CHIMIKA CHRONIKA

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CLINICAL LABORATORY VALUES IN THE AGING POPULATION

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Over the last few decades there has been a significant increase in the average life expectancy due to the decrease in infant mortality and fatal diseases, such as cardiovascular disease and cancer. However, there has been only a marginal gain in the maximum attainable life span. Our main effort, however, should center on the improvement of the functional life span. In this regard, the clinical laboratory can play a major role by defining proper reference ranges for the aging, by identifying disease processes early, and by aiding in the treatment process. --I will report on a study of laboratory values in serum/plasma in the aging, involving sexagenarians to centenarians.

Electrolyte values (Na, K, Cl, CO_2) were remarkably stable and deviated from values in the young adults only in the very old (>90 y). Total Ca values decreased because of lower albumin values, while Ca_i showed no change. Phosphorus values decrease in males but only marginally in females. Kidney function decreased due to decreased filtration rate, which is evidenced by increases in the serum urea nitrogen. Creatinine values remain relatively stable, because of decrease in muscle mass. Uric acid increases were observed in females. Osmolality values increase. Total protein values showed little change, while the nutritional proteins (pre-albumin, albumin, and transferrin) decreased significantly. $Alpha_1$-antitrypsin and haptoglobin increased, while gamma-globulin and ceruloplasmin showed no change. Glucose values increased, but so did insulin and C-peptide. Bilirubin values decreased, while AST and ALT showed only minor changes. ALP increased slightly in males and markedly in females. Cholesterol and HDL-cholesterol increased with age but showed no change in the very old. Creatine kinase and the MB isoenzyme decrease in the aging, but particularly in the very old. B_{12} and folate values decrease, while individual immunoglobulins vary. Thyroxin, T_3, and TBG decrease together with the metabolic rate. The pituitary hormones prolactin, LH, and FSH all showed an increase with age.

Testosterone (free, bioactive, and total) decreases in the elderly, although there is much individual variation, and some individuals have values comparable to those in young adults. The decrease is paralleled by decreased testicular function. In females, there was a corresponding decrease in estrone and estradiol. Decreases in both sexes were seen for dehydroandrosterone and progesterone.

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THE POLYMERASE CHAIN REACTION TECHNIQUE IN MOLECULAR DIAGNOSIS

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The Polymerase Chain Reaction (PCR) technique since its description in 1985 has been increasingly used in molecular diagnosis in a variety of fields in medicine and biomedical research. The PCR technique is performed as an *in vitro* enzymatic synthesis of millions of copies of a specific DNA segment, that is of known sequence. The three main steps i.e. denaturation, primer binding and DNA synthesis represent a single PCR cycle and each step is carried out at a discrete temperature. Repeated cycles of denaturation, primer annealing and primer extention result in the exponential accumulation of a discrete fragment whose termini are defined by the 5' ends of the primers. Its ability to amplify as well as modify a specific target DNA sequence from a complex template in a simple automated procedure, has facilitated many tasks in molecular biology research e.g. cloning and sequencing, thus opening up new areas for experimental investigation. Moreover, the PCR technique had a profound influence on medical research involving clinical and applied medical disciplines. It has been employed to characterize genetic defects, HLA and tissue typing for organ transplantation, determining the sex of human embryos before *in vitro* fertilization, detecting microorganisms, identifying activated oncogenes and the characterization of leukemias and lymphomas among others. Thus the PCR technique is the best suited to help answer the critical diagnostic question concerning the presence of a given DNA sequence in a clinical specimen.

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CYTOKINES : THEIR PHYSIOLOGY AND DIAGNOSTIC SIGNIFICANCE.

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Cytokines are a diverse group of low molecular weight proteins which mediate much of the intercellular signalling required for an integrated response to a variety of external stimuli. These soluble intercellular messenger molecules permit the immune system to interact with the myelopoietic, haemopoietic and neuroendocrine systems. The term cytokines includes the interleukins (IL-1 to IL-16), interferons (IFN α, β, γ), colony stimulating factors and TNFs.

General properties and functions of cytokines :

Cytokines are produced by T cells, mononuclear phagocytes and a variety of other cells in the body, in response to a number of inducing stimuli, i.e. inflammatory or antigenic stimuli or under the influence of other cytokines. The same cytokines are often made by many cell types and individual cytokines often act on many cell types. Cytokine secretion is a brief, self limited event.

Most cytokines act locally, on the same cell that secretes it (autocrine action) or on a nearby cell (paracrine action) by binding to high affinity receptors on the membrane of target cells. Certain cytokines may be produced in sufficient quantities to circulate and exert their action on a distant cell (endocrine action). The binding of a cytokine to its specific receptor results in an altered pattern of gene expression in the target cells. Because of the high affinity that the cytokines and their receptors exhibit for each other, only very small quantities of a cytokine need be produced to elicit a biologic effect.

For many cell types, cytokines serve as growth factors.

Thus, cytokines are secreted when cells are activated by antigen or other cytokines. The cytokines can be functionally organized into groups according to their principal actions : 1) those that promote and mediate natural immunity, 2) those that exert lymphocyte regulatory activity, 3) those that serve to activate inflammatory leukocytes and place these effector cells under T cell regulation and 4) those that act as haematopoietic growth factors.

This complicated network of interacting cytokines functions to modulate and regulate cellular function so that the host may survive in an otherwise hostile environment. Excessive production or actions of cytokines can lead to tissue injury and even death.

Detection of cytokines :

Cytokines in biological fluids (cell culture supernatants, human serum or plasma, synovial fluid) can be detected by their activity in bioassays of cell growth, where they serve either as growth factors or growth inhibitors. To date the use of specific monoclonal antibodies against many individual cytokines has

permitted the development of sensitive and specific radioimmunoassays or enzyme-linked immunosorbent assays. These techniques can detect and quantify various cytokines with great sensitivity. Cytokine production in tissues can be detected by immunohistochemistry and molecular biology techniques. Lately, flow cytometry is also used for the detection of these molecules.

Diagnostic significance of cytokines:

The role of numerous cytokines in the pathogenesis of immuno-inflammatory and infectious diseases is well documented in many instances. However, so far, the relevance of cytokine quantification in biological fluids in clinical conditions remains to be validated. At the present time cytokine determination cannot be used as a diagnostic tool, but merely as a marker of disease activity and severity. The questions "do in vitro determinations provide realistic information on the conditions in vivo ?" and "what cytokines should be measured with a given type of disease and type of clinical trial ?", along with a number of other questions, have still to be answered. As more is understood about the complex interactions of the cytokine network, direct identification of those cytokines involved in tissue pathology and the development of means to selectively control their production or activity may become possible.

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BIOCHEMICAL MARKERS OF BONE METABOLISM

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Age and sex are important factors contributing to biological variation of bone turnover, e.g. children and adolescent have a high bone turnover as well as women in the early menopause.

Changes in bone metabolism are common in different diseases which affect bone humorally (osteoporosis, rheumatoid arthritis, osteoarthritis) or by neoplastic growth. Similar is the action of different medicaments like corticosteroids. X-ray examination is in fact very rough method for evaluation of bone alteration and osteodensitometry is not able to follow gentle changes in bone metabolism.

Biochemical methods may ideally show rapid changes in the breakdown or formation of bone extracellular matrix (ECM) constituents of which collagen is the most abundant. Collagen breakdown results in a mixture of peptides even in free amino acids. In this respect three substances are of interest i.e. pyridinoline (PYD) resp, deoxypyridinoline (DPYD), crosslink elements of cartilage and bone collagens, galactosylhydroxylysine (gal-hyl), which is the predominant product of translational glycosylation of skeletal collagen, and hydroxyproline. All the mentioned substantces cannot be further metabolized or reutilized, and are therefore excreted in urine. Urinary gal-hyl excretion is regarded as a marker of bone resorption in adults as well as as index of skeletal growth in children. Urinary hydroxyproline (Uhyp) determination is of minor value as Uhyp amounts are given not only by bone collagen catabolism but also by Clq catabolism and by diet. PYD and DPYD are determined either by HPLC or ELISA method. Bone collagen comprises practically type I only, its breakdown may be further measured by a urine concentration of cross-linked aminoterminal (INTP) as well as of carboxyterminal telopeptides of type I collagen (ICTP) using ELISA. Osteoclasts activity is also reflected by serum tartrate-resistant acid phosphatase (TRAP) levels.

On the other hand osteoblasts activity is reflected by serum levels of carboxyterminal (PICP) and aminoterminal propetides (PINP), which is three times smaller than PICP. It should be stressed that telopeptides as well as propeptides of collagen type I are not specific markers for bone collagen. Therefore their serum levels may be influenced by other pathological processes like liver cirrhosis, pulmonary fibrosis etc. Further marker of osteoblasts activity are serum alkaline phosphatase and serum osteocalcin (OC) also entitled GLA-protein, which is the major noncollagenous protein of bone dependent on vitamin K. It specifically binds both calcium and hydroxyapatite.

Whereas control group of adult persons had UPYD in average 41.6+10.6 nmol /mmol creatimine and UDPYD 8.1+2.8, the mean value of 94 postmenopausal osteoporotic patients was 109.6+61.7 and that of UDPYD in the same group was 22.8+14.2. Calcitonin treatment caused in many patients normalisation of both these markers. Increased UPYD values reflect intensified bone catabolism. UPYD and UDPYD are increased also in osteoarthrosis, where they signalize that OA destructive process is now localized also in subchondral bone. Our patients with the I or II stage OA showed average UPYD value 43.6, whereas patients in the III stage had average UPYD value 68.8 nmol/mmol creatinine. Rather high UPYD values were found in patients with myeloma multiplex (107.56+). In this disease UPYD values reached over 400.0 nmol/mmol . Determination of UDPYD is unnecessary as correlation coefficient between UPYD and UDPYD in our patients is 0.97. Because we use HPLC for determination of both the substances their urine concentrations are available in one run.

Serum levels of OC only slightly increased in OP patients 13,65, whereas in OA patients were more increased in early stages 9.8 than in the 3 stage (8.6). Patients with myeloma multiplex without treatment showed mean OC value 14.75, which decreased after intensive therapy (9.6). Serum bone alkaline phosphatase was increased up to 42.23 in OA patients (normal up to 22.0), in OP patients after collagen hydrolysate consumption increased up to 67.87.

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ROUND TABLE

HARMONIZATION OF LABORATORY RESULTS WITH THE USE OF REFERENCE MATERIALS. STANDARDIZATION OF ENZYME, CHOLESTEROL AND LIPOPROTEIN DETERMINATIONS

Coordinator: **P. Arzoglou** (Associate Professor of Clinical Chemistry, University of Thessaloniki). Participants: **D. Sgoutas** (Professor of Pathology and Laboratory Medicine, Emory University, USA); **K. Seferiadis** (Associate Professor of Biological Chemistry, University of Ioannina); **G. Ferard** (Professor of Applied Biochemistry, Unversity of Strasbourg, France)

Clinical Laboratorians are concerned with disease correlations, interpretations, problem solving, cost effectiveness, quality assurance and standardization of methods. Since laboratories employ a variety of methodologies , it is not possible or practical to require that all laboratories use the same method, calibrators and controls. The key point to emphasize, however, is that the basis of standardization is traceability: all results should be tied to a defined common accuracy base. This requires that a reproducible analytical base be established to serve as a common denominator that permits laboratories to compare results and monitor analytical performance to ensure continuing accurate and precise results.
Industry's involvement in clinical chemistry has increased the uniformity of test results. Development of automated systems has reduced the number of widely used analytical methods, and concepts such as the National Reference Laboratory System in the U.S.A. have facilitated the traceability of the procedures to a single reference method. Such developments have improved the comparability of results between laboratories and within a laboratory over time. Willingness to work collaboratively to reference several analytical methods to common characterized calibrators had further ensured that results were comparable even when different methods were used.

A concise review of the considerations and recommendations for the ***Standardization of Plasma Lipids and Lipoprotein Measurements***, made by the National Cholesterol Education Program (NCEP) for the measurement of plasma Cholesterol (TC), High-Density Lipoprotein (HDL) cholesterol and Low-Density Lipoprotein (LDL) cholesterol, and Triglycerides (TG) will be presented.
A concerted effort to identify and treat individuals at high risk for coronary artery disease (CAD) because of high blood cholesterol in the U.S.A. and other countries is intended to contribute to lower CAD morbidity and mortality rates. All adults need to know their blood cholesterol level, to be aware of the implications of elevated cholesterol, and to seek the help of a physician should treatment be necessary. In addition, it is necessary in the diagnosis and treatment of hyperlipidemia to assess the distribution of cholesterol among the major plasma lipoproteins, particularly LDL and HDL. Given the central role of the above mentioned lipid classes in the assessment and management of

CAD risk, the NCEP believes that it is important for clinical laboratories to provide precise and accurate measurements for TC, LDL-C and HDL-C and TG. For that reason, the NCEP asked the Laboratory Standardization Panel (LSP) to review and evaluate the current state of reliability of cholesterol testing, promote the uniform interpretation of laboratory results and recommend means to improve the precision and accuracy of TC, LDL-C and HDL-C and TG analysis. The LSP Working Group's recommendations for all these measurements will be presented herein along with some of the issues that the group considered in developing the recommendations. In brief, we shall discuss how for each lipid, lipoprotein,and apolipoprotein, an analytical method was accepted as the basis for universal reference. While it is generally accepted that a definitive method establishes the true value for an analyte, definitive techniques were not widely available to fulfill all of the tasks necessary to provide a point of reference for effective laboratory standardization for all those lipids. Hence, a definitive method was primarily used to validate a more widely used reference method designed for broader use and application. Some reference systems of lipids and lipoproteins are available at present. If reference methods, however, are to serve as the reference point for laboratory standardization, the problem then becomes how to interface the accuracy base with the laboratory community; that is, the base must be transferable on a broad scale. The most rapid and economic means of broadening the transfer of this accuracy base has traditionally been through the use of high-quality secondary reference materials. On the other hand, and in order to properly assess the quality of analytical performance in individual laboratories, meaningful criteria must be established to evaluate the reproducibility of measurements and the deviation from a target value. CDC was the first organization to establish specific performance criteria for TC, TG, and HDL-cholesterol designed to improve the performance of specialized lipid laboratories participating in the CDC-NHLBI Lipid Standardization Program. The performance criteria for specialized lipid laboratories participating in the CDC-NHLBI Lipid Standardization Program, and the NCEP performance goals for clinical laboratories will be presented.

Because of its importance for public health, the accuracy and precision of ***cholesterol*** determination has been of great concern to all international health organisations. The most popular method for cholesterol determinations in all routine laboratories is presently the enzymatic test known as CHOD-PAP. The mean precision of this test is 4% (CV, within run) and up to 6% (CV, between-runs). The method was found to have a bias of ca 5%.
The reference system for cholesterol includes the isotope dilution mass spectrometric definitive method (ID-MS), the Abell-Levy-Brodie-Kendall reference method [J. Biol. Chem., 195, 357 (1952)], the certified pure cholesterol standard and the certified Burchard color reaction but it also includes two equally important steps, hydrolysis of the cholesteryl esters and extraction of cholesterol from sample.
In the laboratory of Ioannina we evaluated the CHOD-PAP method, performed on a Technicon RA-1000 chemistry analyzer and Technicon Set-point calibrator, with the AK method. The analytical performance of the enzymatic method in our laboratory can be considered very satisfactory (within-run

precision for a 6.47 mmol/L sample is 1 %CV and SD = 0.065, between-run precision for the same sample 2.4 % CV and SD = 0.15).
For the AK method, we applied the CDC (Center for Disease Control) modified AK method using NIST (National Institute of Standards and Technology) 911b pure cholesterol calibrator, NIST serum reference material 909a-1 with a certified value of 4.89 mmol/L and four reference sera. The analytical performance ot the AK method in our laboratory, determined with NIST 909a-1 had a precision of 0.42 % CV and SD = 0.02 with a mean value of 4.85 mmol/L. The calculated bias of the AK method versus the certified value was only - 0.8 %. On the other hand, the RA-1000 method against the AK method had a bias of + 4.7 %.
These results show that the AK reference method has a very good accuracy, it does not require expensive instrumentation and can easily be applied to improve the analytical performance of total cholesterol determination in reference laboratories in Greece.

Concerning the determination of ***enzyme catalytic activity***, external Quality Control data show that most often discrepancies are due to the use of various reagents and techniques as well. Accuracy may be transferred via certified reference materials (CRM); many of them have been developed thanks to the European Union's Measurement and Testing Program. These products exhibit four primary characteristics: they are well defined as regards their origin, composition and catalytic properties; they are stable for many years; they have similar properties with their human plasma counterpart; they have been titrated by means of a reference method, wherever available.
CRMs allow validation of routine methods, but also calibration. Various studies on ALP, GGT, amylase, lipase etc have shown that CRMs may be used as calibrators of routine methods. This approach permits to obtain close results with the use of different routine techniques.
However, some limits yet exist:
-Most of commercially available calibrators, control sera etc are not commutable.
-Some techniques are not characterized by the same analytical specificity as reference methods and, consequently, do not allow to obtain an "intertechnique" coherency.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) IN CLINICAL CHEMISTRY: THE PRESENT AND THE FUTURE, NEW APPLICATIONS.

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Abstract

High Performance Liuqid Chromatography (HPLC), as analytical technique, hyphenates the privileges of the separation techniques with the sensitivity, accuracy and repeatability of optical techniques in the instrumental chemical analysis.

HPLC was used as analytical tool in the instrumental chemical analysis thirty years ago, at the University of Yale (USA) by Csaba Horvath. He used the technique in the analysis of amino acids. Since then the technique has been tremendously developed in the fields of research and routine analyses. This is due to the different kinds of samples.

The technique can be used either on line or off line with preconcentration techniques such as, solid phase extraction (SPE) for samples with clinical interest. The technique can be used in the analyses of biological fluids at small volumes 30-40 μl.

This analytical technique can be successfully used in the analyses of different categories of components of clinical interest. HPLC can be applied to the determination of Amino acids, Alkaloids, Antibiotics, Aflatoxins, Barbiturates, Carbohydrates, Catecholamines, Street Drugs, Enzyme Activity, Lipids and Lipoproteins, Proteins, Prostaglandins, Steroids, Tocopherols, Vitamins, etc.

In the international bibliography there is a great number of remarkable papers concerning applications in clinical chemistry.

The future of HPLC in clinical chemistry is very promising, because the technique has to present improvements in the field of instrumentation, micro HPLC, capillary HPLC, nano HPLC etc. There are also improvements in the field of detection, such as diode array and in the field of software and data treatment.

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NMR spectroscopy of biological fluids

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During the last 40 years, nuclear magnetic resonance (NMR) spectroscopy has become one of the most powerful techniques of analytical and structural chemistry. Today, its major impact on clinical medicine is the production of high-quality diagnostic images. It is recognized as a radiological technique which plays an important role in clinical diagnosis.

NMR studies on biological fluids (serum, urine, cerebrospinal fluid etc.) have only been performed in the past decade, as a result of the availability of powerful instrumentation, and have resulted in a number of interesting and important applications in clinical and metabolic biochemistry. The technique allows unusually detailed studies because the determination of metabolite concentrations in whole fluids or the detection of novel, unexpected, metabolites is possible.

Although NMR is less sensitive than other conventional methods, i.e. HPLC, GC, the simplicity of sample preparation and analysis is a particular advantage of the method. Little or no pretreatment of the sample is required and the analysis typically takes only a few minutes . The nondestructive nature of NMR, which is the basis of its in vivo applicability, is also a unique advantage for in vitro analysis. By performing measurements directly on native serum, one can eliminate problems of extraction, recovery, contamination or other artifacts. Because a single spectrum discloses the presence of any detectable compound, NMR may have greatest clinical utility as a "screening" technique in selected situations.

