinová, M. Hanusová

Total of 151 seniors from nursing homes were monitored – seniors received a dose of Imuregen for a period of 3 months. The values of IgG and IgE globins which prove optimisation of the immunity system regulation, were normalised. Significant decrease of the C-reactive protein proves the significant anti-inflammation effects of the product Imuregen. Normalisation of the transferine values confirms the positive adjustment of the iron metabolism. There was also a significant decrease of the SP3 protein values, which are indicators of hormonal dysfunction but also possibly of tumours activity or inflammations. The dynamics of changes of the pre-albumin values confirms significant improvement of nutrition status and an improvement of detoxification processes.
**Verification of Effects of IMUN + in the Sphere of Top-Performance Sports**
Administration of Top-Performance Sports – Prague, RNDr. P. Fořt, CSc.

The extensive study of power demanding sports and endurance types sports confirmed highly positive effects of the product Imuregen mainly in the following spheres:

1. significant improvement of power/strength/stamina parameters, improvement of training capacities
2. significant reduction of sickness rates
3. improvement of the regeneration course
4. protective influence on liver parenchyma from the effects of physical load
5. stimulation of proteo-synthesis

**Use of IMUN + in Patients with Elective Surgery**
Department of Surgery of the Hospital Na Homolce, OKBHI Hospital Na Homolce, Institute of Experimental Medicine, Academy of Science Czech Republic.
MUDr. M. Hladký, MUDr. V. Táborský, MUDr. J. Červinka, MUDr. M. Průcha, MUDr. M. Dostál

The product Imuregen was given to 24 patients in the dose of 2x1 tablet/day 7 days before, and 30 days after surgery. The patients showed a statistically significant decrease of activity values of liver amino-transfers that were significantly pathological at some patients before starting the application of Imuregen. Furthermore, there was statistically significant decrease of the transferine values, which proves positive restoration effects of Imuregen during inflammation. There was also proven a modulation effect of Imuregen in the sphere of cellular immunity due to statistic increase of the amount of CD 3+ lymphocytes in comparison with the control group. The results confirm suitability of Imuregen use as a non-specific supporting product for patients, where it can be possible to expect immunodeficiency in the sphere of cell-mediated immunity.

**Clinical Testing of the IMUN + Product at the Surgery Department**
Hospital in Mladá Boleslav
MUDr. A. Skřivánek

Patients with so-called unhealable defects were selected for clinical tests – i.e. patients with poorly healing fractures, decubites and patients with varicose ulcers or with burns of 3rd degree. After 3 months of serving Imuregen, all the patients showed a significant improvement. While the healing time – i.e. the reduction of the term of treatment – reached 7 to 10 days.

**Immunological Findings and dynamics of Proteins after Application of IMUN +**
Regional Hygienic Station in Ústí nad Labem
DOC. MUDr. J. Richter, CSc.
Patients aged 39 – 58 years with diagnoses of non-plasmatic diseases were given Imuregen in the dose of 2 mg/day for the period of 25 days. Chemical examinations before and after the therapy proved statistically significant increase of pre-albumine values, normalisation of transferine values and values of C – reactive protein of alpha I – antitripsyne. There was also proved an increase in the number of leucocytes, adjustment of distribution of lymphocyte population and normalisation of the share of terminal lymphocytes.
APPLICATION OF IMUREGEN IN MODULATION OF HEALTH

Authors: Richterová S. ¹
Richter J. ²
Turek B. ³

OHS (District Hygienic Station), Ústí nad Labem
Director MUDr. S. Richterová (M.D.) ¹

KHS (Regional Hygienic Station), Ústí nad Labem
Director MUDr. A. Vorderwinkler (M.D.) ²

SZÚ (State Health Institute), Prague
Senior Lecturer MUDr. (M.D.), Csc. ³

The last decade is characterized by the discovery of a series of information on effects of various nutritional, supplementary and supporting preparations which besides simple substitutions are applied mainly in regenerating and immunomodulating processes. They are preparations made from natural materials containing amino acids, polypeptides, nucleotides and minerals inclusive some vitamins and albumens.

These preparations can be used separately as a regenerative and stimulating diet. But the latest findings of research institutes (mainly Japanese and American) show that their significance is far wider, mainly because of effect of individual components mentioned above on many immunoregulative mechanisms. This is caused by the influence of preparations on regeneration of cell substrate and immunity directly through individual components of the preparation. We will inform you about these functions below.
When analyzing preparation Imuregen made by UNIREGEN Co. (approved by the Ministry of Health ČR /CZ Rep./, HEM – 350 – 29.10.1998 – 36469) we found that besides components mentioned by the producer, i.e. minerals, oligopeptides, free amino acids, vitamins, complex bound iron, there is also comparatively high share of dietary nucleotides (3-5 mg/ml).

Dietary nucleotides have recently been related to construction of normal immunity response. But it appears that they can take part in a number of biological functions and that they are basis of genetic codes (DNA, RNA). They also serve as a reserve of energy and mediate hormonal effect (cANP). Effects of dietary nucleotides were followed in detail in literature (mainly by Japanese and American research institutes) and they are given in brief in table 1. It is evident from the data in the table that dietary nucleotides do not only take part in increasing of cellular but also humoral mechanisms and secretion immunity. Of great significance is thought to be the effect on repair of intestinum tenue (thin intestine) and thus not only mechanisms of cellular immunity are influenced but it has definitely been proved their effect on regeneration of microbial flora of intestinal tract, especially after an intensive and long lasting treatment by antibiotics. This concerns also repeated treatment.

The effect of individual amino acids on immunitary mechanisms has been known and described for a longer time. In those works a significant effect is given on repair of antitoxin and also cellular immunity response. For example methionin increases antitoxin response and decreases risk of bacterial infections. Fenylalanin shows practically the same function. Lower levels of arginine, lysine, hystidine are related to substantial decrease of cellular functions and also antitoxin response.
Polypeptides are known to be able to modulate ability of antitoxin immunity. Iron effects on immunity response by direct mechanism. It is known that transferin saturated by iron has significant antivirus effect. Further significant effect is considered to be the influence of iron on utilization of iodine which means, when supplementation of population is used, significant contribution even in solution of inadequate iodine level problem. Here we wish to stress that nucleotides also accelerate utilization of in human organism. The groups of vitamins B can also be applied in regulation of immunity response, mainly in influencing of antitoxin response (thiamin, riboflavin). Both these vitamins have been proved to be linked up with prevention of a number of infectious diseases.

Functional abilities of preparation Imuregen already published some time ago showed significant contribution of dietary nucleotides in nutrition of new-born babies and children. The content of nucleotides in mother milk is very high, while the share of nucleotides in cow milk is very low. This fact has lead a number of manufacturers to supplement those kinds of milk which are used for nutrition of babies and children with mixtures of nucleotides. Some authors speak about humanization of formulas. There also appear works writing about significant contribution of nucleotides application to adults which leads to combination of diets containing nucleotides (Impact).

Examples of this nutrition policy in the world and also in this country lead to considerations on possibility of supplementation of baby (children) food with application of nucleotides and if need be further micronutrients the chronic and general absence of which in our population is known (ascorbic acid, zinc, iron, iodin).

It has been shown that besides indications given for preparation Imuregen because of newly found presence of
nucleotides it is possible to recommend the preparation in a number of clinical diseases – and namely in cases in which we intend to influence fast regeneration of cellular substrate, fast regeneration (repair) of psychical and immunity functions.


(The literature is at the authors disposal)
ANTIDOPPING COMMITTEE OF THE CZECH REPUBLIC
170 00 Praha 7, U Sparty 10
tel./fax 33 37 01 49, E-mail: antidopingvcr@mbox.vol.cz

Distributor - applicant: Uniregen spol. s r.o., Malé Poříčí Na Brzdách 72, 547 03 Náchod
Name of product: Imuregen®
Producer: Uniregen spol. s r.o., Malé Poříčí Na Brzdách 72, 547 03 Náchod

Antidoping Committee of the Czech Republic on the basis of the expertise, according to which the product does not contain forbidden substances,

A G R E E

To the following marking of the product: “Suitable for sportsmen“ without any limitations in the market.

In Prague 1999-10-4

Seal: Antidoping Committee of the Czech Republic

Signature: PhDr. Jaroslav Nekola
Chairman of the Antidoping Committee
Of the Czech Republic
Uniregen Co. Put forward preparation Imuregen (previously approved Juvenil) to the Working Group for children's gastroenterology and nutrition for approval. An expert opinion is required in connection with the change of producer and new law on foods, particularly on foods intended for special nutrition.

Imuregen is intended for stimulation of immunity system. It is produced of beef blood and contains amino acids, peptides, organic salts, bivalent iron. It has already been given to children before under the name Juvenil - see previous expert opinions.

The Working Group have no objections to giving the preparation to children over 1 year, in doses of 10 to 30 drops daily.

MUDr. P. Friihaufl, Csc
Doc. MUDr. O. Pozler, Csc
Doc. MUDr. J. Nevoral, CSc

Working Group for children's gastroenterology and nutrition at the Czech Pediatric Society
PROTOCOL

on expertise of preparation intended for sports nutrition

Applicant: Uniregen spol. s r.o., Malé Poříčí, Na Brzdách 72, 547 03 Náchod

Name of preparation: Imuregen ® (solution 50 ml)

Kind of preparation: Food for special nutrition

Producer: Uniregen spol. s r.o., Malé Poříčí, Na Brzdách 72, 547 03 Náchod

Submitted documentation:
- Application to Antidoping Committee of the Czech Republic for expertise (1 sheet)
- Abstract of Register of Commerce (1 sheet)
- Decision of the Ministry of Health of the Czech Republic, HEM-350-29.10.98-36469 (1 sheet)
- Protocol – Analysis of amino acids etc (2 sheets)
- Protocol – Analysis of Pb, Cd, Hg (1 sheet)
- Protocol – Basic food characteristics (2 sheets)
- Company standard – Imuregen – PN UN-01-98 (5 sheets)

Preparation description: Pellucid, slightly yellowish liquid, soup spices smell, beef bouillon taste.

**Decision**

The present preparation does not create after taking any metabolic or psychic addictive response that could be classified as drug addiction and for this reason it cannot be considered as a doping narcotic substance.

The expertise only concerns the presented sample and the conclusions deduced from it can be applied on other products only if their composition and properties are completely the same as those of the presented product sample.

This expert opinion is a professional pharmaceutical expertise meant for binding decision of the Ministry of Health Authorities of the Czech Republic.

Signature:
Doc. RNDr. Lubomír Opletal, CSc.
Pharmaceutical Faculty Hradec Králové
Department of pharmaceutical botany and ecology

*This Protocol is invalid without the expertise.
EXPERT OPINION (EXPERTISE)

on the dietetic preparation intended as a part of sports nutrition

Name and producer:

**Imuregen ®** (solution, 50 ml)
Uniregen, spol. s r.o., Malé Poříčí, Na Brzdách 72, 547 03 Náchod.
Protocols on composition (PN-UN 01/98) and analysis are enclosed.

Composition of the preparation:

100 g of the preparation contains (mg): mineral substances 40, proteins 35, free amino acids 13, fats 5, saccharides (glucose) 0.4, cholesterol 0.34, Na 13.

Auxiliary substances (%): E 218 (methyl-p-hydrobenzoate) max. 0.1.

Energetic value: 0.8 kJ / 0.19 kcal.

From the mentioned additives the result is one market sort.

Biological activity of preparation components in view of declared effect:

The preparation is declared as a food for special nutrition.

*Imuregen ®* is an extract of dried beef blood prepared by 95% ethanol. This extract after thickening is dispersed in diethylether. Adipose substances are dissolved in this solvent. Mixture of contained substances, i.e. free amino acids (asp acid, threonine, serine, glutamic acid, proline, glycine, alanine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine), oligopeptides, vitamins (thiamin, riboflavin) and other substances are deposited in powder form which is dried and treated again (it is dissolved in water or added as powder trituration into food products).

Detailed analysis of peptide substances (i.e. isolation of individual components and determination of their structure by physico-chemical methods and tracing their biological activity) has not been carried out so far. For this reason it is possible only to describe summary effects of the preparation, the composition of which depends on proveniance of initial material. However it is evident that present oligopeptides are absorbed after peroral application. They are not destructed in digestive chain (although they are of protein character) and show biological activity likewise as it is known for example at ß-casomorphines.

Substances of the preparation accelerate healing processes, reduce time of patient’s convalescence, are effective at tiredness and influence negative impact of stress factors for organism. When taken by sportsmen in combination with other substances, more intensive progress of proteosynthetic responses, faster return to metabolic organism balance and apparently more effective utilization of energy sources (compared to control groups) have been observed. Positive influence on organism immunity has also been found. General improvement of health state and feelings of receivers have also been observed.

Positive effect of substance complex from blood tissue has not only been found with people but also with domestic animals. The tissue extract is able to prevent organism from destruction because it causes its time reduction.

Therapeutic dose is not determined because this material is not classified as medicine.

The producer recommends utilization of the preparation for nutrition of general population including sportsmen as a prophylactic remedy for prevention of deseases development and with sportsmen for improvement of metabolic state of organism.

The declared effect of the preparation is in compliance with biological activity of present components.
**Dosage**:  
The producer recommends 30 to 60 drops daily for adults, 10 to 30 drops for babies and children daily up from 1 year of age. Although it is possible to consume the preparation with other foods, according to judge opinion it is advisable to take it with an empty stomach (half an hour before meal or between meals) because of better biological availability of contained substances.

**Toxicity of components**:  
The main component of the preparation has been approved by certificate HEM. There is no risk of toxicity at mentioned quantity and recommended dosage, it is not harmful for organism from the standpoint of metabolic load or undesirable secondary effects.

**Preparation stability and possibility of decomposition products emergence in view of application form and interaction of individual components**:  
The preparation stability is good, development of significant quantity of disruptive products is not assumed on the condition that the preparation is kept at temperature from 10 to 25 °C in closed container, protected from the sunshine and used not later than 12 months from the date of production.

**Interaction of individual components in organism from the standpoint of undesirable effects**:  
Interactions with exogenously taken substances (medicines and food components) are not known from the literature and are not expected.

**Possibility of preparation abuse – so called doping**:  
Dopping abuse is not real.

**EVALUATION**  
The Standard for the preparation is made up rationally, objectively and corresponds to requirement for products of this kind.  
The product can be recommended without reservation as a food for special nutrition.

**Rectifying proceeding recommendation**  
Rectifying proceeding is not suggested.

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In Hradec Králové 28th September 1999  
Signature:  
Doc. RNDr. Lubomír Opletal, CSc.

Pharmaceutical Faculty Hradec Králové  
Department of pharmaceutical botany and ecology

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RESOLUTION

According to enactment of § 11, par. 2 letter b) and par. 4 Law No. 110/1997 Sb. on food and tobacco products and on change and supplement of related legislation the Ministry of Health of the Czech Republic is agreeable to

sell the food intended for special nutrition in accordance with § 1 par. 1 letter d) Decree No. 336/1997 Sb., suitable for diabetics as well, under the name IMUREGEN, specified in company standard PN UN-01/98, producer UNIREGEN spol. s r.o., Male Porfici, Na Brzdach 72, 547 01 Nachod, under the following conditions: The covering shall include:

1. Notice: „The preparation is suitable for diabetics as well”
„The product is not intended for children under 1 year”

2. Dosage: Adults 60 drops a day
Children 10 - 30 drops a day

Justification

UNIREGEN spol. s r.o., Male Porfici, Na Brzdach 72, 547 01 Nachod, 29th Oct.1998 asked The Ministry of Health of the CR in accordance with enactment of § 11, par. 2, letter b) and par. 3 of the above mentioned Law for the agreement to introduce the product to the market. From the documents enclosed to the application it has been proved that the food cannot threaten the health of persons.

The health unexceptionable nature of the preparation was also examined with to the way of application and necessary health notices, both proposed by the applicant in his application and confirmed by the standpoint of the State Health Institute in Prague of 22nd Oct.1998, reg. No. CZZP 16-2712 I 98 EX 181594, in the standpoint of Doc. MUDr. Nevoral, CSc. (The Czech Pediatric Society) of 26th Nov.1998 and in the standpoint of doc. MUDr. L.Kuzela, DrSc. (The 1nd Internal Teaching Hospital of Faculty Hospital /FN/, Kralovske Vinohrady) offth Oct. 1998.

The applicant shall therefore not only specify the data on the covering according to common binding rules (legal) but also data stated in the decision of the conditions mentioned the food could not be declared suitable for health reasons.

Information: It is possible to appeal against the present Resolution to the Ministry of Health of the CR within 15 days after the date of delivery.

Round Seal: The Ministry of Health

MUDr. Jifi Vytlacil
Chief Hygienist
The Czech Republic
TESTING

THE INSTITUTE OF HYGIENY AND EPIDEMIOLOGY, CZECH
MINISTRY OF HEALTH, DEPARTMENT OF NUTRITION

The Institute carried out the testing of the preparation as required by the Chief Hygienist of the Czech Ministry of Health before approving the standard, and made the following conclusion:

a) The preparation was tested for safety by microbiological and toxicological methods and was found suitable for human consumption without restriction.
b) The biochemical indicators of nitrogen metabolism are within standard, with a 10% reduction of the uric acid content.
c) Functional tests of the cardiovascular system showed a reduction of blood pressure and an improvement of the heart load resistance index.
d) After fourteen days of application of the preparation, the tested persons showed a 19.8% recent memory improvement.
e) The analytical values show an extremely high proportion of free amino acids (51.4%) with a well-balanced spectrum of 11%. Above limit values were found in tryptophan, but only in bonded form, found in free form were leucine, alanine, valine, serine and methionine.

Testing was carried out under the supervision of Prof. Augustin WOLF, M.D., D.Sc.
The purpose of the testing was to prove an increased biological activity of the organism. The preparation was administered to 156 malignant tumour patients, after their causal treatment, as supportive therapy, and to another 12 patients suffering from repeated virus diseases and partial loss of immunity.

The effect of the preparation was evaluated in 15 different tumourous sites according to complete or partial remission and the period of its duration according to the Karnoff Index.

In about 90% of the tested persons there was a complete or partial remission in tumours of the mammary gland, the ovaries, the digestive tract and the rectum, and the malignity index became normalised.

In infaust cases a longer survival was recorded, and there was a strong mitigation, and even complete disappearance of pain, and the administration of generally toxic palliatives could be substantially reduced.

The eight-year clinical study showed that the preparation proved useful for the long-term therapeutical coverage of patients after the completion of protocolar therapy, when the malignant process is usually regarded as being eradicated, without the removal of the causes of the previous illness.

Patients suffering from repeated virus diseases showed a substantial reduction in the recurrence of their disease, by having the immune respons of their organismus increased, as was verified by laboratory testing. On the basis of these long-term preclinical applications it can be said that the administration of this preparation has proved an improvement of the biological activity of the organism.

Testing was carried out under the supervision of Karel ČERNÝ, M.Sc. and Zdeněk REJDÁK, Ph.D.
RESEARCH LABORATORY, TĚCHONÍN, J.E. PURKYNĚ, MILITARY MEDICAL FACULTY, CZECH ARMY.

The purpose of the testing was to find whether it would be possible to use the preparation to influence immunocomplete cell systems and to gain basic information about the immunomodulating potential of the preparation. Its costimulative activity was monitored, and it was found that in concentrations from $1.5 \times 10^{-3}$ to $1.5 \times 10^{-9}$ g/ml the preparation inhibits significantly ($p \leq 0.05$) the lymphoproliferative response of splenic cells to Con A. PHA and LPS added to the cultures in optimum concentrations.

Other tests were carried out to find the proliferative response of splenic cells to mitogens after the administration of the preparation in vivo. The preparation was administered to animals orally during seven days. In this case, to the proliferative response of splenic cells to Con A (5 mg/ml) increased significantly ($p \leq 0.05$) by from 0.015 mg/kg to 15 mg/kg of the daily dose. The response of splenic cells to PHA is significant in the full range of the doses used.

The administration of 15 to 150 mg/kg of the preparation in vivo increases the nonspecific cytotoxic activity of adhering peritoneal cells against the targets K 562 and P 815. The same result was achieved in studying the activity of natural killers in the spleens of mice.

SUMMARY: The testings show that the tested preparation influences the function of the immune system either directly or indirectly by neurohormonal regulation. According to findings on the radiation immunodeficient model, the influence of the preparation on the redistribution of blood-forming cells between the medulla, the spleen and the circulation field may be expected. Thus, the preparation may be described as a substance influencing favourably the organism’s immune response.

Testing was carried out under the supervision of Col. Petr PROPPER, M.D., C.Sc.
The Station studied the immunological findings and the dynamics of proteins after the application of the preparation.

The testing involved 12 tumorous women patients aged from 38 to 58, to whom 2 mg doses of the preparation were administered during 25 days. Blood samples were taken before and after administration, and indicators shown in table 1 were determined. The findings were evaluated by the usual statistical methods.

The findings indicated in table 1 show that the preparation influences mainly the nutrition condition markers. There has been a statistically important increase in prealbumin values, which rose from the lower level of 0.219 g/l to 0.267 g/l. Also, a statistically important normalization of the transferin value occurred. The C-reactive protein alpha 1 – antitrypsine figures also show an important change.

The test has revealed an increase in the leucocyte count, a changed distribution of the lymphocytar population and the normalization of the proportion of terminal lymphocytes. A clinical as well as subjective improvement of the patients was noted while the preparation was administered.

