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Manuscripts and editorial correspondence should be addressed to

M. SAJGÓ, 1052 Budapest, P.O.Box 7, Hungary (biochemistry)
A. NIEDETZKY, 7643 Pécs, P.O.Box 99, Hungary (biophysics)

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ABSTRACTS

22nd MEETING OF THE HUNGARIAN BIOCHEMICAL SOCIETY

25 - 28 August, 1982

DEBRECEN

Foreword

Some thirty years ago the Hungarian Academy of Sciences founded a number of periodicals /Acta/ to provide for the Hungarian scientists the possibility of publication in foreign languages. In the fifties and early sixties if somebody wanted to have a picture of the biochemical and biophysical investigations pursued in Hungary, he found practically all information in this Journal. Times changed and nowadays Hungarian scientists also publish their results in a wide variety of international journals.

It is the first time that the Editorial Board of Acta Biochimica et Biophysica, ASH agreed with the proposal of the Hungarian Biochemical Society to publish the abstracts of the 22nd Meeting of the Society. We think that an overview on what is going on in our fields will be of some interest to our colleagues abroad.

The 22nd Meeting, of course, does not cover all the lines of investigations pursued in Hungary. The contributions are grouped in three symposia and two colloquia of selected topics, and there are also poster presentations from various other fields. We deliberately mixed pure and applied biochemistry emphasising the importance of discussion and exchange of views among scientists working in different environments. We hope that the abstracts of our Meeting will provide a better insight into at least some of the current endeavours of Hungarian biochemists.

Gertrud Szabolcsi

Gertrud Szabolcsi
President
Hungarian Biochemical Society

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POSTERS

CHEMICAL DNA SYNTHESIS

A. Simoncsits, Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.Box 521

During the last few years, the increasing need of synthetic DNA pieces in genetic engineering has greatly stimulated the research in oligonucleotide chemistry. This review lecture summarizes briefly the history of chemical DNA synthesis from the mid-1950's to 1981. The basic principles of the phosphodiester, the phosphotriester and the phosphite--triester methods are discussed.

These approaches require protected monomer, dimer and trimer building blocks. The preparation of these building blocks from the commercially available deoxyribonucleosides or deoxyribonucleotides, and the properties of the most frequently used protecting groups is reviewed. General schemes for the synthesis of DNA pieces containing 10-15 nucleotides by both the liquid-phase and solid-phase methods, as well as the purification and the analysis of the final products are shown.

Among the large number of practical applications of the synthetic DNA pieces in recombinant DNA studies, some important examples /restriction linkers, primers, tools in gene isolation, alteration, etc./ are mentioned. As an alternative to the gene isolation from natural sources, total gene synthesis is also possible starting from single-stranded, chemically synthesized DNA fragments by using the enzymes T4 polynucleotide kinase and T4 DNA ligase.

THE MOBILITY OF THE GENOME. DNA REARRANGEMENT IN PHYLO- AND ONTOGENESIS

P. Venetianer, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521

DNA cloning and DNA sequencing techniques progressed so rapidly during the last decade that our views about the organization of the genome, and the flexibility of this organization have changed dramatically, Besides the classical homologous recombination and mutation, several new mechanisms of change and rearrangement became known. In this lecture some of these mechanisms and their possible biological roles will be discussed; for example: prokaryotic transposons and insertion elements, eukaryotic "jumping genes", retroviruses, cassette-type DNA rearrangements, exon-intron shuffling, rearrangement of immunoglobulin genes, etc.

GENE CLONING IN EUKARYOTIC CELLS

Zs. Fehér, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, H-6701, P.O.B. 521 and Department of Biology, Medical University of Debrecen, Debrecen, H-4012

The following topics will be discussed in this review:

1. Gene cloning in yeast
   a/ Properties of the yeast transformation system
   b/ Types of yeast cloning vectors
   c/ Construction of a gene library
   d/ Possible approaches of selection from a gene library
   e/ Recent results in yeast cloning field
2. Gene cloning in fungi /Neurospora/
   a/ Transformation of Neurospora
   b/ Cloning of the *ga* gene cluster
   c/ Possible vectors for cloning in Neurospora
3. Gene transfer into mammalian cells.

REGULATION OF GENE EXPRESSION IN BACTERIA

Cs. Kari, Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521

The various types of regulation of gene expression in bacteria are summarized briefly. The main types are the following: regulation at the level of transcription and translation; positive and negative control; regulation of initiation, elongation and termination of RNA and polypeptide synthesis; regulation by reversible and irreversible modification of RNA polymerase; control of endoenzyme and exoenzyme synthesis; operon-specific and general control of transcription and translation. Some problems, like structure of promoters and regulation, attenuation, translational control of ribosomal proteins synthesis, regulation of exoenzyme synthesis, etc. are discussed in detail.

REGULATION OF TRANSCRIPTION IN EUKARYOTES

I. Financsek, "Frédéric Joliot-Curie" National Research Institue for Radiobiology and Radiohygiene, H-1775 Budapest

In the regulation of transcription of the three RNA polymerase in eukaryotic cells, conserved DNA sequences, located at fixed distances from the initiation or termination points are to play an important role both in vivo and in vitro.

The ribosomal RNA genes are transcribed by RNA polymerase I. Common sequences were found around the 5' flanking region in the ribosomal genes of relative distant organisms, but their regulative role is still not clear. There are few data concerning the termination signal/s/ of these gene/s/.

The regulator sequences of RNA polymerase II, transcribing the mRNA precursors, seem to be better understood. The so called Goldberg-Hogness and CCAT boxes, located at fixed distances upstream from the initiation point, may not be the only sequences which influence the transcription. Distant regulator signals were found at 1000 base pairs distance or farther from the 5' end of the genes which can drastically alter the rate of transcription. The conserved regions at the termination points of these genes may provide information enough for the termination of the transcription.

The regulator sequences of the initiation of transcription for RNA polymerase III are located inside the gene /at least in the case of 5S RNA gene/ i.e. downstream from the starting point of the transcription. Not only these DNA sequences but also factors that bind to them are involved in the initiation process. Most probably only certain DNA sequences are necessary for the termination of transcription by RNA polymerase III.

NUCLEAR RIBONUCLEOPROTEINS /PRE-mRNP, snRNP/

J. Molnár, Institute of Biology, University Medical School Szeged, H-6720 Szeged, Somogyi-u 4.

Production of mRNA in eukaryotic cells requires several processing steps. These include polyadenylation of their 3′ terminus, formation of a cap structure at the 5′ terminal end and splicing. During the past decade the importance of mRNA processing has evolved. According to several recent reviews the processing of mRNA constitutes a major cellular activity and an integral part of the mechanism of gene expression.

According to electron microscopic and biochemical studies proteins associate with pre-mRNA while it is still being transcribed and the ribonucleoprotein organization of pre-mRNA in the cell nuclei represents a common feature of eukaryotic cells. Intact pre-mRNP particles are considered as polysome--like structures. The polyparticles consist of 30-40 S monomeric subparticles containing a residual stretch of pre-mRNA and a globular protein particle called informofer. The components of the pre-mRNP particles could possibly be involved in the processing of the pre-mRNA, not only as structural /or transport / elements, but taking part in several enzymatic activities as well.

Small nuclear ribonucleoproteins are complexes of small RNAs with proteins, found in eukaryotic cells. They fall into discrete classes, are abundant and are highly conserved across species. The partitioning of snRNPs in various nuclear fractions has been described. Of particular interest are the U1 species. Complementarity between 5′ terminal portion of the U1 RNA and concensus splice junction sequences has led to speculation that U1 RNA might serve to align the splice joints. The hypothetical implication of U1 RNA in the splicing reaction has received some support from experiments in which a lupus antiserum that precipitated RNPs containing U1 RNA was shown to inhibit the proper splicing of adenovirus mRNA.

CHEMICAL SYNTHESIS OF A STRUCTURAL GENE CODING FOR HUMAN VASOPRESSIN

I. Cserpán, Cs. Kari, A. Simoncsits, Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O. Box 521

A 38 base-pair long double-stranded DNA coding for the human peptide hormone Arg-vasopressin has been synthesized. The nucleotide sequence has been designed from the known amino acid sequence of the hormone applying the most favoured codon usage in Escherichia coli. In addition to the coding region, the synthetic gene contains initiation and termination signals as well as appropriate testriction enzyme sites /Eco RI and Bam HI/ at the two ends. Such a design makes it possible to attach the synthetic piece to a cloned gene possessing its own regulatory region and, after expression, to detach the desired oligopeptide from the fused protein by cyanogen bromide cleavage.

The double-stranded DNA was constructed from six oligonucleotides containing 12-14 nucleotide residues, which were synthesized mainly from dimer building blocks by the phosphotriester method of Narang et al. in liquid phase. The oligomers were constructed so that they suitably /5-7 nucleotide residues/ overlap the complementary strands at the ligation points, and the self-complementary sequences were avoided. The individual oligomers were phosphorylated with γ-^{32}P-ATP and T4 polynuclotide kinase. The 5'-phosphorylated oligomers were mixed, annealed and finally ligated with T4 DNA ligase to obtain the double-stranded DNA. The sequence of both strands of the final product were checked by a standard sequencing technique of Maxam and Gilbert, W.

DNA SEQUENCE ANALYSIS OF THE hsp70 HEAT SHOCK GENES IN DROSOPHILA MELANOGASTER

I. Török[1], P.J. Mason[2], F. Karch[2], I. Kiss[1], A. Udvardy[1]

[1]Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, P.O.B. 521, H-6701 Szeged,

[2]Department of Molecular Biology, University of Geneva, 30 quai Ernest-Ansermet, CH-1211 Geneva 4,

D. melanogaster hast two loci /87A7, 87C1/ containing sequences which code for the major 70.000 d heat shock protein /hsp70/. At 87A7 there are two genes in diverging orientation, whilst at 87C1 two tandemly repeated distal copies are separated from a single copy in divergent orientation by about 40 kb /kilobase/ of DNA. Within this 40 kb are found the heat induced genes, interspersed with elements.

We present the DNA sequence of a complete hsp70 gene of the 87A7 type and of the adjacent regions of the two variants from both loci. Using the DNA sequence we were able to deduce the amino acid sequence of the 87A7 variant type. Determination of the 5′ and 3′ end of the hsp70 mRNA in the DNA sequence allowed us to look for regulatory sequences /putative promoter sequence, TATAAATA, and capping site/ of the transcription upstream from the transcriptional initiation site.

Comparing the flanking regions in one locus with those from the other one reveals the presence of several blocks of homologous sequences. These external homologies could be the result of gene conversion or recombination and might help us to understand the evolution of the hsp70 genes.

MOLECULAR CLONING AND EXPRESSION IN E.COLI OF TWO MODIFICATION METHYLASE GENES OF BACILLUS SUBTILIS

A. Kiss, F. Baldauf, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521

Two modification methylase genes of Bacillus subtilis R were cloned in Escherichia coli by using a selection procedure which is based on the expression of these genes. Both genes code for DNA-methyltransferases which render the DNA of the cloning host E. Coli HB101 insensitive to the BspRI endonuclease. One of the cloned genes is part of the restriction-modification system of Bacillus subtilis R, the other one is associated with the lysogenizing phage SP The fragment carrying the SPβ -derived gene directs the synthesis in E. coli of also another methylase activity which protects the host DNA against HpaII and MspI cleavage. Indirect evidence suggests that the BsuRI, HpaII and MspI specific modification activities are encoded by the same gene. No cross-hybridization was detected either between the two B. subtilis genes or between these and the modification methylase gene of Bacillus sphaericus R which codes for an enzyme vith GGCC specificity.

SYNTHESIS OF SMALL MOLECULAR WEIGHT NUCLEAR RNAs OF LIVER WITH DIFFERENT PROLIFERATIVE CAPACITY. POSSIBLE ROLE OF URIDINE-RICH RNAs IN REGULATION OF RIBOSOMAL RNA SYNTHESIS

A. Fónagy, I. Financsek, E.J. Hidvégi, "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, H-1775 Budapest, P.O. Box 101

Synthesis of snRNAs was investigated in various growing stage of regenerating rat liver and Novikoff hepatoma. Proliferation was inhibited by ionizing radiation and cytostatic alkylating agents.

It has been found that 6-12h after partial hepatectomy synthesis of snRNAs was 5-10 times higher in regenerating liver than in control. In growing ascites tumor it decreased gradually, parallel with the declining rate of proliferation. On whole-body irradiation of rats the synthesis increased in liver, in regenerating liver and in hepatoma up to 18h. The most marked increase /7-10 times/ was found in the synthesis of 4.5S, 5S and 10S RNAs. In early stage of growth of hepatoma cells snRNA synthesis increased by 40-50% on treatment with cytostatic hexitol compounds /dibromo-dulcitol, dianhydro-galactitol/ which inhibited tumor growth by 30%. Synthesis of U-rich snRNA was enhanced to the greatest extent, which may be connected with the accelerated rate of rRNA processing after treatment.

DNA was isolated from nucleoli of hepatoma cells, fragmented with restricition enzymes, fractionated on gel, then purified snRNA species were hybridized to the DNA fragments. U_1, U_2, U_3 and 8S RNAs hybridized to three rDNA fragments of nucleolar DNA, while 4S, 4.5S and 5S RNAs did not show hybridization to the same DNA fragments. This suggest that the U-rich RNAs may bind to the ribosomal gene *in vivo*, and are involved in the regulation of synthesis and/or processing of rRNA.

THE"WATER HOLDING CAPACITY" OF THE CHROMATIN

M. Kellermayer, Department of Clinical Chemistry, University Medical School H-7624 Pécs

Gene regulation of eukaryotic cells involves changes in the hydration of chromatin, however, the role of water in this process is not understood. We are attempting to gain insight into the role of water and monovalent ions by studying nuclei isolated from lymphocytes. Nuclei are isolated from bovine thymus glands in a buffered sucrose solution /TSCM/ containing a detergent, Ca^{++}, and Mg^{++}. The water content of the pelleted nuclei was determined after centrifugation /g/ at forces ranging from 1,000 g to 150,000 g. Under these conditions it was found that the water content of the nuclei was constant /65%/ between 40,000 and 150,000 g. When the nuclei /isolated in the TSCM solution/ are pelleted at a low g force, then resuspended in a buffered solution containing 15 meq/l Na^+, K^+, sucrose, Ca^{++} and Mg^{++} /i.e. same osmolarity as the TSCM solution/, and then centrifuged at different speed, it is found that the percentage of water in the pelleted nuclei is increased by 6-8% /i.e., the "water holding capacity" of the nuclei is increased when monovalent ions Na^+ and K^+ are present/. When the nuclei are exposed to a buffered solution containing 75 meq/l Na^+ and K^+, the "water holding capacity" is increased by 18-20%. The relaxation times of the water protons in the nuclear pellets were found to be lower than those for pure water but proportional to the water content of the pellets. These results indicate that monovalent cations may be directly involved in the regulation of chromatin hydration.

COMPARISON OF THE PROPERTIES OF NUCLEAR AND POLYSOMAL POLY/A/--PROTEIN PARTICLES IN RAT LIVER

T. Tomcsányi[+], L. Komáromy[+], J. Molnár[++], A. Tigyi[+],

[+]Department of Biology, University Medical School Pécs, H-7643 Pécs, Szigeti-ut 12

[++] Institute of Biology, University Medical School Szeged, H-6720 Szeged, Somogyi B.-u 6.

Poly/A/-protein particles were prepared from rat liver nuclei and polyribosomes and their properties were compared. Interest is focussed on the "average" poly/A/-protein particles. Knowledge of the structures of these particles may be helpful in understanding the transport processes of mRNA. The properties of the "average" nuclear poly/A/-protein are as follows: Sedimentation coefficient: 16S; buoyant density in CsCl gradient: 1.30 g/cm^3; RNA: protein ratio 1:9; main polypeptide component: M_r 63 000; size of poly/A/:160 nucleotides; periodicity in poly/A/: no periodicity; location of poly /A/ chain in the particle: almost the entire poly/A/ is located on the surface of the particle; diameter of the particle: 13nm. The properties of the "average" polysomal poly/A/-protein particles are as follows: Sedimentation coefficient: 9S; buoyant density in CsCl gradient: 1.36 g/cm^3; RNA: protein ratio: 1:4, main polypeptide: M_r 76 000; size of poly/A/: 136 nucleotides; periodicity in poly/A/: repeating structure with a periodicity of 27 residues; location of poly/A/ chain in the particle: three repeating units are located on the surface, two inside the particle; diameter of the particle: 15nm. Our results shows that nuclear and polysomal poly/A/-protein particles have fundamentally different structures. They differ from each other in every respect, thus during the transport of mRNA from nucelus to the cytoplasm a complete structural reorganization takes place in its 3' end poly/A/-protein particle.

EXTRACTION OF INTRACISTERNAL A-PARTICLES FROM MOUSE EHRLICH ASCITES TUMOR CELLS

B. Bus[1], J. Molnár[1], D.A. Kramerov[2], O.P. Samarina[2]

Institut of Biology

[1] Medical University H-6720, Szeged, Somogyi B.-u 4.

[2] Institute of Molecular Biology, USSR Academy of Sciences, Moscow B-334, Vavilov str. 32. USSR

Ehrlich ascites tumor cells in BALB/c mice contain numerous intracisternal A-particles which are localized within microsomal vesicles when the tumor cells are disrupted by homogenization. Liberation of the particles has been achieved by subjecting microsome suspensions to mechanical shear in the presence of 0.5% of TRITON X-100. The particles were concentrated by sedimentation in sucrose solution and finally banded isopicnically in a sucrose density gradient. Most of the particles were recovered in a density range of 1.20-1.24 g/cm^3. This subcellular fraction was found to posses endogenous reverse transcriptase using its own RNA as template. A high mol. wt. poly/A/$^+$-containing RNA was also purified from this fraction which hybridized to cloned ds RNA sequences.

FINE STRUCTURE OF THE THERMAL DENATURATION PROFILE OF FILAMENTOUS FUNGUS NUCLEAR DNA

Á. Szécsi, A. Dobrovolszky, Research Institute for Plant Protection, Hungarian Academy of Sciences, H-1525 Budapest, P.O.Box 102

Numerical analysis of the thermal denaturation profiles of DNA is a useful tool for examining local fine-structural features or melting modes. The melting modes reflect the denaturation of a segment/or thermosome/ inside the DNA molecule. Thermal denaturation of Fusarium oxysporum nuclear DNA was performed by automatic and continuous increase of the temperature by 0.25°C/min; denaturation was recorded at 270 nm at full equilibrium /30-60°C/. The solvent used for the denaturation reactions contained 50% formamide, 0.015 M NaCl, 0.0015 M sodium citrate, pH 7.0. Changes in absorbance /270nm/ were recorded continuously and automatically with a HP 97-S calculator programmed for automatic sampling of data. Under such conditions, the melting curve /monitoring parameter vs temperature/ exhibits reproducibly fine structural features or melting modes. The numerical analysis of the obtained melting data /absorbance, temperature/ was performed according to the method of Gabárro and Nelder and Mead. The computing procedure was carried out on a HP 9845B desktop computer and a CDC 3300 computer. In this way ten significant peaks can be identified in the thermal denaturation profile of fungus nuclear DNA. Values for the parameters/T_m, Δ T,S/ of the most significant peaks were determined.

IDENTIFICATION OF TWO NEW PROMOTERS PROBABLY INVOLVED IN THE TRANSCRIPTION OF A RIBOSOMAL RNA GENE OF E. COLI

I. Boros, É. Csordás-Tóth, A. Kiss, I. Kiss, I. Török, A. Udvardy, K. Udvardy, P. Venetianer, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521

The DNA sequence in the region preceding the rrnB gene of E. coli was determined until the 1821st nucleotide upstream from the beginning of the sequence coding for mature is S rRNA. In vitro transcription experiments indicated the presence of two new promoters in this region, located more than 1 kb upstream from the known P_1 and P_2 promoters of rrnB. Previous electron microscopic studies demonstrated that these sites bind RNA-polymerase very strongly. In vitro transcription, starting at these sites reads through the entire region into the rrnB gene without termination. A similar uninterrupted transcription into rrnB can be demonstrated in vivo by fusing the DNA containing the new promoters /but not P_1 and P_2/ to the lacZ gene. Thus it seems likely that these promoters /P_3 and P_4/ belong functionally to the rrnB gene and play some role in its regulation of expression.

CONSTRUCTION OF A NEW PLASMID VECTOR FOR THE EXPRESSION OF INSERTED FOREIGN GENES

G. Szabó, S. Erdei, P. Venetianer, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521

We describe the construction of a new plasmid vector which has the following properties: 1. It contains several tandem copies of the strong promoter of the bacterial bla /beta-lactamase/ gene; 2. It contains the translational start codon and the preceding Shine-Dalgarno sequence of the same gene; 3. The coding sequence of the lacZ gene is fused to the above-mentioned signals, thus beta-galactosidase is synthesized under the control of the bla promoter.

Foreign genes can be inserted into three alternative sites of the vector DNA: 1. After the promoters, before the translational start ; 2. After the translational start, before lacZ; 3. Into lacZ.

This way genes can be expressed with a strong bacterial promoter, either with their own translational start, or with the bla translational start, or fused to the beta galactosidase protein. Beta galactosidase, an easily measurable enzyme serves as a quantitative indicator of expression of the inserted foreign gene.

A RECOMBINANT COSMID COMPLEMENTING ino4 MUTATION IN YEAST

Zs. Fehér, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, H-6701, P.O.B. 521; and Department of Biology, Medical University of Debrecen, Debrecen, H-4012

The INO1 gene is the structural gene for the inositol-1-phosphate synthase in yeast. The inositol requiring mutant ino4 is supposed to be a regulatory mutant in inositol biosynthesis. We made an attempt to select to INO4 gene from a yeast gene library constructed by A. Hinnen. This gene library comprises 3000 clones, each containing a fragment of the yeast genome inserted into the EcoRI site of the cosmid pYc1 [HIS3, Ap^R] . The yeast recipient his3, ino1-13, ino4-8 was transformed with the cosmid-pool isolated from the gene library. Recombinant cosmids were recovered from five slow-growing transformants. Each of these cosmids contained the same 10.6 kb EcoRI fragment and transformed ino4-8 with high frequency but not ino1-13. We were able to reisolate the transforming cosmids from the INO4 transformants. The transformants proved to be unstable when propagated on complete liquid medium /YPD/. The physical map of a recombinant cosmid containing only the 10.6 kb insert was established with 7 restriction enzymes.

On the basis of the data we suggest that the cloned 10.6 kb fragment contains the INO4 gene, although the possibility of supression remains to be excluded.

POLYMORPHISM OF THE RESTRICTION FRAGMENTS OF THE ALBUMIN AND ∝-FOETOPROTEIN GENES IN DIFFERENT INBRED STRAINS OF RATS

A. Gál[1], G. Lucotte[2], J.L. Nahon[2], J.M.Sala-Trepat[2]

[1] Inst. Genetics, Biol. Res. Ctr. H-6701 Szeged, P.O.B. 521

[2] Lab. Enzymology, CNRS, Gif sur-Yvette, 91190, France

To study genetic polymorphism at the DNA level the length and number of the restriction fragments of the serum albumin and ∝-foetoprotein /AFP/ genes were compared in different inbred strains of rat. DNA preparations from the liver were digested with EcoRI or HindIII and hybridized to highly labeled, cloned /^{32}P/cDNA albumin and AFP probes.

Seven different fragments containing albumin gene sequences can be identified in the Sprague-Dawley /SD/ rat DNA after EcoRI digestion, while only five were found in the Buffalo/B/ rat. Four of them were identical to those seen in the SD, but the fifth was present only in B. The albumin specific fragments generated by HindIII both in SD and B displayed 4 bands, three of which were common. In the SD x B F_1 hybrid, in both cases, all the bands expected were present. Using the physical map of the SD albumin gene a hypothesis is proposed to explain the mechanism of the transition of one form to the other with three point mutations.

Hybridization of the EcoRI digested DNA with the AFP probe yielded the same banding pattern in both strains. HindIII digestion resulted in 6 different fragments, 4 of them were present in both strains, while 2 were different. In the F_1 hybrid, again, all the 8 bands expected were present. Because the physical map of the AFP gene is not yet published one can make only predictions concerning the possible mechanisms for the transition of one form to the other. Several different strains of the laboratory rats and two transplantable rat hepatomas of B origin were characterized and grouped in this way. The results and their significance are discussed in the lecture.

RESTRICTION CLEAVAGE PATTERN OF THE MITOCHONDRIAL DNA OF A WILD-TYPE YEAST, S. cerevisiae

M. Mink, L. Ferenczy, Department of Microbiology, Attila József University, H-6701, Szeged P.O.B. 428

The mitochondrial DNA /mt DNA/ of S. cerevisiae RXII was analysed by the restriction enzymes Eco RI, Hind II and Bam HI and compared to the patterns and physical maps of mt DNAs of S. cerevisiae MH41-7B /Morimoto and Rabinowitz/ and KL14-4A /Sanders et al./.

In the Eco RI restriction cleavage pattern of mt DNA of RXII seven fragments appared and fragments 5, 9 and 10 were absent. The fragment order in this region for both MH41-7B and KL14-4A mt DNAs is 2-9-5-10 indicating that the mt DNA carrying the recognition sites of Eco RI on fragment 9 were deleted.

Bam HI cleavage resulted in the formation of four fragments instead of five. The second band had a molecular weight of 20 kbp. The order of Bam fragments of MH41-7B and KL14-4A mt DNAs is 3-2, the sum of the molecular weights of them is 28.7 kbp, indicating that the Bam HI site separating fragments 2 and 3 was eliminated and the size of the deletion is about 8-9 kbp. Hind II cleavage showed that fragments 3, 11 and 13 were absent; the fragment order of MH41-7B was in this region 3-13-11 1 and KL14-4A 3-11-1 /fragment 13 was not found/. The molecular weight of the first Hind II fragment of RXII was 19 kbp instead of 17.5 kbp and the sum of Hinc II fragments 3, 13, 11 and 1 of MH41-7B was 26.75 kbp. The difference was about 8 kbp showing that the Hind II sites on fragments 13 and 11 were deleted with the surrounding DNA.

RELEASE OF DNA AND CHANGES IN THE CELL CYCLE IN HUMAN TONSIL LYMPHOCYTE CULTURES

M. Sasvári-Székely, G.[x] Jr. Szabó, J. Taljanidisz, M. Staub, 1st Institute of Biochemistry, Semmelweis University, Medical School, H-1444 Budapest, 8, P.O. Box 260;

[x] Institute of Biophysics, University Medical School Debrecen, H-4012 Debrecen, Nagyerdei krt 98.

Spontaneous release of some DNA has been demonstrated in human tonsillar lymphocytes during culture. In the present work the possible precursor of released DNA and the connection of DNA excretion with the cell cycle were investigated.

We isolated and purified the labelled and released DNA from the culture medium on hidroxiapatite column. It seems to be extrachromosomal DNA as demonstrated by its mobility on agarose slab gel electrophoresis.

For the measurement of cellular DNA in individual cells we applied flow cytometric analysis and flow sorting of ^{3}H--thymidine labelled lymphocytes was also performed with a Becton-Dickinson FACS III system. These experiments revealed, that:

1./ Large cells /sorted according to their light scattering/ are preferentially labelled with ^{3}H-thymidine.

2./ Cells incorporate ^{3}H thymidine not only in S phase of the cell cycle but also in G_1 phase /sorted according to their DNA content, i.e. propidium iodide fluorescene/.

3./ The initially labelled, large cells return into the G_1 phase after 48 hrs of culture without completing the cell cycle.

The changes in the cell cycle during culture and the possible connection with release of DNA will be discussed.

THE ACTIVITIES OF DEOXYCYTIDINE KINASE AND THYMIDINE KINASE ARE DIFFERENT IN VARIOUS LYMPHOID ORGANS

Spasokukotskaja T., Taljanidisz J., Staub M., Sasvári-Székely M. Ist Institute of Biochemistry, Semmelweis University, Medical School, H-1444 Budapest, 8, P.O.Box 260

Autoradiographic studies of various lymphoid tissues have revealed that the distribution of label was different with ^{3}H-deoxycytidine /^{3}H-CdR/ and ^{3}H-deoxythymidine /^{3}H-TdR/ in lymphocytes derived from different organs, but the reasons are not clear yet. The present study was undertaken to examine the possible differences in the phosphorylation of these precursors in various lymphoid tissues. The results show that, the specific enzime activity of thymidine kinase /TK/ is higher in the spleen that in thymus or unseparated tonsillar lymphocytes, and it was the lowest in the T lymphocyte enriched fraction of tonsillar cells. An opposite distribution was obtained with deoxycytidine kinase /dCK/; its specific activity was the lowest in spleen, it was much higher in thymus and in unseparated tonsillar lymphocytes. dCK and TK activities in T cells compared to that in B cell enriched tonsillar lymphocyte fraction were always low. The ratio of dCK to TK was always high in thymus and in tonsillar lymphocytes /2-5/, but it was low in spleen /0.3-0.4/. The enzime activity of DNA polymerase was also measured in these sources, and there was practically no difference between spleen and thymus. The combined activities of DNA polymerase-α , and -β were also the lowest in the T lymphocyte enriched fraction of tonsillar cells. These results suggest that the metabolic pathways of CdR and TdR utilisation for DNA synthesis differ in the lymphocyte population derived from various lymphoid tissues.

REGULATORY EFFECT OF INOSITOL ON MYO-INOSITOL-1-PHOSPHATE SYNTHASE /MIPS/ IN NEUROSPORA CRASSA STRAINS

Á. Kiss, A. Zsindely, M. Schablik[a], M. Szabolcs[b], G. Szabó[a],

Departments of Biochemistry and Biology[a]

[b] Central Research Laboratory, University Medical School of Debrecen, Debrecen, H-4012 P.O.Box 6

We studied the synthesis of MIPS and the immunologically related, enzymatically inactive /silent/ protein as well as the factors affecting the action of the enzyme in Neurospora crassa strains. It has been found earlier that in the wild-type strain enzyme activity decreases parallel with the amount of the enzyme protein, which was determined by rocket immune electrophoresis, in the presence of increasing concentrations of inositol /12.5-100.0 /ug per ml/. Inositol does not affect the production of the "silent" protein in inositol--requiring strains.

Enzyme activity similarly decreased as a function of inositol concentration, whereas the amount of the antigen remained nearly unchanged in 22°C cultures of the thermosensitive mutant strain, which grows at 37°C in the presence of inositol only.

In the heterocaryotic strain, which shows a $50^{+}:50^{-}$ ratio of nuceli, enzyme activity was detectable at 100 /ug/ml inositol concentration yet, whereas of the two antigenic proteins the amount of one was independent of, that of the other protein was reduced by the increased concentration of inositol.

On the basis of our results we assume that inositol or its derivatives inhibit the synthesis of MIPS in the wild-type-strain /regulation of gene functions/. Results obtained with the thermosensitive mutant, however, suggest that regulation on the molecular level is also possible, i.e. the assembly of the precursors to form an active /tetramer/ enzyme may also be influenced by inositol.

IMPAIRMENT OF t-RNA AMINOACYLATION IN BRAIN OF MOUSE IRRADIATED WITH FISSION NEUTRONS IN UTERO

J. Holland, L. Kőrösi, E.B. Bölöni, A. Fónagy, S. Antal, E.J. Hidvégi, "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, H-1775 Budapest. P.O.Box 101

It has been found that exposition of mice to fission neutrons /0.5 Gy neutron + 0.2 Gy gamma/ on days 17-19 of pregnancy resulted in significant loss of whole-body and brain weight by week three after birth. Biochemical investigations showed no change in the labelled precursor incorporation into DNA and RNA of brain and liver. There was, however, 10% decrease of ^{14}C-amino acid incorporation into total brain proteins and 40% into acid soluble nuclear proteins, especially into histones. The mechanism of this specific alteration was investigated in cell free protein synthesizing system. Incorporation of a mixture of ^{14}C-amino acids into proteins of microsomes and mitochondria of irradiated brain and liver did not show any difference as compared to the controls. Nevertheless, the formation of aminoacyl-tRNAs decreased markedly in brain, e.g.: more than 60% in t-RNAs of valine, phenylalanine, arginine and lysin. Aminoacylation ability decreased only slightly in liver. Various combinations of microsomes and adaptor systems /pH 5 fractions/ proved clearly that the impair of aminoacylation ability of brain caused the decrease in protein synthesizing capacity measured in vitro and may be responsible for the disturbance developed in brain in vivo as well.

THE EFFECT OF MICROWAVE IRRADIATION ON THE tRNA-SYNTHETASE SYSTEM OF THE CHICK EMBRYO BRAIN AND LIVER

E. B. Bölöni, L. Ballay, L.D. Szabó, "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, H-1775 Budapest, P.O.Box 101

The biological effect of microwave irradiation on embryonic organism of higher radiosensitivity was studied. White Leghorn chick embryos were incubated at $37.5 \pm 0.5^{\circ}C$ at a relative moisture of 70 per cent. Continuous irradiation /CW/ was brought about with a power density range between 100--300 mW/cm^2 and a frequency of 2450 ± 50 MHz. We have found that embryos irradiated at different days of the embryogenesis /between the 2nd and 19th day of incubation/ with various power density ranges showed differences both in their survival and characteristics of the development as well as in their radiosensitivity. We have found that the radiosensitivity increases reciprocally with the age of embryos.

Effect of the microwave irradiation on the embryonic protein synthesis was investigated with a specific absorption of 220 mW/g at the 15th day of embryonic life. In order to compare some macromolecules taking part in protein synthesis tRNAs and aminoacyl tRNA synthetases were isolated from the brain and the liver of chicken embryos irradiated at the 15th day. tRNAs' acceptor activity and enzyme activity of synthetases showed a small decrease in liver, and about 50 per cent increase in brain. The activity of tRNA seems to be more sensitive than that of the synthetases.

EFFECT OF PNDP ON THE MINOR NUCLEOTIDE CONTENT OF FUSARIUM OXOSPORUM

E.Jakucs, I.Rácz[1], D. Lásztity[1], Department of Microbiology and [1]Department of Plant Physiology of Eötvös L. University Budapest, P.O.Box 324

Although a number of rRNA primary sequences have been determined, little information is avaible on the modified nucleotides which are formed during the post transcriptional maturation of rRNAs.

The minor nucleotide constituents of rRNA from the plant pathogenic fungus, Fusarium oxysporum, were investigated. When the fungus was grown for 8 days in aerated liquid culture the following minor nucleotides were identified from 18S and 25S rRNA: m^6A, m^2A. m^2_6A, m^1G, m^2G, m^2_2G, m^3C, Cm, m^5C, m^3U, Um, cm^5U, rT and Ψ .

RNA was extracted by the phenol method of Kirby, separated by Sepharose 4B chromatography and hydrolysates were analysed by Dowex anion exchange and thin layer chromatography.

PNDP /$\pm$ treo-1-phenyl-2 nitro-1,3-diacetoxy propane/, a new fungicide of unknown biochemical mode of action, caused a great decrease in the minor nucleotide content of the fungus. Only seven types of minor nucleotides were present in the rRNA of fungus grown in 0.08 mg/l PNDP containing medium. A hypermodified nucleotide, mcm^5S^2U, was found in treated, but not in untreated Fusarium.

On comparing the effect of PNDP with that of known protein synthesis inhibitors such as cycloheximide and chloramphenicol, it can be concluded that PNDP acts similarly to cycloheximide, a cytoplasmic protein synthesis inhibitor. The authors suggest that the mode of action of PNDP may be the inhibition of the synthesis of RNA modifying enzymes of Fusarium oxysporum.

Tn7 TRANSPOSON MUTAGENESIS OF A LARGE REGION OF THE pSYM MEGAPLASMID OF RHIZOBIUM MELILOTI 2011

I. Duska, J.S. Julliot[x], Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.Box 521

[x] INRA BP12 31320 Castanet France

A single megaplasmid /MW 400 Mdal/ is common to R.meliloti. Genes controlling symbiotic properties /structural mi f D,H genes; nodule formation/ are located on these megaplasmids /pSym/. For a genetic study of pSym of R. meliloti 2011, we devised a method to mutagenize this plasmid.

Introduction into E. coli of RP4-prime plasmids carrying large segments of pSym: An in vitro constructed RP4-prime carrying a 5Kb DNA fragment of pSym was introduced into this strain. By superinfecting with plasmid pPH1 /in the same IncP1 group as RP4/, we selected clones in which RP4-prime was integrated into pSym by recombination at their homologous DNAs. Such clones were mated with a recA E. coli K12 strain producing transconjugants containing deleted cointegrates composed of RP4 and part of pSym.

Tn7 transposon mutagenesis of a 250Kb region of pSym: A Mu prophage was inserted into the deleted cointegrate pGMI42 to produce a suicide plasmid which was then mutagenized in E. coli with Tn7. The resulting plasmids were transmitted to R. meliloti 2011. The clones selected for Tn7 transfer, which appear at high frequency/10^{-3}/, do not contain any pGMI42::Mu::Tn7 plasmids, but harbour pSym::Tn7 resulting from homologous recombination between pSym and pGMI42::mu::Tn7. Among the Tn7 insertion mutants, 1.5% had symbiotically defective phenotype on the host alfalfa.

DETERMINATION OF UV-INDUCED PHOTOPRODUCTS IN NUCLEIC ACIDS AND NUCELOPROTEIDS BY SPECTROSCOPIC METHODS

A. Fekete, I. Sugár, Gy. Rontó, I. Földvári[x], Biophysical Institute, Semmelweis Medical University, H-1444 Budapest, P.O.B. 263,

[x] Research Laboratory for Crystal Physics, Hungarian Academy of Sciences, H-1502 Budapest, P.O.B. 132

A large number of UV-induced photoproducts have already been identified in nucleic acids and nucleoproteids, but only a few of them could be produced under "in situ" circumstances in considerable amounts. These main photoproducts are responsible for the biological effect of UV light /254nm/ on the mentioned systems.

The aim of our work was to find a selective and non-disruptive method for the quantitative determination of photoproducts which would be sensitive for biologically interisting low doses as well / $<$ 1 kJm^{-2}/. The characteristic changes in the absorption and luminescence spectra due to photoreactions were used to solve this problem. Various methods as vacuum ultraviolet absorption spectroscopy /VUV/,UV difference spectroscopy /UV diff/ and fluorescence emission spectroscopy /fluo/ in different wavelength ranges were found to be optimal for detecting the possible photoproducts:

pirimidine dimers	260 nm/180-190 nm	UV diff/VUV
pirimidine adducts	310 nm/410 nm	UV diff/fluo
pirimidine hydrates	260 nm	UV diff
DNA-protein cross-links	260 nm	UV diff
RNA-protein cross-links	225 nm	UV diff

The spectroscopic data obtained were used to construct dose--effect curves and to develop kinetic models for all important photoproducts.

INVESTIGATION OF NUCLEIC ACID-PROTEIN COMPLEXES BY MELTING TECHNIQUES

K. Tóth, J. Fidy, Gy. Rontó, Biophysical Institute, Semmelweis Medical University, H-1444 Budapest, P.O.B. 263

The structure of the nucleoproteid phage T7 has been studied in our laboratory. Several phase transitions have been identified by microcalorimetry in the temperature range of 25°C - 100°C. Recently we studied the effect of temperature on other physical properties, as UV absorption, CD, fluorescence, light scattering and small angle X-ray scattering. These methods allowed us to characterize the phase transitions by the changes of some structural parameters as superhelical packing of the intraphage DNA, number of H-bonded base pairs, hydrophobicity of the phage-proteins, stacking of nucleic acid bases, size of the bacteriophage. The effect of ionic strength and environment on the phase transition temperatures was also studied. The method was found to be sensitive to slight modifications of the nucleio-proteid structure caused by UV irradiation or addition of some therapeutic drugs.

EFFECT OF LIGHT ON THE $tRNA^{Val}$ ISOACCEPTOR SPECTRA OF WHEAT GERM SHOOT AND ROOT

I. Király, L. Tamás, I. Rácz, D. Lásztity, Department of Plant Physiology, Eötvös University H-1445 Budapest 8. P.O.Box 324

Crude tRNA was prepared from 7-days old wheat seedlings cultivated in different illumination conditions /etiolation, 7, 9, 12, 24, 48 hours and 4 days of illumination/ by the modified phenol method of Kirby.

tRNAS were separated by RPC-5 chromatography then tested for valine acceptor activity. On the basis of changes of the isoacceptor spectra we concluded that

1. The starting point of the light-induction effect may be at the 7th hour of illumination, thus a direct role of photochromes seems to be unlikely.
2. In addition to the pronounced changes of the chloroplast originated tRNAs, the cytoplasmic $tRNA^{VAL}$ pool undergo a demonstrable rearrangement during the greening process.
3. No light induced changes of $tRNA^{VAL}$ spectrum occur in the root.

THE SIGNIFICANCE OF ARACHIDONIC ACID METABOLISM IN THE PHARMACEUTICAL RESEARCH

G. Kovács, Chinoin Pharmaceutical and Chemical Works Ltd.
H-1325 Budapest, P.O.Box 110

Since the late sixties the international pharmaceutical research has been increasingly interested in the metabolism of arachidonic acid /AA/. Among the endogenous metabolites there are drugs in commercial use /e.g. $PGF_{2\alpha}$ or PGE_2/ or on clinical trials /e.g. PGI_2/. Widespread research is going on for analogues with better selectivity and/or longer biological action all over the world. This type of research is demonstrated by the example of the 7-oxo-PGI_2, an original prostacyclin-analogue developed by Chinoin.

Another field of pharmaceutical interest is that of compounds influencing the enzyme systems of AA-metabolism. Among them are molecules with inhibitors and stimulators of phospholipases, cyclooxygenases, peroxydases, PGI_2- and TXA_2--synthetases. The paper is dealing with the most potent compounds described so far and their utilization in the research of the mechanism inflammation and haemostasis.

Special emphasis is plaid on inhibitors of the newly recognized lipoxygenase system with regard to their possible use in the therapy of allergic disorders and inflammation.

TURNOVER OF LIPID-BOUND ARACHIDONATE AND CAPACITY FOR PROSTANOID SYNTHESIS IN THE SHELL GLAND MUSCLE /MYOMETRIUM/ AND ITS GLANDULAR LINING /ENDOMETRIUM/ OF THE LAYING HEN

M. Tóth, G. Asbóth, A. Heiczman, J. Havel, A. Machovitsch
1st Institute of Biochemistry, Semmelweis University of Medicine, H-1444 Budapest 8. P.O.Box 260

Turnover of lipid-bound arachidonate/A/ was estimated from the rate of incorporation of /^{3}H/A *in vitro* into phospholipids /PL/, triacylglycerols /G/ and cholesteryl esters /ChE/ of minced endometrium and myometrium. The main lipid classes were separated with 1D and the various PLs with 2D TLC. Significant amounts of /^{3}H/A were incorporated into the PL and TG fractions while labeling of the ChE fraction was marginal. The labeling of TG fraction did not show tissue-specific differences. However, the endometrium incorporated more than double as much /^{3}H/A into PL as did the myometrium. PSerine and PInositol were labeled significantly faster in both tissues than PCholine and PEthanolamine. Furthermore the tissue difference of specific activities /expressed as cpm/$_/$umol PL/ was 2.4-2.8-fold for PS and PI and 1.4-fold in the case of PC and PE. - Prostanoid synthesis from /^{3}H/A was measured *in vitro* using crude microsomes as enzyme preparation and adrenaline and glutathione as cofactors. Prostanoid synthesis was followed with radio-TLC and specific prostanoid synthesis catalyzed by endometrial and myometrial membranes was compared by means of double reciprocal plots of TxB_2, PGE_2 and $PGF_{2\alpha}$ -synthesizing activities. K_m values did not show tissue specific differences but the V_{max} values revealed a significantly higher prostanoid-synthesizing capacity for myometrial membranes, first of all in the case of PGE_2 where the difference was about 2.5-fold. The finding presented may bear significance in the biochemical mechanism of oviposition.

PROSTANOID SYNTHESIS BY MICROSOMES PREPARED FROM AVIAN UTERUS

G. Asbóth, M. Tóth, 1st Institute of Biochemistry, Semmelweis University of Medicine, H-1444 Budapest 8. P.O. Box 260

To study prostanoid synthesis in the avian uterus /^{3}H/ arachidonate /A/ was incubated with the microsomal fraction of myometrium /M/ and the glandular lining /E/ of the uterus /shell gland/ of laying hens. The products were separated on TLC and identified using four different solvent systems and authentic prostanoids as carriers and markers. The presence of EDTA /1 mM/ in the media both during the preparation of microsomes and incubation was essential. Baseline activity obtained in 0.1 M Tris-HCl /pH = 7.4/, 1 mM EDTA was relatively low, but could be stimulated about 6-fold with 1 mM adrenaline. Under such conditions three stable products of the A cascade were found to be labeled /as % of labeled prostanoids/: TxB_2 /72-83%/ PGE_2 /9-20%/ and $PGF_{2\alpha}$ /5-13%/. This pattern was similar when either E or M was the source of microsomes and was similar at various A concentrations /36 nM - 512 /uM/. M microsomes exhibited 1.6-2.1 fold higher specific activity. Addition of indomethacin /1 /ug/ml/ abolished the conversion of A in both preparations. Glutathione--SH /1 mM/ inhibited the synthesis of all prostanoids, particularly that of TxB_2. Lower concentrations of this cofactor /0.1 mM/ had no effect on TxB_2 synthesis, while PGE_2 production was selectively stimulated 3-5-fold. Various reducing agents /mercaptoethanol, dithiothreitol, dithionit and $SnCl_2$/ were inhibitory. Oxidized glutathions /1 mM/ stimulated PGE_2 synthesis to the same extent as did glutathione-SH /0.1 mM/, indicating that the free SH group may not be involved in this mechanism. These studies demonstrate that TxB_2 is a major metabolite of A in the avian uterus.

POTENTIATION OF ANTIAGGREGATORY ACTIVITY OF PGL_2 BY INHIBITORS OF PHOSPHODIESTERASE IN RABBIT PLATELETS IN VITRO

I. Stadler, Chinoin Pharmaceutical and Chemical Works Ltd. H-1325 Budapest, P.O. Box 110

Antiaggregatory activity of PGI_2, papaverine and phosphodiesterase inhibiting isoquinoline derivatives was tested using rabbit platelats in vitro. 1-/ ∝ -cyano- ∝ -/3-hydroxypropyl-mercapto/ /-methyliden-6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline /P-737/, 1-/ ∝ -cyano- ∝ -/carboxymethyl--mercapto/ /-methyliden-6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline /P-641/ and 1-/3,4-diethoxybenzyliden/-6,7-diethoxy-1,2,3,4-tetrahydro-isoquinoline /$NOSPA^R$/ inhibited platelet aggregation induced by ADP, as did PGI_2, 7-oxo-PGI_2, and papaverine, ID_{50} values of these compounds were: 10 nM PGI_2, 800 nM 7-oxo-PGI_2, 213 /uM papaverine, 147 /uM P-737, 437 /uM P-641, 802 /uM $NOSPA^R$. 1-/ ∝ -cyano- ∝ -/2-carboxy--ethyl-mercapto/ /-methyliden-6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline /P-683/ showed only a slight antiaggregatory effect. When these compounds were used in combination with PGI_2 they potentiated the antiaggregatory effect of PGI_2 and ID_{50} of PGI_2 was decreased in a dose-dependent manner, whereas no potentiating effect could be observed in hemodynamic parameters. Similar effect could be observed on the platelet-3 factor aviability test. The potentiating effect was accompanied with an accumulation of cAMP in the platelets. These effects might be explained by an inhibition of phosphodiesterase and stimulation of adenyl-cyclase by PGI_2. The potentiation effect was studied in "ex vivo" experiments, too. The potentiating activity of P-737 was twofold greater than that of papaverine and for P-641 it was equal to that of papaverine. P-683 and $NOSPA^R$ showed a smaller effect.

EFFECT OF PGI_2 ON THE PHYSICAL STRUCTURE OF SERUM LOW DENSITY LIPOPROTEIDS; A DIFFERENTIAL SCANNING CALORIMETRIC STUDY

S. Virág[1], M. Bihari-Varga[2], É. Gruber[2]

[1] Chinoin Pharm. Ltd., Budapest, Hungary, H-1045 Tó-u 1-5

[2] 2nd Dept. of Pathology of Medical University, H-1091, Budapest, Üllői-u 93

The physical structure /liquid state versus liquid crystalline state/ of cholesterol esters in the LDL molecule seems to be one of the important factors in atherogenesis according to our recent knowledge. The "rigid" liquid-crystalline state of cholesterol esters in the LDL molecule might alter the mobility, rheological properties and transportability of LDL, resulting in a higher deposition rate of cholesterol into the arterial wall.

Recently prostacyclin has been connected with atherogenesis on the basis of thrombogenic theory. Taking into consideration the wide spectrum of biological activities of PGI_2 it was reasonable to study the influence of PGI_2 on the physical properties of LDL cholesterol esters.

Using DSC as basic method for investigation we have studied in vitro and in vivo the influence of PGI_2 and of a more stable analogue /7-oxo-PGI_2/ on the physical behaviour of cholesterol esters in low density lipoproteins. Result show that PGI_2 and 7-oxo-PGI_2 exert a dose-dependent beneficial effect on the physical properties of cholesterol esters in LDL molecule, by promoting their re-arrangement from liquid-crystalline state to liquid state.

PREPARATION OF PROSTAGLANDIN CONTAINING LIPOSOMES /PROSTASOMES/. CHROMATOGRAPHIC ANALYSIS OF STRUCTURE

J. Szebeni, J.H. Breuer[1], S.R. Hollán, V.V. Bezuglov[2], L.D. Bergelson[2], National Institute of Haematology and Blood Transfusion, H-1502 Budapest, P.O.Box 44, [1]Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, P.O.Box 67, [2]Shemyakin Institure of Bioorganic Chemistry, 117988 Moscow, V-334, USSR

Encapsulation of prostaglandins /PGs/ or their derivatives within liposomes /phospholipid vesicles/ or their insertion into the membrane surface may have the following advantages: a model system for the study of PG-cell membrane interactions can be obtained: modulation of the therapeutic action of PGs may be achieved: targeting of liposomes to cells possessing PG-receptors could be accomplished. In our experiments we have incorporated a novel fluorinated PG-derivative, 15-F-$PG_{F2\alpha}$ in egg yolk lecithin liposomes either by cosonicating it with lecithin or by incubating it with sonicated liposomes. The separation of free PG from PG-containing liposomes /prostasomes/ was made by column chromatography on Sephadex G-50 and Sepharose 6-B. By means of double label techniques /labeling PG with ^{3}H and lecithin with ^{14}C/ quantitative comparison of the elution profiles of prostasomes on the different columns could be made and thus the molecular weights and molar compositions of prostasomes could be roughly estimated. Our results indicated that - independently of preparation method - two types of prostasomes have been produced; small prostasomes with high PG content and large prostasomes with low one. The PG/lecithin molar ratios and molecular weights of small and large prostasomes were: 0.13; $6x10^3 < < MW < 4x10^6$; 0.0013; $MW > 6x10^5$, respectively /n = 3-4/. It is suggested that PG molecules become mostly membrane inserted, and two types of PG-lipid interaction play role in the association process.

SOME NEWLY RECOGNIZED CORRELATION OF STEROID HORMONE ACTION, TISSUE LEVEL OF HORMONES AND THEIR BIOSYNTHETIC PROCESSES

T. Fehér, First Department of Medicine, Semmelweis University Medical School, H-1083 Budapest, Korányi Sándor-u 2/A

Recent studies have revealed the high complexity of the interactions of steroid hormones with their receptors. Hormone action and its changes are profoundly influenced, beside the concentration of the hormone in the circulation, by the markedly different level of the steroid hormone in the target tissue, the receptor concentration and by the local transformation of the hormone molecules. As an example, the relations of accumulation, in situ transformation and of some newly recognized effects of steroids in the brain are mentioned.

On the other hand, accumulation of steroids by certain tissues /organs/ may result in changes of molecular processes of steroid metabolism in hormone producing organs. In this respect, the extremely large uptake and structure dependent storage of steroids by the adipose tissue, and the effect of this phenomenon on biosynthetic processes in the adrenal gland seem to be noteworthy.

The hormone dependency of certain tumors is often related to the concentration of their receptors. However, new data suggest that the actual hormone concentration of tumor tissue and that of the surrounding tissues /the "milieu exterieur"/ also influence the tumorogenic processes. As an example of this phenomenon, characteristics of mammary tumors will be discussed.

SEXUAL STEROIDS AND LIVER DAMAGE

J. Gergely, Institute of Pharmacology, University Medical School, Debrecen, H-4012, Nagyerdei krt. 98

Chronic liver damage was induced in rats by CCl_4 treatment. The liver injury was increased by estrone as shown by impaired hexobarbital biotransformation, pathological BSP test value, increased liver weight due to high water content, decrease of protein and glycogen content and loss of microsomal cytochrome P 450. The same parameters demonstrated a protective effect of ovariectomy. This was probably due to the absence of estrogens since progesterone moderated the liver damage. Testosterone exerted a protective effect and inhibited the loss of proteins, which is significant in CCl_4 injury. The beneficial effect of testosterone presented itself after the development of hormonal castration. These results led us to investigate the hepatoprotective hormonal factors. Steroids of strong antiestrogenic and progestogenic action were found to exert palliative effects. Methylestrenolone, a progestogenic and anabolic steroid diminished protein and glycogen loss due to CCl_4 treatment. The most pronounced hepatoprotective effect was exerted by norethindrone, a strong antiestrogen, which prevented the increase of water content and the loss of proteins, cytochrome P 450 and glycogen. Lipid degeneration was demonstrated histologically. The treatment did not cause gain of body weight. Oral administration of the drug was effective. The possible application of norethindrone is considered.

METABOLISM OF ANDROGEN STEROIDS IN DERMAL TISSUE FROM HEALTHY SUBJECTS AND WOMEN WITH HIRSUTISM

I. Faredin, I. Tóth, Endrocrine Unit and Research Laboratory, First Department of Medicine, University Medical School, H-6701 Szeged, Korányi S. fasor 8

Under in vitro condititons, abdominal skin slices from healthy men and women intensively metabolize [4-^{14}C] dehydro epiandrosterone, [4-^{14}C]-5-androstene-3β, 17β-diol and [4-^{14}C] 4-androstene-3,17-dione. The metabolism follows two main pathways in human skin: one of these pathways leads to the biosynthesis of steroids with high androgenic activity /androsterone, testosterone, 5α-dihydrotestosterone/, and the other pathway to inactive derivatives /17-ketosteroids, steroid sulphates/. The skin of healthy men and women is able to transform [4-^{14}C] testosterone to 5α-dihydrotestosterone. All of the more important biochemical processes of the androgen metabolism take place in healthy human skin and Δ^5-3β-hydroxysteroid-dehydrogenase /Δ^5-3β-HSD/, 3α-HSD, 3β-HSD, 17β-HSD, 7α-hydroxylase, 7β-hydroxylase, Δ^5-3β-hydroxy-sulphokinase and Δ^4-5α-reductase are of particular importance in these processes.

In the skin of hirsute women suffering from adrenogenital syndrome, the activities of Δ^5-3β-HSD, 3α-HSD and 17β-HSD are enhanced. On the basis of the results, it is assumed that, besides the pathologically high blood androgen level, the hyperactivities of the enzymes demonstrated in the abdominal skin tissue also play a role in the development of hirsutism.

In idiopathic hirsutism, where the growth of hair all over the body could not be correlated with clinical, urine or blood androgen examinations, the hyperactivities of Δ^5-3β-HSD, 3α-HSD, 17β-HSD and Δ^4-5α-reductase could be demonstrated in the hairy abdominal skin of the patients. It is presumed that these hyperactive enzymes are involved in the production of hyperandrogenism in the skin of women with idiopathic hirsutism.