Proton NMR analysis is of distinct value to clinical diagnosis and therapy of metabolic disease, drug metabolism and intoxication and the monitoring of organ damages (renal, liver). Many inherited metabolic disorders give rise to large amounts of organic intermediates which accumulate proximally to the defective enzyme step. The ability of NMR to detect and identify rapidly, these abnormal intermediates in a wide variety of disorders prior to the initial episode of illness may prevent serious damage such as mental retardation and even death.

The method has been used extensively to detect drug metabolites and elucidate their exact structural features in therapeutic treatment or poisoning episodes.

In situations where renal damage is present, the low molecular weight metabolite profile of the urine is significantly altered, and this is closely reflected in the NMR spectral fingerprint which gives information on the site and mechanism of renal damage, and also may lead to the discovery of novel markers of nephron damage.

In our Laboratory, using a 400 MHz NMR spectrometer, we have identified L-carnitine in serum for studies on lipid metabolism, the pattern of ketone bodies excreted in urine and we have also studied the pattern of excretion of metabolites in paraquat toxicity.

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NEW METHODS AND TESTS IN CLINICAL CHEMISTRY. THE EXAMPLE OF BONE METABOLISM.

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A large number of new diagnostic tests and methodologies have been recently added in the work up of several widespread diseases. Thus, for instance, (a) in the evaluation of ischemic heart disease, we have now cardiac troponins T and I, the myoglobin/carbonic anhydrase III ratio etc, (b) in acute pancreatitis, elastase E-1 and trypsinogen-2, (c) in hyperlipidemias, lipoproteins A and B, Lp(a) and HDL fractions, (d) in obesity, serum leptins, and (e) in septic shoch, the levels of circulating cytokines.

A typical example of the massive entrance of new diagnostic tests and techniques is in the chemical evaluation of bone metabolism. Bone is constantly resorbed (degraded) and formed throughout life at specific foci called "bone remodeling units". In young adults, the rate of bone formation is equal to that of bone resorption. Later in life, especially in the peri- and post-menopausal years, for mostly unknown reasons, the rate of bone turnover appears to accelarate and the two processes cease to be closely coupled, i.e., the rate of bone formation does not keep up with bone resorption. Up to quite recently, we had at our disposal only a limitted number of measurable parameters to estimate the rate of bone turnover. Thus, for the rate of bone formation we had two products of osteoblasts: (a) total serum alkaline phosphatase (TAP) by colorimetry, and (b) total osteocalcin (bone Gla protein) while for the rate of bone resorption (a) urine hydroxy-proline (the break-down product of collagen type I) and (b) tartrate-resistant acid phosphatase (TRAP) measured by colorimetry.

Most new indices of bone turnover are based on measurement of several by-products of the metabolism of collagen type I. Collagen type I is a fibrous proteins produced by osteoblasts. It is the most abudant structural element of bones and forms their basic fabric. Collagen type I is also present in cartilage and other connective tissues. It consists of three polypeptide chains wound together in a helical structure. Two of the chains are identical (α_1 chains) while the third is entirely different (α_2 chain). Each fiber is connected to nearby fibers by pyridinium cross-links. It is the pattern of these cross-links which is characteristic of bone collagen type I, i.e., it differs from that in soft connective tissues.

Some of the new indices of bone formation are: (a) N-terminal extension peptide of collagen type I (N-terminal propeptide, NICP), and (b) C-terminal extension peptide (C-terminal propeptide, CICP). The latter is now available for routine commercial use by RIA or ELISA. (c) intact osteocalcin. Osteocalcin is a monomeric protein of 5800 mol wt; it is produced by osteoblasts and represents approx. 10% of bone non-collagen proteins; the others being the osteopontin, osteonectin, bone sialoprotein, etc. These proteins bind the hydroxyapatite minerals (d) Bone alkaline phosphatase (BAP) by RIA , IRMA, and ELISA.

Some of the new indices of bone resorption are: (a) total pyridinolines and deoxy-pyridinolines in urine following acid hydrolysis and HPLC chromatography (b) free pyridinolines and deoxypyridinolines in urine by ELISA. (c) N-terminal cross-linked peptides (Osteomark, NTX) in urine, (d) C-terminal cross-linked peptides, (CTX, Stillwater, MN) and (e) telopeptide α_1 chain, (CrossLaps). (f) Measurement of interleukin-6 in plasma is currently under evaluation and should not be part of the every day work up of bone metabolism.

In conclusion, we have now at our disposal a multitude of new indices for the chemical evaluation of bone metabolism. Most of these indices can be measured by a multitude of methods, some of them automated. Elevation of these indices in peri- and post-menopausal women indicates increased bone turnover which now appears to be a major determinant of osteoporosis.

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MASS SPECTROMETRY IN CLINICAL CHEMISTRY

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Modern medicine depends upon the collaboration of many scientific disciplines.
The development of these disciplines has contributed, greatly, to the creation of what might be called today «molecular medicine», which will be a most important approach to the diagnosis and treatment of various diseases.
Mass spectrometry and, in particular, the combination of this technique with chromatographic methods, has contributed to the creation of «molecular medicine», either by contributing, among others, to the investigation of metabolic pathways or to the discovery of new biomolecules with specific metabolic functions.
In the fields of clinical chemistry and toxicology mass spectrometry is the method of choice for identification of substances while it is used as a reference method for the validation of other analytical techniques such as immunoassays.
The progress achieved in the field, during the last years, particularly in relation to high mass measurements of biological molecules, techniques of ionization and, also, to sophisticated software used, is impressive.
In addition, we should mention that the wide use of quadrupole mass analyzers has provided simple and cheap bench-top instruments, used effectively in routine analysis.
Such instruments provide great potentials in relation to diagnostic problems.

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SERUM CYTOKINES IN NEWBORN INFANTS

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Background: Cytokines have been characterized as early markers of immune response activation.
Aim: In this study, serum soluble interleukin-2 receptors (sIL-2R) and intercellular adhesion molecule-1 (ICAM-1) values were evaluated in the estimation of neonatal immune system development and maturation in early neonatal period.
Material and Methods: Sixty seven healthy, termed (38.9±1.67 weeks of pregnancy) neonates, 37 girls and 30 boys, mean birth weight 3287±429 gr were included in the study. With their mother's consent, 1 ml of neonatal blood were collected a) in the 1st hour, or during the 1st day (1N) after birth, b) during the 5th day (5N) and c) 40 days (40N) thereafter. In 20 of the cases, maternal serum (MS) before delivery and umbilical cord blood (UCB) serum after delivery were also obtained. The blood samples were collected in pyrogen-free tubes, centrifuged immediately and stored in -20^{o}C until analysed simultaneously. For sIL-2R and ICAM-1 determination, enzyme-immunoassays were performed, using commercially available kits (CELLFREE® IL-2R Test kit και CELLFREE® ICAM-1 Test Kit, T-Cell Diagnostics, Inc, Woburn, Mass, USA).
Results: Both cytokine values showed a significant progressive increase from the UCB to the 40N samples ($p<0.0001$, non paired t-test). In all three neonatal samples, sIL-2R values were significantly higher than those in MS samples ($p<0.0001$, paired t-test, $n=20$). In contrast, ICAM-1 values in UCB and 1N samples were significantly lower than those in MS ($p<0.0001$ paired t-test, $n=20$), in 5N samples, they showed no significant difference with those in MS, while in 40N they were significantly higher than in MS ones ($p<0.001$, non paired t-test). Both sIL-2R and ICAM-1 marker values were dependent on the mode of delivery ($p<0.01$ and $p<0.03$ respectively, one way ANOVA), the lowest being found in the cases with elective cesarean section. Moreover, significantly lower ICAM-1 values were observed in the 1st hour, than those during the 1st day sampling after birth ($p<0.0000$, non paired t-test). A strong correlation was found between either sIL-2R or ICAM-1 values in UCB and 1N ($r=0.80$, $p<0.0006$ and $r=0.50$, $p<0.02$), 1N and 5N ($r=0.76$ and $r=0.60$, $p<0.0000$) and 5N and 40N ($r=0.85$, $p<0.01$ and $r=0.60$, $p<0.03$).
Conclusions: a) The progressive cytokine increase after birth and in early neonatal period may reflect the expansion of newborn immune system in response to environmental influences during the first days of extrauterine life. b) The dependence of sIL-2R and ICAM-1 to the mode of delivery suggests their involvement in the process of labor.

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INTERCELLULAR ADHESION MOLECULE-1 (ICAM-1) IN HUMAN MILK

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Background: Breast milking is one example of the multiple interactions within the mother-infant dyad. Furthermore maternal milk is the ideal source of nutrients and host-defense components for infants protecting not only against infections, but also against inflammation. On the other hand, neonatal immune system is not nul, but only immature and inexperienced and, as it has been recently reported, it is dynamically expanded and developed during the first days of extrauterine life.

Aim: In this study, intercellular adhesion molecule-1 (ICAM-1), an early and reliable marker of immune activation and response was evaluated in colostrum and mature breast milk to examine whether this immunomodulator is a normal constituent of maternal milk with possible physiological action in the protection of the neonate and the development of its immune system in the early postpartum days.

Material and Methods: Colostrum samples were collected up to 2nd day postpartum and mature milk up to the 5th day after delivery from 19 healthy, non-smoking, postpartum women of the 2nd Dept of Obstetrics and Gynecology of Athens University, of mean age 29 (23-36) years, who delivered mature, healthy infants after full-term pregnancies (38-41 weeks), of mean birth weight 3400 (2700-3800) gr. Moreover maternal peripheral blood was drawn before milk collection. Informed consent was obtained from each mother. Blood and milk specimens were centrifuged immediately after collection at 6-10^{o}C for 5 min at 2500 rpm and for 20 min at 2000 rpm respectively. Blood serum and the lower aqueous layer of breast milk, after removal of the upper lipid layer were collected, centrifuged again, aliquoted and stored at -20^{o}C until assay. The determination of ICAM-1 was done by a sandwich type EIA, using a commercially available kit (CELLFREE® ICAM-1 Test Kit, T-Cell Diagnostics, Inc, Woburn, Mass, USA).

Results: Significantly higher concentrations (median, range) of ICAM-1 were found in colostrum (316, 32-1018 ng/ml) than in mature maternal milk (79, 30-416 ng/ml; $p<0.008$, paired t-test, after logarithmic transformation of the values). In contrast, ICAM-1 in all formulas was lower than the detection limit of the method (30 ng/ml). Serum values of ICAM-1 were significantly higher ($p<0.01$) in the first (300, 205-453 ng/ml), than in the 5th day after delivery (117.5, 57-188 ng/ml), while no significant difference was shown between maternal serum ICAM-1 levels and the corresponding levels in both colostrum and mature milk. Values of ICAM-1 in both colostrum and mature milk did not depend on the kind of delivery, maternal age, or parity, whereas a significant correlation was found between a) neonatal birth weight and ICAM-1 in colostrum ($r=0.490$, $p<0.,035$) and b) 1st and 2nd sample of maternal serum ICAM-1 ($r=0.796$, $p<0.05$).

Conclusion: Our findings demonstrate that ICAM-1 is found in considerable amounts in breast milk from the 1st day postpartum, implying possibly its local production in the mammary gland and its physiological importance for the neonate, while this immunoregulator is not detected in all formulas. Thus, breast feeding seems to be very important in the protection of the neonate against infections and the possible activation and development of its immune response.

IL-6 SERUM LEVELS IN CASES WITH LYMPHOPROLIFERATIVE DISORDERS AND PARAPROTEINAEMIAS.

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Interleukin-6 (IL-6), the B cell stimulatory factor 2, has been implicated to play a role in polyclonal B cell activation as well as in the generation of human plasmacytoma and multiple myeloma. The aim of this study was to measure the serum levels of IL-6 in cases of lymphoproliferative disorders with and without homogeneous immunoglobulins (HIgs) as well as in cases of multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS).

Methods: IL-6 was determined in serum samples of 48 cases of B-cell lymphocytic leukaemia (B-CLL) and non Hodgkin's lymphoma (NHL) (24 with and 24 without paraproteinaemia) in 29 cases of MM (13 IgG, 12 IgA, 21 IgD and 2 Bence Jones MM), in 17 cases of MGUS and in 15 normal subjects using a commercial immunoenzymetric assay (Medgenix Diagnostics).

Results: The results are shown in the table. The highest percentage of overt increases of IL-6 serum levels (>50 pg/ml) was seen in cases with B-CLL/NHL with single or multiple monoclonal Igs. Few cases of MGUS or MM studied at diagnosis had high IL-6 levels. The highest values (864 pg/ml, 1010 pg/ml, 1778 pg/ml) were seen in a case of NHL in leukaemic phase, in a IgGκ plasmacytoma and a hairy cell leukaemia respectively.

Conclusion: Clinical parameters of the disease and other biological markers are important in evaluating these heterogeneous findings of IL-6 levels in B-cell neoplasias.

IL-6 (pg/ml)	**Controls**	**B-CLL/NHL without HIg**		**B-CLL/NHL with HIg**		**MGUS**		**MM**	
	N = 15	N = 24 %		N = 24 %		N = 17%		N = 29 %	
0-10	14	8	33,3	2	8,4	12	70.6	21	72,5
11-50	1	10	41,6	10	41,6	3	17,6	3	10,3
>50	0	6	25,1	12	50,0	2	11,8	5	17,2

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DEVELOPMENT OF A SIMPLE AND SENSITIVE ENZYME AMPLIFIED LANTHANIDE LUMINESCENCE IMMUNOASSAY FOR THE DETERMINATION OF TUMOR NECROSIS FACTOR (TNF-α) IN BIOLOGICAL FLUIDS

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Introduction: TNF-α is mainly produced by the monocytes and macrophages and belongs to the group of proinflammatory cytokines. Its major biological actions include the induction of the production of inflammatory compounds, the induction of the expression of adhesion molecules in the endothelial cells as well as the induction of cachexia. The development of reliable and highly sensitive methods for the determination of TNF-α in biological fluids is of great importance for the elucidation of its biological role.
Aim of the study: The development of an simple and sensitive enzyme amplified lanthanide luminescence immunoassay for the determination of TNF-α in serum, plasma or cell culture supernatants.
Reagents: Human rec.TNF-α standard (NIBSC 87/650, Herts, U.K.), monoclonal coating antibody: anti-TNF-α/7 (CLB, Netherlands) , biotinylated monoclonal detection antibody: anti-TNF-α/5 (CLB, Netherlands), polyclonal anti-biotin alkaline phosphatase conjugate (Abbott, IMx/HBsAg assay, USA). 96-microwell plates (Nunc, Denmark). Bovine serum albumin (SERVA, Germany). The phosphate ester of diflunisal, (DIFP) was synthetised and purified in our lab according to the literature.
Method: The developed method is a heterogeneous non-competitive (sandwich type) enzyme immunoassay. Monoclonal anti-TNF-α antibody is coated to the microwell surface by passive adsorption (100μL/well, 2.5μg/mL, incubation 16h, 4°C). BSA 6% (pH=7.4, incubation 3d, 4°C) is used for blocking and then the sample is added (20-100 μL/well, incubation 2h, 30°C). The biotinylated monoclonal detection antibody is added (100μL/well, 0.33μg/mL, incubation 1h, 30°C) followed by the addition of a polyclonal anti-biotin alkaline phosphatase conjugate (100μL/well, 0.03μg/mL, incubation 30min, 30°C). Finally, DIFP substrate solution is added (100μL/well, 0.5mM - incubation 45min - protocol I, or 1mM - incubation 15min - protocol II, 30°C) and the enzymatic reaction is stopped by the addition of NaOH (100μL/well, 0.1M). The product, diflunisal (DIF) forms a highly fluorescent ternary complex with Tb^{3+} and EDTA. The emmited fluorescence is measured with a double beam fluorescence spectrophotometer (Perkin Elmer, M512A) by mixing 180 μL from each well with 700 μL of Tb-EDTA solution (Tb^{3+}:3 mM, EDTA:3 mM, pH=12.6) in the cuvette. Fluorescence is measured either conventionally (λex=284nm, λem=546nm) or by using the Second Derivative Synchronous Fluorescence Spectroscopy technique, (SDSFS).
Results: The assay demonstrates excellent precision (CV<5%), high sensitivity (detection limit~0.2 pg/mL), and good accuracy (recoveries~90-114%). The dynamic range of the assay extends from the detection limit up to 10pg/mL (protocol I) and up to 5000pg/mL (protocol II).
Conclusions: The proposed method is relatively simple, rapid (total incubation time~ 4h) and can be used for the determination of TNF-α in very low concentrations in serum, plasma or cell culture supernatants.

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DEVELOPMENT OF A SIMPLE AND SENSITIVE ENZYME AMPLIFIED LANTHANIDE LUMINESCENCE IMMUNOASSAY FOR THE DETERMINATION OF INTERLEUKIN 6 (IL-6) IN BIOLOGICAL FLUIDS

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Introduction: IL-6 belongs to the group of proinflammatory cytokines and its major biological actions include the induction of the production of acute phase proteins. The development of reliable and highly sensitive methods for the determination of IL-6 in biological fluids is of great importance for the elucidation of its biological role.
Aim of the study: The development of a simple and sensitive enzyme amplified lanthanide luminescence immunoassay for the determination of IL-6 in serum, plasma or cell culture supernatants.
Reagents: Human rec.IL-6 standard (NIBSC 88/514, Herts, U.K.), monoclonal coating antibody: anti-IL-6/16 (CLB, Netherlands) , biotinylated polyclonal detection antibody: anti-IL-6 (CLB, Netherlands), polyclonal anti-biotin alkaline phosphatase conjugate (Abbott, IMx/HBsAg assay, USA). 96-microwell plates (Nunc, Denmark). Bovine serum albumin (SERVA, Germany). The phosphate ester of diflunisal, (DIFP) was synthetised and purified in our lab according to the literature.
Method: The developed method is a heterogeneous non-competitive (sandwich type) enzyme immunoassay. Monoclonal anti-IL-6 antibody is coated to the microwell surface by passive adsorption (100μL/well, 2.5μg/mL, incubation 16h, 4°C). BSA 6% (pH=7.4, incubation 3d, 4°C) is used for blocking and then the sample is added (20-100 μL/well, incubation 2h, 30°C). The biotinylated monoclonal detection antibody is added (100μL/well, 0.33μg/mL, incubation 1h, 30°C) followed by the addition of a polyclonal anti-biotin alkaline phosphatase conjugate (100μL/well, 0.03μg/mL, incubation 30min, 30°C). Finally, DIFP substrate solution is added (100μL/well, 0.5mM - incubation 45min (protocol I), or 1mM - incubation 15min (protocol II), 30°C) and the enzymatic reaction is stopped by the addition of NaOH (100μL/well, 0.1M). The product, diflunisal (DIF) forms a highly fluorescent ternary complex with Tb^{3+} and EDTA. The emitted fluorescence is measured in a double beam fluorescence spectrophotometer (Perkin Elmer, M512A) by mixing 180 μL from each well with 700 μL of Tb-EDTA solution (Tb^{3+}:3 mM, EDTA:3 mM, pH=12.6) in the cuvette. Fluorescence is measured either conventionally (λex=284nm, λem=546nm) or by using the Second Derivative Synchronous Fluorescence Spectroscopy Technique, (SDSFS).
Results: The assay demonstrates excellent precision (CV<5%), high sensitivity (detection limit~0.2 pg/mL), and good accuracy (recoveries~89-117%). The dynamic range of the assay extends from the detection limit up to 100pg/mL (protocol I) and up to 2000pg/mL (protocol II).
Conclusions: The proposed method is relatively simple, rapid (total incubation time~ 4h) and can be used for the determination of IL-6 in very low concentrations in serum, plasma or cell culture supernatants.