Although the set of the tested patients was not fully representative, the unequivocal dynamics of the changes of certain indicators seems to be trustworthy. The dynamics of the changes in those proteins which are dependent on the nutrition condition testifies to the high probability of the favourable effect of the preparation. It seems that like many immunomodulators, the preparation, too, may influence immunomechanisms by the operation of free amino acids or oligopeptides. For example, the primary influencing of prealbumine is probably later projected into a favourable influencing of the lymphocyte population by „thymosin-like“ activity. The reduction of inflammation proteins and trace element carrier proteins will have an equally favourable impact. The presence of trace elements in the preparation is also expected to have a favourable effect. The findings reveal that the studied indicators influencing the immune response mechanisms are showing favourable results after the application of the preparation.

Testing was carried out under the supervision of Prof. J. RICHTER, M. D., C. Sc.
The Institute tested the influence of the preparation on cell cultures and on irradiated cell cultures. The preparation showed a stimulating effect on the cell cultures. The density of the growth of the cell culture is twice to three times higher after adding the preparation than in control cultures. The mitotic activity is much higher than in the control culture: control 3.99%, experimental sample 5.68%. Kidney tissue was used as cell culture.

After irradiating the cell tissues by a subtle dose of ultraviolet radiation, more than 50% of the tissue showed nectaric signs. The tested preparation was applied after 24 hours and the cells began to normalize, the nuclei with nucleoli could be seen distinctly and they showed a good reception of staining agents. There appeared mitoses, which is unequivocal evidence of secondary regeneration. Therefore, the preparation was recommended to the Institute of Clinical and Experimental Medicine for application to persons irradiated after the Chernobyl disaster.

The character of the testing further shows that if worked into a suitable carrier, the preparation will provide protection against ultraviolet radiation and will have a regenerative effect on the skin affected by UV radiation.

Testing was carried out under the supervision of Academicien V. P. KAZNACHEYEYEV.
The purpose of the testing was to establish the biological activity of the preparation. The Institute of Parasitology developed a method of measuring biological activity by means of studying photon emissions with the use of a photomultiplier.

The conclusions made on the basis of the model testing of the preparation is that the preparation influences oxidoreduction and oxidoperoxide conditions, mitigates or eliminates excited energy processes. It behaves like a low-molecular substance with strong penetrating ability. It rapidly increases the energy level of the cell, after which it shows a slow decline in its energy level. After repeated application the preparation improves the general energy balance of the cell.

Testing was carried out under the supervision of Prof. Stefan GRABIEC, D. Sc.
The purpose of the testing was a comparison of the biological activity of the tested preparation with an etalon – the common preparation „Salkoseryl“, produced in the Switzerland. The biotest systém chosen was one based on the reaction of erythrocyte suspension. The assumption was that the biological activity is the greater the larger the proportion of the difference of the selected indicators between the high and low concentration of the etalon in identical conditions. By repeated experiments with the use of the blood of white mice it was found that the studied preparation was 130% more effective than the etalon.

The purpose of further testing was to find a suitable method for measuring the preparation’s standard and to make a comprehensive comparison with the above – mentioned etalon. Eight criteria were used for this purpose. The results, in comparison with the etalon, are shown in the diagrams.

The optimum criteria of measuring the standard of the preparation ares the induction of erythrocyte aggregation (FGA) and the speed of erythrocyte setting. These criteria may be automated and the measuring may be carried out according to a special analyser.

Testing was carried out under the supervision of Georgie CHUICh, M. D., D. Sc.
The testing was carried out on pregnant rats. The experiment had two variants.

a/ The first variant studied the indicator of biological usefulness of the preparation on equal groups of pregnant and non-pregnant rats. Both groups were offered a free choice of pure water and water with a 1% solution of IMUREGEN. The quantity of the liquid consumed was measured daily. An analysis of the measurements has revealed that pregnant rats from the 11th day of gravidity (the whole period of gravidity is 22 days) were showing strong preference for the water with a 1% solution of the preparation (35-40% more in comparison with non-pregnant rats). This shows that in conditions of a free choice, pregnant rats from the second half of the gravidity period prefer to drink a solution containing IMUREGEN.

b/ The second variant studied the influence of the preparation on the weight of pregnant rats and the increase in the level of haemoglobin in the blood of pregnant rats in the newly born rats.

The experimental group was receiving a 1% solution of the tested preparation, and the control group was getting pure water to drink. During the period of gravidity, the animals’ weight and their haemoglobin concentration were studied over 5, 10, 15 and 20 days. It was found that the weight increase was 25% higher in the tested group than in the control group. As from the 15th day, the experimental group showed a 10-15% increase in the haemoglobin content, as compared with the control group. The weight of the newly born rats the experimental group was 18% higher than in the newly born animals in the control group.

Testing was carried out under the supervision of prof. Svetlana ANIKIEVA, M.D., D.Sc.
Application of products Imuregen

In relation to health and disease prevention it is recommended to use only 1 capsule of Imuregen per day, in the weight category up to 50 kg of weight – children’s dose. Prophylactic dose for adult person is 2 capsules. For overweight and obese individuals the dose is doubled, the dose for individuals with critical BMI values of 32.5 and above - the recommended dose is increased to triple. Due to the large loss of zinc in sweat (loses up to 80% zinc), we recommend administration of one tablet of Imuregen Chelated Minerals. The dosage is adjusted according to climatic conditions - at elevated temperatures, additional tablet of Imuregen Chelated Minerals should be taken.

For healthy individuals with increased physical activity (sports activity, wellness gym) we are adding one tablet of Imuregen Eleutheroococcus regularly. During expected increased physical activity, such as sport competition, athletic competition and other sport activities, we recommend 2-3 capsules of Imuregen Eleutheroococcus. It is indeed proven that this drug significantly reduced levels of lactic acid, thereby reducing the feeling of fatigue. In accordance with Australian literature studying various athletic and sport activities (swimming, cycling, rugby, gymnastics), this regime is very efficient and reduces the risk of infections in athletes with high training loads.

Administration of product Imuregen in chronic fatigue syndrome should be differentiated: we distinguish two units of chronic fatigue syndrome. We choose different treatment strategy in CFS induced by EB virus infection and completely different strategy for fatigue syndrome with clear clinical picture, where we first have to eliminate fatigue causes such as: pulmonary diseases, hematologic diseases, metabolic diseases, malignancies, infections of the urinary tract and overtraining syndrome. With CFS EBV infection (definite diagnosis confirmed by PCR technique with the findings of more than 400 particles of EB virus in saliva) we recommend: two capsules of Imuregen or two teaspoons of Imuregen liquid, and two tablets of Imuregen with Beta Glucans preparation and one tablet of Imuregen with Chelated Minerals. The minimum treatment period is six months, then we check the value of the EBV virus in saliva by PCR technique. In the case of negative value of the virus we change the treatment scheme to the normal maintenance dose, as it is used in the healthy population, with paying attention to the weight of the individual and other factors.
In CFS not induced by EBV infection, and excluding the above clinical manifestations, we recommend administration of Imuregen capsules at a dose of at least four capsules a day and two capsules of Imuregen Eleutheroococcus and one capsule of Imuregen with Chelated Minerals. For most cases of CFS, especially where we measure mental stress score (particularly in patients with long-term unclear diagnoses stigmatized often by inadequate treatment), we also give one capsule of Neurofit.

In case of cancer diseases, nucleotides are used as adjunctive treatment which keeps the immune system in best performance. During radiation treatment we take advantage of the ability of pectin fibers to reduce radiation impact and we give Imuregen with fiber – Imuregen V 270. During radiation treatment we recommend to administer at least six capsules a day, then according to the clinical course of the patient’s condition we serve the daily maintenance dose of four capsules of Imuregen V 270.

The initiation phase before the start of chemotherapy, respectively of radiotherapy, is supported by administration of Imuregen with Vit.C+E. We recommend two tablespoons a day for at least fourteen days before beginning of treatment. Increased intake of antioxidants reduces oxidative stress and reduces the risk of side-effects during treatment. We use the same mode in the preoperative phase of all cancer diseases, and the same applications regime at least fourteen days prior to surgery. During recovery time we reduce the dose and serve Imuregen with Chelated...
minerals in doses that we adjust accordingly as per observed plasma concentration values of zinc and foremost the values of iron (we monitor the risk of anemia by monitoring index of the ratio of transferrin receptors to ferritin). After all cancer treatment and in the recovery period we follow patients for the period of at least 4 years (monitoring of tumor markers, inflammatory proteins - CRP, SAA, IL6, orosomucoid, prealbumin and calculated ratio between orosomucoid and prealbumin - CSI Cancer Serum Index). Recovery period requires the same fundamental mode of the administration of Imuregen as in healthy individuals, but always keeping an eye on the current state of the individual, and in the case of finding of inflammatory symptoms, the incidence of viral infections, herpetic infections we apply “fighting ” dose of Imuregen with Beta Glucans with a minimum dose of two tablets twice daily.

The administration of Imuregen in patients with Type -2 diabetes requires the same treatment as the prevention in a healthy population, but it is necessary to monitor number of factors. Particular attention should be paid to diabetics with increased weight, where increased loss of minerals occurs, and also changes in microbial flora of intestinal tract (GALT microbiome ) and the resulting risks of frequent infections of mucosal surfaces. The impact of the above mentioned factors is high demand for reparations/regeneration of mucosal surface. These demands are covered by increased administration of Imuregen. Here we prefer to use Imuregen liquid at a dose of at least two teaspoons a day. As with other infectious states, in acute infections in Diabetics we recommend administration of increase dose of Imuregen liquid at a dose of up to one tablespoon three times daily with a support of one tablespoon of Imuregen with Vit.C+E. The rationale for this procedure is that, the preparation Imuregen not only contains nucleotides, but many other components with antibacterial properties, for example: antimicrobial peptides and proteins, defensins, hepcidins, cathelicidins, lysozymes, calprotectins, etc. The importance of these components of Imuregen culminates in recent years, when there is a continual growth of antibiotics resistant microorganisms and antibiotic treatment of many infections often fails.

Another positive effect of the administration of Imuregen is the influence on growth of mucosal GALT, and positive influencing of bacterial components with an effect, that may affect the metabolism of energy storage, regulation of values of Leptin and its effect on immunoregulatory mechanisms. At this point it is necessary to emphasize the general positive effect, which the long term administration of these Imuregen products have on preventing mild inflammatory reaction. It is known that subclinical and persistent inflammatory stimulation with the presence of relatively low levels of CRP are an inducer of atherosclerotic plaques. With Type -2 diabetics , according to our experience, this risk is many times higher, mainly due to the frequently increased weight, recurrent infections of the respiratory tract and especially infections of urinary tract and in the case of our population is graded by relatively high prevalence of smoking, where smokers are at a high risk of damage to the immune mechanisms due to oxidative stress and exposure to toxics in tobacco smoke.

In inflammations of the joints we always define the origin of the disease and distinguish the administering of Imuregen products: in autoimmune joint disease and in post infectious joint disease (Chronic Borreliosis). In both cases we follow the basic regime for healthy individuals and it is also recommended to use Imuregen with Beta Glucans in the maintenance dose of one tablet daily, and in the event of flare up of the disease- two tablets a day. Other Imuregen products are chosen more or less according to the recommended treatment of the rheumatologist, in which case we follow balancing of oxidative stress with the application of Imuregen with Vit.C+E. at a dose of one teaspoon twice a day.

In diseases resulting from impaired intestinal mucosa, administration of Imuregen is chosen according to the clinical diagnosis. Here are some selected examples of where we use the effects of the Imuregen products: For HP disease after re-treatment of the disease (according to the Maastricht Consensus triple) we try to repair the affected mucous membrane by application of
**Imuregen liquid** one teaspoon twice a day. Continuously monitor for the incidence of HP in the organism (HP ELISA test in the stool). After a period of convalescence we pay attention to maintaining good condition of the immune status by continuous use of the Imuregen preparation as in healthy individuals. Virtually the same process is kept with patients treated by a gastroenterologist for inflammatory disease of GI (ulcerative colitis, Crohn's disease) with the fact, that with these individuals we recommend intermittent application of **Imuregen with Vit.C+E**.

Dysmicrobia manifestations (including those after long term antibiotherapy) we solve by application of **Imuregen with Beta Glucans** at a dose of one tablet twice a day for at least two months and simultaneously we give quality probiotics (multi strain probiotics). With these diseases we base dosage formulations by clinical manifestations of the disease and the patient's symptoms and, of course, we follow the influence of supplementation quality by testing of values of faecal calprotectin.

At this point, we note that all manifestations of inflammatory gastrointestinal tract can be very well monitored by monitoring values of salivary albumin and salivary CRP. (C-Reactive Protein)

Diseases resulting from stress stimuli are handled individually, the optimum is a basic examination of salivary cortisol values for the definition of stress load. It is known that psychos-immunomodulation can control a number of different clinical manifestations. With these patients we recommend Neurofit in higher and long-term doses at least two to four capsules a day for one month, then two tablets daily. We do not forget the possibility of **seasonal affective disorder**, so we regularly monitor the patients and adjust dose accordingly.

In our practice we very often meet with the disabilities of function of liver parenchyma, these are usually post-infectious inflections (hepatitis A, B, C, E, infectious mononucleosis, and others). For these individuals, we recommend use of **Imuregen DNA** at an initiating dose of three capsules a day for a period of one month and then maintenance dose of one capsule twice a day. We realize that the composition of **Imuregen** is similar to number of pharmacological drugs with hepatoprotective effect. In the toxic liver damage (acute ethanol disability) we have used repeatedly mega-doses of Imuregen product with excellent effect and clear impact on the repair of entire spectrum of liver tests, after a six-week Imuregen administration. In these patients, we have used a dose of two **tablespoons** of Imuregen liquid per day throughout the monitored period until normalization of both: the laboratory and the clinical findings. Due to the psychological impacts we have continued in these patients with administration of **Neurofit** at a dose of one capsule twice daily.

Towards the note on the possible effects of the **Imuregen** in terms of increased blood pressure, we state that we cannot confirm your findings. We believe, however, that it would be appropriate to verify this claim, not only with the administration of the preparation, but also with the placebo effect, of course, with corresponding standard measurement technique (repeated BP measurements in thirty minutes intervals).

An interesting chapter is the administration of Imuregen in individuals with allergies. Given that a large proportion of allergic individuals whom we monitor are undergoing oral vaccination regimen using appropriate allergens (allergy monitoring measuring the specific IgE method CAP phadiatop) we have positive experiences with these individuals, with adding of any of available **Imuregen** product in dosage, which corresponds to dose of healthy population, or adding concentrated agent directly into the oral vaccine. In these patients findings of improvement are in both laboratory and clinical.

Fat metabolism (cholesterol, HDL, cholesterol index) can be affected in our opinion, only partially and we do not find even after long-term administration of the preparation significant changes in values. More or less the effect could be achieved by long-term administration of **Imuregen V 270**, however for a really good answer we would have to perform necessary studies with a placebo.
Applications of nucleotides and related products is widely monitored and studied and at present we have at least sixty papers dealing with this issue. I refer to the available database, or we can provide a list of citations of works that deal with this issue. In any case, there are very interesting studies of Australian origin, following application of nucleotides to top athletes, and polar explorers. These studies are supported by high-quality laboratory testing, monitoring especially the parameters of mucosal immunity, which comports with us- we also advocate for non-invasive methods of samples of biological material.

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Abstract

The informational aspects of nucleic acid synthesis have attracted much more attention than the quantitative significance of DNA, rRNA, tRNA, and nucleotide synthesis. Animal and human studies suggest that in energetic terms, 5-10% of the energy used in synthesising tissue protein is expended in manufacturing an appropriate amount of synthetic machinery, that is the ribosome and tRNA. The two sources for synthesis of nucleotides are salvage of nucleotides released by intracellular degradation or derived from the diet, and nucleotides synthesised de novo from amino acids (for example, glutamine) and sugars (glucose). The comparative importance of these two processes is not well defined, but rRNA production requires a high de novo input in cell types with the capacity for rapid division (for example, lymphocytes). The gut is unusual in requiring a ready arterial supply of nucleotides synthesised by hepatic de novo pathways. Animal studies show that an exogenous supply of nucleotides (salvage) can improve liver regrowth, immune responsiveness to a microbial challenge, and gut morphology in diarrhoea models. Humans adapt to dietary nucleotide intake by downregulating de novo pathways. All total parental nutrition regimens, and most enteral regimens lack nucleotides, which may predispose to an inadequate supply of preformed nucleotides to gut and immune cells in the critically ill, artificially fed patient. Unfortunately, there are no clinical studies that answer this point at present.
Abstract

Dietary nucleotides, like glutamine, have attracted attention as a key ingredient missing from nutritional formulae for many years. They are the building blocks of tissue RNA and DNA and of ATP and their presence in breast milk has stimulated research in babies which has indicated that supplementation of infant formula milk leads to improved growth and reduced susceptibility to infection. Animal studies have confirmed some of these data. In particular, dietary nucleotides modulate immune function, promote faster intestinal healing and have trophic effects on the intestine of parenterally-fed rats which are similar to those resulting from glutamine supplementation, but at much lower intakes. Nucleotide supplementation has also been shown to improve some aspects of tissue recovery from ischaemia/reperfusion injury or radical resection. There is, however, a fundamental paradox. The intestine and liver possess powerful homeostatic mechanisms which degrade intake of purines and pyrimidines (i.e. salvage) and replace it with de novo synthesised output. It is possible that peripheral tissues receive only small amounts of nucleotides of dietary origin. Previously, nucleotides have been proposed as being conditionally-essential nutrients that provide an adequate supply of purines and pyrimidines for nucleic acid synthesis in neonates or in the stressed patient. This review explores this puzzle in the light of recent data from nutritional studies and from research into purinergic signalling in the intestine, heart and cells of the immune system. We propose that dietary nucleotides should be considered within a pharmacological and metabolic framework.

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Abstract

Stimulation of ileal epithelium growth and regeneration by dietary nucleotide extracts.

Slízová D¹, Síma P, Richter J, Krš O, Zavadilová J.

Abstract
The gastrointestinal tract epithelium plays an important role not only in digestion and absorption of nutrients, but also in antigen and pathogen signal translocation toward the gut associated lymphoid tissue. Malnutrition in various degrees is recognized as the most common cause of the immune system dysfunction. Research done in the past several years has revealed that dietary nucleotides (dNT) represent an essential compound of nutrition because of their importance in metabolic pathways, energetic processes and nucleic acid synthesis during tissue renewal. Much evidence accumulated suggests that dNT are essential for the growth and maturation of the gut epithelia. In previous experiments we have documented immunoregulative properties of dNT-containing extracts. In this study Balb/c female mice were fed (1) standard diet, (2) dNT-supplemented diet, and (3) dNT-supplemented water for 4 weeks. The supplement in dose of 100 mg/kg/l comprised original extract (Imuregen, Uniregen Ltd., Náchod, Czech Republic). Samples of terminal ileum in each dietary group were removed for histological examination. The length of villi was evaluated by computer morphometry. The highest growth of intestinal villi was observed in group administered dNT-supplemented water. We have found no pathological changes of intestinal epithelium in any experimental group.

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THE QUEST FOR A CURE

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THE BEGINNINGS

As I look back on my life and career and wonder what were the influences and events that shaped them, I realize how little one appreciates what is happening at the time it occurs. The love and support of my parents, which I took more or less for granted while I was growing up, allowed me to take the "road less traveled by" and to persist in the face of considerable obstacles. Who were these parents?

My father, who graduated from New York University School of Dentistry in 1914, had come to the United States from Lithuania as a small boy. My mother came from Russia in 1911 at the age of 14. By the time she was 19, she was married to my father who had then been a dentist for two years. A year and a half later I was born.

Although my mother never had a college education, she was a prodigious reader and the scope of her reading was astounding. My father also loved to read and I can remember many evenings during my childhood when I was exposed to a variety of literature, poetry, history, biography, and fiction, being read aloud by my father.

My mother had more common sense than anyone I have ever known. I am certain she would have been successful in any career she could have undertaken. Although she never complained about the fact that this had not been an option for her in those days, it was apparent that she wanted me to have every opportunity to be independent and to have a satisfying career. This became very important later when I was unable to find a job and continued to rely on my parents for a number of years after graduation from college.

When I was born, we lived in an apartment adjoining the dental office in what was then a middle-class neighborhood in Manhattan, 116th Street and...
Madison Avenue. My brother, Herbert, was born just as I was starting school. Shortly thereafter, we moved to the Bronx, which was then considered a suburb. I loved school. I had a great desire to learn and the mere acquisition of knowledge gave me excitement and pleasure. I had learned to read well before I entered the first grade. It was the custom at that time to skip children who were achieving more than was expected of them. By the time I had finished junior high school, I had been skipped four times and was two years ahead of most of my classmates. This presented some social problems for me during my teenage years.

My high school career was unremarkable. I went to Walton High School, an all-girls school with a good academic curriculum. We were required to take two foreign languages, mathematics, science, English, history—in essence, a college preparatory program. The Wall Street crash of 1929 had changed our life style since my father had lost very heavily in the stock market. By the time I graduated from high school, the depression was at its height and Franklin Delano Roosevelt had just been elected. It is questionable whether my brother or I would ever have gone to college if we hadn’t been living in New York where Hunter College and the College of the City of New York had free tuition, if your high school grades were good enough to qualify you for admission.

The summer before I entered college, my maternal grandfather died of stomach cancer. He had been very close to me during my preschool years. The suffering I witnessed during his last months made a great impression on me. I decided that a worthwhile goal for my life would be to do something to help cure this terrible disease. It was a goal that I never relinquished. I decided I would major in chemistry in college, since that seemed to me to be the best road to the discovery of drugs that could accomplish my goal. I did not know that years later my mother would also die of cancer.