CONCENTRATIONS OF ANDROGENS AND C_{19}-STEROID SULPHATES IN ABDOMINAL SKIN TISSUE OF HEALTHY SUBJECTS AND WOMEN WITH HIRSUTISM

I. Tóth, I. Faredin, Endocrine Unit and Research Laboratory, First Department of Medicine, University Medical School, H-6701 Szeged, Korányi S. fasor 8.

In the abdominal skin tissue of healthy men and women, the highest concentration was that of dehydroepiandrosterone /DHA/, followed in decreasing order by androsterone /And/, 4-androstene-3,17-dione /4-ene-dione/, 5-androstene-3β , 17β --diol, /5-ene-diol/, testosterone /Test/ and 5α -dihydrotestosterone /DHT/. There are more Test and DHT in the skin of men.

As regards the sulphates, the concentration of And-S is highest in the skin of healthy women, while that of DHA-S is highest in men, followed in decreasing order by 5-ene-diol--3-S and Test-S. The concentrations of DHA-s and 5-ene-diol--3-S are much higher in the abdominal skin of men than of women.

In adrenogenital syndrome, the concentrations of all the free androgens are pathologically high, while from among the C_{19}-steroid sulphate esters, the levels of DHA-S and And-S are pathological only.

In idiopathic hirsutism, primarily the concentrations of the highly androgen-active 4-ene-dione, And and Test are pathologically elevated, while the other steroids are either normal or pathologically high. Of the C_{19}-steroid sulphates, DHA-S and And-S occur in enormously large amounts, whereas the other two steroid sulphates give variable results.

The hyperandrogenism assumed in the dermal tissue of patients with hirsutism is confirmed by androgen steroid analysis of the skin tissues.

DYNAMICS OF ADRENAL STEROIDOGENESIS DURING CHILDHOOD AND PUBERTY URINARY METABOLITES OF STEROID INTERMEDIATES AND STEROID HORMONES

L. Kecskés, Central Laboratory, Medical University Pécs, P.O.Box 99

The ontogenetic development of the zona reticularis of the human adren al cortex is completed at the puberty [Dohm, G., Beitr. Path. 150 357 /1973/]. Its development can be followed by the increasing value of $C_{19}O_2$ steroids in the urine. The activity of zona fasciculata and reticularis was inferred from the urinary metabolites of cortisol determined as $C_{21}O_5$ corticoid group and urinary metabolites of steroid interme diates and adrenal androgens determined as $C_{19}C_{21}O_{2-3}$ steroid spectrum by gas chromatography. 34 urinary samples from 29 girls aged 2-14 years were studied. The excretion of different steroids and steroid groups was related to body weights. Before 7 years of age the value of pregnenediol excretion was $3.3^{\pm}1.8$ /ug/kg/day and during the puberty it was elevated to $9.0^{+}6.1$ /ug/kg/day, indicating the increase of pregnenolone pool in the organism. The excretion of pregnenediol did not increase until the last stage of puberty. The increase of pregnenolone pool could neither be attributed to a phenomenon of gonadarche since the rise of pregnenediol excretion started before the puberty, nor to the diminished activity of pregnenolone-16α-hydroxylase as the latter did not seem to play significant role according to our present investigations. The increasing pregnenediol excretion in puberty might be connected with the development of zona reticularis, although the effect of other regulatory factors of the activity of 3β-hydroxy steroid-dehydrogenase cannot be excluded.

KINETIC STUDIES ON THE STEROID HORMONE RECEPTOR INTERACTION

P. Arányi, 2nd Institute of Biochemistry, Semmelweis University Medical School, H-1444 Budapest, P.O.Box 262

In vitro saturation and competition analyses have been used in the past decade in many laboratories to define the molecular requirements for a highly specific interaction between the steroid and its cytoplasmic receptor protein as well as to screen for potent drugs among new analogues. Since in these systems exceedingly long incubation times, were required to reach equilibrium, many of the published results might be inaccurate because of lack of equilibration.

The error may be very large i.e. orders of magnitude and the values obtained in competition tests may be either larger or smaller than the true constants, depending on the relative rates of dissociation of tracer and competitor steroids from their respective complexes. To avoid this it is suggested that the rate constants of the formation and decomposition of the steroid-receptor complexes should be determined rather than the "equilibrium" dissociation constants. Methods of determination of these constants for nonlabeled ligands have been developed in our laboratory. Results of kinetic studies of this kind have been used to determine the structural requirements of complex formation between steroids and the glucocorticoid receptor protein. The methods have also been used for screening, and novel analogues displaying high affinity for glucocorticoid receptors and eliciting glucocorticoid response in target tissues have been found.

HEAT TREATMENT OF THE GLUCOCORTICOID RECEPTOR AND ITS COMPLEXES

K. Tóth, P. Arányi, 2nd Institute of Biochemistry, Semmelweis University Medical School, H-1444 Budapest, P.O.Box 262

Heat stability of the glucocorticoid-receptor protein was investigated in the $0^{\circ}C$ - $44^{\circ}C$ range. Denaturation of the receptor followed first order mechanism. It was preceded by an initial rise of binding activity at low or intermediate temperatures /$0^{\circ}C$ - $27^{\circ}C$/. Arrhenius plot of denaturation gave a straight line revealing that the denaturation mechanism did not change in the temperature range under study.

Heat treatment /e.g. $25^{\circ}C$, 1 hr/ of the steroid-receptor complex resulted in the so called "activation" of the complex. The term means a conformational change. The latter was reflected also by a decreased rate of dissociation. Dependence of this process on the steroid structure was studied. It was found that preformed steroid - glucocorticoid receptor complexes did not change the character of their dissociation upon heat treatment /$25^{\circ}C$, 1 hr/ unless the steroid belonged to the pregnane series and had an 11- -OH substituent. This structure is a known prerequisite of glucocorticoid action. Dissociation of the hydrocortisone- or corticosterone - receptor complex became biphasic upon heat treatment, the second phase of which was characterized by an exceedingly slow dissociation rate. We conclude that the 11- β -OH group is required for the activation of the complex.

CHARACTERIZATION OF THE CYTOPLASMIC ANDROGEN RECEPTOR OF RAT SEMINAL VESICLE

T. Zakár, First Institute of Biochemistry, Semmelweis University of Medicine,H-1444 Budapest 8. P.O.Box 260

Postmitochondrial supernatant /PMS/ prepared from homogenized seminal vesicles of castrated rats contains specific binding sites for androgens. These binding sites, corresponding to the androgen receptor of this accessory sexual gland, have been characterized in several respects.

The high affinity binding of ^{3}H-dihydrotestosterone /^{3}H-DHT/ and ^{3}H-testoster one /^{3}H-T/ to the vesicular androgen receptor was demonstrated by saturation analysis /Scatchard/. The rate constants of association of the two androgens with the receptor were found to be identical, while the rate of dissociation of the T-receptor complex was about three times higher than that of the DHT-receptor complex. When incubated at 0^{o}C in ligand-free form, the receptor was inactivated spontaneously. The inactivation reaction followed first order kinetics, with a rate constant of 0.33 h^{-1}. The ^{3}H-DHT- and ^{3}H-T-receptor complexes sedimented as single, 3-4 S peaks in sucrose gradients containing 0.4 M NaCl. Using a reconstituted *in vitro* system, a PMS dependent and target tissue specific nuclear uptake of androgens has been demonstrated. Under identical conditions about half as much T as DHT was translocated into vesicular nuclei. The steroid specificity of the receptor was studied by measuring the competition of 22 unlabeled steroids with ^{3}H-DHT for receptor binding. According to competitory behaviour, the investigated steroids could be divided into three groups. By correlating the binding characteristics, biological effects and the structure of the test compounds, it was possible to assess /i/ the significance of certain features of steroid molecules in the binding, and /ii/ the effect of different binding kinetics of competitors on the biological activity.

GROWTH-RELATED ALTERATIONS IN THE LIPID METABOLISM OF TUMOR CELLS AND TUMOR-BEARING ORGANISM

T. Kremmer, L. Holczinger, National Oncological Institute, Research Institute of Oncopathology, Department of Biochemistry, H-1122 Budapest, Ráth Gy.-u 5.

One of the fundamental findings of experimental cancer research is that lipids represent both a dinamic energy source and structural constituents of malignant cells. Of particular interest are the growth-related changes in the lipid metabolism of tumor-bearing organism and in the tumor-host relationships. Alterations observed in the lipids of different tumor cell populations maintained in vivo or in vitro as well as of extracellular fluids /blood sera, ascitic plasma/ can be interpreted as changes in the composition and metabolism of a/ fatty acids and glycerolipids, b/ phospho- and sphingolipids and c/ of steril lipids. There are well demonstrable differences e.g. in the various stages of growth of the frequently used Ehrlich ascites carconima cells and in the tumor-bearing host animals as well. From the early phase of implantation a characteristic and continuous endogen lipid mobilizing effect of tumor cells on the host and a parallel and increasing deposit-ion of neutral lipids in the tumor are shown. The alterations of fatty acid transport from the host to the tumor and of the metabolism of phospho/sphingo/lipids and cholesterol can be demonstrated. Structural changes of the tumor cells during growth can also be demonstrated by alterations in the lipid composition and physico-chemical properties of tumor cell cytoplasmic membranes. These observations contribute to the better understanding of the molecular basis of enzymatic, membrane, etc. processes of malignant cell proliferation.

THE ROLE OF PLASMA MEMBRANE PHOSPHOLIPIDS IN THE REGULATION OF LYMPHOCYTE ACTIVATION

M. Szamel, J. Somogyi, K. Resch[1], Inst. of Biochem. Dept. 1 Semmelweis Univ. Med. Sch. Budapest, 1444, P.O.Box 260 and [1] Inst. of Molecular Pharmacol., Hannover Univ. Med. Sch.

The plasma membrane of lymphocytes was shown to contain functional domains bearing the high affinity mitogen receptor in association with $/Na^+ + K^+/$ATPase and lysolecithin acyltransferase, an enzyme responsible for the increased turnover of membrane phospholipids in activated lymphocytes. Phospholipids of isolated thymocyte plasma membranes were modified by means of lysolecithin acyltransferase. Acyltransferase mediated incorporation of palmitic acid or lysolecithin alone had no effect on any of the enzymes tested. Incorporation of linoleic acid or arachidonic acid markedly modulated the activity of membran bound enzymes. The activity of $/Na^+ + K^+/$ATPase was increased, though it returned to the original level after incorporation of high amounts of fatty acids. Concomitantly, the activity of Mg^{2+}-ATPase and γ-glutamyl-transferase were decreased. The activity of lysolecithin acyltransferase itself was also modulated with a peak effect at the same extent of fatty acid substitution required for the modulation of the activity of other enzymes. Strikingly similar changes as after increasing the concentration of polyunsaturated fatty acids were observed in the plasma membrane of mitogen stimulated lymphocytes. The specific inhibitor of $/Na^+ + K^+/$ATPase, ouabain inhibited macromolecular synthesis as well as the incorporation of long chain fatty acids and the selective activation of lysolecithin acyltransferase without altering intracellular potassium levels in stimulated lymphocytes. Our results suggest that acyltransferase mediated changes of membrane phospholipids may underlie the modulation of enzyme activities in activated lymphocytes. Moreover a functional coupling is supposed between $/Na^+ + K^+/$ATPase and lysolecithin acyltransferase which may be involved in the regulation of lymphocyte activation.

ALTERATIONS IN LIPID METABOLISM OF MICE TREATED WITH ENDOTOXIC LIPOPOLYSACCHARIDES AND DETOXIFIED PREPARATIONS

D. Gaál, T. Kremmer, Zs. Bálint, L. Holczinger, National Oncological Insitute - Research Institute of Oncopathology, Department of Biochemistry, H-1122 Budapest, Ráth Gy. u 5.

The influence of different endotoxins /lipopolysaccharides, LPS/ obtained from Serratia marcescens O8 and Escherichia coli O89, and their derivatives /detoxified by partial hydrolysis and irradiation/ on the composition of serum and liver lipids in C57 Black mice was studied. Endotoxic LPS elevated the serum neutral lipids and lipoproteins /fractionated by ultracentrifuge/ particularly the very low density lipoproteins in contrast to the nontoxic preparations, indicating that toxicity is an essential parameter of the hyperlipidemic effect. LPS caused a significant and reversible increase in the liver triglycerides. An elevated lipoprotein lipase /LPL/ activity was detected in 5-10 hours in the sera of experimental animals treated with toxic as well as nontoxic preparations, with or without heparin-pretreatment. In the early phase of action the lipid-lowering and LPL-activating effects of the nontoxic, polysaccharide-rich fraction were simultaneous, while hyperlipoproteinaemia and the rise in serum LPL-activity induced by LPS developed successively.

PHOSPHOLIPID SYNTHESIS IN VARIOUS MICROORGANISMS

P. Biacs, Institute of Agricultural Chemical Technology, University of Technocal Sciences, Budapest, H-1111 Budapest. Gellért tér 4.

Lipid content and composition of various microorganisms change under different cultural conditions and a number of antimicrobal agents influence the synthesis of lipids. The basic biological importance of membrane phospholipids is partly functional, partly structural in life processes of bacteria, yeasts and moulds.

Methicillin at a concentration of 1 microgram/ml causes a significant increase in the amount of all the phospholipids in the exponentially growing cultures of the methicillin-sensitive Staphylococcus aureus 5814S during a 2 h treatment, but the addition of 2 microgram/ml methicillin to the cultures resulted in a significant decrease in the acid phospholipids of the cocci /Rozgonyi and Biacs, 1981/. Cell wall synthesizing enzymes in S. aureus 5814S seem to require for their activity strongly negative-charged membrane lipids with predominance of PG of high fluidity. Our results may be interpreted as these enzymes appear to prefer the prevalence of anteiso-C15:0 acid among the fatty acids of the membrane phospholipids.

Under anaerobic conditions, the plasma membrane of yeasts may be modified by adding lipids. It was found that cholesterol and polyene fatty acids become easily integrated into the membrane of Saccharomyces cerevisiae and substantially change the fluidity of phospholipids /Biacs and Gruiz, 1981/. An increase of medium- and short-chain fatty acids was observed in the fraction of esterified sterols /SE/ and the accumulation of monoene fatty acids /C18:1 and C16:1/ in the PL fraction.

MEMBRANE-LIPIDS OF TWO STRAINS OF TRICHODEMRA VIRIDE WITH DIFFERENT CELLULASE ACTIVITY

K. Gruiz, M. Miklósik, P.A. Biacs, Institute of Agricultural Chemical Technology, University of Technical Sciences, Budapest, H-1111 Budapest, Gellért tér 4.

Some strains of Trchoderma viride are distinguished by their high cellulase activity. Strains of low activity /G/ and high activity /R/ were cultured in shaken flasks using potato-dextrose /PD/ or paper-cellulose /PC/ substrates for 3, 6 and 8 days. Centrifuged mycelia were analysed for lipid content, particularly to sterols and fatty acids. Strains with low cellulase activity /G/ formed 10-12% more mycelia /dry weight/ in both media. The lipid content varied from 6.3% /PD/ to 1.9% /PC/ in strains G, and from 4.6% /PD/ to 2.5% /PC/ in the strain with 3 times higher cellulase activity /R/. Lipid synthesis of both strains was depressed in PC nutrient media. Chromatographic measurements showed more sterols in the lipid-extract of the high cellulase activity /R/ strain. Saponin /aescin, Merck, 10 ppm/ enhanced 2 to 3 fold the cellulase activity and lipid content of the R strain, but higher doses /50 and 100 ppm/ reduced the lipid content to the original level. Exogenous cholesterol was incorporated into the plasma-membrane of mycelia /sterol-esters were detected by thin-layer chromatography of lipid-extracts/, and it protected the membrane structure against saponin.

This phenomenon was more characteristic in the G strain with lower cellulase activity. The sterol content of 20 h cultures was significantly higher than that of the mycelia proliferated for 72 h or more. By adding cholesterol the ratio of C_{14} and $C_{18:3}$ fatty acids increased, whereas a decrease of $C_{18:1}$ and $C_{18:2}$ fatty acids was measured. These phenomena indicated a change in the elasticity of plasma membrane structure.

CELL DAMAGING EFFECT OF ENVIRONMENTAL FACTORS. EXTRAPOLATION OF EXPERIMENTAL DATA TO MAN

L. Varga "F.J.C." National Research Institute for Radiobiology and Radiohygiene, H-1775 Budapest, P.O.Box 101

The role of environmental factors /air-pollution, chemicals drugs, ionizing radiations, radionuclides, infections, etc./ might be rendered probable in the occurrance and development of some diseases. From health hazard aspects, the induction of somatic mutations is considered important which may manifest in the alteration of genetical constitution of cells. For the detection of these effects, quite a number of test-systems have been elaborated /bacterial and mammalian cell systems, animals/ suitable for making examinations on various levels of biological organization /gene- and chromosomal mutations, SCE, micronucleus test, PCC, transformation test, etc./. The assessment of risk might be influenced by some uncertainties. In general, the lower sensitivity limit of the methods mentioned is higher than the environmental concentration level, moreover, the combined effects /additive or synergistic/ of various agents are not fully explored. In the extrapolation of experimental data to human beings the following aspects should be considered:

a/ differences in chromosome arm number, DNA content, effective arm length;

b/ interspecies comparison by using some biological parameters /daily water-loss, calculated factors derived from kinetic data, risk coefficients/;

c/ extrapolation of dose-effect relationship observed at high doses to a lower range.

In the latter case, generally a linear model is applied for the extrapolation of experimental data. According to our results, the validity of linear hypothesis cannot always be accepted, since some radiobiological properties change in different ways.

BIOCHEMICAL MECHANISMS OF LIVER INJURIES INVESTIGATED IN ISOLATED LIVER CELLS

T. Garzó, J. Mandl, Ist Institute of Biochemistry, Semmelweis University, Medical School, H-1444 Budapest, P.O.Box 260

The use of isolated liver cells in the investigation of various functions of the liver became predominant in the last decade and supplanted other technique, e.g. liver perfusion or surviving slices. The various cell types, parenchymal and non-parenchymal liver cells can be studied thus separately with biochemical methods. In contrast to the older techniques it is possible to work at optimal oxigen levels and metabolite supply. The yield of cell preparation may reach 10^8 cells/g liver, thus it is possible to plan rather complex experiments, to vary several parameters simultaneously and yet gain benefits from using so called "self controlled" systems. It follows that isolated hepacytes are very good experimental tools for the investigation of the intrahepatic metabolism of compounds that have specific effects on liver functions: the investigation of drugs and drug interaction represents probably the most important task in this respect. Monooxigenases known to have pivotal role among the enzymes of biotransformation have been studied very widely and intensively and a review of the results obtained using isolated hepatocytes will be presented.

Model liver injuries have been used for a long time in the study of liver functions. D-galactosamine, a hepatospecific agent has also been used for this purpose. Experiments carried out with isolated hepatocytes in this laboratory furnished evidence for the decisive role of amino-glycogen formed in the course of galactosamine metabolism in the early inhibition of protein synthesis following galactosamine treatment. This effect was restricted to parenchymal liver cells, since the protein synthesis of non-parenchymal cells was not affected by galactosamine. The mutual potentiating effect of galactosamine and ethanol, one of the most important agents causing liver damage was demonstrated.

SIGNIFICANCE OF HETEROGENEITY IN BIOCHEMICAL ANALYSIS OF CELL POPULATIONS AND TISSUES

L. Holczinger, National Oncological Institute, H-1122 Budapest, Ráth Gy.-u 7.

It is generally known that organs, tissues and all types of cell populations consist of heterogeneous cells or cell subsets which may differ from each other in several respects /origin, shape, size, number of chromosomes, DNA, RNA, lipid, protein or carbohydrate content, cell cycle etc/. There are also differences in surface charges, osmotic resistance, number and type of surface antigens, receptors and so on. Heterogeneity seems to be a general characteristic of all cell populations either normal or neoplastic.

From biochemical point of view the question is whether biochemical analysis of a certain tissue or cell population as a whole gives reliable informations concerning the biochemical events.

Data will be presented that cell populations separated by different ways from tissues exhibit biochemical characteristics different from each other and from those of the original, non separated tissue. It will also be proved that biochemical characteristics of proliferating cell systems may change during the proliferation period.

BIOCHEMICAL BASIS OF HAEMOSTATIC DISORDERS

R. Machovich, Semmelweis University Medical School, 2nd Institute of Biochemistry, H-1444 Budapest, P.O.Box 262

Maintenance of haemostasic balance depends on the functions of endothelial cells, platelets and blood coagulation-fibrinolytic system. Since several components of coagulation and fibrinolysis are synthesized in the liver, the rate of their synthesis also contributes to the normal haemostasis. The various factors and cells participating in the above reactions, however are not separated from each others. Blood coagulation involves cell-cell, protein-protein and protein-lipid-Ca^{++} cell interactions resulting in 10^4-10^5 fold rate enhancements in some enzyme reactions. One of the critical factors in these extremely complex processes is an enzyme, the thrombin, which /i/ converts fibrinogen to fibrin, /ii/ activates Factor XIII, /iii/ binds to platelets initiating their aggregation and /iv/ interacts with endothelial cells inducing prostacyclin release. In addition thrombin influences its own formation through reactions with prothrombin, Factor V and Factor VIII. Under normal circumstances there is no measurable amount of thrombin present in the circulating blood. Increase of the amount or activity of thrombin, however may result in formation of thrombus inside the vessels leading to thromboembolic disease. Thus, the control of thrombin activity and the regulation of its biological functions are important. The aim of this review is to give a brief survey of our present knowledge of the control of thrombin activity through the regulation of /i/ prothrombin synthesis, /ii/ activation of prothrombin by a cascade mechanism and /iii/ inactivation of excess enzyme by heparin and proteinase-inhibitors of plasma. Furthermore, the interactions of thrombin with platelets, endothelial cells and blood plasma proteins are also discussed.

ENZIMOPATHIES OF AMINO ACID METABOLISM

T. Karsai, Department of Biochemistry, University Medical School, H-4012 Debrecen, P.O.Box 6

In the course of the investigation of inborn errors of metabolism reliable methods have been developed for the detection of pathologic metabolites and for the identification of molecular defects. Problems encountered in the diagnosis of such cases are as follows.

1. Similar clinical signs - defects of different enzymes. Two mentally retarded children of the same parents were examined genetically. Of more than 100 possible enzymopathies hyperglycinemia and hyperglycinuria were detected in the children. A defect of one of 18 enzymes could cause this anomaly. Finally, a deficiency of propionyl CoA carboxylase activity was found.
2. Availability of test samples. Diagnosis is based on the detection of enzyme defects at present. Since most of the enzymes of amino acid metabolism can be found in the liver from where only 20-50 mg samples can be taken by biopsy, specific methods of high sensitivity are required. For this purpose thin-layer chromatographic-videodensitometric microprocesses were developed in our laboratory.
3. Multiplex allelia. A child suffering from cystinuria had defective alleles from both parents. Though he was a biheterozygote, he became sick.
4. Early diagnosis. The aminoacidopathies diagnosed in newborns should be reinvestigated a few months later to avoid unnecessary treatment of the so called transitory hyperaminoacidemias /hyperphenylalaninemia, hypertyrosinemia, etc./.
5. Disregard of symptoms causing no complaint. A patient /male/ treated for rheumatism for decades developed severe arthrosis because the darkening of this urine /alkaptonuria/ was disregarded.

INVESTIGATION OF MICROSOMAL ENZYMES IN VARIOUS MODELS OF LIVER CARCINOGENESIS

M. Gyenes, E. Ujhelyi, K. Lapis, A. Jeney, Zs. Schaff, M.Bencsáth, 1st Institute of Pathology and Experimental Cancer Research, Semmelweis Medical University, 1085 Budapest, Üllői-ut 26.

It has been well established in various laboratories that chemical carcinogenesis comprises of two stages. Following the administration of chemical carcinogens a part of cells is altered /initiation/. But an intense proliferation is also required for the development of the tumor /promotion/. It is known from the studies of Farber, Peraino, Pitot and others that after administration of a chemical carcinogen hyperplastic nodules appear in the liver, which are deficient in G-6--Pase and have increased activity of γ-GTP. Later on hepatocellular carcinoma develops from some of these nodules. In our experiment diethylnitrosoamine /DEN/ was administered to rats as initiator. Promotion stage in the liver was induced in the following manner: stimulation of cell proliferation by partial hepatectomy, differential killing of the non-altered cells by certain chemicals /acetylaminofluorene, CCl_4, thioacetamide/ and stimulation of microsomal enzymes by phenobarbital. The rats were exposed either to an initiator or a promoter or both, and enzyme activities were measured at various time points. Studies on G-6-Pase, N-demethylase activity and cyt.P-450 content showed a decrease in the first 24 hours after DEN administration then they returned to the control values. The first response to the promoters was a slight increase, but later on a prolonged reduction of these enzyme activities occurred. The activity of γ-GTP increased. Our data suggest that enzymatic changes become stable only upon appropriate promotion.

ACTIVITY OF ENZYMES IN MICROSOMES IN PRIMARY HEPATOMA INDUCED BY MC-29 VIRUS

E. Ujhelyi, K. Lapis, I. Földes, K. Nagy, Gyenes M, A. Jeney,
1st Institut e of Pathology and Experimental Cancer Research
Semmelweis Medical University, Budapest VIII. Üllői ut 26

Induction of liver tumours by chemical carcinogens had been performed in 1935 already, however, virus-induced development of liver tumours /in MC-29 virus infected chicken/ was reported first by Lapit et al. in 1973. Later it was also observed that MC-29 virus inoculated into one day old turkeys induced well defined hepatocellular carcinoma in 15-20 days /Schaff et al. 1978/. It is interesting to compare virally and chemically induced primary liver tumours to elucidiate the common and diverse characteristics of the pathogenetical processes. As it has been established in various laboratories, treatment with chemical carcinogens such as nitroso-amines results in drastic alterations of the microsomal enzymes. The question arised whether microsomal enzymes change also during viral carcinogenesis in turkey liver. Therefore N-demethylase, Glucose-6-Pase /G-6-Pase/, gamma glutamyl-transpeptidase /gGT/ activities and the amount of cytochrom P-450 were determined on various days /2-12/following MC-29 virus inoculation of newly hatched turkeys. It was found that at 2 days after MC-29 virus inoculation the activities of all the three microsomal enzymes studied showed a remarkably reduction. However at the time of the appearance of hepatocellular carcinoma most unexpectedly N-demethylase and G-6-Pase activities gradually returned to control values. At the same time gGT activities showed a threefold increase in livers with hepatocellular carcinoma relative to controls, but the amount of cytochrom P-450 did not alter during viral carcinogenesis.

CHANGE OF DNA REPAIR CAPACITY FOLLOWING IN VIVO DNA DAMAGE PRODUCED BY CHEMICAL AND PHYSICAL EFFECTS

L. Gueth, I. Vincze, Department of Biochemistry, National Institute of Hygiene, H-1966 Budapest, Gyáli-ut 2-6.

There are several direct and indirect evidences that DNA repair capacity increases in bacteria and tissue cultures after an in vitro DNA damage. This phenomenon was previously demonstrated in mouse spleen cells after inducing in vivo DNA damage.

In our present work we point out that in mice, after an in vivo DNA damage, DNA repair capacity of spleen cells varies both in time and in extent depending on the physical and chemical effect producing the DNA damage. After in vivo treatment of rats there was a change in the DNA repair capacity /i.e. maximum rate induced by UV light/ of spleen and blood lymphocytes, moreover the dose of UV light necessary to produce half-maximal rate was changed, too.

It is supposed that in DNA repair the rate limiting step of consecutive reactions varies depending on the in vivo treatment. This phenomenon can be considered an adaptation of the cells of the organism to the changing DNA damaging effects of the environment.

REPLICATION AND REPAIR DNA SYNTHESIS IN LIVER AND LUNG OF METHYLCHOLANTHRENE TREATED RATS

G. Sáfrány, A. Tigyi, Department of Biology, University Medical School Pécs, H-7624 Pécs, Szigeti u 12.

During the last 10 years it has become evident that the potential of a chemical to induce cancer depends not only on the nature of genetic damage it causes but also on the rate of cell replication in the target tissue at the time of treatment and on the rate at which the cell repairs DNA damage. There are some experimental results which show that the polycyclic aromatic hydrocarbons induce cancer in lung, but they very rarely cause cancer in liver.

In the present experiments, the effect of a single intraperitoneal dose of 40 mg/kg body weight 3-methyl-cholanthrene /3-MC/ was studied on replication and repair synthesis of DNA in liver and lung of male CFY rats. Replications of DNA in liver was increased 48 hrs after 3-MC treatment. Maximal activity of replication and repair synthesis of DNA in liver and lung was observed 8 days after 3-MC-treatment. After 8 days a slow decrease in the activity of both types of DNA synthesis was observed both in liver and lung.

The activity of DNA polymerase-α was increased both in liver and lung after 3-MC-treatment.

PROTEINS DERIVED FROM HUMAN LYMPHOCYTES INHIBIT THYMIDINE INCORPORATION INTO THE DNA OF TARGET CELLS IN TWO DIFFERENT WAYS

Maria T. Szabó, A. Hrabák, 1st Institute of Biochem. Semmelweis University H-1444, Budapest, P.O.Box 260

Uptake of TdR into the acid soluble cell fraction and its incorporation into DNA was investigated in vitro using ^{3}H-labeled TdR in the presence of two different proteins derived from human tonsillar cells. One of the proteins was extracted from the plasma membrane of cells with deoxycholate; the other was precipitated by ammonium sulphate from the medium of lymphocytes incubated for 4 hours.

The protein extracted from the cell membrane inhibited the incorporation of TdR into DNA both in intact cells and cell homogenates, however, it did not influence the uptake of TdR into the acid soluble cell fraction. The inhibitory effect was not based upon its property to react with TdR directly; it did not bind TdR as shown by equilibrium dialysis experiments and it had no TdR degrading activity.

In the presence of the other protein isolated from the medium of cells not only the incorporation of TdR into DNA was diminished, but the uptake of the nucleoside into the acid soluble cell fraction was also influenced: the K_M value was higher, value for V_{max} was not altered. This protein did not react with TdR directly either.

We suppose that the membrane derived factor inhibits the intracellular DNA synthesis in its target cells without any influence on the entry of nucleoside into the cells.

On the other hand, inhibiton of the incorporation caused by the other factor can be explained by the limited entry of the nucleoside into the cells. The higher K_M value measured in its presence may be related to the carrier mediated transport of TdR or to the activity of TdR-kinase in the cells.
/Abbreviation: TdR = thymidine/

THE EFFECTS OF EMETINE ON THE IMMUNE RESPONSE OF MICE TREATED WITH SHEEP RED BLOOD CELLS

I. Csuka, Á. Soóki-Tóth, K. Mészáros, 1st Institute of Biochemistry, Semmelweis University Medical School, Budapest 1444. P.O.B. 260

Effects of in vivo emetine treatment on the immune response of CFLP mice to sheep red blood cells and on macromolecule synthesis were studied. Four days after the simultaneous administration of emetine and the antigen no lytic antibody synthesis could be demonstrated in spleen cells. The inhibition was less marked /30-40%/, when emetine was administered 72 hours after the antigen injection. It is proposed that emetine inhibits the early stages of the immune response, while the later stages are influenced to a lesser extent.

Emetine is an inhibitor of protein synthesis specific to eukaryotes. Inhibition of protein synthesis of human lymphocytes and Ehrlich ascites cells by emetine has been demonstrated in vitro /Gy. Farkas, F. Antoni et al. 1974, Acta Biochem. Biophys. Acad. Sci. Hung. 9. pp. 63-72./. In isolated mouse spleen cells the incorporation of ^{14}C valine into protein and of ^{3}H-thymidine into DNA was strongly depressed 4, 24, 48 hours after in vivo emetine treatment /33 mg/kg/, then it returned to the control rate at 96 hours. Immunization with sheep red blood cells stimulated protein and DNA synthesis, however simultaneous administration of emetine decreased the incorporation of precursors to the level observed in spleen cells of non-immunized, emetine treated mice. Inhibition of protein and nucleic acid synthesis during the early stage of the immune response blocks the development of the immune response. Our experiments revealed the immunsupressive effect of emetine, which may be applied as a valuable tool in the discrimination between various stages /processing, presentation/ of the immune response.

MECHANISM OF THE BINDING OF THROMBIN TO ENDOTHELIAL CELLS

R. Machovich[1], É. Csonka, [1] Semmelweis University Medical School, 2nd Institute of Biochemistry and 2nd Institute of Pathology, H-1444 Budapest, P.O.Box 262

The interaction of human α-thrombin with mini pig aortic endothelial cells was studied using ^{125}I-labelled enzyme. Equilibrium between bound and free thrombin was attained within one min, and Scatchard analysis indicated two populations of binding sites: a high affinity one of 2×10^{5} sites/cell with a Kd of 6×10^{-8}M and a low affinity one of 2.8×10^{6} sites/cell with a Kd of 7.6×10^{-6}M. Heparin in a 10-fold molar excess of thrombin inhibited the binding by 55%. Modification of two lysine residue of thrombin with pyridoxal-5'-phosphate resulted in a single population of 6.8×10^{5} sites/cell with a Kd of 2.2×10^{-6}M, and heparin did not influence the interaction between modified-enzyme and cell. When the lysine molecule of thrombin involved in heparin binding was protected with heparin against chemical modification, the binding showed similarly to that of the native enzyme: 10^{4} sites/cell with a Kd of 5.4×10^{-8}M and 1.6×10^{6} sites/cell with a Kd of 2.2×10^{-6}M. Since heparin might interfere with both enzyme and cell; the interaction of heparin with endothelial cells was also examined. The results revealed that ^{3}H-heparin bound also to cells with two distinct classes of binding sites: a 1.4×10^{6}sites/cell with a Kd of 3×10^{-7}M and a 1×10^{7} sites/cell with a Kd of 1.4×10^{-4}M. Furthermore, thrombin bound to endothelial cells was released by antithrombin III. On the basis of these and other data in the literature, a model is proposed for the mechanism of the binding of thrombin to endothelial cells.

SPECIFIC BINDING OF THROMBIN ANTITHROMBIN COMPLEX TO HEPATOCYTES

P. I. Bauer[1], J. Mandl, T. Garzó, R. Machovich, 1st and 2nd[1]
Institute of Biochemistry, Semmelweis University Medical School, H-1444 Budapest, P.O.Box 262

Thrombin-antithrombin III complex binds selectively to isolated hepatocytes, whereas antithrombin III alone does not. The binding is time and concentration dependent at 37°C; the apparent K_m value is 0.8 μM. The rate of binding is approx. 1.6×10^5 molecules h^{-1} $cell^{-1}$ at this concentration. At 4°C there is no measurable interaction between the complex and the hepatocyte. The binding is also prevented by pretreatment of cells with trypsin. On the other hand, about 80% of the thrombin-antithrombin III complex bound to hepatocyte is releasable by trypsin digestion. NaF of carboxyatractyloside does not inhibit the process. The interaction of thrombin-antithrombin III complex with hepatocytes seems to be specific, since the complexes of antithrombin III with other proteinases, like trypsin or plasmin, are not bound at the concentrations used. Based on these data, a mechanism for the binding of the inactive, complex form of thrombin to hepatocytes is suggested.

UPTAKE OF ARACHIDONIC ACID AND ITS INCORPORATION INTO PHOSPHOLIPIDS AND TRIACYLGLYCEROLS IN ISOLATED MURINE HEPATOCYTES. THE EFFECT OF THROMBIN-ANTITHROMBIN III COMPLEX

J. Mandl, B. Tanács[1], Z. Spolarics[2], T. Garzó, Ist Institute of Biochemistry and 2nd Institute of Biochemistry[2], Semmelweis University, Medical School, H-1444 Budapest, P.O.Box 260 and [1]Institute of Isotopes of the Hungarian Academy of Sciences, Budapest

The uptake and metabolism of arachidonic acid was investigated in isolated hepatocytes prepared from mouse liver with the collagenase perfusion method /J. Mandl, T. Garzó, K. Mészáros, F. Antoni: Biochem. Biophys. Acta /1979/ 586, 560/. The uptake of arachidonic acid and its incorporation into various lipid fractions was determined with the aid of 5, 6, 8, 9, 11, 12, 14, 15-^{3}H-/n/-arachidonic acid. The individual lipid, phospholipid and prostaglandine fractions were separated with thin layer chromatographic methods.

94-98 per cent of the intracellular arachidonic acid was incorporated into phospholipids and the triacylglycerol fraction following a 60 min incubation at 37^{o}C in a glucose, lactate and albumin containing Krebs-Henseleit bicarbonate buffer solution.

Human thrombin-antithrombin III complex was bound to isolated hepatocytes selectively, while thrombin alone was bound to a small extent only and antithrombin III was not bound at all. The uptake of arachidonic acid of isolated hepatocytes was not altered in the presence of the thrombin-antihtrombin III complex. However, an increased incorporation of arachidonic acid into phospholipids was found in hepatocytes to which the complex was bound. The incorporation of arachidonic acid into phosphatidylcholine and phosphatidylethanolamine was increased while the incorporation into phosphatidyl-inositol was unchanged. No detectable amount of prostaglandine intermediates could demonstrated.

BINDING OF THROMBIN, ANTITHROMBIN-III and THROMBIN-ANTITHROMBIN-III-COMPLEX TO NON-PARENCHYMAL LIVER CELLS AND OTHER MESODERMAL CELLS

T. Spolarics[1], S. Koch, 2nd Institute of Biochemistry[1] and 2nd Institute of Pathology, Semmelweis University Medical School, H-1444 Budapest, P.O.Box 262

Bindig of different coagulation factors to isolated non-parenchymal liver cells, endothelial-, smooth muscle cells and fibroblasts was studied. Non-parenchymal cells were isolated from mouse liver with two methods; pronase digestion and differential centrifugation. Viability of the cells was controlled by Trypan blue exclusion test and by ^{14}C-valine incorporation into proteins. Binding of the coagulation factors to cells was investigated using ^{125}I-labelled thrombin and antithrombin-III. Degradation of the bound proteins was detected by measurement of the acid soluble radioactivity in the medium of the cells. Our results showed that thrombin was bound to the endothelial and the smooth muscle cells selectively, whereas the non-parenchymal liver cells could bind thrombin antithrombin-III and thrombin-antithrombin-III-complex simultaneously. Fibroblasts bound thrombin and complex as well, but the rate of binding was low compared to non-parenchymal liver cells. Furthermore the pronase treated non-parenchymal liver cells degraded thrombin and thrombin-antithrombin-III-complex too. In earlier experiments of this laboratory it has been shown that isolated hepatocytes bound the thrombin-antithrombin-III-complex selectively. In contrast to this finding no selectivity of this type could be demonstrated in the binding of non-parenchymal liver cells.

EFFECT OF PLASMIN ON AORTIC ENDOTHELIAL CELLS

É. Csonka[1], P.I. Bauer, 2nd Institute of Pathology[1], 2nd Institute of Biochemistry Semmelweis University Medical School, H-1444 Budapest, P.O.Box 262

The interaction between endothelial cells and the components of blood coagulation cascade is well documented. However, no data are available on the interaction of endothelial cells with plasmin, the key enzyme of fibrinolysis. Therefore we investigated the effect of plasmin on cultured pig endothelial cells. $[^{125}I]$ -labelled plasmin binds to endothelial cells in a time and concentration dependent manner. The binding is reversible with K_D value of about 1.4×10^{-9}M. Radioactivity bound to the cells dissociates within 2 minutes indicating a rapid equilibrium between the free and bound enzyme.

Chemical modification of the plasmin using phenyl--methyl-sulphonyl fluoride or pyridoxal-5'phosphate reveals that neither the active site nor the heparin binding site are involved in the binding of the enzyme to endothelial cells. When plasmin was added to $[^{3}H]$-arachidonic acid fed endothelial cells a release of radioactivity into the medium was observed. Thin layer chromatography of these samples with two different solvent systems showed that only $[^{3}H]$ -arachidonic acid was released and prostaglandin synthesis was not detectable over the control value.

The physiological role of this binding process is not known at present. We suggest that it may play a role when plasmin is overproduced and the α_2-antiplasmin pool is exhausted. The excess of plasmin is anchored to the surface of endothelial cells until it is inactivated by α_2-macroglobulin or perhaps by antithrombin III. In this way enhanced fibrinolysis may be avoided.

EFFECT OF NONENZYMIC GLYCOSYLATIONS OF LDL ON THE INTERACTION OF ENDOTHELIAL CELL AND LDL

K.G. Büki, P.I. Bauer[1], A.S. Koch[1], 2nd Institute of Biochemistry. 2nd[1] Institute of Pathology Semmelweis University Medical School, H-1444 Budapest, P.O.Box 262

There are several proteins in the blood which are glycosylated during their circulation /M. Brownlee and A. Cerami, Ann. Rev. Biochem. 50 385-432 /1981/ /. The amount of glucose bound to the free NH_2 groups of these proteins is enhanced in diabetes mellitus. The specific binding of LDL /low density lipoprotein/ to endothelial cells needs the -NH_2 group/s/ of the lysine residues of the apoprotein. Therefore we investigated the effect of nonenzymic glycosylation of LDL on the LDL-pig aortic endothelial cell interaction. [^{14}C]-Glucose incorporated in a time and concentration dependent manner into the protein moiety of the LDL. The binding, uptake, degradation of glycosylated, [^{125}I] -labelled LDL was always less than that of the control, nonglycosylated LDL, up to a LDL concentration of 5 mg/ml. The inhibition correlated with the level of glycosylation. The esterification of cholesterol by endothelial cells in the presence of LDL was also investigated using [^{3}H] -oleic acid. The incorporation of [^{3}H] -oleate into the cholesterol-ester fraction was inhibited by 50% in the presence of glycosylated LDL as compared to the cells incubated with normal LDL.

INTERACTION OF HEPARIN WITH LOW DENSITY LIPOPROTEIN

É. Kecskés, K.G. Büki, 2nd Institute of Biochemistry Semmelweis University Medical School, H-1444 Budapest, P.O.Box 262

Heparin forms a complex with human low density lipoprotein /LDL/ in the presence of Ca^{2+} ions. [Estes, J.W., Clin. Pharacokin. 5 204 /1980/] . Raising the ionic strength, the complex dissociates at 0.5 M NaCl. Thrombin or plasmin dissociates the LDL-heparin complex as proved by co-chromatography on Sephadex G200. Heparin, in a complex with LDL, retains its enhancing effect on the rate of both thrombin and plasmin inactivation by antithrombin III. In addition, the binding of LDL to fibroblasts in culture was not influenced by heparin either in equimolar amount or a 100-fold molar excess to the lipoprotein. In conclusion, heparin as well as LDL seems to maintain their biological functions when they are in a complex with each other.

PROPERTIES OF HEPARIN-SEPHAROSE CONJUGATES MADE FROM DIFFERENT HEPARIN LOTS

B. Papp, K.G. Büki, 2nd Institute of Biochemistry, Semmelweis University, Medical School, H-1444 Budapest, P.O.Box 262

The most widely used adsorbent for purification of antithrombin III /AT/ is the heparin-Sepharose affinity gel. We have found that AT does not elute at the same ionic strength from different lots of heparin-Sepharose.

Different lots of heparin from various manufacturers were coupled to the same BrCN-activated Sepharose. The affinity adsorbents were tested by adsorbing AT from plasma and eluting with a salt gradient. Conductivity was measured in the fractions. AT was assayed by thrombin inactivation test.

The various adsorbents had essentially the same capacity for AT, but AT was eluted at different salt concentrations. The elution profiles were also different. Some of them had sharp peaks, others had broad maxima. There was no correlation between the heparin activity /expressed in international units/ and the adsorbents' properties when various heparin lots were examined.

BIOCHEMICAL TESTS FOR GENETIC COUNSELING. DETECTION OF HYPERGLYCINAEMIA. PROPIONYL-CoA CARBOXYLASE DEFICIENCY A RARE METABOLIC DISORDER

S. Szondy, Department of Biochemistry, University Medical School, H-4012 Debrecen, P.O.Box 6

Hyperglycinaemia is caused by a defect in amino and metabolism. It is characterized by high levels of glycine in the serum and urine. Both ketotic and non-ketotic forms occur which can be due to defective function of one of 3 and 6 enzymes, respectively. The deficiency of propionyl-CoA carboxylase activity may lead to severe mental and somatic retardation.

The defective enzyme which caused hyperglycinaemia with severe mental retardation in two children had to be identified because their mother became pregnant again. Then prenatal diagnosis had to determine whether the third child would be healthy.

Repeated thin-layer ion-exchange chromatographic analysis detected glycine in massive amounts in the serum and urine of the two children. The ketoacid content of the urine was found by the Dancis method to correlate with the prevailing clinical state of the children /intermittent ketosis/. The urine contained no fatty acyl-glycine derivatives, but a large amount of propionic acid. The latter is known to accumulate in propionicacidaemia only. Therefore, we advised the laboratory of Human Genetics to measure the activity of propionyl-CoA carboxylase in the amniotic fluid cells. The activity of the enzyme seemed to be normal, thus no clinical abortion was performed. The baby was born healthy one and a half years ago.

CHANGES IN THE ACTIVITY OF ORNITHINE DECARBOXYLASE /ODC/ AND IN THE CONCENTRATION OF POLYAMINES IN RAT LIVER AND KIDNEY DURING ONTOGENESIS

S. Bardócz, P. Rády[1], Cs. Várvölgyi, T. Karsai, [1]Departments of Biochemistry and Hygiene and Epidemiology, University Medical School of Debrecen, H-4012 Debrecen, P.O.Box 6

The activity of ODC /EC 4.1.1.17/ and the concentration of polyamines were studied in rat liver and kidney from the 16th day of foetal life up to the age of 10 months.

1. Both the hepatic and renal activity of ODC decreased continuously from the 16-19th days of foetal life. There was a sharp rise on the 1st day after birth, then the activity of ODC gradually decreased till the age of 1 month, and became nearly constant after that age.
2. The hepatic activity of ODC was higher than the renal one during foetal life. After birth, however, ODC activity was higher in the kidney.
3. When newborn rats were treated with dimethylnitrosamine, a carcinogen, the activity of ODC increased in both the liver and the kidney after the age of 1 month. This increase is ascribed to proliferation induced by dimethylnitrosamine.
4. The concentration of putrescine followed the changes in ODC activity in both organs.
5. Spermidine and spermine concentrations decreased gradually till the age of 1 month, and became constant after that age in both the liver and kidney. The ratio of these compounds was different in the two organs. In adults spermidine concentration was higher in the kidney. Dimethylnitrosamine increased the spermidine concentration more than of spermin in both organs. The increase in the ratio of spermidine to spermine indicated enhanced proliferation.

ISOPROTERENOL-INDUCED RESPONSES IN THE REGIONAL POLYAMINE METABOLISM OF THE RAT HEART

L. Selmeci, S. Bardóczˡ, Z. Egyed, Institute of Pathophysiology Semmelweis University, Medical School, Budapest H-1445 P.O.Box 370 and ¹Department of Biochemistry Debrecen University Medical School, Debrecen H-4012, P.O.Box 6

The present investigations were designed to study the changes in cardiac polyamine /putrescine, spermidine and spermine/ concentration after a single cardiotoxic dose of isoproterenol. Male albino rats from the CFY strain were used. The experimental group received a single dose /25 mg/kg s.c./ of isoproterenol. Controls were injected with physiologic saline. At 24, 48 and 72 hours after isoproterenol treatment the animals were exsanguinated and the hearts were rapidly excised. The left ventricle was separated from the right ventricle and the left ventricle was subsequently sectioned into three parts /basal, medial and apical parts/. The respective parts of two rat hearts were pooled, weighed, homogenized and the supernate was processed for polyamine determination according to Inoue and Mizutani /1973/ with the modification that after in the ion exchange chromatography the samples were dansylated /Seiler, 1973/.

Isoproterenol treatment resulted in substantial alteration of cardiac polyamine metabolism. In the medial part and in the apical tissue the spermidine response exhibited a biphasic pattern with two peaks, one occurred at 24 hours and the second at 72 hours. In the basis of the heart spermidine concentration was significantly increased at 24 hours but returned to control level by 48 hours. The response of the right ventricle was different from that of the left ventricle. Changes of polyamine metabolism observed in this study might be involved in the initiation of cardiac hypertrophy induces by isoproterenol.

PYRUVATE KINASE ACTIVITY IN THE WHITE BLOOD CELLS OF PREMATURE INFANTS

G. Vereb, K. Szücs, G. Balla[1], A. Makay[1], L. Karmazsin[1],
Institute of Medical Chemistry, University Medical School,
H-4012 Debrecen, Bem tér 18/b and [1]Pediatric Department
of the Medical University H-4012 Debrecen, Nagyerdei krt. 98.

Impairment of cellular immune function is accompanied with a decrease of pyruvate kinase /PK/ activity in the mononuclear cells of premature infants or adult patients. However, the reason and mechanism of this decrease is not clear yet.

We have compared the PK activities of mononuclear cells and granulocytes, isolated by density gradient centrifugation from the blood of premature and mature infants and of children older than 1 year. The PK activity in the mononuclear cells of premature infants was 30% and of mature infants 60% of that determined for children. Only slight differences were found between the activities of granulocytes of the various age groups.

Investigating the allosteric regulation of PK of mononuclear cells a great inhibitory effect of phenylalanine was observed /$I_{0.5}$ = 0.1-0.2 mM/. Fructose 1,6 diphosphate /FDP/ added directly into the reaction mixture could hardly reduce this inhibition. However, when the extract of white blood cells was preincubated with FDP for 10-20 min, the inhibitory effect of phenylalanine disappeared almost completely.

On the basus of these observations we suppose that slow structural changes /e.g. association and dissociation/ play an important role in the allosteric regulation and activity of PK in white blood cells.

THE LDH ACTIVITIES AND ISOENZYME PATTERNS OF NORMAL AND PATHOLOGICAL TENDONS

E. Vándor, B.J. Bálint, L. Józsa, National Institute of Traumatology, H-1430 Budapest, P.O.Box 21

The LDH activity and isoenzyme pattern of normal muscle tendons of the lower and of the upper extremities of 12 healthy persons were compared to the same tendons of 4 hypokinetic persons as well as to tendons of 2 persons whose lower extremities became paralysed as a consequence of polio, whereas their upper extremities remained unharmed. The LDH spec. activities of normal tendons are quite high, 406 nM Nad.min^{-1}.protein mg^{-1} in average. The proportion of LDH-5 and LDH-4 isoenzymes are high; the percentual ratio of M and H subunits are 76.6:23.4. There is a positive, significant correlation between the logarithm of the LDH activity and the M %, $r = +0.5992$ and $p < 0.02$. The LDH activities of hypokinetic as well as of polyomyelitic tendons decreased significantly. Their isoenzyme pattern was altered: the proportion of LDH-5 and LDH-4 decreased, the ratio of LDH-3 and LDH-2 increased, the ratio of M and H subunits changed. The higher the LDH activities and the M subunit contents are in the normal tendons, the more they decrease in the pathological state. This relationship is linear. In case of pathological tendons there is no correlation between the logarithm of LDH activities and M %. The tendons of the upper extremities of polyomyelitic persons have practically the same LDH activities and isoenzyme patterns as the tendons of healthy persons and significantly differ from their own paralytic lower tendons. The motion inhibition of various origin leads to similar alterations of tendon LDH activities and isoenzyme patterns.

EFFECT OF NH_3 ON CATECHOLAMINE LEVEL IN DIFFERENT ORGANS, SERUM GOT, GPT, GLDH ENZYME ACTIVITY AND ATP LEVEL OF COMMON CARP /CYPRINUS CARPIO L./

G. Jeney, Z. Jeney, J. Oláh, K. Györe, J. Nemcsók[1], L. Boross[1]
Fisheries Research Institute, H-5541 Szarvas, P.O.Box 47 and
[1]József Attila University, Department of Biochemistry, H-6701 Szeged, P.O.Box 533

The specific mechanism of deleterious effect of ammonia for fishes is not known exactly. For this reason the aim of our study was to investigate those biochemical parameters of different organs and serum, which could indicate necrosis of some tissues /transaminase enzymes and GLDH/, as well as the stress /adrenalin and noradrenalin/ and energy condition of the organism /ATP/.

We determined the changes of adrenalin and noradrenalin level in different organs of young carps, as well as GOT, GPT, enzyme activity and ATP level in serum, elicited by increasing concentrations of ammonia, at different temperatures, pH and oxygen levels.

The levels of adrenaline and noradrenaline were determined according to the method of Anton /1962/. GOT, GPT, GLDH enzyme activities and the ATP level were measured by using standard assay kits /Boehringer/.

The adrenaline and noradrenaline content of different organs /heart, muscle, gills/ increased in direct proportion with ammonia concentration, which reflects the stressor effect of this compound. Ammonia treatment significantly increased the activity of serum GOT, GPT and GLDH, and decreased the level of ATP in the serum. Increase of transaminases shows tissue damages, decrease of ATP level shows the change of energy balance of organism. The damaging effects of ammonia are enhanced by decrease of oxygen content of water and by high temperatures and high pH.

BIOCHEMICAL CHANGES OF VEGETAL RAW MATERIALS AND FOODSTUFFS DURING STORAGE

R. Lásztity, K. Hangyál[1], Department of Biochemistry and Food Technology, Technical University Budapest, H-1521 Budapest, P.O.Box 91 and [1]Research Institute of Sugar Production, H-8000 Szolnok, Vöröshadsereg-u 41.

In the field of food processing and storage there is a growing interest in the more detailed study of respiratory processes. Among the topics of investigations in this field the following problems may be mentioned:

- the effect of external factors,
- the effect of plant variety, and of agrotechnical factors,
- the biochemical regulation and control of respiratory processes,
- the possibility of prediction /calculation/ of respiratory losses based on the knowledge of mechanism and regulation of the respiration,
- elaboration of optimal programs of processing.

The first step of the enzymic browning, the oxydation of polyphenols to quinones is well known. The formation of high molecular weight colouring substances and secondary reactions of quinones, proteins and amino acids need further investigations. The ripening of the fruits is connected with a number of biochemical changes. Most of these biochemical processes are not well known and need further investigation.

SIGNIFICANCE OF POLYPHENOL OXIDASE AND PEROXIDASE IN THE QUALITY OF FOODS OF PLANT ORIGIN

L. Vámos-Vigyázó, Central Food Research Institute, H-1525 Budapest, Herman Ottó-u 15.

The enzymes polyphenol oxidase /PPO/ and peroxidase /POD/ are ubiquitous in the plant kingdom. Their presence gives rise to unfavorable changes during processing and storage of foods of vegetable origin. PPO causes discoloration /enzymatic browning/, losses of vitamin C content and reduced digestibility, while POD action manifests itself in discoloration /carotene bleaching/ and generation of off-flavors. The considerable differences in the properties /substrate specificity, dependence of activity pH and temperature, pH- and temperature resistance, susceptibility to effectors, etc./ of these enzymes present in different genera and varieties render efficient inhibition of their action difficult.

A ten year study carried out in the author's laboratory has been devoted to establish the activities of these enzymes mainly in various fruits and vegetables, the changes in activity during ripening and storage in different conditions, the yearly variations in enzyme levels, the heat resistance of the enzymes, the varietal differences in susceptibility to chemical inhibitors, etc. Some of the most characteristic results are given in detail.

As a result of practical importance it has been established that, in certain conditions, inactivation of POD is not necessary and that limited activities of this enzyme might indicate a better food quality than its total absence.

EFFECTS OF HEAT-TREATMENT AND STORAGE TIME ON THE VITAMIN CONTENT OF VEGETABLE JUICES

M. Gasztonyi, K. Zeletaki-Horváth, Central Food Research Institute, H-1111 Budapest, Budafoki-u 59.

Fruit and vegetable processing by pressing technology results in a marked loss of vitamins, decreasing the nutrition-value of the products.

As a consequence of the heat treatment a very significant occurs in the vitamin C /Jurics, 1970/ and carotene of the products /Balla, 1976; Zoller 1977/.

In the Central Food Research Institute a new enzymatic method was developed /Zetelaki-Horváth, Vas, 1980/ for the solubilization of vegetables and fruits and for the better recovery of vitamins.