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ALTERED ACTIN POLYMERIZATION DYNAMICS IN VARIOUS MALIGNANT CELL TYPES: A NOVEL BIOCHEMICAL INDICATOR REFLECTING MALIGNANT TRANSFORMATION

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Various aspects of cell behaviour, such as changes in volume, locomotion, spreading and cell to cell contact seem to be dependent on the dynamic equilibrium between monomeric and polymerized actin. These cellular functions are probably tightly controlled in non neoplastic cells, while the uncontrolled growth of malignant cells seems to be related to microfilament disorganization. However, disorganization of actin microfilaments studied by morphological approaches, such as immunofluorescent microscopy, can be estimated only semiquantitatively. Thus, searching for alternative approaches to quantitatively study intracellular modifications in the dynamic equilibrium of monomeric and polymeric actin forms in malignant cells we used in the present study the DNaseI inhibition assay, immunoblot analysis and fluorescence measurements. We studied quantitatively changes in the actin polymerization dynamics in primary cultures of normal and malignant human lymphocytes and keratinocytes, normal human endometrial cells, as well as in various leukemic and endometrial adenocarcinoma cell lines. In all tested malignant cells the G/total-actin ratio was found to be 1.4 to 1.8-fold higher, compared to normal cells, indicating that all malignant cells investigated express reduced amounts of polymerized actin (table 1). The above findings were corroborated by fluorescence measurements of the rhodamine-phalloidin-labelled F-actin amounts in normal and neoplastic cells, that showed significantly lower F-actin content in malignant cell preparations. Moreover, the total actin content, as quantitated by the DNaseI inhibition assay and by immunoblot analysis was found decreased by 34-80% in the primary cultures of malignant human lymphocytes and endometrial cells, when compared to the total actin levels in the corresponding normal cells. Proliferation and viability measurements of normal and neoplastic cells in culture, treated equally with cytochalasin B (CB), revealed an increased susceptibility of malignant cells to this anticytoskeletal agent. In conclusion, all investigated malignant cells are characterized by: a) higher G/total-actin ratio, b) decreased F- and total-actin content and c) lower resistance to CB treatment. These quantitatively determined parameters of the actin polymerization state may represent potential biochemical indicators reflecting malignant transformation.

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APPLICATION OF TERBIUM SENSITIZED FLUORESCENCE FOR THE DETERMINATION OF FLUOROQUINOLONE ANTIBIOTICS IN SERUM

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Introduction: Quinolones are synthetic broad-spectrum antibacterial agents, which are active against Gram-positive and Gram-negative organisms. The second generation of quinolones like norfloxacin (NOR), ciprofloxacin (CIP) and pefloxacin (PEF), are characterized by a wider antimicrobial spectrum and significant enhancement of their antibacterial potency. HPLC is the most widely used technique for the determination of quinolones in biological fluids.
Aim of study: The development of a simple, rapid and sensitive fluorometric method for the determination of NOR, CIP and PEF in serum.
Experimental: A model 512-A double beam fluorescence spectrophotometer (Perkin-Elmer, Norwalk, CT, USA), interfaced to an IBM-PC 386DX microcomputer was used. Stock solutions of NOR, CIP and PEF of 10mM and a mixed working solution of Tb^{3+}-TOPO-CPCl-acetate, 3.0mM-1.5mM-0.5mM-0.1M were prepared. The synthetic samples of quinolones were prepared in control serum.
Method: The method is based on the sensitization of terbium ion fluorescence in the presence of a variety of fluoroquinolones and tri-n-octylphosphine oxide (TOPO) as a result of ternary complex formation in weakly acidic aqueous solution (pH ~ 5.5). Weakly acidic aqueous solutions of quinolones show an intrinsic fluorescence (λex~ 280nm , λem~440nm), which effectively decreased in the presence of terbium ions and TOPO, while a new intensive spectral zone of terbium ion fluorescence appeared (λem= 546nm). This phenomenon is well known as sensitization of lanthanide ion fluorescence and is due to the energy transfer from the organic molecule (quinolone) to the emitting terbium ion.
Results: Optimum conditions for the formation of the ternary complexes of quinolone-Tb^{3+}-TOPO have been investigated. The optimum pH ranged from 5.0 to 5.8, for all three quinolones. The most suitable buffer solution was acetate solution 0.1M. The effect of terbium, TOPO and CPCl concentration was studied and their optimum concentrations were determined.
Analytical features. Under optimized conditions the following detection limits were obtained; 1.7 nM, 1.2 nM and 4.4 nM for NOR, CIP and PEF, respectively, while the range of application is 0.5 - 10 μM for all three drugs.
Serum samples. The method has been successfully applied to the determination of NOR, CIP and PEF in 100-fold diluted serum samples or after deproteinization of serum with acetonitrile (serum/acetonitrile = 1/2). The recoveries from serum samples spiked with NOR, CIP and PEF (5.0 - 50.0 μM) were 86.2 ± 2.0% , 107.2 ± 6.3%, and 94.3 ± 5.2%, respectively. Within run and day-to-day precision obtained at 5.0, 25.0 and 50.0 μM of NOR, CIP and PEF were 3.6 - 5.4%, 1.7 - 3.4% and 2.6 - 4.2 % (within run) and 3.3 - 10.5%, 5.2 - 8.4% and 7.6 - 13.7% (day-to-day), respectively. The detection limits of NOR, CIP and PEF were found to be 0.4μM, 0.2μM and 0.8μM (in diluted serum) and 0.2μM, 0.1μM and 0.3μM (in serum after deproteinization with acetonitrile), respectively. The influence of several usually coadministered drugs on the determination of fluoroquinolones in serum has been investigated.
Conclusions: The proposed spectrofluorometric method for the determination of quinolone antibiotics is very simple, rapid and sensitive and could be applied to their determination in serum. The developed detection system of quinolones can be applied for their determination with HPLC.

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A SIMPLE SPECTROFLUOROMETRIC METHOD FOR THE DETERMINATION OF p-AMINOBENZOIC AND p-AMINOSALICYLIC ACIDS IN BIOLOGICAL FLUIDS FOR THE NBT/PABA/PAS TEST.

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Introduction: Since its introduction in 1976, the bentiromide test for the assesment of exocrine pancreatic function has been extensively investigated in terms of sensitivity and specificity. Bentiromide test is based upon specific hydrolysis by pancreatic chymotrypsin of the orally administered synthetic peptide, N-benzoyl-L-tyrosyl-p-aminobenzoic acid (BT-PABA), in the small intestine and measurement of the released p-aminobenzoic acid (PABA) in urine. The amount of PABA excreted in urine in a specific period of time is the measure of pancreatic exocrine function. The specificity of the bentiromide test has been increased by the use of an internal marker and the calculation of the PABA excretion index (PEI), i.e. the ratio of the excreted PABA to that of the marker. The most convenient to patients and hospital staff is the one-day test involving p-aminosalicylic acid (PAS) as a pharmacokinetic marker to determine PEI.
Scope: The development of a simple, rapid and sensitive method for the simultaneous determination of PABA and PAS in urine and for the determination of PABA in serum
Materials: All the fluorimetric measurements were performed with a Model 512 fluorescence spectrometer (Perkin-Elmer Corp., Norwalk, CT) interfaced to an IBM-PC 386DX microcomputer. PABA and PAS stock solutions, 10mM, were prepared as well as mixed working solutions: Tb-TOPO-CPCl-acetate (pH=5,5), 3,0mM-1,5mM-0,5mM-10mM for the measurement of PABA (PABA working solution) and Tb-EDTA-CAPS (pH=12,6), 1mM-1mM-100mM for the measurement of PAS (PAS working solution). Synthetic mixtures of PABA and PAS were prepared in drug-free urine and normal control serum.
Methods: PAS is measured as a ternary complex with terbium and EDTA (λ_{ex}= 324 nm , λ_{em}=546 nm) in alkaline aqueous solution (pH~12.6), while both compounds, PABA and PAS are measured as ternary complexes with terbium and tri-n-octylphosphine oxide (TOPO) (λ_{ex}=292 nm, λ_{em}=546 nm) in weakly acidic aqueous solution (pH~5.5). The concentration of PAS, C_{PAS}, is calculated from the calibration graph, fluorescence intensity (λ_{ex} = 324nm και λ_{em} = 546nm) of PAS-Tb-EDTA ternary complex vs concentration. PABA concentration, C_{PABA}, is calculated from the empirical equation C_{PABA}= $C_{(PABA+PAS)}$ - C_{PAS} x 0,42. $C_{(PABA+PAS)}$ is calculated from calibration graph, fluorescence intensity (λ_{ex} = 292nm και λ_{em} = 546nm) of the ternary complex PABA-Tb-TOPO vs concentration.The measurement of PABA και PAS in urine and serum has been performed after alkaline hydrolysis of the samples.
Results: The spectral characteristics of the ternary complexes were studied and optimum conditions for their formation (pH, concentration of terbium, TOPO and CPCl) have been investigated. The detection limits are 0.07 μM and 0.02 μM for PABA and PAS , while the range of application are 0 - 10 μM and 0 - 40 μM (final concentration), respectively.
Urine samples. We performed a detailed study on the effect of urine matrix as well as of hydrolysis process on the analytical signals for PABA and PAS either in PABA- or PAS-working solutions. Analytical-recovery experiments for PABA and PAS were performed in spiked urine and serum samples. Recoveries ranged from 96 to 110% while within- and between-run precision was found to be between 1,0 and 5,5%. Interference from several drugs and other compounds was also tested. The method has been successfully applied to the determination of PABA in serum samples after their deproteinization with acetonitrile and alkaline hydrolysis.
Conclusions: The proposed fluorimetric method for the determination of PABA and PAS is simple, sensitive and rapid and could be used for the NBT/PABA/PAS test.

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DETERMINATION OF PHENYTOIN IN HUMAN HEAD HAIR

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INTRODUCTION

Phenytoin (diphenyldantoin, PHT) is effective as antiepileptic and as myocardial anti arrhythmic Because it show a comparatively narrow therapeutic range (10 - 20 μg/ml), there is a requirement for regular monitoring of PHT concentrations in blood. The purpose of this study was to evaluate the range of carbamazepine and valproic acid levels in head hair of patients receiving systematically these drugs.

MATERIAL - METHODS

Hair samples from 7 (4 male and 3 female) patients aged between 14 and 77 years old and suffering mainly from epilepsy, in a single therapy form or in combination with other drugs, were selected for the study. The specimens were cut to two pieces, 2cm length per piece, from hair root. Hairs of healthy individuals were collected, to be used as a blank in recovery experiments of PHT from standard solutions (40μg/ml from ABBOTT). For the quantitative determination of the above substances, immunochemical technique was employed using an Abbott Analyser (TDx) and reagents.

Hair preparation

A. 50 - 100 mg of hairs were diluted in 3ml of NaOH 1N solution, heated for 1h at 100°C, vortexed into a tube and cooled at room temperature (dissolution of hair). Drops of HCl 6N were added, pH adjusted at 9 and 1ml of a pH 9 buffer solution was added. 5ml of diethyl ether were added, vortex for 1 - 2 min, centrifuged and the organic phase (supernatant) was separated into a new test tube. The solvent evaporated, the precipitant was re-dissolved in 300μL of physiological liquid (0.9% NaCl) and PHT was assayed by FPIA in Abbott TDx.

B. As previously but 5ml of dichloromethane were added.

C. As previously but pH adjusted at 7.

RESULTS

Table 1: Recovery of PHT from standard solutions (40μg/ml) by the procedures A, B and C (n=4).

Standard (μL)	PHT (μg)	Procedure A (μg)	(%)	Procedure B (μg)	(%)	Procedure C (μg)	(%)
0	0	ND*		ND*		ND*	
125	5.00	2.525 ± 0.313	50.5				
250	10.00	7.139 ± 0.273	71.4	6.794 ± 0.155	67.9	5.003 ± 1.051	50.0
375	15.00	9.443 ± 0.473	63.0				
500	20.00	10.986 ± 0.217	54.9				

*None detected; less than the sensitivity of the assay (0.5μg/ml).

Table 2: Results from PHT measuring in patient head hair, employing hair preparation procedure A.

Sample	Age (years)	Sex	Dose (mg/24h)	Dose duration	Hair concentration (μg/g of hairs)		Serum concentration (μg/ml)
					1st piece	2nd piece	
1	39	female	300	chronic	26.44	13.73	7.15
2	57	male	200	chronic	5.38	4.40	5.78
3	20	female	200	chronic	10.39	9.88	4.07
4	14	male	45	1.5 months	ND*	ND*	0.21
5	77	male	200	1 month	2.87	ND*	6.28
6	25	female	300	2 months	21.55	9.00	15.57
7	69	male	100	1.5 year	5.25	3.94	1.83

*None detected; less than the sensitivity of the assay (0.5μg/ml).

DISCUSSION

According to recovery studies of the standard solutions, there is no significant difference ($p<0.05$, as found by t test) between the procedure A and B. There are positive results from the experiments involving patients' hair(Table 2). Generally, there is reduction on drug concentrations from the 1st to 2nd piece, this is due to the drug degradation to its metabolites over time. The drug absorption by hair is depended on the patients' age, but is not depended on blood concentrations. It is obvious that we have been enlighten with respect to the analysis methods of PHT in head hair, but however, some further study on the subject is required.

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MAXIMAL PERCENTAGE CHANGE IN PLASMA VOLUME CAUSED BY ATHLETIC ACTIVITY

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Differentiations of maximal percentage change in plasma volume (CPV) have been studied on non-athletes or athletes of the same discipline, but no collective study with various sports has been done and there was no comparison between sports in identical conditions of maximal exercise. We studied differentiations of CPV and of changes in content (CC) of basic biochemical partameters which are associated with osmolality, such as urea, glucose, albumin, electrolytes, lactate in different athletic disciplines.

Method: 73 men (9 non athletes as control, 11 elite basket ball players, 21 soccer - players, 4 endurance athletes, 18 tae kwon do athletes, 8 body-builders) exercised on an electric cycloergometer, according to a protocol of maximal effort. CPV and CC of parameters we measured, were calculated with one method which uses hematocrit and hemoglobin (Dill-Costill, Strauss, Elington). Glucose, urea, albumin, electrolytes (sodium, potassium), hemoglobin, hematocrit were measured according to standardized procedures from blood drawn from an antecubital vein. Hematocrit was measured in dublicate after microcentrifugation, while hemoglobin with Drabkin's method. Red blood cell count was measured on an hematologic analyzer and erythrocyte indices (MCV, MCH, MCHC) were calculated. Osmolality was measured with an osmometer (freezing point depletion technique) Blood pressure was measured with a sphygmomanometer.

Results: CPV was found identical among sports and in the total sample of athletes was -14.3 ± 3.8% and did not differ from control where it was found -15.2±3.2%. Osmolality increased (4%) after maximal exercise, while osmolal content decreased (-10.3% in athletes and -12.5% in control) Concentration of sodium increased in athletes (1.3%) and in control (3.8%), while sodium content decreased (-12.7% and -12% respectively). Albumin concentration increased (13.9% in athletes and 12.9% in control) and albumin content decreased -2.5% and -4.2% respectively.

Differentiations were found in CC of separated parameters, like the CC of urea which was greater in a wider grouping of anaerobic sports (Group II) in relation to a wider grouping of aerobic sports (Group I), and the CC of glucose was greater in aerobic sports. Osmolality was greater in group I than osmolality of group II, while the control group was placed in between. Differentiations were also noticed and in the correlations of osmolality with biochemical and hematologic parameters. We found an unexpected blunting of osmolality correlations to sodium in the aerobic group and an opposite sign correlation to hematological parameters between group I and group II (positive correlation to hematocrit in group I, while negative in group II).

Correlations of Work differed among the two groups and in group I we found a correlation to CC of glucose. MCV differed in its correlations and in group II MCV correlated with CC of urea, while in group I did not.

Conclusions: In order to get a more full picture of changes during exercise, changes of concentrations of biochemical parameters must be accompanied by study of CC. Although there was no proof of existence of differentiations in CPV between different athletic disciplines, the factors connected to it, like the CC's and osmolality, differ and are influenced by the metabolic character of each sport. The relative increase of osmolality in aerobic sports is related to a lesser CC of urea in this discipline. CC of glucose is greater in aerobic sports and this reflects higher mobilization of carbohydrates in these sports.

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DETERMINATION OF SIALIC ACIDS IN BIOLOGICAL FLUIDS BY ION-PAIR REVERSED-PHASE LIQUID CHROMATOGRAPHY.

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Sialic acids are a family of 9-carbon carboxylated 2-keto sugars widely distributed in nature. The most commonly occuring sialic acid is N-acetylneuraminic acid. Elevations of serum sialic acids have been reported in patients with malignant melanoma and cancers of the brain, gastrointestinal and gynecologic system and their determination is useful in monitoring tumour progression or regression during therapy. Increased excretion of free N-acetylneuraminic acid in urine is observed in Salla disease and infantile free sialic acid storage disease and increased urinary levels of conjugated N-acetylneuraminic acid were found in sialidosis.

In the present work, an ion-pair reversed-phase high performance liquid chromatography for the separation and determination of N-acetylneuraminic acid, N-glycolylneuraminic acid, 2-deoxy-2,3-dehydro-N-acetylneuraminic acid, N,9-O-diacetylneuraminic acid and cytidine-5'-monophospho-N-acetylneuraminic acid, with UV detection at 215 nm, is described. Ion-pair reversed-phase liquid chromatography was carried out on C_{18} column using an aqueous solution of triisopropanolamine 60 mM, pH 3.50 adjusted with concentrated phosphoric acid as the eluent. The flow rate was 0.60 ml/min. The mixture of five sialic acids is separated within 17.3 min and amounts of 0.10-5.0 nmole were required for the quantitative determination of the sialic acids. The proposed method is suitable for the determination of sialic acids in serum and urine with an average analytical error of 3.8%.

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EVALUATION OF MARKERS OF BONE FORMATION IN CHILDREN WITH METABOLIC BONE DISORDERS

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Osteogenesis Imperfecta (OI) and β-homozygous Thalassemia (β-Th) heritable diseases which are associated with serious metabolic bone diosrders. The aim of the present study was to evaluate the phase of bone formation by sensitive markers of bone collagen formation and mineralization of bone matrix. The patients which were studied: a) 20 patients with β-Th (Group A), 14 patients with ITh (Group B) and 13 patients with OI (Group C) aged 2-17 years old. In Serum samples were measured: 1) The c-carboxyl-terminal propeptide of type I procollagen (PICP) which appears to be an effective marker of collagen formation. 2) The osteocalcin concentration (OC) and the total alkaline phosphatase (ALP) both of which appear to be specific markers of bone mineralization. The PICP and OC were measured by the enzyme-linked immunosorbent assay (ELISA) of Metra Biosystems, while the ALP by an ordinary enzymatic method. Results: 1) The PICP was found to be statistical significantly low ($p<0,01$) in all the groups of patients. 2) The OC and the ALP were found to be statistically high in groups A and B ($p<0,05$) while there was not any statistically significant difference in group C. Conclusions: 1) In patients with β-Th, ITh, and OI was observed low collagen synthesis and thus low bone matrix as it can be seen from the decreased PICP serum concentration. 2) In patients with β-Th nd ITh the low collagen synthesis was combined with high activities of mineralization of bone matrix of bones, as it can be seen from the increased OC and ALP serum concentrations. 3) In patients with OI increased bone mineralization activity was not observed. The findings of this study can be probably lead to an effective treatment in the metabolic bone disorders of these patients.

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THE LOW DENSITY LIPOPROTEIN SUBFRACTION PROFILE IN PATIENTS WITH MYOCARDIAL INFARCTION.

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INTRODUCTION: Low density lipoprotein (LDL) consists of discrete subfractions with different physicochemical and metabolic characteristics. The heterogeneity of LDL particles is related to different degree of atherosclerotic risk. Dense LDL which possesses diminished resistance to oxidative stress, may be potentially more atherogenic. The aim of the present study is to determine the LDL subfraction profile in patients with myocardial infarction.

MATERIALS-METHODS : 26 male patients with old myocardial infarction (6-17 months) were selected from the Department of Cardiology of Evangelismos Hospital. Age 56.00±3.81. Control subjects were 26 healthy male volunteers. Age 55.19±3.72. Three LDL subfractions LDL1, LDL2, LDL3 were isolated from plasmas by KBr density gradient ultracentrifugation. Total cholesterol, triglycerides, and phospholipids were determined by automated enzymatic methods. Protein by the method of Lowry.