Hunter College was an excellent school and we had a remarkably large class of chemistry majors, about 75, for a woman’s college. Most of the women were planning to teach, but a few of us were determined to be laboratory scientists. We were undoubtedly a little naive not to realize what was awaiting us on graduation in 1937. The depression was still with us and the few available jobs or graduate school assistantships were definitely not for women. What had made me think that graduating “summa cum laude” would open any doors for me to a research laboratory?

After an entire summer of job-hunting and of sometimes being told, “We have never had a woman in the laboratory; we think you would be a distracting influence,” my perseverance began to falter. I decided I had better go to secretarial school and learn something useful. After six weeks of secretarial school, I was rescued by the New York Hospital School of Nursing. They offered me a job as a laboratory assistant, helping to teach the Biochemistry
course for nurses. It was only for a trimester and I would be unemployed again at the end of the three months, but I didn’t hesitate. My secretarial career was over.

Several months after this reprieve, I felt fortunate to obtain a job working for nothing for a young chemist, Alexander Galat, whom I had met socially. He had been given a laboratory in a small pharmaceutical company as partial payment for a diagnostic test he had invented. After six months he was permitted to pay me $12 per week. By the time I left, I had one and a half years of good laboratory experience, was earning $20 per week, and had saved enough to go to graduate school at New York University for my Master’s degree. I was able to do the research for my thesis on a part-time basis, while teaching chemistry and physics in the New York City high schools. My teaching career, as a so-called “permanent substitute,” lasted two years. I enjoyed it some of the time, especially when I had good students, but I was still waiting for the opportunity to get into research.

World War II finally did what I had been unable to do on my own: it opened the doors for women to work in chemistry laboratories. While the men were away, employers had to take the “risk.” My return to the laboratory was as a quality control chemist in a food laboratory. Testing the acidity of pickles, measuring the color of mayonnaise and determining the concentration of sugar in preserves was not very glamorous, but I was earning money and I was learning to use instrumentation I had never seen in college. After a year and a half, it was time to move on.

I was offered a job in a new research laboratory at Johnson and Johnson in New Brunswick, New Jersey. One of their vice presidents had decided that the company should go into the pharmaceutical business and had hired an experienced organic chemist, Alan Pierce, to head the laboratory. I was one of his two assistants. We were soon synthesizing sulfonamides and I was beginning to feel that I was finally on my way. Six months later there was a change in vice presidents and the decision was made not to go into the pharmaceutical business after all. I was told I could stay on, measuring the tensile strength of sutures. That was not quite what I had in mind for my future. I began job-hunting again.

THE OPPORTUNITIES

Introduction to Nucleic Acids

My job interview with George Hitchings at Burroughs Wellcome Co. was unusual in that, instead of asking me a lot of questions about myself, Hitchings proceeded to tell me what he was doing. I had not heard much about nucleic acids before, nor did I know what purines and pyrimidines were. The idea
that one might possibly interfere with cell division by making antimetabolites of the structural bases of nucleic acids sounded exciting. Hitchings apparently thought my scholastic record and laboratory experience were sufficient to fulfill his requirements for a chemist and promised to call me in a week. I went home excited at the prospect that I might get this job and determined to hold off accepting any other offers (of which there were then two) until I had heard from George Hitchings. A week later the offer came. On June 14, 1944, I went to work at the Wellcome Research Laboratories at a yearly salary of $2600.

My first assignment was to make 2-thioparaxanthine (2-mercapto-6-hydroxy-1,7-dimethylpurine), which someone wanted to test for its antithyroid activity. This involved following the methods of Emil Fisher described in the old German literature. Fortunately, my knowledge of German was adequate, but the reactions involved were difficult. In particular, the first step, which involved heating theobromine with phosphorus oxychloride for six hours in a sealed glass tube at 160° C and rocking the tube every hour to facilitate solution of the solid, was a bit frightening. The glass tubes were encased in metal pipes and heated in a gas-lit oven. It was a hot summer and our laboratory was not air-conditioned. Moreover, there were dryers for a dextromaltose product directly below our laboratory and the temperature of the floor was generally over 100° F. The work-up of the reaction mixture was also difficult and there were multiple steps thereafter. Nevertheless, by the end of July, I had successfully synthesized 1.5 grams of 2-thioparaxanthine.

During that first summer I learned as much as there was to learn about purines from the sparse literature on the subject. The synthetic papers were mainly in German, the works of Traube, Fisher, Isay, and Biltz published in the early 1900s and a few English papers by Bogert and Davidson. I devoured the book on Nucleic Acids by Levene & Bass and realized how little was really known about the structure or biosynthesis of the nucleic acids. The arrangement of the nucleotides in nucleic acid was still being debated in the early 1940s. Were they linked through the sugars or through the phosphate moieties? Was the sequence the same in all nucleic acids, with a tetr successful organic synthesis which involved heating theobromine with phosphorus oxychloride for six hours in a sealed glass tube at 160° C and rocking the tube every hour to facilitate solution of the solid, was a bit frightening. The glass tubes were encased in metal pipes and heated in a gas-lit oven. It was a hot summer and our laboratory was not air-conditioned. Moreover, there were dryers for a dextromaltose product directly below our laboratory and the temperature of the floor was generally over 100° F. The work-up of the reaction mixture was also difficult and there were multiple steps thereafter. Nevertheless, by the end of July, I had successfully synthesized 1.5 grams of 2-thioparaxanthine.

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in 1944 that DNA prepared from one strain of pneumococcus could transform another.

Since so little was known at that time about the enzymes and metabolic pathways by which the nucleic acid bases were biosynthesized or incorporated, it was almost an act of faith that one might be able to synthesize antimetabolites of these bases that would actually be utilized by cells, or that could prevent the utilization of essential metabolites. Nevertheless, it was known that certain lactic acid-forming bacteria could grow and multiply on defined media if one of the four “natural” purines (adenine, guanine, xanthine, hypoxanthine) was supplied together with thymine. Thus, these bacteria obviously had the pathways for the interconversion of adenine and guanine and for the amination of hydroxypurines. The enzymes responsible for these interconversions were not isolated or named until the 1950s. Alternatively, one could supply an acetone powder of liver, the so-called “L. casei factor” and the microorganism would make its own purines and thymine. The structure of this growth factor, folic acid, was not known until 1946. By the time I arrived in the laboratory, Hitchings, with the aid of a young woman, Elvira Falco, whom he had enticed away from the Bacteriology laboratory, had set up an assay with *Lactobacillus casei* in which one could test a compound for its ability to substitute for or to antagonize a purine, thymine, or folic acid. This test system was valuable, not only for the identification of inhibitors of cell division, but for the accumulation of a body of knowledge from which one could deduce the existence and structural requirements of enzymes not yet known and pathways not yet elucidated.

After a few months in the laboratory, I was making other purines besides thioparaxanthine, as well as pyrimidines and pteridines. Some of these were actually compounds that had never been described before and I felt the excitement of the inventor who creates a “new composition of matter.” The pteridines presented a real challenge to a young chemist. They were not crystalline, were highly insoluble, could not be re-crystallized from water or organic solvents and had no melting points. They could be synthesized from 4,5-diaminopyrimidines by closure of the pyrazine ring with diketo compounds. The 4,5-diaminopyrimidines were also intermediates for the synthesis of purines.

One of the instruments that we sorely lacked in those days was a photoelectric spectrophotometer. We did have an old Bausch and Lomb spectrometer, which had a carbon arc as a light source. The relative absorption of a solution at various wavelengths was recorded on a photographic plate, with the solvent as control. One had to match the density of silver grains visually to determine the degree of absorption. My first paper published from Hitchings’ laboratory was one dealing with “The Ultraviolet Absorption
Spectra of Thiouracils." I still look at that paper with some amusement when I see the peculiar irregularities in the curves resulting from the methodology we had used. Our first Beckman DU Spectrophotometer, obtained in 1946, was cause for great celebration.

**Leukemia**

After a little more than a year in the laboratory, the biochemistry of nucleic acids became more challenging to me than the mere synthesis of new compounds. The compound that was to teach me much about purine utilization and interconversions, as well as to excite me by its activity against human leukemia, was 2,6-diaminopurine, which I synthesized in 1948. The synthesis had been described in the German literature in the early 1900s. It seemed like such a simple analog, a possible antagonist of either adenine or guanine. According to *L. casei*, diaminopurine was an adenine antagonist, since the only purine that could reverse the inhibitory activity of high concentrations of diaminopurine was adenine. Somewhat surprisingly, diaminopurine also had the characteristics of a folic acid antagonist, since its effects could be reversed by folic acid. This fact, together with data on a number of pyrimidines and pteridines, led us to the conclusion that the 2,4-diaminopyrimidine moiety could confer antifolate properties, no matter what else was attached to the molecule. This finding was to prove an excellent lead into the development of the antimalarial and antibacterial diamino pyrimidines by others in our laboratory (1).

In 1947 Hitchings had established a link with the Sloan-Kettering Institute, then headed by Cornelius Rhoads. This connection enabled us to send compounds there for antitumor testing in mice and was the basis of many collaborative efforts in the following decade. Joseph Burchenal soon found that 2,6-diaminopurine had activity against a mouse leukemia and could produce increases in survival similar to those with methotrexate, although at a higher dose. Diaminopurine also showed activity against vaccinia virus, a DNA virus, in Randall Thompson’s hands at the University of Indiana.

After Fred Philips had studied the toxicology of diaminopurine in animals and Burchenal had found that it produced remissions in two adults, one with acute myelocytic and one with chronic myelocytic leukemia, interest in the compound intensified. One of the remissions lasted over two years, during which time the young woman gave birth to a child. Unfortunately, she then relapsed and was resistant to further therapy. Two other patients failed to respond at all. Although most patients could not tolerate diaminopurine because of nausea and vomiting, it seemed as though we were on the threshold of a "breakthrough."

In the next few years we spent a great deal of time studying the utilization
and interconversion of purines, nucleosides, and nucleotides in \textit{L. casei}, in collaboration with M. Earl Balis and George Brown, using radioactive precursors. Of great help was the investigation of the diaminopurine-resistant strain of \textit{L. casei} that we had isolated. From these studies \cite{2} we concluded that: “The biochemical nature of the mutation involved in diaminopurine resistance would appear to involve the alteration of an enzyme system which deals, normally, with the incorporation of adenine,” and that the “assumption is that diaminopurine is incorporated into an analogue of the metabolite via the direct pathway by which adenine is incorporated and that it is the diaminopurine-containing analogue that is the active inhibitor.” Two years later, Arthur Kornberg described the isolation and properties of adenine phosphoribosyltransferase and our enzyme had been identified.

The period of the 1950s was a golden age in nucleic acid biochemistry. It was the time when John Buchanan, G. Robert Greenberg, and their groups unraveled the multiple steps of purine biosynthesis and found that inosinic acid (hypoxanthine ribonucleotide) was the first purine compound formed in the biosynthetic pathway. The double helical structure of DNA was proposed by Watson and Crick. Kornberg described the enzymatic synthesis of DNA by DNA polymerase. Every Spring meeting of the Federation of American Societies for Experimental Biology revealed new and astonishing information about nucleic acid synthesis.

Excited by the wonderful knowledge that was unfolding, I decided to continue my formal education and obtain a PhD degree. Needless to say, I had no thought of giving up my job. The only solution was to attend graduate school on a part-time basis. Brooklyn Polytechnic Institute was the only school offering graduate classes in Chemistry at night. For two years I went to Brooklyn several nights a week, which involved a long commutation from Tuckahoe during rush hour, and a one hour subway ride back home to the Bronx after class, late in the evening. Suddenly, this came to an end. The Dean informed me that if I was really “serious” about my graduate work, I would have to give up my job and go to school full time: I told him this was not possible. My attempt to obtain a PhD was over. For years I wondered whether I had made the right decision. Now I know I did.

While the biochemical studies occupied some of my time, I continued to synthesize a variety of purines, pyrimidines, and pteridines. One of the synthetic procedures that was to play an important part in my work was thiation, the direct exchange of sulfur for oxygen in heterocyclic systems. In 1943, Carrington had described the thiation of hydantoins with phosphorus pentasulfide in an inert, high-boiling solvent, tetralin. I used the method successfully for a number of pyrimidines and quinazolines, but had much more difficulty applying it to the purines. Part of the problem lay in the
insolubility of guanine, xanthine, and hypoxanthine in an organic solvent like tetralin. When the reaction took place, the product coated the starting material and no further reaction occurred. There was also the problem of separating thioguanine from guanine or 6-mercaptopurine from hypoxanthine. Unlike pyrimidines, purines were difficult to chlorinate, and that route to the mercapto compounds was unsatisfactory at that time.

With persistence and some changes in solvent and reaction conditions, I finally succeeded in isolating and purifying thioguanine and 6-mercaptopurine. Both compounds behaved like purine antagonists in \textit{L. casei}. Our attention centered on 6-mercaptopurine (6-MP) at first, since it was the easier one to synthesize. When it was tested at Sloan-Kettering in early 1951 against Sarcoma 180, Donald Clarke reported that the tumor was not only inhibited but, in many of the mice, it completely regressed or proved to be nonviable on transplantation. The compound was also active on several mouse leukemias. Burchenal was extremely anxious to test 6-MP in children with acute leukemia.

In those days children with acute leukemia had a mean life expectancy of 3 months if untreated. With the use of cortisone and the antifolate methotrexate in 1949, that average survival time was increased to 6 months. By 1953, Burchenal and his group had extended the mean survival time of leukemic children to one year by the addition of 6-MP to their treatment. By the end of 1953 the drug had been approved by the FDA for the treatment of acute childhood leukemia. At a three-day symposium sponsored by the New York Academy of Sciences in 1954, much of the data on 6-mercaptopurine was reported (3).

It took years before the work of a number of different groups revealed all of the loci of action of 6-MP. One of our early insights was based on studies with a 6-MP-resistant strain of \textit{L. casei}. In 1953, we deduced that, since the 6-MP-resistant mutant was unable to grow with hypoxanthine as the purine source, 6-MP and hypoxanthine must require the same enzyme for utilization. In addition, we suggested “that a hypoxanthine-containing metabolite may be an intermediate in the conversion of adenine to guanine in \textit{L. casei}, and the transformation to a guanine-containing substance is viewed as a possible site of action of 6-mercaptopurine” (4). The identification of inosinic acid as the first purine nucleotide formed by de novo biosynthesis had not yet been made. The many loci of action of 6-MP ribonucleotide, its conversion to thioguanosine, and the subsequent incorporation of thiodeoxyguanosine into DNA have been reviewed many times (See refs. in 5, 6). As new chemotherapeutic drugs joined the armamentarium for fighting leukemia, 6-MP remained one of the mainstays in the multi-drug regimens, passing from being a remission-inducing agent to one still used in maintenance therapy. Thioguanine (6-TG), which
was developed several years later, found its niche in the treatment of acute myelocytic leukemia in adults, particularly in combination with cytosine arabinoside.

Within a year of the time 6-MP became an accepted treatment for acute childhood leukemia, it became apparent that this drug was not going to be the cure we had all hoped for. Relapses occurred and patients often no longer responded to 6-MP and methotrexate. These were heart-breaking times. We seemed to be so close to the solution and yet the ultimate goal eluded us. We still had much to learn about leukemia, its persistence in "privileged" sites such as the central nervous system, about the need to continue treatment long after remission had occurred, and the importance of multiple drug regimens to prevent or overcome resistance.

Our approach to improving upon 6-MP was, in hindsight, somewhat simplistic. We would make a variety of derivatives of 6-MP and explore their structure-activity relationships, study the mechanism of resistance to 6-MP, and attempt something that was quite unusual at that time, investigation of the pharmacokinetics and metabolic fate of 6-MP.

In 1950, the separation of nucleic acid bases, nucleosides and nucleotides by ion-exchange and paper chromatography had been reported by Waldo Cohn and C. E. Carter. Radioactive sulfur had become available and I was able to synthesize $^{35}$S-6-MP from 6-chloropurine. The radioactive samples were counted with a Geiger flow counter, necessitating drying each sample on planchets to make infinitely thin films. The ion-exchange columns were bulky and the flow rates variable; the separations frequently took days. Nevertheless, after some metabolism studies of 6-MP in mice by Samuel Bieber and myself, the radioactive drug was studied in several leukemic patients, in collaboration with Leonard Hamilton of the Sloan-Kettering Institute (3).

Our methodology did not permit us to do pharmacokinetics in the way it can be done now with high pressure liquid chromatography and mass spectrometry. However, fractionations of the urine samples on Dowex-1 and Dowex-50 ion-exchange columns led to the isolation and identification of a number of metabolic products. We began to understand the various catabolic reactions to which 6-MP was subject, e.g. methylation and oxidation of the sulfur, removal of the sulfur to form inorganic sulfate, and oxidation of the purine ring on the 2 and 8 positions.

The methodology improved with the advent of scintillation counters, but we did not get our first high pressure liquid chromatograph until 1969. The metabolic studies were to play an important role in our understanding of species differences in the disposition of the thiopurines and consequent differences in therapeutic effectiveness. The large number of structural modifications of 6-MP and 6-TG that were made and tested in our laboratory
in the 1950s were made largely on an empirical basis. We did not yet have
the kind of knowledge of the substrate and inhibitor specificities of the purine
and pyrimidine metabolizing enzymes that was acquired later.

Two derivatives made with the specific intention of providing masked forms
of 6-MP and 6-TG were their 6-S-(1-methyl-4-nitro-imidazolyl) derivatives,
azathioprine (Imuran®) and thiamiprine (Guaneran®). These compounds were
expected to be vulnerable to nucleophilic attack between the sulfur on the
purine and the methyl-nitroimidazole ring because of the ortho-nitro substit­
uent. Hopefully, this might happen preferentially inside leukemic cells,
releasing 6-MP and 6-TG. These derivatives were as active as 6-MP and 6-TG
against Adenocarcinoma 755 in mice, but appeared to be less toxic and,
therefore, to have a better therapeutic index. We enlisted the collaboration of
R. Wayne Rundles at Duke University to study their metabolism and
antileukemic activities in man (6, 7). Although our laboratories were some
500 miles apart, the blood and urine samples were flown back and forth
between LaGuardia Airport and Raleigh-Durham with regularity, usually
packed in Rundles’ little black leather doctor’s bag. These studies showed
that these prodrugs were indeed converted to 6-MP and 6-TG, and were as
active as these thiopurines in chronic myelocytic leukemia. Although some
quantitative differences were discerned between the metabolism of 6-MP and
azathioprine in man, there was no indication of an improved chemotherapeutic
index in leukemia.

With Rundles we were able to test the clinical efficacy of a number of other
substituted derivatives of 6-MP and 6-TG that had shown good antitumor
activity in mice. In patients with chronic myelocytic leukemia who were not
very ill, efficacy could be evaluated within a few weeks, while the metabolic
studies were in progress. Patients who did not respond to the new derivative,
as measured by the granulocyte count, were given 6-MP or 6-TG to test
whether the leukemia was responsive to these effective thiopurines. The
6-alkylthiopurines, which had shown good activity in the mice, were inactive
in man, although the patients responded well to 6-MP or 6-TG. The reason
for this discrepancy soon became evident. In mice, the alkylthio groups were
being dealkylated to the free thiopurines. In man, the alkylthio groups were
being oxidized to form alkylsulfinyl groups or were removed from the purine
ring completely (7). Thus, with studies in a relatively few patients, we
eliminated the need for extensive clinical trials of inactive compounds.

Immunosuppression and Transplantation

In 1958, we were unexpectedly precipitated into the field of immunology.
Robert Schwartz, working with William Dameshek in Boston, had obtained
some 6-MP from us and had tested it for its ability to suppress the antibody
response in rabbits to bovine serum albumen. Their rationale was that "immunoblasts," which were formed when lymphocytes responded to the challenge of a foreign protein, closely resembled leukemic lymphoblasts. Therefore, a drug that worked against acute leukemia might have an effect on the immunologically stimulated lymphocytes. And indeed it did. Schwartz worked out the conditions of timing and dose that could produce immunological tolerance in rabbits. In 1958, an understanding of the immune response was in its infancy. There were large lymphocytes and small lymphocytes, but it was not at all clear which were more important in the immunological response. Plasma cells and macrophages were known, but no one had yet discovered B cells and T cells, let alone different kinds of T cells. Schwartz imbued us with his own enthusiasm and convinced us of our obligation to set up an immunological screen to test some of our other antimetabolites for this interesting property. In the meantime, he offered to test compounds in his rabbit system under code numbers without knowledge of the chemical structures. Bieber, our "in house" biologist, set up a screen for immunosuppression, which consisted of injecting sheep red cells into the tail vein of mice and examining the mice for their antibody response a week later.

Having a test for immunosuppressive activity turned out to be extremely useful, not only for what it taught us about the immune response, but because it revealed some interesting differences between rabbits and mice with respect to azathioprine. In mice, azathioprine was more active than 6-MP in suppressing the immune response, whereas in rabbits azathioprine was inactive. This surprising discrepancy was explained later when we found that azathioprine was metabolized differently in the rabbit than in the mouse or man. Because of high concentrations of aldehyde oxidase in the rabbit, extensive oxidation occurred on the purine ring at position-8, before the compound could be split to 6-MP. Thus, the product released after oxidation and reaction with glutathione was 6-mercaptop-8-hydroxypurine, which is biologically inert. Fortunately, mouse and man are similar in their metabolism of azathioprine.