It was found in previous experiments that endo-poly-galacturonase treatment increased the recovery of β- carotene in the carrot juice as compared to earlier methods /Gátai, Zetelaki-Horváth, 1977/. It is important to know the rate of decomposition of the vitamins during heat treatment and storage for the development of a good production technology. This is why ascorbic acid and β-carotene content of the vegetable juices were examined. Carrot juice, enriched by 30 mg vitamin C per flask, was used as model material. A 12 and 30 per cent decrease in the β-carotene content was measured after flash pasteurization and after heating for 20 minute at 85°C resp. The decrease in the vitamin C content of the samples was more than 4% after 20 minute pasteurization.

No vitamin C was detected in the carrot juice after 8 weak storage at 25°C, while about 40 per cent of the original value was detectable when samples were stored in a refrigerator. The decrease in the β-carotene content of the samples was significantly lower than that of vitamin C. After a 6 month storage at 25°C, about 50 per cent loss of the original β- carotene content was found.

INVESTIGATION OF SOME BIOCHEMICAL CHANGES IN APPLES AND PEARS DURING STORAGE

P. Merész, K. Jamrich, A. Susits, Department of Biochemistry and Food Technology, Technical University Budapest, H-1521 Budapest, P.O.Box 91

The elaboration of new, more adequate forms of storage needs better knowledge of biochemical changes of fruits during storage. The main purpose of research work to be reported is the investigation of enzymes playing important role in the biochemical changes of fruits.

The activity of poliphenoloxidases, different dehydrogenases and pectinases, the quantity of organic acids was measured in 3 apple varieties: Starkinson, Golden Delicious, Idared and 2 pear varieties: Hardenport, Nemeskrasszán. The fruits were stored in non-controlled and controlled atmosphere at the Department of Fruit Growing, University of Horticulture.

The methods used were the following. The activity of PPO was determined by the increase in extinction at 400 nm, by automatic analyser type "Contiflo", with catechol substrate. The common effect of pectinases was characterized by the decrease in viscosity of pectin solution determined in viscosimeter type Ostwald-Canon-Fenske. The activity of dehydrogenases was determined by the change of fluorescence as a result of the increasing quantity of NADH. The measurements of composition of organic acids were carried out by GC type Chrom 41. The first results of the investigations show a good correlation between some biochemical changes and quality characteristics and the pectinase activity. The elucidation of the correlations between polyphenoloxydase and dehidrogenases activity and the quality characteristics of fruits needs further, more detailed investigations.

BIOCHEMICAL CHANGES TO EDIBLE MUSHROOMS DURING STORAGE

E. Vadon-Györey, D. Törley, Technical University Budapest, Department of Biochemistry and Food Technology, H-1502 Budapest, P.O.Box 91

Changes of the proteins of edible mushrooms were examined under following storage conditions: storage at room temperature without controlling the humidity of the air, storage at room temperature, at 72% relative humidity, storage in a refrigerator at $4^{\circ}C$. The cell-sap obtained by crushing the fruiting bodies was analyzed by polyacrylamide gel electrophoresis. The protein patterns, the dehydrogenase and polyphenoloxidase activities of certain protein bands were established. The pherograms and the zymograms showed the increase of the number of the isoenzymes and of the enzyme activities during storage.

CHANGES IN COMPONENTS OF AETHEROLEUM MENTHAE PIPERITAE DURING STORAGE

É. Nagy, I. Seres, Institute of Pharmacognosy Semmelweis Medical University, H-1085 Budapest, Üllői u 26.

Medical and industrial application of species of mint is of great significance. In food industry spearmint oil is used for flavouring chewing gum, liqueur and sweets. Its most important characteristics, from the point of view of application, are its taste and smell.

The aim of our studies was to examine the changes in the oil during storage. The volatile oil was subjected to the effects of heat and light. We kept the oil at different temperatures between 40 and 160°C centigrade for 1 to 6 hours. Illumination was carried out with a xenon lamp, the oil being in dark or white glasses at room temperature for 48, 120 and 240 hours. A 240-hour illumination is similar to exposition to natural light during a 5-year-storage under ordinary circumstances. In order to demonstrate the changes we applied thin-layer chromatography, and the relative quantities were determined using a densitometer type ERI 65 equipped with automatic extinction-registrator and integrator appliance.

Analysing the densitograms we obtained a number of data on the changes of the components. We observed an increase of the menthon content on the effect of illumination. This finding seems to be important because menthon has a toxic effect on the liver.

OXIDATIVE CHANGES IN IRRADIATED FOODSTUFFS

B. Matkovics, Biological Isotope Laboratory, "A.J." University Szeged, H-6701, Szeged 1, P.O.Box 539

Preservation of food by irradiation is an important alternative.

In the presence of O_2, gamma-rays lead primarily to the formation of active oxygen radicals in living tissues. At the request of the Central Food Research Institute /Budapest/, we have begun an investigation of superoxide dismutase /EC 1.15.1.1/, which takes part in the dismutation of superoxide radicals /O_2^-/, and of catalase /EC 1.11.1.6/ and peroxidase /EC 1.11.1.7/, which are involved in the decomposition of H_2O_2. In addition to these peroxide metabolism enzymes, we have studied the changes in lipid peroxidation, glucose, ascorbic acid, etc. in various plant materials /onion, potato and mushroom/ at different times following gamma-irradiation.

ANTIBIOTICS AND RECENT DEVELOPMENT OF THEIR RESEARCH

A. Szentirmai, Institute for Drug Research, H-1325, Budapest, P.O.Box 82

110 years ago Roberts noticed an antibacterial effect caused by Penicillium glaucum culture. More that 50 years ago Fleming demonstrated that the filtered broth of Penicillium notatum inhibited the growth of Streptococcus and 10 years later the "Antibiotic Era" has started by the therapeutic utilization of penicillin. At present the annual consumption of antibiotics of the world amounts to 25.000 tons and out of this 75% belongs to the β -lactam penicillin and cephalosporin derivatives, 20% to the tetracyclines and the remaining 5% is shared by macrolides, fungistatic polyenes, aminglycosices and ansa-chain antibiotics. These compounds, based on their bacterial spectra provide a powerful tool against the most frequently occuring infections diseases. However, further efforts have to be taken to discover new antibiotics having better therapeutic applicability /high oral absorption, prolonged blood level, stability against different chemical and physiological effects/, moreover to improve their activity against resistant bacteria. For broadening of bacterial spectra, more than 20.000 derivatives /mostly penicillins and cephalosporins/ were synthesized and tested. These results at the same time indicate the limits of chemical modifications. Decisive change in this field can only be achieved if the traditional screening methods will be replaced by entirely new ones. Since the most effective drugs of easily accessible strains became known during the past decades, therefore noval and effective compounds can only be hoped for as secondary metabolites isolated from new types of rarely occuring microorganisms. This would necessitate the elaboration of more sensitive and specified screening methods and the development of techniques for precise and rapid elucidation of chemical structures which would result in new, useful antibiotics.

BETA-LACTAM ANTIBIOTICA AND THEIR BIOSYNTHESIS

K. Marossy, BIOGAL Pharmaceutical Works H-4042 Debrecen, Pallagi u 13.

Among the beta-lactam antibiotics the penicillins, containing penam structure, cefalosporins, with cephem structure and thienamycin, containing carbapenam skeleton, are the most important from the economical point of view for BIOGAL.

Penicillin G is synthesized by P. chrysogenum from α--amino-adipic acid, cysteine, valine and the precursor of the side chain phenyl-acetic acid. Penicillin biosynthesis has been shown to involve the utilization of the intact C_5-skeleton, together with the aminonitrogen and all six methyl hydrogen atoms, during incorporation of the of L-valine into the intermediate tripeptide δ -/L- α -aminoadipyl/ -L-cysteinyl-D-valine /ACV/ and the latter as a prerequisite of the subsequent oxidative cyclisation step leading to formation of the penam S-C/2/ bond. The γ -L-glutamyl-L-cysteinyl synthetase has been postulated to be the key enzyme of the synthesis of the ACV-tripeptide.

The ACV-tripeptide was detected in cefalosporin producing C. acermonium too. This precursor is converted into penicillin N → deacetoxycephalosporin C → cephalosporin C. The role of cysteine was studied during the biosynthesis of thienamycin, but the whole pathway has not been known in detail. Depending on the microorganism, the carbon source regulatory effect may be exerted. For example saccharose is favored over glucose by the penicillin producer P. chrysogenum. The molecular mechanism of carbon catabolite regulation may be related to growth rate control of antibiotic biosynthesis.

THE AMINOGLYCOSIDE ANTIBIOTICS; CORRELATION BETWEEN THEIR BIOSYNTHESIS AND CELL FUNCTIONS

Gy. Vargha, Gy. Barabás, Institute of Biology, University Medical School, H-4012 Debrecen

The aminoglycoside-type antibiotics represent a well distinquishable class of secondary metabolites with antibacterial activity. Their applicability for clinical use underlines the importance of search for new or modified members of these antibiotics. This need is also supported by their more or less toxic nature and the developing resistance of bacterial strains against these substances. In order to find new aminoglycoside antibiotics by rational biological manipulation, the knowledge of their biosynthetic routes is highly required. We briefly summarize some general aspects of the biosynthesis of these antibiotics in this paper. The chemical structures of the aminoglycosides are closely related. The existance of common constituents in these compounds suggests similar or partially identical biosynthetic pathway for the different aminoglycosides. Our data are also presented concerning the relationschip between the biosynthesis of streptomycin and neomycin and that of the cell wall constituents. On the basis of the discussed structural similarities, protoplast fusion technique seems to be suitable for the biological synthesis of new aminoglycosides with modified structures.

GENETIC ENGINEERING AS A POTENCIAL TOOL FOR INCREASED ANTIBIOTIC PRODUCTION

G.B. Kiss, I.Ott[1], Institute of Gentics, Biological Research Centre, Hungarian Academy of Sciences, H-6701, Szeged, P.O. Box 521, [1]Research Institute for Pharmaceutical Chemistry, H-1325 Budapest, P.O.Box 82

Actinomycetes produce several thousand antibiotics of which about 70 have significant commercial application in human and veterinary medicine and agriculture. Extrachromosomally replicating plasmids are involved in the production of some antibiotics. Their role in this process can be direct but more often indirect. Whether or not the genes coding for antibiotic determinants are located on the chromosome or on plasmids, recombinant DNA technology would add a new dimension to the analysis of the biochemistry and genetics of the secondary metabolism of actinomycetes and would also facilitate the development of industrial strains with increased antibiotic yield, or capable of making new antibiotics. Recent results achieved in the development of molecular cloning vectors suitable for isolation and expression of particular genes in actinomycetes will be presented.

HOMOGENITY OF HUMAN HEMOGLOBIN α-CHAIN

J. Földi, Institute of Haematology and Blood Transfusion
H-1502 Budapest, P.O.Box 44

The human hemoglobin α-chain is the product of a duplicated gene, i.e. the α-chain is coded by two structural loci per haploid genom. The independent evolution of the two gene--copies may lead to their divergence and to the heterogeneity of their protein products. The products of the other duplicated globin genes are heterogeneous. However we have not had unequivocal data on homogeneity of the human α-chain. The original primary structure study showed the homogeneity on the chain, but the methods were not adequate to detect the heterogeneity. At the same time our earlier comparative immunological study indicated the heterogeneity of this chain. In the present work the primary structure and the postsynthetic modification of the α-chains of a patient were studied. The α-genes of the patient coded two abnormal and the normal α-chain, which were separable by ion exchange chromatography. The sequence study was performed with a Beckman 890 sequencer. For the determination of total sequence of the three variants a new strategy of sequencing, a new method for separation of amino acid phenylthiohydantoins and a new procedure for automated sequencing of small peptides in liquid phase were developed. On the basis of the sequence determination of each of the three chain variants we conclude that the human hemoglobin α-chain is homogeneous. The nonenzymatic glycosylation of the α-chains was followed by measuring the quantity and distribution of incorporated /^{14}C/-glucose after in vitro incubation of intact red blood cells. The differences found in glycosylation seem to explain why the immunological methods showed the heterogeneity of the homogeneous α - chain.

RELATION BETWEEN THE STRUCTURE OF THE HINGE REGION AND THE RELATIVE POSITION OF THE Fab AND Fc PARTS IN IgG MOLECULES

I. Simon, F. Kilár, P. Závodszky, G.A. Medgyesi[1], Institute of Enzymology, Hungarian Academy of Sciences, H-1502 Budapest, P.O.Box 7 and [1]National Institute of Haematology and Blood Transfusion, H-1502, Budapest, P.O.Box 44

Human IgG molecules can be classified into four subclasses. The main structural difference between the subclasses is in their hinge region.

Since our knowledge about the steric structure and functional role of the hinge is incomplete and contradictory, we compared the structure of IgG's belonging to the four subclasses. Small angle X-ray scattering measurements were aimed to give accurate, direct results on the radius of gyration as well as the precise differences between the radii of gyration of the various subclasses. For the latter a novel difference method of high sensitivity was applied. Based on hydrodynamic and small angle scattering measurements the following statements can be made:

1. The value of the radius of gyration /R/ obtained by us for the IgG1 subclass is 4.9 ± 0.2 nm. This value is significantly smaller than published in the literature, however it is quite large for a molecule of 150000 molecular weight /for an ideal sphere of this size R = 2.8 nm/. This value suggests that there is a close contact between the Fab and Fc parts in the human IgG1 molecule.

2. Surprisingly the value of R for the IgG3 subclass is 20 per cent smaller than that of the IgG1 which has a shorter hinge region. Our data contradict the suggested extended polyproline helix hinge model. It looks likely that the long hinge peptide is folded back between the Fab and Fc parts of the molecule.

3. Two different cross section factors were measured for all four subclasses.

THE ROLE OF THE INTEGRITY OF INTERHEAVYCHAIN DISULFIDE BOND IN THE COMPLEMENT BINDING ABILITY OF RABBIT IgG

Török, J., Lakatos, S., Závodszky, P., Institute of Enzymology Hungarian Academy of Sciences, H-1502 Budapest, P.O.Box 7

The two symmetrical halves of the IgG molecule are held together by the interheavychain disulfide bond and noncovalent forces. Reduction of the disulfide bond leads to changes in certain functional properties e.g. in the binding of the first subcomponent /Clq/ of the complement. In the experiments where complement binding was abolished, samples alkylated with iodoacetamide were used. Only slight differences were observed between reduced and alkylated and native IgG molecules in their conformations and physico-chemical parameters. The functional consequences of reduction and alkylation can be explained in various ways: the lack of disulfide bond, conformational changes in the CH2 domain, distortion in the relative position of the domains because of the bulky alkylating agent.

We determined the conditions for preferential reduction of the heavy-heavy interchain disulfide bond, with minimal cleavage of the heavy-light and intrachain disulfide bonds. Then we prepared molecules alkylated with iodoacetamide and methyliodide with a yield of 90 per cent. The samples were checked by SDS-gel electrophoresis and in the analytical ultracentrifuge, and the half-molecules proved to be bound to each-other by noncovalent forces only.

To control the complement binding ability of the various antiovalbumin IgG samples Clq was isolated from pooled rabbit serum. ^{125}I labeled Clq was used in the binding experiments.

To distinguish between the possible distortive effect of the bulky acetamide group and the lack of the disulfide bridge in itself on the complement binding of rabbit antiovalbumin IgG, we compared the Clq binding ability of native, reduced and alkylated with iodoacetamide, and reduced and alkylated with methyliodide IgG samples.

QUANTITATIVE ANALYSIS OF INTERACTING ENZYME SYSTEMS: THE DISSOCIABLE GLYCEROPHOSPHATE DEHYDROGENASE /GDH/ IS COMPLEXED WITH ALDOLASE

J. Ovádi, R.I. Mohamed Osman, J. Batke, Institute of Enzymology, Hungarian Academy of Sciences, H-1502 Budapest, P.O.Box 7

Covalent binding of fluorescein isothiocyanate /FITC/ does not influence the enzymatic activity of GDH. The dissociation constant for dimer-monomer equilibrium of FITC-labelled GDH is 4 /uM, which dramatically increases in the presence of substrates /25 /uM/, indicating a substrate-induced dissociation. Comparison of these values with that calculated from the initial velocities of enzymatic reaction suggests that the substrate-induced dissociation of dimer is slow with respect to the catalytic reaction.

We have carried out the theoretical and experimental analysis of the complex formation of FITC-labelled GDH and aldolase. Both monomeric and dimeric GDH interact with aldolase /K_{dimer} = 0.2 /uM and $K_{monomer}$ = 1 /uM/. The velocities of the formation of enzyme complexes are very small in comparison with those of both enzymatic reactions and that of self-dissociation of GDH. Interaction between aldolase and GDH may direct the carbon flow from glycolysis toward lipid synthesis.

DEMONSTRATION OF STERICAL CHANGES AT THE ACTIVE CENTRE OF BUTYRYLCHOLINESTERASE /BuChE/ USING THE METHOD OF "REVERSIBLE-IRREVERSIBLE" DOUBLE INHIBITION

J. Gaál, F. Bartha, J. Batke, Institute of Enzymology, Hungarian Academy of Sciences, H-1502 Budapest, P.O.Box 7

The 2-chloro-12-/2-piperidino-ethyl/-dibenzo/d,g/ /1,3,6/ dioxazocin-HCl [EGYT-2347] , a new specific inhibitor of BuChE is characterized kinetically. EGYT-2347 inhibits non-competitively the hydrolysis of butyrylthiocholine iodide catalyzed by BuChE /K_i = 0.15 /uM, at 37°C in 0.1 N Tris-HCl, pH 7.5/.

Further, the affinity of the serine-OH at the active center towards the irreversible inhibitor of diisopropyl--phosphofluoridate /DFP/ was tested in the presence and absence of EGYT-2347. The BuChE-EGYT-2347 comples does not react /or it is several orders of magnitude less reactive/ with DFP probably because of the structural changes caused by the binding of EGYT-2347.

The simultaneous application of a non-competitive reversible inhibitor/EGYT-2347/ and a competitive irreversible inhibitor /DFP/ seems to be a useful approach to study the steric changes at the active center of BuChE.

EFFECT OF SUBSTRATES ON THE FLUORIMETRIC PROPERTIES OF ANS-LABELED 3-PHOSPHOGLYCERATE KINASE /PGK/

M. Vas, J. Batke, Institute of Enzymology, Hungarian Academy of Sciences, H-1502 Budapest, P.O.Box 7

Pig muscle 3-phosphoglycerate kinase /PGK/ was labeled with the fluorescent dye, 1-anilino naphthalene-8-sulfonate /ANS/. Bindig of ANS to PGK apparently does not influence the binding of substrated to the enzyme. In addition, the labeled enzyme seems to be fairly active. Intensity of the fluorescence emission of the enyzem-bound ANS is increased and its maximum is blue shifted compared to the free ANS. - Both the fluorescence emission and the fluorescence polarization of the enzyme-bound ANS is variously influenced by the different substrates. Titrations were carried out with both 3-phosphoglycerate /PG/ and MgATP. It was found that PG was bound more loosely /K = $= 2\text{-}3\text{x}10^{-4}$ M/ to the PGK.MgATP biner complex compared to the free enzyme /K = $2\text{-}3\text{x}10^{-5}$ M/. Essentially the same is valid in the case of MgATP, too. While the binding of MgATP to the free PGK can be described by two different dissociation constants /$K_1 = 2\text{-}3\text{x}10^{-5}$M/ and $K_2 = 3\text{-}5\text{x}10^{-4}$M/, in the case of PGK-PG biner complex only a single, low affinity MgATP binding site /K = $1\text{-}2\text{x}10^{-4}$ M/ can be detected.

It may be concluded that the conformational effects of both PG and MgATP are important in respect of PGK function.

THE INTRINSIC SUBSTRATE SPECIFICITY OF A CYCLIC NUCLEOTIDE INDEPENDENT "HISTONE KINASE"

T. Romhányi, J. Seprődi, G. Mészáros, A. Faragó, 1st Institute of Biochemistry, Semmelweis University Medical School, H-1444 Budapest, P.O.Box 260

Phosphorylation of proteins by a series of protein kinases plays an important role in the regulation of cellular processes. Studies on the substrate specificity of the cAMP dependent protein kinase have led to the concept that protein kinases recognize determinants of the amino acid sequence around the serine phosphorylated. However little information is available on the substrate specificity of the cyclic nucleotide independent protein kinases. We have separated a type of cyclic nucleotide independent histone kinase /HK/ from the cytoplasm and nucleus of human lymphocytes /Farago et al., BBA, 1973, 297, 517 and ibid, 1974, 370, 459/ and from the extract of bovine thymus. As we have reported the HK phosphorylates Ser-32 of calf thymus H2b histone and a synthetic peptide containing the amino acid sequence of H2b from Gly-26 to Lys-34 but it does not act on Ser-36 which is phosphorylated preferentially by the catalytic subunit of the cAMP dependent protein kinase /Romhányi et al., BBA, 1982, 701, 57/.

The tryptic phosphopeptide obtained from calf thymus H1 histone phosphoryleted by HK contained the Ser 108 of H1. This result indicated that the HK investigated by us was identical with that designated by Langan as HK II /Fed. Proc. 1971, 30, 1089/. Comparing the amino acid sequences around the phosphorylated serines of the different substrates we concluded that HK recognized substrates containing lysine located two positions toward the COOH terminus from the serine. Synthetic peptides: Gly-Gly-Gly-Gly-Ser-Arg-Lys-Gly and Glp-His-Trp-Ser--Tyr-Lys-Leu-Arg-Pro etilamid were also phosphorylated by HK. However, the latter peptide was not phosphorylated any more when Lys was substituted for Gly or D-Lys.

SPECIFIC INHIBITION OF GRANULOCYTE ELASTASE BY PEPTIDE ALDEHYDES

G. Cs.-Szabó, E. Széll[1], P. Elődi, Department of Biochemistry University Medical School, H-4012 Debrecen, P.O.Box 6, and [1]Institute for Drug Research, H-1325 Budapest, P.O.Box 82

Two substrates, Boc-DPhe-Pro-Nle-pNA and its analogue with a P_1 Val residue, have been designed for human granulocyte elastase on the basis of regression analysis of kinetic data obtained for several peptide substrates. Both had a good Michaelis constant, though their enzymatic reactivity was low.

On this basis, 5 peptide aldehyde inhibitors, each with a sequence analogous with the substrate, were synthesized by routine peptide chemical procedures: 3 tripeptides /P_1 = Nle, Val or Ala/ and 2 tetrapeptides extended by an N-terminal Pro residue /P_1 = Nle or Val/. The aldehyde group was obtained from the appropriate dimethyl-pyrazolide by $LiAlH_4$ reduction. The N-terminus was protected by a Suc group to improve solubility. The effects of the inhibitors were studied both with pancreatic and with granulocyte elastase and chymotrypsin. Low molecular weight esters /Boc-Ala-Np and Bz-Tyr-OEt/ were used as substrates.

The two chymotrypsins were inhibited only by the P_1 = = Nle derivative, and only to a small extent, /$K_{I,gran.}$ = = 1.4×10^{-3} M; $K_{I,panc.} = 2.75 \times 10^{-3}$ M/. Increasing the chain length in the Nle-aldehyde gave an inhibitory effect better by an order of magnitude. All aldehydes proved to be effective competitive inhibitors for the elastases. The most effective inhibitor for the granulocyte elastase was Suc-DPhe-Pro-Val--aldehyde /$K_I = 4 \times 10^{-5}$ M/. It produced 10 times stronger inhibition than the other aldehydes. This inhibitor proved to be specific for the granulocyte elastase, since it did not inhibit either of the two chymotrypsins, and was 100 times less effective for the pancreatic elastase /$K_I = 1.4 \times 10^{3}$ M/.

HYDROLYSIS OF PEPTIDE SUBSTRATES CONTAINING AMINOPHOSPHONIC ACIDS BY PROTEOLYTIC ENZYMES

G. Ősapay, K. Ősapay[1], CHINOIN Pharmaceutical and Chemical Works, H-1325 Budapest, P.O.Box 110, and [1]EGYT Pharmaco-chemical Works, H-1475 Budapest, P.O.Box 100

A number of aminophosphonic acid analogues of natural amino acids have been isolated recently from living organisms. The synthesis and coupling to peptides of these aminophosphonic acids have been carried out only in the past few years, and their behaviour towards proteolytic enzymes is hardly mentioned in the literature.

We have synthetized some carbobenzoxy aminophosphonic acid ethyl esters and we have incorporated these analogues into a number of di- and tripeptides at different positions /15 compounds/. The normal peptides have also been prepared:

Cbz-Gly-Leu-OEt, Czb-Gly-Phe-OEt, Cbz-Gly-β-Ala-OET, Cbz-Ala-Ala-OET, Cbz-Ala-Phe-OEt, Cbz-Phe-Gly-OEt, Cbz-Gly-Leu-Gly-OEt.

The analogous peptide pairs were submitted to enzymatic hydrolisis under identical conditions. In our experiments papain and in some cases chymotrypsin were used.

The enzyme reactions were carried out at 25°C, pH 6.7 and 7.7, with substrate concentration of 0.5-1.0 mM, enzyme concentration 0.5-2.0 /uM. The enzymatic hydrolysis of the above substrates was followed spectrophotometrically using ninhydrin and o-phtalaldehyde.

Carbobenzoxy-amino acid esters are hydrolyzed by papain, but the latter of the phosphonamide bond towards acidic hydrolysis is much smaller than that of the corresponding peptide amide bond. The rate of these enzyme reactions depends on the structure of substrates.

ASSAY OF PROTEASES WITH CHROMOGENIC NAPHTHOL- AND NAPHTHYLAMINE-DERIVATIVES

F. Teichmann, Department of Biochemistry, University Medical School, H-4012 Debrecen, P.O.Box 6

Diazonium salts form coloured compounds with enzymatically hydrolyzed naphthol- and naphtholamine-derivatives used as chromogenic substrates. The methods generally applied for the assay of proteases have the following drawbacks: 1. naphthol is photosensitive and also inhibits the enzymes, therefore it should be converted to a stable coloured compound right after formation, 2. the low solubility of some substrates, naphthol-AS and naphthol-ASD necessitates the use of a detergent during the enzyme reaction, and 3. no continuous measurements can be performed.

For my studies I used trypsin, chymotrypsin and proteinase-K, 8-chromogenic substrates and 3 diazonium salts. Some of the substrates showed remarkable self-decomposition at high pH. The plot of $t_{1/2}$ versus pH was exponential. The diazonium salts had no effect on enzyme activity. Of the detergents tested BRIJ 35 proved to be the best for colour development, even though it had a detectable adverse effect on enzyme activity. Further, I determined the rate of coupling of diazonium salts to the naphthol derivatives at various concentrations. Fast Garnet reacted readily, but the colour was instable. Fast Blue RR gave a permanent colour even at high pH. The molar extinction coefficient of the coloured products ranged between 25 000 and 40 000 $cm^2 \times mol^{-1}$. The values of K_M and V_{max} were also determined.

The new method based on the simultaneous application of the compounds participating in the colour development permitted continuous registration of enzyme activity and gave valuable experiences useful in staining procedures demonstrating enzyme activity after acrylamide gel electrophoresis.

INVESTIGATION OF THE SUBSTRATE-BINDING SITE OF THE β-LACTAMASE FROM BACILLUS CEREUS

L. Kiss, P. Nánási, Biochemical Institute L. Kossuth University, H-4010 Debrecen

By catalyzing the hydrolysis of the β-lactam ring in penicillins and cephalosporins, the β-lactamases defend growing bacteria against these antibiotics. Resistance is of obvious clinical importance hence a great interest has been arisen in the mechanism of action of lactamases. Although there is now good evidence that the hydroxyl group of serine-70 /Cohen et al. 1980, Biochemistry, 14 3996/, is at the catalytic site, the group/s/ involved in the binding of the substrate is not known. Knowledge of the substrate binding groups would provide a good possibility for the synthesis of new β-lactamase resistant β-lactam derivatives. We investigated the role of the tyrosine groups in the binding of the substrate. The tyrosine groups were modified with N-acetylimidazole. Five groups could be modified in native conformation. The kinetic analysis showed in the case of benzylpenicillin that the K_m value increased 5 fold and V_{max} was decreased due to the modification of tyrosines.

These results suggest that in contrast to previous data /Wolozin et al. 1982, Biochim. Biophys. Acta 701 1534/ the tyrosine side chains may have a role in the binding of the substrate or in the maintenance of active conformation. Further investigations are in progress.

DEGRADATION OF CYCLODEXTRINS WITH ASPERGILLUS ORYZAE α--AMYLASE

I. Jodál, J. Harangi, A. Lipták, P. Nánási, J. Szejtli[1], Institute of Biochemistry, Kossuth Lajos University, H-4010 Debrecen, P.O.Box 55 and [1]Chinoin Pharmaceutical Works, H-1325 Budapest, P.O.Box. 110

It is well-known that cyclodextrins can be hydrolized with α-amylases of various origin. However, no precise data have been reported about the nature and proportion of the maltooligomers liberated during the hydrolysis.

The present studies were aimed at the investigation of the mechanism of the degradation of α-, β- and γ-cyclodextrins by the α-amylase from Aspergillus oryzae. The progress of the enzymatic hydrolysis, performed under appropriate conditions, was monitored by measuring the change of the amount of reducing groups. The nature of the hydrolysis products /different membered maltooligomers/ and their proportion in the mixture were studied by liquid chromatographic method.

The results show that there are great differences in the rate of hydrolysis of α-, β- and γ-cyclodextrins by the α-amylase from Aspergillus oryzae. These results are supported by the data of liquid chromatographic and kinetic measurements.

Further studies are in progress for explaining the great differences observed between the rate of hydrolysis of the three different ring-membered cyclodextrin-substrates.

LIMITED PROTEOLYSIS OF GLYCOGEN PHOSPHORYLASE BY SUBTILISIN

V. Dombrádi, B. Tóth, P. Gergely, G.Bot, Institute of Medical Chemistry, University School of Medicine, H-4026 Debrecen, Bem tér 18/B

Limited proteolytic digestion of rabbit skeletal muscle phosphorylase a and b was undertaken at two pH values /7.0 and 8.5/ by subtilisin immobilized to Sepharose 4B. The effect of ligands /substrates, activators and inhibitors of phosphorylase/ was investigated by following the changes in /i/ enzyme activity of phosphorylase measured in the presence and absence of AMP, /ii/ in the SDS gelelectrophoretic patterns of phosphorylase, /iii/ in the ^{32}P-content of ^{32}P-labeled phosphorylase a.

The phosphorylated a form of phosphorylase was found to be more susceptible to proteolysis by subtilisin than the nonphosphorylated b form, especially at pH = 7.0. Phosphorylase a lost its activity when treated with subtilisin. All the ligands tested inhibited the digestion of phosphorylase a, while AMP and glucose 1-P accelerated the proteolysis of phosphorylase b. In some cases /in the presence of glucose 6-P, glucose or caffeine/ a transient activation of phosphorylase b was observed during the proteolytic degradation.

LIGAND'S EFFECT ON THE DEPHOSPHORYLATION OF HEART AND SKELETAL MUSCLE SPECIFIC PHOSPHORYLASES

K. Szücs, G. Vereb, G. Bot, Institute of Medical Chemistry, University Medical School, H-4026 Debrecen, Bem tér 18/b

Dephosphorylation of heart and skeletal muscle specific isophosphorylase a-s, prepared from pig and rabbit were studied, comparing the influence of various allosteric effectors on the rate of dephosphorylation. The rate of the dephosphorylation was assayed by following the decrease of the phosphorylase a activity, and by measuring ^{32}P liberation from ^{32}P-labelled phosphorylase a. The phosphorylase-phosphatase reaction was characzerized by a pseudo first order rate constant in both cases.

Dephosphorylation of heart specific isoenzymes - without ligands - appeared to be 4-5 times slower than that of muscle specific ones. Glucose 6-phosphate /G-6-P/ in physiological concentrations /0.1-1 mM/ increased /2-3 fold/ the rate of dephosphorylation of heart specific phosphorylases only. AMP in a concentration of 10^{-5} M inhibited the dephosphorylation of both enzyme types. In case of heart specific phosphorylases 0.5-1 mM G 6-P abolished the inhibition caused by 10^{-5} M AMP, moreover it enhanced the rate of dephosphorylation significantly/as compared to the reaction without ligands/. Contrarily, using skeletal muscle enzymes as substrates, 5-10 mM G-6-P had no significant reducing effect on the AMP inhibition.

Considering the effects of various ligands /AMP, G-6-P, glucose, glycogen, caffeine, adenosine, inosine/ on the dephosphorylation we conclude that the nucleotide site of heart and skeletal muscle specific phosphorylases are structurally different.

EFFECT OF FRUCTOSE ADMINISTRATION ON INTERCONVERSION OF PHOSPHORYLASE A INTO PHOSPHORYLASE B

B. Tóth, Institute of Medical Chemistry, University School Debrecen, H-4026 Debrecen, Bem tér 18/b

In hereditary fructose intolerance and also in normal animals the ingestion of fructose produces marked hypoglycaemia. Administration of fructose results in the accumulation of fructose-1-P /F-1-P/ and elevates the concentration of active phosphorylase in normal liver and kidney. The mechanism by which fructose administration activates liver phosphoylase is still unknown.

We have investigated the effect of F-1-P and other metabolites formed from fructose on the dephosphorylation /inactivation/ of phosphorylase by phosphatase. F-1-P was the only metabolite which inhibited the dephosphorylation of phosphorylase. Fructose, glucose and glucose-6-P did not influence the inhibitor effect of F-1-P. The inhibition of phosphatase by F-1-P has been investigated by kinetic studies and found to be competitive with respect to phosphorylase. It is known that phosphorylase is also inactivated by trypsin in an irreversible manner. F-1-P was also inhibitory on the inactivation of phosphorylase by trypsin, while glucose and glucose-6-P abolished its inhibitory effect. We have investigated the effect of F-1-P on the dephosphorylation of ^{32}P-histone and p-nitrophenyl-phosphate /PNPP/. F-1-P inhibited both the dephosphorylation of ^{32}P-histone and PNPP; glucose and glucose-6-P did not influence the effect of F-1-P.

According to our results the inhibition of phosphatase by F-1-P results in the accumulation of active phosphorylase. This effect of F-1-P is specific and the inhibition is not influenced by other metabolites.

THE ROLE OF GLUCOSE, AMP AND POLYAMINES IN THE CONTROL OF GLYCOGEN METABOLISM

Á. Illés, B. Tóth, Institute of Medical Chemistry, University Medical School Debrecen, H-4026 Debrecen, Bem tér 18/b

Glycogen phosphorylase /EC 2.4.1.1/, key enzyme of glycogen metabolism, exists in two interconvertible forms: catalytically active a and inactive b. Phosphorylase a /phosphorylated at Ser-14/ is dephosphorylated by phosphoprotein phosphatase. This reaction is influenced by several ligands inhibiting or activating the rate of dephosphorylation.

We have investigated the AMP inhibition the conversion of phosphorylase a into b and it was found that 5 /uM AMP caused 50% reduction of reaction rate. Physiological concentration of glucose, a well-known activator of the dephosphorylation, slightly decreased the inhibitora effect of AMP. Other ligands tested /glucose-6-P, glycogen, fructose etc./ were ineffective. The inhibition caused by AMP was relieved by 1 mM of caffeine.

Polyamines, in the concentration occuring in vivo, inhibited the dephosphorylation of phosphorylation of phosphorylase a. Combination of glucose with polyamines resulted in total reversal of AMP inhibition. It seems that polyamines are those naturally occuring ligands which can control the intracellular level of phosphorylase a through the opposite action of AMP and glucose.

POSSIBLE REGULATORY MECHANISMS OF TRANSGLUTAMINASE ACTIVITY IN CELLS: ROLE OF CA^{2+}, CALMODULIN AND PHOSPHOLIPIDS

L. Fésüs, A. Horváth, H. Muszbek, Dept. Clinical Chemistry Univ. Sch. Med. Debrecen, H-4012 P.O.Box 40

Transglutaminases catalyze an acyl-transfer reaction /with the concomitant release of NH_3/ between peptide bound glutamine residues and primary amines including the ε-amino group of lysine residues in appropriate peptides. In the latter case an isopeptide bond called ε-/γ-glutamyl/ lysine is formed. Though transglutaminase activity has been demonstrated in the cell membrane, cytoplasma and nucleus of mammalian cells, its function is not known.

We have purified cellular transglutaminase from guinea pig liver and measured its activity either by the determination of ^{14}C-putrescine incorporated into casein or using our recently developed kinetic assay in which the released NH_3 is continuously monitored by a GLDH-NADH system.

The purified enzyme requires at least 0.5-1.0 mM Ca^{2+} for its catalytic activity, a Ca^{2+} concentration far above the steady state level found intracellularly. Since transglutaminase activation usually follows a Ca^{2+} influx into the cells, the multifunctional Ca^{2+}-binding regulatory protein, calmodulin, was a possible candidate to mediate Ca^{2+} action. However, we could not find a complex formation between brain calmodulin and transglutaminase and the enzyme activity was not modified by calmodulin under any circumstances.

After addition of natural phospholipids to transglutaminase, a concentration-dependent inhibition was observed reaching a 70 per cent inhibition of original activity as a maximal effect. When the addition of Ca^{2+} to the enzyme preceded that of phospholipids, the inhibition was the same. Neither tritonization nor freezing and thawing could release the enzyme from the influence of phospholipids.

CHANGE IN MOLECULAR FORMS OF ACETYLCHOLINESTERASE IN EXPERIMENTAL ALLERGIC NEURITIS

Z. Rakonczay, G.K. Molnár[1], P. Kása, Central Research Laboratory, Medical University, H-6720 Szeged, Somogyi u. 4. and [1]Department of Neurology, University of Turku, SF-20520 Turku 52

The morphological and clinical symptoms of the human Guillain-Barré syndrome /GBS/ can also be induced experimentally in rabbit, and the aetiology and pathomechanism of the disease can be studied.

In our research work on this subject we examined the changes in the molecular forms /4S, 6S, 10S and 16S/ of acetylcholinesterase /AChE: EC 3.1.1.7/ in the central and peripheral nervous systems of rabbits with experimental allergic neuritis /EAN/.

It was found that in the regions studied /spinal cord, ventral and dorsal roots, spinal ganglia, n. ischiadicus/, the quantity of 10S AChE is significantly /$p < 0.05$/ decreased, while that of the soluble 4S form is considerably elevated, in the motor axons starting from the ventral horn of the spinal cord,whereas elsewhere /e.g. spinal ganglia/ the various forms of AChE /4S, 10S, 16S/ all display a decreased activity.

The results led us to assume that in the course of the demyelinization in experimental allergic neuritis the proteolytic enzymes primarily exert their effect on the 10S AChE localized on the surface of the axon.

INFLUENCE OF INDUCTION ON FUNCTIONAL COOPERATION OF LIVER MICROSOMAL MONOOXYGENASE ENZYMES

B. Schoket, I. Vincze, Department of Biochemistry, National Institute of Hygiene, H-1966 Budapest, Gyáli-u 2-6.

Microsomal cytochrome P-450-linked monooxygenase system plays a major role in the metabolism of chemical carcinogens and of other xenobiotics. 3-methylcholanthrene and phenobarbital were given intraperitoneally to male Wistar rats. Time course and dose dependence of the induction was examined by measuring quantity of cytochrome P-450 and cytochrome b_5 as well as activities of NAD/P/H-dependent flavoproteins in liver microsomal cytochrome P-450 linked monooxygenase system. Composition of the supramolecular complex is changed by the inducers: induction of cytochrome b_5 as well as of NAD/P/H dependent reductases is quantitatively different from the induction of cytochrome P-450 both in time dependence and in their proportions. Results obtained by measuring benzo/a/-/pyrene monooxygenase activity of the supramolecular enzyme system in the presence of NADPH and NADH, resp. indicate that NADPH-dependent electron transfer through cytochrome P-450 and NADH-dependent electron transfer through cytochrome b_5 must be in interaction with each other. Our experimental results suggest that the composition of the multienzyme complex is modified during induction, and the functional cooperation of the components is also modified simultaneously.

PREPARATION AND PROPERTIES OF IMMOBILIZED HORSE SERUM CHOLINESTERASE

K. Kovács, B. Szajáni, L. Boross[1], Reanal Factory of Laboratory Chemicals, H-1441 Budapest 70, P.O.Box 54 and [1]Department of Biochemistry, Attila József University, H-6701 Szeged, Középfasor 52

Experiments have been carried out to prepare immobilized forms of horse serum cholinesterase /EC 3.1.1.8/ by covalent attachment to polymeric supports. We have found that the cholinesterase could be immobilized most effectively on a polyacrylamide type matrix having carboxylic functional groups activated by water-soluble carbodiimide. The activity of immobilized enzyme was about 100 units/g solid. As an effect of immobilization the pH optimum for catalytic activity was shifted from pH 7.6 to pH 8.0. The apparent optimum temperature for the catalytic activity of the immobilized form was nearly identical to that of the soluble cholinesterase. The substrate specificity was not significantly altered by the immobilization. With butyrylthiocholine iodide as substrate, the $K_{m,app}$ value was markedly lower than that of the soluble enzyme. The immobilization did not improved significantly the heat stability of the enzyme. Heat inactivation of immobilized cholinesterase appeared to be a complex process of at least two separate steps.

Immobilized cholinesterase has a potential application in the environmental /water and air/ pollution control as a functional part of monitoring systems based on its susceptibility towards organophosphate, organosulfate and carbamate types of insecticides.

EFFECTS OF IMMOBILIZATION ON THE CATALYTIC PROPERTIES AND STABILITY OF STARCH-DEGRADING ENZYMES

K. Ivony, G. Kalmár, B. Szajáni, Reanal Factory of Laboratory Chemicals, H-1441 Budapest 70, P.O.Box 54

Two industrially important starch-degrading enzymes, glucoamylase /EC 3.2.1.3/ and cyclodextrin glycosyltransferase /EC 2.4.1.19/ have been immobilized by covalent attachment to a synthetic polymeric support possessing polyanionic character. It offered an opportunity to investigate the effects of immobilization to the same type of support on the catalytic properties and stability of different enzymes. The pH optimum for catalytic activity of immobilized glucoamylase was relatively close to that of the soluble enzyme. However, in the case of cyclodextrin glycosyltransferase the pH optimum was shifted to acidic direction, from pH 5.9 to pH 5.4, as a result of immobilization. The apparent optimum temperature for the catalytic activity of immobilized glucoamylase was found at 60°C that is about 5°C lower than that of the soluble enzyme. The immobilized cyclodextrin glycosyltransferase showed a broad apparent optimal temperature range between 40°C and 60°C whereas the soluble enzyme had a relatively sharper maximum around 60°C. In spite of the hydrophylic microenvironment, the conformational stability of glucoamylase was not improved by the immobilization. On the contrary, cyclodextrin glycosyltransferase showed a significantly enhanced heat stability in its immobilized form.

Our data demonstrate clearly that the enzyme structure has a definite role in the modification of catalytic properties and stability caused by immobilization process.

ISOLATION AND CHARACTERIZATION OF STREPTOMYCES FRADIAE ALDOLASE

L. Kandra, P. Nánási, Institute of Biochemistry, Kossuth Lajos University, H-4010 Debrecen, P.O.Box 55

Aldolase from Streptomyces fradiae was purified by fractionation with ammonium sulfate and gel filtration on Sephadex G-150 colum. The purified enzyme was homogeneous on electrophoresis. The enzyme catalyzes the cleavage of fructose-diphosphate and its activity was measured by the determination of the liberated triose phosphates using chemical methods.

According to the results it seems that Streptomyces fradiae aldolase differs from muscle aldolase with respect to mechanism of action and general characteristics. In the presence of substrate it cannot be reduced by $NaBH_4$ but can be inhibited with EDTA. Therefore, metal ions may play an important role either in the structure or mechanism of action of the enzyme. Monovalent cations increase the activity, thus according to the above properties the enzyme may be included in class II of aldolases.

The aldolase from Streptomyces fradiae exhibits increased sensitivity toward K^+ ions. Heat treatment results in desensitization. In the absence of K^+ ions the enzyme loses its activity on freezing or freezee-drying.

The kinetic behaviour of the enzyme differs from the Michaelis-Menten type reaction and it shows a sigmoidal saturation curve. Its unusual kinetic properties refer to an allosteric enzyme.

INACTIVATION OF CARBONIC ANHYDRASE WITH VITAMIN B_6 IN VITRO

A. Csiba, Department of Medicine and Clinical Pharmacology, Municipal Hospital Péterfy, H-1441 Budapest, Péterfy Sándor u 14

Carbonic andhydrase /EC.4.1.1.2/ has a molecular weight of about 29 000 and contains one zinc atom per molecule. This enzyme catalyzes mainly hydration of CO_2 and dehydration of carbonic acid or bicarbonate ion. Both human carbonic anhydrases B and C are inhibited by monovalent anions, by acetazolamide, and by alcohols.

Human carbonic anhydrase was prepared by the method of Armstrong et al. /1966, J. Biol. Chem. 241 5137/. The preparation did not contain haemoglobin as contamination. Carbonic anhydrase isoenzymes have not been isolated. Enzyme activity was assayed as the esterase action following the hydrolysis of p-nitrophenyl acetate /Whitney et al. 1967, J. Biol. Chem. 242, 4212/. The procedure is as follows: 54.3 mg of p-nitrophenyl acetate was dissolved in 3 ml of acetone; water was added slowly with vigorous stirring to a final volume of 100 ml /3 mM p-nitrophenyl acetate/. This solution was prepared freshly each day. In a spectrophotometric cell, 1,0 ml of this solution was mixed with 1.9 ml 0.1 M triaethanolamine buffer /pH = 7.0/ and 0.1 ml enzyme - inhibitor mixture /1 : 1 v/v/, with rapid stirring /test system/. The increase in absorption was measured at 405 nm in a Specord UV-VIS /Zeiss/ spectrophotometer at 25°C for 5 min. Blank: the same mixture, without enzyme. Activity of the enzyme preparation was 73.47 U/M protein. Inhibitor constants of Vitamin B_6 and acetazolamide were determined according to Dixon. The calculated k_i were 1.01×10^{-4} M /Vitamin B_6/ and 8.20×10^{-8} M /acetazolamide/. The inhibitions were noncompetitive. Vitamin B_6, a chelating agent, reversibly binds Zn^{2+} and other divalent cations.

THE SPECIFIC NATURA OF DRUG BINDING SITES AT HUMAN SERUM ALBUMIN

M. Simonyi, I. Fitos, Central Research Institute for Chemistry, Hungarian Academy of Sciences, H-1525 Budapest, P.O.Box 17

Despite of widespread views regarding the most abundant protein is plasma as exhibiting non-specific binding properties, several lines of evidence point to the specific character of albumin binding sites:

- the binding of endogenous compounds /e.g. lipids, tryptophan, bilirubin/ to albumin is of physiological significance;
- saturation can be observed in some cases of high affinity binding;
- several drugs compete for albumin binding sites with endogenous compounds or with other drugs;
- the binding of certain types of chiral compounds is stereoselective.

The specific binding of drugs to albumin has specific implications in the medical treatment.

i/ Administration of certain drugs may be counter-indicated by the physiological state of the patient.

ii/Combined application of drugs leads to interactions at the level of serum binding that may be of clinical importance.

iii/ Stereoselective binding of racemic drugs to albumin results in stereoselective distribution.

Examples are given to illustrate some of the above points.

BINDING OF REVERSIBLE AND IRREVERSIBLE LIGANDS TO RAT BRAIN OPIATE RECEPTOR/S/

M. Szücs, G. Tóth, S. Benyhe, P. Zeman, M. Wolleman, J. Szécsi[1], K. Medzihradszky[1], Inst. of Biochem. Biol. Res. Center of the Hung. Academy of Sciences, H-6701 Szeged, P.O.Box 521 and [1]Central Research Institute for Chemistry of the Hungarian Academy of Sciences, H-1525 Budapest,P.O.Box 17

Pharmacological and biochemical evidences support the existence of multiple opiate receptors. However, the correlation of the biochemically demonstrated high- and low-affinity binding sites and the pharmacologically defined receptor subtypes / μ, δ, κ / is not known yet.

In the present report the characteristics of ^{3}H-naloxone binding are examined in rat brain membrane preparation. Both at the high- and at the low-affinity site morphine and Leu--enkephalin competed potently for ^{3}H-naloxone binding sites. A hydrazone derivative of naloxone, naloxazone showed even a somewhat higher affinity than naloxone, but the phenylhydrazone derivative proved to be a much less effective competitor havin an IC_{50} of 5 /uM.

In experiments where membranes were preincubated with ligands and extensively washed, 10^{-5}M naloxazone caused a 44%, naloxone-phenylhydrazone a 23% irreversible inhibition of the high affinity ^{3}H-naloxone binding. Similar results were found with the chloromethyl derivative of D-Ala^{2}-Leu^{5}-enkephalin. Detailed analysis of the ^{3}H-saturation curves shows that in the presence of 10^{-5}M enkephalin-chlorometyl ketone the high affinity site is selectively blocked. Experiments in which the membranes were preincubated in the presence of both naloxazone and enkephalin chloromethyl ketone revealed that the two compounds might act partly through the same population of binding sites.

PREPARATION OF 1,7,8-^{3}H-DIHYDROMORPHINE OF HIGH MOLAR ACTIVITY

G.Tóth, F. Sirokmán, M. Szücs[1], S. Benyhe[1], Isotope Laboratory of BRC, H-6701 Szeged, P.O.Box 521, and [1]Institute of Biochemistry BRC, H-6701 Szeged, P.O.Box 521

Tritiated morphine derivatives of high molar activity have played central role in revealing the mechanism of opiate receptor-ligand interactions. The most generally used alkaloide type opiate receptor agonist, dihydromorphine is available at high molar activity, but the preparation is made in two tritiation steps. Our aim was to synthesize multiple labeled tritiated dihydromorphine of very high molar activity in one step.

1-Iodocodeine was prepared by the reaction of codeine, chloramin T and NaI according to Liebman et. al /J.Org. Chem. 43/4/737/1978/. 1-Iodomorphine formed by demethylation of 1-iodocodeine with boron tribromide was a good precursor for the preparation of 1,7,8-^{3}-dihydromorphine. 1-Iodomorphine was tritiated with tritium gas, using PdO catalyst. The pure material was isolated from the reaction mixture by TLC. Beside 1,7,8-^{3}H-dihydromorphine which was the main product, 1-^{3}H--morphine was also formed in 3%. The two products could be separated from each other. The specific activities were 67,9 Ci/mmol and 19.1 Ci/mmol, respectively. The products were homogeneous in 3 different TLC systems.

The potency of the labeled ^{3}H-dihydromorphine was tested in displacement experiment performed in rat brain membrane preparation. At 1 nM ^{3}H-DHM concentration the unlabelled morphine showed an IC_{50} of 2 nM, which is in a good agreement with data in the literature.

INHIBITION OF VARIOUS BETA-LACTAMASES BY PENICILLANIC ACID DERIVATIVES

L. Benesch, J. Remportné-Rádóczi, I. Stadler, G. Kovács, J.Cs. Jászberényi[1], F. Hernádi[2], CHINOIN Pharmaceutical Works, H-1325 Budapest, P.O.Box 110, [1]Research Group of Antibiotics of the Hungarian Academy of Sciences, L. Kossuth University H-4010 Debrecen, P.O.Box 22, [2]Department of Pharmacology, Chemotherapy Section, University Medical School Debrecen, H-4012 Debrecen, P.O.Box 12

We have studied the beta-lactamase inhibiting activity of some penicillanic acid/PA/ derivatives [6- β -bromoPA/I./; 6- β -iodoPA /II./; PA 1,1 dioxide/III./; 6- ∝ -bromoPA 1,1-dioxide /IV./; 6- ∝ -chloroPA 1,1-dioxide /V./] on beta--lactamases of different origin. Enzyme activity measurement was carried out by a modification of the method of O'Callaghan et al. /1972, Antimicrob.Ag. Chemother. 1 283 /. The kinetic parameters of the inhibitory effects of the compounds were determined. The kinetic constants of beta-lactamase I. produced by Bacillus cereus in case of Nitrocefin substrate were K_m = 83.3 /uM; V_m = 40 /uMmin^{-1}. The enzyme inhibiting potency of the tested compounds, assessed on the basis of 50% inhibition, was /in decreasing order/ as follows: II. = 4; III. = 20; I. = 40; IV. = 430; V. = 550 /uM. The same values obtained for the compounds with beta-lactamase produced by Escherichia coli J53 RP4 were: K_m = 111 /uM; V_m = 140 /uMmin^{-1}; the I_{50} of the compounds were: II. = 0.5; III. = 7; I. = 100; IV. = 500; V. = 600 /uM. On the basis of the kinetic parameters studied, the tested compounds showed competitive or partial competitive inhibition of the beta-lactamases tested.

BIOTRANSFORMATION OF ACETYL-SECONDARY GLYCOSIDES IN DIGITALIS LANATA LEAF DICS

B. Lenkey, P. Nánási, P. Tétényi[1], Institute of Biochemistry L. Kossuth University, H-4010 Debrecen, P.O.Box 55 and [1]Research Institute for Medicinal Plants, H-2011 Budakalász, P.O.Box 11

The transformation of lanatoside glycosides /Nánási et al., 1980. Acta Horticulture 96 57/ and that of secondary ones /Lenkey et al. 1981, Planta Medica 43 409/ were studied in our previous investigations using a vacuum infiltration technique on dics of leaves of Digitalis lanata. Both lanatoside glycosides and secondary ones were transformed into each other as well as into other glycosides. Transformations between the glycosides of digitoxigenin and those of digoxigenin were very rapid. 16 β hydroxilation was also shown /it needs formation of gitoxigenin and its glycosides/ when we studied the transformation of lanatoside A and C or digitoxin and digoxin.

Transformations of acetyl-secondary glycosides were also studied. By the use of vacuum infiltration technique, we applied the ^{14}C-labelled precursor glycosides biosynthesized from ^{14}C-progesterone using intact Digitalis lanata plants. Glycosides of digitoxigenin and digoxigenin were synthesized from both acetyl-digitoxin and acetyl-digoxin with the same intensity and the synthesis of glycosides of gitoxigenin from these glycosides was very slow. Glycosides of gitoxigenin were synthesized very quickly when the acetyl-gitoxin have been the precursor and at this time the formation of glycosides of digitoxigenin and digoxigenin were equivalent and slower than synthesis of glycosides and gitoxigenin. Transformations of acetyl-secondary glycosides into secondary glycosides were more intensive than into lanatoside ones.These experiments also indicated that the formation and metabolism of glycosides of gitoxigenin follow a pathway partly different from those of other glycosides.

RADIOCHROMATOGRAPHY OF DEPRENYL METABOLITES

L. Tóthfalusy, K. Magyar, H. Kalász[1], Department of Pharmacodynamics Semmelweis University of Medicine Budapest, H-1089 Budapest, Nagyvárad tér 4 and [1]Department of Pharmacology Semmelweis University of Medicine Budapest, H-1089, Budapest, Nagyvárad tér 4

Deprenyl /phenyl-isopropyl-methyl-propinylamine/ was labeled with radiocarbon. Rats were treated with 5 mg/kg deprenyl by i.v. administration and their urine was collected for 24 hours. Urine samples were hydrolyzed at pH = 1 and by 6 N HCl. The samples were prepurified by gel chromatography on Sephadex G-15 gel column using 0.01 N HCl as eluent. The radioactive fractions were collected and deprenyl metabolites were analysed by HPLC on silica gel column using a "dynamic reverse phase system".

Five radioactive metabolites were found, one of them has amphoteric characteristics which can be transformed into a polar basic product by strong acidic /6 N/ hydrolysis.

Three other products have weak basic character and occur in non-conjugated form. Further analysis and identification of the products by thin-layer chromatography is in progress.