RESULTS : The chemical composition of each subfraction as well as the mass expressed as % of total LDL mass, are shown in the table.

LDL subfraction	Chem.Composition,%	Controls	Patients	Statistics
LDL1	Cholesterol	50.33±2.02	48.59±0.61	t=4.19, P<0.0001
(density range,	Triglycerides	4.08±0.20	4.44±0.09	t=8.36, P<0.0001
1.030-1.033 g/ml)	Phospholipids	20.62±1.30	21.56±0.29	t=3.58, P<0.001
	Protein	24.97±2.43	25.41±0.51	ns
	Mass, % of total	**33.18±12.9**	**15.29±3.69**	**t=6.75,P<0.0001**
LDL2	Cholesterol	48.19±2.5	48.09±0.48	ns
(density range,	Triglycerides	4.15±0.25	4.42±0.06	t=5.29, P<0.0001
1.033-1.040g/ml)	Phospholipids	20.91±0.93	21.06±0.26	ns
	Protein	26.75±2.28	26.43±0.58	ns
	Mass, % of total	**47.02±10.63**	**44.97±9.75**	**ns**
LDL3	Cholesterol	44.75±3.63	43.57±1.52	ns
(density range,	Triglycerides	5.99±0.58	5.90±0.20	ns
1.040-1.045g/ml)	Phospholipids	18.11±1.32	19.46±0.72	t=4.61, P<0.0001
	Protein	31.16±1.32	31.06±0.72	ns
	Mass, % of total	**19.80±6.93**	**39.75±10.94**	**t=7.86,P<0.0001**

Values (except of density) are mean±SD. Statistical analysis was performed by the t-test. $p<0.05$ was considered as significant.

CONCLUSION: Patients with myocardial infarction have a Phenotype B versus Phenotype A of the healthy volunteers . Regarding the chemical composition the more pronounced differences among the two groups were observed in the light LDL1 subfraction.

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LIPIDAEMIC AND LIPORPOTEIN PARAMETERS IN PATIENTS WITH MYOCARDIAL INFARCTION

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INTRODUCTION - PURPOSE : It is known that high plasma concentrations of lipoproteins LDL, VLDL & Lp(a) are risk factors for the myocardial infarction, while high levels of HDL and apo A-I have a protective role. In the last few years many efforts have been made to investigate the protective role of HDL and especially for HDL subfractions (HDL-2, HDL-3) and LpA-I.
The aim of the present study is to determine these parameters in patients with myocardial infarction and relate them with the risk factors [Lp (a) & apoprotein B-100 (apoB)].
MATERIALS - METHODS: Estimates were made on two groups. Firstly on a sample of 50 men with ages between 49-62 years old, healthy, non smokers, free of cardiovascular disease who formed the control group. Secondly on a group of 92 men with ages between 45-62 years old, who had developed myocardial infarction 6 -17 months ago.
Total cholesterol (CHOL) was determined by the automated enzymatic method CHOD-PAP. HDL-cholesterol was determined by the automated enzymatic method "Cholesterol Enzymatique". Apoprotein B-100 (apoB) and apoprotein A-I (apoA-I) were measured nephelometrically. Triglycerides (TG) were determined by the automated enzymatic method "G.P.O - TRINDER", HDL-2/HDL-3 by double precipitation with dextran sulfate - MgCl2, lipoprotein (a) (Lp(a)) by micro-Elisa and lipoprotein A-I (LpA-I) by electrophoretic technique.
RESULTS :The table summarizes the findings (mg/dl) and the t-test analysis.

	PATIENTS $\bar{x}\pm sd$	**CONTROLS** $\bar{x}\pm sd$	**STAT. ANALYSIS**
AGE	55,52 ± 3,97	54,62 ± 3,49	t=1,41
HDL	36,84 ± 6,84	43,20 ± 5,53	t=6,11, p <0,001
Chol.	228,73 ± 40,04	228,00 ± 34,51	t=0,11
apoB	141,10 ± 41,41	124,34 ± 30,87	t=2,73 p<0,01
apoA-I	134,67 ± 29,46	150,48 ± 26,86	t=3,23 p<0,01
Lp(a)	32,70 ± 31,83	11,82 ± 4,03	t=6,19 p<0,001
TG	152,09 ± 71,19	134,30 ± 50,54	t=1,73 p<0,1
LpA-I	0,46 ± 0,09	0,52 ± 7,90	t=0,05
HDL-2	12,77 ± 2,88	17,72 ± 3,45	t=8,68 p<0,001
HDL-3	24,07 ± 5,26	25,48 ± 3,13	t=2,01 p<0,05
LDL	161,47 ± 35,61	157,94 ± 29,80	t=0,63

CONCLUSION :It is concluded that there are statistically significant differences between the two groups for the HDL, apoB, apoA-I, Lp(a) HDL-2 and HDL-3 while the most significant of them can be observed in the HDL-2, Lp(a) & HDL which could be considered useful markers for prediction of myocardial infarction.

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PAF-ACETYLHYDROLASE ACTIVITY IN LDL SUBFRACTIONS OF PATIENTS WITH FAMILIAL HYPERCHOLESTEROLEMIA BEFORE AND AFTER OXIDATION IN VITRO

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Human plasma platelet-activating factor acetylhydrolase (PAF-AH) is a potent antiinflammatory enzyme associated with lipoproteins. In normolipidemic population, the LDL-associated PAF-AH is preferentially distributed in the dense subfraction, the more susceptible to oxidation in vitro. We studied the distribution of PAF-AH activity in LDL subfractions of 13 patients with heterozygous familial hypercholesterolemia (FH) and 13 age and sex matched healthy normolipidemic volunteers, before and after oxidation. The lysophosphatidylcholine (lyso-PC) production during oxidation was also studied. Three LDL subfractions were isolated by density gradient ultracentrifugation and oxidized for 3h in the presence of 16 μM Cu^{2+}. PAF-AH activity was measured by the TCA precipitation method and lyso-PC levels were measured by phosphorus analysis and expressed as lyso-PC/sphingomyelin molar ratio (lyso-PC/Sph). Results are shown in the table:

	Normolipidemic volunteers			FH patients		
	LDL1	LDL2	LDL3	LDL1	LDL2	LDL3
PAF-AH 0h	2.8±1.0	4.5±1.0	14.6±3.0	6.1±2.5	9.9±4.4	18.5±3.8
PAF-AH 3h	1.8±0.8	2.0±1.2	5.8±1.9	2.7±0.6	4.3±2.7	9.7±3.6
Lyso/Sph 0h	0.15±0.02	0.15±0.03	0.16±0.03	0.15±0.06	0.16±0.08	0.16±0.05
Lyso/Sph 3h	0.27±0.04	0.39±0.03	0.42±0.06	0.40±0.18	0.46±0.08	0.55±0.11

Values are the mean±SD. $P<0.05$ for all the values among the subfractions in each group as well as between the 2 groups except the lyso-PC/Sph 0h.

Conclusion: PAF-AH activity in LDL subfractions of FH patients was higher than in normolipidemic volunteers. During oxidation the enzyme activity was decreased in both groups but remained higher in FH patients. This difference results in higher lyso-PC production in FH patients which could contribute to the enhanced atherogenicity observed in those patients.

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ABSTRACT

LIPOPROTEIN PROFILES IN DIFFERENT TYPES OF TRAINING

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High physical activity is one of the factors most widely proved to be associated with high HDL concentrations.
With the understanding of the role of HDL as a risk factor for cardiovascular disease new interest was given to the investigation of the influence of physical exercise and training on this lipoprotein parameter. An elevation of HDL-C has been documented in a variety of sports disciplines. Different types of exercise and consequently athletic specialities can be separated - as far as metabolism is concerned - in two large groups aerobic and anaerobic.
The rationale of this study based on experimental results on athletes (n=554) with respect on the ratio factor RF of lipoproteins concentrations, low density lipoproteins (LDL/HDL) or total cholesterol to high density lipoproteins (TC/HDL), led us to propose the RF as a useful parameter in assessing lipid adaptation to physical training, according to different athletic specialities or type of sports.
From cross sectional studies in our laboratory with different athletic specialities and sports with respect to lipid profiles (total cholesterol, triglycerides, and lipoproteins HDL-LDL-VLDL) the following presentation of results is made and we compare the lipid and lipoprotein profiles of elite athletes, divided in groups of 25 specialities forming 4 significant unities (n=554) to a sedentary one (n=63) as control.
The sequence of athletic specialities is observed in order of increasing RF (TC/HDL) values. Controls RF was used as limit ratio value (RF=3.98). Endurance sports such as team games (football, basketball, volleyball), as well as short and long distance running and cycling show favourable high HDL and low RF values, $3.02\pm0.75^{***}$ and $3.36\pm0.74^{***}$ respectively, compared with sedentary people. In non endurance anaerobic and strength sports such as wrestling, boxing, fencing, sprint and jumping lipoprotein values are found to be nearer to controls (non athletes) $3.60\pm0.84^{***}$.

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VARIATIONS OF SERUM Lp(a) LEVELS IN ELDERLY WITH TYPE II DIABETES MELLITUS

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Lipoprotein Lp(a) has been recently indentified as an important independent risk factor for the development of coronary heart disease or cerebrovascular ischemic attack.
Aim: a) To compare Lp(a) serum levels between diabetic patients and normal subjects, b) To correlate the alterations of Lp(a) levels in diabetics with various parameters which may aggravate the prognosis of the disease.
Subjects: Serum level of Lp(a) were measured in 65 patients with type II diabetes (28 males and 37 females, mean age 69 $\pm$ 11 years) and were compared to 63 normal controls. The Lp(a) values were also correlated with the presence of hypetension, vascular complications, insuline treatment, body mass index (BMI), blood lipid profile (CHO, TG, HDL, $ApoA_1$), renal function, metabolic control of the disease (fasting blood glucose, HbA_1C) and serum Mg levels. Methods of statistical analysis: Mann-Whitney U, Multiple regression stepwise analysis.
Results: 1) The serum levels of Lp(a) in diabetic patients did not differ from those of the control group (P= 0,34), 2) Although there was no significant association between Lp(a) and HbA_1C (P= 0,78) an inverse currelation was shown between fasting blood glucose and Lp(a) levels (P= 0,0078), 3) It was not found any difference in serum Lp(a) in diabetics with high blood pressure or obesity, in smokers, in patients under insuline therapy or those with vascular complication.
Conclusions: We did not identify an elevation of Lp(a) in diabetic patients or a correlation of Lp(a) with any adverse prognostic factor studied.

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THE EFFECT OF SELECTED ATHLETIC ACTIVITIES ON ADOLESCENT LIPID PROFILES

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Different types of exercise and consequently sport activities are expected to affect lipid and lipoprotein blood levels. With the appreciation of the role of high density lipoproteins (HDLc) as a protecting agent for cardiovascular disease a new impetus was given to this investigation of lipid metabolism and our aim was to observe short and long term effects of exercise to adolescent lipid profiles.

The subjects, healthy high school students of both sexes (n=160, where ♂= 85 and ♀= 75), 12.5 ± 0.5 years of age, voluntarily participated in the study. They were divided randomly in two equal groups, a ball games group (A) and a control (B). The students of group B attended a 3 hours weekly Physical Education class, and those of group A attended in addition a 4-8 h weekly training in ball games. Sixty of the students (29 of group A and 31 of group B) continued for 18 months the program. Six and 18 months from the start of the program participants were tested and retested.

At the first test, comparing intergroup differences, it was found that students of group A (compared to group B) had lower mean values of body mass index (BMI) (-6%, $P<0.05$) and plasma Total cholesterol/HDLc ratio (-14.4%, $P<0.05$) and higher values of aerobic power (+32%, $P<0.001$), plasma HDLc (+8%, $P<0.05$), apoproteins as ApoA1 (+8%, $P<0.001$) and ApoA1/ApoB ratio (+9%, $P<0.05$). At the retest the students of group A continued to show higher aerobic power (+12%, $P<0.05$). Comparison in the same group, after test and retest, disclosed a progressive decrease of plasma ApoA1 (-8%, $P<0.01$) and of ApoA1/ApoB ratio (-12%, $P<0.001$) in group A, while in group B after the retest body weight was increased (+10%, $P<0.01$) and plasma triglycerides were decreased (-23%, $P<0.05$).

Although ball games, compared to physical education program alone, seem to increase in the short-term aerobic power (as the field 20m shuttle run test showed) and to improve blood lipid profiles in boys and girls, however in the long term seem to have a steady effect on the aerobic power and an incosistent one on lipid profiles. In conclusion the more prolonged sport activities in ball games have a constant positive impact on aerobic power, but not a constant beneficial effect on lipid profiles in adolescents.

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REFERENCE VALUES OF ESSENTIAL TRACE ELEMENTS IN BLOOD SERUM OF A HEALTHY BULGARIAN SUBPOPULATION

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Introduction

The endeavour to advance reference values for trace elements in plasma or serum of healthy individuals promptly reveals existence of profound controversy. It is obvious the necessity of determination of reference values for particular country or geographical region.

Material and Methods

We determined concentration of selenium, manganese, zinc, copper, iron and magnesium in blood serum of 345 apparently healthy Bulgarian individuals (184 men and 161 women). We used electrothermal atomic absorption spectrometry with graphite furnace (ETASS), Zeeman background correction and matrix modification for determination of selenium and manganese, and flame atomic absorption spectrometry (FAAS) for determination of zinc, copper, iron and magnesium. The reference limits were estimated according the approved recommendations of IFCC, using REFVAL statistical package.

Results and Discussion

The estimated reference limits are as follows: Se: 423-1123 nmol/l; Zn: 9.4-24.6 µmol/l; Cu: 10.7-26.0 µmol/l; Fe: 9.8-34.7 µmol/l and Mg: 0.67-1.03 mmol/l. The serum concentration of selenium of Bulgarian subjects is much lower than those in USA and Canada. It is also lower than those found in different European countries. It is somewhat near to the reported for Greece. Recently there are reports for selenium levels in Turkey and Yugoslavia which are similar to those found in Bulgaria. That means that probably all Balkan peninsula is a selenium deficient region. We have studied also the influence of gender and age on selenium concentration. Women have lower selenium level with statistically significant difference $p<0.01$. We found only on man a slight correlation between serum selenium level and age $r=0.2$, $p<0.02$.

We also found gender differences in the results for zinc and iron.

Recently the most often reported reference values for Mn in blood serum are between 9.1-18.2 nmol/l (0.1-1 µg/l). We observed a mean concentration of Mn in blood serum in the above mentioned limits. Our findings are in agreement with the statement of Versieck and Cornelis in their book for reference values of trace elements, that the concentration of Mn in serum really too low. The similarity of the values recently reported from different authors suppose that the exposition with Mn in the modern industrial society is similar in different countries.

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CKMB MASS OR ACTIVITY? COMPARISON OF TWO AUTOMATED METHODS FOR THE DETERMINATION OF CK-MB.

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In this study we compared the performance of the CK-MB mass assay of Dade Stratus II analyzer with the CK-MB activity assay of Dupont Dimension analyzer by parallel measurements of serum samples from 342 Acute Myocardial Infraction (AMI) and non-AMI patients in our hospital. The results from the Stratus analyzer showed a poor correlation with those obtained with Dimension technique but the CK-MB values from the Stratus agreed with those of other enzymes like CK, LDH, ALAT, ASAT and the ECG of the patient. Therefore we believe that the CK-MB mass assay is very useful in the early detection of myocardial infarction in correlation with newer biochemical markers (i.e. Troponin I), whereas the results of the CK-MB activity assay must always be evaluated together with other enzyme markers.

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EVALUATION OF CARDIAC TROPONIN I AND MASS CKMB AS MARKERS OF MYOCARDIAL INJURY IN CORONARY ARTERY BYPASS GRAFTING

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Cardiac troponin I (cTnI) has been proposed as a sensitive and specific assay to detect myocardial injury. This protein belongs to the troponin complex which is involved in the regulation of actin-myocin binding during muscle contraction and is composed of troponin I (TnI), troponin T (TnT) and troponin C (TnC). TnI (MW=29kD) is the component which inhibits the ATPase activity of myosin which blocks myosin from moving along the filament. The Stratus cTnI assay is an automated fluorimetric enzyme immunoassay which employs two mouse monoclonal antibodies specific for the TnI cardiac isotype and a six-point stored calibration curve. To determine the ability of this assay to measure myocardial injury during coronary artery bypass grafting (CABG), serial cTnI and mass CKMB (using also the Stratus II analyzer) were measured prior to and at regular intervals following surgical intervention in 250 serially collected blood samples of 60 patients in comparison with 255 serially collected blood samples of 33 patients admitted to the cardiology unit of our hospital with acute myocardial infarction (AMI). The relative increase in cTnI over baseline after CABG was an early event but was lower than the observed increase in AMI and presented one peak of 30 ng/ml 50 h after the beginning of the operation. The relative increase in cTnI over baseline during AMI started 50 minutes after the admission of the patient to the hospital and presented two peaks of 45 ng/ml after 12 and 96 h. The mass CKMB increase after CABG and in AMI was an early event but significantly lower in CABG than the observed increase during AMI and in the CABG group stayed increased for 3.5 days longer than in the AMI group. The correlation between the values of the cTnI and mass CKMB was better in the CABG group (r=0.750) than in the AMI group (r=0.334). On average in both groups cTnI stayed increased for 10 days longer than CKMB. We conclude that the cardiac troponin I (cTnI) is a highly specific marker of myocyte damage and a more sensitive indicator of successful myocardial recanalization than CKMB.

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DIRECT LDL-CHOLESTEROL ASSAY: COMPARISON OF THE RESULTS OBTAINED WITH THOSE CALCULATED BY THE FRIEDWALD FORMULA

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In this study LDL-Cholesterol was measured in fresh serum samples from 100 patients of our center both directly using the LDL assay by Sigma and indirectly by calculation with Friedwald formula. The direct LDL assay measures the LDL fraction after precipitation of the HDL and VLDL fractions. The precipitation is done by using polyclonal antibodies of the corresponding apolipoproteins bound on latex particles. The LDL is finally measured on the Dupont Dimension biochemical analyzer using a modified HDL protocol according to manufacturer instructions.

These preliminary results obtained from the direct LDL assay were relatively higher than those calculated with the Friedwald formula. The difference is in the order of 7.12%.

We believe that a further investigation of the factors which interfere with the direct LDL assay would help to evaluate this method better in comparison with the indirect method of calculation of the LDL-Cholesterol.

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THE INFLUENCE OF hCG AFP AND uE3 POPULATION STATISTICAL PARAMETERS ON BIOCHEMICAL SCREENING FOR CROMOSOMAL ANOMALIES IN THE SECOND TRIMESTER OF PREGNANCY

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INTRODUCTION: The algorithm for calculation of Down's syndrome risk in the second trimester of pregnancy, uses the statistical parameters: mean, SD, and r, of hCG, AFP and uE3 MoMs for pregnancies with healthy as well as with Down syndrome embryos. In 1988, Wald et al reported for the first time these population statistical parameters for healthy and affected embryos. In 1992, the same author reported a second, modified set of population statistical parameters for ultrasound dated pregnancies.
AIM: The aim of this study was the comparison of screening results for Down's syndrome using risks calculated with each one of these two sets of population statistical parameters.
MATERIAL AND METHODS: The material of the study was two groups of pregnancies. The control group included 359 singleton pregnancies from 15th to 24th week of pregnancy with mean age 29.4 years and range 17-43 years. Pregnancy outcome was known (healthy neonate) in 83% of these pregnancies. The group of affected pregnancies included 17 pregnancies from 17th to 24th week of pregnancy. Nine of these cases had embryos with trisomy 21, 4 with trisomy 18 and by 1 pregnancy with trisomy 13, del.(7), r(13) and 47, XXY (Klinefelter syndrome). hCG and AFP were measured by MEIA method on Abbott's IMx analyser. uE3 was measured by FPIA method on Abbott's TDx-FLx analyser. In both groups the risk for trisomy 21 was calculated by means of a software designed by us. Risks were calculated using the population statistical parameters of Wald 1988 (DRold) as well as of Wald 1992 (DRnew). Comparison of screening results with the two risk factors was performed by ROC curves.
RESULTS: Using 1:275 as risk cut-off, both risk factors detected 67% (6/9) of embryos with Down syndrome but DRnew gave 7.5% false positive results compared to 3.9% with DRold ($p=0.053$). The area under ROC curve was 0.935 for DRold and 0.894 for DRnew. DRold was negative in all four cases with trisomy 18 in contrast to DRnew that was positive in one of these cases. Both risk factors were positive for del.(7) and 47,XXY, while both were negative for r(13) and trisomy 13.
CONCLUSION: The selection of population statistical parameters influences the results of biochemical screening for Down's syndrome raising the number of false positive results. Laboratories should be informed for this effect and select statistical parameters that fit better population they serve.