The story of how Schwartz's report on the immunosuppressive activity of 6-MP inspired a young surgeon, Roy Calne, in England, to try 6-MP to prevent kidney transplant rejection in dogs has been told before (5, 8). Calne visited us on his way to spend a year with the transplant surgeon, Joseph Murray, at the Peter Bent Brigham Hospital in Boston, and we gave him azathioprine to try on dog kidney transplants. I often wonder whether we would have given him that compound if we had not already done the metabolic experiments with azathioprine in mouse and man or studied its immunosuppressive activity in mice. Was it Fate? The use of azathioprine to prevent kidney transplant rejection in man became a reality in 1962. The rest is history. It was 16 years
before the next successful immunosuppressive agent for use in kidney transplants, cyclosporin, became available. Over 200,000 kidney transplants have now been performed worldwide, and heart, liver, lung, pancreas, and bone marrow transplants are also common. Azathioprine is also a recognized treatment for rheumatoid arthritis.

It has often been asked whether azathioprine has any real superiority over 6-MP as an immunosuppressant. That question really cannot be answered by in vitro tests since, in the absence of red blood cells, the conversion of azathioprine to 6-MP is very slow. In a variety of in vivo test systems, the difference between the two drugs is not large but is always in favor of azathioprine, i.e. immunosuppressive activity occurs at a lower dose and the maximum tolerated dose is higher. This difference may be due to pharmacological factors such as the slow release of 6-MP from the red blood cells after the reaction of azathioprine with glutathione. However, it is more likely that azathioprine reacts with other sulfhydryl and amino groups on cell membranes or enzymes, thereby blocking some receptors, in addition to releasing 6-MP.

**Gout and Hyperuricemia**

One of the principal catabolic products of 6-MP is its oxidation product, thiouric acid. We had shown in the early metabolic studies in 1954 that this compound was formed by the enzyme xanthine oxidase. Doris Lorz had studied this enzyme in depth from 1950 to 1956, examining it for both substrate and inhibitor specificity. The time now seemed ripe to determine whether inhibitors of this enzyme could function in vivo and could change the catabolic fate of 6-MP. We chose 4-hydroxypyrazolo(3,4-d) pyrimidine (allopurinol), a compound synthesized by Falco in the mid-50s, for our first in vivo trials in mice. This compound was a potent inhibitor of xanthine oxidase, had no cytotoxic activity on bacteria or tumors, and was well tolerated by the mice. Had we tested a number of the other available inhibitors (as we did subsequently), we would have concluded that this approach was not viable. In mice and in dogs, the oxidation of 6-MP to thiouric acid was markedly inhibited by concomitant use of allopurinol. This increased the antitumor and immunosuppressive activities of 6-MP three- to fourfold. Toxicity was also increased but, in mice, there was an improvement in the therapeutic index. In man, the effect of allopurinol on the metabolism of 6-MP was similar to that in the mouse and the antileukemic efficacy of 6-MP was also increased three- to fourfold, but there appeared to be no change in the therapeutic index. Our initial disappointment gave way to excitement at the thought that a nontoxic in vivo inhibitor of xanthine oxidase might be useful for inhibiting uric acid formation. Indeed, in one of Rundles' leukemic patients in whom the combination of 6-MP and allopurinol was studied, the levels of serum and urinary uric acid did decrease.
The disease in which excess uric acid is a major problem is gout. The next clinical studies followed quickly in collaboration with Rundles and James Wyngaarden at Duke (5, 9). The findings of efficacy were unequivocal. Uric acid tophi dissolved under treatment, serum and urinary uric acid decreased, and side effects were minimal. Although allopurinol has a short half-life in the serum, its oxidation product, oxypurinol, has a long half-life, about 18–24 hr. Moreover, oxypurinol is also a potent xanthine oxidase inhibitor, so that enzyme inhibition is prolonged and the drug needs to be given only once a day. Vincent Massey found that oxypurinol binds very tightly to the reduced form of xanthine oxidase and is released only after reoxidation of the enzyme. With Alfred Gutman and T’sai-Fan Yu, we showed that oxypurinol is reabsorbed in the proximal kidney tubule in a manner similar to uric acid. This accounts for its long plasma half-life. Hypoxanthine and xanthine, the oxypurine intermediates in purine degradation, are excreted by the kidney at close to glomerular filtration rate, or are reutilized for ribonucleotide synthesis. In 1966, the year that allopurinol was approved by the FDA, a symposium held in London under the auspices of the Heberden Society brought together many of the investigators from various parts of the world to present their experiences with allopurinol (10). A new and effective treatment for gout had been launched.

Expanding the Horizons

Although by this time our laboratory had been responsible for a number of important chemotherapeutic agents, only two enzymes of nucleic acid metabolism had been studied in any depth: xanthine oxidase and dihydrofolate reductase. The 2,4-diaminopyrimidines synthesized by Elvira Falco, Peter Russell, and Barbara Roth had led to the antimalarial pyrimethamine and to the wide-spectrum antibacterial trimethoprim (1). When James Burchall joined the department in 1962, he began the isolation and characterization of the dihydrofolate reductases from many different species. As the differences between these unfolded, the particular sensitivities of the enzyme from different sources to different diaminopyrimidines, which had previously been delineated empirically, began to be understood. Moreover, it laid the foundation for the search for new selective dihydrofolate reductase inhibitors. In 1966 we hired Thomas Krenitsky, a young biochemist whose postdoctoral work had been involved with uridine phosphorylase. Our program to investigate in detail the enzymes of purine and pyrimidine metabolism was now launched in earnest. This investigation was to give us, in the years that followed, an understanding of the substrate and inhibitor specificities of the phosphotransferases, phosphoribosyltransferases, kinases, oxidases, and deaminases, as well as of the enzymes of nucleotide interconversion. This would
help us to understand and capitalize on some of the exciting selective activities that we later encountered with protozoa and viruses.

In 1967 Hitchings was promoted to Vice President in charge of Research. The Biochemistry Department was divided into the Department of Experimental Therapy, of which I became the head, and the Department of Microbiology headed by Burchall.

A year later the announcement was made that Burroughs Wellcome Co. would be vacating the premises at Tuckahoe, New York, and moving in 1970 to a new research facility to be constructed in Research Triangle Park, North Carolina. The factory would also move to North Carolina. Both facilities would be much larger and, thus, we could begin to recruit additional staff, which had not been possible in our inadequate laboratory space. While we were able to squeeze a few new people into the Tuckahoe laboratories during the last year before our move, many joined us in North Carolina. The new building was not ready when we arrived and waiting for a few new laboratories to be available each week required a good deal of patience and stamina. Fortunately, the universities in the area, Duke University, the University of North Carolina at Chapel Hill, and North Carolina State University, were able to make some space available for our chemists for that first year. The rest of the research staff coped as well as it could.

A sad feature of the move for me was the loss of almost all of a group of young, dedicated, talented women who had worked tirelessly with me to study the metabolism of 6-mercaptopurine, thioguanine, azathioprine, and allopurinol. They were unable to move to North Carolina because of family obligations. I also lost several of my young chemical assistants, some of whom went on to graduate school, some to other jobs in the New York area.

Within a year or two of the move, my department had greatly expanded. Krenitsky's section of Enzymology had added three PhD's, Richard Miller, James Fyfe, and Thomas Spector. A section of Metabolic Studies had been formed, with Donald Nelson and Paulo de Miranda as senior investigators, with Thomas Zimmerman soon to follow. There was an Immunology Section, with Gerald Wolberg and Richard Quinn, and a Chemistry Section with Janet Rideout and Lowrie Beacham. We also established a tissue culture laboratory, headed by Naomi Cohn. The knowledge, expertise, and dedication of this team and those who joined them in the 1970s, was responsible for the work which followed.

In addition to a large increase in staff, there was an upgrading of our instrumentation and facilities. We bought our first high pressure liquid chromatograph in 1969; within five years we had four of them. Recording infra-red and ultraviolet spectrophotometers, nuclear magnetic resonance, and mass spectrometry were taken for granted and computerization of instruments
became commonplace. The revolution in methodology had made some of our early experiments seem archaic and it became almost embarrassing to publish the data obtained by those old methods.

The exploration of new fields to conquer with our antimetabolites continued, together with basic studies of mechanism of action and resistance, pathways of metabolism, transport, and attempts to regulate immunological reactions.

**Leishmaniasis**

I would like to digress somewhat at this point from the chronological sequence to relate our experiences with allopurinol in an area into which we were thrust unexpectedly in the mid-1970s. Joseph Marr, who was then in the Department of Medicine in St. Louis, called one day to inform me that he had found allopurinol to be toxic to leishmania, a protozoan which causes a disease, leishmaniasis, that is widespread in tropical countries. I was skeptical at first. Our previous studies with allopurinol had shown it to be amazingly inactive with respect to any of the anabolic pathways of nucleic acid synthesis. Krenitsky had found it to be a substrate for purine nucleoside phosphorylase, but the allopurinol ribonucleoside formed accounted for only 10% of an allopurinol dose in humans, was inert, and was excreted in the urine. Allopurinol was a very poor substrate for human hypoxanthine phosphoribosyltransferase (HPRT) and the amount of allopurinol ribonucleotide formed in rat tissues was so low that it could only be detected after using large intravenous doses of $^{14}$C-allopurinol of high specific activity. Even under the latter conditions, allopurinol was not incorporated into the nucleic acids. Marr's findings with leishmania were, therefore, intriguing and challenging. They required an explanation. We set up a productive collaboration with Marr during which we learned a great deal about the difference between protozoan and mammalian enzymes (See refs. in 5). In our group the principal players were Nelson, Krenitsky, and Spector.

The first surprise was that the leishmania could convert allopurinol very efficiently to a ribonucleotide. In fact, the amount of allopurinol ribonucleotide formed on incubation of allopurinol with the promastigote forms of *L. donovani* or *L. braziliensis* for 24 hr in culture was more than three times the concentration of ATP in these protozoa. This was due to very high levels of a leishmanial HPRT with a lower $K_m$ and higher $V_{max}$ than for the mammalian enzyme. Even more unexpected was the finding that the leishmania had converted allopurinol ribonucleotide to the corresponding amino analogue, and formed 4-aminopyrazolo(3,4-d)pyrimidine mono-, di- and triphosphates. These latter compounds (APP-P, APP-DP, and APP-TP) are not formed in
mammalian cells. Studies on the aminating enzyme, succinoadenylate (SAMP) synthetase, showed that the leishmanial enzyme could indeed convert allopurinol ribonucleotide to the succinylamino derivative, which is then converted by SAMP lyase to the amino compound. The mammalian SAMP synthetase does not act upon allopurinol ribonucleotide. The toxic compound is apparently the APP-TP since it can act as a substrate for the leishmanial RNA polymerase and be incorporated in the RNA.

Another unusual finding with the leishmania was its ability to utilize allopurinol ribonucleoside and convert it directly to the nucleotide, a reaction not found in man. Therefore, it seemed possible that allopurinol ribonucleoside could be used as a treatment for leishmaniasis in place of allopurinol. This substitution might be an advantage since allopurinol ribonucleoside is quite soluble and is excreted in the urine essentially unchanged in man, whereas allopurinol is converted quite rapidly to oxypurinol, which is inactive on leishmania. Unfortunately, we were unable to test allopurinol ribonucleoside in the available rodent models for leishmaniasis since rodents, unlike man, oxidize allopurinol ribonucleoside to oxypurinol ribonucleoside, which is inactive.

Another protozoan that is responsible for a widespread tropical disease is *Trypanosoma cruzi*, the etiologic agent of Chagas' disease. Like the leishmania, *T. cruzi* can also convert allopurinol to APP-TP, which is toxic to the parasite. The clinical efficacy of allopurinol in the treatment of Chagas' disease (11) and in cutaneous leishmaniasis has recently been demonstrated (12). Our excursion into the biochemistry of protozoa was intriguing and useful.

**Herpesvirus Infections**

Another field, which we had only touched upon in 1949, that of antiviral chemotherapy, was inviting. The current dogma was that one could not hope to interfere with the replication of viral DNA without toxicity to the DNA of the host cell. Idoxuridine (5-iodouracil deoxyriboside) had found utility as a treatment for herpetic keratitis by topical application to the eye. The compound was, however, too toxic and too metabolically unstable to be given parenterally. In 1968 there was a report that adenine arabinoside, isolated from the fermentation filtrates of a strain of streptomyces, had activity against DNA viruses. This rang a bell. Since 2,6-diaminopurine had shown antiviral activity against vaccinia virus, a DNA virus, and all our studies on diaminopurine had shown it to be a close analog of adenine, perhaps 2,6-diaminopurine arabinoside would also have antiviral activity. Although we did not at that time have an antiviral screen operating in-house, our colleague John Bauer, in the Wellcome Research Laboratories in the U.K., had kept a number of
antiviral screens going, in vitro and in vivo. We synthesized 2,6-diaminopurine arabinoside and, in 1969, sent it off to Bauer to test. The answer came back by telegram. The compound was active against herpes simplex virus and against vaccinia virus in mice. A new field was open to us!

The synthesis and antiviral studies of a number of purine arabinosides were expanded. The synthetic work was performed by Janet Rideout, Bauer and Peter Collins continued the antiviral screening in the U.K. Of particular interest was the finding by de Miranda that 2,6-diaminopurine arabinoside was deaminated in vivo to guanine arabinoside, which was as potent an antiviral as the diamino derivative (13). This was good news since the deamination of adenine arabinoside resulted in a large decrease in antiviral potency. Our move to North Carolina disrupted the work temporarily, although the delay turned out to be a blessing in disguise. Better compounds were just beyond the horizon.

When we moved, our previous head of Organic Chemistry retired and we acquired a new head, Howard Schaeffer, who came from the University of Buffalo. Schaeffer had for years been studying the substrate and inhibitor specificities of the enzyme adenosine deaminase. He had found a good inhibitor, erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) and had also found that a portion of the riboside moiety of adenosine could be altered to an acyclic side chain without elimination of substrate activity. He continued to expand the series of acyclic nucleosides when he came to Burroughs Wellcome. The compounds were sent to the U.K. for antiviral screening. The activities of both EHNA and 9-(2-hydroxyethoxymethyl)adenine against the herpes simplex virus (HSV) were interesting and warranted further synthetic efforts. Schaeffer and Lilia Beauchamp extended the series both with respect to the heterocyclic base and the side chain. The 2,6-diamino-9-(2-hydroxyethoxymethyl)purine was more active than the adenine derivative. Moreover, as had happened in the arabinoside series, the diaminopurine compound was deaminated in vivo to the guanine derivative. No one could have anticipated that this guanine derivative (acyclovir) would be 100 times more active than the diamino compound. Indeed, the diamino derivative was active because of its conversion to the guanine analog by adenosine deaminase. In the presence of an adenosine deaminase inhibitor, it was inactive.

From the time we first knew that we had an exciting new antiviral agent in hand, a large group of diligent and devoted scientists worked as a team to obtain both the basic and practical information needed to make the compound a successful treatment for herpesvirus infections. The first challenge was to discover the reason for the high degree of selectivity of acyclovir (originally called acycloguanosine). Its ability to inhibit replication of herpes simplex viruses, types 1 and 2, (HSV-1, HSV-2) and varicella zoster virus (VZV) at
concentrations between 0.1 μM–4 μM, while the 50% inhibitory values for the cells in which the viruses were grown were 300 μM–3000 μM, posed an important challenge. The answer would undoubtedly yield important information about the biochemical differences between herpes viruses and mammalian cells.

To do the kind of mechanistic and metabolism studies that we had in mind, we established a virus laboratory in our building in 1975 and hired Phillip Furman and later Karen Biron, as well as several junior scientists to undertake the viral studies. They worked closely with the other members of the Department. Our colleagues in the U.K. continued to explore the activity of acyclovir in a variety of animal models and with a variety of viruses.

The first definitive experiments on mechanism of action were those which showed that 14C-acyclovir was converted in HSV-infected cells to three new radioactive metabolites. These appeared in a high pressure liquid chromatogram of the cell extract in the region of the nucleoside mono-, di-, and triphosphates. These compounds were not formed to any noticeable degree in uninfected cells. The identity of these new compounds was relatively easy to establish since they were convertible enzymatically to the original acyclovir (ACV).

Some elegant biochemistry by Fyfe identified the first enzyme in the conversion of ACV to its phosphates as a herpes virus-specified thymidine kinase. Although this enzyme was known to exist, its substrate specificity had never been investigated to any extent. The idea that an acyclic nucleoside analog of guanosine could serve as a substrate for this enzyme would certainly not have seemed likely to any knowledgeable chemist. Yet it was unquestionably true. When the viral enzyme was isolated and purified, its ability to phosphorylate ACV was confirmed. The cellular thymidine kinase isolated from uninfected Vero cells was inactive on ACV. Thus, the first major basis for the selectivity of the compound was established.

The substrate specificity of the herpes virus-specified thymidine kinase was examined in detail by Paul Keller. A certain latitude in the size and nature of the acyclic side chain was permissible but a terminal hydroxyl group was essential. The enzyme did not tolerate changes in the purine base to any extent, e.g. xanthine, adenine or 2,6-diaminopurine with a 9-(2-hydroxyethoxy)methyl side chain were not substrates. However, 2-methylthio-6-aminopurine with the same side chain was a substrate for the herpes thymidine kinase. Interestingly, it did not have antiviral activity. This finding reminded us that phosphorylation to a monophosphate was a necessary but not a sufficient requisite for antiviral activity.

The remaining steps in the activation of ACV to a triphosphate were investigated by Richard and Wayne Miller. They found that the cellular
guanylate kinase transformed ACV monophosphate to the diphosphate and that several cellular enzymes could convert the di- to the triphosphate.

The next selective step in the activity of ACV was the effect of its triphosphate on the viral and cellular DNA polymerases. Furman, Biron, and St. Clair conducted this investigation. The viral DNA polymerases of HSV-1, HSV-2, and VZV proved to be much more sensitive to inhibition by ACV triphosphate than were the cellular α and β DNA polymerases. Moreover, the viral polymerases were able to incorporate ACV into the growing viral DNA molecule, thereby causing chain termination, since ACV lacked a 3'-hydroxyl group. There was also an inactivation of the viral DNA polymerase by ACV triphosphate that was not seen with the cellular enzymes.

While the synthetic and mechanistic studies were progressing, there was much to be learned about the therapeutic potential of acyclovir in animal model systems, and its absorption, tissue distribution, metabolism, pharmacokinetics, and toxicology. When we published the first two papers in *Proceedings of the National Academy of Sciences* and *Nature* in late 1977 (14) and early 1978 (15), the studies had been going on for over three years. While the first two papers did not appear to attract a great deal of attention, the thirteen posters presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy in October 1978 created a major stir. I remember the occasion well for personal as well as professional reasons. I was in the process of recovering from back surgery, which had been performed at the beginning of September. The surgeon thought it inadvisable for me to fly to Atlanta for the meeting on October 1st. I assured him that nothing could keep me away, even if, as did happen, I had to be carried onto the plane on a pallet and taken off in a wheel chair. We had waited too long for this moment and I was not going to miss all the fun. My back eventually healed anyway.

One reason for the excitement at the 1978 meeting was that we had finally shown that antiviral drugs could be selective and that one could capitalize on the differences between the viral and cellular enzymes. Within months, all the large pharmaceutical companies had initiated antiviral programs and the competition became fierce. Perhaps it was a good thing for, when the AIDS epidemic broke several years later, scientists were already geared up to think that antiviral chemotherapy was not impossible.

The pharmacological properties of ACV were better than we could rightfully have expected: it was metabolically stable and was excreted in the urine, essentially unchanged, in man; was distributed in all tissues, including the brain; and had a plasma half-life of about 3 hr. Its oral absorption varied greatly from species to species, being very good in the mouse and dog, poor in the rat, and intermediate (about 15–20%) in man. The monkey absorbed almost none of the drug orally and the plasma half-life was very short. The
data on pharmacokinetics and metabolism in different animal species was very helpful in understanding why the drug had to be given at much higher doses in some animal model systems for herpes virus infections than in others (5, 16).

As we had hoped and anticipated, ACV was nontoxic at doses that far exceeded those needed for antiviral activity. The thorough toxicology work-up by Walter Tucker and his team was very reassuring and was important to convince the skeptics that an effective antitherpetic agent really could be safe.

During the next few years we had many collaborators who were outstanding in the preclinical and clinical evaluation of the antiviral activities. Bauer and Collins tested acyclovir in a number of herpes virus infections in mice and rabbits; Earl Kern examined its activity in HSV-2-infected guinea pigs; Ken Soike tested its effect on the Simian varicella zoster virus in monkeys. The intravenous pharmacokinetics in man was investigated by de Miranda, Harvey Krasny, and Steven Good, in collaboration with Richard Whiteley, Paul Lietman, and James Connor. The early clinical trials against genital herpes infections were conducted by Lawrence Corey.