METABOLISM OF 1,3-DIPHENYL-ISOPROPYLAMINE /DIPA/ IN RATS

M. Pálfi-Ledniczky, K. Jemnitz, J. Szammer, L. Ötvös,
Central Research Institute for Chemistry of the Hungarian Academy of Sciences, H-1525 Budapest, P.O.Box 17

1,3-diphenyl-isopropylamine /DIPA/ was used as modell compound to study the activity of mixed-function mono-oxygenase system of liver, so it was important to learn the in vivo metabolism of DIPA.

DIPA and its metabolites are excreted within a few days time. The urine /0-24 hr/ contain 50% and feces 25% of the dose applied, in the bile /0-5 hr/ 10% of the administered compound appeared. 90% and 98% of DIPA and its metabolites are present in conjugated form, mostly as glucoronides, in urine and bile, respectively. The metabolites were separated by TLC, identified by GC, GC-IR and GC-MS.

On the base of the structure of the metabolites excreted we might propose a metabolic pathway for DIPA. The major metabolite is a compound hydroxylated in the aromatic ring, the minor metabolites are formed by deamination. The rate of the in vivo hydroxylation and deamination of the aromatic ring is in agreement with the results of in vitro experiments studying the formation of MI complex and NADH consumption.

ENZYMATIC CLEAVAGE OF DEOXYURIDINE ANALOGUES BY PYRIMIDINE NUCLEOSIDE PHOSPHORYLASES

Zs. Veres, I. Szinai, A. Szabolcs, G. Dénes, L. Ötvös, A. Jeney[1], Centralt Research Institute for Chemistry, Hungarian Academy of Sciences, H-1525 Budapest, P.O.Box 17., and [1]First Institute of Pathology, Semmelweis Medical University, H-1085 Budapest, Üllői u 26

It was found that some 5-substituted derivatives of 2'-deoxyuridine have antiviral /5-ethyl,-isopropyl,-bromovinil-2'-deoxyuridine/ or antitumor activity /5-hexyl-2'--deoxyuridine/. The appropriate 5-alkyl pyrimidine bases have no, or very weak biological activity. Therefore the cleavage of the N-glycosidic bond by pyrimidine nucleoside phosphorylases in these nucleosides results in losing the biological activity of the parent compounds.

The investigation of the phosphorolysis of nucleosides as a function of chemical structure may lead to the better knowledge of the substrat specificity and designing more potent drugs.

We have studied the cleavage of nucleosides by enzymes isolated from mouse liver, rat intestinal epithelium and NK/Ly ascites cells. The enzyme assay was carried out according to the method of Yamada.

The cleavage rate of 5-bromovinil-2'-deoxyuridine is similar to that of 2'-deoxyuridine. The rate of phosphorolysis of 5-alkyl-2'-deoxyuridines is related to the length of the side chain in position 5. The longer the side chain is the slower the rate of reaction. The rate of enzymatic cleavage of nuceloside analogues containing branching side chain is hardly or not detectable in our system. 5-alkyl-2,2'-anhydro--deoxyuridines are not substrates of these enzymes.

METABOLISM OF 5-ETHYL-2'-DEOXYURIDINE AND 5-HEXYL-2'-DEOXYURIDINE IN MICE

I. Szinai, Zs. Veres, M. Bihari, V. Kemény, A. Szabolcs, L. Ötvös, Central Research Institute for Chemistry, Hungarian Academy of Sciences, H-1525 Budapest, P.O.Box 17

Biotransformation of 2-^{14}C-labelled 5-ethyl-2'-deoxyuridine /EdU/ and 5-hexyl-2'-deoxiuridine /HdU/ was investigated in healthy and NK/Ly ascites tumorous mice. It was decribed that EdU has antiviral activity and HdU is active against Ehrlich and NK/Ly tumours and Novikoff hepatoma. Since 5-ethyl- and 5-hexyl-pyrimidine bases do not show any biological activity, the study of metabolism of EdU and HdU seems very important.

The metabolites were isolated by extraction, gel and adsorption chromatography from plasma, liver and ascites fluid, and identified by mass spectroscopy and infrared spectorscopy. We identified 5-ethyl-uracil as the only metabolite of EdU in liver and ascites fluid of tumour bearing mice. In healthy animals 5-ethyl-uracil and a methylated 5--ethyl-uracil were found. The cleavage of N-glycosidic bond /biotransformation of nucleoside to base/ were followed by TLC and measuring of radioactivity of these compounds. Decomposition of HdU was faster in the plasma of control animals than in tumorous mice. In addition to 5-hexyl-uracil as a main metabolite of HdU we identified the hydroxylated and the methylated bases and nucleosides as minor metabolites. The derivatives of HdU mentioned above were not detectable in healthy animals. We have observed that the biotransformation of EdU and HdU varied essentially from the catabolism of uridine and thymidine. Investigation of metabolism of nucleoside analogues might help to understand their biological activity and the design new drugs.

INFLUENCE OF THE FORMATION OF STEROID-CYCLODEXTRIN COMPLEXES ON THE EFFICIENCY OF STEROID BIOCONVERSIONS

E.N. Udvardy, G. Hantos, Zs. Vida, Chemical Works of Gedeon Richter, Microbiological Research Laboratory, H-1475 Budapest 10, P.O.Box 27

In the processes of steroid bioconversion the low solubility of substrate or products can be a limiting factor in increasing advantageously the concentration of the substrate and enhancing the rate of the reactions. Cyclodextrins can form inclusion complexes of different composition and stability with steroid compounds and so they can increase considerably the solubility of steroids in the aqueous media. The β-cyclodextrin is capable to influence the reactions of steroids in bioconversion processes by the following ways:

- resulting a higher solubility of steroids
- accelerating the dissolution rate of steroids present in solid particles
- preventing the formation of complexes of steroids with the microbial cells or enzymes which could interfere with the bioconversion
- restricting or stimulating some of the concomitant reactions in the bioconversion system.

Examples will be presented to illustrate the usage of β-cyclodextrin in microbial dehydrogenation of hydrocortisone and methyl-testosterone and hydroxylation of cortexolone derivatives.

FORMATION OF DIHYDROXY-CARDENOLIDE WITH STREPTOMYCES PURPURASCENS

K. Albrect, Gy. Máté, N.E. Udvardy[1], T. Láng, Institute for Drug Research H-1325, P.O.Box 82 and [1]Microbiological Research Laboratory Gedeon Richter Chemical Works Ltd., H-1475 Budapest, P.O.Box 27

Digitoxin is hydroxylated with Str. purpurascens into 12β and 7β -monohydroxydigitoxin and into 7β , 12β -dihydroxydigitoxin. The dihydroxy compound was formed only via 7β hydroxydigitoxin.

To explain the phenomenon we propose a mechanism based on Brannon's and Kieslich's postulates for enzymatic steroid hydroxylation and on measurements on Dreiding's molecular model of cardiac aglycon.

The fixation possibilities of cardenolide on an enzyme surface and the agreement of the proposed mechanism with the observations of other authors are discussed.

MIKROBIAL TRANSFORMATION OF CARDIAC GLYCOSIDES

Gy. Máté, K. Albrecht, N.E. Udvardy[1], T. Láng, Institute for Drug Research, H-1325 P.O.Box 82 and [1]Microbiological Research Laboratory, Gedeon Richter Chemical Works Ltd. H-1475 Budapest, P.O.Box 27

Five of 1000 Streptomyces isolated - identified as Streptomyces purpurascens - was able to transform Lanatosid-A to digoxin.

The medium used for culturing the strain contained 2% groundnut meal and 3% glucose and its pH was adjusted to 7.0 prior to sterilization. Temperature was 32°C both for growth and for fermentation. The substrate - 1-5 g per litre of medium - was introduced as a methanolic solution. The transformation was monitored by TLC.

Besides digoxin we found 7β -OH-digitoxin and 7β -OH--digoxin too. We detected purpurea-A, acetyldigitoxin, acetyldigoxin and digitoxin as intermediates. All of them proved to be precursors of digoxin and 7β -OH-compounds.

The activities of the enzymes participating in the transformation were decreasing in the following order: deacetylase, β-glycosidase, hydroxylase.

The elaborated industrial procedure yields o.5 gl^{-1} digoxin in a five-day fermentation.

IMPROVEMENT OF CLAVICEPS PURPUREA BY MUTAGENIC TREATMENT OF PROTOPLAST

K. Olasz, T. Gaál, K. Zalai, Gedeon Richter Chemical Works Ltd., Microbiological Research Laboratory, H-1475 Budapest, P.O.Box 27

For a long time it has been a problem whether heterokaryotic state is a necessity for alkaloid productivity of Claviceps purpurea.

The conclusion has a fundamental effect on the way by which the productivity of that strain can be increased. The efficiency of mutagenic program is higher if there is some alkaloid production in homokaryotic state too, but if the producing strain is necessarily heterokaryotic to be able to produce we have to use in vivo recombination methods besides mutagenesis.

To investigate this question we selected uninucleated protoplasts of C.purpurea MNG 0088 strain on the basis of their size.

The colonies regenerated from these protoplasts didn't differ from the parental strain in productivity so we concluded that the alkaloid production of this strain doesn't depend on the heterkaryotic state. Consequently we chose mutagenic treatment to improve this strain.

Purposefully we treat the smaller protoplasts of the new homokaryotic strain with physical and chemical mutagenic agents, so we can expect the promt expression of any mutation occured.

Protoplasts are more sensitive to some mutagens used than intact cells and even at the same survival rate the distribution curve of their alkaloid production measured in submerged cultures reveals wider scattering for the treated protoplasts than for treated intact cells, because in the latter case complementation can always occur among nuclei.

IMMOBILIZATION ON AKRILEX GELS THROUGH BENZOQUINONE

M. Kálmán, L. Boross, A. József University, Department of Biochemistry, H-6701 Szeged, P.O.Box 533

In enzyme immobilization and affinity sorbent preparation techniques the activation of supports is of crucial importance. Reagents used have to form stable bonds and little nonspecific adsorption. Supports have to be stable chemically and mechanically, and resistant to attack by enzymes. Low nonspecific adsorption is another important requirement. Taking into account the requirements mentioned above we preferred the utilization of polyacrylamide gels for immobilization. A new method had been developed for the activation of polyacrylamide gels in our laboratory. The activation by benzoquinone is a relatively simple and inexpensive way to produce an active support being capable for coupling ligands having $-NH_2$, -OH or -SH groups. The optimal conditions for the activation of Akrilex gels by benzoquinone were elaborated. The binding capacity of activated gels were tested using bovine serum albumin. Under optimal conditions 1 g of dry activated gel could bind about 60 mg protein from the bovine serum albumin solution of 20 mg/ml. The coupling of small ligands /glucose and γ-amino butiric acid/ and some enzymes were also studied. 1 g of dry activated gel could bind about 50 /umol small ligand. The coupled amounts of glyceraldehyde-3-phosphate dehydrogenase, aldolase, glucose, oxidase, catalase and trypsin were similar to that of bovine serum albumin. The remaining activity of the coupled enzymes depended strongly on the type of the enzymes.

A SHORT CARACTERIZATION OF A METHANOL UTILIZING MIXED BACTERIUM CULTURE ORIGINATING FROM BOVINE GASTRIC SYSTEM

A. Krámli, Institute of Medical Chemistry, Medical University Szeged, H-6720 Szeged, Dóm tér 8

We investigated a methanol utilizing mixed culture of microorganisms, originating from bovine gastric system with respect to steroid transformation, the possibility of completing with overculturing protozoa, and technical problems of the protein product. Now we report the results achieved in 1978-80.

We have isolated the mixed culture from bovine rumen and reticulum in a nutritive solution, containing 5 to 10% methanol. This was re-cultured with 2 to 3% methanol in large amounts. We isolated several strains from the mixed culture, and collected data concerning their reproduction at rising methanol concentrations.

Five different types of cultures were caracterized morphologically. Four of them can grow at a methanol concentration higher than 3%, and one tolerates methanol but shows no growing. We have also found some other strains which utilize or tolerate methanol.

In the mixed cultures several sorts of strains live together, forming a stable, resisting culture. Stability is proved by well characterizable individual properties. On the basis of the above-discribed observations, investigation into the balance of the mixed bacterium cultures in general seems to be desirable, too. Similarly, it is not desirable to revive the investigations into own antibiotica and redox-potential.

LYSINE FERMENTATION ON HYDROL

B. Janzsó, J. Végh[1], Budapest Technical University, H-1521 Budapest, Gellért tér 4; and BIOGAL Pharmaceutical Works, H-4042 Debrecen, Pallagi u 13

There are two basic factors of an economical lysine fermentation: a well producing strain and a cheap source of raw materials. The aim of our investigations was to achieve both of these conditions. In the beginning we were in the possession of a polyauxotrophical strain. We succeeded to improve its productivity by the development of its amino-ethylcysteine resistance. Nitrosoguanidine and UV-rays were used as mutagen factors. The productivity of the new strain was increased by about 30-40%. The possible carbon sources in the industrial fermentative lysine production are: molasses /sugar-beet, sugar-cane/, polysaccharide-hydrolysates /hydrol/, alcohols /methanol, ethanol/, organic acids /acetic acid/ and hydrocarbons /paraffines/. As far as Hungary is concerned the molasses and hydrol are the cheap ones. The results with hydrol proved to be very promising. At first the optimal composition of the broth for the lysine fermentation was determined: hydrol 20%, corn steep liquor 5%, $/NH_4/_2SO_4$ 3.5%, K_2HPO_4 0.05%, KH_2PO_4 0.05%. We are able to obtain a lysine level between 60-100 g/l in a laboratory fermenter of 5 litres working volume. The best result was 160 g/l when the carbon source was given subsequently. In the most favourable case even a conversion of 50% could be reached. At present we are dealing with the scale-up problem: the best result we could reach till now in a 60 l fermenter was 50 g/l/60 hours. In Hungary hydrol is a by-product of the crystalline dextrose production. We continue the experiments at present with molasses.

CHANGES IN THE RATE OF ENZYMATIC BROWNING, POLYPHENOL OXIDASE ACTIVITY, O-DIHYDROXYPHENOL CONCENTRATION AND PEROXIDASE ACTIVITY DURING AFTER-RIPENING OF APRICOTS

I. Gajzágó, V. Nádudvari-Márkus, L. Vámos-Vigyázó, M. Kerék[1]
Central Food Research Institute, H-1525 Budapest, P.O.Box 76
and [1]Cegléd Research Station of the Research Institute of Fruits and Ornamental Plants, Budapest

In experiments carried out for four years eith ten apricot cultivars, the values of polyphenol oxidase /PPO/ activity and o-dihydroxyphenol /ODP/ concentration showed extremely great variations both with the year and with the cultivar. It was assumed that changes in the state of maturity occurring during the investigations might be the reason of the variations.

Samples of two apricot cultivars picked at canning maturity were, therefore, after-ripened for 2 weeks at 5°C in normal atmosphere and PPO as well as peroxidase /POD/ activities, ODP content and the rate of enzymatic browning /BA/ were determined right after picking and after one and two weeks of storage.

At picking, the values of all the parameters except BA were similar in the two cultivars. PPO activity showed but minor fluctuations during storage, ODP content increased slightly in one of the two cultivars and remained at the initial level in the other. POD activity increased 3.5 and 7fold, respectively, while BA decreased to 33% and 23% of the initial value.

POD activity has been found to increase also during the storage of apples and carrots. However, the changes in the values of PPO activity and ODP content were slight as compared to those found in apricots. Thus, the latter cannot be accounted for by changes in the state of maturity.

CHROMATOFOCUSING METHOD FOR THE PURIFICATION AND SEPARATION OF CELLULASE ENZYME COMPONENTS

M. Szakács-Dobozi, Central Food Research Institute, H-1525 Budapest, Herman Ottó u 15. P.O.Box 75

A cellulolytic enzyme complex produced by microbial fermentation was purified and separated by chromatofocusing in laboratory experiments. The isoelectric points of the components desalted on a Sephadex G-25 column were determined by Flat Bed IEF /Pharmacia/. Since the pI values cover the range pH 9.05-4.4, the separation of the components was accomplished in several steps on a PBE 94 chromatofocusing column. A gradient of pH 9-6 was formed by equilibrating the column with 0.025 M pH 9.4 ethanol-amine - acetic acid as starting buffer and eluting with Polybuffer 96 - acetic acid at pH 6.0. The separated enzyme fraction was the mixture of two components with different pI values, and it had C_x and cellobiase activities. To separate the other components a gradient of pH 6-4 was formed by equilibrating the column with 0.025 pH 6.2 histidine-HCl as starting buffer and eluting with Polybuffer 74 hydrochloric acid at pH 4.0.

The first peak that was not bound out of the separated 10 fractions had all the four activities /FPA, C_1, C_x, cellobiase/, four other peaks were found to contain mostly C_x, and low cellobiase activities, one peak had only C_x, and four other fractions had cellobiase activity only.

Further experiments are required with narrower pH gradients to achieve a more complete separation of the components.

UTILIZATION OF AN IMMOBILIZED LACTASE PREPARATION FOR WHEY TREATMENT

V. Nádudvari-Márkus, E. Kiss, Central Food Research Institute, H-1525 Budapest, Herman Ottó ut 15

Whey is a valuable by-product of the dairy industry. Its utilization as food is gaining importance. In order to make it available to lactose intolerant individuals /about 10-15% of the Hungarian population/ its lactose content has to be converted to the monosaccharides glucose and galactose. The use of the enzyme β-galactosidase in an immobilized form might reduce the costs of lactose hydrolysis and make the process economical.

A commercial lactase preparation from Aspergillus niger /Lactase LP^{R}, Rapidase, France/ has been immobilized without further purification by adsorption on a phenol-formaldehyde resin /Dia-Prosim, France/ and cross-linking with glutaraldehyde. Conversion was monitored by the measurement of glucose formed using the GOD-Perid Test Combination /Boehringer, Mannheim, FRG/. The influence of flow-rate and temperature were studied.

The solution of Lactase LP was repeatedly used for immobilization as in the first step only about 7% of the activity were bound to the carrier along with 90% of the protein content of the solution. When operating a column /50 cm^3/ of immobilized enzyme at 50°C and 60°C at flow rates of 0.1-9.0 $cm^3 min^{-1}$, an inverse linear relationship was obtained between the degree of hydrolysis and substrate flow-rate. Total conversion was achieved at 60°C at flow-rates below 0.5 $cm^3 min^{-1}$. At 40°C conversion varied according to a maximum curve and maximum degree of hydrolysis /80%/ was achieved at a flow-rate of about 0.5 $cm^3 min^{-1}$. The conversion required for lactose intolerant diets /70-75%/ could be achieved at 60°C at a flow-rate of about 4.5 $cm^3 min^{-1}$.

ENZYME ACTIVITIES OF WHEATS /Triticum aestivum/

A.Párkány-Gyárfás, Central Food Research Institute, H-1525 Budapest, Herman Ottó u 15 P.O.Box 76

Tissue enzymes influence the quality of foods prepared from the milling products of wheat. Amylase is considered to be the quality determining enzyme. According to data from the literature, peroxidase might favorably affect dough properties in breadmaking.

It was, therefore, considered necessary to investigate enzyme activities in samples of identical wheat varieties grown at different locations. We compared the amylase and peroxidase activities of early and mid-season ripening varieties os wheats grown in four regions of the country.

The Phadebas Amylase Test was used to measure amylase activity in the extracts. Peroxidase activity was determined using hydrogen peroxide substrate in the presence of the hydrogen donor o-phenylene diamine.

The investigation of the wheat varieties showed that out of the four locations, the highest amylase and the lowest peroxidase activities were found in the wheat samples from the region of Debrecen. The same varieties grown at Iregszemcse were characterized by low amylase and high peroxidase activities.

It was established that in all four regions peroxidase activities were higher in early than in mid-season ripening varieties. If the peroxidase activity of a variety is high, its amylase activity is, in the majority of cases, low and vice versa.

LIPOIC ACID AND METHIONINE CONTENT OF YEAST

Muayad A. Aly, B. Mátrai, A. Halász, Central Food Research Institute, H-1525 Budapest, Herman Ottó u 15

Mutants with increased levels of sulphur containing amino acids produce smaller colonies on sulphur deficient media than the parental strain do /Okanishi M. et al. 1970, Candia Journal of Microbiol. 16 1139/.

Hiltz et al. /1959, Biochemische Zeitschrift 332 151/ showed that the yeast cell reduces sulfate via several intermediates to hydrogen sulfide. Activation sulfate is reduced to sulfite by the catalytic action of sulfate reductase which contains ∝-lipoic acid as a prostheric group. This means that lipoic acid can be regarded as a cofactor in methionine synthesis. Therefore the relationship between the methionine content and lipoic acid content of some yeast strains was studied. Our results showed that a significant relationship does exist between the two components. Lipoid acid content was higher in yeasts of high methionine content. Aeration and addition of sulphur to the medium increased the concentration of both components.

QSAR-STUDIES ON PHOTOSYNTHESIS INHIBITING 4-HYDROXY-BENZONITRILES

Z. Szigeti, T.Cserháti[1], Department of Plant Physiology, Eötvös University, H-1445 Budapest, P.O.Box 324 and [1]Research Institute for Plant Protection, H-1525 Budapest, P.O.Box 102

It is known that 3,5-disubstituted 4-hydroxy-benzonitriles inhibit the photosynthetic electron transport chain on the reducing side of photosystem II. The herbicidal effectivity of the 3,5-dihalogeno- and the 3-nitro 5-halogeno--4-hydroxy-benzonitriles depends on the halogenic substituents and decreases in the following order: I > Br > Cl. It is not clearly understood, what kind of molecular parameters are responsible for the effectivity order observed.

The inhibitory effect of some substituted 4-hydroxy--benzonitriles on the photosynthetic activity was determined in 8 different in vivo and in vitro systems. To obtain informations about their lipophilicity and adsorptivity under various conditions the thin-layer chromatographic behavior of benzonitriles were studied on reversed phase, on silica gel, on aluminium-oxide and on cellulose at differ ent pH values and ion concentrations.

The benzonitriles - containing highly hydrophilic substituents - have a higher lipophilicity in ionic environment. This phenomenon can be explained by the strong intramolecular forces between the substituents. The good correlation measured between R_M values at pH 5 and 8 proves that in this interval the pH does not affect the relative lipophilicity of the benzonitriles. The relative lipophilicity measured in ionic and ion free environment does not correlate linearly with the biological effectivity. The majority of biological activities correlates rather with the adsorptivity of the compounds. This fact indicates that the adsorption processes influence considerably the biological effectivity, the adsorptive interaction of benzonitriles and the target can be of hydrophilic character.

EFFECT OF MAGNESIUM ON THE RESPIRATION OF PLANTS

S.A. Kiss, J.F. Török, Borsod Chemical Works, Dept. of Agrochemie, H-3702 Kazincbarcika

Respiration rates of different plants /bean, maize, cucumber, sorrel/ were measured by the Warburg apparatus. The experiments were carried out in the dark to eliminate the photosynthesis processes which disturb the respiration measurements.

The treatments of plants were as following: 1./ spraying of plants with 1% $MgSO_4$ solution, and 2./ absorption of this solution by the plant seedlings. The spraying was finished when a thin, continuous layer of solution was formed on the leaves' surfaces. The respiratory measurements were made on cut leaves 24 hours after treatment. For absorption the leaves were kept in the $MgSO_4$ solution for 24 hours. Control plants and leaves were treated with distilled water.

Our results showed a decreasing of 30-40% in respiration rate in plants treated with magnesium. The possible reason of this decrease will be discussed.

EFFECT OF CHANGING THE K/Ca RATIO IN THE MEDIUM ON THE GROWTH OF RASPBERRY CALLUSES CULTURED IN VITRO

M. László, Institute for Fruit and Ornamental Plant Growing, H-9431 Fertőd

The adequate ratio of minerals plays a very important role in the nutrition of plants. The change of the ratio of nutritients influences the activity of phytohormones, thus that of cytokinins too. High K/Ca ratio induces high cytokinin activity in the shoot- and root-meristem /H. Göring et al. 1976, Biologische Rundschau Jg. 14 177/. Relationship between cytokinin content or activity and K/Ca ratio seems important because the former stimulates the synthesis of several substances /protein, betacyanin, lignin/.

The concentration of K and Ca were changed in the medium /T. Murashige et al. 1962, Physiol. Plant. 15 473/ of callus cultures obtained from the shoot tip meristem of raspberry cultivar Malling Exploit. Cytokininfree medium was used because the raspberry callus proved to be cytokinin autotroph /M. László 1981, Proc. 21st Hung. Annu. Meet. Biochem., Veszprém aug.24-27, 149-150./. Calluses were growing at 27^{o}C in the dark. Their wet weight was determined after a growth period of 30 days. Results showed that the optimal interval of K/Ca ratio was between 3 and 10. Maximal growth was experienced at a ratio of 6.5. Since the increase of callus weight was mostly due to cell division stimulated by cytokinins, we may suggest that the change of K/Ca ratio influences the synthesis or activity of cytokinins in raspberry callus-test too.

NEW METHOD TO MEASURE THE AVAILABILITY OF PESTICIDES IN FORMULATIONS

T. Cserháti, É. János, Research Institute for Plant Protection Budapest, H-1525, Herman Ottó u 15

Formulation facilitates the application and enhances the biological efficiency of pesticide molecules. The inert carriers, solvents or dispergants, anionic and nonionic tenzides - often present in pesticide formulation - can accelerate the solution of active ingredients which hardly soluble in water. They can promote the spreading on the surface of the target organisms, the penetration through the lipophilic membranes and the mobility inside the target organisms; that is they can modify availability. We developed a new method to measure the availability of active ingredients in different formulations. The pure and formulated active ingredients were suspended in a 2% warm agarose solution in a concentration of 5 mg active ingredient/g agarose, then the suspension was poured into a glass tube with an internal diameter of 3.7 mm. When the agarose rod hardened it was blowed out of the tube and a piece of 2 mm was cut and weighted. The agarose rod was submerged into distilled water of 5 cm^3 which was changed at definite intervals. The concentration of active ingredient in the aqueous solutions was determined by a suitable method /spectrophotometry, gas chromatography, high performance liquid chromatography/. The logarithm of concentration in the agarose rod was plotted versus diffusion time. The mass transfer coefficient related to the availability can be calculated from the linear part of the plot. A higher mass transfer coefficient means a higher availability. It has been established that the mode of preparation and the ageing and storage of agarose rod has considerably influenced the numerical value of the mass transfer coefficient. The coefficient of variation of the method was found to be under 10%.

ORGANIC MASS OF PLANTS AND THE CONVERSION OF ENERGY

R. Nehéz, Research Institute for Cereal Production, H-6701 Szeged, P.O.Box 391

The base of profitable agricultural production constitutes clear-headed energy-economy, this goes for energy produced by living organisms too.

On basis of biosynthetic courses of vegetal organic matters was initiated of me measuring numbers for the maximal masses are equivalent /only equivalent/ to simple carbohydrate unit /bioeconomical minimal equivalent, 1/ME/ /Nehéz et al. 1971 MTA Agrártud. Közl., 30 229/. The 1/ME value is termed in energy as biochemical energy effectivity in comparison with energy of simple carbohydrate unit /Nehéz et al. 1981 Proc. 21st Hung. Annu. Meet. Biochem. 148-148/.

The mass effectivity and energy effectivity are presented in case of starch 0.90 and 1.00, merely generally of vegetal carbohydrate 0.87 and 0.98, fiber 0.80 and 0.95, oil 0.34 and 0.86, protein /from forms ^{+V}N, ^{+VI}S/ 0.40 and 0.90, just as protein /from ^{-III}N, ^{-II}S/ 0.57 and 1.22, respectively.

These values are employed for solving of several problems of plant genetics and plant breeding.

The greatest yield masses are associated never with their highest oil or protein %-s in practice of the growing. In chemical bonds of oil and protein matters is stored the bounded sun-energy in much less mass as be like in starch. The simultaneous increasing of the highest degree of yields and of oil or protein proportion is limited - with stoichiometrical definiteness of compounds and unavoidable losses of biosyntheses together - by vegetal energetical causes too. Since the energy effectivity of vegetal carbohydrates, oils and proteins are not very different, the increase of energy effectivity is most practicable by increasing of mass-yields.

STRUCTURAL INVESTIGATION OF RADIATION-INDUCED AGGREGATES OF RIBONUCLEASE

Gy. Hajós, H. Delincée[1], Central Food Research Institute, H-1525 Budapest, Herman Ottó u 15 and [1]Institut für Biochemie, Bundesforschungsanstalt für Ernährung, D-75, Karlsruhe

Bovine pancreatic ribonuclease irradiated in solution with ^{60}Co- γ -rays /Mee et al., 1972 Radiat. Res. 52 588/ have been studied in order to investigate the nature of chemical the forces the aggregates are formed by. The aggregates were isolated by thin-layer gel filtration. On the basis of polyacrylamide gel electrophoresis and thin-layer gel filtration with sodium dodecyl-sulfate we have concluded that these aggregates are linked by covalent bonds.

Incubation of the aggregates with 6M urea and 2% mercaptoethanol followed by thin-layer gel filtration showed these covalent bonds to involve both disulfide bridges and non-sulfur containing bonds. On the basis of densitometric measurements the proportion of the latter was found to be about 55 /$\pm$5/ %.

The existence of covalent bonds not containing sulfur was also confirmed by isoelectric focusing /Delincée et al., 1975 Int. J. Radiat. Biol. 28 565/: by this method, definite differences were established between the proteolytic hydrolysates of the reduced aggregates and the reduced monomer of ribonuclease.

PROTEOLYTIC ACTIVITY OF CRUDE PORCINE GUT EXTRACT

A. Halász, I. Szalma-Pfeiffer, Central Food Research Institute Budapest, H-1525 Budapest, Herman Ottó u 15

Fresh porcine gut was minced three times in a meat grinder. Proteolytic activity was determined from the suspension /2% dry weight content/ after a one-hour-extraction at 30°C. The proteolytic activity was measured by the Anson method and expressed in haemoglobin equivalent per gram /$PA_G\ g^{-1}$/.

The effect of ultrasonic treatment was investigated with a Labsonic equipment /300 W/ after treatment for 1x5 min, 3x5 min and 5x5 min, using ice water cooling. The proteolytic activity of the crude extract was in every case lower than that of the minced control sample.

The pH value and ionic strength of the solution used for extraction had a great influence on the proteolytic activity of the crude extract and the specific proteolytic activity as well: in case of 0.9% NaCl solution the optimum was at pH 8.0, and with distilled water, at pH 5.5.

The proteolytic activity of the crude extract was dependent on the concentration of the sample. The original sample showed the lowest activity increased from 9.72 to 24.06 PA_G He g^{-1}.

At a substrate concentration of 1% the pH optimum for the crude extract was at pH 1.5. In the temperature range of 23°C-80°C the best resulrs were obtained at 50°C. The SDS gel-electrophoretogram of the crude sample had three protein bands, their proteolytic activity was investigated according to AOKI et al. /1981/.

DIFFERENTIATION MARKERS OF ISOLATED RUMINAL EPITHELIAL CELLS

S. Neogrády, F. Kutas, P. Gálfi[1], Department of Physiology, University of Veterinary Sciences, H-1400 Budapest, P.O.Box 2 and [1]Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1581 Budapest, P.O.Box 18

Ruminal epithelial cells isolated by fractionated trypsinization were characterized by phase contrast microscopy and electron microscopy. The fractions consisting for the most part of str. corneum and str. granulosum cells and the fractions consisting mainly of str. spinosum and str. basale cells were separated and assayed for alkaline phosphatase /AP/ activity by the method of Hanna et al./1979 I. Supramol. Structure 11 451/ and lactate dehydrogenase /LDH/ activity by the method of Kornberg /1955, Methods in Enzymology 1 441/.

The fractions consisting of str. corneum and str. granulosum cells showed AP and LDH activities of 2.19 ± 0.22 and 1.15 ± 0.48 kat/mg protein, while those consisting of str. spinosum and str. basale cells showed activities of 0.22 ± 0.20 and 40.08 ± 6.35 kat/mg protein, respectively.

Comparison of the information emerging from enzyme activity assays with the electron microscopic findings revealed a high degree of differentitation in cells exhibiting a high AP activity and a low degree of differentiation in those with a high LDH activity. The opposite tendencies of AP and LDH activity changes have truly portrayed the degree of differentiation of ruminal epithelial cells and can thus be utilized as markers of that property also.

EFFECT OF FLUORINE IN POULTRY

M. Cs. Németh, Institute of Medical Chemistry, Medical University Szeged, H-6720 Szeged, Dóm tér 8

In 1979, we have reported on the effect of fluorine and its antagonism to silicon in connection with the solidity of eggshell, in an experiment of poultry feeding. In the course of this we observed that as a result of fluorine administration the number of the so-called "watered" eggs increased all at once. We examined therefore the egg proteins.

The food of Shaver's layer-hybrid groups was supplemented for seven months with fluorine in toxic quantity. We followed with attention the egg production and determined the total protein in serum and isolated, homogenized white of egg, with Biuret's reaction.

The effect of fluorine began to show itself in the slow, gradual decrease of egg production from the fourth month to the end of the experiment. Production fell back from 90% to 40%. Then the total protein in serum was lower by 18%, and in the white of egg it was lower by 19%, as compared with the control.

Our earlier conclusion that silicon is an antagonist of fluorine, is supported by our observation that the simultaneous administration of silicon in addition to fluorine, moderated the effect of the latter in every respects.

These observations complete the earlier data on the effect of fluorine. Further, these results call attention to the potential hazards of fluorine application both in humans and animals.

MYOSIN POLYMORPHISM IN SINGLE FIBERS OF SKELETAL MUSCLE

K. Pintér, K. Mabuchi[1], F. Sreter[1], J. Gergely[1], Biochemistry Department, Eötvös Loránd University, H-1088 Budapest, and [1]Boston Biomedical Research Institute, Boston, USA

Single fibers isolated from different rabbit and human muscles have been analysed for myosin light chains and isozymes by gel electrophoresis under denaturing /SD/ and native /in presence of Na-pyrophosphate/ conditions. The single fibers have been divided into two or three portions in order to carry out the gel electrophoreses and histochemical examination /diaphorase staining/.

Pyrophosphate gels reveal differences between the fast subtypes 2A and 2B. There is a shift in the relative intensity among myosin isozyme bands - the middle band /LC_1LC_3 heterodimer/ is the prominent band in 2B fibers, while the most intense band in 2A fibers is the slowest one /LC_1LC_1 homodimer/. Isozymes found in 2A fibers have mobilities that differ from those of 2B fibers suggesting the presence of different heavy chains. Fibers used for the former studies have been isolated from fairly pure 2A or 2B type muscles. Isozymes in fibers of mixed muscle show less difference in migration velocity of the 2A and the 2B type isozymes yet there is the same shift in their relative intensities. Humam 2A and 2B fibers are similar to their rabbit counterparts obtained from mixed muscle.

Human fibers exhibit considerable promiscuity with respect to fast and slow light chains, and isozyme bands likely to correspond to hybrids have been observed. Similar results have been obtained by examining fibers of masseter and electrically stimulated tibialis ant. muscle of rabbit.

In case of rabbit slow myosin, where two different light chains /LC_{1a}, LC_{1b}/ analogous to the alkali light chains /LC_1, LC_3/ of fast myosin exist, we have shown the presence of three isozymes analogous to those of fast myosin.

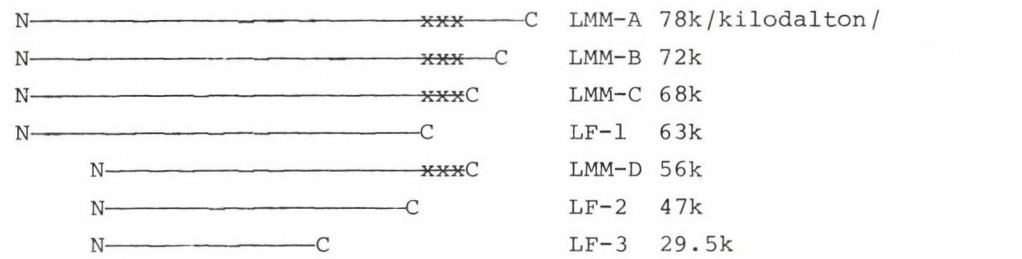
THE SUBSCTRUCTURE OF LIGHT MEROMYOSIN. LOCALIZING A REGION RESPONSIBLE FOR THE FILAMENT FORMATION OF MYOSIN

L. Nyitray, M. Bálint, G. Mócz, L. Szilágyi, R.C.Lu[1], Department of Biochemistry, Eötvös Lóránd University, H-1088 Budapest, Puskin u 3. and [1]Department of Muscle Research, Boston Biomedical Research Institute, 20 Staniford Street Boston, Mass. 02114

Light meromyosin /LMM/ obtained limited proteolysis is responsible for the filament formation of myosin.

It has been shown earlier that well-defined fragments are formed from LMM by limited tryptic digestion /Bálint et al. /1968/ J. Mol. Biol. 37 317/. We have examined the mechanism of tryptic fragmentation of LMM by means of $NaDodSO_4$--polyacrylamide gel electrophoresis and partial sequence-analysis of the fragments isolated by gel filtration. The solubility of the fragments was investigated, too.

On the basis of the gel pattern and of the identified sequences the following fragmentation scheme of LMM is suggested:



LMM-A, -B, -C, -D and LF-1, -2, -3 correspond to insoluble and soluble fragments at low ionic strength, respectively. The region indicated by asterisks in the sketch is responsible for the low ionic strength insolubility. We conclude that this part of LMM may play a significant role in the assembly of myosin into filaments.

COMPARISON OF THE MYOSIN HEAVY CHAIN PEPTIDES FROM MUSCLES WITH DIFFERENT FUNCTION

Ö. Takács, F. Guba, J. Gergely[1], Institute of Biochemistry, University of Medical Sciences, H 6701 Szeged, P.O.Box 415 and [1]Boston Biomedical Research Institute, Department of Muscle Research, 20 Staniford Street, Boston, Mass. 02114 USA

It is well known that the muscles with different function contain myosin isozymes. The electrophoretic pattern on SDS gels shows the existence of characteristic light chain composition. The heterogeneity of heavy chains has not been studied systematically yet. Therefore, authors isolated the heavy chains from pure white, red and cardiac muscles. For comparison one dimensional SDS gels according to Laemmli /Nature 277 680 /1970// and two dimensional peptide maps were made according to O'Farrel /J. Biol. Chem. 250 4007 /1975//. The isolated heavy chains were split in two different ways:

a./ Cyanogen bromide treatment,

b./ Enzymatic digestion using V8 protease of S. aureus.

On SDS gels the red and cardiac myosin heavy chain peptides are very similar, showing only slight differences - mainly in their relative distribution.

In contrast, the peptides generated from white heavy chain differ from the red or cardiac ones qualitatively, too. The CNBr procedure, being carried out in an extremely denaturing medium seems to be highly insensitive to conformational factors, whereas the procedure involving careful enzymatic digestion, relatively small structural differences could be greatly amplified by the conformational differences caused by them. Thus, peptides generated by V8 protease show profound differences in the 2-D maps indicating marked differences in primary structure of white, red and/or cardiac myosin heavy chains.

PREPARATION OF HUMAN CARDIAC MYOSIN FROM ATRIAL AND VENTRICULAR MUSCLES

E. Moravcsik, S. Fazekas[1], V. Székessy-Hermann[1], Cardiovascular Surgical Clinic and [1]2nd Institute of Biochemistry, Semmelweis University Medical School, H-1088 Budapest, Puskin u 9

Human cardiac KCl-myosin was prepared from atrial and ventricular muscles. The purification included gelfiltration on Sepharose 4B column. It was confirmed that the preparations of atrial and ventricular myosins have different lipid content, total phosphate and amino acid phosphates contents. Difference of the molecular weight could not be demonstrated by molecular sieving technique, nevertheless, a significant deviation was found in the phosphate saturation of the atrial and ventricular myosins. The saturation of myosin depends on ATP and Mg concentration and incubation time. The maximum saturation was reached after about 2 min incubation on the effect of 1 mM ATP and 10 mM $MgCl_2$ at pH 7.3. The phosphate saturation could be influenced by serotonin, caffeine and Cu ions. The amino acid phosphates of samples were separated and determined.

CALCIUM TRANSPORT IN SARCOPLASMIC RETICULUM MEMBRANE

J. Domonkos, L. Heiner, M. Vargha, jr., Research Laboratory of Neurological Department, University Medical School Szeged, H-6701 Szeged, P.O.Box 397

The study of the Ca^{2+}transport of sarcoplasmic reticulum /SR/ vesicles was performed by determination of phospho-enzyme /EP/ formation / γ-^{32}P incorporation from ATP/, ATP-ase activity and Ca^{2+}-uptake / ^{45}Ca, membrane-filtration/.

In addition to Ca^{2+}-dependent EP formations above saturating Ca^{2+} concentration Mg^{2+}-dependent EP formation is also observed. On the other hand, below or at the saturating Ca^{2+} concentration Mg^{2+} promotes the EP decomposition. The ATPase activity /EP decomposition/ induced by release of cations from the binding sites of enzyme requires the presence of a proton mobilizing /buffer/ system, but there is no need of this for EP formation. The peak of Mg^{2+} /basic/ ATPase activity is at much lower buffer concentration, than that of Mg^{2+}-activated Ca^{2+} /extra/ ATPase activity. Changes in the Ca^{2+}-uptake depending on buffer concentration are similar to the changes in Mg^{2+}-ATPase and not in Ca^{2+}-ATPase activity. In the presence of albumin - which is also a proton mobilizing compound and it cannot enter SR vesicles - Mg^{2+}-ATPase is active only, and neither Ca^{2+}-ATPase activity nor Ca^{2+}-uptake can be observed.

The results suggest that 1/ the Me^{2+}/H^{+} exchange plays intrinsic role in the Ca^{2+}-transport, 2/ the release of Mg^{2+} from binding sites takes place easier and earlier than that of Ca^{2+}, 3/ the Mg^{2+} /basic/ATPase has an important, or even "pacemaker" role in Ca^{2+}-uptake, 4/ the basic ATPase - as one of the initial steps in Ca^{2+}-uptake - functions on the outside surface, while the Ca^{2+}-ATPase responsible for the decomposition of Ca.EP coupled to basic ATPase, is on the inside of SR vesicles.

EFFECTS OF ISCHEMIA CAUSED BY LIGATURES AND SECTION OF THE ABDOMINAL AORTA ON THE METABOLISM OF DIFFERENT KINDS OF SKELETAL MUSCLES

Á. Altorjay, K. Sas, I. Sohár, L. Dux, F.Guba, Department of Biochemistry Medical University, H-6701 Szeged, P.O.Box 415

The ligature of the artery is a generally used method for causing hypoxia. Similar to the cases of myotoniy /Sohár et al. /1977/ Acta biol. med. germ. 36 1621/ and immobilization /Sohár et al. /1981/ Proc. 21st Hung. Meet., 53/, the degradation of tissues is often accompanied by hypoxia, it seemed to be reasonable to examine the effects of ligatures of the abdominal aorta on the metabolism of skeletal muscles.

In our experiments 80 CFY male rats /230±10 g body weight/ were used. LDH activity, the distribution of LDH isoenzymes and the quantity of L-lactate and pyruvate were determined in the slow-oxydative type m. soleus, the fast glycolytic m.gastrocnemius and m. semimembranosus on the 1st, 2nd, 4th, 7th and 14th days after the operation and the degradation of the muscles was studied by histochemical method.

In both types of muscles, the LDH activity and the quantity of L-lactate and pyruvate were decreased. A decrease in the H/M ratio of LDH isoenzymes was observed. The lowest values were found on the 2nd and 4th days.

It can be suggested that hypoxia produced by the ligatures and section of the abdominal aorta can be regarded as a model of hypoxia in skeletal muscles not later than 5th day after the operation since later the metabolite and enzyme leakage begins to stop and the blood circulation returns to normal.

EFFECT OF ISCHEMIA ON PROTEINASE ACTIVITIES IN SKELETAL MUSCLE

I. Sohár, Á. Altorjay, J. Varga, K. Sas, F. Guba, Department of Biochemistry Medical University, Szeged, H-6701 Szeged, P.O.Box 415

Previous investigations showed that the proteolytic enzyme activities increased in skeletal muscles after various treatments. It was supposed that one of the causes of activation could be the hypoxia. This hypothesis could be supported by the results of Decker. Tischler and Chua obtained opposite changes as a result of hypoxia and anoxia in heart and skeletal muscles.

Hypoxia of rat skeletal muscles was produced by ligature of the abdominal aorta /preceding abstract/. The activities of cathepsin D, L and chymotrypsin like proteinases of soleus and semimembranosus muscles were measured after surgey on the 1^{st}, 2^{nd}, 4^{th} or 7^{th} day. The activity of cathepsin L increased markedly on the fourth day, the changes of cathepsis D activity were similar, but the activity of chymotrypsin increased only slightly in the soleus muscle. These changes were more expressed in the slow-oxidative type soleus muscle.

Results from this study indicate that higher proteolytic enzyme activities are responsible for the muscle protein breakdown in ischemia. The cause of activation may be the changed condition during hypoxia. The lower pH value and the reduced redox potential can activate the lysosomal cystein proteinases /cathepsin L,B,H/ having pH optima around pH 6. Their catalytical centre is active in the presence of free -SH groups. Further activation is probably due to the splitting of peptide- inhibitors bound to the /inactive/ proteinases. This reaction may be catalyzed by the proteinases activated earlier.

EFFECT OF LONG TERM PROTEIN FREE DIET ON THE PROTEINASE ACTIVITIES IN SKELETAL AND HEART MUSCLES

L. Odobasic, I. Sohár[1], S. Mehikic, F. Guba[1], Department Biochemistry Medical Faculty University of Tuzla, Irceva BB, 45000 Tuzla, Jugoslavia and [1]Department of Biochemistry Medical University, H-6701 Szeged, P.O.Box 415

Protein as food serves as supply for energy and for amino acids which are building blocks of protein in the tissues. On the effect of protein free diet the body and muscle weight decreased. It is known that the muscle protein synthesis is slower in this state. We examined in this study the proteolytic enzyme activity changes in soleus, semimembranosus and heart muscles during long term protein free diet /10 days, 3, 6, 9 weeks/. 250 g $\pm$ 10 g CFY male rat were used for experiments /10 animals a group/. The activities of lysosomal cathepsin D, L and extralysosomal 1 mM Ca^{++} activated neutral proteinase /CANP/, chymotrypsin-like /cathepsin G/ and alanine aminopeptidase were determine /I. Sohár et al., /1981/ Adv. Phys. Sci. 24 381/. The activity of CANP was two times higher at the third and sixth weeks of diet. The lysosomal enzyme activities increased even more in the soleus muscle at the sixth week of experiment. The activity of alanine aminopeptidase and cathepsin G decreased during the period of examination.

We can conclude that during protein free diet some proteinase can be activated or the catabolism of proteinases is slower than that of the other muscle proteins. The lysosomal proteinases and the CANP are probably involved in the faster protein degradation in muscle during long term protein free diet.

EFFECT OF LONG-TERM PROTEIN-FREE DIET ON THE ENERGY METABOLISM IN SKELETAL AND HEART MUSCLES

I. Takáts, L. Szekeres, I. Sohár[1], F. Guba[1], L. Odobasic[b], Department of Pharmacology, University Medical School Szeged, H-6701 Szeged, P.O.Box 115, [1]Department of Biochemistry, University Medical School Szeged, H-6701 Szeged, P.O.Box 415, and [b]Department Biochemistry Medical Faculty, Tuzla, Jugoslavia

A study was undertaken to elucidate the effect of a long-term protein-free diet on the energy metabolism in skeletal and heart muscles of rat. Male CFY type rats weighing 200-250 g were kept on protein-free diet for 3 and 9 week respectively. The creatine phosphate /CP/, ATP, ADP,AMP and lactate content of different type of muscles /m. soleus, m. semimembranosus and myocardium/ were determined enzymatically in anaesthetized, thoracotomized and artificially respirated animals /I. Takáts et al. /1979/ in Pharmacological control of heart and circulation /Eds. L. Tardos, L. Szekeres and J. Gy. Papp/ Pergamon Press and Akadémiai Kiadó, p. 71/.

CP content of m. semimembranosus increased significantly even after 3 week. Such alterations were not observed in CP concentration of m. soleus and of the myocardium. The ATP concentration of m. soleus was slightly decreased. The myocardial high-energy phosphate content did not change. After 9 week the lactate level of the heart tissue increased significantly.

These results indicate that after 3 or 9 week diet the possible initial alterations /decrease of energetic state, increase in lactate concentration/ were compensated. This phenomenon was most expressed in the fast, glycolytic type muscle. During the protein depletion the preservation of the energetic state requires more intensive anaerob-metabolism in the oxydative skeletal and heart muscle.

MODIFICATION OF MODEL AND BIOMEMBRANES BY HYDROGENATION WITH WATER-SOLUBLE CATALYSIS

L. Vigh, F. Joó[1], Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.Box 521 and [1]Institute of Physical Chem. KLTE, H-4010 Debrecen

A manipulation which directly changes the fatty acid core of the membranes is the hydrogenation of the unsaturated double bonds in the presence of catalysts. A water-soluble catalyst, a sulphonated triphenyl phosphine complex of rhodium $RhCl/mSP\phi_2/_3$, was tested on model membrane system by Madden et al. We compared this rhodium catalyst with a newly developed water-soluble rutenium catalyst, $RuCl_2/mSP\phi_2/_2$.

While the Rh complex showed hardly any activity up to 20°C, there was about 17% loss in the relative number of double bonds using the Ru complex at 20°C after incubation of soya bean lecithin liposomes for 1 hour. The initial reaction rate was also higher with $RuCl_2/mSP\phi_2/_2$ than with $RhCl/mSP\phi_2/_3$ at 20°C and Ru complex was consistently more active during the investigated period. Both catalysts were markedly selective in saturation of polienic fatty acids: the Ru complex was more effective not only in saturating linolenic and linoleic acid, but also converting oleate to stearate. The reaction rate of hydrogenation continuously decreased with increasing sterol/ /phospholipid molar ratio.

Preliminary experiments with chloroplast thylakoid and microsomal fractions of spinach leaves demonstrated that modulation of fluidity of biomembranes by hydrogenation could be effected with $RuCl_2/mSP\phi_2/_2$. The method opens possibilities to investigate the contribution of the esterified fatty acids as far as saturation is involved in the regulation of membrane--bound enzymes, which is thought to take place possibly via an effect on the fluidity of the microenvironment near the active site of the enzymes.

EFFECT OF BETA-RECEPTOR AGONISTS AND ANTAGONISTS ON THE STRUCTURE OF RAT LIVER PLASMA MEMBRANE

Gy. Nemecz, T. Farkas, Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, 6701 Szeged, P.O.Box 521

Binding of antagonists to the beta-adrenergic receptor is largely entropy driven, with a small enthalpy component. On the other hand, the binding of agonists is associated with a large decrease in enthalpy which permits a high decrease in entropy.

The different binding characters might be reflected by changes of the membrane structure. Electron spin resonance studies on liver plasma membranes probed with a low concentration of 5-nitroxid stearate /12.3/ demonstrated that the agonists /norepinephrin, isoproterenol/ increased the ordered state of the membrane lipids. Whereas the antagonist /propranolol/ increased the lipid fluidity, as indicated by a decrease in the orfer parameter S. We didn't find any difference between the transition break points.

These data indicated that the binding characters are in connection with architectural changes in the membrane system.

ON THE ROLE OF GUANYL NUCLEOTIDES IN HORMONE ACTIVATION OF ADENYLATE CYCLASE /AC/ IN GRANULOSA CELLS /GC/

A. Takáts, F. Hertelendy[1], 1st Institute of Biochemistry, Semmelweis University Medical School, H-1444 Budapest, P.O.Box 260 and [1]St. Louis University Medical Center, 1325 S. Grand Blvd, St. Louis MO 63104, USA

We studied by two experimental approaches the role of Gpp/NH/p in the activation of AC in chicken GC membranes isolated from mature ovarian follicles. In the first we found that enzyme activity /ACI/ in GC membranes obtained before the endogenous LH surge /A/, was maximally activated by Gpp/NH/p whereas after the LH peak /B/ nucleotide sensitive enzyme activity /ACII/ was significantly lower. Addition of saturating conc. of LH left-shifted the dose response curves of both ACI and ACII decreasing the K_{act} for Gpp/NH/p but it did not increase the V_{max} of ACI. On the other hand LH "superactivated" ACII yielding V_{max} similar to that of ACI. In the second we measured the binding of ^{3}H-Gpp/NH/p to GC membranes of A and B. ^{3}H-Gpp/NH/p saturation of a yielded a biphasic curve in the Scatchard analysis indicating two binding sites /K_{d1}: 0.284 /uM, K_{d2}; 1.96 /uM/ whereas the saturation of B yielded a straight line with a K_d of 0.224 /uM that was similar to the K_d of high affinity binding site of A. Moreover, the binding capacity of B was significantly lower than that of A. In the presence of LH /5 /ug/ml/ a second, low affinity Gpp/NH/p binding site appeared in the B. On the other hand the hormone had no effect on the saturation profile of A. Preincubation of GC membranes with LH /10 /ug/ml for 60 min/ did not affect ^{3}H-Gpp/NH/ p binding to B whereas the same treatment caused the disappearance of the low affinity binding site of A. We suggest that the low affinity binding site for guanyl nucleotides plays a key role in the hormone regulation of AC in granulosa cells.

INHIBITION OF MITOCHONDRIAL SUBSTRATE ANION TRANSLOCATORS BY A SYNTHETIC AMPHIPATHIC POLYANION

T. Kőnig, I. Stipani[1], F. Palmieri[1], 2nd Institute of Biochemistry, Semmelweis University Medical School, H-1088 Budapest, Puskin u 9 and [1]Institute of Biochemistry, Faculty of Pharmacy, Center for the Study of Mitochondria and Energy Metabolism, C.N.R., University of Bari, I-70126 Bari, Via Amendola, 165/A

The effect of a polyanion /a copolymer of methacrylate, maleate and styrene in a 1:2:3 proportion, average molecular weight: 10.000/ on substrate anion translocators of rat liver mitochondria has been studied. The polyanion inhibits the dicarboxylate, oxoglutarate, tricarboxylate and adenine nucleotide translocators in a concentration dependent manner. The activity versus inhibitor concentration curves are sigmoidal. The polyanion inhibits the oxoglutarate and tricarboxylate translocators competitively. On the contrary, the inhibition of the adenine nucleotide translocator by the polyanion is of the mixed-type. The K_i values of the polyanion calculated from Lineweaver-Burk plots are the following: oxoglutarate translocator: 4.0 /uM; tricarboxylate translocator: 1.2 /uM; adenine nucleotide translocator: 1.3 /uM using ADP as substrate and 0.8 /uM with ATP. It is concluded that the polyanion after binding to the outer surface of the inner membrane acts 1./ primarily, by increasing its negative charge, thus repelling competitively the negatively charged substrate from the active centre; 2./ the sensitivity of the translocators towards the polyanion depends on the number of negative charges of their substrate; 3./ in the case of the adenine nucleotide translocator the polyanion interacts also either with other site/s/ of the translocator or with the membrane lipids close to it, thereby decreasing its mobility in a non-competitive manner.

PROTECTION AGAINST LIPIDPEROXIDATION BY SUBSTRATE OXIDATION OF MITOCHONDRA

L. Mészáros, Á. Németh, 2nd Institute of Biochemistry, Semmelweis University Medical School, H-144 Budapest, P.O.Box 262

The well-known role of radicals in tissue damage manifests itself mainly in the process of lipidperoxidation /LPO/. The physiologically most important source of radicals in the liver for the LPO is the microsomal NADPH-cytochrome P450 system. Results presented here show an interaction between the process of LPO and the mitochondrial substrate oxidation. Measuring malondialdehyde formation as a product of LPO we found that: 1. The NADPH dependent LPO of a mitochondrial preparation from rat liver was decreased by addition of respiratory substrates such as β-OH butyrate and succinate. Succinate appeared to be the most effective /90% of inhibition/. 2. Using a reconstituted system containing microsomes and mitochondria it was shown that the inhibition by mitochondrial substrate oxidation also affected the microsomal LPO. A decreased microsomal LPO was also detected using submitochondrial particles instead of mitochondria. 3. Addition of succinate reduced the extent of NADPH dependent. LPO also in homogenate of rat liver. 4. Using specific inhibitors it was shown that the reduction of the segment of electron transport chain between the rotenone- and the antimycin-sensitive sites /probably CoQ/ was responsible for the inhibition of LPO. This latter is in agreement with the data of Mellors and Tappel / J. Biol. Chem. 241 4353. 1966/ that reduced CoQ is an effective antioxidant. On the basis of our findings it is supposed that mitochondria can play an effective role in the protection against LPO in the cell.