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A DECADE OF CARRIER DETECTION AND PRENATAL DIAGNOSIS OF HAEMOPHILLIA IN GREECE

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Advancements in DNA technology have offered a valuable tool for genetic counceling in families with history of haemophillia during the last decade by increasing certainty about carriership and by offering first trimester prenatal diagnosis. The availability of several polymorphic markers within or closely linked to FVIII and FIX genes,and the presence of variable number of tandem repeats (VNTRs) within introns of FVIII provide a very useful way to tracking the defective gene within already affected families. The application of methods that detect directly the molecular defect add substantially to the informativity by elucidating cases of sporadic haemophillia or allowing definite diagnosis for families otherwise non informative. Since 1985, 278 (221 A,57 B) potential or obligate carriers of haemophillia belonging to 161 (136 A, 25 B) pedigrees have been studied in our Centre for carrier detection. The use of 4 RFLPs, 2 VNTRs and direct analysis of the defect by PCR or Southern Blot, have offered a total informativity of 98.6% (57.5 obligate car, 20.8% carriers, 20.3% non carriers, 1.4% undiagnosed) for haem. A. The detection of the following RFLPs for haem. B (TaqI, DdeI, XmnI, HhaI) by PCR and SSCP of all of the exons of the gene have offered a total informativity of 89% (53% obl. car., 20% car., 16% non car., 11% non inf). Prenatal diagnosis has been requested for 94 pregnancies so far. Initial testing in fetal blood sample in the second trimester has been substituted in 1986 by molecular analysis of chorionic villi in the first trimester. Forty six females and 19 healthy males were born. Another 9 haemophilic boys were born because their parents chose not to terminate pregnancies. Two spontaneous abortions occured and eighteen male foetus pregnancies were terminated (15/18 foetus affected, 1/18 non informative, 2/18 abortion regardless of the result) (A DNA sample bank of 90% of Greek haemophilics is now available in our Centre). Conclusively during the last decade we have withnessed dramatic changes in the detection of carriers and feasibility of prenatal diagnosis.

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LABORATORY INFORMATION SYSTEM IN A GREEK HOSPITAL

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The Laboratory Information System (LIS) described here has been installed in a new Cardiac Surgery Hospital and is based on a SUN Server running under UNIX Operating System which is connected with 5 smart terminals and with 7 workstations (PC's) running a specialized application under Windows 3.1. These workstations are connected with laboratory analyzers and control the flow of requests and results between the analyzers and the server. They also run the quality control of the tests and constrain the results of the last few days locally for safety reasons. The on-line instruments include a Cobas-Mira, a Cobas-Fara and a Dupont-Dimension biochemistry analyzers, a Coulter-MAXM and a Coulter-JS hematology analyzers, an AMES Clinitek 100 Urine-Analyzer and a Behring Nephelometer BN-100. Independently, a Hospital Information System (HIS) with terminals in all the wards and hospital sections, sends test requests to the LIS server which distributes them to all the appropriate analyzers and prints out worklists for the non-automated sections. The moment one analyzer gives a result for a test, this request is canceled for all the other analyzers. Hence, the workload can be processed freely in any one of the available instruments of the section. With the use of a personal password, the results are validated either locally from the LIS terminals and/or the HIS terminals or remotely via a modem connection, directly or via the INTERNET, by the authorized laboratory personnel and then are directed to the HIS.

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AN ORIGINAL SCORING AND RANKING SYSTEM OF CLINICAL CHEMISTRY LABORATORIES PARTICIPATING IN THE GREEK NEQAS FOLLOWING A TWO-YEAR CYCLE OF OPERATION.

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The Greek National External Quality Assessment Scheme (NEQAS) has completed a two-year cycle of successful operation. Participants in the scheme are 150 laboratories, state and private, from Greece and Cyprus. In 12 two-monthly expeditions, patricipants have determined 18 of the most frequently ordered analytes in control sera of normal and abnormal concentrations and received 12 eighteen-page report booklets containing the statistical evaluation of their individual results as compared to overall performance. Interpretaion of data is facilitated through the use of graphic presentations, namely distribution histograms, Levey-Jennings charts and Youden plots.

In order to improve its flexibility and provide more information, the Greek NEQAS software underwent several modifications, the main feature being the development of an original scoring and ranking system. For each analyte, a given laboratory is graded from 0 to 10 according to the deviation of its individual results from the respective target values as expressed in terms of Standard Deviation Index (SDI) units. For example, a grade of 10 is assigned to laboratories with an SDI ranging from 0 to 0.2, a grade of 9 is given to those with an SDI from over 0.2 to 0.4 etc, and finally, grade 0 includes all values over 2 SDI units. The overall performance of a participant is shown on a table (Table I) in which the rows correspond to the 18 analytes of the programme and the columns correspond to the 12 expeditions of control material. In the column that follows, the mean for each analyte is presented. The last column contains the ranking of the laboratory for a specific analyte. In the last row, the totals of each column are inserted and the last cell on the right gives the position of the individual laboratory among the participants in the scheme. In this way, participants can follow their individual performance across time and have a general idea of their status among Greek laboratories.

Table I

1st CYCLE	FINAL EVALUATION											CODE NUMBER 200		
ANALYTE	SCORE													RANK
	1	2	3	4	5	6	7	8	9	10	11	12	Mean	
ALBUMIN	9	5	8	7	5	4	7	0	7	8	9	7	5.63	**87 / 132**
CALCIUM	4	7	5	8	9	6	8	9	8	5	4	8	7.00	**63 / 129**
GLUCOSE	10	8	8	8	0	6	8	6	8	8	10	8	6.75	**63 / 142**
POTASSIUM	7	9	5	9	7	7	4	0	9	5	7	4	6.00	**70 / 126**
CREATININE	9	8	9	10	10	8	2	6	10	9	9	2	7.75	**24 / 142**
TOTAL PROTEIN	8	10	7	9	7	8	6	9	9	7	8	6	8.00	**26 / 135**
SODIUM	8	8	9	1	10	2	9	7	1	9	8	9	6.75	**63 / 124**
UREA	8	9	9	9	9	10	1	8	9	9	8	1	7.88	**37 / 142**
URIC ACID	7	9	9	9	9	9	9	7	9	9	7	9	7.38	**39 / 135**
TRIGLYCERIDES	4	5	7	8	6	5	6	8	8	7	4	6	6.13	**74 / 135**
BILIRUBIN	8	7	7	10	10	8	6	7	10	7	8	6	7.75	**34 / 131**
CHOLESTEROL	7	4	5	8	10	5	7	3	8	5	7	7	6.13	**75 / 134**
ALP	9	1	9	8	9	5	9	8	8	9	9	9	7.25	**50 / 133**
γ-GT	8	3	1	3	10	4	4	3	3	1	8	4	4.50	**101 / 136**
LDH	7	-	9	8	-	8	5	8	8	9	7	5	7.50	**53 / 132**
CK	10	-	6	9	-	4	4	9	9	6	10	4	7.00	**61 / 131**
SGOT	7	10	10	6	10	10	9	9	6	10	7	9	8.88	**9 / 142**
SGPT	8	5	4	2	8	4	4	5	2	4	8	4	5.00	**37 / 142**
TOTAL	**7.6**	**6.7**	**7.0**	**7.3**	**8.0**	**6.2**	**5.3**	**5.7**	**7.3**	**7.0**	**7.6**	**5.3**	**6.80**	**61 / 142**

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DETERMINATION OF PANCREATIC AMYLASE BY IMMUNOINHIBITION ON THE AUTOMATED CHEMISTRY ANALYSER OLYMPUS AU-560.

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Increased levels of total α-amylase are not always related to pancreatic disorders because the salivary glands and other tissues also produce amylase. On the other hand, the presence of high levels of pancreatic isoamylase (p-isoamylase) in the serum is a clear indication of pancreatic disease because the isoenzyme is only produced in this organ and disappears after pancreatectomy. Until recently the determination of p-isoamylase was performed after selective inhibition with wheat germ lectin. The recently developed immunoinhibitory method of determination uses a specific antibody to inhibit the salivary isoamylase (s-isoamylase). This method is also used in the new assay of MEDICON HELLAS for p-isoamylase which was adapted and introduced in our laboratory for the analyzers OLYMPUS AU-560 and Technicon RA-1000. The samples tested (n=200) were all from our hospital patients having physiological or elevated levels of total α-amylase.

The correlation between total amylase from OLYMPUS and MEDICON was very good [T-AMY(OLYMPUS) = 1.12 x T-AMY (MEDICON) - 8.5, r = 0.998, range: 15-1500 U/L]. A good correlation exists also between total and pancreatic amylase from MEDICON on the analyzers RA-1000 and OLYMPUS AU-560 [T-AMY(RA) = =0.985 x T-AMY(OLYMPUS) + 8.2, r = 0.984, range: 15-1500 U/L, P-AMY(RA) = =1.040 x P-AMY(OLYMPUS) - 0.26, r = 0.994, range: 10 - 280 U/L]. When used on gel electrophoresis (ISOAMYL kit, Analis BECKMAN) the MEDICON reagent for p-isoamylase shows complete inhibition of the salivary isoenzyme. Comparison of values between the p-isoamylase on OLYMPUS and the electrophoresis kit also shows a good correlation [P-AMY(BECKMAN) = 0.998 x P-AMY(OLYMPUS) + 0.35, r = 0.94, n = 40].

The results show that the commercial preparation for the determination of p-isoamylase in serum from MEDICON is an easy, fast and automated method for this test, and exhibits high sensitivity and specificity for pancreatic diseases as compared to the total α-amylase assay.

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HIGH RESOLUTION ^{1}H-NMR SPECTROSCOPY OF SERUM: SEPARATION OF L-CARNITINE RESONANCE

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The analysis of biological fluids by high resolution nuclear magnetic resonance(NMR) spectroscopy, is a recent application of NMR to clinical chemistry[1]. Many important endogenous metabolites and xenobiotics of low molecular weight can be detected and quantified simultaneously in one measurement. The experimental procedure is rapid, nondestructive to the sample and requires little or no pretreatment. The ^{1}H-NMR spectra of biofluids (e.g. serum, urine, cerebrospinal fluid) can be very informative yielding " fingerprints" of the metabolic state of the body and allow the investigation of pathological problems. The screening of unusual or abnormal metabolites and toxins are some of the applications of the method.
L-Carnitine is a carrier molecule in the transport of fatty acids from the cytoplasm into the mitochondria. Carnitine deficiency is observed in patients on hemodialysis and in certain muscle diseases.^{1}H-NMR spectroscopy has been used to detect L-carnitine in glucose-free fluids such as urine and muscle extract[2,3]. In serum, the complex spectrum of glucose in the region 3.20-3.95 ppm obscures many important metabolites, including L-carnitine. The aim of the study was the separation of the resonances of L-carnitine and glucose without additional steps of chemical isolation.
Serum samples of healthy volunteers were deproteinized by centrifugation over filter, lyophilized and redissolved in D_2O. NMR spectra were recorded on a BRUKER AMX400 spectrometer .
The most intense resonance of L-carnitine is due to the N-methyl groups containing 9 protons. In ambient temperature this signal is hidden by the double -double resonance of the βC_2 proton of glucose. We applied temperature variation to separate these two resonances . By lowering the temperature, the L-carnitine resonance moves slightly downfield and the best separation is obtained at 19 degrees Celsius.

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SOLID-PHASE EXTRACTION STUDY AND PHOTODIODE ARRAY RP-HPLC ANALYSIS OF XANTHINE DERIVATIVES IN HUMAN BIOLOGICAL FLUIDS

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ABSTRACT

An automated reversed-phase high-performance liquid chromatography (HPLC) - photodiode array method using a multi linear gradient elution is described for the simultaneous analysis of nine xanthines: xanthine, 7-methylxanthine, 3-methylxanthine, 1-methylxanthine, isocaffeine, theobromine, paraxanthine, theophylline and caffeine. The separation method development was based on mobile-phase optimisation and off-line solid-phase extraction (SPE) from human biological fluids: blood serum and urine. Eluent consisted of 0.05 M CH_3COONH_4 and methanol (90:10 v/v) changing to (70:30 v/v) over a period of 20 min. Identification of xanthines was achieved by photodiode - array detector and quantitation was performed at 270 nm. Isocaffeine was used as internal standard at a concentration of 3.06 ng/μl. High extraction recoveries were achieved from Merck RP-18 cartridges using 1% hydrochloric acid as eluent, requiring small volumes, 40 μl of blood serum and 100 μl of urine. The separation of xanthines was achieved on octylsilica, using a Silasorb C_8, 10μm, 250x4.6 mm i.d. analytical column thermostated at 32 ^{0}C and proved to be highly selective, sensitive, reproducible, accurate and rapid regarding the nine compounds. Detection limits ranged from 2 to 3 ng for 20 μl injected volume while linearity holds up to 20 ng/μl for each compound.

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EVALUATION OF THE HPLC ANALYSIS OF HYALURONAN IN THE DIAGNOSIS OF HUMAN MALIGNANT MESOTHELIOMA

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Mesothelioma is a malignant neoplasm of mesothelium. It is a primary serosal tumor which arises from the cavities of pleura, peritoneum and pericardium. Biologically, the mesothelioma must be a sarcoma, but most often it displays an epithelial morphology, and therefore its recognition from lung adenocarcinomas and pleural metastases is difficult. The sarcomatous nature of mesothelioma, however, is evident from the secretion of macromolecules in the matrix of the tissue. One such extracellular mesenchymal macromoledule is hyaluronan (HA). This polysaccharide is present in most connective tissues and belongs to the glycosaminoglycan family. It consisted of the repeating disaccharide unit: →4-β-D-glucuronic acid-(1→3)-N-acetyl-β-D-glucosamine1→. HA is produced by malignant mesotheliomas in excessive amounts and this has been widely used for diagnostic purposes. However, the determination of HA by histochemical methods is difficult, mainly due to the presence of interfering substances. It is therefore of great interest to develop highly sensitive and accurate methods utilizing HPLC and enzymes that specifically cleave HA.

In the present study pleural and peritoneal fluids have been analyzed. HA in the collected effusions was precipitated with 4 vol. of 50% ethanol and then digested with an equi-unit mixture of chondroitinases ABC, AC and chondo-4- and 6-sulfatases. The HA-derived nonsulfated Δ-disaccharides were separated on an Econosphere NH_2 5U column eluted in 9 mM phosphate buffer, pH 2.55 [1]. The detection was performed at 232 nm and the determination by using external standards of HA-derived Δ-disaccharides. The detection limit of the method was lower than 0.1 μg hyaluronan-derived uronic acid / ml.

The results of the analyses showed that a cutoff of 75 μg/mL of hyaluronate-derived uronic acid would ensure the 100% specificity for a malignant mesothelioma. The posterior probability of a mesothelioma is markedly increased even with a cutoff value of 25 μg/mL. Mesothelioma cases, evaluated cytologically, with hyaluronate content below this limit was only 20% (false-negative values) [2].

The proposed HPLC method for the HA determination in effusion seems to be a useful assay in the diagnosis of the malignant mesothelioma. The simplicity of the assay, due to that the material to be analyzed is obtained without surgery, and the high sensitivity of the HPLC method makes it suitable for routine determinations. Furthermore, the HA determinations in all effusions may furthermore results in the detection of a tumor at an earlier stage than that recognized clinically.

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SCREENING TEST FOR THE DIAGNOSIS OF HAEMOGLOBINOPATHIES AND DIABETES FROM BLOOD SPOTS ON HUMIDIFIED PAPER BY CATION EXCHANGE HPLC

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The last 25 years dried blood spots are used for the diagnosis of hereditary diseases as hyperthyroidism, phenylketonuria and enzymopathies. Today dried blood spots are used as well for the screening of haemoglobinopathies and the structural characterization of abnormal haemoglobin variants at protein and gene level (Wajcman et al.)[1]. In the case of haemoglobin, a small piece of the blood spot is cut and analyses performed by electrofocusing, either on agarose, or on polyacrylamide gels and by anion or cation exchange HPLC. These methods are suitable for the diagnosis of abnormal haemoglobin variants, mainly of HbS, but their efficacy in the case of thalassaemias is limited. The aim of this communication is to evaluate a cation excange HPLC based method for the screening of haemoglobinopathies, thalassaemias and diabetes from blood spots collected on humidified filter paper (HFP).

Material: 50 random blood specimens were used in this study. Blood was collected in K3EDTA vials, while blood drops (60-80μl) were applied on filter papers.

Methods: filter papers were dipped in H2O, placed in plastic bags and stored at 4°C until used. Then they were eluted from the paper by the addition of 400μl solution containing 7mM KCN, 24mM potassium phthalate and 2ml/l saponin pH 5.5. The haemolysates were prepared in 1/20 dilution in H2O. Cation HPLC analysis was performed by the Variant Bio-Rad system, using the "thalassaemia short" program.

Results: main results are summarized (median ± SD) as below:

	K3EDTA	Spot	r	F ratio	p
HbA2	2.6 (2.0-5.8)	2.7 (2.0-6.4)	0.977	698	0.000
HbF	0.7 (0.0-74.7)	0.8 (0.0-75.6)	0.999	87447	0.000
HbA1c	6.3 (3.1-7.5)	6.4 (3.8-7.8)	0.954	340	0.000
HbA1d	3.9 (1.9-5.5)	4.1 (2.4-5.5)	0.884	118	0.000

(*r* is the correlation coefficient, *F* the variance ratio of F-distribution and *p* the significance level). The results showed a significant correlation between haemoglobin values observed in liquid and paper sampling. This correlation is more significant in the case of HbA2 and HbF. This is due to the program of chromatographic analysis that was used.

Conclusions: The described methodology has the following advantages: is not aggressive for the population tested; the sample collection, handling, storage and shipment are considerably easy; it is suitable for screening test of large populations as students, soldiers etc. and characterized by accuracy and reproducibility. In our opinion, these preliminary results lead to the conclusion that this methodology can constitute a national pilot program for screening thalassaemias, haemoglobinopathies and diabetes.

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IDENTIFICATION OF HUMAN HEMOGLOBIN AND GLOBIN CHAIN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) : APPLICATION IN PRENATAL DIAGNOSIS OF THALASSEMIA AND THE CHARACTERIZATION OF HEMOGLOBIN VARIANTS

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Introduction : Prenatal diagnosis of Thalassemia is routinely performed in our days in the 10th-12th week of gestation by mutation analysis of DNA extracted from the chorionic villy or the amniotic fluid. However, for a small number of couples, prenatal diagnosis in the first trimester of gestation is not possible due to non-informative DNA of the parents or due to late identification of the carrier's mutation. In these cases, prenatal diagnosis is carried out by analyzing fetal blood samples, obtained during the 20th week of gestation. For that reason, it is clearly understood that the diagnosis should be the faster possible.

Aim : We have outlined a fast and reliable methodology for the identification of the common hemoglobins, using cation exchange high performance liquid chromatography (HPLC). This method minimizes ths time needed for one sample's analysis to four minutes. In addition, combination of cation exchange and reverse phase HPLC allows the fast characterization of hemoglobin variants. Using this approach we identified three α-globin chain variants for the first time in our population.