In September 1981, Burroughs Wellcome Co. held a three-day international symposium on acyclovir in Washington, D.C., under joint sponsorship with the National Institute of Allergy and Infectious Diseases. The proceedings of that symposium were published as a supplement to the *American Journal of Medicine* in 1982 (16). The 77 papers that were presented covered chemistry, mechanism of action, antiviral spectrum, preclinical pharmacology and toxicology, efficacy in animal models, antiviral efficacy in the normal and in the immunocompromised host with mucocutaneous, ocular, and genital herpes infections. In 1982, the FDA approved the ointment and intravenous formulations of ACV (Zovirax)® and several years later the oral form. The number of indications for the use of ACV has continued to increase and now include mucocutaneous herpes infections, genital herpes, herpes encephalitis, shingles, and chicken pox. Prophylactic use of the drug has been of great help in preventing the activation of latent virus in immunosuppressed patients, e.g. those undergoing bone marrow or organ transplantation or cancer chemotherapy. In patients with genital herpes who suffer from frequent recurrences, prophylactic use of ACV has markedly reduced the number of recurrences or prevented them entirely (17). Antiviral chemotherapy has come of age.

**THE PRIZE**

In 1983 I had to make a decision whether to retire or go on working: I chose to do both. With acyclovir now launched, I thought it might be a good time to retire as head of the Department before a new drug turned up that I couldn’t
bear to leave. The members of the group were such excellent scientists and I knew they would all go on to do well, with or without me. For years I had the pleasure of being an orchestra conductor of some fine musicians. They could indeed make beautiful music together. I was fortunate that I could leave the group in the capable hands of Tom Krenitsky, while I remained at Burroughs Wellcome as a Scientist Emeritus and consultant. I felt I had the best of both worlds.

The first year of my “retirement” was a very busy one and made me realize that I would probably never have time to wonder about what to do next. I was president of the American Association for Cancer Research that year and had just been appointed to the National Cancer Advisory Board for a six-year term. I was also serving on a Steering Committee for Filariasis for the World Health Organization. There was still time to go to scientific meetings and give lectures around the world. I was also in the happy position of remaining close to the work going on at Burroughs Wellcome, watching the expansion of the antiviral program in particular. It was satisfying to watch the same team that had done so well with the development of ACV now expand and proceed to use their skills to develop azidothymidine (AZT, zidovudine, Retrovir®) for the treatment of AIDS.

In mid-October 1988, to my complete surprise, I was notified that I had been awarded the Nobel Prize in Physiology or Medicine, together with George Hitchings and Sir James Black. People often ask whether this wasn’t what I had been aiming for all my life. Nothing could be farther from the truth. It had never occurred to me that I might be considered for this award. My rewards had already come in seeing children with leukemia survive, meeting patients with long-term kidney transplants, and watching acyclovir save lives and reduce suffering. The Nobel Prize was a wonderful international recognition not only for me but for our whole team, for whom it was a great morale booster.

The Nobel Prize ceremony itself is, of course, a memorable event, and the week of celebration that surrounds it is, as my five-year old grandniece described it, a fairy tale. It was wonderful to be able to share the occasion with eleven members of my family, my brother’s children and grandchildren. Unfortunately, my brother was ill at the time and could not come.

What I did not anticipate was how much the receipt of the Nobel Prize would change my life. Suddenly, I was greatly in demand by the press, television, universities, committees, and Boards. Honors began to come in a steady stream. I was elected to the National Academy of Sciences, the National Inventors’ Hall of Fame, National Women’s Hall of Fame, and received the National Medal of Science. I now have many honorary degrees, but I still remember the feeling of pride when I received the first two, in 1969, from
George Washington and Brown Universities. They represented the PhD I had never received and the vindication of my parents’ faith in me. Unfortunately, neither of them lived long enough to see it.

PASSING THE TORCH

Shortly after my retirement, I was asked to be a Research Professor of Pharmacology and Medicine at the Duke University Medical Center. I agreed to work with a third-year medical student each year on a research project. The students are allowed this optional year of research in place of course work. The students have worked in the laboratory of Henry Friedman in the Department of Pediatrics, while I have acted as mentor, helping to delineate the problem, design the experiments, analyze the results, and plan the next steps. We have worked on brain tumor biochemistry, pharmacology and chemotherapy both in vitro and in human xenografts in nude mice. It has been challenging for me and, I think, for the students. They are all so bright, so motivated and so energetic that I find myself needing to read and study along with them. I have had seven students to date, three women and four men, and all have done exceedingly well in their final year and in their medical residencies. It is the kind of teaching I thoroughly enjoy and I hope to continue it as long as I am able.

As I look about me and see young people shying away from science, I feel an almost missionary zeal to do something to change that trend. My hope is that I can not only help students already committed to science, but that I can spread the news among the younger generation that science is fun. I would like to see them experience the same excitement and fulfillment that I have had in my career. However, I try not to minimize the difficulties they may encounter or the perseverance it may require. If the goal is worthwhile and its pursuit enjoyable, the reward will be inevitable.

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Modulation of the immune response mediated by dietary nucleotides

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Dietary nucleotides have been reportedly beneficial, especially for infants, since they positively influence lipid metabolism, immunity, and tissue growth, development and repair. Rapidly proliferating tissues, such as the immune system or the intestine are not able to fulfill the needs of cell nucleotides exclusively by de novo synthesis and they preferentially utilize the salvage pathway recovering nucleosides and nucleobases from blood and diet. In the present review we describe the modulatory effect of dietary nucleotides on the immune system together with some of their effects on gut-associated lymphoid tissue. Dietary nucleotides influence lymphocyte maturation, activation and proliferation. Likewise, they affect the lymphocyte subset populations in both the small intestine and blood. Moreover, they are involved in enhancing macrophage phagocytosis and delayed hypersensitivity as well as allograft and tumour responses. In addition, they contribute to the immunoglobulin response in early life, having a positive effect on infection. In fact the incidence and duration of acute diarrhoea is lower in infants fed supplemented-nucleotide formulas. The molecular mechanisms by which dietary nucleotides modulate the immune system are practically unknown. Dietary nucleotides have been shown to enhance the production and the genetic expression of IL-6 and IL-8 by foetal small intestinal explants. Dietary nucleotides may influence protein biosynthesis as well as signal membrane transduction mediated by the interaction of exogenous nucleosides and their receptors may also contribute to modulate the expression of a number of genes, some of which can directly affect the levels of intestinal cytokines.


Keywords: dietary nucleotides; immunity; gut-associated lymphoid tissue

Introduction

Nucleotides are low molecular weight intracellular compounds, which play key roles in nearly all biochemical processes. Nucleotides are naturally present in all foods of animal and vegetable origin as free nucleotides and nucleic acids. Concentrations of RNA and DNA in foods depend mainly on their cell density. Thus, meat, fish and seeds have a high content of nucleic acids and milk, eggs and fruit have relatively lower levels. The total content of RNA oscillates between 50 and 400 mg/100g for animal viscera, 80 and 350 mg/100g for marine foods and 140–490 mg/100g for dry leguminosae. Soluble nucleotides are present in milk from various mammals, contributing up to 20% of the non-protein nitrogen and nucleotide patterns are species specific (Gil & Uauy, 1995). Although nucleotide deficiency has not been related to any particular disease, dietary nucleotides have been reported to be beneficial for infants since they positively influence lipid metabolism, immunity, and tissue growth, development and repair (Carver & Walker, 1995; Sánchez-Pozo et al, 1999; Gil, 2001).

Rapidly proliferating tissues, such as the immune system or the intestine are not able to fulfill the needs of cell nucleotides exclusively by de novo synthesis and they preferentially utilize the salvage pathway, recovering nucleosides and nucleobases from blood and diet. An exogenous supplement of these compounds through the diet may be essential to sustaining growth and to maintain the cellular function in these tissues (Uauy et al, 1996; Gil, 2001).

The modulator effects of nucleotides on the intestinal and immune tissues have been traditionally considered as separate issues. However, nowadays it is well known that both are closely related, not only because a significant proportion of intestinal cells are of immune origin but also because...
intestinal epithelial cells can produce immunomodulatory molecules, such as cytokines, which regulate the immune response (Walker, 1996). Following these considerations, in the present review we describe the modulator effect of dietary nucleotides on the immune system together with some of their effects on gut-associated lymphoid tissue. Finally, we discuss the potential molecular mechanisms by which dietary nucleotides influence the immune response.

### Nucleotide effects on lymphocyte maturation, activation and proliferation

The terminal deoxynucleotidyl transferase (TdT) enzyme has been referred to as an index of the immaturity of lymphocytes. Mice fed on a nucleotide-free diet have shown a higher percentage of TdT-positive cells proceeding from the thymus and the spleen than those fed on a diet supplemented with RNA, adenine or uracil, suggesting that dietary nucleotides could stimulate the maturation of lymphoid cells. The suggested mechanism proposes that dietary nucleotides exert a predominant effect upon the initial phase of antigen processing and lymphocyte proliferation suppressing the uncommitted T lymphocyte responses, as demonstrated by higher levels of TdT for undifferentiated lymphocytes in primary lymphoid organs in mice fed a nucleotide-free diet (Kulkarni et al., 1989). On the other hand, a regulatory role of dietary nucleotides in immunohematopoiesis has also been proposed (Rueda & Gil, 2000).

Dietary nucleotides have been reported to enhance the maturation of enterocytes both at weaning and during adult life in rats (Uauy et al., 1996). Since enterocytes are not only absorptive cells but they are also involved in processes related to the immune response, namely in cytokine production and antigen presentation, it might be assumed that dietary nucleotides may contribute to the maturation of the gut-associated lymphoid system.

There is numerous evidence demonstrating that exogenous nucleotides increase the proliferative response to T cell dependent mitogens (PHA, ConA, PWM) whereas no effects are seen when B cell dependent mitogens are used; this has been reviewed extensively in previous works (Kulkarni et al., 1989; Jyonouchi et al., 1994; Carver & Walker 1995; Gil et al., 1997; Rueda & Gil, 2000; Gil, 2001).

In animal models stimulated with allogenic spleen cells, dietary nucleotides enhance the lymphoproliferative response, particularly during the recovery of protein-energy malnutrition and it has also been reported that in nucleotide-starved rats the parenteral administration of a mixture of nucleotides and nucleosides (OG-VI) determined an increase in the lymphoproliferative response to ConA in spleen cells. It has also been demonstrated that Balb/c and DBA/2 mice present an increase in the popliteal lymph node blastogenic response to antigens, allogens and mitogens when they are fed with a diet supplemented with a mixture of nucleosides and nucleotides (Kulkarni et al., 1989; Gil et al., 1997, Rueda & Gil, 2000; Gil, 2001).

### Dietary nucleotides and lymphocyte subpopulations

It has been reported that there are no major differences in the T and B lymphocyte subpopulations between mice fed on diets enriched in RNA, uracile or adenine, compared with those fed a diet without nucleotides. The effects of dietary nucleotides on lymphocyte subset populations in preterm infants have been reported recently: CD4+ cells from children fed the nucleotide formula showed a significantly higher percentage than those fed the standard formula at 10 days of life (Navarro et al., 1999).

### Modulation of the macrophage phagocytic activity by dietary nucleotides

A number of reports have related dietary nucleotides and macrophage activity. In mice inoculated with S. aureus, the phagocytosis of microorganisms was lower in those that were fed on a nucleotide-free diet than in those fed on a diet supplemented with RNA or adenine. Dietary nucleotides enhance the interaction of macrophages and T cells, explaining the higher susceptibility of mice fed a nucleotide-free diet to Candida infection (Kulkarni et al., 1989).

### Nucleotide modulation of the delayed hypersensitivity and allograft and tumour responses

The first studies worked out to determine the effects of dietary nucleotides showed that mice fed a nucleotide-free diet and previously challenged with an intravenous stimulus of sheep red blood cells (SRBC) exhibited an enhanced delayed cutaneous response when these cells were injected in the mouse legs. An increase in the delayed hypersensitivity response to SRBC and to DNFB in BALB/c and DBA/2 mice fed a diet supplemented with a mixture of nucleotides and nucleosides has also been reported (Kulkarni et al., 1989; Carver & Walker, 1995; Rueda & Gil, 2000).

One of the most studied models to ascertain the influence of dietary nucleotides on immunity is the evaluation of the response of the host against allografts. The duration of heart allografts implanted on mouse ear was shown to increase when the diet was devoid of nucleotides; the addition of yeast RNA to the diet resulted in a reduced period of allograft survival. Likewise, the use of cyclosporine as an immunosuppressor agent in mice had a synergic effect when combined with a nucleotide-free diet, leading to a higher period of allograft survival (Kulkarni et al., 1989). However, no differences were seen when mice were inoculated with a fibrosarcoma or the LSTRA syngenic lymphoma, which is highly aggressive (Navarro et al., 1996).

Natural killer (NK) cells are one of the main populations involved in the immune response against transformed cells. The activity of NK cells is increased in the mice fed a diet supplemented with nucleotides respect to those fed a diet without nucleotides. Likewise, Carver has shown in human
newborns, that NK cell activity at the second month of life is increased in infants fed formula supplemented with nucleotides (Carver & Walker, 1995).

**Modulation of immunoglobulin production by nucleotides**

Experiments carried out in mice fed a nucleotide-free diet for 3 weeks have shown a profound decrease of specific antibody responses to T cell-dependent antigens and a retained response to T cell-independent antigens and lipopolysaccharide (Jyonouchi et al., 1994). Likewise, a mononucleotide–nucleoside mixture used in experimental total parenteral nutrition, restored the humoral immune responses to T cell-dependent antigens in mice fed a nucleotide-free diet. However, this solution did not show any effect on the in vitro specific antibody production in response to T cell-dependent antigens. More recently, our work group has reported in BALB/c mice that the addition of nucleotide mixtures to a nucleotide-free diet resulted in an increase in the response of haemolytic IgG-forming cells induced by previous immunization with sheep erythrocytes; when the diet was supplemented with single nucleotides, AMP, GMP or UMP increased the IgG response whereas CMP and IMP had no effect. GMP was the only nucleotide that increased the haemolytic IgM-forming cell response (Navarro et al., 1996).

In recent years, our group has done a series of studies to determine the influence of dietary nucleotide supplementation to infant formulas on the levels of circulating antibodies in preterm infants. Total serum IgM and IgA levels increased significantly for the first 3 months of life, whereas no differences were detected for serum IgG; levels of IgE were undetectable (Gil et al., 1997; Navarro et al., 1999). Another study with preterm infants showed higher concentration of specific IgG against α-casein and β-lactoglobulin for the first month of life in newborns fed a low-birth-weight infant nucleotide-supplemented formula (Martinez-Augustin et al., 1997). In a third study, the influence of dietary nucleotide supplementation on the recovery of infected and malnourished children has been evaluated (Rueda & Gil, 2000). Refeeding after malnutrition did not produce any significant changes either in serum total and specific immunoglobulins against α-casein and β-lactoglobulin or in saliva IgA levels (Martinez-Augustin et al., 1997). Finally, a recent study has also shown that dietary nucleotides may modulate the immune response in normal infants, enhancing the production of specific IgG against low response antigens, namely H. influenzae type b, a bacterium responsible for meningitis episodes in early infancy (Pickering et al., 1998).

**Dietary nucleotides and defence against infection**

Animals injected intravenously with *C. albicans* or *Staphylococcus aureus* and fed a nucleotide-free diet had a significantly lower survival rate than mice fed RNA, adenine or uracil supplemented diets. On the other hand, intraperitoneal administration of a nucleoside–nucleotide mixture for 14 days was associated with reduced translocation of Gram-negative enterics to the mesenteric lymph nodes and spleen in comparison to control animals. The extent of the damaged mucosa was greater in controls and these animals were more susceptible to the lethal effects of the endotoxin lipopolysaccharide from *E. coli*, which suggests that dietary nucleotides may block the bacterial translocation by preventing endotoxin-induced mucosal damage (Kulkarni et al., 1989; Carver & Walker, 1995, Gil, 2001).

One of the potential mechanisms by which nucleotides reduce the incidence of infection is the modulation of the intestinal microflora. Our group reported for the first time that nucleotide supplementation to an infant formula reduced the counts of enterobacteria and increased the counts of bifidobacteria in the fecal microflora (Gil & Uauy, 1995; Uauy et al., 1996). These results suggested that nucleotides could act as prebiotics favouring the proliferation of the beneficial flora and inhibiting that of potential pathogens. These results support clinical findings showing a low incidence of acute diarrhoea in infants fed nucleotide supplemented formula in developing (Brunser et al., 1994) and developed countries (Pickering et al., 1998).

**Potential mechanism of action of dietary nucleotides**

It has been proposed that dietary nucleotides exert effects upon cellular immune functions by acting on the T-helper/inducer population with the predominant effect on the initial phase of antigen processing and lymphocyte proliferation. The suggested mechanism would be the suppression of uncommitted T-lymphocyte responses as demonstrated by higher activities of deoxynucleotidyl transferase, a marker of undifferentiated lymphocytes, in primary lymphoid organs of mice fed a nucleotide-free diet (Kulkarni et al., 1989).

Another hypothesis is that exogenous nucleotides may modulate T-helper (Th) cell-mediated antibody production (Jyonouchi et al., 1994). It has been suggested that dietary nucleotides may favour the balance of T cell differentiation to Th-2 cells, which are mainly involved in the B-cell response and in the suppression of pro-inflammatory reactions induced by Th-1 cells.

The molecular mechanisms by which dietary nucleotides modulate the immune system are practically unknown. It has been suggested that the small intestine should play a key role in the regulatory effects of nucleotides upon the immune response. The gut-associated lymphoid tissue can initiate and regulate T cell development and may act as a thymus analogue (Walker, 1996). Dietary nucleotides have been shown to enhance the production and the genetic expression of IL-6 and IL-8 by foetal small intestinal explants when challenged with IL-1 beta, the response being nucleotide concentration dependent. Furthermore, the addition of AMP to the culture media resulted in the suppression of crypt cell proliferation followed by the restoration of
differentiation and the induction of apoptosis across the human small intestinal epithelium (Sánchez-Pozo et al., 1999). Dietary nucleotides may influence the protein biosynthesis by regulating the intracellular nucleotide pool. In addition, signal membrane transduction mediated by the interaction of exogenous nucleosides and their receptors may also contribute to modulate the expression of a number of genes, some of which can directly affect the levels of intestinal cytokines (Figure 1).

References
IMUREGEN
Producer: Uniregen s r.o. Co., Na ch o d

The customer - (UNIREGEN s r.o. Co., Na ch o d) delivered for analyses the sample of food for special nutrition IMUREGEN in quantity 4 x 50 ml in bottles of brown glass, content 50 ml, with plastic cover, label with the name of product, number 4 and information on dry matter concentration (100 g.1-1).

Free amino acids were determined in the laboratory VU servis s r.o., Pohoi'elice using the method of chromatography on ionexes (AAA) in analyser arrangement for AA in hydrolysis product.

Further components of the sample were determined in the laboratory of Clinical Biochemistry MEDSERVICE s r.o. Co., Brno in the system usual for blood analyses.

The results of both laboratories were corrected to the found real dry matter content (g/100 of the preparation IMUREGEN - see the Protocol). The results were then converted into dilution corresponding to the final product (i.e. 1:100, dry matter content 100 mg/100 g of Imuregen preparation.

The results corrected like this and given in tables 1 and 2 were used as data for determination the composition of Imuregen preparation.

The working protocols are an integral part of this Protocol.
Table 1. Free amino acids content in Imuregen preparation (final product)

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</tbody>
</table>

Table 2. Components content in Imuregen preparation (final product)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Unit</th>
<th>Component</th>
<th>Quantity</th>
<th>Unit</th>
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<tr>
<td>Na</td>
<td>12.88</td>
<td>mg/100g</td>
<td>urea</td>
<td>0.92</td>
<td>mg/100g</td>
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<tr>
<td>K</td>
<td>3.29</td>
<td>mg/100g</td>
<td>urea</td>
<td>52.76</td>
<td>ug/100g</td>
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<tr>
<td>Ca</td>
<td>5.21</td>
<td>ug/100g</td>
<td>glue.</td>
<td>0.39</td>
<td>mg/100g</td>
</tr>
<tr>
<td>Mg</td>
<td>1.91</td>
<td>ug/100g</td>
<td>uric. acid</td>
<td>52.95</td>
<td>ug/100g</td>
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<tr>
<td>Fe</td>
<td>12.01</td>
<td>ug/100g</td>
<td>P</td>
<td>42.16</td>
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<td>Li</td>
<td>4.83</td>
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<td>bi li.</td>
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<tr>
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<td>trig. lye</td>
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<td>alb.</td>
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<td>gamma</td>
<td>4.90</td>
<td>mg/100g</td>
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</tr>
</tbody>
</table>

Brno 20th September 1998

Officia l Seal: INSTITUTE OF PROPHYLACTIC MEDICINE
Masaryk University, Faculty of Medicine
662 44 Brno, 662 44 Brno, 662 44 Brno, 662 44 Brno

Signature: RNDr. Jiří Totussek, CSc. Laboratory Chief
Nucleotides

What are nucleotides?

Nucleotides are ubiquitous compounds in the cells of all living organisms and play a critical role in many biological processes.

Nucleotides are found in all cells and nuclei are formed after cleavage of nucleic acids. They are the building blocks of nucleic acids DNA and RNA, which are bearers of heredity. They are also part of many important enzymes necessary for nutrition and energy metabolism.

Chemically nucleotides are ubiquitous low molecular weight compounds consisting of six-membered or five-membered purine pyrimidine nitrogenous bases and pentose (ribose or deoxyribose) together with one or more phosphate groups. From the pyrimidines are cytosine (2-hydroxy-4-amo-pyrimidine), uracil (2,4-dihydroxypyrimidine), and thymine (5-methyl-2,4-dihydroxypyrimidine) of purines adenine (6-aminopurine) and guanine (2-amino-6-hydroxypurines). If present in the molecule nucleotide ribose, regards ribonucleotide, if present deoxyribose, regards deoxyribonucleotide.