INVESTIGATION OF THE INITIAL PHASE OF MICROSOMAL LIPID PEROXIDATION

I. Venekei, Ágnes Kittel, I. Horváth, 2nd Institute of Biochemistry, Semmelweis University Medical School, H-1444 Budapest 8, P.O.Box 262

During the study of microsomal lipid peroxidation /LPO/ induced by various Fe-complexes we obtained results which contradict the findings of others using NADPH-citochrome P450 reductase incorporated into liposomes /Svingen et al., The Journal of Biol. Chem. 254 5892 1979/. Investigating the LPO increasing effect of c-hem -9-pep /HP-9/ we observed that in the presence of NADPH the slow rate of formation of malondialdehyde MDA, 21 pmoles/min./mg prot. was doubled after 10-15 min.. We examined the effect of Fe-complexes on the MDA formation in these two time periods. We determined the MDA production of the suspension of mouse liver microsomes /Ruege and Aust, Methods in Enzymol. 52 306, 1978/ in the presence of NADPH and regenerating system.

We found that 1./ the MDA formation in both periods was increased by ADP Fe^{2+}, ADP Fe^{3+}, EDTA-Fe^{2+} more than ten fold; 2./ EDTA-Fe^{3+} doubled the MDA formation in the first period, while inhibited it in second one; 3./ HP-9 given in the first period acted similarly to the addition of EDTA-Fe^{3+}, while giving in the second one it increased MDA formation similarly to that caused by either ADP-Fe^{2+} or EDTA-Fe^{3+}.

Our results make possible a further, detailed study of the mechanism of microsomal LPO. We suppose - especially on the basis of our results obtained with HP-9 - that in the first phase NADPH causes an enhanced reduction of endogenous Fe-complexes, which enables the initiation of LPO.

EFFECT OF HEM-NONAPEPTIDE ON THE ACTION OF NADPH-CYTOCHROM C /P-450/ REDUCTASE

M. Kramer, I. Horváth, 2nd Institute of Biochemistry, Semmelweis Medical School, H-1444 Budapest, P.O.Box 262

Previously we found /Horváth et al., unpublished/ that the tryptic fragment of cytochrom c hem-nonapeptide /HP/ caused lipid peroxidation /LPO/ in rat liver microsomes. The oxygen consumption commenced immediately after starting the reaction while, in contrast to the ADP-Fe-enhanced LPO, malondialdehyde production was appearant only 10-15 min later. Since the initial rapid O_2 consumption presumably could be attributed to the interaction between the reoxidable HP /Baba, Y., Mizushima, H., Watanabe, H., Chem. Pharm. Bull. 16 763 1968/ and NADPH cit.c /P-450/ reductase /E.C. 1.6.2.4./, the key factor in LPO, we investigated the effect of HP on the rate of the reductase-catalyzed NADPH oxidation. We found that 1. in 0.3 M K-phosphate buffer /pH 7.7/ containing NADPH /20 /uM/, HP /20 /uM/ and purified reductase /Digman, J.D., Strobel, H.W.: Biochem. Biophys. Res. Comm. 63 845 1975 /, intensive NADPH oxidation was observed. The rate of oxidation with HP was higher than that found with cytochrom c alone. 2. NADPH oxidation in the presence of HP could be strongly inhibited by superoxide dismutase. 3. In Tris HCl buffer /0.1 M, pH 7.7/ the oxidation rate was much slower than in phosphate buffer. 4. Addition of cytochrom c to the HP containing system inhibited the oxidation in a concentration dependent manner. A kinetic analysis of the NADPH oxidation by computer modeling revealed that it was not of the Michaelis-Menten type but followed a rather complicated route. This might be caused by the complex influence on the reaction mechanism of the various oxygen containing free radicals produced in the process. A further characterization of the reaction is in progress.

INTERACTION OF NADH: CYTOCHROME b_5 REDUCTASE WITH CYTOCHROME C HEAM NONAPEPTIDE

M. Végh, I. Horváth, 2nd Institute of Biochemistry, Semmelweis Medical School, H-1444 Budapest, P.O.Box 262

We found earlier that haem nonapeptide /"hp"/ prepared from cytochrome c by proteolysis was able to induce lipid peroxidation in liver microsomes, and possibly also influenced microsomal drug metabolism. So it was of interest to investigate the effect of hp on both NADPH: cytochrome c /P_{450}/ reductase and NADH: cyt. b_5 reductase. NADH: cyt. b_5 reductase [E.C. 1.6.2.2.] was prepared by the method of Mihara /Meth. Enz. 52 1978 102./ from Triton solubilized rat liver microsomes. Enzyme activities were measured spectrophotometrically at 25°C, in 0.15 M Tris-HCl, pH 7.5. We found that

1. The reductase catalysed NADH oxidation and oxygen consumption in the presence of hp, even in the absence of cyt. b_5, or any other electron acceptor. This process was inhibited by KCN.
2. This NADH oxidation was diminished in the presence of superoxide dismutase; catalase had no significant effect.
3. Hp had no influence on the rate of enzymatic NADH oxidation in the presence of cyt. b_5. On the other hand hp reoxydized reduced cyt. b_5, similarly to reduced cyt. c /Baba Y. et al., Chem. Pharm. Bull. 16 1968 763./.
4. Cyt. c added without cyt. b_5, inhibited the hp-induced enzymatic NADH oxidation.
5. Hp suppressed the O_2^- dependent reduction of nitrotetrazolium blue /O_2^- was generated by xanthine oxidase/.

From these results we conclude that

1. Hp binds both dioxygen and superoxide anion,
2. Hp can reduce dioxygen,
3. consequently, reduced dioxygen may participate in various free radical-chain reactions.

DIFFERENT BEHAVIOUR OF T AND B LYMPHOCYTES CAUSED BY SURFACE MODIFICATION

A. Hrabák, M.T. Szabó, Institute of Biochemistry Department I. Semmelweis University Medical School, H-1444 Budapest, P.O. Box 260

Human tonsillar lymphocytes were not damaged by 0.01% deoxycholate /DOC/. A protein fraction inhibiting DNA synthesis was solubilized by this treatment /Hrabák et al., BBA, 602, 87-96, 1980/.

The PHA and Con A-stimulated DNA-synthesis of tonsillar lymphocytes was not influenced by DOC-treatment. The mitogenic stimulation of the non-adherent /T-cell enriched/ fraction separated on nylon wool column was enhanced by DOC.

The ^{51}Cr-uptake of the lymphocytes was diminished after incubation in Eagle's medium; 0.01% DOC-treatment resulted in a more considerable decrease in this uptake. Similar observations were made in separated cell fractions: the decrease was more pronounced in B lymphocytes. T-cells could be labeled significantly better by $Na_2{}^{51}CrO_4$ than B-cells.

The pre-treatment of lymphocytes in isotonic medium or in 0.01% DOC at 37°C caused a change in the distribution of cells on nylon wool: the number of non-adherent cells was increased. This effect may be explained by the modification of the cell surface: a great part of the surface structures responsible for the cell adherence was shed and a number of cells lost their capacity to adhere to nylon wool. This can be proven by the enhanced 5'-nucleotidase activity in the non-adherent population; this enzyme is characteristic of adherent cells.

PROSTAGLANDIN BINDING ABILITY OF BLOOD CELLS

L. Kőrösi, J. Holland, K. Wágner, "FJC" National Research Institute for Radiobiology and Radiohygiene, H-1775 Budapest, P.O.Box 101

The ability of human blood erythrocyte, lymphocyte and thrombocyte to bind different prostaglandin derivatives: prostaglandin-E_2, prostaglandin-$F_{2\alpha}$, tromboxane was investigated. At 37°C blood cells bound the above prostaglandin derivatives labelled with tritium in a time-dependent manner. A method was elaborated for the study of the binding conditions. The radioactivity of bound prostaglandins was measured by liquid scintillation.

The binding ability of erythrocytes was retained even after a new days of storage. The binding ability was not influenced either by different anticoagulants applied or by the composition of nutritive medium. Erythrocytes bound only prostaglandin-E_2, no activity was measured in case of PG-$F_{2\alpha}$. Haemolysed erythrocytes lost their binding ability. No difference was found in the binding ability of the individual and mixed blood cells of donors. Similarly, lymphocytes bound ^{3}H-PGE_2, though binding was also measured with an other prostaglanding isomer, $F_{2\alpha}$.

Under similar experimental conditions, tromboxane-B_2 binding was demonstrated in trombocyte-rich cell suspension. The effect of ^{60}Co-γ irradiation on binding ability was also studied.

RELATIONSHIP BETWEEN CHANGES IN LIVER CELL MEMBRANE POTENTIAL AND THE DEVELOPMENT OF CHRONIC HEPATIC PRPHYRIA

F. Kószó, Cs. Siklós, N. Simon, Department of Dermatology and Venerology, University Medical School Szeged, H-6701 Szeged, P.O.Box 480

Decrease in activity of uroporphyrinogen decarboxylase alone seems to be not sufficient to explain the pathomechanism of the development of urorporphyrinuria characterizing chronic hepatic porphyrias and damage to the hepatocyte membranes appears to be important. To test our hypothesis rats were chronically poisoned with a diet containing 0.2% hexachlorobenzene, and during the treatment, porphyrin excretion and the liver cell membrane potential of the animals were measured. In response to the treatment the liver cell membrane potential fell rapidly; from the 8th day on, the change was significant. All the time of the complete development of uroporphyrinuria /6-8 weeks/ the membrane potential fell from the control value of -51.0 ± 0.35 mV /mean $\pm$ S.E.M./ to about -30 mV. Further chronic treatment /a total of 11 months/ caused an additional membrane depolarization of only 2-3 mV; after day 60 the decrease was no longer significant. In this period the porphyrin excretion changed at most only slightly; its composition no longer alter ed. On day 150 of treatment, the poisoning was stopped in 5 animals. After 1 month, only the total porphyrin excretion was decreased appreciably in these animals; the dominant uroporphyrinuria and the liver cell membrane potential were practically unchanged. Although a membrane potential decrease is not specific for porphyria, the considerable deterioration in the function of the membrane barrier in response to the hexachlorobenzene treatment nevertheless might promote the departure of the water-soluble uroporphyrin from the cell, and hence the development of uroporphyrinuria.

ISOLATION OF A DNA UPTAKE-STIMULATING FACTOR FROM THE CULTURE MEDIUM OF NEUROSPORA CRASSA

Gy. Tóth, M. Schablik, M.F-Német, G. Szabó, Institute of Biology, University Medical School of Debrecen, H-4012 Debrecen, P.O.Box 1

It has been demonstrated earlier /M. Schablik and G. Szabó 1981 FEMS Microbiol. Lett. 10 395-397/ that during the early stationary phase of growth the culture medium of the slime strain of Neurospora crasse /FGSC 1118/ contains a heat- and protease-sensitive substance which stimulates the uptake of exogenous DNA /Mol. wt.: 3×10^7/. This substance has been designated DUSF /DNA uptake stimulatin factor/. Its chemical structure was then unknown; its effect seemed to resemble that of the competence factor of bacteria. The aim of the present study was to develop a method for the isolation of DUSF and to analyse its chemical structure.

Slime cells were harvested from a 48-h culture of Neurospora crassa by centrifugation. Proteins were removed from the supernatant by batchwise adsorption on a Cellulose P-11 resin. The proteins were eluted from the resin by salt gradient elution. To detect DUSF, the amount of ^{3}H-labelled DNA taken up by the Neurospora cells was measured in a reaction mixture of standard composition. Fractions containing DUSF were concentrated by ultrafiltration. The preparation was let through a molecule-sieving Bio-gel A /0.5 m/ column. The activity of DUSF corresponded to a small protein peak. This preparation, however, contained eight components as shown by gel-electrophoresis. The active DUSF was separated by preparative polyacrylamide gel electrophoresis. The molecular weight of DUSF, estimated by molecular sieving, was 2.4×10^5. DUSF is a slightly acidic protein, with an isoelectric point between pH 4.5 and 5.5 as determined by isoelectric focusing.

The preparation of homogeneous DUSF renders the detailed chemical analysis and the study of the DNS uptake-stimulating effect of DUSF possible.

THE EFFECT OF ALTERATIONS IN THE LIPID COMPOSITION AND FLUIDITY OF THE PLASMA MEMBRANE ON THE ASSIMILATION AND TRANSPORT OF VARIOUS CARBOHYDRATES IN CANDIDA ALBICANS

M. Pesti, Research Institute, Seed Production and Trading Company, H-6601 Szentes, P.O.Box 41

Various types of sterol mutants of Candida albicana were isolated. Significant differences were found in the composition of their fatty acid, phospholipid and sterol contents. The membrane-bound chitin synthase activity in the total membrane fractions from two examined ergosterol deficient mutants was significantly higher in comparison to that in the ergosterol-producing strain /M. Pesti et al., Curr. Microbiol. 5 178, 1981/. These strains exhibited a higher plasma membrane order parameter, as measured by intercalated fatty acid spin probe /L.I. Horváth, M. Pesti, Proc. 21st Hung. Annu. Meet. Biochem. Veszprém, 249, 1981/.

In media containing 0.01% of the carbon source, most of the sterol mutants could assimilate glycerol, α-methyl-D--glucoside, DL-lactic acid, L-sorbose, L-arabinose and ribitol to only a significantly reduced extent as compared with the parental strains, or not at all. The same results were experienced in shaken cultures, too. In this case, however, about a 10 times higher concentration of the carbon sources resulted in the same growth curve of the sterol mutants as that of the parental strain. This observation was confirmed by measurement of respiratory activity using the Warburg respirometer and by determining glycerol uptake applying /2-^{3}H/-glyerol. As regards the absorption spectra of the cytochromes, no differences were found between the ergosterol deficient mutants, designated erg-2 /ATCC 44831/ and erg-16 ATCC 44830/, and their parental strain, designated 33 erg^{+} /ATCC 44829/.

MERCURY INHIBITION OF K^+-UPTAKE SYSTEM IN ROOTS

C. Bujtás, E. Cseh[1], Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, H-1525 Budapest, P.O.Box 35 and [1]Department of Plant Physiology, Eötvös University, H-1445 Budapest, P.O.Box 324

K^+ absorption of excised low salt, wheat roots was measured using $^{86}Rb^+$ as radioactive tracer. Uptake period was 20 minutes. Experimental solutions contained always 5.10^{-4} mol.dm^{-3} $CaSO_4$ and 10^{-4} mol.dm^{-3} KCl.

Concentration dependence of the effect of Hg^{2+} was examined. Hg^{2+} is a strong inhibitor of K^+-uptake of wheat roots. After 10 minutes in 10^{-5} mol.dm^{-3} Hg^{2+}, the K^+-uptake is about 30% of the control value.

On the basis of the time course of the inhibition, of results of washing the inhibited roots with control solution and from experiments combining Hg^{2+} and cysteine treatment it is concluded that Hg^{2+} probably cannot penetrate into the cells and it affects the plasmalemma. The inhibition of K^+-uptake might be caused by blocking of -SH groups, as no inhibition was found when excess cysteine was used together with Hg^{2+} treatment and uptake can be restored by washing the Hg^{2+}-treated roots with cysteine. Other amino acids /gly, ala, ser/ have not such effect.

The elucidation of the exact role of the -SH groups in K^+-uptake system of the roots needs further experiments.

EFFECT OF LINCOMYCIN ON THE DEVELOPMENT OF THYLAKOIDS

É. Sárvári, Department of Plant Physiology, Eötvös University, H-1088 Budapest, Muzeum krt 4/a

Bean seedlings were treated with lincomycin /a specific inhibitor of protein synthesis on 70S ribosomes/ during greening. It caused a marked reduction in the amount of chlorophyll, especially that of chlorophyll-a. Stroma thylakoids were more sensitive to lincomycin than grana. The amount of the chlorophyll-a containing chlorophyll-protein complexes /i.e. P700-chlorophyll-protein complex and CP_a- the reaction centres and tightly bound light-harvesting antennae of photosystem I and photosystem II, respectively/ decreased in the treated thylakoids. Absorption spectra proved a reduction in the amount of chlorophyll-a forms absorbing at longer wavelength, too. In spite of these facts the photosystem I activity /measured as methylviologen reduction or light-induced absorbance change at 700 nm/ of treated membranes changed only moderately, moreover, we observed a more pronounced, long wavelength fluorescence emission, characteristic to photosystem I. The photosystem II activity /dichlorophenol-indophenol reduction with water or diphenylcarbazide as electron donors/, however, was very low. Gel electrophoretic investigation of the protein pattern of treated membrane fragments showed a reduction in the amount of apoproteins of the above mentioned chlorophyll-protein complexes and in several other polypeptides belonging to the photosystem II particle. These results support the concept of the chloroplastic synthesis of reaction centre apoproteins. The synthesis and/or assembly of photosystem II seems to be strongly affected by lincomycin treatment, too.

THE SYNTHESIS AND TRANSPORT OF MUSCARINIC ACETYLCHOLINE RECEPTORS IN THE RAT STRIATUM

K. Gulya, P. Kása, Central Research Laboratory, Medical University, H-6720 Szeged, Somogyi ut 4

We have examined the synthesis and transport of muscarinic acetylcholine receptors /mAChR/ on the basis of their specific binding of ^{3}H-quinuclidinyl benzylate /^{3}H QNB/ in the striatum of 15 days old and adult rat /Yamamura, H.I., Snyder, S.H. /1974/ Proc. Nat. Acad. Sci. USA 71 1725/. The subcellular fractions were separated by flotation in a discontinuous Ficoll gradient /Salvaterra, P.M., Mahler, H.R., Moore, W.J. /1975/ J. Biol. Chem. 250 6469/. The receptor binding capacities were compared to the activities of choline acetyltransferase /CAT/ and acegylcholinesterase /AChE/ in all fractions. The AChE activity was estimated by the spectrophotometric procedure of Ellman et al. /Ellman, G.L., Courtney, K.D., Andres, V.Jr., Featherstrone, R.M. /1961/ Biochem. Pharmacol. 7 88/, while the enzyme activity of CAT was measured radioenzymatically according to the method of Fonnum /1969, Biochem. J. 125 465/.

The microsomal fraction of 15 days old rat has a fourfold amount of mAChR compared to the same fraction of adult rat. The high specific ^{3}H-QNB binding of this fraction refers to the perikaryonal synthesis of mAChR. The ^{3}H-QNB binding in the synaptosomal fraction of 15 days old rat demonstrates the rapid axonal transport of mAChR from the site of synthesis to the synaptic membrane.

EFFECT IF KAINIC ACID ON ACETYLCHOLINE STEADY-STATE LEVEL OF THE CENTRAL NERVOUS SYSTEM

D. Budai, G. Szepesy, K. Gyula, P. Kása, Central Research Laboratory, Medical University, H-6720 Szeged, Somogyi ut 4

The effect of kainic acid on some inhibitory neurons of the central nervous system is well known. There is no information, however, on the changes of the colinergic system /choline; acetylcholine; choline acetyltransferase, CAT, EC. 2.3.1.6; and acetylcholine esterase, AChE, EC. 3.1.1.7/ of excitatory neurons in several regions of the brain /striatum, cortex, hippocympus, cerebellum/.

In our present study the effects of subcutaneously administered kainic acid /8 mg/kg/ were tested 5 days after injection. Our results demonstrate a significant increase in the steady-state level of acetylcholine and a decrease in free choline content. These changes were not accompanied by significant variations in CAT and AChE activities.

EFFECT OF COLCHICINE TREATMENT ON THE LEVELS AND DISTRIBUTIONS OF TRACE METALS IN THE HIPPOCAMPUS OF RAT

P. Szerdahelyi, Centralt Research Laboratory, Medical University H-6720 Szeged, Somogyi ut 4

Intrahippocampal injection of colchicine in to rats induces the degeneration of granule cells in the dentate gyrus. Our previous histochemical investigations based on sulphide--silver impregnation revealed that trace metals can be demonstrated mainly in the granule cells of the area dentata and in their mossy fiber terminals. Our intention was to clarify how the level and the localization of hippocampal trace metals change following the action of colchicine. It was found that intrahippocampal colchicine treatment induces a temporary disappearance of the trace metal staining of the regio superior pyramidal cells, while there is a considerable reduction in the staining of the granule cells of the area dentata and the mossy fiber neuropil.

Simultaneously, in contrast to the histochemical results quantitative examination of the trace metal levels showed that colchicine treatment does not lead to depletion of zinc, iron and copper from the hippocampal formation.

The combined atomic absorption spectrophotometric and histochemical investigations suggest that there is no correlation between the trace metal staining and the quantitative amounts of the trace metals.

ACTIVITY OF SOME ENZYMES IN NICKEL-DEFICIENCY

M. Szilágyi, S. Szentmihályi, M. Anke[1], Research Institute for Animal Nutrition, H-2053 Herceghalom, and [1]Karl Marx Univerzität Leipzig, Wissenschaftsbereich Tierernährungschemie, DDR-6900 Jena, Dornburger Str. 24.

Ni was proved to be essential for animals. Ni-deficiency has caused growth retardation, increased mortality of mothers and their offsprings. Haemoglobin and haematocrit levels have been significantly lower, and the activities of some dehydrogenase and transaminase enzymes have been decreased in rat /Kirchgessner et al., Bioin. Chem. 6, 55, 1976/. The physiological role of nickel is not yet established.

In our present work we publish the results of clinical-chemical examination of blood, heart, liver and kidney samples of goats fed Ni-deficient /0.1 mg/kg Ni/ and control /5 mg/kg Ni/ diets. The parameters under examination were: activity of SDH, LDH, α-HBDH, AST, ALT, ALD, CPK, ChR enzymes.

The following parameters were found to be decreased significantly in the Ni-deficient animals. In the serum: SDH, ALD; in the hart: SDH, α-HBDH, AST, ALT, CPK, in the liver: SDH, ChE; in the kidney: α-HBDH and CPK.

Our results and other studies suggest that Ni may be involved in protein- and carbohydrate metabolism.

URINARY EXCRETION OF MODIFIED RIBONUCLEOSIDES BY CANCER PATIENTS IN POSTOPERATIVE ^{60}Co RADIOTHERAPY

J.P. Pallos, P. Keresztes, E. Csató, L.D. Szabó, Frederic Joliot-Curie National Research Institute for Radiobiology and Radiohygiene, H-1775 Budapest, P.O.Box 101

Ionizing radiations alter the metabolism of DNA and RNA molecules, too. Urine contains many types of their metabolic end products. Reports on increased urinary excretion of nucleic acid derivatives by patients with malignant disease exposed therapeutically to ionizing radiation /Buric L., Dienstbier, Z. Radiobiol. Res. Radiother. p. 227 IAEA Vienna 1977/ and animal experiments /Szabó L.D. et al., COSPAR 23rd Meeting Abstracts, p. 514 1980/ support the suitability of deoxynucleosiduria as biochemical indicator of irradiation.

Ribonucleic acids are known to be modified after they have been transcribed from DNA. Their structurally transformed constituents do not enter in the salvage pathways and excretion is possible. Cancer patients excrete modified ribonucleosides in higher amounts /Gehrke, C.W. et al., Cancer Res. 39, 1150 1979/.

We present the relationship found between the time and cose of irradiation and the increase of some modified ribonucleosides in the urine of therapeutically irradiated patients. The postoperative twenty-days radiotherapy of 14 patients consisted of fractionated local ^{60}Co-γ-irradiation on every second day with 20-25 Gy total dose. We applied immobilized phenylboronic acid affinity chromatography for removel of ribonucleosides from urine and reverse-phase HPLC for analysis. Time and dose dependent increase in urinary excretion of 1-methylinosine and 1-methylguanosine was observed.

EFFECTS OF ENDOTOXIN AND DETOXIFIED ENDOTOXIN ON THE RIBONUCLEOSIDEURIA OF RATS

P. Keresztes, J.P. Pallos, E. Csató, L.D. Szabó, L. Bertók
"Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, H-1775 Budapest, P.O.Box 101

Endotoxin, a high molecular weight polysaccharide produced by pathogenic or non-pathogenic gram-negative bacteria is a toxic agent. Its action is accompained by acute reactions such as fever, leukopenia, followed by leukocytosis and eosinopenia /Westphal O., Int. Archs. Allergy appl. Immun. 49, 1-43 1975/.

Cytotoxicity of endotoxin is generally accepted. Toxic attack is followed by catabolism of RNAs of cytosol. Non-reutilisable minor components of RNA are present in urine under normal conditions, too /Gehrke C.W. et al., Cancer Research 39, 1150-1153, 1979/. Changes in urinary concentration of minor ribonucleosides indicate the cell death.

Three groups of rats /3 animals each/ were treated with 400 /ug of endotoxin, 0.08 /ug endotoxin + 5 mg Pb-acetate as sensitiser and 400 /ug of detoxified endotoxin, respectively. Induced ribonucleosideuria was followed by affinity chromatography and reverse-phase high performance LC analysis of pooled daily urine.

Significant increase in excretion of β -pseudo-urine and N_2^2-dimethyl-guanosine have been observed in two groups treated with endotoxin as compared with animals treated with endotoxin detoxified by irradiation /Bertok, L., Kocsár, L. Izotóptechnika 11, 543 1972/.

A NEW METHOD FOR THE PRODUCTION OF TMS DERIVATES OF METHYLATED NUCLEIC ACID BASES AND THEIR GC EXAMINATION

E. Csató, P. Keresztes, J.P. Pallos, D. Knausz[1],"Frederic Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, H-1775 Budapest, P.O.Box 101 and [1]Faculty of Analytics of Eötvös Lóránd University School, H-1088 Budapest, Muzeum krt. 4

N-methylated nucleic acid bases are the so-called minor components of tRNA and ribosomal RNA, formed after the biosynthesis of nucleic acid on the effect of methylase enzymes.

In the urine of tumour bearing patiens, higher amounts of N-methylated bases can be detected than in the urine of healthy persons /Borek E., Cancer Res. 31, 596 1971/. The elaboration and application of effective analytical methods might play an important role in the early diganosis and therapy of malignant tumours.

In the experiments gas-chromatographic analysis of trimethylsilyl /TMS/ derivatives of N-methylated bases was performed. New TMSIC /trimethylsilyl-carbamidate/ type silylating agent was used for the formation of derivatives synthesized at the Eötvös Lóránd University. The optimal conditions of silylation were determined for the following bases: k.NeAde, 2-MeAde, 5-MeAde, 7-MeGua, 3 MeCyt, 5-MeCyt and compared with methods described by other authors. The new method is reliable, simple and quick. Their presence in the urine of tumour bearing patiens either before or after their radiotherapy will be discussed.

A SENSITIVE FILM-DETECTION METHOD FOR THE QUANTITATIVE DETERMINATION OF ^{3}H LABELLED COMPOUNDS IN THIN-LAYER ION--EXCHANGE CHROMATOGRAPHY

A. Guttmann, L. Fehér, K. Magyar, Central Isotope Laboratory, Semmelweis University of Medicine, H-1089 Budapest, P.O.Box 370

Thin-layer chromatography /TLC/ is a general and important method for the study of metabolism of drugs. Out of the different kinds of TLC one type, namely, the ion-exchange TLC proved to be very useful. One of the advantages of the method is that the separation is based upon the polar characteristics of compounds. On the other hand sensitivity of ^{3}H autoradiography is increased by orders of magnitude as a result of the scintillation property of polystyrene matrix as it has been shown in our last experiments. This fact makes possible to avoid the fluorographic sensitisation treatment. In the case of fluorography, the treatments by PPO in organic solvent or by Na silicylate in water dissolve and blur the spots, so the resolution is decreased. In our experiments with standard substances, ^{3}H labelled amino acids were separated on silica gel and ion-exchanger /based on polystyrene matrix/ layers then the plates were treated by PPO and Na--salicylate, respectively, and were placed on Agfa Gevaert Mamoray RP3 X-ray films. The exposures were carried out at $+20^{\circ}C$ or $-70^{\circ}C$ for 24 hours and background fog absorbance was set at 0.25 or 0.5 by flashing. Our results suggest that the measurement of the amount of ^{3}H-labelled compounds can be made about 150 times more sensitive by using polystyrene than with silica gel plates if the so called film detection is applied. The increase of sensitivity was not significant with ^{14}C labelled compounds.

INVESTIGATION OF ANTITHROMBIN-III PURIFIED BY AFFINITY CHROMATOGRAPHY

R. Vasileva, M. Jakab, F. Haskó, P. Dobó, National Institute of Haematology and Blood Transfusion, H-1502 Budapest, P.O.Box 44

Purification of Antithrombin-III /AT-III/ for experimental and therapeutic purposes was carried out by affinity chromatography according to the method of M. Wickerhauser et al. /1979/. The blood plasma was partially purified with polyethyleneglycol/average molecular weight: 4000/ at 20% concentration to precipitate the major part of the nondesired proteins. The AT-III from the supernatant was gained through a batch adsorbtion and elution procedure with Heparin-Sepharose /in a plasma:gel ratio of 50:1/. For the elution of AT-III 0.02 M, pH 7.3 phosphate buffer was used with an increasing NaCl concentration. The eluate was pasteurized at pH 8.0 and 60°C for 10 hours with the addition of 0.5 M Na-citrate after concentration and desalting. The superfluous Na--citrate was removed by dialysation and the solution containing At-III was sterile filtered and freeze-dried. The mobility of AT-III determined with immunoelectrophoresis was equal to α_2-macroglobulin. The active protein in the plasma and in the purified AT-III preparation were compared with two-dimensional immunoelectrophoresis. The AT-III in the plasma shows a high active peak and two inert minor peaks. The purified AT-III gives a smaller active peak and instead of the two minor inert peaks one higher inert peak could be observed. During pasteurization the biological activity of AT-III is decreasing. On the basis of the thrombin inhibition activity of our AT-III preparation and the AT-III quantity determined with radial immunodiffusion. We estimated that the yield of our procedure was 20% of the AT-III of the original plasma only.

RAPID PROCEDURE FOR DETERMINATION OF TOTAL PORPHYRINS IN URINE

L. Szécsényi-Nagy, A. Csiba, Municipal Hospital Péterfy, H-1441 Budapest, Péterfy Sándor u 14

A rapid procedure has been adapted in which an anion--exchange resin and spectrofluorometry are used to determine total porphyrins in urine /Sobel, C., Cano, C., Thiers, R.E. /1974/ Clin. Chem. 20 1397/.

Varion AT-660 anion-exchange resin /chloride form, Nitrokémia - Hungary/ was used without pretreatment. For column preparation, an appropriate amount of resin is suspended in distilled water and poured into the columnt /K16/20, Labor MIM - Hungary/ to give a 1.6 x 1.0 cm resin bed. After excess water has flowed through, the column is ready for use. A 5 ml aliquot of urine is then pipetted into the reservoir of the column and allowed to flow through the anion-exchange resin. Uncharged materials are washed through the column with three 5 ml portions of distilled water. These washes are discarded. Porphyrins are eluted from the resin by adding four 5 ml portions of 3 N HCl. The eluent solutions are combined, their fluorescence is measured at 646 nm while scanning with the excitation monochromator from 340 to 440 nm. The "peak" height is a measure of the total porphyrin concentration. Working standard of coproporphyrin in 3 N HCl was prepared from porphyrin stock solution at concentrations ranging from 0 to 0.15 mg/liter. Coproporphyrin stock solution was prepared from coproporpyrin - carboxymethylester /Calbiochem/ according to Martinez and Mills /1971, Clin. Chem. 17 199/.

Ten normal urine samples contained an average of 72 /ug porphyrin per liter/range 20,0 - 150,0 /ug/liter/. Five urine samples contained an average of 770 /ug of porphyrin per liter/range 520 - 1100 /ug/liter/. Variation coefficients were 55,47 and 29,64%, respectively.

A SIMPLE METHOD FOR THE QUANTITATIVE DETERMINATION OF XYLITOL BY HPLC

P. Kertész, B. Szőke[1], K. Nikolics[1], Gy. Kéri[1], Clinic of Conservative Dentistry, Semmelweis University Medical School, H-1088 Budapest, Mikszáth tér 5 and [1]1st Institute of Biochemistry, Semmelweis University Medical School, H-1088 Budapest, Puskin u 9.

Xylitol seems to be one of the most effective sugar substitutes in caries prevention, because the potential cariogenic microorganisms have no or limited ability to utilize it. Therefore a new method, HPLC combined with refractometric detection was worked out for separation and quantitative determination of xylitol.

A Waters HPLC apparatus and differential refractometer was used. The separation was carried out on /u-Bondapak-NH_2 column /Waters/.

We were able to separate xylitol from xylose, ribose, fructose, mannitol, sorbitol, glucose, galactose, sucrose and lactose. Linear correlation was found between the xylitol content of injected samples and the detector response in the range of 10-100 /ug of xylitol. The sensitivity of our method is two orders of magnitude higher than the methods presented earlier. On the other hand the samples can be tested in native form without any previous treatment in contrast to other methods, e.g. gas-chromatography.

Summarizing our results a simple, highly reproducibile method was developed, which is fast, sensitive and it seems to be a useful determination for serial tests, too.

NON-ENZYMATIC GLYCOSYLATION OF PROTEINS

L. Klujber, Zs. Baranyai, J. Gabulya, R. Szücs, Department of Pediatrics, University Medical School of Pécs, H-7623 Pécs, József Attila utca 7

Under physiological and pathological circumstances non--enzymatic glcosylation is an important postsynthetic modification process.

The rate and speed of glycosylation depends mainly on relative amount of the carbohydrate, quality of the proteins and the carbohydrates, and a lesser extent it is influenced by temperature and pH. Under optimal conditions 1 mol of serum albumin binds 3 mols of glucose. However, rate and speed of the glycosylation is also altered by modification of active sites of the protein, like free amino groups.

Both in vitro and in vivo glycosylation begins rapidly, what is followed by a slow process toward equilibrium. It was found that within 2 min /37^{o}C, pH 7.4/, albumin reacts with 40 percent of the glucose. Glucose loading experiments showed that in humans the amount of serum-glycosydes begins to rise after 30 min. to reach its maximum level at the 90th min.

According to our observations, in blood samples of healthy and diabetic persons the amount of protein-glycosydes fall more quickly than it is expected by their half-life, namely it returns to the basic level after 2 to 4 days. In vitro experiments showed that the degree of glycosylation can be reduced by 50 to 90 percent by xylosylation and carbamylation. On the basis of these data it is suggested that glyc osylation is not an irreversible process.

To obtain reliable results the method is a crucial factor. We found that measurement of 5 hydroxymethyl-furaldehyde obtained after total hydrolisis of proteins is the best analytical procedure.

LONG-WAVELENGTH FORMS OF PROTOCHLOROPHYLL IN VIVO AND IN MODEL SYSTEMS

B. Böddi, F. Láng, Department of Plant Physiology Eötvös Loránd University, H-1445 Budapest, P.O.Box 324

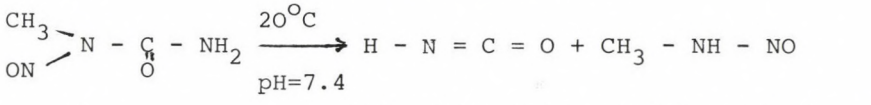
The knowledge of the molecular structure of different photosynthetic pigment forms is very important to understand the primary processes of photosynthesis. This work was aimed at the study of the origin of the long wavelength absorption and fluorescence bands existing in the spectra of etiolated leaves. Model systems were constructed from spectroscopically pure protochlorophyll /Pchl/ and were examined by absorption, fluorescence and circular dichroism /CD/ spectroscopy. The solid films treated with vapour of water, acetone or ammonia and the micellar solutions of Triton X-100 made possible to prepare several long-wavelength forms of Pchl. The positions of the absorption and fluorescence bands of these forms were very similar to those present in vivo in the etiolated leaves: 668, 675, 689 and 697 by absorption and 678, 684, 696 and 705 nm by fluorescence spectroscopy. In addition to these forms, also the well-known Pchl forms with absorption maxima at 628, 635, 640 and 650 nm were observed. The data of the CD spectroscopy showed that the long-wavelength forms in our model systems are probably formed via $\pi - \pi$ interactions which exhibit optically inactive electronic transitions.

It is supposed on the basis of these results that at least some of the long-wavelength absorption and fluorescence bands of etiolated leaves are probably due to molecular aggregates of Pchl which may have a similar structure to that of the compounds in our model systems.

SPONTANEOUS FORMATION OF L-HOMOCITRIULLINE IN NEUTRAL MEDIUM FROM L-LYSINE AND N-METHYL N-NITROSO-UREA; IN VIVO AND IN VITRO INHIBITION OF FORMATION BY L-ASCORBIC ACID

L. Trézl, I. Rusznák, T. Szarvas[1], G. Török, M. Börzsöny[2], Budapest Technical University, Institute of Organic Chemical Technology, H-1521 Budapest, Müegyetem rakpart 3, [1]Institute of Isotopes of the Hungarian Academy of Sciences, H-1525 Budapest, Konkoly Thege u, P.O.Box 77 and [2]National Institute for Hygiene, H-1106 Budapest, Gyáli u 2

N-methyl-N-nitroso-urea /MNU/ is a particularly toxic and carcinogenic compound in animal organisms. Under physiological circumstances /in neutral medium/ this compound is assumed to decompose spontaneously into isocyanic acid and methyl-nitrosamine without enzymatic activation.



MNU isocyanic acid methyl-nitrosamine

The released toxic isocyanic acid carbamoylates the ε- NH_2 groups of proteins and nuclein bases. The assumed spontaneous carbamoylation reaction could be demonstrated in model reactions in neutral solution of 0.01 mol labelled lysine /L--lysine-6-^{3}H/ /pH = 7.4/ even at 20°C under the effect of MNU. The released isocyanic acid gets bound to the ε- NH_2 group of lysine carbamoylates it and homocitrulline is formed. Homocitrulline was identified by ^{13}C-NMR spectroscopy, thin layer chromatographic analyses, comparison to known test materials.

In model reactions the formation of homocitrulline could completely be inhibited by L-ascorbic acid if the molar ratio of ascorbic acid was higher than that of MNU. In vivo experiments were carried out on 18-21 g CFLP/LATI mice and the effect of L-ascorbic acid preventing acute toxicity was proved if ascorbic acid was added intragastrically in a dose of 5 g/kg before adding 100 mg/kg MNU i.p. The 50% mean survival increased from 5.4 days to 8.9 days.

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Role of Fructose-1-Phosphate in the Regulation of the Dephosphorylation of Glycogen Phosphorylase *a*

GY. BOT, E. KOVÁCS, B. TÓTH, V. DOMBRÁDI, P. GERGELY

Institute of Medical Chemistry, University Medical School, Debrecen, Hungary

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Fructose-1-P inhibits the dephosphorylation of muscle and liver phosphorylase *a* by phosphatase. Fructose-1-P also inhibits the dephosphorylation of phosphorylated histone and p-nitrophenyl phosphate by the same phosphatase. Fructose-1-P has an inhibitory effect on the tryptic digeston of phosphorylase *a*, but is ineffective when the esterase activity of trypsin is assayed with a synthetic substrate. On the basis of the above findings the effect of fructose-1-P on the phosphatase reaction can be attributed to its binding to both the phosphatase and phosphorylase *a*. The inhibiton of the dephosphorylation of phosphorylase *a* by fructose-1-P is not affected by the presence of glucose, glucose-6-P or fructose. It is suggested that the fructose-1-P accumulated (either as a result of hereditary fructose intolerance or after the administration of fructose to normal animals) maintains a high level of phosphorylase *a* in the liver by inhibiting phosphorylase phosphatase.

Introduction

The covalent modification of phosphorylase (EC 2.4.1.1) controls the breakdown of glycogen in the tissues. The phosphorylated form *(a)* is the catalytically active species while the dephosphorylated one *(b)* is inactive. Protein phosphatase catalyses the dephosphorylation of phosphorylase *a* by hydrolysing a phosphate group covalently bound to the Ser-14 residue of phosphorylase *a*. This process also regulates the concentration of phosphorylase *a*. Therefore its control plays an important role in glycogen metabolism. Several possibilities emerge for the control of the dephosphorylation process and the effects of various small molecules (ligands) are probably the most important (Bot, Dósa, 1971; Martensen et al., 1973a, b; Detwiler et al., 1977).

AMP was the first ligand the inhibitory effect of which on the dephosphorylation of phosphorylase *a* has been recognized as early as 1951 by Sutherland. The effect of several ligands has been investigated up to date and in certain cases the mechanism of inhibition is also known (Molan et al., 1964; Martensen et al., 1977). Phosphate-containing compounds are the most effective inhibitors (e.g. glucose-1-P, mannose-1-P, glycero-P, UDPG etc.).

Recent data have shown that the intracellular concentration of fructose-1-P could reach high levels following the administration of fructose. In hereditary fruc-

tose intolerance the ingestion of fructose produces a profound hypoglycaemia. This hypoglycaemia cannot be relieved by the administration of glucagon. Large doses of fructose cause hypoglycaemia in normal animals, too (reviewed by Van den Berghe, 1977). Administration of fructose results in the accumulation of fructose-1-P in normal liver (Burch et al., 1969) and kidney (Burch et al., 1980).

The present work provides evidence that fructose-1-P inhibits the dephosphorylation (inactivation) of phosphorylase *a* by protein phosphatase. Fructose produces a large increase in the concentration of fructose-1-P (18 mmole/kg of liver: Burch et al., 1969), which is high enough for the complete inhibition of phosphatase. This results in the accumulation of phosphorylase *a*.

Methods

^{32}P-labeled rabbit skeletal muscle phosphorylase *a* was prepared with a specific activity of 55 U mg^{-1}. Liver phosphorylase was prepared in *b* form (Stalmans et al., 1974) and converted into the phosphorylated *a* (specific activity 29 U mg^{-1}) form with purified phosphorylase kinase and [1-^{32}P] ATP. Rabbit liver protein phosphatase was prepared according to the method of Brandt et al. (1975). Rabbit skeletal muscle protein phosphatase was purified by the method of Lee et al. (1980) in the presence of protease inhibitors.

Dephosphorylation of [^{32}P]-phosphorylase *a* by phosphatase was carried out at 37 °C in 40 mM Tris, 10 mM mercaptoethanol, pH 7.4. The ligands were dissolved in the same buffer and added to the incubation medium at the indicated concentrations. Eight μM phosphorylase was used in most experiments and 2 – 8 μM in the kinetic investigations. The reaction was started by the addition of phosphatase at a concentration which did not induce more than 20% dephosphorylation of phosphorylase *a* in 10 min. Aliquots (100 μl) were removed at intervals and added to 100 μl of 0.2% bovine serum albumin. The reaction was immediately stopped by 1 ml 10% trichloroacetic acid. After centrifugation 1 ml supernatant was taken for radioactivity counting as described previously (Bot et al., 1975). Initial reaction rate was determined from a plot of [^{32}P] released from phosphorylase versus reaction time. Trypsinization was carried out under the same conditions, using 8 μM phosphorylase and 0.2 μM bovine pancreas trypsin.

The alkaline phosphatase activity of the protein phosphatase was assayed according to Li (1979) at pH 8.2 using p-nitrophenyl phosphate (PNPP) as substrate, while the phosphohistone phosphatase activity was measured according to Killilea et al. (1978) at pH 7.2 using [^{32}P] labeled histone I A. Trypsin activity was assayed by the method of Schwert and Takenaka (1955).

Results

Administration of fructose produced a large increase in the concentration of fructose-1-P and a smaller increase in glucose and glucose-6-P in the liver of rats (Burch et al., 1969). First we have checked the effect of these metabolites on the

dephosphorylation by purified muscle phosphatase of muscle phosphorylase *a*. It is seen that fructose-1-P was the only metabolite formed from fructose that inhibited the release of ^{32}P from phosphorylase *a* (Fig. 1A). The others had no inhibitory effect. Glucose and glucose-6-P even stimulated dephosphorylation. We studied whether the inhibition by fructose-1-P was altered by other metabolites. It is seen from Fig. 1B that fructose, glucose or glucose-6-P did not influence the inhibitory effect of fructose-1-P.

Preparations of purified muscle phosphatase dephosphorylated not only phosphorylase *a* but other phosphoproteins (histone, casein) as well and had alka-

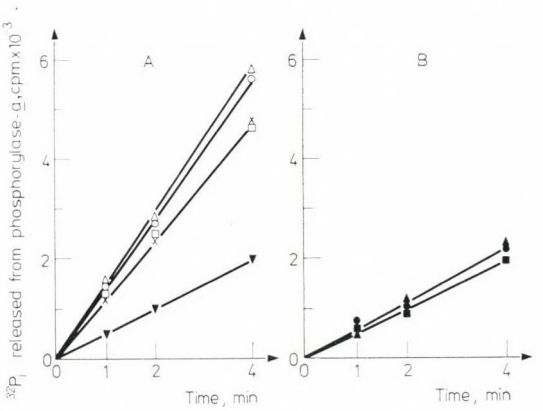

Fig. 1. Effect of metabolites on the dephosphorylation by phosphatase of muscle phosphorylase *a*. Additions (A): none (×), 5 mM glucose (○), 5 mM glucose-6-P (△), 20 mM fructose (□), 10 mM fructose-1-P (●); (B): 10 mM fructose-1-P + 5 mM glucose (●), 10 mM fructose-1-P + 5 mM glucose-6-P (▲), 10 mM fructose-1-P + 20 mM fructose (■)

line phosphatase activity, too. Table 1 summarizes the effect of ligands on the activity of protein phosphatase. The activities of phosphorylase phosphatase (Fig. 1) as well as those of histone phosphatase and alkaline phosphatase were inhibited by fructose-1-P. This inhibition was not influenced by the presence of other metabolites. It is known that muscle phosphorylase *a* is also inactivated by trypsin in an irreversible manner. A hexapeptide containing the phosphorylated serine residue (Ser-14) is split off during trypsinization (Graves et al., 1968). The results obtained with limited tryptic digestion of phosphorylase *a* are also summarized in Table 1. Fructose-1-P also inhibited the inactivation of phosphorylase *a* by trypsin and glucose as well as glucose-6-P, cancelled its inhibitory effect.

Kinetic studies of fructose-1-P inhibition by muscle phosphorylase showed (Fig. 2) that fructose-1-P was a competitive inhibitor with respect to phosphorylase *a*. The Dixon plot (Fig. 3) has an upward curvature and straight lines were obtained when the reciprocal values of reaction rate were plotted against the square of fructose-1-P concentration. The inhibitory constant corresponded to 5 mM of fructose-1-P.

Table 1

The effect of ligands on the activity of protein phosphatase assayed with various substrates and on the tryptic digestion of muscle phosphorylase a

Experimental details are given in Methods and the activities are expressed as percentages of the reaction rates measured in the absence of ligands

Metabolite	Reaction rate (%)			
	Protein	Phosphatase	Activities	Tryptic digestion of phosphorylase *a*
	Phosphorylase *a*	Histone	PNPP	
None	100	100	100	100
Fructose-1-P				
5 mM	64	86	80	91
10 mM	47	66	70	70
20 mM	28	49	51	62
Fructose-1-P 10 mM				
+ Glucose 5 mM	65	68	60	115
+ Glucose-6-P 5 mM	50	54	55	165
+ Fructose 20 mM	48	66	57	73
Glucose 5 mM	126	100	101	125
Glucose-6-P 5 mM	127	65	70	180
Fructose 20 mM	105	103	100	103

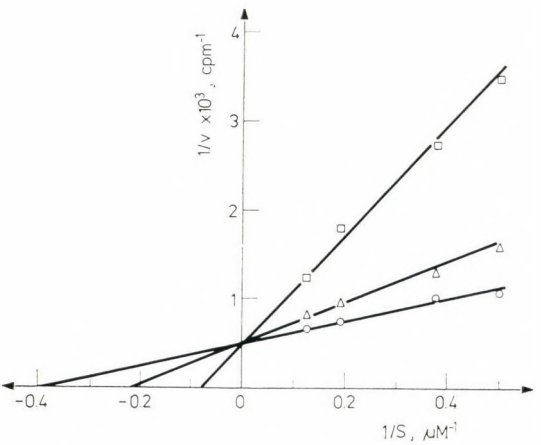

Fig. 2. Kinetics of the inhibition by fructose-1-P of the dephosphorylation by phosphatase of muscle phosphorylase *a* Lineweaver-Burk plot with respect to [^{32}P] phosphorylase *a* at 0 mM (○), 4 mM (△) and 10 mM (□) fructose-1-P

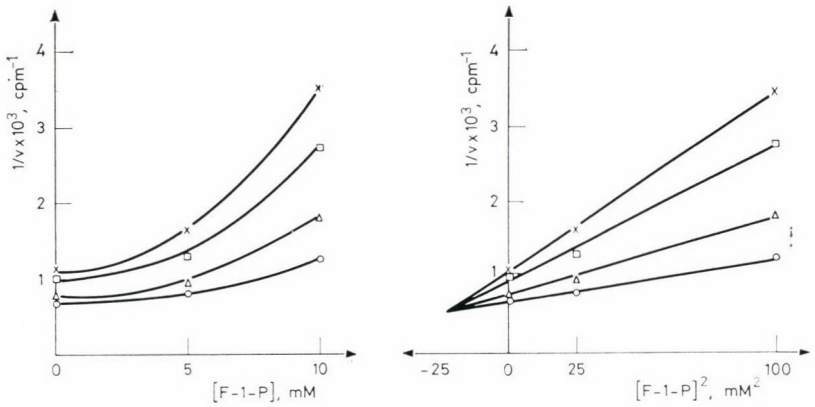

Fig. 3. Inhibitory effect of fructose-1-P on the dephosphorylation of muscle phosphorylase *a*. Data were taken from Fig. 2 and plotted according to Dixon. Phosphorylase *a* concentrations were 8 μM (○), 5.3 μM (△), 2.65 μM (□) and 2 μM (×)

We also investigated the effect of fructose-1-P on the dephosphorylation or liver phosphorylase *a* (Table 2). Fructose-1-P inhibited the inactivation by liver phosphatase of liver phosphorylase *a*. Glucose, glucose-6-P and fructose did not influence the inhibition caused by fructose-1-P. These data indicate that fructose-1-P decreases the activity of phosphatase toward both liver and muscle phosphorylase *a*.

Table 2

Dephosphorylation of rabbit liver phosphorylase a by rabbit liver protein phosphatase in the presence of various metabolites

Dephosphorylation of phosphorylase *a* is characterized by the initial reaction rates and these are expressed as percentages of the reaction rates measured in the absence of metabolites

Metabolite	Reaction rate (%)
None	100
Glucose 5 mM	119
Glucose-6-P 5 mM	97
Fructose 20 mM	100
Fructose-1-P 5 mM	74
10 mM	50
Fructose-1-P 10 mM + Glucose 5 mM	55
Fructose-1-P 10 mM + Glucose-6-P 5 mM	45
Fructose-1-P 10 mM + Fructose 20 mM	51

Discussion

The mechanism of fructose-1-P inhibition observed in the dephosphorylation by phosphatase of muscle phosphorylase *a* is complex. The use of alternative substrates (histone, PNPP) of phosphatase and the tryptic digestion of phosphorylase *a* suggest that fructose-1-P can act on two sites. The convex character of Dixon plots also indicates that two loci of inhibition exist. One site of fructose-1-P action is on phosphorylase *a* as it has been demonstrated that fructose-1-P inhibits trypsinization, too. Control experiments proved that fructose-1-P did not affect trypsin activity assayed by the hydrolysis of N-benzoyl-L-arginine ethyl ester. The second site is on phosphatase since fructose-1-P inhibited the dephosphorylation by protein phosphatase of different phosphosubstrates (phosphorylase *a*, histone and PNPP). The inhibitory capacity of fructose-1-P was different in these three probes: dephosphorylation of histone and PNPP was less inhibited by fructose-1-P than that of phosphorylase *a*. These observations indicate that fructose-1-P inhibits protein phosphatase and among the substrates of phosphatase resulting in a more pronounced inhibition it can only modify the structure of phosphorylase *a*. The different effects of glucose and glucose-6-P on the fructose-1-P inhibition observed in the tryptic digestion and dephosphorylation of phosphorylase *a* (Table 1) further confirm the existence of two types of fructose-1-P binding sites (i) the inhibition caused by fructose-1-P on phosphorylase *a* is transient, since glucose or glucose-6-P can cancel it (tryptic digestion), (ii) fructose-1-P causes a permanent inhibition of phosphatase, since glucose or glucose-6-P cannot cancel its effect (dephosphorylation of phosphosubstrates).

It is difficult to localize the binding site of fructose-1-P on phosphorylase *a*. There are at least two possibilities. One is the active site, since fructose-1-P inhibits the enzymatic activity of liver and muscle phosphorylase *a*, and it is competitive with respect to the substrates (Kaufmann, Froesch, 1973; Thurston et al., 1974). Nucleotide binding site can also bind fructose-1-P by its phosphate moiety. Two distinct fructose-1-P binding sites per monomer of muscle phosphorylase *a* were recently deduced from X-ray crystallographic data (Fletterick, Madsen, 1980).

Fructose-1-P also inhibited the dephosphorylation of liver phosphorylase *a*. The effect of fructose administration on the enzymes of glycogen metabolism has been investigated. In the early studies it was found that fructose decreased the content of liver phosphorylase *a* (Thurston et al., 1974; 1975; Van den Berghe et al., 1973). An increase in the activity of liver phosphorylase *a* by fructose was reported in perfused rat liver (Walli et al., 1975; Jakob, 1976) and in isolated hepatocytes (Van de Werve, Hers, 1979; Ciudad et al., 1979; 1980) when the enzymic activity tests were performed shortly after the fructose administration. Infusion of fructose resulted in an increase in the activity of protein kinase, elevated the levels of cyclic AMP (Miller, 1978), but the activity of liver phosphorylase kinase was unchanged (Van de Werve, Hers, 1979).

According to our results the activation of protein kinase and phosphorylase kinase is not necessary for the transient increased activity of phosphorylase *a* upon

administration of fructose. Inhibition of dephosphorylation can increase the amount of phosphorylated protein without any effect on the rate of the phosphorylation reaction. Fructose administration produces large amounts of fructose-1-P in the liver and kidney (Burch et al., 1980) which, in turn, leads to high levels of phosphorylase *a* in these tissues. This effect of fructose-1-P is specific, since its inhibitory effect is not influenced by other metabolites (glucose and glucose-6-P). Fructose-1-P also inhibits the enzymatic activity of phosphorylase *a* formed (Kaufmann, Froesch, 1973; Thurston et al., 1974) preventing the production of glucose from glycogen.