Materials and methods : Hemoglobin analysis is carried out using cation exchange HPLC (column CX-300) and a NaCl gradient. The time needed for the efficient separation of the hemoglobin fractions is only four minutes. Globin chain analysis is achieved using reversed phase HPLC (column C-8) with a ACN/Methanol gradient. Globin chains are then separated using the CM-23 chromatography and digested with DPCC-treated trypsin. The tryptic peptides are separated using reversed phase HPLC (column C-18), using a TFA/ACN gradient. The primary sequence of the unknown peptide is determined with a peptide sequencer (Applied Biosystems). Finally, DNA sequencing was performed in selected cases for the characterization of the nucleotide change which resulted in the amino-acid substitution.

Results and discussion : Cation exchange HPLC is a powerul methodology for the characterization of human hemoglobins in terms of simplicity and reproducibility. In addition it is the least time consuming method, with only four minutes duration. Our results is in excellent correlation with the results obtained by CM-23 chromatography (r=0.975), meaning that cation exchange HPLC could be used for the prenatal diagnosis of Thalassemia. In addition, combination of cation exchange and reversed phase HPLC allowed the characterization of hemoglobin variants for the first time in the hellenic population, such as Hb Setif (α94 Asp→Tyr), Hb Hasharon (α47 Asp→His) and Hb G-Bristol (α68 Asn→Lys) in combination with sickle cell anemia (Hb S). Finally combination of reversed phase HPLC and DNA sequencing allowed the characterization of an unknown γ-globin chain variant, which was named Hb F-Lesvos (Gγ75 Ile→Thr). The later proves that HPLC is a very important diagnostic tool in an analytical laboratory, which offers in both routine analysis and research of hemoglobinopathies.

Aknowledgements : The authors would like to thank the scientific and technical staff of the Units of Prenatal Diagnosis and Prevention of Thalassemia, especially Dr. M. Xenaki for providing the samples. This work was partially supported from a research grant from the Ministry of Health (Aγ837/91 to M.N.P.).

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DENATURING GRADIENT GEL ELECTROPHORESIS : A POWERFUL TOOL FOR THE DETECTION OF NUCLEOTIDE SUBSTITUTIONS IN THE β-, γ- AND δ-GLOBIN GENES REGION

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Introduction : The molecular analysis of genetic diseases relies on several methodologies which allow detection of the molecular defect responsible for the disease. The molecular basis of β- as well as δ-Thalassemia in the hellenic population is highly heterogenous, thus demanding application of a modern and reliable approach for the detection of the respective mutations. Until now, a number of approaches have been described for the identification of the disease-causing mutations, but most of them were either complicated, laborius or unsafe, due to radioactivity. Quite recently, the introduction of the polymerase chain reaction (PCR) gave birth to easier and safer techniques for the identification of mutations and polymorphisms.

Aim : In this communication we describe the application of the DGGE methodology for the characterization of molecular defects in the β-, δ- and γ-globin genes region, responsible for β- and δ-Thalassemia and Hereditary Persistence of Fetal Hemoglobin respsectively. This approach is easy, reliable, less time consuming and safe, thus it could be easily adopted by a clinical or research laboratory for the identification of nucleotide substitutions.

Materials and Methods : Isolation of genomic DNA is achieved from blood leucocytes. The β-, γ- and δ-globin genes regions were amplified independently, using specific pairs of primers for each region. The selection of the respective primer set is based on the results and data, provided by a specific algorithm (DGGE-MELT software, MIT contract #5078S to M.N.P.), which simulates the melting behaviour of a specific DNA fragment, when electrophoresed through a denaturing gradient gel. Following amplification, the PCR products are electrophoresed through a acrylamide gel with linearly increased gradient of denaturing agents, such as urea and formamide. UV transillumination of the DGGE gel reveals specific electrophoretic patterns for the respective mutations. Finally, DNA sequencing was performed directly on a PCR product for the characterization of an unknown mutation.

Results and Discussion : Application of the PCR-DGGE methodology for the characterization of β-thalassemia mutations revealed the 13 most frequent mutations in our population and further 10 rare mutations, most of which are reported for the first time in Greece. Furthermore, DGGE analysis in adult cases with elevated levels of fetal hemoglobin showed the characteristic pattern of the Greek type of HPFH mutation and further a new type of HPFH, namely the Cretan type. DGGE is now routinely applied in our laboratory for the β-Thalassemia and HPFH cases instead of the PCR-ASO methodology. In addition, DGGE analysis in cases of δ-Thalassemia in combination with DNA sequencing revealed two novel mutations, in the promoter region of the δ-globin gene (-30 T→C) and in the acceptor splicing site of the first intervening sequence (IVS I-3'ss G→C). The role of these mutations in δ-globin gene's expression is now under study with transient expression assays. The above results clearly shows that DGGE is a modern and powerful tool for the rapid screening of mutations in thalassemic cases in our population and the identification of novel mutations and polymorphisms.

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EVALUATION OF CARDIAC TROPONIN T AS A MARKER OF CARDIAC TISSUE NECROSIS.

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Cardiac Troponin T (cTnT) is a 37KDa polypeptide that binds the troponine complex (TnT, TnI, TnC) to tropomyosin molecules. cTnT has small but distinct differences in its aminoacid sequence from skeletal TnT. Its level in plasma is proportional to the amount of the myocardial damage.

We compared the sensitivity of cTnT and CK-MB in revealing cardiac tissue necrosis in four different groups of patients.

1st group: 28 acute myocardial infraction cases (AMI) diagnosed with ECG. cTnT and CK-MB were measured in samples taken the first and the fourth day after admission in order to evaluate the thrombolytic therapy and the reperfusion of the artery ($cTnT_1$ = 15,04 ngr/ml, $cTnT_4$ = 5,37 ngr/ml).

2nd group: 48 patients with unstable angina (UA).

3r group: 25 patients with angioplasty (PTCA). cTnT was measured before and afterwards ($cTnT_B$ = 0,039 ngr/ml, $cTnT_A$ = 0,476 ngr/ml).

4th group: 25 elderly patients (age=75,6 years) with ischemic cardiopathy diagnosed with pulmonary oedema (PO).

Group	A (AMI)	B (UA)	C (PTCA)	D (PO)
No of patients	28	48	25	25
cTnT	100	75	52	84
CK-MB	93	21	12	16

Percentage of elevated levels of cTnT and CK-MB (cTnT > 0,2 ngr/ml, CK-MB > 10%).

We measured cTnT levels using an anosoenzymatic sandwich method with two monoclonal antibodies (M_7 and 1B10, Boehringer Mannheim) on ES-300 system. The calibration curve exceeds from 0,00 to 16,60 ngr/ml. We also measured CK-MB on a Cobas-Fara analyser (Sclavo Diagnostics).

cTnT in comparison to CK-MB has a higher sensitivity in revealing myocardial tissue damage. All patient groups have pathological cTnT levels in percentage higher than that of CK-MB. Every patient with pathological cTnT has also elevated CK-MB levels.

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EVALUATION OF TOTAL AND PANCREATIC AMYLASE DETERMINATION METHODS FOR THE DIAGNOSIS OF ACUTE PANCREATITIS

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The routine diagnosis of Acute Pancreatitis (AP) still poses a diagnostic problem. For this reason a large number of laboratory tests have been used. In this effort we have tried using determinations of total amylase (T-AMS) and P-isoamylase (P-AMS) by a biochemical method that employs selected salivary isoenzyme blockers, as well as the P/T% factor deriving from them. As a basic verification method for AP diagnosis we used AMS isoenzyme electrophoresis.

Scope of this work is to evaluate the usefulness of the above parameters for the routine diagnosis of AP and calculate the sensitivity and specificity of each one separately, as well as their combination.

78 patient samples were examined, belonging to two groups:

1. 43 samples with AP and
2. 35 with other abdominal disorders,

and the samples of 30 healthy witnesses were examined as well.

Tests were run on the Technicon RA-XT biochemistry analyzer at 37°C to determine:

1. T-AMS with a biochemical method using Ethylldene-4-nitrophenyl-a-D-maltoheptaoside as substrate and
2. P-AMS with a biochemical method with immunoinhibition of salivary AMS by:
   - MAK<H-S-AMY>M-Tu88E8-IgG(BR) and
   - MAK<H-S-AMY>M-Tu66C7-IgG(BR) antibodies and the same as above substrate.

All sera were electrophoresed using the Isoamyl/Beckman/Belgium method.

Statistic calculations were performed using three biochemical indicators: T-AMS, P-AMS and P/T% for the evaluation of our results. The table below lists our results:

RESULTS

(Best cut-off value)		**(ROC curve)**		**– "and/or" strategy – Closest approach criterion**	
T-AMS	380 u/l	P-AMS	0,891	(P) or (P/T%)	0,151
P-AMS	280 u/l	P/T%	0,822	(P) or (T)	0,164
P/T%	80%	T-AMS	0,678	P-AMS	0,198
				T-AMS	0,304
				(P) and (P/T%)	0,343
				(P) and (T)	0,365

These results prove that:

1. there are marginal decision values for each test,
2. P-AMS determinations present the best diagnostic value, as defined by ROC curves, while
3. the (P) or (P/T%) combination, based upon "and/or" strategy is the most dependable means for routine AP diagnosis.

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METHODS TO STUDY IN VITRO LIPOLYSIS WITH HIGH SUBSTRATE CONCENTRATIONS IN A NON-ALKALINE ENVIRONMENT

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Since fats represent a substantial part of our diet, the process of lipolysis is very important in digestion. Dietary lipids consist almost entirely of triglycerides.
Pancreatic lipase is optimally active in normal postprandial duodenal conditions (presence of calcium, NaCl, conjugated bile salts, colipase...). Because of its irreversible denaturation below pH 5, substitution therapy in e.g. cystic fibrosis has therefore limited effects. Some interest has been taken in the use of microbial lipases as potential lipase substituents in enzyme therapy. We want to investigate the kinetics of the lipase of the fungus Rhizopus javanicus. Often microbial lipases are inhibited by the large bile salt concentrations in the duodenum since they cannot be activated by a cofactor such as colipase. We followed the kinetics of the Rhizopus javanicus lipase in vitro, taking physiological conditions into account. Therefore, a method was developped to determine all lipid fractions in the reaction mixture in the presence of high amounts of triglycerides.
In a second study, we want to investigate lipolysis of butter fat or randomised butter fat with pancreatic lipase. The potency to increase plasma cholesterol levels in man is reduced by randomisation of butter fat. Here, only the monoglyceride(MG)fraction was of interest since the proportion of α- and β-MG should influence the synthesis of triglycerides after absorption in the intestine and the cholesterolemic and atherogenic effect of the dietary fat. We developped a method to determine the MG isomers taking care that isomerisation does not occur during preparation of the sample.
For the first study, the reaction mixture consisted of an emulsion with purified olive oil (mainly triglycerides) and HPMC (100g oil and 10g HPMC/l), $CaCl_2$ (10mM), NaCl (100 mM) and a mixture of pure conjugated bile salts in physiological proportions (0 to 8 mM). At pH 6 400µl of enzyme solution (crude Rhizopus javanicus extract in 1% NaCl) was added. The pH was kept constant and samples of 0.6 ml were taken at different time points. After quantitative extraction of all lipids, the extracts were brought on SPE aminopropyl cartridges (J.T. Baker®) and sequential elution of triglycerides, diglycerides, monoglycerides and fatty acids was carried out. All fractions were taken to dryness and after transesterification to methyl esters quantification with CGC was performed. Internal standards were monoC14:0, diC15:0, triC17:0 and C17:0 FFA .
In the second study, MG isomers could be extracted from a reaction mixture containing an emulsion with butter fat and HPMC. No acid was used in the extraction solvent. For the separation of α and β MG, TLC plates impregnated with boric acid were used. The developping solvent was chloroform/aceton/methanol (73/25/2). Large amounts of triglycerides were eliminated before TLC with column chromatography with silica treated with boric acid. The MG containing fraction was eluted with chloroform/aceton (3/2). Solvents had to be removed under vaccuum in silanised glass vials to avoid isomerisation. Analysis of methylesters occured in the same way as described above.
The Rhizopus javanicus lipase is not inhibited by bile salts at pH 6 in the presence of high amounts of olive oil presented as a fine emulsion. In contrast, without bile salts the activity of the enzyme is almost 4 times lower. Even, with a concentration of 8 mM bile salts, no significant inhibition occurs. The use of SPE in combination with CGC shows many advantages above the standard pH-stat method in determining the kinetics of a microbial lipase.
Also the method to quantify α and β monoglycerides of butter fat, proves to be valuable. No isomerisation occurs during extraction and separation but to obtain reproducible results, extreme care must be taken to avoid transformation of β to α MG.

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L-ASPARAGINASE OF *THERMUS THERMOPHILUS* :AN ENZYME WITH CLINICAL INTEREST

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L-asparaginase is the enzyme that catalyses the hydrolysis of L-asparagine to L-aspartic acid and ammonia. The clinical action of L-asparaginase is attributed to the reduction of circulating L-asparagine, which is nesessary for the growth of certain neoplastic cells(1,2). L-asparaginase therapy is complicated by immunological reactions and various side effects(2).One of the side effects was ascribed to the capability of most L-asparaginases to hydrolyse L-glutamine and to limit serum levels of this amino acid. Therefore, for the clinical studies i) a glutaminase-free L-asparaginase and ii) an L-asparaginase with less side effects, will be more advantageous.

We have recently shown that L-asparaginase of *Tetrahymena pyriformis* is a multi-subunit enzyme exhibiting protein kinase activity as well. Both native and dephosphorylated L-asparaginase show antiproliferative activity on three breast cancer cell lines (T47D, MCF7 and BT20) and on Walker 256 cells(3). The present study describes the purifucation and characterisation of L-asparaginase from a thermostable microorganism, *Thermus thermophilus*, which is grown at 65°C and therefore its enzymes are very stable during purification.

The optimum pH of assaying L-asparaginase from *Thermus thermophilus* is 10 and presents a Km for L-asparagine 5mM. Purification of L-asparaginase from this microorganism can be achieved as follows:

a) Ammonium sulfate fractionation (20-40% saturation)
b) Anion exchange chromatography (DE-52)
c) Hydrophobic chromatography (Phenyl Sepharose)
d) Pseudoaffinity chromatography (Cibacron Blue Sepharose) and
e) Pseudoaffinity chromatography (Reactive Red Agarose).

L-asparaginase shows a molecular weight of 230kDa on gel filtration (Sephacryl S-300 Superfine).

The enzyme upon these steps is purified 4500 fold and its specific activity reaches a value of 450IU/min/mg protein. L-asparaginase of *Thermus thermophilus* is a thermostable enzyme and its activity increases from 37°C to 78°C. Its activity is stable for many days at room temperature and for many weeks at 4°C. The purified enzyme does not require metals, while Zn^{++}, Ca^{++} and Mn^{++} present inhibitory activity. L-asparaginase does not hydrolyse D-asparagine or L-glutamine and its reaction is inhibited by L-aspartic acid.

Since L-asparaginase has been used as anticancer agent, the aim of this work is to find an L-asparaginase with better antiproliferative activity. Our initial work is very encouraging sinse L-asparaginase does not present L-glutaminase activity. It is therefore of primary importance that larger amount of the enzyme be prepared.

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SELECTIVE INHIBITION OF CARBOXYLESTERASE IN PANCREATIC LIPASE ASSAYS

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From the known methods of determination of lipolytic activity the continuous monitoring titrimetric method appears the most advantageous, due mainly to the ability to use high concentrations of physiological substrates. Amongst others, the great range of values over which the method is linear and the broad spectrum of pathological values that is covered by this method must be emphasized. On the other hand, the titrimetric pH-stat method has the disavantage of determining also the products of the carboxylesterase activity on the substrate.

The goal of this study was the finding of a selective inhibitor of carboxylesterase towards pancreatic lipase in sources that contain both enzymes.

The human pancreatic juice and an extract of rabbit pancreas were used as enzyme sources, as well as comercially available carboxylesterase and purified lipase from rabbit pancreas.

The inhibitors that were tested were: Woodward's K reagent, ethoxyformic anhydride, p-chlorobenzoic mercury, benzamindine, PMSF and its combinations with taurocholate, taurodeoxycholate, cholate, deoxycholate and glycocholate sodium salts.

The effects of the inhibitors were studied after their preincubation with the sample and with two different methods, for the selective determination of the activities of the two enzymes.

Carboxylesterase activity was determined by a photometric method employing p-nitrophenyl acetate as substrate and exibiting no lipase interference, as ascertained. Lipase activity was determined by a turbimetric method exhibiting no carboxylesterase interference.

Among the inhibitors tested, ethoxyformic anhydride and Woodward's K reagent in high concentrations demonstrate almost total inhibition of both enzymes. On the other hand p-chlorobenzoic mercury does not significantly inhibit carboxylesterse, while benzamidine does not seem to exert any effect.

PMSF when used alone exibits a much lesser inhibition than when used in combination with bile salts. A prerequisite is the preincubation of the sample with the bile salt and the further incubation with PMSF. From the different time combinations tested the best results were obtained after a 45 minute incubation of the sample with the bile salt followed by a 60 minute incubation with PMSF. From the various bile salts studied sodium taurocholate in combination with PMSF proved the most selective inhibitors, i.e. 95% inhibition of carboxylesterase and only 16% inhibition of lipase. The combinations of PMSF with the rest of the bile salts show also selective but less effective inhibition of carboxylesterase, ranging from 87 to 92% for esterase and 22 to 71% for lipase.

From the aforementioned results it can be concluded that selective inhibition of esterase is achieved following incubation of samples with sodium taurocholate and PMSF. This may lead to enhanced specificity of lipase assays.

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COMPARISON OF TWO RADIOIMMUNOASSAYS, Amerlex AND SORIN, FOR THE DETERMINATION OF DEHYDROEPIANDROSTERONE SULFATE (DHEA-S)

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Reference values (R.V.) in hormone determinations depend on the nature of reagents used in different commercial preparations thus making difficult to compare results between different laboratories. The " reference values" proposed by the manufacturer are usually based on a limited number of healthy individuals and should be verified before introducing the specific test In the laboratory. We compared two common radioimmunoassays for the determination of DHEA-S, a good indicator of adrenal androgen production, Amerlex and SORIN, for their between-runs reproducibility and the proposed reference values by the two companies.

For the evaluation we used 100 laboratory samples and 30 samples from apparently healthy individuals from the hospital personnel of both sexes between 20 and 50 years of age for the verification of the R.V.'s. The reproducibility performance was calculated from control sera 1 and 2 (from Amerlex) and control serum 3 (from SORIN).

The correlation between the two assays was found to be relatively good [DHEA-S(SORIN) = 0.481 x DHEA-S(Amerlex)+15, r=0.9379, range = 59 - 7147 ng/ml] but the two assays gave different values for the samples tested. The following table shows a classification of the samples tested according to the proposed R.V.'s of the two commercial preparations:

		DHEA-S (Amerlex)			
		<R.V.	Between R.V.	> R.V.	Total
DHEA-S (SORIN)	< R.V.	**8**	26	3	37
	Between R.V.	-	**28**	28	56
	> R.V.	-	-	**1**	1
	Total	8	54	32	94

As shown in the table, only a small number of samples (37 out of 94) agrees in their characterization by the two assays. Besides, only 20 out of 30 (67%) for the Amerlex kit and 15 out 30 (50%) for the SORIN kit of the apparently healthy individuals tested are within the range of reference values proposed by the respective manufacturer. If we revise the range of the R.V.'s so as to incorporate 95% of the healthy population tested we obtain for SORIN a mean value of 1440 ng/ml and range 740-2600 ng/ml and for Amerlex a mean value 3070 ng/ml and range 1500-5500 ng/ml. With these corrected reference ranges the disagreement between the two assays is now limited to 4 out of 94 samples. The CV (%) ranges from 4.24 to 9.50 for Amerlex and from 8.62 to 10.33 for SORIN (n=8).