Nomenclature of nucleotides

<table>
<thead>
<tr>
<th>Base</th>
<th>Nucleophlic</th>
<th>Nucleotide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>Adenosine (A)</td>
<td>Adenosine 5'-monofosfát (AMP) a</td>
</tr>
<tr>
<td>Guan</td>
<td>Guanosin (G)</td>
<td>Guanosin-5'-monofosfát (GMP)</td>
</tr>
<tr>
<td>Hypoxanthin</td>
<td>Inosin (I)</td>
<td>Inosin-5'-monofosfát (IMP)</td>
</tr>
<tr>
<td>Cytosine</td>
<td>Cytidin (C)</td>
<td>Cytidin-5'-monofosfát (CMP)</td>
</tr>
<tr>
<td>Uracil</td>
<td>Uridine (U)</td>
<td>Uridin-5'-monofosfát (UMP)</td>
</tr>
<tr>
<td>Thymin</td>
<td>Thymidin (dT) b</td>
<td>Thymidin-5'-monofosfát (dTMP)</td>
</tr>
</tbody>
</table>

a AMP is sometimes called adenylic acid (adenylate). b This is reserved for thymidine deoxyribonucleoside, therefore abbreviation dT.

What is our body needs?

The food which have support all physiological functions, must be represented in adequate proportions of low molecular substance (trace elements, minerals, vitamins, etc.), And high molecular weight substances (carbohydrates, lipids, proteins). Only in the period of growth and aging, exhaustion or diseases are some components of priority (increase intake of certain trace elements, vitamins and proteins), sometimes restricted (carbohydrates and saturated lipids). It turned out that it is under the above circumstances rising requirements of the organism to some other low molecular weight substances, which are indeed present in the normal diet, but not in sufficient quantities. They are mainly nucleotides.

Dividing cells in the human body needs $10^9$ nucleotides. From this data we can deduce how many nucleotides in an organism is only required for cell division in quiescent tissue regeneration, restoration
of skin, mucosal epithelium, and hematopoiesis. Previously experts thought that the body can generate enough of them even in case of increased needs, but it turned out that it is necessary to supply extra food. It is therefore necessary to ensure their intake during childhood growth of the organism, in adulthood and old age for tissue regeneration, during convalescence after illness or surgery, especially for a full course of the immune response. It is appropriate to increase their income as a precaution during the expected epidemic of infectious diseases.

Nutrition and Immunity

Interconnection terms nutrition and immunity is particularly recently the subject of basic research. It turns out that lifestyle changes in recent decades, including changes in nutritional status has an impact on a number of diseases that are mediated immune responses.

Disease resistance and quality and length of human life rests on three pillars: mental stability, reasonable and adequate physical activity and a balanced diet (nutrition). Over the past 150 years was extended in industrialized countries, life expectancy, mainly due to the introduction of public health measures in the 19th century, having resulted in the spread of the deadliest pandemics and epidemics of infectious diseases, and in no small part thanks to the discovery of effective drugs such as chemotherapy and antibiotics. However, at least since the middle of last century, began professional and general public to realize that lifestyle in these countries brings the negatives: the declining population immunity to infectious diseases and an increase in allergic, autoimmune, cardiovascular, neurodegenerative diseases and cancer. The inversely related to the aforementioned triad: the ever-increasing trend of engagement work is accompanied by increased mental stress, lack of time for active relaxation (physical activity) and changing eating habits (preference technologically modified food), which is often compensated by increased consumption of food products and support drug.

Genes regulating metabolic functions of the human body have evolved over millions of years and are still adapted to the way of feeding our prehistoric ancestors. Eating habits fundamental changes accompanying the modern lifestyle may not always be in conformity with genetically given metabolism.

Types of food from the Paleolithic hunter gatherer-society has changed dramatically. It is estimated that our ancestors diet contained over current our diet 10-15 times more fiber, 5-10 times more n-3 polyunsaturated fatty acids and antioxidants, and 3 times more protein and potassium salts. In contrast, today receives 10 to 20 times more of sodium chloride and at least 4 times more saturated and 2 times more monounsaturated fatty acids.

To this must be added the consumption of "high-energy" but "nutritionally empty" food and lack of exercise. The consumption of fats has increased from 20% in the 19th century to today 50%, at the expense of low-energy diet, whose intake decreased over the same period by half.

Mutual relations of the immune system and nutritional components are essential for growth, development and health of the individual.

The role of nucleotides in the prevention and immunity

Twenty years ago, noted the symposium the American Society for Nutrition, that is biochemical or physiological reason for it to be nutritionally nucleotides considered essential, because in nitrogen
metabolism play a negligible role. New research yielded unambiguous demonstration that the nucleotides are important from many points of view indispensable nutritional component. The organism is a food can be used directly, without having to construct high energy de novo. Their insufficient food intake may result not only larger variability range of immune functions but mainly reflected in the function of liver, heart and intestinal tract. In childhood, then delays the development of the CNS, immune system and overall growth.

**Nucleotides in child nutrition**

Importance of nucleotides in nutrition excel, when you realize that when they divide each cell must create a new nucleotides, which are then composed an exact copy of nucleotides to daughter cells. Admittedly cells can produce by themselves, but the formation of new nucleotides of the elements and simple compounds is very energy intensive. Most of these substances require cells rapidly growing tissues and blood production, as well as some cell types that are responsible for defense against infection. Also, each of starvation or prolonged administration and fiduciary unbalanced diet is reflected particularly in the development of brain centers that are responsible for the development of intelligence. It is not accidental that in malnourished children who grow up in areas long affected by various natural disasters, their mental development lags.

For nutrients, including nucleotides in the diet, however, is to tissues and the brain is still a long way to go. First, it must be appropriately digested and processed to transition to the blood which is conveyed to the target tissues. We know that newborns and infants are not able to eat normal food. Their digestive system must also evolve, which also needs nucleotides. The situation is even more complex. Digestive system and especially the gut, are accompanied by a highly specialized tissue that is populated by cells that guard the "purity" of the internal environment of the body. They are different types of cells absorbing any foreign particulate matter, including micro-cells that produce effective antimicrobial agents and, ultimately, cells producing antibodies. All these diverse cells are collectively referred to immunocompetent cells, abbreviated immunocytes, because it forms the immune system, which is responsible for the destruction of all harmful bacteria and parasites that when they get further into the blood, can cause serious illness. The intestine is from this perspective, the largest organ of our immunity is logical, because the easiest way to germs get into the body with food. The immune system of the intestine is developing most rapidly during the first two years after birth. Therefore it is very important that especially during this period the child received full diet containing adequate amounts of nucleotides.

Between 1993 to 1997, held its first comparative study on infant care facilities in the USA, Japan and Spain, which confirmed the important role of nucleotides for premature and artificially fed babies. Those which received food fortified nucleotides quickly drove weight deficit and had higher levels of antibodies in serum. Even more immunostimulatory effect of nucleotides reflected in malnourished children and children who have suffered long-term diarrhea as a result of intestinal infections. In the group fed with diet containing nucleotides condition rapidly improved and also increase the amount of protective compounds in serum.

The best food for newborns and infants breast milk. Nucleotides are present in breast milk (among other substances) in relatively large concentrations. The first study, which showed the presence of various grafts nucleic acids in the form of POLYNT, NT, nucleosides, or their various compounds in breast milk dates back to 1960. Most of these substances, ie mixtures, often referred to as "total available nucleotides" contains colostrum (colostrum), 50-60 mg.l⁻¹. During breast-feeding their content in milk decreases for about 3 months at 30 mg.l⁻¹. The total concentration of the individual components depend
on the nutrition of the mother. Over time, as the child becomes another diet, their content in milk decreases.

If we bear in mind the role they play nucleotides in infant feeding, breast milk can not totally replace cow milk. Breast milk contains up to 5 times more usable nucleotides compared to cow's milk. Preschool children and older children already receive a normal diet that includes adequate supply of nucleotides (and other food ingredients) necessary for their growth. The situation is different in patients after an illness or injury and recovering children, eventually. children living in areas affected by industrial air pollution in urban areas or high traffic. It is with these children, whose immune system is subjected to permanent stress, it would be necessary to increase the daily ration supplemented with nucleotides appropriately food preparations. (Sima, P. Nucleotides in child nutrition. Nutrition and Food, 2001, vol. 56, no. 1, . 4-5).

Research and studies

- Growth and Differentiation
- Response to inflammation
- Intestinal flora
- Intestinal hem
- Effect of supplementation of nucleic acid solution on the intestinal mucosa in parenteral nutrition
- Protein malnutrition in connection with an acute bacterial infection
- Lipoproteins
- Effects on liver parenchyma
- Immunological effects
- Use of arginine, omega-3 fatty acids and nucleotides in the enteral nutrition

Nucleotides in the elderly nutrition

We know that to a healthy life need varied diet containing essential components (proteins, fats, carbohydrates), as well as vitamins, mineral substances and many other substances (flavonoids, probiotics, prebiotics). However foods are always present also nucleic acids DNA and RNA, the carrier of the gene in cell nuclei, which are also part of important enzymes. Internationally known as "dietary nucleotides".

Less already known that in various diseases, either infectious or chronic, but especially during aging, or aging requires a sick organism increased intake nucleotides. Their importance will stand out, when you realize that when they divide each cell must create a new nucleotides, which are then consist of a subsidiary cell exact copies of DNA and RNA. Cells can nucleotides they can, however, create their own, but their formation from the elements and simple compounds is very energy intensive. The body is therefore "learned" effectively take advantage of ingested food.

Nucleotides are crucial for tissue repair, especially in the aging organism. On one single cell dividing them consumes a billion. It can deduce how many nucleotides in an organism is only required for cell division when replacing cells of the skin and mucous membranes as well as for blood formation. For illustration, only the cells of the intestinal mucosa in man replaced daily average of about 300 g.
However, there is increasing demand for nucleotides during the immune response when the cells divided vigorously responsible for defense against infection.

The aging body no longer requires such as food intake for youth. The total volume of the diet of many elderly decreases to 20%. Accordingly, they should share the missing nucleotide supplement in the form of dietary supplements. Already, because due to weakened immune function seniors are more susceptible to infectious diseases and slower to heal. This also applies to support the treatment of chronic diseases, especially for speeding up recovery after illness or surgery.

Dietary supplements containing nucleotides correspond to the objectives and nutritional recommendations of the World Health Organization, which are included in the "Nutrition for Health in the 21st Century" aimed at reducing infectious, cardiovascular, neurodegenerative diseases and cancer, and improving the quality of life in old age.

**Importance of nucleotides for aging body:**
- slow down aging, because they facilitate tissue repair;
- protects against infectious diseases, because they increase the readiness of immunity;
- support the treatment of chronic diseases;
- accelerates recovery.

**Nucleotides protect aging body and enhance the quality of life of seniors.**

The number of people older than sixty years already long climbs worldwide. The discoveries of modern medical science, new, hitherto unknown drugs with high efficiency, eradication of pandemic infectious diseases, improvement of communal hygiene, and medical oversight of food and drinking water were reflected in both the decrease in mortality and in life expectancy.

According to the World Health Organization in 2000, this aged and elderly survived worldwide to 600 million people and an estimate for 2025 is that the number of seniors will exceed more than a billion. In industrialized countries are the fastest growing part of the senior population is people over eighty years. It means that age takes a relatively large part of the time span of human life, but also that in the near future seniors will occupy an increasing share in the overall population.

The World Health Organization therefore regularly publishes a number of resolutions and challenges, which draws attention to the health problems of aging. UN General Assembly decided that from 1991 on 1 October celebrated "International Day of Older Persons".

The European Union declared 2012 the 'European Year for Active Ageing and Solidarity between Generations ", whose central idea was that life does not end after the sixties that active seniors are playing and will play in modern society increasingly important role.

The programs promoting the values of active aging is connected, the Czech Republic a number of projects that are focused on health stays, fitness programs and education for the elderly. The essence of all these activities is to inform people and health professionals given the increase in life expectancy must be accompanied by an increase in quality of life, and that this phase of life could be lived in good health and actively as possible.

Also, members of younger must realize that they have a high probability to live to an even greater age, which will certainly want to live as actively and health.

The most important and relatively simplest way to achieve active aging adhering to the principles of
good nutrition and lifestyle from an early age. In old age is to be expected with the dual threats to health: obesity, or vice versa malnutrition.

**Significant changes that accompany aging?**
- physiological: changing the composition of body tissues, decreasing muscle mass (about 20 percent)
- Energy: reduced physical strength and endurance performance (seniors have reduced demands on energy intake by 20-30% ) decreases heat production.

**Which basic principles must nutrition programs for seniors respect?**
- adjust food intake changes accompanying aging;
- appropriate diet to slow the degenerative processes and enhance tissue regeneration organism;
- respect the nutritional and energy requirements of an aging organism
- respect century cultural eating habits.

**Author**

**Dr., Petr Sima, PhD.** - immunologist
deals with the evolution of immunity and the influence of nutrition on immunity.

Since r. 1967 until the present time is still working in the Institute of Microbiology ASCR. In addition, he worked in the Bay of Kotor in Yugoslavia at the Institute for Research on marine animals, Basel Institute for Immunology in as well as a specialist haematologist-immunologist at the University Hospital in Angola Luanda. It carries Medals JEPurkyně for the development of life sciences and has authored or co-authored more than 200 scientific publications. Since 1990 is an investigator and co-investigator 19 grant projects. He is a member of Czech and international scientific societies.
Nucleotides. A review and current advances

The endogenous supply of nucleotides is maintained de novo synthesis and the salvage pathway. Because there are metabolically costly process, it is more efficient to use already formed nucleotides. This is particularly true in rapidly dividing tissues, such as intestinal and lymphoid tissues, which require nucleotides for the synthesis of nucleic acids. One DNA replication requires at least $10^9$ nucleotide molecules. An exogenous source of nucleotides, such as dietary supplement, could optimize the tissue function by sparing the cost of de novo synthesis or salvage. This may be especially important during periods of rapid grows.

Dietary nucleotides can improve the grows and differentiation of the intestinal cell lines, the response to injury of the intestinal track and positively influence the bacterial flora in infants. In the intestine of breast fed infants, bifidobacteria were predominant, while gram-negative bacteria predominate in those of infants fed cow’s milk based formula. Breast milk contains about hundred times the level of nucleotides present in cow’s milk. Nucleotides supplemented milk increased HDL and decreased VLDL in infants, suggesting that nucleotides enhance lipoprotein synthesis particularly in the intestine.

Feeding nucleotides to infants improve hepatic function and restoration and has been associated with increased polyunsaturated fatty acids in erythrocytes, suggest in that nucleotides play a role in the conversion of 18C essential fatty acids to 20C-22C very long chain polyunsaturated fatty acids.

In humans, rapidly proliferating lymphocytes require an exogenous supply of nucleotide for optimal function even if de novo synthesis and salvage pathways have been demonstrated. Lymphocytes seem to require dietary nucleotides for normal maturation and function. In mice and humans, a nucleotide free diet significantly depressed IgM and IgG antibody production from spleen cells. Host resistance to bacterial and fungal infection decreases on nucleotide free diets. In human, parenteral and/or peroral solutions with nucleotides given to postoperative cancer patients improve immune function and infectious complications and length of hospital stay was reduced compared to a control group.

A large number of clinical studies on human infants has been conducted to investigate in particular the gastrointestinal and immunological effects of dietary nucleotides. Beneficial effect of dietary nucleotides on mucosal regeneration, immune function have been demonstrated. “Immunonutrition” is a term which has been adopted to describe diets that contain
several additives. Som in the form of glutamine. Arginine, fish oils and nucleotides alone or in combination.

Results of an intervention study- Iodine supplementation of Gipsy children for a period of three month with humanized milk (nucleotides and polypeptide) has regulating effect in the iodine metabolism as well as in secretory immunity. In supplemented children a significantly lower frequency of diseases and shorter periods of morbidity were recorded.

Ústí nad Labem 15.11.2002.
NUCLEOTIDES

What Are Nucleotides?

Introduction

> What Are Nucleotides? & What Functions Do They Serve in the Body?

Genes, Chromosomes, and Junk DNA?

What Are The Differences Between RNA And DNA?

Where Do Nucleotides Come From?

What Are Nucleotides?

Nucleotides are molecules comprised of a sugar molecule, a phosphate unit, and a nitrogenous (nitrogen-containing) base. They are found everywhere within the cells of our bodies, including the nucleus and the cytoplasm. Some of these nucleotides function as cellular messengers for communication from the outside to the inside of the nucleus, like cyclic adenosine monophosphate (AMP). Others are used to store energy like adenosine triphosphate (ATP). There are nucleotides that function as physiological mediators, like adenosine diphosphate (ADP) which is produced during muscle contraction and necessary for platelet aggregation, and uracil diphosphate (UDP) which has a role in the body’s detoxification process. The nucleotides that we will be discussing in this book, however, will be those necessary for the construction of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

The nucleotides used by the body to create DNA and RNA are made up of a five-carbon sugar (ribose), a phosphate group (one phosphorus and three oxygen atoms), and one nitrogen-containing base. In the case of DNA, found in the nucleus of all cells and responsible for transmitting genetic information, one of four bases will be used to create the nucleotides: adenine (A), guanine (G), cytosine (C), or thymine (T). And the ribose molecule has had one oxygen atom removed from it so it is more correctly termed deoxyribose.

In the case of RNA nucleotide production, the sugar molecule remains as ribose and three of the nucleotide bases; adenine, guanine, and cytosine, are still used, but the base uracil (U) replaces thymine.

Let’s take a look at the following diagrams 1 and 2. Perhaps this will make it a little clearer. Diagram 1 consists of an example of a DNA molecule on the left and drawings of the RNA and DNA nitrogenous bases on the right. The dark circled P (DNA molecule) is the phosphate group and the five-sided figure with the S is the sugar (deoxyribose) molecule. Attached from one of each of these is one base pair represented by an A, T, G, or C. Larger diagrams of the nitrogenous bases representing their chemical structure are to the right of the DNA molecule. You’ll notice that the guanine and adenine are created with two carbon-nitrogen rings. They have been termed purines. The single-ringed molecules, cytosine, thymine, and uracil are called pyrimidines.
When the nitrogenous base is attached to the sugar molecule it is now called a **nucleoside**. And a nucleotide is a nucleoside with the phosphate unit attached.

### Five-sided sugar (pentose) + Nitrogenous base → Nucleoside

Example: Uracil + Ribose → Uridine

### Nucleoside + Phosphate Group → Nucleotide

Example: Uridine + One phosphate → Uridine monophosphate

I’ve created a little chart below to, hopefully, add a little more clarity.

<table>
<thead>
<tr>
<th>Base</th>
<th>Nucleoside</th>
<th>Nucleotide</th>
<th>Abbreviation</th>
<th>Used to construct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ sugar</td>
<td>+ one phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>adenosine</td>
<td>adenosine monophosphate</td>
<td>AMP</td>
<td>DNA and RNA</td>
</tr>
<tr>
<td>Guanine</td>
<td>guanosine</td>
<td>guanosine monophosphate</td>
<td>GMP</td>
<td>DNA and RNA</td>
</tr>
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<td>Cytosine</td>
<td>cytodine</td>
<td>cytodine monophosphate</td>
<td>CMP</td>
<td>DNA and RNA</td>
</tr>
<tr>
<td>Thymine</td>
<td>thymidine</td>
<td>thymidine monophosphate</td>
<td>TMP</td>
<td>DNA only</td>
</tr>
<tr>
<td>Uracil</td>
<td>uridine</td>
<td>uridine monophosphate</td>
<td>UMP</td>
<td>RNA only</td>
</tr>
</tbody>
</table>

### Diagram 2

You may have noticed in Diagram 1 that the nucleotide bases bind to one another in a very specific fashion. In DNA molecules, adenine and thymine (A-T) bind to one another as does guanine and cytosine (G-C). With RNA,
uracil takes the place of thymine and, instead, it binds with adenine (A-U). Purines bond only with pyrimidines. The two types can never bond to the same type. In other words, pyrimidines bases can not bond to other pyrimidines bases. This specific pairing of nucleotides helps to insure the correct passing on of genetic information from the mother cell to the daughter cells.

Looking again at Diagram 1, you’ll notice that as the nucleotide bases bond, the sugar molecule and the phosphate units form a **backbone** on each side, and the entire complex starts to take on the appearance of a ladder. After the bases have bonded, they are called **base pairs**, and form the rungs of the ladder while the phosphate-sugar backbones create the rails.

Now imagine stacking 3 billion base pairs one upon another, and you’ve created a typical DNA molecule. As the DNA molecule forms, it takes on a twisted, or corkscrew, appearance known as a **double-stranded helix**. Please take a look at Diagram 3. Once again you’ll notice that the rungs of the ladder are created using nucleotides with the base pairs guanine and cytosine (G-C) and adenine with thymine (A-T).

![Diagram 3: Genes, Chromosomes, and Junk DNA?](http://nucleotideresearch.com/chptr01.html)
NUCLEOTIDES

Where Do Nucleotides Come From?

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Where Do Nucleotides Come From?

Dietary nucleotides, as their name implies, come from our diets. These are termed exogenous since they are created external to our body from various food sources. Our body can also create nucleotides. These are referred to as endogenous since they are created within. The creation of new nucleotides is referred to as de novo synthesis. For dietary sources of nucleotides, please refer to the following chart.