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References

Bot, Gy., Dósa, I. (1971) Acta Biochim. Biophys. Acad. Sci. Hung. *6* 73–87
Bot, Gy., Varsányi, M., Gergely, P. (1975) FEBS Lett. *50* 351–354
Bot, Gy., Kovács, E., Gergely, P. (1977) Acta Biochim. Biophys. Acad. Sci. Hung. *12* 335–341
Brandt, H., Capulong, Z. L., Lee, E. Y. C. (1975) J. Biol. Chem. *250* 8038–8044
Burch, H. B., Max, P., Chyu, K., Lowry, O. H. (1969) Biochem. Biophys. Res. Commun. *34* 619–626
Burch, H. B., Chol, S., Dence, C. N., Alvey, T. N., Cole, B. R., Lowry, O. H. (1980) J. Biol. Chem. *255* 8239–8244
Ciudad, C. J., Massagué, J., Guinovart, J. J. (1979) FEBS Lett. *99* 321–324
Ciudad, C. J., Massagué, J., Salavert, A., Guinovart, J. J. (1980) Mol. Cell. Biochem. *30* 33–38
Detwiler, T. C., Gratecos, D., Fischer, E. H. (1977) Biochemistry *16* 4818–4823
Fletterick, J. R., Madsen, N. B. (1980) Ann. Rev. Biochem. *49* 31–61
Graves, D. J., Scharfenberg Mann, S. A., Philip, G., Oliveira, R. J. (1968) J. Biol. Chem. *243* 6090–6098
Jakob, A. (1976) Mol. Cell. Endocrinol. *6* 47–58
Kaufmann, U., Froesch, E. R. (1974) Eur. J. Clin. Invest. *3* 407–413
Killilea, S. D., Aylward, J. H., Mellgren, R. L., Lee, E. Y. C. (1968) Arch. Biochem. Biophys. *191* 638–646
Lee, E. Y. C., Silberman, S. R., Ganapthi, M. K., Petrovic, S., Paris, H. (1980) Advances in Cyclic Nucleotide Research *13* 95–131
Li, H. C. (1979) J. Biol. Chem. *254* 3368–3374
Martensen, T. M., Brotherton, J. E., Graves, D. J. (1973a) J. Biol. Chem. *248* 8323–8328
Martensen, T. M., Brotherton, J. E., Graves, D. J. (1973b) J. Biol. Chem. *248* 8329–8336
Miller, T. B. (1978) Biochim. Biophys. Acta *540* 151–161
Nolan, G., Novoa, W. B., Krebs, E. G., Fischer, E. H. (1964) Biochemistry *3* 542–551
Schwert, G. W., Takenaka, Y. (1955) Biochim. Biophys. Acta *16* 570–575
Stalmans, W., Lallux, M., Hers, H. G. (1974) Eur. J. Biochem. *49* 415–427
Sutherland, E. W. (1951) in Phosphorous Metabolism (Mc Elroy, W. D., Glass, B. eds) Vol. 1, pp. 53–61 J. Hopkins Press, New York
Thurston, J. H., Jones, E. M., Hauhert, R. E. (1974) Diabetes *23* 597–604
Thurston, J. H., Jones, E. M., Hauhert, R. E. (1975) Biochem. J. *148* 149–151
Van den Berghe, G. (1977) Curr. Top. Cell. Reg. *13* 97–135
Van den Berghe, G., Hue, L., Hers, H. G. (1973) Biochem. J. *134* 637–645
Van de Werve, G., Hers, H. G. (1979) Biochem. J. *178* 119–126
Walli, A. K., Birkmann, L., Schimassek, H. (1975) Biochem. Soc. Trans. *3* 1039–1042

Correspondence: Gy. Bot
Institute of Medical Chemistry
H-4026 Debrecen, Bem tér 18/B

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‚Role of Pyridoxal 5'-Phosphate in the Regulation of the Interconversion of Phosphorylase *a* into Phosphorylase *b*

B. TÓTH, P. GERGELY

Institute of Medical Chemistry, University Medical School, Debrecen, Hungary

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Removal of piridoxal 5'-phosphate from phosphorylase *a* resulted in a product with substantially different properties as a substrate for phosphatase. The dephosphorylation of apophosphorylase *a* was still inhibited by AMP, IMP, glucose 1-phosphate and uridine-diphosphate-glucose, but the inhibition was not influenced by glucose, caffeine or glycogen. The kinetics of inhibition were found to be noncompetitive in the dephosphorylation of apophosphorylase *a*, whereas competitive in the dephosphorylation of phosphorylase *a*. Inorganic phosphate inhibited the interconversion of both forms by phosphatase in the same manner.

Glucose 6-phosphate, an activator of the dephosphorylation of phosphorylase *a*, inhibited the dephosphorylation of apophosphorylase *a*. Our results suggest that phosphate-containing ligands have an inhibitory effect on the interconversion of apophosphorylase *a* into *b* by phosphorylase phosphatase and that one of the sites of action is probably the interconverting enzyme, phosphatase itself.

Introduction

Glycogen phosphorylase (EC. 2.4.1.1) contains one mole of pyridoxal 5'-phosphate per mole of protomer linked to the enzyme via a Schiff base to Lys-679. PLP was also shown to be located in a hydrophobic packet (Graves, Wang, 1972). Recent evidence (summarized by Heimreich and Klein, 1980) supports the suggestion that the 5'-phosphate group plays an important role in catalysis. Removal of PLP results in an inactive protein with different structural characteristics, especially in its tendency to dissociate to monomers (Hedrick et al., 1966). Reconstitution with PLP restores the native structure and ligand binding interactions with a return of catalytic activity. Recent studies show that the coenzyme is in close proximity to the binding site for the substrate, glucose-1-P (Parrish et al., 1977; Fletterick, Madsen, 1980; Jenkins et al., 1981).

Enzymatic interconversion of glycogen phosphorylase by phosphorylase kinase and phosphorylase phosphatase (EC. 3.1.3.17) is an important mechanism for the regulation of phosphorylase activity. In response to nervous or hormonal signals, phosphorylase *b* is converted to phosphorylase *a* by the action of phospho-

Abbreviations: glucose-1-P, glucose-1-phosphate; glucose-6-P, glucose-6-phosphate; PLP, pyridoxal 5'-phosphate; UDPG, uridine-diphosphate-glucose.

rylase kinase which results in the incorporation of a single phosphate per monomer at Ser-14. Phosphorylase *a* is the enzymatically active species and its inactivation is catalyzed by phosphorylase phosphatase. Both processes are controlled by the presence of ligands that inhibit or activate the rate of interconversion. Various small molecules (ligands) can inhibit the dephosphorylation of phosphorylase *a* by phosphatase. (Martensen et al., 1973a; Detwiler et al., 1977.) Using another substrate, phospho-tetra-decapeptide derived from phosphorylase *a*, the mechanism of inhibition is also known in the case of certain ligands (Nolan et al., 1964; Martensen et al., 1973a). Glucose and caffeine can stimulate the dephosphorylation of phosphorylase *a* and their effect is increased by lower temperatures (Bot, Dósa, 1971; DeBarsy et al., 1972; Bailey, Whelan, 1972; Martensen et al., 1973b; Detwiler et al., 1977; Bot et al., 1978). It has been demonstrated that glucose (DeBarsy et al., 1972; Bailey, Whelan, 1972) and caffeine (Bot et al., 1977; Whithers et al., 1979) can dissociate tetrameric phosphorylase *a* into a dimeric form. Dimeric *a* is considered as a substrate of phosphatase (Bot, Gergely, 1972). The dephosphorylation reaction is also activated by glucose-6-P (Hurd et al., 1966a, b; Holmes, Mansour, 1968; Bot, Dósa, 1971; Martensen et al., 1973b; Detwiler et al., 1977).

Activators can suspend the effect of inhibitors on the dephosphorylation of phosphorylase *a*. Both glucose and caffeine decrease the inhibition of the phosphatase reaction by AMP or IMP (Bot, Dósa, 1971; Martensen et al., 1973a, b; Bot et al., 1977, 1978).

Earlier results suggested that the influence of ligands on the dephosphorylation of phosphorylase *a* depended on the presence of coenzyme. Glucose, caffeine and glucose-6-P had no effect on the dephosphorylation of apophosphorylase *a* (Graves et al., 1975; Graves et al., 1977; Yan et al., 1979). AMP, a well-known inhibitor of the dephosphorylation of native phosphorylase *a*, still inhibited the inactivation of apophosphorylase *a* (Yan et al., 1979).

From X-ray crystallography and other physicochemical probes, information is now available about ligand binding sites in phosphorylase (see the reviews of Fletterick and Madsen, 1980; Dombrádi, 1981; Jenkins et al., 1981). We have studied the effect of ligands on the dephosphorylation of native phosphorylase *a* and apophosphorylase *a* catalyzed by phosphorylase phosphatase. We report results which show the importance of PLP in the dephosphorylation of phosphorylase. We also describe some of the experiments carried out to get a deeper insight into the control of this dephosphorylation process.

Materials and methods

Rabbit skeletal muscle phosphorylase *b* was prepared according to the method of Fischer and Krebs (1962) and phosphorylase kinase was purified by the method of Cohen (1973) [^{32}P]-phosphorylase *a* was made by the procedure of Bot et al. (1975). The preparation was recrystallized three times and nucleotide contamination was removed by repeated treatment with Norit A. The ratio of absorbancies

of phosphorylase *a* at 260 and 280 nm was 0.56, indicating the absence of nucleotides (Helmrich et al., 1967). The phosphorylase preparation had a specific activity of 54–56 units per mg and an activity ratio of higher than 0.85 in the absence and presence of AMP, as measured by the procedure of Illingworth and Cori (1953). The specific radioactivity of [^{32}P]-phosphorylase *a* ranged from 3 to 3.5×10^5 cpm per mg protein.

[^{32}P]-apophosphorylase *a* was prepared from [^{32}P] phosphorylase *a* by the procedure of Shaltiel et al. (1969). Freshly prepared apophosphorylase *a* was used throughout the experiments. The apoenzyme had less than 1% residual activity observed with native enzyme and was reconstituted by treatment with a 5-fold excess of PLP at 30 °C for 20 min to 90–95% of the activity observed with native phosphorylase *a*. The excess of PLP was removed by passing the reconstituted enzyme through a Sephadex G25 column equilibrated with 40 mM Tris-10 mM β-mercaptoethanol (pH 7.4). The reconstituted enzyme was also used as a substrate in the phosphatase reactions.

Phosphorylase phosphatase (phosphatase) was prepared according to the method of Brautigan et al. (1980). The preparation was stored at −25 °C in 40 mM Tris – 10 mM β-mercaptoethanol – 2 mM EDTA buffer pH 7.0 and 50% glycerol. Prior to use glycerol was removed by dialysis against 100 vol. of 40 mM Tris – 10 mM β-mercaptoethanol, pH 7.4 at 4 °C.

Protein was assayed by the method of Lowry et al. (1951) or spectrophotometrically at 280 nm using an absorbance of 13.2 for a 1% solution of phosphorylase (Buc, Buc 1968).

The rate of dephosphorylation of [^{32}P]-phosphorylase *a* or [^{32}P]-apophosphorylase *a* was measured as follows. [^{32}P]-labelled substrates (2.2–8.7 μM based upon a subunit molecular weight of 97 500) were preincubated with or without ligands for 5 min at 37 °C in 40 mM Tris – 10 mM β-mercaptoethanol, pH 7.4. The dephosphorylation reaction was started by the addition of phosphatase whose concentration was chosen such that in 10 min it should not induce more than 50% dephosphorylation of the substrates. Total reaction volume was 600 μl. Aliquots (100 μl) were removed at intervals and mixed with 100 μl of 2% bovine serum albumin. The reaction was stopped immediately by the addition of 1 ml of 10% trichloroacetic acid. After centrifugation (3000 × *g*, 5 min) 1 ml of the supernatant was taken for counting the radioactivity. Inorganic [^{32}P]-phosphate released was counted by Cerenkov radiation as described previously (Gergely et al., 1976). The rate of dephosphorylation was characterized by the initial reaction velocities obtained. The mean of three independent experiments is given, the errors were less than ±5%.

Equilibrium dialysis experiments were carried out in an MSE Dianorm apparatus operating with macrocells (working volume 1 ml) and Spectrapor membranes (m.w. cut off: 12 000–14 000). The enzyme compartment contained 8.7 μM phosphorylase *a* or apophosphorylase *a* and the ligand compartment [U-^{14}C]-adenosine 5'-monophosphate (The Radiochemical Centre, Amersham) in the absence or presence of 50 mM glucose in 40 mM Tris – 10 mM β mercaptoethanol buffer, pH

7.4. The cells were rotated gently (4–5 rpm) for 4 h at 22 °C. Control experiments proved that equilibration of AMP across the membrane was complete after 4 h. Aliquots of the solutions on either side of the dialysis membrane were taken and the radioactivity was determined by scintillation counting.

Results

Inhibition of the dephosphorylation of apophosphorylase a *by ligands*

To investigate the influence of PLP on the action of inhibitors on enzymatic dephosphorylation, the effects of ligands on phosphorylase *a* and apophosphorylase *a* were compared. The ligands tested were those which are known to be bound to phosphorylase *a* at a well-defined site.

As shown in Fig. 1, 100 μM AMP almost completely blocked the liberation of [$^{32}P_i$] from phosphorylase *a* and 3 mM phosphate caused about 50% inhibition of the dephosphorylation reaction. Removal of PLP decreased the rate of dephosphorylation (initial velocites were 740 nmoles of [$^{32}P_i$] released/min for phosphorylase *a* and 235 nmoles of [$^{32}P_i$] released/min for apophosphorylase *a*, respectively). The per cent change in the rate of dephosphorylation measured in the presence of phosphate was found to be independent of substrates, since 3 mM phosphate also inhibited the dephosphorylation of apophosphorylase *a* by 50%. Figure 1 also indicates that the dephosphorylation of apophosphorylase *a* was less affected by AMP, but still inhibited by 100 μM AMP.

Figure 2 illustrates the kinetics of inhibition by AMP. It is seen that AMP gave noncompetitive kinetics with respect to apophosphorylase *a*. Dixon plots also confirmed the above kinetics, and a K_i of 51 μM was obtained. The results of Fig. 3 show that inorganic phosphate was a competitive inhibitor with respect to apophosphorylase *a*. On the basis of Dixon plots a K_i of 3 mM was found.

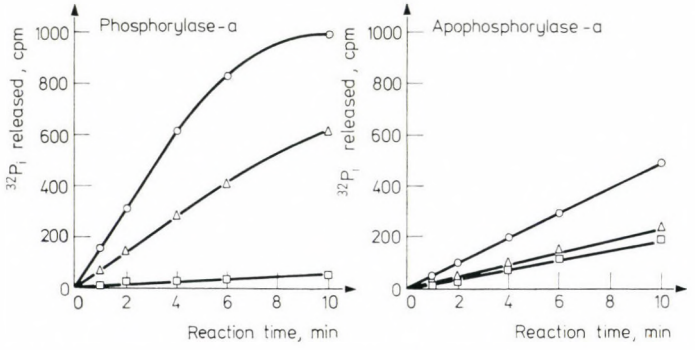

Fig. 1. Effect of AMP and phosphate on the dephosphorylation of phosphorylase *a* and apophosphorylase *a* by phosphatase. The reaction was carried out as described in Methods. Dephosphorylation of ^{32}P-labelled substrates in the absence (○) and presence of 0.1 mM AMP (□) or 3 mM phosphate (△)

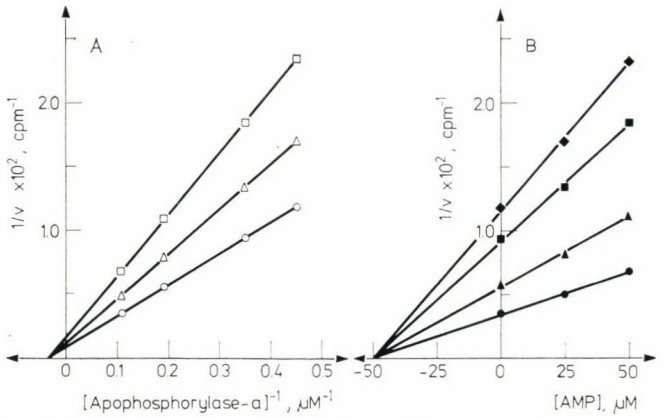

Fig. 2. Kinetics of the inhibition by AMP of the dephosphorylation of apophosphorylase *a* by phosphatase. A, Lineweaver—Burk plot with respect to [^{32}P]-apophosphorylase *a* in the presence of no (○), 25 μM (△) or 50 μM (□) AMP. B, Dixon plot with respect to AMP (from the data of Fig. 2A) at apophosphorylase *a* concentrations of 8.7 (●) 5.8 (▲), 2.9 (■) or 2.2 (◆) μM

Table 1 summarizes the inhibitory constants of ligands tested in the dephosphorylation of phosphorylase *a* and apophosphorylase *a*. As shown the kinetics of inhibition changed in the case of AMP, IMP and glucose-1-P. The dephosphorylation of apophosphorylase *a* was less sensitive to ligands, a considerably higher amount of inhibitor was needed for the same inhibition. Inorganic phosphate was an exception, K_i values were the same for both substrates and the kinetics of inhibition were also identical.

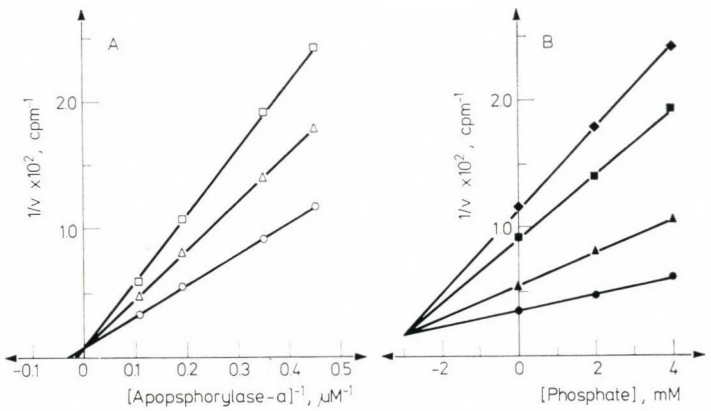

Fig. 3. Kinetics of phosphate inhibition in the dephosphorylation of apophosphorylase *a* by phosphatase. A, Lineweaver—Burk plot with respect to [^{32}P]-apophosphorylase *a* at 0 mM (○), 2 mM (△) and 4 mM (□) phosphate. B, Dixon plot with respect to phosphate (from the data of Fig. 3A) at apophosphorylase *a* concentrations of 8.7 (●), 5.8 (▲), 2.9 (■) and 2.2 (◆) μM

Table 1

Inhibitors of the dephosphorylation of phosphorylase a *and apophosphorylase* a

Inhibitor	Phosphorylase *a*		Apophosphorylase *a*	
	K_i (mM)	Type of inhibition	K_i (mM)	Type of inhibition
AMP	0.004	competitive	0.05	noncompetitive
IMP	0.08	competitive	3	noncompetitive
Glucose-1-P	2	competitive	10	noncompetitive
UDPG	2.5	competitive	30*	—
P_i	3	competitive	3	competitive

* The kinetics of UDPG inhibition were not investigated, therefore the K_i value corresponds to the concentration of inhibitor that caused 50% inhibition.

Activation of the dephosphorylation of apophosphorylase a *by ligands*

Table 2 demonstrates that neither glucose nor caffeine stimulated the dephose phorylation of apophosphorylase *a*, and that glucose-6-P was inhibitory. Glucos- and caffeine became activators of the dephosphorylation of phosphorylase *a* reconstituted by PLP, but glucose-6-P still inhibited the reaction. Table 2 also compares the data of the dephosphorylation of native phosphorylase *a* indicating that all

Table 2

Effect of glucose, caffeine and glucose-6-P on the dephosphorylation of apophosphorylase a, *PLP-reconstituted phosphorylase* a *and native phosphorylase* a

The experimental procedure was identical with that described in Methods. The initial reaction rates of dephosphorylation are given in nmole ^{32}P released/min (see the values in brackets) and expressed as percentage of the reaction rate assayed in the absence of ligands

Ligand	Rate of dephosphorylation		
	Apophosphorylase *a*	PLP-reconstituted phosphorylase *a*	Phosphorylase *a*
None	100 (235)	100 (725)	100 (740)
Glucose 20 mM	100	123	127
80 mM	101	133	136
Caffeine 1 mM	102	118	118
10 mM	99	121	121
Glucose-6-P 5 mM	78	86	130
20 mM	45	53	111

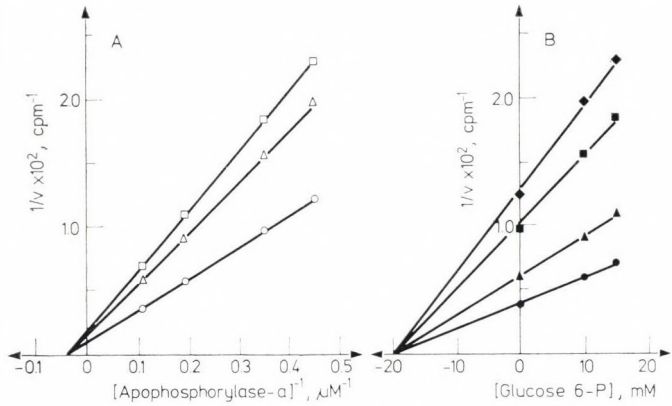

Fig. 4. Kinetics of glucose-6-P inhibition of the dephosphorylation of apophosphorylase *a* by phosphatase. A, Lineweaver—Burk plot with respect to ^{32}P-apophosphorylase *a* at 0 mM (○), 10 mM (△), and 15 mM (□) glucose-6-P, B, Dixon plot with respect to glucose-6-P (from the data of Fig. 4A) at apophosphorylase *a* concentrations of 8.7 (●), 5.8 (▲), 2.9 (■) and 2.2 (◆) μM

three ligands increased the reaction rate. However, it is remarkable that glucose-6-P was less effective at a concentration of 20 mM than 5 mM (Fig. 4).

Combined effects of inhibitors and activators

The suspension of the effect of inhibitors by activators has less been studied for the dephosphorylation of phosphorylase *a* and no data are available for the combined effect of ligands on the inactivation of apophosphorylase *a*. The dephosphorylation of phosphorylase *a* is under a complex allosteric regulation, in particular by nucleotides, carbohydrates and phosphate-esthers. Therefore, the effects of these ligands on phosphatase activity were investigated.

Several general features emerge from the data of Table 3. AMP acted in the micromolar range, whereas all other inhibitors tested gave 50% inhibition at concentrations in the millimolar range. Inorganic phosphate proved to be as inhibitory for the dephosphorylation of phosphorylase *a* as for that of apophosphorylase *a*. Glucose and caffeine suspended the inhibitions of phosphatase reaction for phosphorylase *a* with the exception of phosphate. Glucose-6-P was less effective in suspending various inhibitions. The inhibitions observed in the dephosphorylation of apophosphorylase *a* were not suspended by the addition of either glucose or caffeine, and glucose-6-P increased the inhibition.

Effect of glucose on the amount of AMP bound to phosphorylase

Figure 5 shows the results of equilibrium dialysis experiments. The amount of AMP bound to phosphorylase is plotted against the total AMP concentration in the dialysis cell. It is seen that the amount of AMP bound to phosphorylase *a* was

Table 3

Effect of activators and inhibitors on the dephosphorylation of phosphorylase a *and apophosphorylase* a

Data are expressed as percentage of the reaction rate assayed in the absence of ligands

	Inhibitor		Activator Added			
			None	Glucose (20 mM)	Caffeine (1 mM)	Glucose-6-P (5 mM)
[^{32}P]-phosphorylase *a*	AMP	5 μM		*83*	*98*	*103*
	IMP	50 μM	65	*101*	*100*	*103*
	Glucose-1-P	2 mM	50	*93*	*96*	61
	UDPG	2 mM	55	*89*	*93*	63
	P_i	4 mM	35	38	38	23
[^{32}P]-apophosphorylase *a*	AMP	100 μM	44	44	45	38
	IMP	5 μM	43	45	45	40
	Glucose-1-P	10 mM	21	52	53	46
	UDPG	20 mM	64	65	64	59
	P_i	4 mM	39	38	39	18

less in the presence than in the absence of glucose, in accordance with the finding of Helmreich et al. (1967). The affinity of AMP for apophosphorylase *a* was diminished to that observed for phosphorylase *a*. The presence of glucose did not influence the binding of AMP to the apoenzyme. Caffeine was also ineffective on the binding of AMP to apophosphorylase *a* (not shown).

Discussion

Muscle phosphorylase *a* can exist as a dimer or a tetramer depending upon the temperature, pH and ionic strength. Only dimeric phosphorylase *a* and not the tetrameric one can be considered as a substrate of phosphatase (Bot, Dósa, 1971; Bot, Gergely, 1972; Martensen et al., 1973a; Bot et al. 1977). Apophosphorylase *a* behaves as a typical associating-dissociating system occurring as a monomer above 30 °C (Shaltiel et al., 1969). To simplify interpretation of our kinetical investigations, all experiments were carried out at 37 °C and pH 7.4. Under these conditions, both forms of phosphorylase show only one peak with a sedimentation coefficient corresponding to the dimeric form (phosphorylase *a*) and monomeric one (apophosphorylase *a*), respectively.

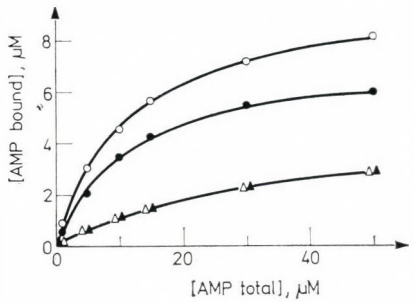

Fig. 5. Binding of AMP to phosphorylase *a* (○, ●) and apophosphorylase *a* (△, ▲) in the absence of glucose (○, △) and in the presence of 50 mM glucose (●, ▲) as studied by equilibrium dialysis. Experimental conditions are given in Methods

Phosphorylase *a* is a better substrate of phosphatase than the apoenzyme. On the basis of kinetic data the V_{max} of apophosphorylase *a* is reduced by 50% and the K_m increases 10 fold (30 μM) comparted to the kinetic parameters of the native phosphorylase *a* (Graves et al., 1975). Earlier investigations of Graves et al. (1975) and Yan et al. (1979) have shown that the coenzyme is also important for the activation of dephosphorylation reaction by glucose and caffeine, but PLP is not essential for the inhibition caused by AMP. Our data are in accordance with these observations demonstrating the role of PLP in the control of the dephosphorylation reaction.

Dephosphorylation of apophosphorylase *a* is inhibited by phosphate containing ligands similarly to the inactivation of phosphorylase *a* by phosphatase. However, the apoenzyme is less sensitive to the inhibition, 5 to 20 times as much inhibitor is needed for the same inhibitory effect to be achieved. The only exception is inorganic phosphate which acts in the same concentration on the dephosphorylation of both substrates. Martensen et al. (1973a) suggested that the inhibition caused by phosphate is directed by phosphatase rather than phosphorylase *a*. This would explain why the inhibitory constants are identical even if the phospho-substrates are different. Inhibition by other ligands could be interpreted as follows.

Muscle phosphorylase *a* has well-defined binding sites as deduced from X-ray crystallographic data and other physicochemical investigations (for references, see Fletterick and Madsen, 1980; Dombrádi, 1981; Jenkins et al., 1981). We assume that the ligands that are inhibitory in the dephosphorylation reaction are those which can bind to the nucleotide site of phosphorylase. This site is about 30 Å apart from the catalytic site where PLP is covalently linked to Lys 679. Binding of AMP (or IMP) to the nucleotide site causes conformational changes in the molecule decreasing the availability of the phosphoryl group (attached to Ser 14) at the NH_2-terminal end of phosphorylase *a* to phosphatase (or trypsin). In the unliganded form the phosphoryl group is hydrogen bonded to Arg-69 and Arg-43' of the symmetry-related monomer (Fletterick et al., 1979). Several other amino acid residues

are also involved in the interaction of AMP with the nucleotide site. Tyr-75 and an arginine residue are important in this respect (Li et al., 1977; Lee, Benisek, 1976). The interaction between AMP and Tyr-75 causes the movement of Arg-69 (both residues are in the same helix), and this response may remove Ser 14-phosphate from the favoured position. It is possible that the nucleotide binding site is modified with the removal of PLP from the enzyme. Ser 14-phosphate is surrounded by positive charges which may provide the binding site for phosphatase (Fletterick et al., 1979). The K_m for apophosphorylase *a* is 30 μM while that for phosphorylase *a* is 3 μM and the maximal valocities for the two substrates are similar showing the conformational changes on the NH_2-terminal part of the molecule. The nucleotide binding site is also located at this part of phosphorylase. Therefore some modifications may occur in this region after PLP-removal.

The X-ray diffraction work has also shown that the nucleotide binding site is remarkably indiscriminate in binding phosphoryl ligands. Thus the inhibitory effect of other phosphate-containing ligands (IMP, UDPG, glucose-1-P) could be explained similarly to that of AMP inhibition. Our data show that the dephosphorylation of apophosphorylase *a* is less inhibited by ligands and the kinetics of inhibition are also different for the dephosphorylation of holoenzyme and apoenzyme. The ligands tested so far inhibited the dephosphorylation of phosphorylase *a* and apophosphorylase *a* in a competitive and non-competitive manner, respectively (see Table 2). This difference might be due to the fact that the binding of phosphate-containing compounds to the nucleotide site of apophosphorylase is decreased as it was proved by equilibrium dialysis studies using AMP. On the other hand, phosphatase may also contain phosphate binding site(s) which would operate when the concentration of ligands is increased. It is interesting to note that phosphate inhibited the dephosphorylation of holo and apoenzyme with the same K_i and the kinetics of the inhibition were also identical. Our data confirm the previous report of Martensen et al. (1973a) that the inhibition caused by phosphate is directed by phosphatase rather than phosphorylase. The phosphate binding site of phosphatase could bind ligands with phosphate moiety (AMP, glucose-1-P, UDPG etc), causing a change in the kinetic pattern of the dephosphorylation reaction. This would also explain why glucose-6-P, an activator of the dephosphorylation of phosphorylase *a*, inhibits the dephosphorylation of apophosphorylase *a*. Direct action of these ligands on phosphatase is confirmed by dephosphorylation experiments using alternative substrates (phosphohistone and p-nitrophenyl-phosphate) of phosphatase (unpublished observation).

The coenzyme, pyridoxal phosphate is indispensable for the activation of the dephosphorylation reaction. Earlier studies have demonstrated the lack of glucose (Graves et al., 1975) and caffeine (Yan et al., 1979) activation in the dephosphorylation of apophosphorylase *a*, and our results confirm these data. We have also tested the effect of activators on the inhibition of the phosphatase reaction. It is known that AMP inhibition observed in the dephosphorylation of phosphorylase *a* is suspended by glucose or caffeine (Martensen et al., 1973a; Bot et al., 1977; Bailey, Whelan, 1972; DeBarsy et al., 1972). Glucose and caffeine can suspend the inhibi-

tory effect of other ligands (Table 3). Inorganic phosphate is the only exception, neither glucose nor caffeine can suspend its inhibitory effect.

Neither glucose nor caffeine influences the dephosphorylation of apophosphorylase *a* in the presence of various inhibitors. It seems that the active site, which binds glucose, and the nucleoside site, which binds caffeine in the phosphorylase, are considerably modified upon the removal of PLP. It is known that the nucleoside site is in close proximity to the active site (10 Å, Fletterick, Madsen, 1980) and PLP is involved in the structure of the active site. Our equilibrium dialysis data prove that glucose expels AMP from native phosphorylase *a* but does not affect the AMP bound to apophosphorylase *a*.

Glucose-6-P is considered as an activator of the dephosphorylation of phosphorylase *a* (Bot, Dósa, 1971; Martensen et al., 1973b; Detwiler et al., 1977). Indeed, it enhances the rate of the phosphatase reaction (see Table 2) and suspends the inhibitory effect of AMP and IMP (Table 3). However, glucose-6-P is an inhibitor in the dephosphorylation of apophosphorylase *a*. It is known that glucose-6-P also binds to the nucleotide site of phosphorylase (Fletterick, Madsen, 1980) and acts as an inhibitor in the phosphatase reaction of phosphorylase *a*. Its inhibitor character is masked in the case of phosphorylase *a*. Glucose-6-P can expel AMP from phosphorylase (Busby, Radda, 1976), since it binds to the same nucleotide binding site as AMP. AMP is firmly bound to phosphorylase *a* (Kastenschmidt et al., 1968), and purified preparations of phosphorylase *a* may contain various amounts of AMP impurities. Glucose-6-P "activates" the phosphatase reaction by expelling AMP. The activating effect of glucose-6-P is apparent and decrease upon the rigorous removal of AMP. The preparation of apophosphorylase *a* involves also a gel filtration step (Sephadex G25) and it seems that removal of PLP from phosphorylase facilitates the removal of AMP impurities. This explains why PLP-reconstituted phosphorylase *a* is inhibited by glucose-6-P. The activating effect of glucose and caffeine has still remained in the phosphatase reaction of the reconstituted enzyme proving that they operate with a different mechanism.

A comparison of the actions of ligands the phosphatase reaction points to a characteristic difference in the binding sites of phosphorylase. Ligands bound to the nucleotide site are all inhibitory in the dephosphorylation reaction. These ligands also contain a phosphate moiety which is involved in the interaction of specific amino acid residues sorrounding the nucleotide binding site.

Ligands which occupy other binding sites are not inhibitory in the phosphatase reaction and can suspend the inhibitions of the dephosphorylation of native phosphorylase *a* caused by other ligands linked to the nucleotide site.

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References

Bailey, J. M., Whelan, W. J. (1972) Biochem. Biophys. Res. Commun. *46* 191—197
Bot, Gy., Dósa, I. (1971) Acta Biochim. Biophys. Acad. Sci. Hung. *6* 73—87
Bot, Gy., Gergely, P. (1972) FEBS Lett. *24* 7—10
Bot, Gy., Varsányi, M., Gergely, P. (1975) FEBS Lett. *50* 351—354
Bot, Gy., Kovács, E., Gergely, P. (1977) Acta Biochim. Biophys. Acad. Sci. Hung. *12* 335—341
Bot, Gy., Kovács, E., Pólyik, E., Gergely, P. (1978) Acta Biochim. Biophys. Acad. Sci. Hung. *13* 189—192
Brautigan, D. L., Picton, C., Fischer, E. H. (1980) Biochemistry *19* 5787—5794
Buc, M. H., Buc, H. (1968) in 4th FEBS Symposium in Regulation of Enzyme Activity and Allosteric Interactions, Universitetsforlaget, Oslo, 109 pp. (eds. Ekvamme, E., Phil, A.)
Busby, S. J. W., Radda, G. K. (1976) Curr. Top. Cell. Reg. *10* 89—160
Cohen, P. (1973) Eur. J. Biochem. *34* 1—14
DeBarsy, T., Stalmans, W., Laloux, M., DeWulf, H., Hers, H. G. (1972) Biochem. Biophys. Res. Commun. *46* 183—190
Detwiler, T. C., Gratecos, D., Fischer, E. H. (1977) Biochemistry *16* 4818—4823
Dombrádi, V. (1981) Int. J. Biochem. *13* 125—139
Fischer, E. H., Krebs, E. G. (1962) Methods in Enzymol. *5* 369—373
Fletterick, R. J., Sprang, S., Madsen, N. B. (1979) Can. J. Biochem. *57* 789—797
Fletterick, R. J., Madsen, N. B. (1980) Ann. Rev. Biochem. *49* 31—61
Gergely, P., Vereb, Gy., Bot, Gy. (1976) Biochim. Biophys. Acta *429* 809—816
Graves, D. J., Wang, J. H. (1972) in The Enzymes, Vol. 7 pp. 435—482 (ed. Boyer, P. D.) Acad. Press, N. Y.
Graves, D. J., Carlson, J. R., Skuster, J. R., Parrish, R. F., Carty, T. I., Tessmer, G. W. (1975) J. Biol. Chem. *250* 2254—2258
Graves, D. J., Parrish, R. F., Uhing, R. J., Korytnyh, W. (1977) Proceedings of the 11th FEBS Meeting, Vol. 42 pp. 185—194 (ed. Esmann, V.) Pergamon Press, Oxford
Hedrick, J. L., Shaltiel, S., Fischer, E. H. (1966) Biochemistry *5* 2117—2125
Helmreich, E., Michaelides, M. C., Cori, C. F. (1967) Biochemistry *6* 3695—3710
Helmreich, E., Klein, H. W. (1980) Angew. Chem. *19* 441—455
Holmes, P. A., Mansour, T. E. (1968) Biochim. Biophys. Acta *156* 275—284
Hurd, S. S., Teller, D., Fischer, E. H. (1966 *a*), Biochem. Biophys. Res. Commun. *24* 79—84
Hurd, S. S., Novoa, W. B_3, Hickenbottom, J. P., Fischer, E. H. (1966 *b*) Methods of Enzymol. *8* 546—550
Illingworth, B., Cori, G. T. (1953) Biochem. Prep. *3* 1—9
Jenkins, J. A., Johnson, L. N., Stuart, D. I., Stura, E. A., Wilson, K. S., Zanatti, G. (1981) Phil. Trans. R. Soc. Lond. *293* 23—41
Kastenschmidt, L. L., Kastenschmidt, J., Helmreich, E. (1968) Biochemistry *7* 4543—4550
Lee, Y. M., Benisek, W. F. (1976) J. Biol. Chem. *251* 1553—1560
Li, E. C. Y., Fletterick, R. J., Sygusch, J., Madsen, N. B. (1977) Can. J. Biochem. *55* 465—473
Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. *193* 265—273
Martensen, T. M., Brotherton, J. E., Graves, D. J. (1973 *a*) J. Biol. Chem. *248* 8323—8328
Martensen, T. M., Brotherton, J. E., Graves, D. J. (1973 *b*) J. Biol. Chem. *248* 8329—8336
Nolan, C., Novoa, W. G., Krebs, E. G., Fischer, E. H. (1964) Biochemistry *16* 4818—4823
Parrisch, R. F., Uhing, R. J., Graves, D. J. (1977) Biochemistry *16* 4824—4831
Shaltiel, S., Hedrick, J. L., Pocker, A., Fischer, E., H. (1969) Biochemistry *8* 5189—5196
Withers, S. G., Sykes, B. D., Madsen, N. B., Kasvinsky, P. J. (1979) Biochemistry *18* 5342—5348
Yan, S. C. B., Uhing, R. J., Parrish, R. F., Metzler, D. E. Graves, D. J. (1979) J. Biol. Chem. *254* 8253—8269

Correspondence: B. Tóth
Institute of Medical Chemistry
H-4026 Debrecen, Bem tér 18/B

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Immunological and Allosteric Identity of Heart-Specific Glycogen Phosphorylase Isoenzymes from Various Mammals*

GY. VEREB, Kornélia SZŰCS, GY. BOT

Institute of Medical Chemistry, University School of Medicine, Debrecen, Hungary

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Heart-specific and skeletal muscle-specific isophosphorylases were purified to homogeneity from bovine, rabbit, pig and dog hearts and from the skeletal muscle of these species, respectively.

Rooster serum against pig heart-specific phosphorylase markedly inhibited all heart-specific isophosphorylases (1 μl antiserum caused a 17—34 mU inhibition) without appreciable inhibitory effect on skeletal muscle-specific ones.

Heart-specific enzymes have an obligatory and high sensitivity to glc-6-P inhibition either in the dephosphorylated (*b*) or the phosphorylated (*a*) form, showing an $I_{0,5}$ value of 0.8—2.2 mM and 0.2—0.3 mM, respectively. In the *b* form, most of them have a 1.5—3 fold higher sensitivity to AMP activation in comparison with the skeletal muscle enzymes.

These results indicate a common characteristic structural and functional identity of heart specific phosphorylases, different from the skeletal muscle-specific ones.

Introduction

Glycogen phosphorylase (EC 2.4.1.1), the starting enzyme of glycogenolysis exists in multiple forms in several mammalian hearts: a skeletal muscle specific phosphorylase, a heart specific one and their hibrid was distinguished first by DEAE-cellulose chromatography (Yunis et al., 1962) and later by gel electrophoresis (Davis et al., 1967).

Some allosteric properties of heart-specific phosphorylase *b* prepared from rabbit (Schliselfeld et al., 1970), pig (Hanabusa, Kohno, 1969; Will et al., 1970; Will, Krause, 1972; Vereb, Bot, 1972) and human heart (Will et al., 1974) have been described, however, a detailed comparison of heart-specific phosphorylases has not been carried out.

Attempts were made also for an immunological characterization of phosphorylase isoenzymes isolated from the heart (Yunis et al., 1962; Schliselfeld, 1973; Yonezawa, Hori, 1975). Our previous experiments indicated an inhibitory effect of

Abbreviations: glc-1-P, glucose 1-phosphate; glc-6-P, glucose 6-phosphate; GEM buffer, 15 mM glycerol 2-phosphate, 1 mM EDTA, 10 mM 2-mercaptoethanol, pH 6.8

* Part of the material of this article was reported at the 28th International Congress of Physiological Sciences, Budapest, 1980.

the antiserum against rabbit skeletal muscle phosphorylase on the activity of various skeletal muscle-specific phosphorylases existing in mammalian hearts. This inhibition was useful in the detection and quantitative evaluation of the skeletal muscle-specific phosphorylase in these hearts (Vereb et al., 1977).

Here we present data about the common immunological character of heart-specific phosphorylases from various mammals. In addition, by comparing some enzymological parameters of heart- and skeletal muscle-specific phosphorylases (especially of their *a* forms) we report a characteristic allosteric sensitivity of heart-specific phosphorylases as a further proof of their functional identity.

Materials and methods

Skeletal muscle-specific phosphorylases were prepared in the *b* form from bovine, pig, dog and rabbit skeletal muscle according to Fischer and Krebs (1958). Pig skeletal muscle phosphorylase *b* which does not crystallize was further purified by affinity chromatography on AMP-Sepharose as reported by Dombrády and Vereb (1978).

Heart-specific phosphorylase from rabbit and dog heart was isolated by DEAE-cellulose chromatography according to Davis et al. (1967) and from pig and bovine heart by affinity chromatography on AMP-Sepharose as reported by Vereb et al. (1978) using for the elution of the pig and bovine enzyme 2 mM and 5 mM glc-6-P in GEM buffer (15 mM glycerol 2-phosphate, 1 mM EDTA, 10 mM 2-mercaptoethanol, pH 6.8), respectively.

Phosphorylases isolated on DEAE-cellulose were further purified on AMP-Sepharose in a similar way, using 10 mM AMP for elution. The preparation were concentrated on Amicon PM-30 membrane and dialyzed against GEM buffer, pH 6.8. Their specific activities varied between 30–45 U/mg protein. Protein was determined by the biuret method of Gornall et al. (1949). Each of the phosphorylase preparations (either in the *b* or in the *a* form) was treated with charcoal to remove adsorbed nucleotides (2 × 1 mg Norit A (Serva) per mg of enzyme protein).

Conversion of phosphorylase b into phosphorylase a was performed according to Krebs and Fischer (1962) using rabbit muscle phosphorylase kinase prepared as reported by Cohen (1973) up to the step of Sepharose chromatography.

The activity of phosphorylase *b* and *a* was measured by the method of Illingworth and Cori (1953) in the presence of 1 % glycogen, 16 mM glc-1-P, 10 mM glycerol-2-phosphate, 0.5 mM EDTA, 5 mM 2-mercaptoethanol, pH 6.8, at 30 °C, with and without 1 mM AMP, respectively. One enzyme unit is defined as the amount of phosphorylase which liberates 1 μmol P_i from glc-1-P per min. Inorganic phosphate was determined according to Taussky and Shorr (1953).

Antiserum against pig heart-specific phosphorylase was obtained by immunization of roosters. Immunization and immunotitration were performed as described by Vereb et al. (1977). The antiserum was stored at −20 °C or in freeze-dried state and used without any further purification. Details of immunotitration are described in the legend to Fig. 1.

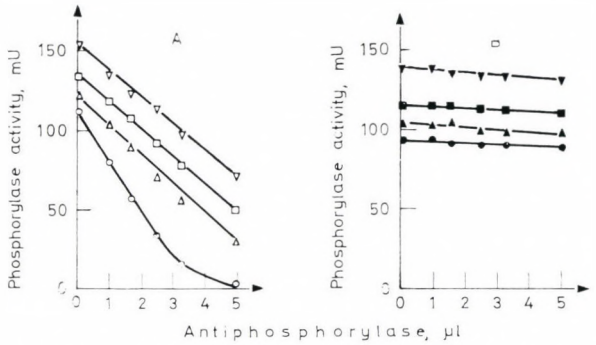

Fig. 1. Effect of antiphosphorylase against pig heart-specific phosphorylase on the activity of various isophosphorylases. Heart-specific or skeletal muscle-specific phosphorylases (prepared as described in Materials and Methods) in the *b* form were incubated with a given amount of serum from immunized rooster in the presence of GEM buffer and 5 µl serum from non-immunized rooster (total volume 0.2 ml, 30 °C, 10 min) and the remaining activity was assayed as described in Methods. A) Heart-specific phosphorylases (open symbols); B) Skeletal muscle-specific phosphorylases (closed symbols); ▽—▽, ▼—▼ bovine, □—□, ■—■ rabbit; △—△, ▲—▲ dog, ○—○, ●—● pig

Results and discussion

First we compared the immunoinhibition of heart-specific and skeletal muscle-specific phosphorylases (Fig. 1).

It can be seen that rooster antibodies *against pig heart-specific phosphorylase* have an inhibitory effect on the activity of all heart-specific phosphorylases. This inhibition proved to be proportional to the quantity of antibodies added, up to 70–80 per cent inhibition as can be revealed from the inhibition curve of pig heart-specific phosphorylase. Considering the linear part of the inhibition plots, 1 µl of antiserum inhibits 17 mU, 18 mU, 20 mU and 34 mU of heart specific phosphorylase from rabbit, bovine, dog and pig, respectively. This value proved to be independent of the quantity of the phosphorylase in the inhibition mixture (assayed between 50–200 mU).

On the other hand, the various skeletal muscle-specific phosphorylases can hardly be inhibited by antibodies against pig heart-specific phosphorylase (1 µl of rooster serum inhibits only 1–2 mU of activity).

The observed difference in the inhibitory effect of anti-pig-heart-specific phosphorylase serves as a further support to the different immunochemical properties of heart-, and skeletal muscle-specific phosphorylases in addition to the findings with antisera against skeletal muscle phosphorylase (Yunis et al., 1962; Vereb et al., 1977). On the other hand, our results indicate a common structural organization of various mammalian heart-specific phosphorylases which is responsible for the immunoinhibition, similarly to the class specifity of mammalian or avian skeletal muscle phosphorylases (Jókay et al., 1958).

Table 1

Kinetic constants of phosphorylase b from various mammals

Phosphorylases were prepared as described in Materials and methods. K_m for glc-1-P was determined in the presence of 1% glycogen and 1 mM AMP; glc-1-P was varied between 2–16 mM. Determination of K_m for glycogen was performed in the presence of 16 mM glc-1-P and 1 mM AMP; glycogen was varied between 0.04–1%. $A_{0.5(AMP)}$ represents the AMP concentration causing half-maximal activation as determined from a Hill plot of AMP saturation in the presence of 16 mM glc-1-P and 1% glycogen. $I_{0.5(glc\text{-}6\text{-}P)}$, the concentration of glc-6-P causing a 50% inhibition was determined at 1 mM AMP, 16 mM glc-1-P and 1% glycogen. Each value represents an average of two or more determination

Source	$K_{m(glc\text{-}1\text{-}P)}$, mM		$K_{m(glycogen)}$, %		$A_{0.5(AMP)}$, mM		$I_{0.5(glc\text{-}6\text{-}P)}$, mM	
	Skeletal muscle	Heart	Skeletal muscle	Heart	Skeletal muscle	Heart	Skeletal muscle	Heart
Rabbit	6.0	6.0	0.03	0.05	0.105	0.03	4.5	2.2
Pig	13.0	7.0	0.06	0.070	0.11	0.075	no inhibition	0.9
Bovine	16.0	7.5	0.07	0.05	0.25	0.14	30.0	0.75
Dog	5.5	8.3	0.03	0.06	0.07	0.12	4.4	1.8

The available enzyme preparations made it possible also to compare the kinetic parameters of various phosphorylases. Table 1 shows the K_m values for the substrates glc-1-P and glycogen, as well as the concentration of AMP and glc-6-P required for the half-maximal activation and inhibition of the phosphorylase *b* preparations ($A_{0.5(AMP)}$ and $I_{0.5(glc\text{-}4\text{-}P)}$, respectively).

The skeletal muscle-specific phosphorylases can be classified into two different groups on the basis of their affinity for glc-1-P with a K_m value of 6 mM (rabbit, dog) or 13–16 mM (pig, bovine). These data are consistent with previous observations (Assaf, Yunis, 1973; Schliselfeld, 1973; Hanabusa, Kohno, 1969). The $K_{m(glc\text{-}1\text{-}P)}$ values of heart-specific phosphorylases do not show such a variation, they are rather similar to the group of skeletal muscle phosphorylases having a K_m value of 6–8 mM.

On the basis of the K_m values for glycogen (0.03–0.07% for skeletal muscle enzymes and 0.05–0.07% for heart-specific enzymes) there is no characteristic and significant difference between the two groups of phosphorylases.

The AMP concentration causing half-maximal activation ($A_{0.5(AMP)}$) vary between 0.07–0.25 mM for the skeletal muscle enzymes and 0.03–0.14 mM for the heart-specific ones. In most cases heart-specific phosphorylase has a 1.5–3 fold higher affinity for AMP than the corresponding skeletal muscle enzyme, in agreement with the observation of Schliselfeld et al. (1970) concerning the rabbit phosphorylases; but dog phosphorylases show an opposite sensitivity.

A characteristic difference exists between the two groups with respect to the inhibitory effect of glc-6-P. The concentrations of glc-6-P required for a 50% inhi-

Table 2

Effect of substrates and glc-6-P on the activity of phosphorylase a from various mammals

Phosphorylase *b* preparations as listed in Table 1 were converted to phosphorylase *a* with rabbit skeletal muscle phosphorylase kinase (see Methods). K_m for glc-1-P and glycogen was determined as described in the legends to Table 1 but in the absence of AMP. $I_{0.5(glc\text{-}6\text{-}P)}$ was determined in the presence of 16 mM glc-1-P and 1% glycogen, without AMP. Each value represents an average of two parallel determinations

Source	$K_{m(glc\text{-}1\text{-}P)}$, mM		$K_{m(glycogen)}$, %		$I_{0.5(glc\text{-}6\text{-}P)}$, mM	
	Skeletal muscle	Heart	Skeletal muscle	Heart	Skeletal muscle	Heart
Rabbit	6.0	9.7	0.025	0.05		0.25
Pig	4.5	9.5	0.05	0.06	No inhibition	0.20
Bovine	6.0	4.5	0.05	0.04	(up to 5 mM)	0.20
Dog	8.0	8.0	0.03	0.035	(glc-6-P)	0.30

bition of skeletal muscle enzymes show a great variation, in accordance with our earlier findings (Bot et al., 1971). Rabbit and dog enzymes can be inhibited at the highest degree ($I_{0.5}$ are 4.5 mM and 4.4 mM, respectively) and the pig enzyme cannot be inhibited by glc-6-P at 1 mM AMP concentration. In contrast, the values of $I_{0.5(glc\text{-}6\text{-}P)}$ for heart enzymes are in all cases quite low, varying between 0.8–2.2 mM.

Previously we have demonstrated the competitive nature of glc-6-P inhibition with AMP on pig heart-specific phosphorylase and we have made use of this competition for specific elution during affinity chromatography on AMP-Sepharose (Vereb et al., 1978; Dombrádi et al., 1979). Earlier the great difference between the inhibition of the skeletal muscle and heart-specific phosphorylase from pig has been explained solely by assuming insensitivity of the skeletal muscle enzyme to glc-6-P. Our present investigations indicate a pronounced and characteristic sensitivity of all heart-specific phosphorylases in the *b* form to glc-6-P (including the pig enzyme as well), in contrast to the skeletal muscle enzymes.

A comparison of the *a* forms presents a further opportunity to differentiate between the two groups of phosphorylases (Table 2).

Table 2 shows that all phosphorylases of the two groups in the *a* form havea nearly identical K_m value for glc-1-P, varying between 4–8 mM. Similarly, there is no characteristic difference in the K_m value for glycogen; they range essentially between 0.03–0.05% for both types of the enzymes.

A unique difference could be observed, however, in their inhibition by glc-6-P; skeletal muscle-specific phosphorylases were not inhibited up to 5 mM glc-6-P, whereas heart-specific phosphorylases showed an $I_{0.5}$ value of 0.2–0.3 mM.

This high sensitivity to glc-6-P seems to be a common characteristic of the *a* form of the heart-specific phosphorylases. Consequently, this metabolite can play

a regulatory role in the glycogenolysis of the heart muscle and may have a physiological significance in controlling the glycogen breakdown also when phosphorylase exists in the phosphorylated *(a)* form, because the concentration of glc-6-P in heart muscle varies in the range of 0.2–2 mM depending on the metabolic state (Opie, 1976).

Comparing the metabolite regulation of the *b* form of isophosphorylases, the heart-specific enzymes show a higher sensitivity to the activating effect of AMP. This feature is not striking (and appears as a tendency), but the obligatory and significant sensitivity to glc-6-P inhibition of the *b* form and particularly of the *a* form calls our attention to a difference in their structure as compared to that of skeletal muscle phosphorylases.

The common characteristic allosteric regulation and common immunochemical reactivity of the heart-specific phosphorylase, isolated from various mammalian hearts, suggests a common genetic control of these enzymes, differing from that of skeletal muscle-specific enzymes. Such an idea is supported by findings in patients with skeletal muscle phosphorylase deficiency (Miranda et al., 1979).

Function differentiation of phosphorylase can be followed during phylogenesis; an increase in affinity for substrates and activator (AMP) is characteristic of muscle phosphorylases (Yonezawa, Hori, 1976). Our observation show no appreciable difference between the heart- and skeletal muscle-specific phosphorylases with respect to their affinity for substrates, suggesting a similarity of their catalytic sites. But the characteristic structural properties of the nucleotide site, which is involved in the binding of glc-6-P and is responsible for glc-6-P inhibition could be a tool in investigating the differentiation of heart-specific phosphorylases during phylogenesis.

References

Assaf, S. A., Yunis, A. S. (1973) Ann. N. Y. Acad. Sci. *210* 139–152
Bot, G., Pólyik, E., Vereb, G. (1971) Acta Biochim. Biophys. Acad. Sci. Hung. *13* 189–192
Cohen, P. (1973) Eur. J. Biochem. *34* 1–14
Davis, C. H., Schliselfeld, L. H., Wolf, D. P., Leavitt, C. A., Krebs, E. G. (1967) J. Biol. Chem. *242* 4824–4833
Dombrádi, V., Vereb, G. (1978) Acta Biochim. Biophys. Acad. Sci. Hung. *13* 7–12
Dombrádi, V., Vereb, G., Bot, G. (1979) Int. J. Biochem. *10* 905–908
Fischer, E. H., Krebs, E. G. (1958) J. Biol. Chem. *231* 65–71
Gornall, A. G., Bardawill, C. J., David, M. M. (1949) J. Biol. Chem. *177* 751–766
Hanabusa, K., Kohno, H. (1969) J. Biochem. *66* 69–76
Illingworth, B., Cori, G. T. (1953) in Biochem. Prep. (Snell, E. E. ed.), Vol. *3*, pp. 1–9, Wiley, New York
Jókay, I., Bot, G., Szilágyi, T. (1958) Acta Physiol. Acad. Sci. Hung. *14* 155–161
Krebs, E. G., Fischer, E. H. (1962) in Methods in Enzymol. (Colowick, S. F., Kaplan, N. O., eds.) Vol. *5*, pp. 373–376, Acad. Press, New York
Miranda, A. F., Nette, E. G., Hartlage, P. L., DiMauro, S. (1979) Neurology *29* 1538–1541
Opie, L. H. (1976) Circ. Res. *38* Suppl. *1* 52–74
Schlisefeld, L. H. (1973) Ann. N. Y. Acad. Sci. *210* 181–191
Schlisefeld, L. H., Devis, C. H., Krebs, E. G. (1970), Biochemistry *9* 4959–4965

Taussky, H. H., Shorr, E. (1953) J. Biol. Chem. *202* 675—695
Vereb, G., Bot, G. (1972) Acta Biochim. Biophys. Acad. Sci. Hung. *7* 35—46
Vereb, G., Gergely, P., Bot (1977) Acta Biochim. Biophys. Acad. Sci. Hung. *12* 237—244
Vereb, G., Dombrádi, V., Bot, G. (1978) Int. J. Biochem. *9* 13—16
Will, H., Krause, E.-G., Wollenberger, A. (1970) Biochem. Biophys. Res. Commun. *40* 7—14
Will, H., Krause, E.-G. (1972) Acta Biol. Med. Germ. *28* 919—933
Will, H., Krause, E. G., Böhm, M., Guski, H., Wollenberger, A. (1974) Acta Biol. Med. Ger. 149—160
Yonezawa, S., Hori, S. H. (1975) J. Histochem. Cytochem. *23* 745—751
Yonezawa, S., Hori, S. H. (1976) J. Biochem. *79* 1109—1111
Yunis, A. A., Fischer, E. H., Krebs, E. G. (1962) J. Biol. Chem. *237* 2809—2815

Correspondence: GY. VEREB
Institute of Medical Chemistry
H-4026 Debrecen, Bem tér 18/B

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Effect of Plaster Cast Immobilization on the Turnover Rates of Soluble Proteins and Lactate Dehydrogenase Isoenzymes of Rabbit M. Soleus

I. ÉDES, E. DÓSA, I. SOHÁR, F. GUBA

Department of Biochemistry, University Medical School Szeged, Szeged, Hungary

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In atrophysed muscle the decreases of the activity of LDH izoenzymes can be explained partly by a 15 per cent decrease of the enzyme synthesis and partly by a 25 per cent increase in its catabolism. The synthesis rate of soluble proteins barely changed during immobilization. In the atrophysed muscle the decrease of the amount of soluble proteins could be almost exclusively interpreted in terms of a 25 per cent enhancement of degradative processes.