The results indicate that it is essential to verify the proposed reference intervals of a new method before introducing it in the laboratory along with its analytical performance and cost of analysis. We finally propose that the method used along with the laboratory value and reference range for each determination, should always be indicated.

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METHODOLOGICAL CONSIDERATIONS IN THE DETERMINATION OF PLASMA CATECHOLAMINES, SEROTONIN AND THEIR METABOLITES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION.

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INTRODUCTION: The simultaneous determination of catecholamines (CA: nor-epinephrine, dopamine), serotonin (5-HT) and their metabolites (DOPAC, HVA, 5-HIAA) in biological fluids, has long posed a challenge to the analyst. A series of methods were developed in the last decade including Radioenzymatic (R.I.A.) and High Performance Liquid Chromatography but clearly none fulfills all the requirements of the ideal analytical technique. The determination of the biogenic amines and their metabolites poses technical problems, like the low concentration, good separation, time and cost of the analysis.

AIM OF THE STUDY: The aim of the present study was to review and compare most chromatographic methods available and to develop a simple and rapid analytical method for the simultaneous determination of CA, 5-HT and their metabolites in plasma.

MATERIAL AND METHODS: BAS chromatographic system with a BAS (LC-4C) electrochemical detector, a Chrompack guard column (Chromsep HPLC Column Glass, 50 mm, Spherisorb 5 μm ODS 2) and the reversed phase Chrompack analytical column (5 μm ODS, 100X3.0 mm ID). The following mobile phases were tested: 1) 2,8 L (21,0 g citrate, 54,0 g sodium citrate, 3,65 g sodium octyl sulfate, 1,11 g Na_2EDTA, 165 ml acetonitrile), pH=5,0, 2) 1 L (14,7 mM sodium dihydro-phosphate, 30 mM sodium citrate, 27,0 μM Na_2EDTA, 1 ml triethylamine, 2,6 mM sodium octyl sulfate, 80 ml acetonitrile, 5 ml tetrahydrofuran), pH=3,5 και 3) 1 L (25,0 mM sodium dihydro-phosphate, 50 mM sodium citrate, 27,0 μM Na_2EDTA, 10,0 mM diethylamine.HCl, 2,2 mM sodium octyl sulfate, 30 ml methanol, 22 ml dimethylacetamide), pH=3,2.

PLASMA PREPARATION: Platelet rich or poor plasma was obtained by different centrifugations, sample absorption in activated alumina or boric acid gel was used.

RESULTS-CONCLUSIONS: Our results showed: a) the sample preparation is critical for the analytical recovery of the substances determined. Boric acid gel compared to alumina gave an analytical recovery 10-15% higher and the procedure of sample preparation was simpler, faster and has increased sensitivity, b) the comparison of mobile phase resolution showed that the use of mobile phase No 2 allows the simultaneous determination of plasma CA, 5-HT and their metabolites but delays the analytical estimation (25-35 min.). The concentration of surfactant (sodium octyl sulfate) is critical for the resolution and is related with time of analysis. The organic modifier is also very important for the time of analysis and the retention time of each substance under analysis.

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TREATMENT OF WILSON'S DISEASE: PROBLEMS AND PERSPECTIVES

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Wilson's disease (hepatolenticular degeneration) represents one of the most severe manifestations of copper toxicity in human. It occurs with a remarkably uniform worldwide prevalence of 30 per million. It is an inherited disease its the name, derives from the work of the neurologist S.A.K.Wilson who observed a familial disease of the liver and the central nervous system.

For the treatment of patients suffering from Wilson's disease many substances have been used; such as British anti-Lewisite (BAL), penicillamine, triethylenetetramine, inorganic zinc and recently tetrathiomolybdates, the evaluation of tetrathiomolybdates in the treatment of Wilson's disease has shown promising results.

BAL is known to increase the urinary excretion of copper but requires painful injections and is therefore impractical over a long term therapy. Penicillamine acts by reductive chelation, by reducing the copper bound to protein, thereby reducing the affinity of the protein for the copper and allowing penicillamine to bind the copper which is then urinary excreted. The main disadvantage of penicillamine is its toxicity. An alternative drug, triethylenetetramine, has been developed for patients who exhibit penicillamine toxicity. It is also a chelator, acting primarily by enhancing urinary excretion of copper.

Zinc is an anticopper agent which has been developed in the United States as zinc acetate and in Europe as zinc sulphate. Zinc acetate offers more favorable gastrointestinal tolerance. Zinc acts by inducing intestinal cell metallothionein which binds copper with a high affinity and holds it until the intestinal cell is sloughed into the stool and excreted by feces. Zinc blocks not only the uptake of copper in food but the reabsorption of endogenously secreted copper from saliva and gastric juice. One of the major advantages of zinc is its very low level of toxicity. It is suggested for the treatment of the presymptomatic patients, pregnant women and in the maintenance treatment.

Recently tetrathiomolybdates were suggested as an alternative treatment as a result of observations in ruminants who suffered from chalcosis-copper toxicosis-showed very promising results.

Tetrathiomolybdates have 2 mechanisms of action:

- First, they form a complex with copper and proteins in the intestinal tract and prevents absorption of copper.
- Second, the absorbed tetrathiomolybdates form a complex with copper and albumin in the blood rendering the copper unavailable for cellular uptake.This drug has previously been used in the maintenance treatment of a limited number of patients who were intolerant of penicillamine and triethylenetetramine, and has began an evaluation of tetrathiomolybdates for the initial treatment of neurologically affected patients.

The analytical methods that have been developed in our laboratory for the determination of tetrathiomolybdates and molybdates-the latter consist the oxidation product of the former- in blood plasma are the following:

I) Differential pulse voltammetric determination of tetrathiomolybdates.

II) Simultaneous differential pulse voltammetric determination of tetrathiomolybdates and molybdates. The principal advantages can be summarized as follows:

- Their determination in blood plasma is possible after removal of blood plasma proteins, after treatment with trichloroacetic acid or after ultrafiltration.
- Under the optimum conditions, the determination of 1 ng/ml of tetrathiomolybdates is possible. While relative standard deviation is lower than 4%.
- The methods allow the discrimination between tetrathiomolybdates and molybdates as well free, labile and bound tetrathiomolybdates and molybdates.

We conclude that the above mentioned methodoly can be applied in the monitoring of Wilson's disease, at least as far as it concerns the evaluation of the optimum therapeutic dose of tetrathiomolybdates.

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EVALUATION OF PROGESTERONE DETERMINATION ON THE AXSYM/Abbott ANALYZER: COMPARISON WITH THE DELFIA / WALLAC METHOD.

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The automatic hormone analyzer AXSYM/Abbott is a random access instrument capable of performing simultaneously up to 15 different hormone and tumor marker tests. The range of tests performed for reproductive endocrinology was recently enriched by the addition of progesterone (PRG) determination. Our laboratory was selected by the company to perform the required performance tests on the analyzer and to compare results with the in-house methodology (DELFIA / WALLAC) for the new test.

For the statistical evaluation we followed the NCCLS guidelines, protocol EP5-T2, with AXSYM kits made available to us by the company and the commercial PRG kits from DELFIA. For within-run and between-runs reproducibility tests we used the L, M and H control sera from Abbott Diagnostics. One hundred (100) serum samples from our Hospital were used to compare the two methods.

The results are shown in the following table:

	Control serum	AXSYM		DELFIA	
		Mean value x	CV (%)	Mean value x	CV(%)
Within-run	L	1.083 (*)	4.24	0.987	7.09
	M	5.843	2.85	5.750	5.27
	H	22.610	4.66	27.830	6.64
Between-runs	L	0.984	8.47	0.991	16.40
	M	5.552	6.81	6.078	7.34
	H	21.806	9.64	27.930	4.38

(*) All values are expressed in ng/ml

The two methods compare very well [PRG(AXSYM) =1.139 x PRG(DELFIA) -0.08, r= 0.993, range of values: 0.11-40.0 ng/ml, n=100]. We noticed a small degree of inconsistency in the higher (H) values between the two methods. In addition, we calculated the sensitivity of the new method and found it to be 0.17 ng/ml (x + 2SD of the 0 ng/ml sample).

We conclude that the new PRG determination on AXSYM has at least the same analytical characteristics as one of the previous methods on the market and that it can be used as a routine determination in the laboratory.

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EVALUATION OF CHEMILUMINESCENT IMMUNOASSAYS FOR T3, T4, TSH IN S300, BYK-SANGTEC AND CORRELATION WITH RIA IN USE

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In an attempt to automate the routine hormone assays of our lab, chemiluminescent immunoassays for T3, T4, TSH in S300, Byk-Sangtec were evaluated and the results were correlated with the RIA in use (T3, T4: Amersham, TSH: Cis International). The results are presented at the following tables

1) Linearity test

	1:1	1:2	1:4	1:8	1:16	1:32	1:64
TSH	>100	59.0	60.8	61.6	65.6	73.6	89.6
T4	19.9	18.0	18.8	24.6	20.2	-	-
T3	>800	920	817	676	-	-	-

2) Recovery test

TSH			T4			T3		
expected value	obtained value	% recovery	expected value	obtained value	% recovery	expected value	obtained value	% recovery
0.13	0.17	131	2.3	2.1	91	30	<30	-
0.4	0.57	143	4.7	4.2	89	100	83	83
1.2	1.4	120	9.3	9.0	97	190	183	95
4.0	3.6	90	15.5	15.6	101	390	344	88
12.5	11.9	96	24.9	24.9	91	780	647	83
25.0	25.8	103						
50.0	50.5	101						
100.0	83.1	83						

3) **Reproducibility test within and between assays**

	Within assay		Between assays	
T3	%CV at different levels	2.8 - 7.4	CV%	6.5
T4	>>	3.2 - 4.6	>>	6.5
TSH	>>	1.75 - 3.8	>>	5.2

4) **Linear regression and bias for paired serum samples**

	n	Slope	Y-Intercept	r	Z score	Bias
TSH	257	0.9660	- 0.1790	0.9852	- 0.6525	yes
T4	171	0.8173	1.2326	0.8638	- 2.2	no
T3	203	0.9025	26.39	0.8192	5.3	no

In conclusion, concerning the T3,T4,TSH in S300, Byk-Sangtec, reproducibility and accuracy were satisfactory and there was good correlation with the RIA in use, especially for T4, TSH.

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SEROLOGICAL AND IMMUNOHISTOCHEMICAL DETECTION OF CA 15-3 IN BREAST CANCERS AND THEIR RELATION WITH OTHER PROGNOSTIC FACTORS

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Introduction: CA 15-3 is a breast cancer associated antigen that is found in the serum of most patients with advanced breast carcicona. Elevated values of CA 15-3 correlate with metastatic tumor burden and with the course of the disease. CA 15-3 is a high molecular weight carbohydrate antigen recognized by two different monoclonal antibodies 11 5D8 and DF_3.

Purpose: The purpose of this study is to investigate if there is any possible correlation between the distribution of the monoclonal antibody DF_3 in tumor cells of breast cancer and the serum values of the same antigen in breast cancer patient preoperatively, so the clinical course of the patient can be monitored more effectively and can be established the prognostic value of this tumor marker.

Material and Methods: Thirty six patients with operable breast carcinoma were treated by partial or radical mastectomy, and their serum values of CA 15-3 are known preoperatively. Twenty eight of them were infiltrating ductal carcinoma, 6 were of lobular carcinoma, one medullary carcinoma and one case tubular cancer. In all of them the degree of differentiation, the size of the tumor, the lymph node infiltration and the levels of hormonal receptors were known. In addition the c-neu amplification of these tumors was done immunohistochemically. Serum CA 15-3 antigen was determined by a sandwich Elisa Kit supplied by Abbott Diagnostics (Mabs DF_3 and 115D8). The upper limit of the reference range was set at 35IU/mL. Immunohistochemical detection was performed in paraffin embedded tissue using the monoclonal antibody DF_3 (Hist Cis *Biointernational*) using a *streptavidin-biotin Method* (*Biogenex Super Sensitive Kit*).

Results: Immunohistochemical pattern of CA 15-3 in normal tissue showed a positive staining in the apical border of the epithelial cells and no staining in any other part of the tissue. Breast carcinoma displayed not only an apical and membranous staining but a strong cytoplasmic staining in some of the cases. We devided the cases in two groups, one with cytoplasmic detection of CA 15-3 in over 60% of the tumors cells and the other group with membranous detection of CA 15-3 in over 60% of the tumor cells. There was good correlation between cytoplasmic staining and elevated serum values of CA 15-3 (over 35IU/mL, $p<0.005$). There was no correlation seen between the degree of differentiation and the serum values of CA 15-3. On the contrary, cytoplasmic localization of this antigen and degree of differentiation had a good correlation showing a trend of increasing percentages (Gr I: 28,6%, GrII: 64,3%, GrIII: 85,7%). There is no correlation found between lymph node infiltration and serum levels of CA 15-3 as well as with the immunohistochemical pattern of the same antigen. The receptor status and the serological or immunohistological detection of CA 15-3 did not show any correlation. The tumor size doesn't have any correlation with CA 15-3 expression not serological nor immunohistochemically. There is a good correlation between the amplification of c-neu oncogene and cytoplasmic detection of CA 15-3 in tumor cells in a percentage of 69,3% versus 25% which is the percentage of membranous detection of CA 15-3 and c-neu amplification ($p<0,05$).

Discussion: The immunohistochemical pattern of CA 15-3 in breast cancer gives us some valuable information about qualitative characteristics of the tumor, such as the degree of differentiation and biochemical behaviour. The good correlation found between cytoplasmic localization of CA 15-3 and elevated serum values (>35 IU/mL),, helps us to conclude that the use of serological and immunohistological detection of CA 15-3 simultaneously is a useful prognostic tool for the management of breast cancer patients. The good correlation between c-neu amplification and cytoplasmic localization of CA 15-3, shows that perhaps certain changes that occur on the tumor cell membrane are responsible for the cytoplasmic detection of CA 15-3 that leads to elevated serum values.

Chimika Chronika, New Series, 25, 156 (1996)

THE CONTRIBUTION OF BIOCHEMICAL MARKERS CASA AND CA 125 IN THE PRE-OPERATIVE ASSESSMENT OF ADNEXAL TUMORS

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CASA (Cancer Associated Serum Antigen) is a relatively new biochemical tumor marker, used in pre-operative evaluation and monitoring of ovarian cancer.

It detects the glycoprotein polymorphic epithelial mucin (PEM) the product of MUC-1 gene. PEM is guite distinct from CA 125 antigen, biochemically.

The purpose of this study is the pre-operative differential diagnosis of endopyelic tumors with the help of both, CASA and CA 125.

To this end, we measured the values of these two markers in the sera of 72 patients before the operation and we compared the findings with the results of the histological examination. Cut-off values for CASA and CA 125, were 4 U/ml and 35 U/ml respectively.

The sensitivities of CASA and CA 125 for the detection of ovarian cancer were 60 and 82% respectively. In the few cases where the more sensitive CA 125 was falsely negative for the presence of the disease, CASA was also negative. On the contrary, in 6 cases where CA 125 was falsely positive (endometriosis, teratoma etc). CASA was truly negative.

In conclusion, the high specificity of CASA makes this marker very useful in the differential diagnosis of endopyelic tumors, especially in combination with CA 125.

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METHOD FOR THE DETERMINATION OF THE LEVELS OF VITAMIN D METABOLITES, 25-OHD, 24,25-$(OH)_2$D AND 1,25-$(OH)_2$D IN THE SAME SAMPLE OF SERUM OR PLASMA

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Introduction

The determination in blood, of the levels of the three main vitamin D metabolites [25-OHD, 24,25-$(OH)_2$D and 1,25-$(OH)_2$] can be useful in basic research, in the study of bone metabolism and in clinical diagnosis.

Purpose

The purpose of the present study was the development of a new method suitable for paediatric use. The method should be easier, less time consuming and less expensive than the already existing ones and adopted to existing equipment. Finally the method should be capable of measuring all three main metabolites of vitamin D, 25-OHD, 24,25-$(OH)_2$D and 1,25-$(OH)_2$D in the same relatively small volume of serum or plasma

Methods

The method involves acetonitrile extraction of the serum or plasma sample (0.5-1.0mL) and preliminary purification of the vitamin D metabolite fraction on Sep-Pak C_{18}. mini columns. The columns are washed with water and 70% (v/v) methanol in water . The vitamin D metabolite fraction is eluted with acetonitrile. The separation of the three metabolites is carried out by straight phase high performance liquid chromatography (HPLC) on a Zorbax Sil column with eluting solvent hexane: 2-propanol: methanol 95:10:3 (v/v). The losses during the chromatographic separation are estimated with the use of [^{3}H] internal standards. The levels of 25-OHD and 24,25-$(OH)_2$D are determined by a competitive protein binding (CPB) assay with the use of normal human serum D binding protein. The levels of 1,25-$(OH)_2$D are determined with a radioreceptor assay employing 1,25-$(OH)_2$D receptor from calf thymus.

Results

The recovery of the metabolites after the chromatographic separation in a series of 15 samples (n=15) was 64.6 ± 5.8 % for 25-OHD, 61.1 ± 4.5 % for 24,25-$(OH)_2$D and 60.6 ± 3.1 % for 1,25-$(OH)_2$D (mean ± SD).

When 9 aliquots of the same human serum were assayed, the mean ± SD values were 16.6 ± 1.3 ng / mL for 25-OHD, 1.20 ± 0.07 ng / mL for 24,25-$(OH)_2$D and 22.6 ± 1.5 pg / mL for 1,25-$(OH)_2$D. The coefficients of variation were respectively 7.8, 5.6 and 6.7%.

When a normal human serum sample was assayed in nine (n=9) serial assays the mean (± SD) values were for 25-OHD 23.8 ± 2.5 ng/mL, for 24,25-$(OH)_2$D 1.53 ± 0.20 ng/mL and for 1,25-$(OH)_2$D 24.4 ± 2.1 pg/mL. The coefficients of variation were 10.5, 12.9 and 8.7% respectively .

Conclusions

A new method for the determination of the three main metabolites of vitamin D has been developed in this study. The method is adopted for paediatric use and existing equipment and is based in modification and combination of existing techniques taking advantage of new chromatographic techniques such as the use of Sep-Pak C_{18} instead of the LH-20 which are time consuming, require large amounts of solvents and give lower recoveries. The determination of 25-OHD and 24,25-$(OH)_2$D is carried out with the use of human D binding protein instead of rat protein. The use of the calf thymus receptor for 1,25-$(OH)_2$D contributes to the specificity of the determination. The method is less time consuming than others.

The results about the repeatability and reliability of the method, as shown by the evaluation of the method, were satisfactory fulfilling the criteria set originally.

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GENOTYPING OF THE APOLIPOPROTEIN E POLYMORPHISM IN THE GREEK POPULATION

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Apolipoprotein E (apoE) is a plasma glycoprotein with many functions. It constitutes an important element for the lipoprotein metabolism by acting in IDL as a ligand for the LDL receptor, it participates in the nervous cell regeneration, in the immunoregulation, in the regulation of intracellular cholesterol and steroid origin in the adrenal cells, and as an activator or regulator of the liver lipase and the LCAT. ApoE is found in the amyloid plaques of Alzheimer and Creutzfeldt-Jacob diseases, as well as in other types of brain and systematic amyloidosis.

The apoE gene is localized on chromosome 19. The polymorphism of this gene is expressed in the general population with the three more common alleles ε2, ε3, and ε4. Therefore there exist 3 homozygous (ε2/ε2, ε3/ε3, ε4/ε4) and 3 heterozygous (ε3/ε2, ε4/ε2, ε4/ε3) forms. From the three alleles ε3 is considered as the normal one.

The incidence of ε4 allele in other populations ranges between 10-20%. In comparison with allele ε3 the ε4 exists in higher ratio in groups of patients with ischemic heart and Alzheimer diseases. So far, the ε2/ε2 genotype presents as diagnostic marker for patients with hyperlipidemia type III.