Dietary Sources of Nucleotides

(milligrams/100 grams)

Source Adenine Guanine Total Purines RNA

Beef liver 62 74 197 268
Beef kidney 42 47 213 134
Beef heart 15 16 171 49
Beef brain 12 12 162 61
Pork liver 59 77 289 259
Chicken liver 72 78 243 402
Chicken heart 32 41 223 187
Fresh seafood 8 185 411 341
Clams 14 24 136 85
Mackerel 11 26 194 203
Salmon 26 80 250 289
Sardines 6 118 245 243
Squid 18 15 135 100
Dried legumes 17 14 56 356
Split peas 88 74 195 173
Lentils 54 51 162 140
Blackeye peas 104 82 222 306
Pinto beans 46 39 144 485
Nucleotides are some of the largest molecules synthesized by our cells, and their creation requires many substrates, many steps and huge amounts of energy. They biosynthesis of nucleotides is under very tight control since energy is wasted when making too much, and DNA replication and cellular metabolism is slowed down when making too little. Our cells are also very sensitive to the amount of free nucleotides floating within the nucleus or the cytoplasm. The cell will always choose to use the nucleotides that have already been created before synthesizing new ones. Cellular energy is conserved and DNA replication is enhanced by making sure that your cells are constantly supplied with exogenous nucleotides.
NUCLEOTIDES

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What Are The Differences Between RNA And DNA

- The 3 nitrogenous bases of both remain the same (adenine, guanine, and cytosine) except DNA uses thymine as the 4th base, and RNA uses uracil. RNA is composed using the 5-carbon sugar, ribose. And, DNA uses deoxyribose.
- DNA contains double strands of nucleotides, while RNA has only one strand. Please refer to Diagram 4.
Diagram 4

Where Do Nucleotides Come From?
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Genes, Chromosomes, and Junk DNA
Each cell in our body contains a lot of DNA. In fact, if our cells were enlarged to the size of an aspirin pill, our DNA would be 10,000 meters long. This is equivalent to a 10 K run or the length of 109 football fields! The DNA is wrapped around specialized proteins, and this DNA-protein is tightly packed to form chromosomes. Every cell in your body has two sets (one from Mom and one from Dad) of 23 chromosomes for a total of forty-six. Every living thing on the planet contains relatively the same DNA. It is the genetic material in all forms of life. This means that the DNA of all organisms, from bacteria to onions to gorillas, is made of molecules using the same kinds of instructions. However, the number of chromosomes is quite different. Please take a look at the following chart.

Organism Number of chromosomes

Human 46
Chimpanzee 48
Carp 104
Gorilla 48
Dog 78
Chicken 78
Frog 26
Housefly 12
Mosquito 6
Onion 16
So we have 1 trillion cells in our bodies with 46 chromosomes found in each nucleus. Each set of chromosomes contains 50,000 to 100,000 genes carried in 3 billion nucleotide pairs of DNA. So what are the genes? A gene consists of a specific sequence of nucleotides found on a strand of the DNA molecule. It is a segment of DNA that contains the information necessary for the creation of a sequence of amino acids essential for a specific enzyme or other protein. The sum total of the genes contained in an organism’s full set of chromosomes is termed the genome. Some of these proteins are created through the action of only one gene. Other, more complicated, proteins are created using several genes. A gene can be made up of only a few hundred nucleotide bases or it may require thousands of bases. In the 1980’s researchers learned that only 28% of human DNA is made up of genes and only 5% of the nucleotide bases found within these genes actually contain coding sequences for proteins. In fact, humans have more non-coding DNA than any other species studied thus far. The non-coding DNA that makes up about 98% of the human genome was formerly known as junk DNA, since its functions had not been discovered.

Recently investigators have discovered that some of this junk DNA found at the ends of the chromosomes, known as telomeres, is involved in cellular aging and controls the number of times that a cell can divide. Other non-coding segments of DNA have been found to switch on and off certain genes, and function as regulators influencing other different gene activity. There are some researchers who theorize that some of the junk DNA is deactivated genetic material from our earlier ancestors. Maybe one day we will find that it truly serves a purpose and really isn’t junk afterall.

RNA and Protein Synthesis Whereas DNA’s primary role is passing on exact genetic information through cell division, and providing templates for protein synthesis, RNA is concerned with protein synthesis only. I think most of us tend to think of muscles, ligaments, or tendon tissues when we think of proteins, but in reality they are much more than that. For example, hemoglobin, found in our red blood cells, is a protein that transports oxygen throughout our bodies. Collagen is the most abundant protein in the body and is a component of all connective tissues in the body. Hormones are also proteins that are produced and released from glands. These proteins, or hormones, travel to specific sites and help to regulate all tissue and organ function. Other proteins include enzymes. Some enzymes are digestive and help break down foods into usable forms, while other metabolic enzymes function as chemical modulators and cause specific chemical
reactions to take place, while increasing the rate at which these reactions occur. All protein synthesis occurs in the cytoplasm of the cell within organelles called ribosomes. Remember that the DNA is found only in the nucleus. So how does this information get from the inside of the nucleus to the ribosomes found outside of the nucleus? This is accomplished by making copies of certain portions of the DNA that provide a template for specific amino acids and proteins. These copies, known as the messenger RNA (mRNA), are created using free-floating nucleotides found within the nucleus. The nucleotides are grouped in groups of three each or triplets. These triplets are also known as codons. The mRNA can travel through the nuclear membrane to the ribosome. The mRNA can be made up of anywhere from 5,000 to 200,000 nucleotides. The process of creating mRNA from DNA templates is called transcription.

The final process, known as translation, is accomplished by a different type of RNA known as the transfer RNA (tRNA). Unlike mRNA, tRNA is located outside of the nucleus, in the cytoplasm, and carries information for only one amino acid. Once the mRNA reaches the ribosome, the tRNA, carrying three nucleotides specific for an amino acid, deposits these nucleotides on the corresponding codon. As the second tRNA deposits its group of nucleotides, it is bound with the first group to form chains of triplets to form amino acids. As the amino acids are bound together, polypeptides and longer-chained proteins are formed. In the case of the amino acids tryptophan and methionine, only one mRNA codon is required for their creation. Other amino acids, like arginine, leucine, and serine require six mRNA codons. For example purposes, I've listed the essential amino acids and the base pair sequences required for their synthesis. The essential amino acids are those that can not be manufactured by our body and must be obtain from our diets. For fun, cover up the DNA triplet section and see if you can give the corresponding nucleotide. Remember that DNA uses thymine and RNA uses uracil.

**What Are The Differences Between RNA And DNA?**
Dr. Josef Richter has been for many years leading Czech scientists, specialist in Immunology and Oncology. (Now retired – still adviser & consultant for Minister of Health of Czech republic) He was director of prestigious Institute of Health in city: Usti nad Labem in Czech Republic. He and his institute were representing Czech Republic in World Health Organisation (WHO). He also kept private office until recently, for the most prominent Czech citizens, politicians actors etc, including former president of Czech Republic Mr. Vaclav Havel (who himself was taking preparation Imuregen for many years)

Dr. Richter was chosen by Ministry of Health to conduct very comprehensive study in the year 2001 – 2003, **Name of program:** *Protective and immunomodulative influence of supplementing RNA, comparing of clinical testing and experimental model.*

He was free to choose his own assistants/participants. He selected the most prominent and experienced Czech doctors/scientists, among them names like:

**Co-Researchers:**
- RNDr. Petr Šíma, CSc. BÚ Academy of Sciences Prague
- MUDr. Ivan Pfeifer, CSc. Charles University Prague

**Special assistance:**
- RNDr. Vlastimil Král, CSc.; MUDr. Dalibor Jílek, CSc.; Ing. Ivana Stiborová;
- MUDr. Stanislava Richterová; Jitka Pešková; Mgr. Lucie Dobiášová, Health Institute Ústí nad Labem
- MUDr. Dagmar Slížová, Institute of anatomy LFUK Hradec Králové

After long consideration Dr. Richter and his team decided to test **Czech product Imuregen** and compare it to top rated German made medical product: *Torula.* They conducted 2 year testing program on humans and in laboratory. After conclusion of the clinical studies this team of scientist under Dr. Richter leadership recommended Czech product *Imuregen* to Ministry of Health as an excellent and very economical product to increase immunity in general population of Czech Republic. For your information – company Uniregen was absolutely not involved in this Ministry grant and testing. They only provided their product Imuregen after it was chosen by team of scientist as the best suitable product for testing. (The whole study including graphs and pictures is available)

Dr. Sima was leader of the team residing in Czech Academy of Sciences and his team was conducting laboratory testing on mice for this grant.

Dr. Richter has been using product Imuregen in his own research and practise for many years and has been conducting independent clinical studies on Imuregen. (attached additional PDF document)
In essence, man must eat. We are made by nature or by God, - living organism, and we have to receive some information in the form of food. And in our diet are certain ingredients. The basic components are proteins, then are fats, carbohydrates, etc, as they were gradually discovered. At the beginning of the last century were vitamins, then came the nuance such as flavonoids, which are basically vegetable dyes and that was the effect, which was then in the 80s of last century called French paradox, which meant that the Mediterranean nations, in particular the French drink wine and have also the lowest number of heart attacks and cardiovascular disease. It was discovered that in flavonoids are numerous substances which attracted great attention. Main are quercitrin and resveratrol. Contrary the Finns in the north, who had milk diet and severe fatty meats were known in Europe with their history of cardiovascular disease. But once they voluntarily switched to a diet of Mediterranean people, cardiovascular disease in Finland was reduced to general average level. Sometime in the 80's of last century someone came up with the idea that there is another ingredient that is mostly negated even by nutritional specialists and these are called nucleotides or dietary nucleotides. These are actually nucleic acids or enzymes, which are not nucleotides, it is DNA in the nucleus, RNA in mitochondria and in a number of enzymes and nobody really recognize that it is logical that if we eat any food we eat actually cells, so we eat cores where are the nucleic acid. There is a great intense research since the 80s, which is now essentially closed, as research of vitamins, but unfortunately it still did not get into awareness or experts that the body needs those nucleotides. A certain researches in their group LERLEIKA, I think it is an American group, devoted enormous attention and found out that actually the whole period of development, the organism learned to use these dietary nucleotides as material for cell division, for regeneration. It's still better, as I always tell the students that you build the house faster from the panels than from brick, cement, water, etc. So nucleotides are actually sort of building panels for ours dividing cells. And what it means dividing cells? It means recovery of all cells like: skin, intestinal mucosa, lung epithelium. Unfortunately the old cells die, the body is removing them, and the cell must divide. The French had figured out, even without knowing that those nucleotides will one day become a nutritional supplement,- Roax I think did it in 1975, that one cell on average need 10 to power of nine of nucleotides, which means one billion. So where the body has to take it from? it will not be “slapping it of cement, sand and water”, but it will take it from the food straight across grafts, as oldilo or polionukleotids. Single nucleotide is such, than it is oldilo that is more nucleotides in a row or polionukleotids, that are all grafts of DNA, RNA. But as I say, it’s for regeneration and from it follows logically the next thing. It is not only child’s body that needs to grow tissues, muscles, bones, nervous system, but also older people need it. Because as people are older, there are now hundreds of millions of old people and it will continue to increase, so there it comes to protecting the quality of life, not only extended the age, but also to preserve its quality. And those older people also need nucleotides for the tissue repair. And now we get to immunity. If cell meets some pathogen, it is generally called an antigen, it receives such impulse that begins to divide. And for the cells to produce antibodies against some bacterial infection or the flu, it takes several generations of cells, we measured it once, that generational time of any cell which will produce antibodies is approximately 10-12 hours. This means that the cell gets to the stage of precursor, then further development and further and after 36 to 72 hours organism is able to respond to the infection, the disease. This means that it needs supply of nucleotides for the division of a hundred millions of lymphoid cells, to make it quickly. And these are the two reasons that totally justify that professionals start to take dietary nucleotides as other nutrients entirely seriously, which is still not happening and that those experts recommended that the nucleotides are very important in three periods of life. Nucleotides are absolutely irreplaceable in growth period, the period of infection and the recovery from the illness, even postoperatively or after chemotherapy and during aging. Of course, we need proteins, we need vitamins, but those are catalysts that help, but the nucleotides have to be taken into account for their importance.
Q: When you are talking about 90 years of research of nucleotides and Imuregen patent dates back to the 50s. This means that they actually found empirically that something works and did not know that it is a nucleotide?

No, they had it from other sources abroad, sometimes they noticed that, when you get some food rich in it, like caviar. I was once in Russia studying and colleague told me, that his grandfather ate this. And when the grandson broke his leg, it quickly healed. And because they are actually cell nuclei, there is plenty of nucleotides. Meat is especially useful. As those who do not eat meat, vegetarians, make big mistake, because even if in beans, peas, lentils, legumes are generally nucleotides almost as much as in meat, but they are less available to the body. Before they are digested, and split, at that time the body loses a lot of energy. Whereas in the thousands or hundreds of thousands of years of human evolution we have learned to eat meat. We are actually the only ape that eats meat and meat made us that we grew nervous system that we’re talking etc. And even that we are two-legged, because that effort to stay on two legs is much greater than keep on four legs. But to be on two legs, allowed us to gain a huge perspective, looked into the prairie, see the prey etc., so it is a very big accomplishment.

And I will get back to Imuregen. Imuregen was invented by Prague pharmacist who had it patented and isolated them from bovine blood without knowing that nucleotide are there. And because blood is a source of nucleotides, not only caviar, but the blood, that's why Maasai are so beautiful, healthy, because when they open the artery of the cattle to drink the blood o it has good results. But the preparation is perhaps 100 years old and even older because even the famous German chemist von Liebig had recommended broth made from the meat. so if someone is recovering from an illness, or perhaps after an injury, what will he get? He will get beef soup, which is full of the nucleotides. And imuregen is the same.

I got some information from Japan, the Japanese use the nucleotides to promote health, immune support etc. The Chinese have taken it apparently even before the Olympics, because it’s not illegal doping, so they were feeding their athletes with it and won many medals. I would stop here, to explain because we always said support of growth, and support of immunity and increase of immunity and it is a bit distorted, because if we will increase immunity, we could chase the immune system into a circle, it could get exhausted. If infection should occurred, the immune system would already be exhausted. It is better to talk about supporting the immune system readiness, preparedness, expectations for the infection, and I think that this is what those nucleotides are doing, so I think it should be interpreted this way.
Dietary Nucleotides and Early Growth in Formula-Fed Infants: A Randomized Controlled Trial

WHAT'S KNOWN ON THIS SUBJECT: Dietary nucleotides are nonprotein nitrogenous compounds found in high concentrations in breast milk. A high nucleotide intake has been suggested to explain some of the benefits of breastfeeding, compared with formula feeding, and to promote infant growth.

WHAT THIS STUDY ADDS: Results of the study revealed that nucleotide supplementation increases weight gain and head growth in formula-fed infants. Therefore, nucleotides may be conditionally essential for optimal infant growth in some formula-fed populations.

abstract

BACKGROUND: Dietary nucleotides are nonprotein nitrogenous compounds that are found in high concentrations in breast milk and are thought to be conditionally essential nutrients in infancy. A high nucleotide intake has been suggested to explain some of the benefits of breastfeeding compared with formula feeding and to promote infant growth. However, relatively few large-scale randomized trials have tested this hypothesis in healthy infants.

OBJECTIVE: We tested the hypothesis that nucleotide supplementation of formula benefits early infant growth.

PATIENTS AND METHODS: Occipitofrontal head circumference, weight, and length were assessed in infants who were randomly assigned to groups fed nucleotide-supplemented (31 mg/L; n = 100) or control formula without nucleotide supplementation (n = 100) from birth to the age of 20 weeks, and in infants who were breastfed (reference group; n = 101).

RESULTS: Infants fed with nucleotide-supplemented formula had greater occipitofrontal head circumference at ages 8, 16, and 20 weeks than infants fed control formula (mean difference in z scores at 8 weeks: 0.4 [95% confidence interval: 0.1–0.7]; P = .006) even after adjustment for potential confounding factors (P = .002). Weight at 8 weeks and the increase in both occipitofrontal head circumference and weight from birth to 8 weeks were also greater in infants fed nucleotide-supplemented formula than in those fed control formula.

CONCLUSIONS: Our data support the hypothesis that nucleotide supplementation leads to increased weight gain and head growth in formula-fed infants. Therefore, nucleotides could be conditionally essential for optimal infant growth in some formula-fed populations. Additional research is needed to test the hypothesis that the benefits of nucleotide supplementation for early head growth, a critical period for brain growth, have advantages for long-term cognitive development. Pediatrics 2010;126:e946–e953

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KEY WORDS
nucleotides, infant growth, occipitofrontal head circumference

ABBREVIATIONS
SGA—small for gestational age
OFC—occipitofrontal head circumference
CI—confidence interval

Dr Singhal was the principal investigator and main author; and Ms Clough and Ms Jenkins were responsible for data collection, which was supervised by Ms Lanigan and Ms Kennedy. All authors contributed to study design and preparation of the final manuscript for submission.

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Breastfeeding has benefits for growth and development, but whether these effects are attributable to sociobiological differences between breastfed and formula-fed infants or to the nutritional composition of breast milk remains controversial. Several nutrients in breast milk have been suggested to contribute to its biological advantages over cow’s milk–based formulas, including a higher concentration of nucleotides. Nucleotides are nonprotein, nitrogenous compounds that play key roles in many biological processes and are suggested to be conditionally essential nutrients in infancy. However, although nucleotides have been added to some infant formulas for many years, their benefits in humans remain uncertain.

The supply of nucleotides provided through de novo synthesis and less metabolically costly salvage pathways is thought to be insufficient for optimal function of rapidly growing tissues such as those in the gastrointestinal, lymphoid, and hematopoetic systems. These tissues have limited capacity for de novo nucleotide synthesis, and although a lower nucleotide intake may not result in a clinical deficiency syndrome, an exogenous supply of nucleotides to rapidly growing tissues is important for their optimal function. Consistent with this hypothesis, nucleotide supplementation in rats has been shown to enhance growth and maturation of the gut, increase villous height, mucosal protein, and disaccharidase activity in the proximal intestine, and enhance gut mucosal recovery after experimentally induced diarrhea. Dietary nucleotides also increase the gene expression of certain gastrointestinal enzymes, and increase postprandial blood flow in mesenteric arteries of infants. Theoretically, therefore, trophic effects of nucleotides on the gut, along with benefits for immune function and incidence of diarrhea, could have advantages for infant growth, a hypothesis supported by results of animal studies.

In humans, however, results of randomized studies have not revealed a beneficial effect of nucleotide supplementation on growth of formula-fed infants. Nevertheless, nucleotide intake was shown to affect biomarkers that could influence catch-up growth in malnourished children and to increase growth in weight, length, and head circumference in infants born small for gestational age (SGA). Nucleotide supplementation may therefore enhance infant growth at least in some vulnerable populations. Here we report the findings from a study in which we investigated the effects of nucleotide supplementation on colonic microbiota and the incidence of diarrhea, and on infant growth. We were particularly interested in the effect of nucleotides on head growth, suggested to be greater in breastfed infants and related to long-term cognitive function.

SUBJECTS AND METHODS

Study Population

Research nurses recruited infants from 4 hospitals (2 each in Leicester and Nottingham, United Kingdom) between 1999 and 2002. Healthy singletons who were born at ≥37 weeks’ gestation without congenital abnormalities, and who had already commenced formula feeding were eligible (n = 200). A reference group of breastfed infants, identified at birth, were eligible if they were still breastfeeding at 8 weeks (n = 101). Informed and written consent was obtained from the mothers of the infants, and the study was approved by a national ethics committee.

Study Design

As soon as possible after birth, formula-fed infants were randomly assigned to be fed with a nucleotide-supplemented infant formula (31 mg/L) (n = 100) or a control formula with <5 mg/L nucleotides (n = 100). A random permuted-block design, stratified according to center (Leicester or Nottingham), was allocated by an independent statistician, and allocation was concealed by the use of numbered, sealed, opaque envelopes. Infants not withdrawn from the study continued to receive the assigned formula milk until the age of 5 months. Research staff and mothers were blind to the identity of the formula.

The composition of supplemented formula was based on the concentration of free nucleotides and nucleosides in human milk as reflected in European regulations in force at the time of the study. Supplemented formula contained: cytidine monophosphate (15 mg/L), adenosine monophosphate (6 mg/L), uridine monophosphate (5 mg/L), inosine monophosphate (3 mg/L), and guanosine monophosphate (2 mg/L), whereas the control formula (Farley's First Milk) had <3 mg/L measurable cytidine monophosphate. The formulas (manufactured by H. J. Heinz Company Ltd, Hayes, United Kingdom) were identical except for their nucleotide concentrations (Table 1) and met European guidelines for formula composition.