The accelerated catabolism is most probably due to the proteolytic enzymes activated by immobilization.

Introduction

Plaster cast immobilization of the actively working muscle provides an excellent opportunity to study the relationships between contractile activity and protein metabolism. In the course of atrophy resulting from muscle immobilization, the amount of soluble proteins (Takács et al. 1977, Sohár et al., 1977), and that of oxidative and glycolytic enzymes decrease and the metabolism in differently functioning muscles tends to get very similar (Édes et al., 1980).

Among the glycolytic enzymes, the amount and composition of lactate dehydrogenase (LDH, EC. 1.1.1.27) become altered and the metabolism of muscle tissue accommodates to the new conditions. The relative or absolute shift in the metabolism of a given tissue is always accompanied by the formation of a new isoenzyme pattern (Kowalewski, 1972). As a consequence of their central position in the intermediary metabolism, isoenzymes possess a key role in the regulation of aerobic and anaerobic metabolism of tissues.

In this work we studied the influence of immobilization-induced changes in the anabolic and catabolic processes on the synthesis and degradation of soluble proteins and LDH izoenzymes.

Materials and methods

Animals

Male New Zealand rabbits, weighing 2500 g on the average, were used. Right hind limbs of the animals were fixed in extension with circular plaster casting. Rabbits of the same breed, weight and sex served as controls.

Abbreviations: LDH, lactic dehydrogenase

LDH turnover assays

Following two weeks in plaster casting the animals were given 700 μCi ^{3}H-leucine (specific activity 1.4 Ci/mmol), i.v., in isotonic salt solution. At various intervals afterwards the plaster was removed, the animals exsanguinated and the m. soleus of the prepared limb was dissected. Connective tissue and fat were removed from the muscles. Homogenization was performed in 50 mM phosphate buffer, pH 7.2, containing 0.1 per cent Triton X-100. Subsequently, the samples were centrifuged at 100 000 $\times$ g for 60 min. The clear supernatant fluids were pooled and dialysed for 2 hours, against 0.2 M phosphate buffer + 0.5 M NaCl.

LDH was isolated by means of affinity chromatography (O'Carra, Barry, 1972) on a substituted Sepharose 4B column. This method was based on the finding that substituted Sepharose 4B – in the presence of NADH – specifically binds LDH isoenzymes. Eluted LDH fractions were concentrated and, from the individual samples, 0.1 – 0.2 ml aliquots were removed and directly dissolved in 5 ml toluol/Triton X-100 cocktail.

Activities were expressed in dpm-s. Decrease of radioactivity was plotted and half life ($t_{1/2}$), as well as synthesis and decomposition ratio constants (k_s, k_d) were calculated. Presuming that steady state was attained, $k_s = k_d E$ (Dölken, Pette, 1974) where k_s is the synthesis ratio constant, k_d is the decomposition ratio constant and E is the intracellular concentration of the enzyme. k_d can also be calculated from the half life: $t_{1/2} = \ln 2/k_d$.

Turnover of soluble proteins

From the 100 000 $\times$ g supernatant fluids, soluble proteins were precipitated by 10 per cent trichloracetic acid and centrifuged for 5 min at 3000 $\times$ g. Pellets were washed with 70 per cent acetone, solubilized with 0.2 M NaOH and the activity was measured in toluol/Triton X-100 cocktail. Radioactivities were determined in a Packard Tri-Carb Scintillation Counter.

Protein concentrations were determined by the microbiuret method (Goa, 1953).

Measurement of LDH activity

Activities of this enzyme were determined by monitoring spectrophotometrically the decrease of NADH concentration in the course of the reaction. To calculate the protein content of LDH, 400 U/mg was taken as the specific activity of the enzyme.

Results

As a result of plaster cast immobilization the amount of total LDH isoenzymes decreased (Fig. 1). This effect was most prominent in the first two weeks of immobilization and from the 14th day on, the amount of isoenzymes was stabilized

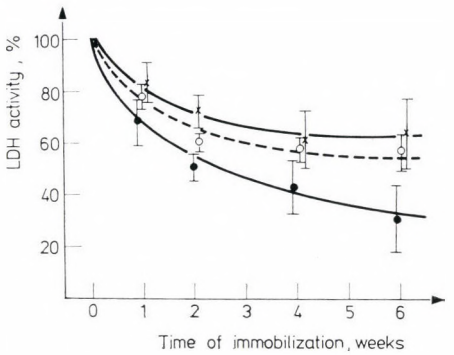

Fig. 1. Effects of immobilization on total LDH activity, on soluble protein content and on muscle weight. Values are the means of three different experiments and are given as the percentage of the control (100%) ± S.D. ×—×: LDH activity, ○—○: soluble protein content, ●—●: muscle weight

at about the 60 per cent level. The slope of the curve indicating the total amount of soluble proteins exhibited a similar pattern: on day 14 it decreased to the 55 per cent of the initial value and remained practically unchanged.

Turnover rates were assayed two weeks after the immobilization. By this time the intracellular amounts of LDH isoenzymes and soluble proteins reached a new steady state again, but at a lower level that is, the ratio of protein anabolism and catabolism again reached equilibrium.

Figure 2 depicts the radioactivities of soluble proteins of both the normal and immobilized muscles, as a function of time. In the normal m. soleus the maximal radioactivity in the soluble proteins could be detected 6 hours after the administration of the isotope. For the immobilized muscle this time was somewhat shorter. The half-lives ($t_{1/2}$) of soluble proteins were 2 days for the normal and 1.6 days for the immobilized muscle. Because of the enhanced reutilisation of the labeled amino acids, the half lives were calculated from the first phase of the curves.

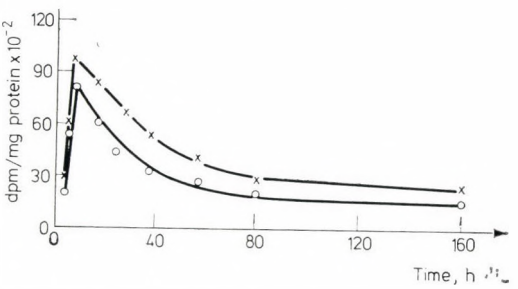

Fig. 2. Radioactivities of soluble proteins as a function of time. Values are the means of three different experiments. ×—× normal muscle; ○—○ immobilized muscle. Values are given in dpm/mg units

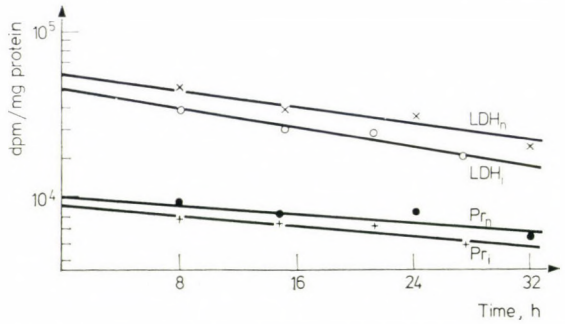

Fig. 3. Changes in the radioactivities of soluble proteins and LDH izoenzymes. LDH_n and LDH_i are the radioactivities in the normal and immobilized muscle. Pn_r and Pr_i are the activities of soluble proteins in the normal and immobilized muscle, expressed as dpm/mg protein units. Values are the mean of three separate experiments. Straight lines were fitted using the least square method

Figure 3 shows the decrease of radioactivity of LDH as a function of time, in normal and immobilized muscle. The half life calculated from the curves was 1.25 days for the normal and 1 day for the immobilized m. soleus. We attempted to determine the half lives of LDH izoenzymes (LDH-1, LDH-2, etc.), separately, but, due to the low radioactivities, we failed to isolate the required amount.

In Table 1 we summarize the half lives, the values of ratio constants between the synthesis and decomposition rates, as well as tissue concentrations of the soluble proteins and LDH izoenzymes.

Table 1

Characteristics of the turnover of soluble proteins and LDH isoenzymes

k_d: constant of decomposition ratio, $t_{1/2}$: half life. E: tissue concentration, k_s: constant of synthesis ratio

	k_d (day)	$t_{1/2}$ (day)	E ($mg \times g^{-1}$)	k_s ($mg \times g^{-1} \times day$)
LDH normal muscle	0.554	1.25	0.377	0.208
immobilized muscle	0.693	1.00	0.255	0.176
Soluble protein normal muscle	0.346	2.00	53.4	18.5
immobilized muscle	0.433	1.60	41.5	18.0

Discussion

M. soleus in the rabbit contains mainly slow oxidative fibres which are under constant tone. The turnover rate of soluble proteins in the oxidative m. soleus is high, as compared to that of the rapid glycolytic muscles (Dölken, Pette, 1974). The rapid turnover of proteins in the m. soleus can be interpreted by means of the high proteolytic activity of the muscle (Goldspink et al., 1970, Pluskal, Pennington, 1973).

Degradation of soluble proteins of the muscle becomes accelerated under both anabolic and catabolic circumstances (Waterlow et al., 1978; Goldspink, 1977). Waterlow et al. (1978) observed an enhanced protein degradation during the development of the muscle, in muscle hypertrophy, as well as under muscle atrophy resulting from starvation and protein deficiency. Most critical in the pathogenesis of the immobilization-elicited muscle atrophy is, according to Goldspink (1977) the decrease of protein synthesis and the increase in protein degradation. Our results show that, as a response to plaster cast immobilization, the catabolism of soluble proteins in the rabbit m. soleus increases, accompanied by a slight decrease of their synthesis rate. Half-lives of the different glycolytic enzymes in this muscle are in the range of 0.8 – 1.3 days (Dölken, Pette, 1974). This is shorter than the average value for soluble proteins. Dölken and Pette (1974) found a 24 hours long half life for LDH in rabbit m. soleus, while we obtained 30 hours. The observed amino acids in the two studies (^{3}H-leucine and ^{14}C-leucine, respectively).

Immobilization enhanced the catabolism of LDH izoenzymes: the k_d value increased from 0.54 to 0.69. Accompanying this growth of the k_d constant, the value of the k_s constant also changed, indicating a decrease of the synthesis rate of LDH.

Another effect pointing to the slowing down of protein synthesis elicited by immobilization is the change in the nucleic acid content of the muscle. As a result of plaster casting, the total RNA content, as well as the amount of RNase inhibitor, decreases with a concomitant increase alkaline RNase activity (Kiss, Guba, 1979).

The increased catabolism that develops upon plaster casting can be interpreted in terms of the activation of the proteolytic enzymes of the muscle. According to Sohár et al. (1980) the activities of lysosomal proteolytic enzymes (chatepsin B, D, E and L) are substantially increased in the atrophysed m. soleus. The activities of the cytoplasmic proteolytic enzymes were not significantly influenced by immobilization. In the oxidatively working m. soleus the activities of the lysosomal enzymes were significally higher than those of the rapid-glycolytic muscles.

References

Dölken, G., Pette, D. (1974) Hoppe-Seyler's Z. Physiol. Chem. *355* 289

Édes, I., Sohár, I., Mazareán, H., Takács, Ö., Guba, F. (1980) Acta Biochim. et Biophys. Acad. Sci. Hung. *15* 305

Goa, J. (1953) Scand. J. Clin. Lab. Invest. *5* 218

Goldspink, D. F. (1977) J. Physiol. *264* 267
Goldspink, D. F., Harris, J. B., Park, D. C., Pennington, R. J. (1970) Enzym. Biol. Clin. *11* 481
Kiss, Zs., Guba, F. (1979) FEBS Lett. *108* 185
Kowalewski, S. L. (1972) Die Isoenzyme der Lactatdehydrogenase, p. 56. Georg Thieme Verlag, Stuttgart
O'Carra, P., Barry, S. (1972) FEBS Lett. *21* 281
Pluskal, M. G., Pennington, R. J. (1973) International Res. Communications System 73—11 3—10—30
Sohár, I., Nagy, I., Takács, Ö., Kovács, Zs., Guba, F. (1980) Mechanism of Muscle Adaptation to Functional Requirements, Editors: Guba, F., Maréchal, G., Takács, Ö. p. 213 Pergamon Press, Oxford
Sohár, I., Takács, Ö., Guba, F. (1977) Acta Biol. Med. Germ. *36* 1621
Takács, Ö., Sohár, I., Pelle, T., Guba, F., Szilágyi, T. (1977) Acta Biol. Acad. Sci. Hung. *28* 213
Waterlow, J. C., Garlick, P. J., Millward, D. J. (1978) Protein turnover in mammalian tissues and in the whole body, p. 625. North-Holland Publishing Company, Amsterdam
Wroblewsky, F., La Due, J. S. (1955) Proc. Soc. Exp. Biol. Med. *90* 210

Correspondence: I. Édes
Department of Biochemistry,
H-6725 Szeged, Korányi fasor 6

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Adenosin-5'-diphosphate Penetration into Synaptosomes Isolated from Rat Cerebral Cortex

A. CSILLAG, M. KÁLMÁN, Zsuzsanna CSATÓ*

Department of Anatomy I and Department of Biochemistry I*, Semmelweis University Medical School, Budapest, Hungary

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Rat cortical synaptosomes responded to externally added ADP by a respiratory increase in the presence of various substrates. The incorporation of ^{3}H-ADP was rapid in the first 5 minutes of incubation and was followed by a further slow influx. The amount of ^{3}H-ADP incorporated considerably exceeded that of basal uptake and did not depend on the presence of EGTA. As revealed by EM autoradiography, the major part of incorporated radioactivity of the fraction was found in synaptosomes. The results suggest that ADP can enter intact synaptosomes. This process might serve as a possible mechanism for the removal of ADP in the synaptic environment. The data fit in with other findings according to which adenine nucleotides released from and taken up into nerve endings can play a modulatory role in synaptic transmission.

Introduction

It has been known for some time that isolated nerve ending particles (synaptosomes) are appropriate model systems for studying several aspects of synaptic transmission, excitability and transport phenomena. Previous works have shown that synaptosomes are surrounded by a resealed membrane derived from the interrupted axolemma (Gray, Whittaker, 1962; Whittaker, 1968). They are known to behave as osmometers (Whittaker, 1969; Keen, White, 1971) and show an energy-dependent accumulation of ions and other substances (Bradford, 1969; Escueta, Appel, 1969; Blaustein, Goldring, 1975; White, 1975; Levi, Raiteri, 1976), which indicates that the plasma membrane can serve as an efficient permeability barrier. Its functions resemble in many respects those of neuronal membranes in general but some findings indicate that synaptosomal membranes differ in permeability from those of other parts of the neuron. For example, nerve membranes are thought to be impermeable to adenine nucleotides (Caldwell et al. 1960). It appears, however, both from respiration studies (Verity, 1972) and from direct measurements of adenine derivative movements (Kuroda, McIlwain, 1974; Meunier, Morel, 1978) that the plasma membrane of isolated nerve endings can, under certain conditions, be permeable to adenine nucleotides. There is direct evidence for ATP and adeno-

Abbreviations: EM, electron microscope; EGTA, ethylenelglycol-bis-(2 aminoethyl)-tetraacetic acid; RCR, respiratory control ratio.

sine being released from synaptosomes on stimulation (Kuroda, McIlwain, 1974; Meunier et al. 1975; White, 1978). The present work is an account of investigations on the influx of ADP into synaptosomes using different experimental approaches.

Materials and methods

Synaptosomes were isolated from rat cerebral cortex by the method of Hajós (1975). The particles collected from the 0.8 M sucrose layer of gradients were diluted with ice-cold distilled water to 0.4 M and sedimented at 20 000 × *g* for 15 min. The pellets were resuspended in 0.32 M sucrose. The fractions were regularly checked by electron microscopy: routinely, the purity of synaptosomal fractions was 80–90%. The contamination by free mitochondria was 10–20%. The oxygen consumption of the fractions was measured polarographically, using a Clark-type oxygen electrode (Radelkis, Budapest), at 37 °C. For uptake studies, samples of synaptosomes were incubated in a shaking water-bath at 37 °C. The medium was identical to that used in the respiration studies. The final volume was 0.7 ml from which 0.5 ml aliquots were withdrawn, transferred into Eppendorf centrifuge tubes and spun in a bench centrifuge for 30 s. The supernatants were removed by narrow-tipped Pasteur pipettes making sure that the pellets were not touched. Then the surface of pellets was given two washes of 0.32 M sucrose (containing 1 mM $CaCl_2$), the last washing solution was sucked from the tubes by a Pasteur pipette and the tube wall was wiped with a small tissue tampon. The pellets were extracted overnight in 0.5 ml of NCS Tissue Solubilizer, 300 μl aliquots were mixed with 5 ml of Instagel scintillant, kept in the dark for 24 hours and counted in a Beckman LS-355 or in a Packard Tri-Carb liquid scintillation counter. Protein was determined according to Lowry et al. (1951). For EM autoradiography, samples of synaptosomes (1 mg/ml) were incubated in media identical to those used in uptake studies (except that 185 kBq of ^{3}H-ADP was present) for 6 min, at 37 °C. Incubation was terminated by the addition of Karnovsky's fixative (Karnovsky, 1964), after which synaptosomes were sedimented, the pellets were washed twice with 0.32 M sucrose and twice with medium. Samples were postfixed in 1% OsO_3, embedded in Durcupan and processed for EM autoradiography. Sections of light gold interference colour were cut, placed on glass slides (pre-coated with celloidin), dipped in Ilford L 4 emulsion (1 : 4 dilution) and exposed for 5 weeks. The slides were developed with Kodak Microdol-X, the sections were floated on the surface of distilled water, picked up on copper grids and stained with uranyl acetate and lead citrate.

[2-^{3}H] Adenosine-5'-diphosphate was purchased from the Radiochemical Centre, Amersham. All other chemicals were of the highest purity available.

Results

Synaptosomes respiring in a sucrose-saline medium responded to externally added ADP by an increased rate of respiration (Table 1). The highest respiratory control values were observed with pyruvate *plus* malate as substrates. The effect of ADP on respiration required the presence of EGTA. This compound enhanced the response to ADP when added either before or after ADP (traces A and B of Fig. 1). The effect of EGTA could be prevented by Ca^{2+}, and this Ca^{2+}-inhibition was reversed by the presence of excess EGTA (trace C of Fig. 1).

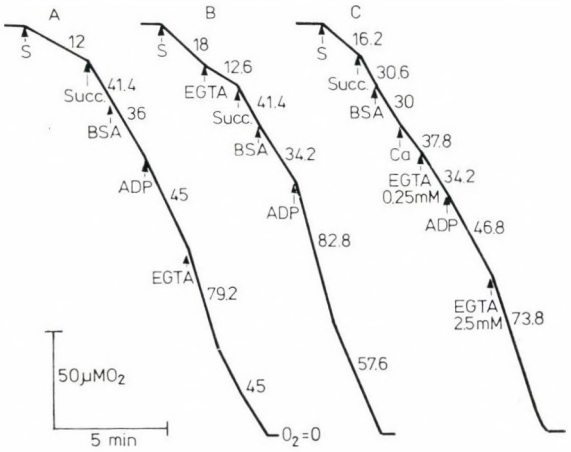

Fig. 1. Effects of EGTA and Ca^{2+} on the respiration of synaptosomes. Medium: 160 mM sucrose, 64 mM NaCl, 4 mM KCl, 0.8 mM $MgCl_2$, 6.4 mM Tris-P_i (pH 7.4) in 2.2 ml final volume. Additions: S — 50 μl sample containing 2.6 mg protein, succ — 6.9 mM Na-succinate, BSA — 1.4 mg/ml bovine albumin, ADP — 225 μM adenosine diphosphate (trisodium salt), EGTA (if concentration not indicated) — 0.25 mM ethylene glykol-bis-(2 aminoethyl)-tetra-acetic acid (tris salt, pH 7.4), Ca — 0.9 mM $CaCl_2$. The figures above the tracings represent respiratory rates in nA 0/mg/min

Table 1

Oxygen consumption of synaptosomes in the presence of various substrates and ADP

Respiratory rates are expressed in nmol O_2/mg/min, as the mean ± S.E.M. (n). RCR values were calculated by dividing respiratory rates measured in the presence of ADP (225 μM) by those measured in its absence. Concentrations of substrates: pyruvate — 8 mM, malate — 4 mM, glutamate — 8 mM, succinate — 4—8 mM

Substrate	No ADP	ADP	RCR
pyruvate + malate	19.7± 2.3 (5)	53.2± 5.8 (5)	2.7± 0.06
succinate	15.8± 1.5 (6)	33.5± 2.9 (6)	2.2± 0.20
glutamate ± malate	14.8± 1.9 (6)	27.4± 3.7 (6)	1.9± 0.20

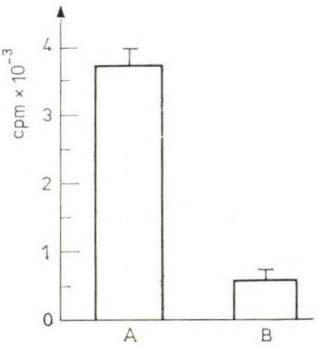

Fig. 2. Incorporation of ^{3}H-ADP into synaptosomes. Fifty μl samples of synaptosomes (containing 1.4 mg protein) were suspended in 0.7 ml of medium identical to that in Fig. 1 except that 7 mM Na-succinate was present (for further details see Methods). ^{3}H-ADP was added to both A and B at 37 kBq/ml and cold ADP to B only, at 250 μM concentration, at zero time. Specific activities: A — 703 kBq/nmol; B — 0.15 kBq/nmol. Final concentrations of ADP: — A — 0.053 μM; B — 250 μM. The samples were incubated for 6 min. Each column represents the mean ± S.E.M. of five parallel counts

The measurements of labelled ADP uptake show that by decreasing the specific activity of ^{3}H-ADP (keeping the concentration of radioactivity in the medium unchanged) the amount of incorporated radioactivity fell to a level (Fig. 2) not exceeding the inulin-penetrable space (as determined in preliminary experiments). The uptake values of labelled ADP were found to be similar in the presence of EGTA or with no EGTA but Ca^{2+} present (Fig. 3). The time course of ^{3}H-ADP incorporation showed a rapid exponential rise in the first 5 minutes of incubation after which a further slow increase could be observed (Fig. 4). We have experienced in previous studies that synaptosomes underwent gradual morphological deterioration after about 20 minutes of incubation at 37°, hence incubation times longer than 20 min were not used.

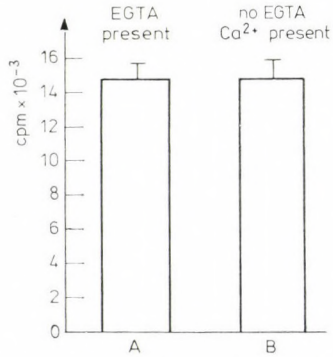

Fig. 3. Lack of effect of EGTA on the incorporation of ^{3}H-ADP into synaptosomes. Medium as in Fig. 2 except that 0.28 mM EGTA was present in A and 1 mM $CaCl_2$ was present in B. 107 kBq/ml ^{3}H-ADP was added at zero time. The samples were incubated for 5 min. Columns A and B represent the mean ± S.E.M. of four and five parallel counts, respectively

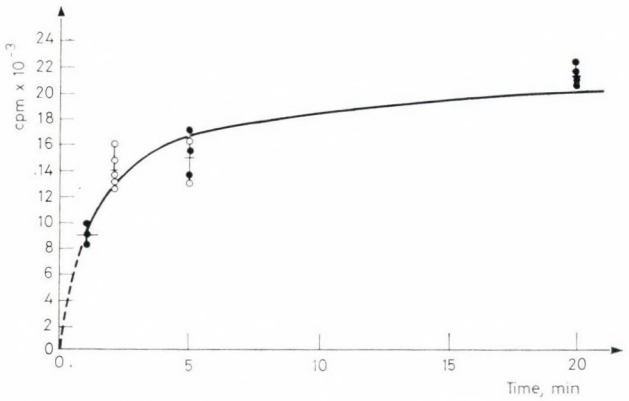

Fig. 4. Time-dependent incorporation of ^{3}H-ADP into synaptosomes. Medium as in Fig. 2. 107 kBq/ml ^{3}H-ADP was added at zero time. The results of two experiments are plotted (indicated by open and filled circles), each point on the graph representing a separate measurement

The distribution of silver grains on autoradiograms of synaptosomal fractions labelled with ^{3}H-ADP showed that out of 400 labelled particles 53.2 $\pm$ 1.5% were synaptosomes as calculated from four parallel counts. A typical example of labelled synaptosomes is shown in Fig. 5. As expected, the rest of label was found predominantly over free mitochondria.

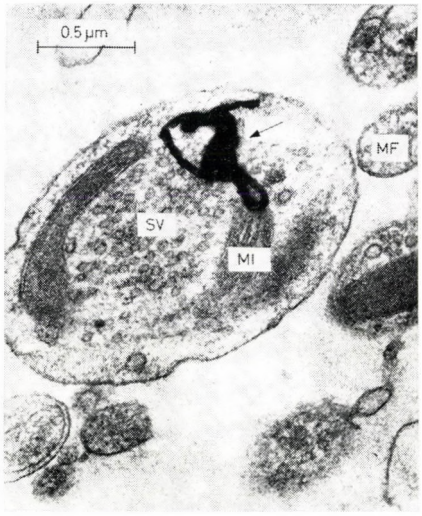

Fig. 5. EM autoradiogram of a typical synaptosome incorporating ^{3}H-ADP as indicated by the silver grain (arrow). MI = intraterminal mitochondrion, MF = free mitochondrion, SV = synaptic vesicles

Discussion

The transient rise of the respiratory rate on addition of ADP is a well-known reflection of mitochondrial energy-conservation and the acceptor control phenomenon. If synaptosomal fractions are also able to produce this effect, their "intraterminal" mitochondria must have direct access to externally added ADP through the synaptosomal plasma membrane. Verity (1972) measured high respiratory control ratios in his synaptosomal fractions but did not discuss the problem of ADP penetration. He also found an absolute requirement of EGTA for RCR values to be sufficiently high. As this author used Ficoll for preparing synaptosomes his results may not be directly comparable with those obtained in fractions prepared by sucrose methods. In our hands, synaptosomes prepared on a sucrose gradient were able to respond to ADP by a two-to-threefold increase of respiratory rate using various substrates. The values may appear low but one has to take into consideration that the "intraterminal" mitochondria are surrounded by a cytoplasmic environment where, due to various ATPase activities, the ATP/ADP ratio may never be high enough to elicit a genuine state 4 respiration. The $Na^+ - K^+$-ATPase may be primarily responsible since its activity has been clearly demonstrated by the energy-dependent K^+-accumulation of the fraction under conditions similar to those of respiration measurements (Hajós et al., 1977, Csillag, Hajós, 1980). As the value of the denominator of RCR fraction (state 4 rate) is relatively high, the value of the fraction becomes relatively low. Respiratory control ratios around 2 were observed in rat brain synaptosomes by Sitkiewicz et al. (1980).

The EGTA-dependence of respiratory control was also confirmed. This raised the question of a possible direct effect of EGTA on plasma membrane permeability, since chelators may increase the permeability of biological membranes (Settlemire et al., 1968). The studies on labelled ADP uptake did not support this type of effect because the values were similar with or without EGTA. Thus, EGTA presumably acted here at the mitochondrial level as a stabilizer of mitochondrial coupling. It is well known that the best preparations of brain mitochondria can be obtained with EGTA or EDTA present in the isolating media (Clark, Nicklas, 1970) and it appears that chelators might have a similar effect on the "intraterminal" mitochondria of synaptosomes as well. The rate of incorporation of ^{3}H-ADP into synaptosomes might reflect the time course of restitution of ionic equilibrium in particles. For K_+, an equilibrium was normally attained between 5 and 10 min of incubation (Csillag, Hajós, 1980). Presumably, in the first, most active phase of ion transport the turnover of adenine nucleotides is high, whereas in the equilibrium phase it falls to a lower steady-state value. The marked effect of decreasing the specific activity of ^{3}H-ADP on the amount of label incorporated argues against the presence of a considerable non-specific binding or adsorption of the isotope to particles.

Views on the permeability of synaptosomal plasma membrane to adenine nucleotides have been rather divided. Some authors accept it as a matter of course (Verity, 1972), others either do not discuss this possibility or reject it. For example, Logan and Waters (1976) interpret their findings on exogenous ATP hydrolysis as

the presence of at least 80% inside-out particles among synaptosomes rather than reckoning with an ATP influx through intact synaptosomal membrane. Our fractions were regularly checked by electron microscopy and consisted of 80–90% synaptosomes with well recognizable internal structure. Therefore, contamination by inside-out particles was rather unlikely. The results of EM autoradiography indicate that synaptosomes participate to a considerable extent in the incorporation of radioactivity from ^{3}H-ADP. Thus, the possibility that synaptosomes are inactive elements in the responses described can be excluded. Although the involvement of free mitochondria in the uptake of radioactivity may not be negligible, their role in the respiratory responses is presumably less important. The morphological configuration of the majority of free mitochondria does not correspond to that of intact brain mitochondria with high RCR values (Hajós, Csillag, 1978). Also, the lack of respiratory increase on addition of Ca^{2+} (Fig. 1 C) – which is expected to affect free mitochondria but not intraterminal ones – argues against the competence of free mitochondria in the controlled respiration of our synaptosomal fraction.

The present study indicates that ADP enters intact synaptosomes in amounts sufficient to induce a respiratory response. Its entry is presumably influenced by the turnover of intrasynaptosomal adenine nucleotides. Whether this process has any physiological role is as yet unknown. The transport of adenine derivatives has been reported to be relevant to purinergic synaptic mechanisms (Nurnstock 1972) or to a modulatory role of adenosine and related compounds in the release of other transmitters (Ginsborg, Hirst, 1972; Ribeiro, Walker, 1975; Vizi, Knoll, 1976; Clanachan, Paton, 1977; Sawynok, Jhamandas, 1976). It is generally accepted that synaptosomes show the highest permeability to adenosine, whereas the phosphorylated forms have been found to penetrate to a lesser degree in Torpedo synaptosomes (Meunier, Morel, 1978). These authors found high ATPase activity and a lack of degradation mechanisms for ADP although the latter was not confirmed by Zimmermann et al. (1979). It appears that, in rat cortical synaptosomes, a direct influx might contribute to the elimination of ADP from the extra-synaptic environment.

References

Blaustein, M. P., Goldring, J. M. (1975) J. Physiol. (Lond.) *247* 589–615
Bradford, H. F. (1969) J. Neurochem. *16* 675–684
Burnstock, G. (1972) Pharmac. Rev. *24* 509–581
Caldwell, P. C., Hodgkin, A. L., Keynes, R. D., Shaw, T. I. (1960) J. Physiol. (Lond.) *152* 561–590
Clanachan, A. S., Paton, D. M. (1977) Br. J. Pharmac. *59* 534P
Clark, J. B., Nicklas, W. J. (1970) J. Biol. Chem. *245* 4724–4734
Csillag, A., Hajós, F. (1980) J. Neurochem. *34* 495–503
Escueta, A. V., Appel, S. H. (1969) Biochemistry *8* 725–733
Ginsborg, B. L., Hirst, G. D. S. (1972) J. Physiol. (Lond.) *224* 629–645
Gray, E. G., Whittaker, V. P. (1962) J. Anat. (Lond.) *96* 79–88
Hajós, F. (1975) Brain Res. *93* 485–489

Hajós, F., Csillag, A. (1978) Acta Morph. Acad. Sci. Hung. *26* 107–113
Hajós, F., Csillag, A., Kálmán, M. (1977) Exp. Brain Res. *33* 91–99
Karnovsky, M. J. (1964) J. Cell. Biol. *27* 137A
Keen, P., White, T. D. (1971) J. Neurochem. *18* 1097–1103
Kuroda, Y., McIlwain, H. (1974) J. Neurochem. *22* 691–699
Levi, G., Raiteri, M. (1976) Int. Rev. Neurobiol. *19* 51–74
Logan, J. G., Waters, G. A. (1976) J. Physiol. (Lond.) *256* 30P
Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. biol. Chem. *193* 265–275
Meunier, F. M., Israel, M., Lesbats, B. (1975) Nature *257* 407–408
Meunier, F. M., Morel, N. (1978) J. Neurochem. *31* 845–851
Ribeiro, J. A., Walker, J. (1975) Br. J. Pharmac. Chemother. *54* 213–218
Sawynok, J., Jhamandas, K. H. (1976) J. Pharmac. Exp. Ther. *197* 379–390
Settlemire, C. T., Hunter, G. R., Brierley, G. P. (1968) Biochim. Biophys. Acta *162* 487–499
Sitkiewicz, D., Skonieczna, M., Krzywicka, K., Dziedzic, E., Staniszewska, K., Bicz, W. (1980) J. Neurochem. *34* 614–626
Verity, M. A. (1972) J. Neurochem. *19* 1305–1317
Vizi, E. S., Knoll, J. (1976) Neuroscience *1* 391–398
White, T. D. (1975) J. Neurochem. *24* 1037–1042
White, T. D. (1978) J. Neurochem. *30* 329–336
Whittaker, V. P. (1969) In "The Synaptosome. (Handbook of Neurochemistry, Vol. 2)" pp. 327–364 (A. Lajtha ed.) Plenum Press, New York
Zimmermann, H., Dowdall, M. J., Lane, D. A. (1979) Neuroscience *4* 979–993

Correspondence: A. CSILLAG
Department of Anatomy I.
Semmelweis University Medical School
H-1094 Budapest, Tűzoltó-utca 58

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Uridine-rich Low Molecular Weight RNAs of the Nucleolus and the Nucleus Hybridized with the Ribosomal DNA of Novikoff Hepatoma Cells

(Short Communication)

Anna FÓNAGY, I. FINANCSEK, E. J. HIDVÉGI

"F. Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary

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Nucleolar DNA of Novikoff hepatoma ascites tumor cells was digested with the restriction enzyme EcoRI. 45*S* pre-ribosomal RNA was isolated from the nucleoli, labeled with ^{125}I in vitro and hybridized to the restriction fragments of nucleolar DNA. Three fragments of molecular weight 3.0, 4.3 and 7.8 megadaltons respectively, containing ribosomal RNA genes were detected.

4*S*, 4.5*S*, 5*S*, U1 and U2 low molecular RNAs from the nucleus as well as 5.8*S*, U3 and 8*S* low molecular weight RNAs from the nucleolus were isolated, purified and labeled with ^{125}I in vitro. Hybridization was performed with these RNA species to the restriction fragments of ribosomal DNA. In addition to the expected 5.8*S* RNA, also U1, U2, U3 and 8*S* RNAs hybridized to the ribosomal DNA fragments. No hybridization occurred with 4*S*, 4.5*S* and 5*S* RNAs.

Hybridization of ribosomal DNA to the U3 and 8*S* RNAs, localized specifically in the nucleolus, suggests that these low molecular weight RNA species play a role in the regulation of transcription and/or processing of pre-ribosomal RNA in the nucleolus.

The nucleotide sequences of LMW RNAs from the cell nucleus and nucleolus have been determined (Busch et al., 1982). The biological functions of these RNAs, however, are unknown. Evolutionary conservation of primary sequences of U-rich LMW RNAs (Branlant et al., 1980) and common antigenic determinants of U-rich LMW ribonucleoprotein particles (Lerner, Steitz, 1979; Lerner et al., 1980) suggest special functions in the cell nucleus. Based on these data several hypotheses have been advanced for the function of LMW RNAs, (1) organization of nuclear structures (Sekeris, Niessing, 1975; Miller et al., 1978; Pederson, Bhorjee, 1979), (2) processing and splicing of primary transcripts of pre-mRNA (Deimel et al., 1977; Northemann et al., 1977; Lerner et al., 1980; Rogers, Wall, 1980; Yang et al., 1981) and (3) regulation of transcription (Ringuette et al., 1980).

Our previous experiments suggest that the synthesis of certain LMW RNAs is in correlation with the synthesis of rRNA (Fónagy, Hidvégi, 1975a; 1975b; Fónagy et al., 1981). It is known that the transcription of rRNA (Busch, Smetana,

Abbreviations: LMW RNA, low molecular weight RNA; U-rich LMW RNAs, uridine-rich low molecular weight RNAs; pre-mRNA, precursor of messenger RNA; pre-rRNA, precursor of ribosomal RNA; rDNA, ribosomal DNA; noDNA, nucleolar DNA; SDS, sodium dodecyl sulfate

1970) and the first steps of the maturation of pre-rRNA (Quagliarotti et al., 1970; Perry, 1976) occur in the nucleolus and that the U3 LMW RNA is localized solely in the nucleolus (Busch et al., 1982). The present experiments suggest that U3 and 8*S* LMW RNAs play a role in the transcription and/or processing of rRNA in the nucleolus. A preliminary account of this finding has already been reported (Financsek et al., 1980).

Novikoff hepatoma ascites tumor cells were used five days after transplantation of 10^7 cells into 100–120 g CFY female rats. Nuclei were isolated by the citric acid procedure (Higashi et al., 1966). For the isolation of nucleoli, the nuclei were first isolated by homogenization in 2.0 M sucrose and nucleoli were released by disruption of the nuclei by sonification as previously described in detail (Hidvégi et al., 1971). DNA was extracted from the isolated nucleoli by a modified procedure of Marmur (1961) and digested with EcoRI enzyme in 0.05 M Tris-HCl buffer, pH 7.5 containing 10 mM $MgCl_2$.

Total RNA was extracted at 65 °C from the purified nuclei or nucleoli by the SDS-phenol procedure (Steele, Busch, 1967) and fractionated first by sucrose density gradient centrifugation. Fractions containing LMW RNA and 45*S* pre-rRNA were pooled and precipitated. 45*S* pre-rRNA was further purified by sucrose density gradient centifugation. LMW RNA species were separated and purified by two subsequent electrophoreses on 10%, 1 cm diameter, 12 cm long polyacrylamide gels at 4 °C, 4 mA/gel for 12 h according to Loening (Fónagy, Hidvégi, 1975a). After electrophoresis the gels were stained with 0.2% methylene blue in 0.4 M acetate buffer. Bands were excised, and RNA was extracted in 0.02 M glycine buffer, pH 9, containing 0.05% SDS (Dolja et al., 1977). Residual polyacrylamide and methylene blue were removed by filtration through Schleicher and Schuell, Ba85 selectron filter. RNA was labeled with ^{125}I in vitro by using the original Commerford procedure (1971) as modified by Teraba and McCarthy (1973).

noDNA was digested with the restriction enzyme EcoRI and fragments were separated by electrophoresis on 1%, 0.5 cm diameter, 20 cm long agarose gel at 4 °C, 40 mA/gel for 16 h. DNA fragments were transferred to filter according to Southern (1973) and hybridized with ^{125}I-labeled RNAs. Hybridizations were carried out in 2 × SSC buffer (SSC is 0.15 M sodium chloride, 0.015 M trisodium citrate) at 65 °C for 16 h. After hybridization the filters (Schleicher and Shüll, BA 85) were washed 4 to 5 times with 2 × SSC, 0.1% SDS and 0.2% sodium pyrophosphate at 50 °C for a total of 1 to 2 h. They were than placed in contact with film (Forte) for 12 h.

The autoradiography in Fig. 1 shows three fragments of noDNA represented by 3.0, 4.3 and 7.8 megadalton molecular weights. These fragments were similar to the rDNA described for mouse (Tiemer et al., 1977; Mishima et al., 1978) and chinese hamster (Stambrook, 1978). Purified and ^{125}I-labeled LMW RNAs from the nucleus or nucleolus were hybridized to these EcoRI fragments of rDNA. Fig. 1 shows that U1 and U2 RNAs of the cell nucleus and 5.8*S*, U3 and 8*S* RNAs of the nucleolus hybridized to the three rDNA fragments. Fig. 1

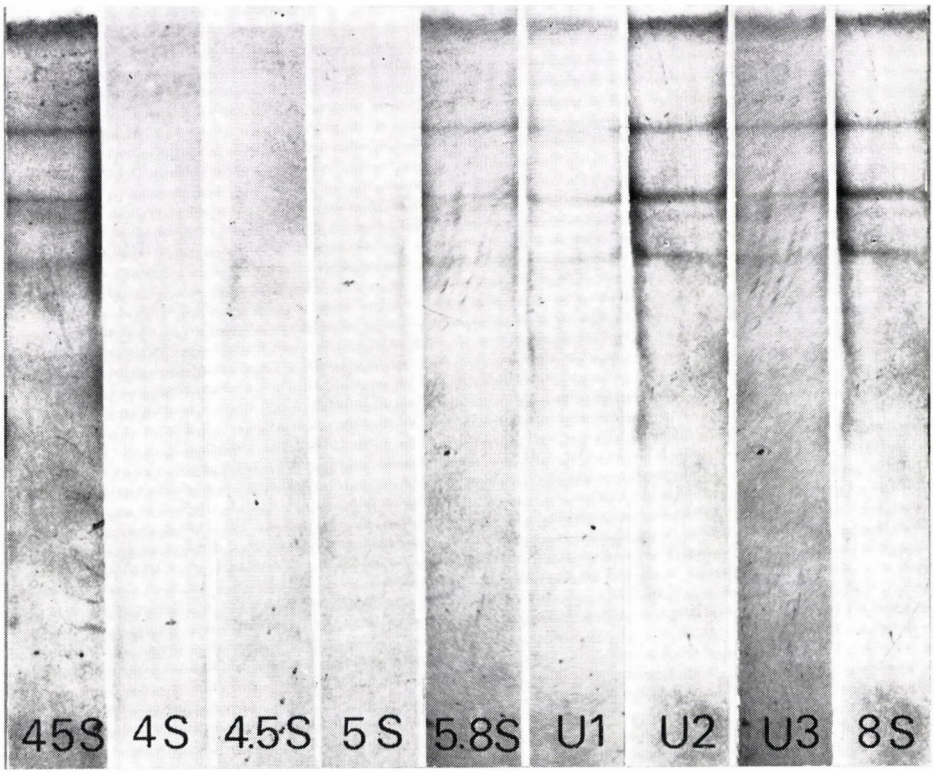

Fig. 1. Hybridization of various LMW RNA species of the nucleolus or nucleus to the EcoRI fragments of rDNA. EcoRI fragments of noDNA were separated by electrophoresis on 1% agarose gel (20×0.5 cm) with 40 mA/gel for 16 h, transferred to nitrocellulose filter according to Southern (1973) and hybridized with isolated and purified ^{125}I-labeled RNA species. *Autoradiography:* slabs left to right: nucleolar 45*S* pre-ribosomal RNA, nuclear 4*S* RNA, nuclear 4.5*S* RNA, nuclear 5*S* RNA, nucleolar 5.8*S* RNA, nuclear U1 RNA, nuclear U2 RNA, nucleolar U3 RNA and nucleolar 8*S* RNA

also demonstrates that no hybridization occurred to rDNA fragments with 4*S*, 4.5*S* and 5*S* RNAs of the cell nucleus.

Competitive hybridization was carried out in the presence of excess cold 45*S* pre-rRNA, between ^{125}I-labeled 8*S*, U-rich LMW RNAs and rDNA fragments. It was found that cold 45*S* pre-rRNA competed for the same rDNA fragments as both 8*S* RNA and U-rich LMW RNAs of the cell nucleus. The formation of specific hybrids is supported by the findings that (1) the melting profiles of rDNA hybrids either with 45*S* pre-rRNA or 8*S* RNA or U-rich LMW RNAs were different and that (2) decreasing the salt concentration or increasing the temperature during hybridization did not abolish the reaction of rDNA with 8*S* or U-rich LMW RNAs.

These experiments strongly support the notion that there is sequence homology between rDNA and certain LMW RNA species, such as U1, U2, U3, 5.8*S* and 8*S* RNAs, but not between rDNA and 4*S*, 4.5*S* and 5*S* RNAs.

Hybridization found between 5.8*S* RNA and rDNA was expected as the gene of 5.8*S* RNA is known to be localized in the ribosomal gene (Speirs, Birnstiel, 1974; Walker, Pace, 1977).

Engberg et al. (1974) found no hybridization between U1 or U3 LMW RNAs and rDNA of baby hamster kidney cells. The contradiction between the data of Engberg et al. (1974) and ours can be interpreted by the fact that the DNA in the experiment of Engberg et al. (1974) did not originate from the nucleolus and that caesium chloride gradient centrifugation used for the separation of DNA did not resolve specific sequences as in our experiments in which restriction fragments were used.

Hybridization of 8*S* and U3 RNAs to rDNA is in agreement with earlier data. 8*S* RNA was isolated first by Prestayko et al. (1970) by heating isolated nucleolar 28*S* RNA of Novikoff hepatoma cells. Similar association to 28*S* RNA was found for U3 RNA (Prestayko et al., 1971) for 5.8*S* RNA (Pace et al., 1977) and for 5.5*S* RNA in rattlesnake liver (Giorgini, DeLucca, 1976). These RNAs are localized in the nucleolus and it was suggested that they serve as transport molecules during maturation of pre-rRNA (Prestayko et al., 1970; Zieve, Penman, 1976).

Recently, a hypothesis for the role of U-rich LMW RNAs has been advanced by Lerner et al. (1980). The sequences of 43 known pre-mRNAs at their exon-intron boundaries showed homology with the 5' end of U1 LMW RNA. On the basis of this finding it was proposed that U1 LMW RNA may play a role in the maturation processing of pre-mRNA. This idea may be extended to pre-rRNA maturation processing. The sequence of the ribosomal processing site of rDNA in mouse (Urano et al., 1980), human (Financsek et al., 1982a) and rat (Financsek et al., 1982b) cells has been determined. Recently, Michot et al. (1982) reported the sequence of the internal transcribed spacer of mouse rDNA. It has been shown that sequence homology exists between part of U3 RNA of rat and the mouse internal transcribed spacer sequence at the cleavage site of 32*S* pre-rRNA. Consequently, U3 RNA might take part in the processing of 32*S* pre-rRNA to 28*S* rRNA and 5.8*S* RNA.

In conclusion these experiments suggest that U-rich LMW RNAs, especially U3 and 8*S* RNAs in the nucleolus, may play a role in the regulation of rRNA synthesis either at the transcription level and/or during the maturation of pre-rRNA.

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References

Branlant, C., Krol, A., Ebel, J. P., Lazar, E., Gallinaro, H., Jacob, M., Sri-Widada, J., Jeanteur, P. (1980) Nucleic Acids Res. *8* 4143

Busch, H., Reddy, R., Rothblum, L., Choi, Y. C. (1982) Ann. Rev. Biochem. *51* 617

Busch, H., Smetana, K. (1970) The Nucleolus, Academic Press, New York

Commerford, S. L. (1971) Biochemistry *10* 1993

Deimel, B., Louis, C., Sekeris, C. E. (1977) FEBS Lett. *73* 80

Dolja, V. V., Negruk, V. I., Novikov, V. K. (1977) Anal. Biochem. *80* 502

Engberg, J., Hellung-Larsen, P., Frederiksen, S. (1974) Eur. J. Biochem. *41* 321

Financsek, I., Fónagy, A., Hidvégi, E. J. (1980) Abstr. ICCB 2nd Meeting, Europ. J. Cell. Biol. *22* p. 59

Financsek, I., Mizumoto, K., Mishima, Y., Muramatsu, M. (1982*a*) Proc. Natl. Acad. Sci. USA *79* 3092

Financsek, I., Mizumoto, K., Muramatsu, M. (1982*b*) Gene *18* 115

Fónagy, A., Hidvégi, E. J. (1975*a*) Biochem. J. *146* 357

Fónagy, A., Hidvégi, E. J. (1975*b*) Proc. FEBS 9th Meeting, Biochemistry of the Cell Nucleus (edited by Hidvégi, E. J.) Vol. *33* p. 181, Publ. Hung. Acad. Sci. Budapest

Fónagy, A., Jeney, A., Szamos, J., Institóris, L., Hidvégi, E. J. (1981) Acta Biochim. et Biophys. Acad. Sci. Hung. *16* 151

Giorgini, J. F., DeLucca, L. (1976) Nucleic Acids Res. *3* 165

Hidvégi, E. J., Prestayko, A. W., Busch, H. (1971) Physiol. Chem. Physics *3* 17

Higashi, K., Narayanan, K. S., Adams, H. R., Busch, H. (1966) Cancer Res. *26* 1582

Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L., Steitz, J. A. (1980) Nature *283* 220

Lerner, M. R., Steitz, J. A. (1979) Proc. Natl. Acad. Sci. USA *76* 5495

Marmur, J. (1961) J. Mol. Biol. *3* 208

Michot, B., Raynal, F., Bachellerie, J. P. (1982) Abstr. ECCB 1st Meeting, Biol. Cell *45* p. 301

Miller, T. E., Huang, C. Y., Pogo, A. O. (1978) J. Cell Biol. *76* 692

Mishima, Y., Sakai, M., Muramatsu, M., Kataoka, Y., Honjo, T. (1978) Proc. Japan Acad. *54* 657

Northemann, W., Scheurlen, M., Gross, V., Heinrich, P. C. (1977) Biochem. Biophys. Res. Commun. *76* 1130

Pace, N. R., Walker, T. A., Schroeder, E. (1977) Biochemistry *16* 5321

Pederson, T., Bhorjee, J. S. (1979) J. Mol. Biol. *128* 451

Perry, R. P. (1976) Ann. Rev. Biochem. *45* 605

Prestayko, W., Tonato, M., Busch, H. (1970) J. Mol. Biol. *47* 505

Prestayko, W., Tonato, M., Lewis, B. C., Busch, H. (1971) *246* 182

Quagliarotti, G., Hidvégi, E. J., Wikman, J., Busch, H. (1970) J. Biol. Chem. *245* 1962

Ringuette, M., Liu, W. C., Jay, E., Yu, K. K. Y., Krause, M. O. (1980) Gene *8* 211

Rogers, J., Wall, R. (1980) Proc. Natl. Acad. Sci. USA *77* 1877

Sekeris, C. E., Niessing, J. (1975) Biochem. Biophys. Res. Commun. *62* 642

Southern, E. M. (1973) J. Mol. Biol. *98* 503

Speirs, J., Birnstiel, M. L. (1974) J. Mol. Biol. *87* 237

Stambrook, P. J. (1978) Chromosoma *65* 153

Steele, W. J., Busch, H. (1967) Methods in Cancer Research (edited by Busch, H.) Vol. III. p. 61, Academic Press, New York

Teraba, A., McCarthy, B. J. (1973) Biochemistry *12* 4675

Tiemer, D. C., Tilghman, S. M., Leder, P. (1977) Gene *2* 173

Urano, Y., Kominami, R., Mishima, Y., Muramatsu, M. (1980) Nucleic Acids Res. *8* 6043

Walker, T. A., Pace, N. R. (1977) Nucleic Acids Res. *4* 595

Yang, V. W., Lerner, M. R., Steitz, J. A., Flint, S. J. (1981) Proc. Natl. Acad. Sci. USA *78* 1371

Zieve, G., Penman, S. (1976) Cell *8* 19

Correspondence: A. FÓNAGY
"F. Joliot–Curie" National Research Institute for
Radiobiology and Radiohygiene
H-1775 Budapest, Pentz Károly utca 5

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Microwave Method for Determining Dielectric Parameters of Living Biological Objects II. Study of Ionic Water Binding

S. MISIK,* G. MASSZI**

* Research Institute for Viticulture and Enology, Kecskemét, and ** Biophysical Institute, Medical University, Pécs, Hungary

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The dielectric constant and the loss of diluted KCl and NaCl solutions of near-physiological concentrations (0.1 and 0.2 mol) were determined at 6.3 GHz measuring frequency in a temperature range of 20—50 °C by our cavity resonator technique — partly in view of examining ionic water binding, and partly to control the accuracy of the method.

Ionic water binding is greatly dependent on the quality of the ion and it is independent of temperature in the range of 20—50 °C. The activation energy values of the measured electrolyte solutions are close to that of pure water, so there is a close connection between ion-movements and the dipole rotation of water. According to our data the ionic conductivity measured at 6.3 GHz agrees with low frequency conductivity (±10 per cent).

Introduction

Microwave dielectric parameters of diluted electrolyte solutions have been determined by different microwave measuring methods several times in recent years, but the interpretation of the results is not unanimous.

Hasted et al. examined the dielectric properties of water and diluted electrolyte solutions by methods measuring damping factor, reflection coefficient and using cavity resonator technique (Hasted et al., 1948; Hasted, Roderick, 1958; Hasted, 1961, 1972; Collie et al., 1948; Haggis et al., 1952). Pottel and Lossen (1967) determined dielectric parameters in a very wide frequency range (0.5—38 GHz) by measuring the attenuation. Masszi and Örkényi (1967) determined the conductivity of KCl solutions in 1.8—4.0 GHz frequency range by measuring the damping factor and the standing wave ratio.

Le Petit et al. (1977) used the cavity resonator technique together with a Time Domain Reflectometry system and measured the dielectric parameters of diluted KCl solutions with different concentration, and the relaxation frequency of the same solutions.

We have developed a microwave cavity resonator technique for measuring the dielectric parameters of water, diluted solutions and biological probes (Misik et al., 1978), and have determined the parameters of electrolyte solutions partly for controlling the accuracy of the measuring method, partly for studying ionic water binding.

Material and method

The ε' dielectric constant and ε'' dielectric loss of diluted KCl and NaCl solutions (0.1 and 0.2 mol, i.e. close to physiological concentrations), were determined by microwave cavity resonator technique between 20–50 °C at 6.3 GHz, and the results were compared with the literary data.

The dielectric parameters of electrolyte solutions were calculated by a relative method – measuring the capillary tube with probe and water, and taking into account the well-known theoretical ε' and ε'' values of water.

Results and discussion

Figures 1 and 2 show the temperature dependency of the dielectric constant and the loss of electrolyte solutions.

It is known from the literature that the strongly polar properties of ions upset the structure of water, so ions destroy water structure. At the same time a multilayer hydrate cover is being formed around the ions. Owing to these effects the dielectric constant of electrolyte solutions is linearly decreasing by the increase of concentration, while their dielectric loss is almost linearly increasing (Hasted et al., 1948; Haggis et al., 1952; Hasted, 1961; Pottel, 1965; Kraeft, 1965). Figures 1 and 2 demonstrate that our measurements confirmed this statement.