For the above reasons it is necessary to know the distribution of apoE genotypes in the Greek population, as the incidence of hyperlipidemia as well as the Alzheimer disease is sufficiently high.

The objective of the present study is to characterize the apoE genotypes in the Greek population and to compare the results with the data from other countries.

The material consists of 200 blood samples of adult blood donors from the National Blood Center of the Nikea Regional General Hospital. The methods consist of DNA isolation from blood, polymerase chain reaction/PCR amplification, digestion of the products with restriction enzyme, agarose gel electrophoresis and photography. Statistical analysis is applied to the results.

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A LIPOPROTEIN-ELECTROPHORESIS DECISION SUPPORTING AND FILING SYSTEM

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Background: An electrophoretic pattern evaluation and decision supporting system focused at the present on Lipoprotein-gel automated diagnosis has been developed.
Methods: The two-dimensional gel electrophoresis images are digitized by using a commercially available PC-linked scanner and the optical density distribution curve, along the symmetry axis of the electrophoresis pattern is formed. According to a case-based reasoning procedure, the acquired curves form each time a "diagnostic vector" $d_j = dj\ (a_{1j}, a_{2j},...,a_{kj})$. This vector is compared to a set of i diagnostic vectors $D_i = D_i\ (a_{1i}, a_{2i},...,a_{ki})$, i = 1,2,...,m, which correspond to the already evaluated by the medical expert cases and constitute a continuously expandable reference knowledge base. By defining an appropriate comparison metric M, for the n-th patient, a diagnostic proposal is displayed by the system, by appointing to that case, the proposal attached to the vector Di that minimizes the metric $M(d_j-D_i)$, i=1,2,...,k.

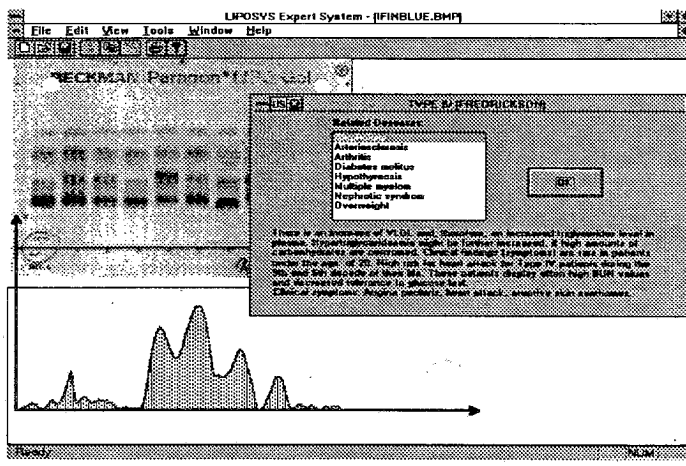

Figure 1: A typical active window of the system displaying the density-curve of an Lipoprotein-Electrophoresis, Diagnostic-Proposal (Fredrickson IV) and suggestions for further clinical investigation.

Results: The knowledge-base has been formed by introducing 104 evaluated cases, kindly allocated by Prof. Dr. H. Schmidt-Gayk, University of Heidelberg and has been laboratory tested. Within this gel-set, used as test-samples, the success of the system is 100%. Tests performed using random gels, from various laboratories show success between 70-95 %, depending on the user.
Conclusion: The system suits for the formation of a comprehensive and easily accessible paperless electrophoresis-image record, for supporting remote reasoning, through modem linking, as well as, for medical training purposes. The low cost of the system, since the application software can run on any at least 386DX/40 MHz processor, (it is recommended the use of at least 486DX2 with 8Mb RAM) and of the scanner (at least 400 dpi, recommended 1200 dpi), its flexibility and universality make it affordable for any low-budget Institution. The system is open to improvement and extension. Further Knowledge Bases are under creation, for the evaluation of protein and haemoglobin electrophoresis (Mediterranean anaemia diagnosis).

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CORRELATION OF RESULTS FROM DIMENSION / SMS-Dupont (D) and RA/XT-Technicon (R) CHEMICAL ANALYZERS

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The use of different chemical analyzers with large throughput presupposes intense quality control assesment and close correlation of the results. In our study we performed internal quality control and correlation of results from a network of DIMENSION / SMS-Dupont (D) and RA/XT Technicon (R) analyzers with open and fixed chemistry channels. Control sera (Ciba Corning) of normal and abnormal concentrations were employed for the measurements of end point, Glucose (GLU), Uric Acid (UA), Total Protein (TP), zero order (SGPT, CK, LDH, γ-GT) and first order, Urea (U), analytes.The study was performed over a period of 20 consecutive days under usual working conditions.

A. ***NORMAL CONCENTRATIONS - EARLY MEASUREMENTS***

	GLU (mg/dl)		U (mg/dl)		UA (mg/dl)		TP (g/dl)		SGPT (U/l)		γ-GT (U/dl)		CK (U/l)		LDH (U/l)	
analyzer	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R
mean	86	85	29	28	5.4	5.5	5.4	5.5	26	29	35	29	227	234	269	274
SD	2.0	3.3	1.7	1.6	0.12	0.21	0.11	0.13	1.7	2.0	2.5	1.5	5.9	4.1	9.4	6.9
CV %	2.3	3.8	5.9	5.8	2.2	3.8	2.0	2.4	6.6	6.9	7.1	5.1	2.6	1.8	3.5	2.5
r	0.62		0.14		0.17		0.06		0.46		0.1		0.3		0.2	

B. ***ABNORMAL CONCENTRATIONS - EARLY MEASUREMENTS***

	GLU (mg/dl)		U (mg/dl)		UA (mg/dl)		TP (g/dl)		SGPT (U/l)		γ-GT (U/l)		CK (U/l)		LDH (U/l)	
analyzer	D	R	D	R	D	R	D	R	D	R	D	R	D	R	D	R
mean	285	281	95	98	9.2	9.1	4.2	4.6	82	98	106	96	476	493	792	810
SD	9.8	12.1	4.9	2.4	0.2	0.4	0.15	0.14	5.8	1.6	2.8	2.8	15.2	16.6	20.1	31.2
CV %	3.4	4.3	5.2	2.5	2.2	4.4	3.6	3.0	7.1	1.6	2.6	2.9	3.2	3.4	2.5	3.6
r	0.46		0.15		0.20		0.33		0.38		0.001		0.09		0.19	

CONCLUSIONS

1.The measurements of different chemical analyzers show good accuracy and precision.

2.Accurate and precise results derived from different analyzers do not correlate one each other.

3.Attention must be paid on the fixed - type analyzers where standardization and calibration of the open channel chemistries are specifically required.

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LEAD AND CADMIUM BLOOD REFERENCE VALUES

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Introduction

Lead and cadmium are toxic metals widely distributed in the modern city environment. The concentration of these metals in biological fluids corresponds to the environmental pollution levels and varies significantly with geographical area, demographic factors (such as sex and age), as well as the population's smoking and feeding habits. Lead and cadmium toxicity in humans has been the subject of many studies. The terms *maximum exposure value* and *quality control limits* signify the critical levels over which clinical symptoms may appear. In evaluating subclinical symptoms and their effective prevention, it is essential to know the concentration of these metals in human blood (reference values) as measured in healthy, non-professionally exposed adults of the same district.

The aim of the present study is to determine the reference values of lead and cadmium in the blood of healthy adults in Athens.

Materials and Methods

Blood samples were obtained from 507 healthy adult Athenians (272 men and 253 women), non-professionally exposed to toxic metals. During blood sampling, the patients answered a questionnaire specifically developed for the purpose of the study. The lead and cadmium concentration in whole blood was determined by Atomic Absorption Spectroscopy. The Fernadez method was used for the measurement of lead concentration, while the protocol suggested by IUPAC was employed for the determination of cadmium. Special attention was paid during blood sampling and analysis to prevent potential infection. Statistical analysis was performed by using the REFVAL program as suggested by EPTV of IFCC for the determination of reference values in clinical chemistry.

Results and Conclusions

The lead concentration in men's blood (106.7 + 21.1 μg/l) is higher than in women's (65.3 + 31.2 μg/l), probably due to the different number of erythrocytes in their blood and the different proportion of fatty tissue per body weight in the two sexes.

Since emissions from vehicles using leaded gasoline are a major source of lead pollution, the statistically significant increase ($p < 0.001$) in the blood lead concentration observed in the residents in the center of Athens is probably the result of heavier traffic in this area. Blood lead values did not correlate with age ($r=0.009$), probably because most lead entering the human organism is stored in the bones, while only 0.5-1% enters blood circulation.

Blood cadmium concentration is the same in men and women. Since tobacco leaves have a high cadmium content, and cadmium enters the human body mainly through the respiratory system, blood cadmium levels are considerably affected by smoking. The average blood cadmium level for smokers (1.5 μg/l) is approximately twice as much as the one for non-smokers. The increase is directly proportional ($r=0.58$) to the total number of cigarettes smoked during a day as well as in a lifetime, although cadmium levels do not correlated with age ($r=0.001$).

Blood lead levels did not correlate with residence location, which is explained by the absence of this metal in automobile fuel.

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DEVELOPMENT AND EVALUATION OF A COMPETITIVE ENZYME-LINKED ASSAY METHOD (ELISA-TYPE) FOR MEASURING BIOTIN IN HUMAN SERUM

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Biotin is a water-soluble vitamin found in tiny amounts in all living cells, whereby it catalyzes important metabolic reactions as coenzyme of carboxylases. Biochemical malfunctions connected with biotin metabolism in human have been associated with several serious clinical syndromes. However, it has not been completely elucidated whether biotin level fluctuations in human can be used as a reliable indicator for diagnosing or monitoring the above syndromes, or whether these fluctuations are associated with the appearance of other diseases. In this study we describe a rapid, simple, sensitive and reliable enzyme-linked assay method for determining biotin in human sera, which can be applied to the clinical monitoring of patients. According to the method, microtitre plates are at first incubated (18h, 22°C) with 100 μL of a biotinylated bovine γ-globulin, 2 μg/mL, in sodium carbonate buffer pH 9.2, 0.05M. This incubation step can be performed independently of the analysis time, since the immobilized compound remains stable for at least 2 months at 4 °C. The plates are washed with phosphate buffer pH 6.5, 0.04M, containing NaCl, 7.2 g/L (washing solution A), incubated (1h, 22°C) with 200 μL of a bovine serum albumin solution, 10 g/L, in sodium bicarbonate buffer pH 8.5, 0.1M, containing NaN_3, 0.5 g/L (blocking solution), and then washed three times with washing solution A containing Tween 20, 0.05%, v/v (washing solution B). Then, the plates are incubated (30min, 22°C) with 100 μL of equivolume mixtures of: i)streptavidin - horseradish peroxidase solution, 20 ng/mL, in buffer A containing BSA, 36 g/L, and ii)standard biotin solutions (5-640 ng/L), or unknown samples, in the same buffer. The mixtures of the above solutions are preincubated for 15min at 22°C. Following the above incubation, the plates are washed three times with the washing solution B and incubated in dark with a substrate (ABTS) solution, 1 mg/mL, in citrate/phosphate buffer pH 4.4, 0.1M, containing H_2O_2, 0.003% (30min, 22°C). Finally, the optical absorbance is read in an ELISA reader (405 nm) and the biotin concentration in unknown samples is determined from the standard curve (dynamic range: 5-640 ng/L). The method is sensitive (2 ng/L), accurate [i)recovery of exogenous biotin concentrations, 100-114%, ii)linear recovery of high endogenous biotin concentrations in serial dilutions (1:2 to 1:8), 91-117%], precise (intra- and inter-coefficient of variation: 1.6-3.9% and 3.7-7.2%, respectively), independent of the sample protein concentration (addition of up to 8% BSA in pooled human sera has no effect on the analysis), with a non-specific binding value (blank) < 3%. Determination of biotin in serum samples obtained from 68 apparently healthy adult individuals, 35 pregnant women at the final trimester of pregnancy, and 40 patients under chronic haemodialysis treatment were 66-600 ng/L (mean 223 ng/L), 60-360 ng/L (mean 186 ng/L), and 0.56-1.62 μg/L (mean 1.11 μg/L), respectively. These values are in accordance with those previously reported in the literature and confirm the clinical reliability of the method. Among the advantages of the method are: avoidance of radioisotopically labelled tracers, simple analytical protocol, short assay time, high sensitivity, broad working range of the standard curve -suitable for the determination of biotin in human sera-, stability of the assay reagents for a period of at least 12 months, during which no significant inter-assay variation is observed, and versatility for measuring biotin in various biological samples, due to the assay protein-independence. The assay reagents are commercially available or can be easily prepared in Hospital Laboratory. Apart from its excellent analytical characteristics, the method described is among those few which have been experimentally validated for their capability in the assessment of biotin in human sera.

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EVALUATION OF PROGESTERONE, ESTRADIOL, PROLACTIN, LH, FSH, HCG T4, T3U AND TSH ON THE IMMULITE CHEMILUMINESCENT IMMUNOASSAY ANALYZER.

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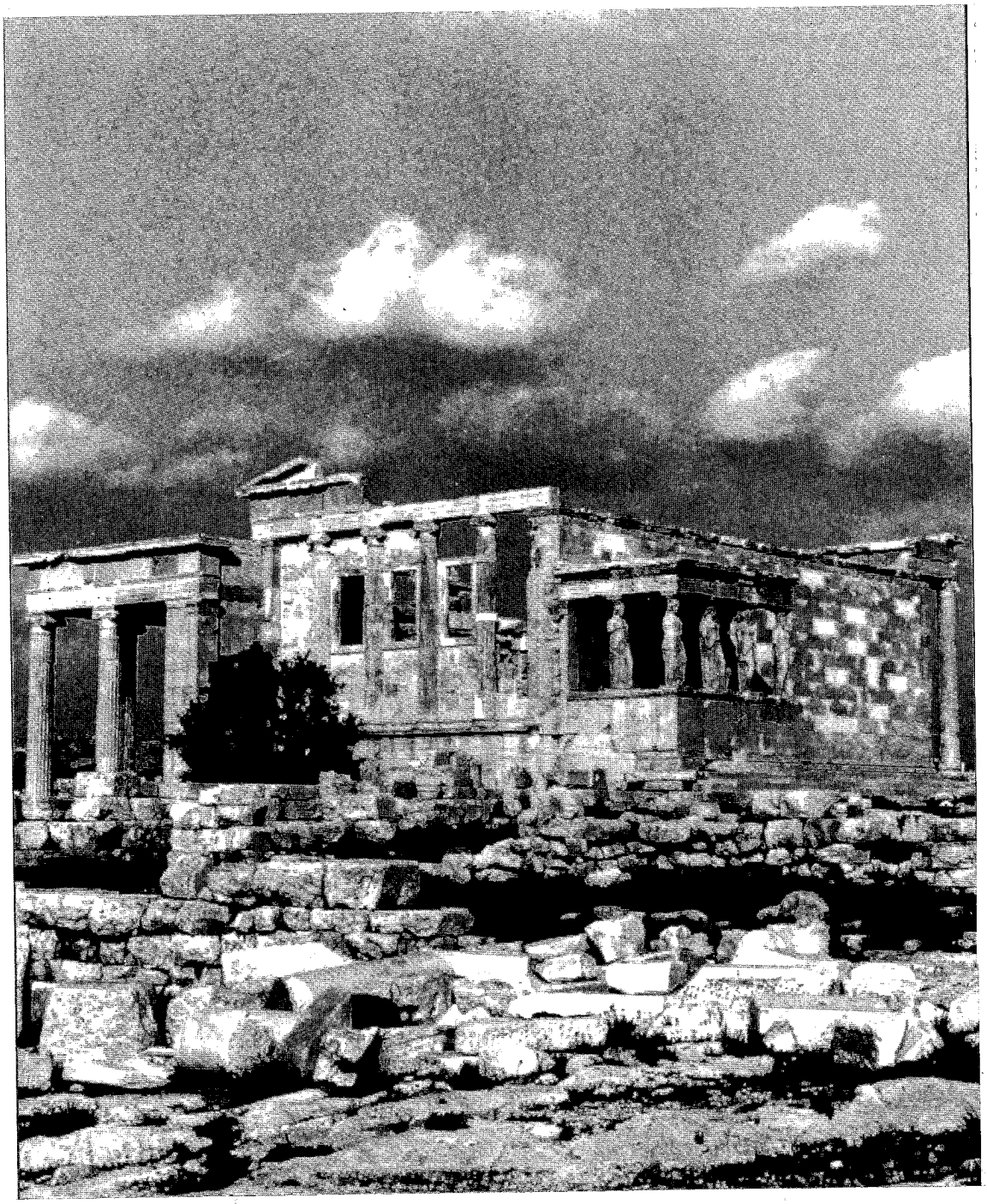
We evaluated DPC/Cirrus (Randolph, NJ 07869) Progesterone (PROG), Estradiol (E2), Prolactin (PRL), LH, FSH, HCG, Thyroxine (T4), T_3 Uptake (T3U) and Third Generation TSH (hTSH) assays. These methods were compared to Diagnostic Products Corp. (Los Angeles, CA 90045) Double Antibody E2 RIA, Coat-A-Coat PROG, PRL, T3U and T4 RIA, Serono (Allentown, PA 18103) LH, FSH RIA, Serono IRMA PRL, HCG RIA and BioRad (Hercules, CA 94547) IRMA TSH RIA. IMMULITE is a random access instrument which utilizes chemiluminescent technology. Sample and alkaline phosphate-labeled reagent are incubated with an antibody-coated bead. After incubation the bead is washed and dioxetane substrate is added. The enzyme conjugate bound to the bead cleaves the substrate to form a chemiluminiscent product which is measured. Within-run precision studies (n=10) gave the following CVs at 3 concentrations (low, medium and high): PROG (15.7,7.9,6.8%), E2 (16.7,6.1,7.4%), PRL (4.7,2.8,5.0%), LH (7.9,3.6,6.7%), FSH (3.9,4.2,3.4%), HCG (9.2,7.5,4.9%), T4 (8.5,4.8,4.5%), T3U (3.1,3.7,3.3%) and hTSH (3.9,5.0,3.7%). Between-run precision studies (n=10) gave the following CVs at 3 concentrations : PROG (17.9,6.9,13.3), E2 (15.2,7.2,8.9 %), PRL (5.9,7.0,5.8%), LH (11.9,6.1,7.3%), FSH (6.2,8.9,7.3%), HCG (13.4,6.2,7.5%), T4 (15.6,7.7,7.3 %) T3U (4.5,4.0,3.1%) and hTSH (5.0,8.4,7.7%). Percent recovery averaged from 81 to 100 % for all assays. Least squares analysis of the results where x is the comparison assay and y is the IMMULITE value gave the following results: for PROG (n=184), slope (s) =0.94, y-intercept (y) = -1.347 and correlation coefficient (r) = 0.99; E2 (n=56), s = 0.70, y = 5.71, r = 0.97; PRL (n=57), s=0.73, y =1.67, r = 0.91; LH (n=54), s = 1.37, y= -1.29, r= 0.96; FSH (n=79), s = 1.27, y= -3.19, r= 0.99; HCG (n=55), s = 0.84, y= 0.202, r= 0.94; T4(n=62), s = 0.84, y= 0.76 r= 0.87; T3U (n=62), s= 0.944, y= 2.42, r= 0.70, and for hTSH (n=62), s = 0.80, y= 0.16 and r= 0.96; We conclude that the IMMULITE immunoassay system will provide clinically reliable results. The precision of some of the assays is higher than the RIA comparison methods.

CONTENTS



ΟΛΥΜΠΙΑΚΗ ΑΕΡΟΠΟΡΙΑ

ΕΡΕΧΘΕΙΟΝ

Hellas

ΕΛΛΗΝΙΚΟΣ ΟΡΓΑΝΙΣΜΟΣ ΤΟΥΡΙΣΜΟΥ