Clinical, social, demographic, and anthropometric data were collected in formula-fed infants at the time that they were randomly assigned to formula groups (baseline) and in breastfed infants at the age of 8 weeks. Social class identification was coded according to the registrar general’s classification and was based on the occupation of the parent who provided the main financial support for the family. Weight was measured by using electronic scales (Seca, Hamburg, Germany) that were accurate to 1 g, and length was measured by using a Rol
TABLE 1  Nutritional Composition of Infant Formulas

<table>
<thead>
<tr>
<th>Typical Values</th>
<th>Per 100 mL as Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td></td>
</tr>
<tr>
<td>Kj</td>
<td>284</td>
</tr>
<tr>
<td>kcal</td>
<td>68</td>
</tr>
<tr>
<td>Protein, g</td>
<td>1.5</td>
</tr>
<tr>
<td>Whey protein, g</td>
<td>0.9</td>
</tr>
<tr>
<td>Casein protein, g</td>
<td>0.6</td>
</tr>
<tr>
<td>Carbohydrate (lactose), g</td>
<td>7.0</td>
</tr>
<tr>
<td>Fat, g</td>
<td>3.8</td>
</tr>
<tr>
<td>Linoleic acid, mg</td>
<td>350</td>
</tr>
<tr>
<td>α-Linolenic acid, mg</td>
<td>44</td>
</tr>
<tr>
<td>γ-Linolenic acid, mg</td>
<td>33</td>
</tr>
<tr>
<td>Long-chain polyunsaturated fatty acid, mg</td>
<td>26</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>39</td>
</tr>
<tr>
<td>Chloride, mg</td>
<td>40</td>
</tr>
<tr>
<td>Copper, μg</td>
<td>42</td>
</tr>
<tr>
<td>Iodine, μg</td>
<td>4.5</td>
</tr>
<tr>
<td>Iron, mg</td>
<td>0.6</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>5.2</td>
</tr>
<tr>
<td>Manganese, μg</td>
<td>3.4</td>
</tr>
<tr>
<td>Phosphorus, mg</td>
<td>27</td>
</tr>
<tr>
<td>Potassium, mg</td>
<td>57</td>
</tr>
<tr>
<td>Sodium, mg</td>
<td>17</td>
</tr>
<tr>
<td>Zinc, mg</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin A, μg</td>
<td>100</td>
</tr>
<tr>
<td>Thiamin, μg</td>
<td>42</td>
</tr>
<tr>
<td>Riboflavin, μg</td>
<td>55</td>
</tr>
<tr>
<td>Vitamin B6, μg</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin B12, μg</td>
<td>0.1</td>
</tr>
<tr>
<td>Biotin, μg</td>
<td>1.0</td>
</tr>
<tr>
<td>Folic acid, μg</td>
<td>3.4</td>
</tr>
<tr>
<td>Niacin, mg</td>
<td>0.7</td>
</tr>
<tr>
<td>Pantothenic acid, mg</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin D, μg</td>
<td>6.9</td>
</tr>
<tr>
<td>Vitamin E, μg</td>
<td>1.0</td>
</tr>
<tr>
<td>Vitamin K, μg</td>
<td>0.5</td>
</tr>
<tr>
<td>Choline, mg</td>
<td>2.7</td>
</tr>
<tr>
<td>Taurine, mg</td>
<td>4.8</td>
</tr>
<tr>
<td>Magnesium, mg</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Nucleotide composition of nucleotide-supplemented formula: cytidine monophosphate, 15 mg/L; uridine monophosphate, 5 mg/L; adenosine monophosphate, 6 mg/L; guanosine monophosphate, 2 mg/L; and inosine monophosphate, 3 mg/L. Measurable nucleotide in control formula: cytidine monophosphate, 3 mg/L.

Statistical Analysis

The primary efficacy study outcome was the effect of nucleotide supplementation on stool microbiota and incidence of diarrhea. Infant growth was both a safety and efficacy outcome. Anthropometric measurements were expressed as SD scores (z scores) calculated by using published centiles. Growth was assessed as attained size at ages 8, 16, and 20 weeks and as the change in size between these ages. To allow comparison with previously published data, growth was also expressed as the rate of gain per 24-hour period, the number of nighttime waking periods, pacifier use, and episodes of colic (according to mother’s interpretation of crying symptoms). Research nurses verified all recorded information at each home visit.

RESULTS

A total of 301 infants were recruited, and of these 88 of 100 infants fed nucleotide-supplemented formula, 85 of 100 infants fed control formula, and 96 of 101 breastfed infants were still breastfeeding at the age of 5 months (Fig 1). Infant participation the study was discontinued for reasons that were not known or were social and nonclinical. Mothers of 5 infants (1 infant in the nucleotide-supplemented formula group and 4 in the control-formula group) removed them from the study because of perceived problems with the milk, such as apparent hunger (n = 2) or vomiting (n = 3). Mothers changed the diets of these infants to formulas different from those used in the study. Mothers who discontinued their infant’s participation in the study did not allow follow-up visits, so these infants were not included in analyses performed when participating infants were 5 months old.

Both trial formulas were well tolerated, and no serious adverse events were reported. The time spent crying, incidence of colic, night-time wake periods, and use of pacifiers did not sig-
significantly differ between randomized groups. No infant had a diagnosis of persistent diarrhea or other chronic illness likely to affect growth.

Nucleotide-supplemented formula and control-formula groups were closely matched at the time of randomization (Table 2). There were no significant differences between randomized groups in incidence of eczema or asthma, use of antibiotics during the follow-up period (data not presented), or volume of formula intake (Table 3). The mean age at which solid food was introduced was ~14 weeks for formula-fed infants and 15 weeks for breastfed infants.

Infant Growth in Randomly Assigned Formula-Fed Groups

Infants randomly assigned to be fed with a nucleotide-supplemented formula had greater OFC at ages 8, 16, and 20 weeks than infants fed with control formula (Table 3) (mean difference in z scores at the age of 8 weeks: 0.4 [95% confidence interval (CI): 0.1–0.7]; P = .005) and at 20 weeks of age (adjusted mean difference: 0.2 z scores [95% CI: 0.1–0.4]; P = .005) and at 20 weeks of age (adjusted mean difference: 0.2 z scores [95% CI: 0.1–0.4]; P = .05). No infant had a diagnosis of persistent diarrhea or other chronic illness likely to affect growth.

Mean difference: 0.2

Nuclear-supp

FIGURE 1
Progress of trial to 5 months of age.

Table 2 Subject Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Randomized Formula-Fed Groups</th>
<th>Breastfed Reference Group</th>
<th>Comparison of 3 Dietary Groups, P^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (N = 100)</td>
<td>Nucleotide Supplemented (N = 100)</td>
<td></td>
</tr>
<tr>
<td>At birth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender, % (n)</td>
<td>56 (56)</td>
<td>61 (61)</td>
<td>51 (51)</td>
</tr>
<tr>
<td>Weight, g</td>
<td>3458 ± 527</td>
<td>3439 ± 590</td>
<td>3468 ± 507</td>
</tr>
<tr>
<td>Weight, z score</td>
<td>0.3 ± 1.0</td>
<td>0.1 ± 1.2</td>
<td>0.1 ± 1.0</td>
</tr>
<tr>
<td>Gestation, wk</td>
<td>39.2 ± 1.3</td>
<td>39.5 ± 1.4</td>
<td>39.6 ± 1.1</td>
</tr>
<tr>
<td>SGA, % (n)^b</td>
<td>5 (5)</td>
<td>15 (15)</td>
<td>10 (10)</td>
</tr>
<tr>
<td>Social class, nonmanual labor, % (n)</td>
<td>39 (39)</td>
<td>41 (41)</td>
<td>81 (81)</td>
</tr>
<tr>
<td>Mother with academic degree, % (n)</td>
<td>8 (8)</td>
<td>8 (8)</td>
<td>52 (52)</td>
</tr>
<tr>
<td>Cesarean birth, % (n)</td>
<td>40 (40)</td>
<td>32 (32)</td>
<td>31 (31)</td>
</tr>
<tr>
<td>At randomization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, d</td>
<td>7.5 ± 2.8</td>
<td>7.1 ± 2.6</td>
<td>—</td>
</tr>
<tr>
<td>Weight, g</td>
<td>3457 ± 510</td>
<td>3457 ± 595</td>
<td>—</td>
</tr>
<tr>
<td>Weight, z score</td>
<td>−0.6 ± 1.0</td>
<td>−0.5 ± 1.2</td>
<td>—</td>
</tr>
<tr>
<td>Length, cm</td>
<td>50.3 ± 2.2</td>
<td>50.5 ± 2.8</td>
<td>—</td>
</tr>
<tr>
<td>Length, z score</td>
<td>−0.7 ± 1.1</td>
<td>−0.5 ± 1.4</td>
<td>—</td>
</tr>
<tr>
<td>OFC, cm</td>
<td>35.2 ± 1.3</td>
<td>35.3 ± 1.5</td>
<td>—</td>
</tr>
<tr>
<td>OFC, z score</td>
<td>−0.3 ± 1.0</td>
<td>−0.1 ± 1.1</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are mean ± SD analyzed by using the Student’s t test or percent (n) analyzed by using the χ^2 test. There was a slight loss of n for some variables (<2%). — indicates data not applicable.

* The 3 dietary groups were compared by using analysis of variance for continuous data and χ^2 for categorical data.

b There were no significant differences between infants in the randomized formula-fed groups except for the number of infants born SGA (defined as birth weight <10th centile for gestation and gender) (P = .02).
fed with control formula at 8 weeks of age but not at 16 and 20 weeks (mean difference at 8 weeks of age: 0.3 \( z \) scores [95% CI: 0.01–0.7]; \( P = .04 \)) (Table 3). This difference remained after adjustment for potential confounding factors (gender, socioeconomic status, and gestation) together with weight \( z \) score at randomization (adjusted mean difference: 0.2 \( z \) scores [95% CI: 0.04–0.4]; \( P = .02 \)). Length did not significantly differ between randomized formula-fed groups at any age (Table 3).

Infants fed nucleotide-supplemented formula showed greater increases in weight and OFC between the time of random assignment to formula group and 8 weeks of age than infants fed control formula (mean difference for weight: 0.3 \( z \) scores [95% CI: 0.05–0.4]; \( P = .02 \)) (mean difference for OFC: 0.2 \( z \) scores [95% CI: 0.05–0.4]; \( P = .01 \)) (Table 3), even after adjustment for potential confounding factors (as above), together with anthropometric variables at randomization (adjusted mean difference for weight: 0.2 \( z \) scores [95% CI: 0.04–0.4]; \( P = .02 \)) (adjusted mean difference for OFC: 0.2 \( z \) scores [95% CI: 0.07–0.4]; \( P = .005 \)). Changes in \( z \) scores for weight and OFC between 8 and 16 or 16 and 20 weeks of age did not significantly differ between randomly assigned formula-fed groups (Table 3).

The rate of gain in OFC in the first 8 weeks (as analyzed according to Cosgrove et al\(^{22} \)) was greater in infants fed with nucleotide-supplemented formula (mean [SD]: 16.6 [3.6] mm/OFC at baseline per week) than controls (15.6, 3.1 mm/OFC at baseline per week) (mean difference: 1.0 mm/OFC at baseline per week [95% CI: 0.04–2.0]; \( P = .04 \)). Similar findings were obtained for the rate of weight gain in the first 8 weeks (mean [SD]: 79.4 [23.7] vs 72.5 [22.2] g/kg weight at baseline per week in nucleotide-supplemented and control formula groups, respectively; mean difference: 7.0 g/kg weight at baseline per week [95% CI: 0.2–13.7]; \( P = .04 \)). The rate of length gain did not significantly differ between randomized formula-fed groups (data not shown).

### TABLE 3 Growth in Infancy

<table>
<thead>
<tr>
<th></th>
<th>Formula Fed ((N = 100))</th>
<th>Control ((N = 100))</th>
<th>(P)</th>
<th>Breastfed ((N = 101))</th>
<th>Comparison of 3 Dietary Groups, (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Completed study, % ((n))</td>
<td>85 (85)</td>
<td>88 (88)</td>
<td>.4</td>
<td>96 (96)</td>
<td>.07</td>
</tr>
<tr>
<td>Age at final visit, wk</td>
<td>20.5 ± 1.3</td>
<td>20.4 ± 1.4</td>
<td>.6</td>
<td>20.3 ± 1.4</td>
<td>.8</td>
</tr>
<tr>
<td>Age solids introduced, wk</td>
<td>14.0 ± 2.3</td>
<td>13.8 ± 2.3</td>
<td>.3</td>
<td>15.4 ± 1.8</td>
<td>&lt;.001&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>Volume of formula intake, mL/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age 8 wk</td>
<td>783 ± 185</td>
<td>827 ± 174</td>
<td>.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age 12 wk</td>
<td>847 ± 198</td>
<td>849 ± 199</td>
<td>.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age 20 wk</td>
<td>739 ± 180</td>
<td>753 ± 187</td>
<td>.6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Anthropometric variables**

<table>
<thead>
<tr>
<th></th>
<th>Age 8 wk</th>
<th>Age 16 wk</th>
<th>Age 20 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>5.0 ± 0.6</td>
<td>6.5 ± 0.8</td>
<td>7.3 ± 0.9</td>
</tr>
<tr>
<td>Weight, (z) score</td>
<td>−0.1 ± 1.0</td>
<td>0.05 ± 1.0</td>
<td>−0.01 ± 1.0</td>
</tr>
<tr>
<td>Length, cm</td>
<td>56.5 ± 2.4</td>
<td>56.8 ± 2.7</td>
<td>56.8 ± 2.5</td>
</tr>
<tr>
<td>Length, (z) score</td>
<td>−0.1 ± 1.0</td>
<td>−0.1 ± 1.2</td>
<td>−0.1 ± 1.0</td>
</tr>
<tr>
<td>OFC, cm</td>
<td>38.8 ± 1.2</td>
<td>39.5 ± 1.4</td>
<td>41.3 ± 1.3</td>
</tr>
<tr>
<td>OFC, (z) score</td>
<td>−0.06 ± 0.9</td>
<td>0.4 ± 1.0</td>
<td>−0.03 ± 1.0</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>15.4 ± 1.4</td>
<td>16.3 ± 1.9</td>
<td>17.2 ± 1.6</td>
</tr>
</tbody>
</table>

**Change in \(z\) score between randomization and 8 wk**

<table>
<thead>
<tr>
<th></th>
<th>Weight</th>
<th>Length</th>
<th>OFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in (z) score</td>
<td>0.5 ± 0.7</td>
<td>0.7 ± 0.7</td>
<td>0.3 ± 0.7</td>
</tr>
</tbody>
</table>

**Change in \(z\) score between 8 and 16 wk**

<table>
<thead>
<tr>
<th></th>
<th>Weight</th>
<th>Length</th>
<th>OFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in (z) score</td>
<td>0.04 ± 0.5</td>
<td>0.03 ± 0.5</td>
<td>−0.2 ± 0.5</td>
</tr>
</tbody>
</table>

**Change in \(z\) score between 16 and 20 wk**

<table>
<thead>
<tr>
<th></th>
<th>Weight</th>
<th>Length</th>
<th>OFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in (z) score</td>
<td>0.04 ± 0.3</td>
<td>0.07 ± 0.6</td>
<td>0.05 ± 0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD analyzed by using the Student\'s \(t\) test and percent (\(n\)) analyzed by using the \(\chi^2\) test. — indicates data not applicable.

The 3 dietary groups were compared by using analysis of variance and Bonferroni corrections for continuous data and \(\chi^2\) test for categorical data.

Post hoc analyses: breastfed versus control formula: \(P < .001\); \(P = .001\); breastfed versus nucleotide-supplemented formula: \(P < .001\); \(P = .001\); control formula versus nucleotide-supplemented formula: \(P = .04\).
Other Analyses

The effect of nucleotide supplementation on OFC was not a consequence of greater weight gain alone. Thus mean OFC of nucleotide-supplemented infants was greater than controls at 8 weeks after adjustment for potential confounding factors (as above), OFC z score at randomization, together with weight z score at 8 weeks (adjusted mean difference: 0.2 z score [95% CI: 0.01–0.3]; P = .04). Similarly, infants fed nucleotide-supplemented formula had a significantly greater increase in OFC z score from randomization to the age of 8 weeks even after adjustment for potential confounding factors, OFC z score at randomization, together with the change in weight z score between this period (adjusted mean difference: 0.2 z scores [95% CI: 0.01–0.3]; P = .03).

OFC was greater in infants given nucleotide-supplemented formula than controls after adjustment for birth weight z score (see above) or adjustment for the number of infants born SGA (birth weight <10th centile for gestation and gender) (adjusted mean difference: 0.6 z scores [95% CI: 0.2–0.9]; P = .003). However, the effect of nucleotide supplementation on head growth was greater in those born with lower birth weight: the interaction randomized formula-fed group × birth weight z score was statistically significant for z-score change in OFC in the first 8 weeks (P = .04) but not for z-score change in weight in the first 8 weeks (P = .9).

Comparison With Breastfed Infants

In secondary analyses, OFC z score at 8 weeks of age was greater in breastfed infants than infants fed control formula (P = .04), but did not significantly differ from that of infants fed nucleotide-supplemented formula (P = .9) (Table 3). Weight z score at 8 weeks of age did not significantly differ between the 3 dietary groups (P = .1).

DISCUSSION

The presence of nucleotides in breast milk, but not in unsupplemented formula, is suggested to have benefits for health including improved somatic growth. Our findings are consistent with this hypothesis and suggest that nucleotide supplementation of formula increases infant weight gain and head growth. Nucleotides could therefore be conditionally essential in some formula-fed populations. Importantly, the effect of nucleotide supplementation in increasing OFC was independent of changes in body weight, which suggests a preferential effect of nucleotide supplementation on head growth. Because the rapid increase in head size in early infancy reflects an increase in brain volume during a critical period in development and is related to higher cognitive function later in life, these data raise the hypothesis that nucleotide supplementation of infant formula could have advantages for long-term cognitive development. Theoretically, therefore, higher nucleotide concentrations found in human milk compared with most formulas may contribute to the benefits of breastfeeding for early head growth and hence later cognitive development. Our data were consistent with those of Cosgrove et al., who found substantial and clinically important effects of nucleotide supplementation for weight gain and head growth in formula-fed infants born SGA. The effect size (difference in OFC growth rate between nucleotide-supplemented infants and controls of ~2 mm/m OFC at baseline per week) was greater than in our study (1 mm/m OFC at baseline per week), probably because of greater catch-up growth in infants born SGA. Nevertheless, even this smaller effect in our study resulted in greater OFC in nucleotide-supplemented compared with control-formula–fed infants at 8 weeks (by 0.4 z scores), a substantial effect for populations that amounts to ~8% of the population variation in head size and is similar to the 10% difference in growth rates attributable to nucleotides according to Cosgrove et al. This effect, although greatest at 8 weeks of age, remained at 20 weeks, which suggested a longer-term benefit of nucleotide supplementation on head growth. In contrast, differences in weight between infants in randomly assigned formula-fed groups were not apparent at ages 16 and 20 weeks, probably because of the addition of other dietary sources of nucleotides in both groups with the introduction of complementary feeding (at the age of ~14 weeks). Nucleotide supplementation seemed to ameliorate the disadvantage of formula feeding compared with breastfeeding for head size at the age of 8 weeks. Because breastfed infants may have faster head growth than formula-fed infants this finding suggests that a lower concentration of nucleotides in formula compared with breast milk may limit the optimal rate of head growth in some formula-fed infant populations. This effect of nucleotides may be greatest in infants born SGA, as suggested previously, and was supported by our finding of significantly greater head growth in infants born SGA who were fed with nucleotide-supplemented formula. In contrast to our data, and to other reported data from infants born SGA, results of previous randomized trials have not shown advantages of nucleotide-supplemented formula for somatic growth. There are several potential explanations for this difference, although few data to support a particular hypothesis. One theory is that nucleotides are conditionally essential during periods of rapid growth and hence nucleotide-supplemented formula may have greater effects on...
growth in populations with faster postnatal growth, such as infants born SGA. However, postnatal growth is determined by genetic and environmental factors, other than size at birth, including the degree that maternal characteristics affect fetal growth. Therefore, an inherently faster rate of postnatal growth could explain growth-promoting effects of nucleotides in the current population, a hypothesis supported by the faster weight gain in the first 8 weeks in infants fed control formula relative to the UK reference population (by 0.5 SD) (Table 3).

Another hypothesis to explain the variation in reported benefits of nucleotide-supplemented formula for somatic growth is the existence of a threshold intake, above which additional dietary nucleotides have little effect on growth. Control formulas used in previous studies contained 10 mg/L inherent nucleotides (compared with <3 mg/L in our study), an amount that may have obscured any advantage of nucleotide supplementation at higher concentrations. Differences in study findings may also be attributable to population differences in absorption, use, and metabolism of nucleotides or beneficial effects of nucleotides on gastrointestinal microflora, although little evidence exists to support this hypothesis.

A lack of evidence for the mechanism of the beneficial effects of nucleotide supplementation is a limitation of our study. Nucleotides may act as flavor enhancers and promote growth by increasing formula intake, but like previous investigators, we found no evidence for this. Nucleotide supplementation could have protein-sparing effects. For instance, Uauy calculated that daily nucleotide synthesis requirements could consume up to 10% of protein requirements in infancy. Nucleotides also enhance immune function and so could improve growth by reducing illness episodes, although, again, we found no evidence to support this hypothesis. A more probable mechanism is a beneficial effect of nucleotides on the gastrointestinal tract, which could enhance epithelial function and nutrient absorption by a trophic effect or via an increase in intestinal blood flow.

CONCLUSIONS
Among the few human populations to have a minimal nucleotide intake are infants fed formula without nucleotide supplementation. Our findings of a beneficial effect of nucleotides on head growth in formula-fed infants, together with the previously described benefits of nucleotide supplementation for immune function and diarrhea prevention, support the addition of nucleotides to all cow’s milk–based infant formulas. However, controversy exists as to the benefits of faster weight gain, and the slower weight gain that occurs in breastfed infants compared with formula-fed infants may be beneficial. Nevertheless, the advantage of nucleotide supplementation for increased head growth during a critical period for brain development may have important long-term implications for formula-fed infants.

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REFERENCES

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DOI: 10.1542/peds.2009-2609

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