The difference between the dielectric constants of diluted electrolyte solutions and pure water can be well examined by the help of the so-called dielectric decrement (= ε'-decrease for 1 mol of KCl or NaCl) (Table 1). According to the table the dielectric decrement is independent of temperature between 20–50 °C, it is greatly dependent on the quality and less dependent on the concentration of the electrolyte. The higher decrement value of NaCl is due to the radius of Na^+ being

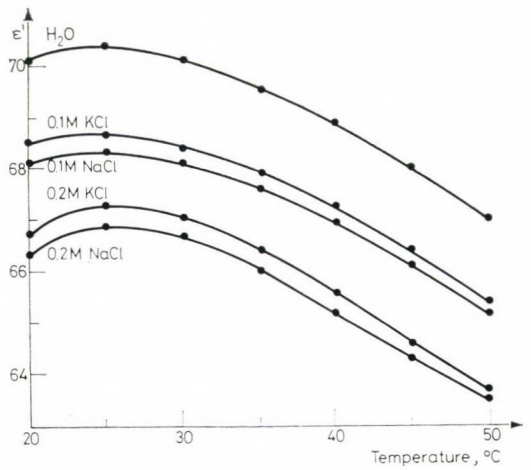

Fig. 1. Temperature dependency of ε' values of water and diluted electrolyte solutions

Table 1

Dielectric decrement of salt solutions

Solution \ °C	20	25	30	35	40	45	50	mean
0.1 M KCl	17.0	16.5	16.5	17.0	16.0	16.5	15.5	16.4
0.2 M KCl	17.0	15.5	15.0	16.0	16.25	16.5	16.5	16.1
0.1 M NaCl	20.0	20.0	19.5	20.0	19.0	19.0	17.5	19.3
0.2 M NaCl	19.25	17.25	16.75	17.5	18.0	18.0	17.75	17.8

smaller than the radius of K^+, so the amount of hydrated water is bigger around it (Giese et al., 1970).

The activation energy of water can be calculated from the temperature dependency of dielectric parameters. The Arrhenius equation is valid for relaxation frequency (Hedvig and Zentai, 1965):

$$f_r = \frac{c}{\lambda_s} = A \cdot e^{-\frac{E}{RT}}, \tag{1}$$

where A is the constant depending on the probe's quality; R is the universal gas constant; T is the absolute temperature; E is the activation energy; c is the velocity of light; λ_s is the relaxation wave-length.

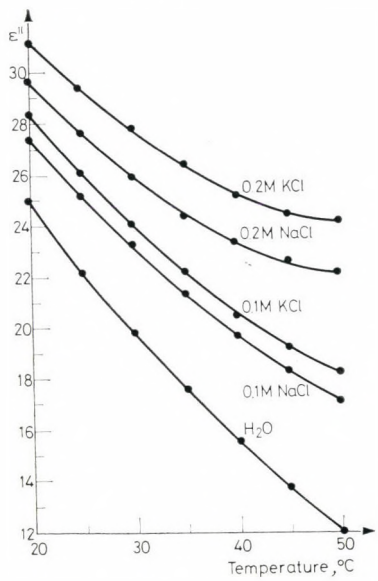

Fig. 2. Temperature dependency of ε'' values of water and diluted electrolyte solutions

So:

$$\frac{1}{\lambda_s} = \frac{A}{c} \cdot e^{-\frac{E}{RT}}. \tag{2}$$

According to the literature (Haggis et al., 1952) the dielectric loss of water can be determined for any frequency by a simple Debye-equation (Debye, 1929):

$$\varepsilon'' = \frac{\varepsilon_s - \varepsilon_\infty}{1 + \left(\frac{\lambda_s}{\lambda}\right)^2} \cdot \frac{\lambda_s}{\lambda} \tag{3}$$

(ε_∞ is the dielectric constant measured at a mm-long wave-length; ε_s is the static dielectric constant of water; λ_s is the relaxation wavelength; λ is the wavelength of 6.3 GHz frequency).

Since λ (= 4.76 cm) of 6.3 GHz measuring frequency is bigger than λ_s ($\approx$ 1.5 cm), relation (3) can be written in the following form

$$\varepsilon'' = (\varepsilon' - \varepsilon_\infty) \cdot \frac{\lambda_s}{\lambda} \tag{4}$$

with less than 10 percent mistake (Schwan, 1965), from where

$$\frac{1}{\lambda_s} = \frac{\varepsilon' - \varepsilon_\infty}{\varepsilon''} \cdot \frac{1}{\lambda}. \tag{5}$$

Contracting (2) and (5), the following equation can be used to describe the temperature dependency of dielectric parameters:

$$\log \frac{\varepsilon' - \varepsilon_\infty}{\varepsilon''} = -\frac{E}{2 \cdot 3\, R} \cdot \frac{1}{T} - \log \frac{c}{A \cdot \lambda}. \tag{6}$$

On the basis of equation (6) $\log \frac{\varepsilon' - \varepsilon_\infty}{\varepsilon''}$ is linearly changing in function of $1/T$ and E activation energy can be determined from the rise of the line illustrating this change. By this calculation we got values 18.1 $\pm$ 1.3 kJ/M as the activation energy of dipole rotation of pure water, which agrees with literary data (e.g. Sandus, Lubnitz, 1961; Pottel, Lossen, 1967).

The activation energy of salt solutions can be calculated from the temperature dependency of the equivalent conductivity of salt. First the specific conductivity of the solutions should have been calculated on the basis of

$$\sigma = \Delta\varepsilon'' \cdot \varepsilon_0 \cdot \omega, \tag{7}$$

where $\Delta\varepsilon'' = \varepsilon''_{\text{salt}} - \varepsilon''_{\text{water}}$; $\varepsilon_0 = \frac{1}{4\pi \cdot 9 \cdot 10^{11}}$ A s/V cm

is the dielectric constant of vacuum; and $\omega = 2\pi f_0$ is the angular-frequency.

Equivalent conductivity means the conductivity of a solution volume in which electrolyte equivalent to 1 g is solved (Erdey-Grúz, 1963):

$$\Lambda = \frac{\sigma_{\text{salt}}}{c} \cdot 10^3 = \frac{\sigma_{\text{solution}} - \sigma_{\text{water}}}{c} \cdot 10^3 \tag{8}$$

where c is the molarity of the solution.

The activation energy can be calculated from the temperature dependency of equivalent conductivity (Glasstone et al., 1941):

$$\Lambda = \Lambda_0 \cdot e^{-\frac{E}{RT}}, \tag{9}$$

from where

$$\log \Lambda = -\frac{E}{2.3\,R} \cdot \frac{1}{T} + \log \Lambda_0 . \tag{10}$$

If $\log \Lambda$ is described in function of $\frac{1}{T}$, the values of activation energy are:

0.1 M KCl:	17.0 ± 0.2 kJ/mol
0.2 M KCl:	16.4 ± 0.4 kJ/mol
0.1 M NaCl:	18.9 ± 0.4 kJ/mol
0.2 M NaCl:	20.2 ± 0.4 kJ/mol

Conclusions drawn – on the basis of a comparison with the values 18.1 ± 1.3 kJ/M of water – from the values of activation energy:

- K^+ causes a so-called negative, Na^+ causes a so-called positive hydration (Giese et al., 1970);
- the activation energy is dependent on concentration;
- the activation energy values of electrolyte solutions are close to those of water, i.e. there is a close connection between ion-movements and dipole rotation.

In Table 2 we compared our measured Λ values to some Λ values from the literature that were results of measurements at sound frequency (Falkenhagen,

Table 2

Λ values of electrolyte solution at low and high frequency

	Low frequency	Measured	% of change
0.1 M KCl 25 °C	129.0	132.0	+ 2.3
0.1 M KCl 50 °C	194.5	214.0	+10.0
0.1 M NaCl 25 °C	106.7	100.0	− 6.3

1953). The results of this comparison contradict the theory of Little and Smith (1955), according to which the conductivity of electrolyte solutions would increase with an increase in frequency, and between 10 and 20 GHz it would be 4–5 times higher than the value at sound frequency. The reason for the increase is supposed to be that at sound frequency ions would actually move together with their hydrate cover, and the neighbouring molecules could hinder this movement. At high frequency when there is no obvious ion-movement, the lack of this "hindering" effect could result in an increase of conductivity. In our experiments, however, confirming the results of Masszi and Örkényi (1967), no such an increase in conductivity was noticed, since the ionic conductivity measured at 6.3 GHz agreed with low frequency conductivity with a deviation not exceeding ±10 per cent.

References

Collie, C. H., Hasted, J. B., Ritson, D. M. (1948) Proc. Phys. Soc. London *60* 145
Debye, P. (1929) Polare Molekeln. S. Hirzel Verlag, Leipzig
Erdey-Grúz, T. (1963) A fizikai kémia alapjai. Műszaki Könyvkiadó, Budapest
Falkenhagen, H. (1953) Elektrolyte. S. Hirzel Verlag, Leipzig
Giese, K., Kaatze, U., Pottel, R. (1970) J. Phys. Chem. *74* 3718
Glasstone, S., Laidler, K. J., Eyring, H. (1941) The Theory of Rate Processes. McGraw-Hill Co., New York
Haggis, G. H., Hasted, J. B., Buchanan, T. J. (1952) J. Chem. Phys. *20* 1452
Hasted, J. B., Ritson, D. M., Collie, C. H. (1948). J. Chem. Phys. *16* 1
Hasted, J. B., Roderick, G. W. (1958) J. Chem. Phys. *29* 17
Hasted, J. B. (1961) Progress in Dielectrics *3* 103
Hasted, J. B. (1972–73) Water; a Comprehensive Treatise (ed. F. Franks). Plenum Press, New York
Hedvig, P., Zentai, G. (1965) Kémiai szerkezetkutatás rádióhullámokkal. Műszaki Könyvkiadó, Budapest
Kraeft, W. D. (1965) Z. Phys. Chem. *230* 368
Le Petit, J. P., Delbos, G., Bottreau, A. M., Dutuit, Y., Mazat, C., Cabanas, R. (1977) J. Microwave Power *12* 335
Little, V. F., Smith, V. (1955) Proc. Phys. Soc. London *68* B 65
Masszi, G., Örkényi, J. (1967) Acta Biochim. Biophys. Acad. Sci. Hung. *2* 69
Misik, S., Masszi, G., Torma, I. (1978) Acta Biochim. Biophys. Acad. Sci. Hung. *13* 201
Pottel, R. (1965) Ber. Bunsenges. Physik. Chem. *69* 363
Pottel, R., Lossen, O. (1967) Ber. Bunsenges. Physik. Chem. *71* 135
Sandus, O., Lubnitz, B. B. (1961) J. Phys. Chem. *65* 881
Schwan, H. P. (1965) Ann. N. Y. Acad. Sci. *125* 344

Correspondence: S. Misik

Research Institute for Viticulture and Enology,
H-6000 Kecskemét, Kisfái 182, Hungary

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Investigation of Hydration of Macromolecules II. Study of Ethylene-glycol and 1-4-dioxane Solutions by Dielectric Method

L. KOSZORÚS, G. MASSZI

Biophysical Institute, Medical University, Pécs, Hungary

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Microwave conductivity and permittivity of aqueous solutions and of solutions containing 0.02 N $MnSO_4$, of 1–4 dioxane or ethylene glycol were measured at a frequency range of 2.2–3.5 GHz at 30 °C. The conductivity of 20 kHz was also measured at the same temperature. According to our data:

1. The relaxation wavelength of water increases together with the increase of the concentration of non-electrolyte.

2. The own relaxation wavelength of ethylene glycol molecule is ~3.3 cm in infinite dilution, and it increases together with the increase of concentration.

3. The concentration dependence of micro-viscosity of bulk water can be estimated from the relaxation wavelength of water. If microviscosity is taken into account, the calculated ion mobility agrees with the measured data.

Introduction

One of the problems in biophysical research is to explore the connection between water-structure and biological organization. Microwave investigations bring information about the so called "vibrationally averaged" structure existing for a picosecond time (Eisenberg, Kautzmann, 1969). According to former examinations, the dielectric relaxation wavelength of bulk water increases under the effect of hydrophilous groups of biological materials and macromolecules, which indicates that there are structures surviving much longer in bulk water, than in free water (Cook, 1951; Masszi, 1972a; Masszi et al., 1976; Kaatze, 1978; Masszi et al., 1980).

According to the Debye theory (1929) the relaxation wavelength is proportional to the microviscosity of water. This conclusion is corroborated by modern microwave investigations (Collie et al., 1948; Grant, 1957). Examinations of ion-mobility and self-diffusion prove, that the microwave relaxation wavelength of water in macromolecular solutions changes proportionally with the microviscosity of bulk water (Masszi, 1972b; Inzelt, Gróf, 1976).

For a detailed analysis of the problem solutions of macromolecules were chosen in which identical water-binding groups recur periodically. The aim of our present examination was to perform microwave examinations of ethylene glycol (the homologous building-stone of poly-ethylene glycol), and of aqueous solutions of dioxane.

Material and method

Solutions of volume rate of $\Phi = 0.05, 0.10, 0.15, 0.20, 0.25$ and 0.30 were made from ethylene glycol (E. Merck A. G., Darmstadt, pro analysi) and dioxane (Fine Chemical Producing Co-operative, Budapest, puriss.) liquids, adding water clarified by ion exchanger and 0.02 N $MnSO_4$ solution. The purity and the ionic conductivity of the solutions was checked by conductivity measurement of 20 kHz. The conductivity of the water clarified by ion exchanger is smaller than 10^{-2} mS cm^{-1} (10^{-3} Ω^{-1}m^{-1}), the conductivity of ethylene glycol and dioxane solutions is smaller than $3 \cdot 10^{-2}$ mS cm^{-1} ($3 \cdot 10^{-3}$ Ω^{-1} m^{-1}).

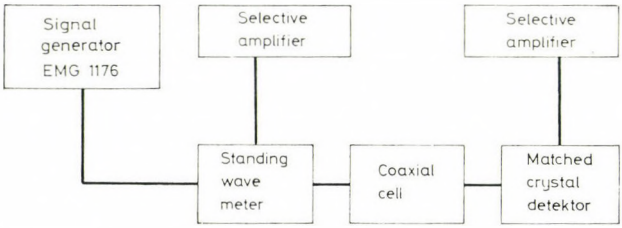

Fig. 1. Block diagram of the apparatus

Determination of microwave parameters was traced back to measurements of attenuation coefficient (α) and standing wave-ratio (r) in a range of frequency of 2.2 to 3.5 GHz (Masszi, Örkényi, 1967; Masszi, 1970).

A block diagram of the equipment is shown by Fig. 1. By using a cell of appropriate length ($l = 2, 4, 8$ cm), and by power matching of the crystal unit we can achieve that the input resistance of the cell is not influenced by the output resistance of the cell, and the matched crystal produces a signal proportional to the square of the voltage leaving the cell.

In this case the attenuation coefficient (α) is:

$$\alpha = \left[\left[\ln \left(\frac{U_0}{U} \cdot \frac{r(r_0 + 1)^2}{r_0(r + 1)^2} \right) \right] \frac{1}{2l} + \alpha_0 \right. \tag{1}$$

where U_0 and U refer to the voltage leaving the cell in the case of pure water and the substance to be measured, while r and r_0 are the standing wave-ratios in cases of cells filled with water and with the substance to be measured, respectively, l is the length of the cell, α_0 is the attenuation coefficient of water.

The complex refractivity is ($n^* = n - j\kappa$), its real part is

$$n = a \left[Z + \sqrt{Z^2 + \left(1 + \frac{k^2}{a^2} \right)} \right] \tag{2}$$

where

$$Z = \frac{r^2 + 1}{2} \qquad a = \frac{Z_{02}}{Z_{01}}$$

Z_{02} is the wave impedance of the cell, Z_{01} is that of the coaxial section leading to the cell.

The imaginary part of the complex refractivity is:

$$\kappa = \frac{15}{\pi f} \alpha \tag{3}$$

where f is frequency in GHz.

Microwave permittivity (ε') and conductivity (σ) can be calculated by the following formulas:

$$\varepsilon' = n^2 - \kappa^2 \tag{4}$$

$$\sigma = \frac{100}{6\pi} n\alpha [\text{mS cm}^{-1}] . \tag{5}$$

The measurements were performed at 30 °C, the temperature of the cell was stabilized with an accuracy of 0.1 °C.

The microwave parameters of the water of a temperature of 30 °C were calculated on the basis of Hasted's general work (Franks, 1972), according to which:

$$\begin{aligned} \varepsilon_0 &= 76.8 \\ \varepsilon_\infty &= 4.2 \\ \tau &= 7.2 \text{ ps } (\lambda_{s_0} = 1.36 \text{ cm}) \\ \alpha_* &= 0.012 \text{ rad} \end{aligned} \tag{6}$$

and

$$\varepsilon^* = \frac{\varepsilon_0 - \varepsilon_\infty}{1 + j(\omega t)^{1-\alpha_*}}$$

The microwave parameters calculated from equation (6) are summarized in Table 1. (If the initial data of the calculation were: $\alpha_* = 0$ and $\varepsilon_\infty = 5$, the difference was 0.2 to 0.25 per cent at ε', and 2 to 3 per cent at α and σ.)

Figures 2 and 3 show the microwave parameters of enthylene glycol solutions measured directly and determined according to formulas (1) to (5).

The relaxation wavelength was determined by two mathematical methods:

1. In the approximate calculation, the so-called "relaxation wavellength for long wavelength" calculation (Budó, 1949) it was taken into account, that

$$1 \gg \left(\frac{\lambda s}{\lambda}\right)^2$$

Table 1

Dielectric parameters of water at 30 °C (Franks, 1972)

f: frequency, ε': permittivity; *n:* refractivity; α_0: attenuation coefficient; σ: conductivity

f [GHz]	ε'	n	α_0 [cm]	σ[mS cm^{-1}]
2.2	75.9	8.723	0.1934	8.95
2.5	75.7	8.712	0.2488	11.50
2.8	75.4	8.701	0.3109	14.35
3.0	75.3	8.693	0.3559	16.41
3.2	75.1	8.684	0.4039	18.61
3.5	74.7	8.670	0.4812	22.13

and so the following approximate equation can be set up for conductivity

$$\sigma = \frac{(\varepsilon_0 - \varepsilon_\infty)\lambda s}{54} \cdot f^2 + \sigma_{0m} \tag{7}$$

according to which conductivity is in a linear connection with the square of frequency. Matching a straight line to the points of measurements with the method of the smallest squares, λ_s was determined from the rise of the straight line.

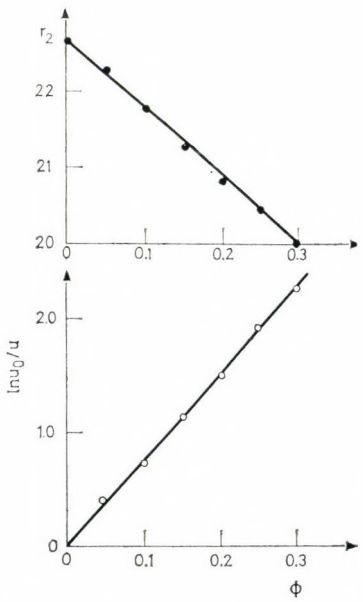

Fig. 2. Data of measurements of ethylene glycol solutions at 30 °C, at frequency of 3.5 GHz

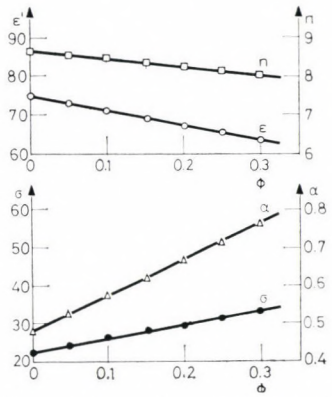

Fig. 3. Dielectric parameters determined from the data of Fig. 2

2. For Debye-equations set up in the form of

$$\varepsilon = \frac{\varepsilon_0 - \varepsilon_\infty}{1 + \left(\frac{\lambda_s f}{30}\right)^2} + \varepsilon_\infty \tag{8}$$

$$\frac{f}{\sigma - \sigma_{0m}} = \frac{54\,\lambda s}{900(\varepsilon_0 - \varepsilon_\infty)} f^2 + \frac{54}{(\varepsilon_0 - \varepsilon_\infty)\lambda_s} . \tag{9}$$

The σ_{0m}, λ_s and ε_0 parameters, for which the amount of the square of the difference between the data calculated from equations (8) and (9) and the measured data is the minimum, were found by a computer program. From equation (9) with the knowledge of $(\varepsilon_0 - \varepsilon_\infty)\lambda_s$ can be determined from the coefficient of f^2 and also from the constant. The difference between the two λ_s was always smaller than 2 per cent. The values calculated from the constant are shown by Tables 2 to 5.

Table 2

Dielectric data of dioxane solution at 30 °C

Φ — volume rate of dioxan, ε_0 — static dielectric constant, λ_{s_1} — "relaxation wavelengths for long wavelengths", λ_{s_2} — relaxation wavelength determined by Debye's equation, σ_{0m} — constant conductivity in the microwave range, η_m — microviscosity of bulk water (η_{water} = = 0.798 cP)

Φ	ε_0	λ_{s_1} [cm]	λ_{s_2} [cm]	σ_{0m} [mS cm^{-1}]	η_m [cP]
0.05	73.3±0.3	1.37	1.43	0.62	0.839
0.10	69.4±0.3	1.49	1.57	0.21	0.921
0.15	65.5±0.3	1.62	1.72	−0.24	1.009
0.20	61.5±0.3	1.74	1.87	−0.52	1.097
0.25	57.4±0.4	1.90	2.08	−1.00	1.220
0.30	53.2±0.5	2.05	2.32	−1.54	1.361

Table 3

Dielectric parameters and micro-viscosity of dioxane solutions diluted with 0.02 N $MnSO_4$ solution at 30 °C

Symbols are the same as in Table 2.

Φ	ε_0	λ_{s_1} [cm]	λ_{s_2} [cm]	σ_{0m} [mS cm^{-1}]	η_m [cP]
0.05	72.8±0.7	1.41	1.48	2.99	0.868
0.10	69.5±0.5	1.52	1.60	1.25	0.939
0.15	65.5±0.5	1.60	1.70	1.49	0.998
0.20	61.0±0.7	1.70	1.84	0.96	1.080
0.25	57.6±0.7	1.88	2.06	−0.02	1.209
0.30	54.1±0.9	2.05	2.29	−0.86	1.344

Table 4

Dielectric data of ethylene glycol solutions at 30 °C

Symbols are the same as in Table 2

Φ	ε_0	λ_{s_1} [cm]	λ_{s_2} [cm]	σ_{0m} [mS cm^{-1}]
0.05	74.1±0.6	1.51	1.59	−0.25
0.10	72.1±0.7	1.66	1.78	−0.25
0.15	70.0±0.8	1.82	1.97	−0.08
0.20	68.1±0.9	1.97	2.16	0.08
0.25	66.2±1.2	2.14	2.38	0.09
0.30	64.4±0.4	2.33	2.65	−0.13

Table 5

Dielectric data of ethylene glycol diluted with 0.02 N $MnSO_4$ solution at 30 °C

Symbols are the same as in Table 2

Φ	ε_0	λ_{s_1} [cm]	λ_{s_2} [cm]	σ_{0m} [mS cm^{-1}]
0.05	73.9±1.1	1.55	1.64	1.57
0.10	72.0±1.1	1.70	1.82	1.33
0.15	70.0±1.1	1.86	2.01	1.10
0.20	68.2±1.3	2.08	2.21	0.86
0.25	66.2±1.5	2.19	2.43	0.62
0.30	64.4±1.6	2.37	2.68	0.39

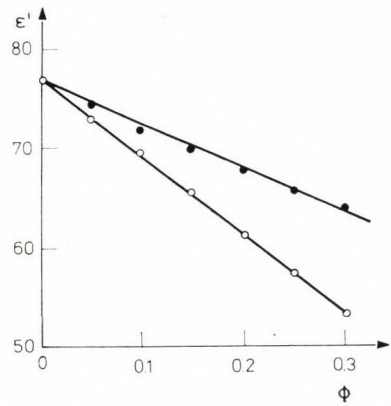

Fig. 4 Concentration-dependence of dielectric constant of ethylene glycol (– · —) and dioxane (– ○ –)

Concentration-dependence of ε_∞ was left out of consideration, and matching was performed by $\varepsilon_\infty = 4.2$. A change of $\varepsilon_\infty \pm 0.5$ results in a change of λ_s smaller than the measuring error.

Considering the approximations and the fact that our measurements are performed in the initial phase of the whole dispersion range the 1st method can be regarded as to a first approximation of the relaxation wavelength (λ_{s_1}), and the 2nd method – as to a closer approximation (λ_{s_2}).

Results

1. There is a significant increase in the relaxation wave-length in the case of an increase of concentration of either dioxane or ethylene glycol.

In the case of a dioxane solution, as the dioxane molecule has no own dipole moment, the relaxation wavelength unambiguously corresponds to the relaxation wavelength of water. In accordance with the Debye equation (1929) the relaxation wavelength is proportional to the micro-viscosity of water (Grant, 1957):

$$\lambda_{s_0} = C \frac{\eta_{m_0}}{T} \tag{10}$$

where C is a constant which is connected to molecular and structural characteristics, T is the absolute temperature, η_{m_0} is the microviscosity of pure water. Applying relation (10) for dioxane solutions, microviscosity of the bulk water of the solution is

$$\eta_m = \frac{\lambda_{s_0}}{\lambda_s} \eta_{m_0} . \tag{11}$$

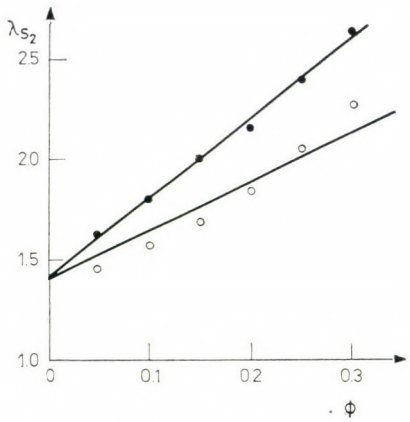

Fig. 5. Concentration-dependence of the relaxation wavelength of ethylene glycol (—·—) and dioxanes(— ○ —)

Considering that the viscosity of water (η_{water}) corresponds well to the microviscosity of water, which can be calculated from the Debye equation (Collie et al., 1948)

$$\eta_m = \frac{\lambda_{s_0}}{\lambda_s} \eta_{water} \cdot \tag{12}$$

The values of microviscosity calculated from equation (12) are indicated in the 6th column of Tables 2 and 3.

2. The dielectric constants of diethylene glycol solutions are greater than those of dioxane solutions (Fig. 4). This indicates that diethylene glycol, the static dielectric constant of which is 33.2 at 30 °C (Franks, 1972), is able for rotation in the microwave field, and its own relaxation wavelength can be found in the microwave range. This is supported by the concentration dependence of the relaxation wavelength (Fig. 5). The figure suggests that the relaxation wavelength of an ethylene glycol solution is made up of two components: one of them is the relaxation wavelength of water which is approximately equal to the relaxation wavelength of water in the dioxane solution, the other is the relaxation wavelength of ethylene glycol itself.

This conclusion is corroborated by low-frequency measurements. Microwave parameters of solutions diluted with 0.02 N $MnSO_4$ solution agree with the corresponding parameters of the pure solution; this suggests, that the slight ion-content does not cause a significant change either in the dielectric constant or in the relaxation wavelength. The σ_{0_m} frequency-independent conductivity agrees with the conductivity corresponding to the ion-content with an accuracy of ± 0.5 mS cm^{-1}. The correct determination of σ_0 was carried out by a conductivity measurement of 20 KHz. Conductivity decreases when concentration increases (Fig. 6). This decrease can be ascribed to two causes.

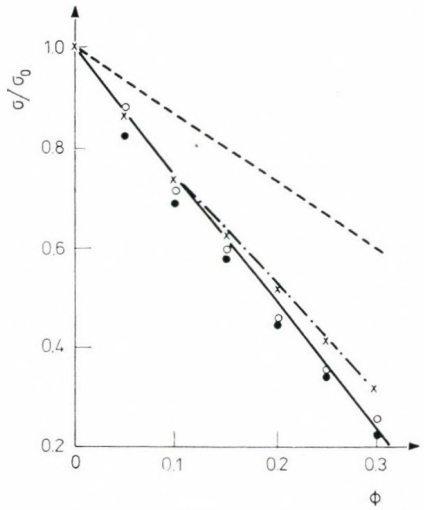

Fig. 6. Concentration dependence of relative conductivity in ethylene glycol and dioxane solutions with 0.02 N $MnSO_4$ (—·— ethylene glycol; —○— dioxane; --- according to Fricke's theory; —×— curve determined considering micro-viscosity

a) Obstruction effect (Fricke, 1924).

b) Increase of the microviscosity of water (Masszi, 1972).

In ethylene glycol and dioxane solutions the relative conductivity $\left(\frac{\sigma_0(\Phi)}{\sigma_0}\right)$ depends on the concentration nearly in the same way in both solutions.

The results of older and newer theories describing the concentration dependence of relative conductivity can be summarized in a simple formula (Robinson, Stoks, 1954):

$$\frac{\sigma_0(\Phi)}{\sigma_0} = 1 - 1.5\Phi\,. \tag{13}$$

If formula (13) is completed by the change of relative micro-viscosity of water, the data of measurements are well approximated by the equation

$$\frac{\sigma_0(\Phi)}{\sigma_0} = \frac{\lambda_{s_0}}{\lambda_{sd}}(1 - 1.5\Phi) \tag{14}$$

(Table 6, Fig. 5).

The slighter deviation can be accounted for by the association between Mn^{++} and SO_4^{--} ions (Ernst, Truka, 1937; Pottel, 1969) and by the ion and water binding of non-electrolyte.

3. The own relaxation wavelength of ethylene glacol (λ_{se}) can be estimated with the aid of "relaxation wavelength for long wave". In this case:

$$\lambda_{se_1} = \frac{\Delta\varepsilon_d\lambda_{sd_1} + \Delta\varepsilon_e\lambda_{se}}{\Delta\varepsilon_d + \Delta\varepsilon_e} \tag{15}$$

Table 6

Comparison of concentration dependence of relative conductivity with the theoretical values

$\sigma_0 = (1.86 \pm 0.08)$ mS cm^{-1} at 30 °C

Φ	$\frac{\sigma^0(\Phi)}{\sigma_0}$ dioxane	$\frac{\sigma_0(\Phi)}{\sigma_0}$ ethylene glycol	$1-1.5\Phi$	$\frac{\lambda_{S_2}}{\lambda_{S_0}}(1-1.5\Phi)$
0.05	0.88	0.83	0.93	0.88
0.10	0.71	0.69	0.85	0.73
0.15	0.60	0.58	0.78	0.62
0.20	0.46	0.45	0.70	0.51
0.25	0.35	0.34	0.63	0.41
0.30	0.26	0.22	0.55	0.32

where:

$$\Delta\varepsilon_d = \varepsilon_{0_d} - \varepsilon_\infty$$

$$\Delta\varepsilon_e = \varepsilon_{0_e} - \varepsilon_{0_d}.$$

Index e denotes the data of ethylene glycol solution, index d denotes those of dioxane solution).

When setting up equation (15) we started from the supposition that the difference between the dielectric constant of ethylene glycol and dioxane solution arises from the own dielectric accessories of ethylene glycol.

By substituting the data

$$\frac{\Delta\varepsilon_d}{\Delta\Phi} = -77.8 \qquad \frac{\Delta\lambda_{S_{d_1}}}{\Delta\Phi} = 2.3$$

$$\frac{\Delta\varepsilon_e}{\Delta\Phi} = -40.5 \qquad \frac{\Delta\lambda_{S_{e_1}}}{\Delta\Phi} = 3.3$$

calculated from the data of Figs. 3 and 4 we obtain:

$$\lambda_{S_e} = 1.2\,\Phi + 3.3 \text{ [cm]}$$

and (16)

$$\lim_{\phi \to 0} \lambda_{S_e} \to 3.3 \text{ (cm)}.$$

So the relaxation wave-length of ethylene glycol molecule increases with the concentration in a linear way in the approximation.

4. On the basis of equation (16) in the case of infinite dilution $\lambda_{S_e} = 3.3$ cm ($\tau = 17.5$ ps), and these data give an opportunity for estimating the proportions of ethylene glycol.

According to the Debye (1928) equation:

$$a = \sqrt[3]{\frac{kT\tau}{4\pi\eta F_e}} \tag{17}$$

where k is the Boltzmann constant, T is the absolute temperature, τ is the relaxation time, η is the viscosity of water, F_e is the factor characterizing the shape of the molecule. With the knowledge of the distances of binding and the conformation of ethylene glycol (Karlsson, 1980) the shape of ethylene glycol can be approximated by an ellipsoid of rotation, the axis of rotation of which is

$$a = 3.3 \text{ [Å]}$$

and the minor axis perpendicular to it is:

$$b = 2.5 \text{ [Å]}$$

$\frac{a}{b} = 0.76$, and the shape factor belonging to it is: $F_e = 0.63$ (Budó et al., 1939).

Taking this value into consideration, the axis calculated from equation (17) are:

$$a = 4.5 \text{ [Å]} \qquad (3.9 \text{ [Å]})$$
$$b = 3.4 \text{ [Å]} \qquad (3.0 \text{ [Å]})$$

The data in brackets are the values calculated with the aid of the Powles-correction treated in the discussion.

Discussion

1. The relaxation time determined with dielectric measurements

$$\tau = \frac{\lambda_s}{2\pi c} \tag{18}$$

($c \rightarrow$ light velocityl)

was considered as equal to the time-constant of the decay of dielectric polarization (T), which is determined by the following function:

$$\alpha(t) = \frac{\varepsilon_0 - \varepsilon_\infty}{T} e^{-\frac{t}{T}} \tag{19}$$

where $\alpha(t)$ is the dielectric polarization, t is the time. According to the theoretica examinations of Powles (1953):

$$T = \frac{3\,\varepsilon_0}{2\,\varepsilon_0 + \varepsilon_\infty}\,\tau\,. \tag{20}$$

In the examined range the multiplication factor of τ varies from 1.46 to 1.44, thus its neglection causes 1 per cent error, at the maximum, in the proportion of relaxation times.

The Powles correction means a change of $\frac{1}{\sqrt[3]{1.5}} = 0.87$ times in the calculation of the radius of ethylene glycol molecule. Either considering the correction, or not, a greater value is obtained for the size of the molecule than the one which could be obtained by calculations on the basis of distance of binding. This is compatible with the presumption that the ethylene glycol molecules bind 1 or 2 water molecules, and rotate together with them in the alternating electric field.

2. The process of relaxation in water can also be described with the aid of a mathematical model, according to which water is made up of two components:

(*a*), hydration water, the relaxation wavelength of which is constant and greater than that of free water;

(*b*), bulk water, the relaxation wavelength of which agrees with that of free water (Kaatze, 1978, Kaatze et al., 1978).

According to this model, ethylene glycol in aqueous solution corresponds to hydration water. However, the results obtained for ethylene glycol solutions differ from the dielectric characteristics of hydration water in two respects: The dielectric constant of ethylene glycol solutions is distinguishable from the dielectric constant of free water, and at higher ethylene glycol concentrations – as a result of stronger molecular interactions – the relaxation wave-length increases. The lack of these two phenomena in the case of hydration water makes it difficult to accept the similarity between ethylene glycol in aqueous solution and the model of hydration-water.

The relaxation wavelength of water is taken as an average, for the determination of its distribution measurements have to be performed in a wider range of frequency.

3. According to the examinations of Pauly and Schwan (1966), Pauly (1972) the ionic conductivity in haemoglobin solutions is lower than the theoretically expected value. In order to explain the phenomenon it is supposed that the space between macromolecules may be regarded as a narrow capillary, in which the powers damping the motion of ions are greater than in free water. If this presumption is right, the damping effect has an influence not only on the motion of ions, but also on dipole rotation, and an increase of the relaxation wavelength can also be expected (Operan, Vincze, 1972). Measurements of viscosity (Churajev et al., 1970) really show, that the viscosity of water in micro-capillaries increases. The present examination reveals a similar phenomenon not in colloide, but in real solutions, which suggests that the role of specific hydrophilous groups also have to be taken into account in understanding the phenomenon.

Acknowledgement

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References

Budó, A., Fischer, E., Miyamoto, S. (1939) Physik Z. *40* 337
Budó, A., (1949) J. Chem. Phys. *17* 686
Churayev, N. V., Sobolev, V. D., Zorin, Z. M. (1970) Special Discussions of the Faraday Society *1* 213
Collie, C. H., Hasted, J. B., Ritson, D. M. (1948) Proc. Phys. Soc. London *60* 145
Cook, H. F. (1951) British H. Appl. Phys. *2* 295
Debye, P. (1929) Polare Molekeln. S. Hirzel, Leipzig.
Eisenberg, D., Kauzmann, W. (1969) The Structure and Properties of Water. Clarendon Press, London, Oxford University Press, New York.
Ernst, E., Truka, J. (1937) Phys. Z. *31* 103
Franks, F. (1972) Water; a comprehensive treatise. Plenum Press, New York — London Vol. 1. Chap. 7. J. B. Hasted. Liquid Water. Dielectric properties p. 277 Vol. 2. Chap. 7. J. B. Hasted. Dielectric properties p. 423
Fricke, H. (1924) Phys. Rev. *24* 575
Grant, E. H. (1957) J. Chem. Phys. *26* 1575
Inzelt, Gy., Gróf, P. (1976) Acta Chim. Acad. Sci. Hung. *88* 13
Kaatze, K. (1978) Progr. Colloid Polymer Sci. *65* 214
Kaatze, K., Göttman, O., Podbielski, R., Pottel, L., Treveer, U. (1978) J. Phys. Chem. *82* 112
Masszi, G., Örkényi, J. (1967) Acta Biochim. Biophys. Acad. Sci. Hung. *2* 69
Masszi, G. (1970) Acta Biochim. Biophys. Acad. Sci. Hung. *5* 321
Masszi, G. (1972a) Acta Biochim. Biophys. Acad. Sci. Hung. *7* 341
Masszi, G. (1972b) Acta Biochim. Biophys. Acad. Sci. Hung. *7* 349
Masszi, G., Inzelt, G., Gróf, P. (1976) Acta Biochim. Biophys. Acad. Sci. Hung. *11* 45
Masszi, G., Tigyi, J., Koszorús, L. (1980) Int. Conf. on Water and Ions in Biological Systems. Bucarest
Operan, R., Vincze, J. (1972) IV. Internat. Biophys. Congr. Moscow, Vol. III. p. 449
Pauly, H. (1973) Biophysik *10* 7
Pauly, H., Schwan, H. P. (1966) Biophys. J. *6* 621
Pottel, R. (1965) Ber. Bunsenges. phys. Chem. *69* 363
Powles, J. G. (1953) J. Chem. Phys. *21* 633
Robinson, R. A., Stokes, R. M. (1959) Electrolyte Solutions, Butterworths, London

Correspondence: L. Koszorús

Biophysical Institute, Medical University,
H-7624 Pécs Szigeti út 12.

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Is the Difference of Vapour Pressure the Driving Force of Real Osmotic Water Transport?

F. VETŐ

Biophysical Institute, Medical University, Pécs, Hungary

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Some textbooks and communications explain water transport simply by the difference of vapour pressure of water. Our measurements performed in a clay-cylinder osmometer through $Cu_2Fe(CN)_6$ membrane prove that, in cases of complex solutions with components of dissimitar potentials to permeate, or in cases of temperature gradients, the direction and speed of the osmotic volume flow is independent of the difference of vapour pressure. The real water transport is a polyfactorial function of the cross-effects and transport coefficients determined by the characteristics, interactions of thermodynamic driving forces of the given system. There is no simple physical model applicable for every cases of water transport.

Introduction

In a former communication I wrote: "For demonstrating that on the basis or the simplified vapour pressure conception we contradict the facts, further experiments were performed in systems of D_2O and normal watery solution" (Vető, 1974a). As it is shown by these experimental data, a volume flow of concentrated D_2O with a lower vapour pressure into normal aqueous solution with a higher vapour pressure takes place in osmometers through a $Cu_2Fe(CN)_6$ membrane. Such volume flows in the direction of higher vapour pressure were measured several times also in systems of normal aqueous solutions containing different solutes (multicomponent systems) (Vető, 1974b).

This is opposed by " . . . the conception describing the difference in vapour pressure of the solvent on the two sides of the semipermeable membrane as the driving force" (Ernst, 1975). The quantitative connection for this is:

$$\Delta\pi = \frac{RT}{v} \ln \frac{p_2}{p_1},$$

where p_2 and p_1 is the vapour pressure of the water in the solutions, v is the molar volume of water, R is the gas constant, T is the absolute temperature, $\Delta\pi$ is the difference of osmotic pressure. Some textbooks and some other communications explain water transport either simply by the difference of vapour pressure, or exclusively by the difference of chemical potential of water. It must, however, be stressed that these explanations are correct only in the case, if the membrane is

ideally semipermeable (i.e. it lets through only the solvent, e.g. H_2O), and if the system is isothermic. Otherwise, even a slight excess of hydrostatic pressure can drive the concentrated sucrose solution through a glass filter "membrane" towards pure water, though in sucrose solution both the chemical potential and the vapour pressure of water are smaller than in pure water. Further, thermoosmotic water flow from pure cold water into pure warm water – not only in the opposite direction! – has also been demonstrated (Alexander, Wirtz, 1950; Haase, de Greiff, 1965; Haase et al., 1970; Dariel, Kedem, 1975; D'Ilario, Canella, 1977), though the vapour pressure is greater in the warmer water (the chemical potential is smaller).

In the present experiments we wished to gain further data for the question whether the real water transport processes can be interpreted by the difference of vapour pressure as the only driving force. Our measurements were performed in order to decide the question unambiguously. The results showed again, that merely from the difference of vapour pressure we cannot conclude either to the extent, or even to the direction of water transport in real systems.[+]

The following questions were examined:

1. Is the speed of osmosis in our systems, in isothermic circumstances proportional to the difference of osmotic pressure and concentration? ($J_v = -L_p \sigma \Delta\pi$; J_v is the volume flow, L_p is the hydraulic conductivity, σ is the reflection coefficient). This was, at the same time, a test for the suitability of osmometers.

2. What is the direction of flow when H_2O and D_2O are set against each other and D_2O is the warmer of the two, i.e. it has a greater vapour pressure? How does the direction of ΔT (and the consequent difference in vapour pressure) influence the velocity of $H_2O - D_2O$ osmosis?

3. What is the direction and speed of thermoosmosis in the models in the case when H_2O of different temperatures (vapour pressures) or solutions of equal concentrations but different temperatures are set against each other?

4. How the direction and velocity of osmosis can be affected by an increase in solution temperature on one side only, in cases when concentrated D_2O and normal aqueous solutions are set against each other? What is the role of the direction of ΔT and the temperature of the membrane (the average temperature)?

5. How does the magnitude and direction of ΔT influence osmosis in the case when H_2O (or thin solution) and more concentrated normal aqueous solutions are set against each other? What is the role of ΔT and of the temperature of the membrane?

6. How does the velocity of osmosis change as plotted against the temperature of the whole system? Is it simply proportional to the absolute temperature, or is it different? How large is the energy of activation of osmosis? Does it coincide with the heat of evaporation of water (Spanner, 1954; House, 1974)?

[+] A part of these results was reported at the annual joint meeting of Hungarian Biophysical, Biochemical and Physiological Societies in Pécs, in 1977. Abstract: F. Vető, Acta Physiol. Acad. Sci. Hung. *52* 117, 1978.

Methods

The previously used fired clay cylinder osmometers (4 ones: a, b, c, d; their surface: 180 cm^2) were used with $Cu_2Fe(CN)_6$ membranes (Ernst, Homola, 1952; Vető, 1974a). Ion exchanged water (HERCO, $\gamma < 5$ μS/cm) and chemicals of analytical purity were used for the solutions. The heavy water (D_2O) was of 99.75 per cent purity, it had been made for the purpose of spectroscopy. The osmometer took place in the inner waterspace of an ultra thermostate. By this arrangement the temperature of the outer liquid space was set to the desired value. Water of the desired temperature was continuously perfused by an other ultra thermostate through the metal heating-cooling element taking place in the centre of the osmometer. By this arrangement the temperature of the inner solution-space was kept at the desired value. The temperature of the solutions was measured by copper-constantan thermocouples placed on the outer and inner surfaces of the wall of the clay cylinder (membrane). The volume change was read off from calibrated, graduated, thin glass tubes (pipettes of 1 ml). Hydrostatic pressure differences did not occur in these experiments. The vapour pressure value of the solutions was calculated from the data of appropriate tables (Kohn, 1965; Wexler, 1976), and it was measured with a Hewlett-Packard vapour pressure osmometer (type 301 A) when it was necessary. A measurement with the pair of solutions under examination in our model lasted for 4 to 10 hours or for some days when it was necessary, and the data (volume, temperature) were read off at every half hour during the day. After changing the solutions rinsing was performed several times for 1/2 to 2 days; when identical solutions were used we waited for the equilibrium to set in before the measurements. The average of the measured data and the standard error of mean, as well as the regression coefficient of the time-dependence of volume change were calculated in the usual way, and in the diagrams these data were indicated.

Results

The results of measurements are shown by the graphs and diagrams.

1. According to the data shown by Fig. 1 the linearity between the velocity of osmosis and the difference of concentration is limited. Our models worked appropriately as osmometers.

2. According to the data in Fig. 2 – as it is known already – when there is no difference in temperatures H_2O which has a greater vapour pressure flows into concentrated D_2O of a smaller vapour pressure. If, however, D_2O is warmer – despite the fact that its vapour pressure is greater – H_2O of much smaller vapour pressure flows into it more quickly. If H_2O is warmer, i.e. it has a much greater vapour pressure, the velocity of osmosis does not change essentially as compared to the previous situation. (The cross-hatched columns indicate the cases of osmosis from a place of lower vapour pressure to a place of higher vapour pressure.)

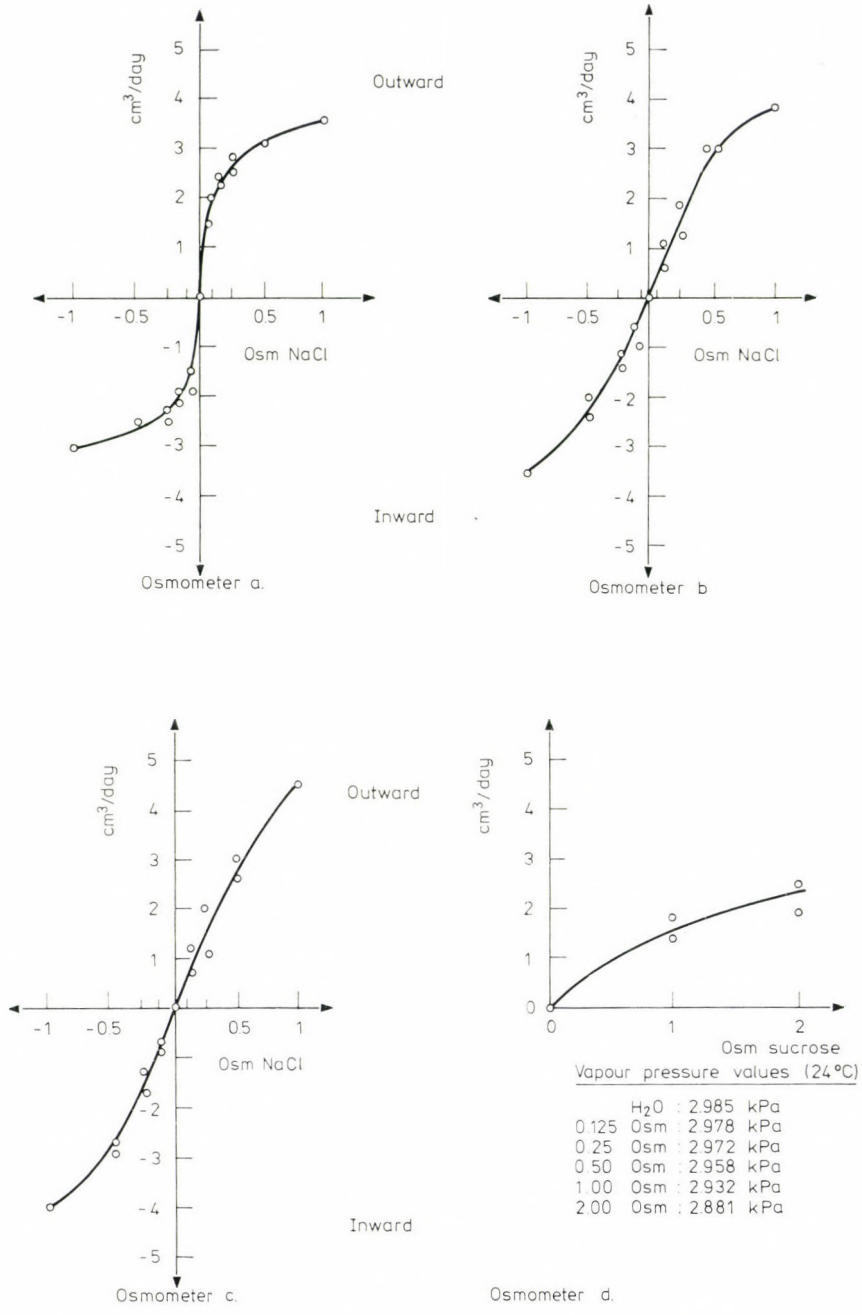

Fig. 1. Velocity of osmosis from H_2O into solution at 24 °C plotted against Δ_c

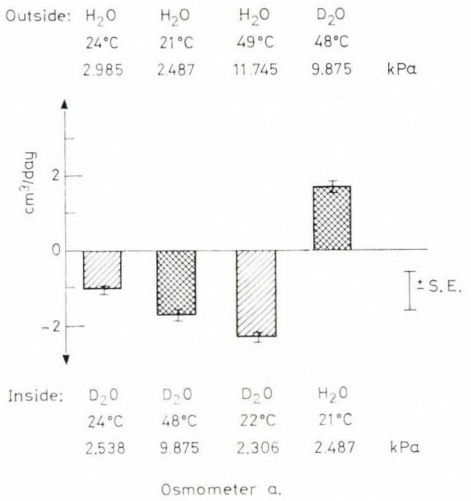

Fig. 2. The osmosis of H_2O into D_2O is independent of the direction of the difference of vapour pressure

3. The thermoosmotic effect is very small despite the great differences in vapour pressure. The first two columns of Fig. 3 show the thermoosmotic effects from warm liquids to cold ones, the last column shows those effects from cold liquids to warm ones, the latter effects are statistically significant, though their absolute value does not appear to be important.

4. As it is shown by Figs. 4, 5, 6 the osmosis of D_2O into normal aqueous solutions, considering either its direction or its velocity is independent of the difference of vapour pressures. Irrespective of ΔT it is always D_2O that flows into the solution. Thus, if the temperature and vapour pressure of the aqueous solution is increased unilaterally in relation to D_2O, the direction of osmosis is not reversed, moreover the flow becomes much quicker in the direction of the greater vapour pressure. While the ΔT and the difference of vapour pressure do not play a part here, the temperature of the membrane (i.e. the average temperature) determines the velocity of osmosis. This is especially well demonstrated by the last 3 columns of Figs. 5 and 6.

5. The osmosis of cold normal water (H_2O) into NaCl solution is the quicker, the warmer the NaCl solution is and consequently, the greater its vapour pressure is. The warmer temperature of water – its vapour pressure being much greater than that of NaCl solution – does not increase velocity (Fig. 7). If the more concentrated sugar solution is warmer (and, thereby, its vapour pressure is much greater) than the less concentrated solution on the opposite side the velocity of osmosis is twice as great as it used to be under isothermic conditions (Fig. 8 and 9). On the basis of the vapour pressure conception an intensive thermoosmosis could be expected in this case to the opposite direction. Further columns of Figs 8

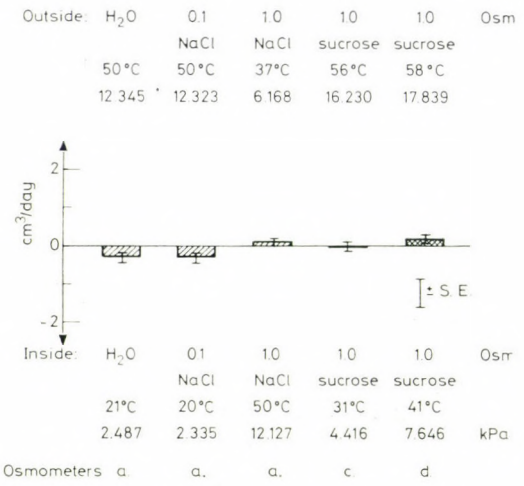

Fig. 3. The greatness of thermoosmotic effects

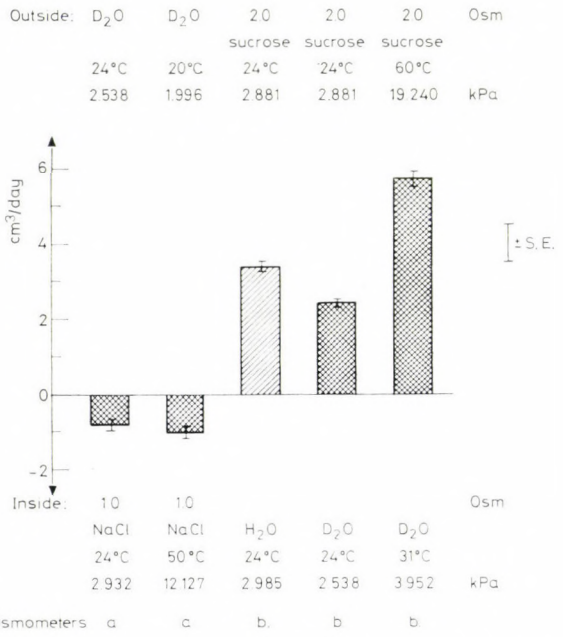

Fig. 4. Osmosis of D_2O into normal aqueous solutions having greater vapour pressure

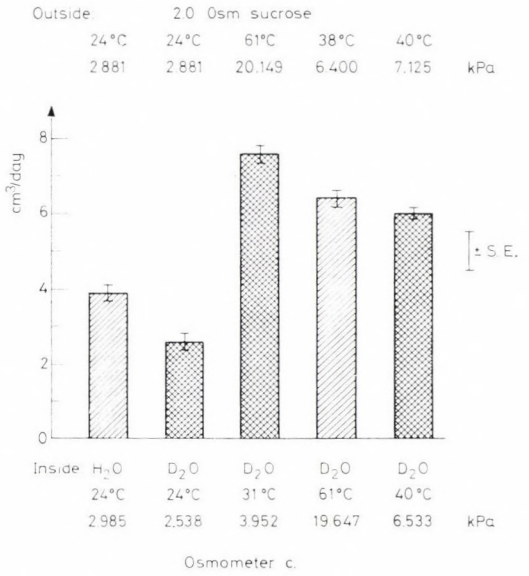

Fig. 5. Osmosis of D_2O into a normal aqueous solution having greater vapour pressure

and 9 prove, that the difference of vapour pressures plays no part in these cases, but the average temperature may be a determining factor.

6. The velocity of osmosis is not simply proportional to T, but it increases more quickly (Fig. 10) and its energy of activation is 24 kJ mol^{-1}. $L_p\sigma$ is not independent of the temperature. In our case the activation energy of osmosis is only 55 per cent of the evaporation heat of water.

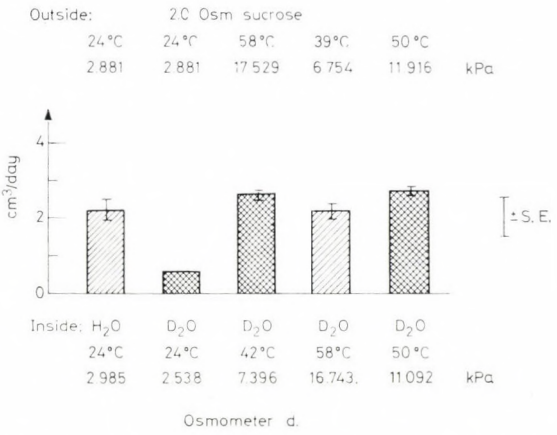

Fig. 6. Osmosis of D_2O into a normal aqueous solution having greater vapour pressure

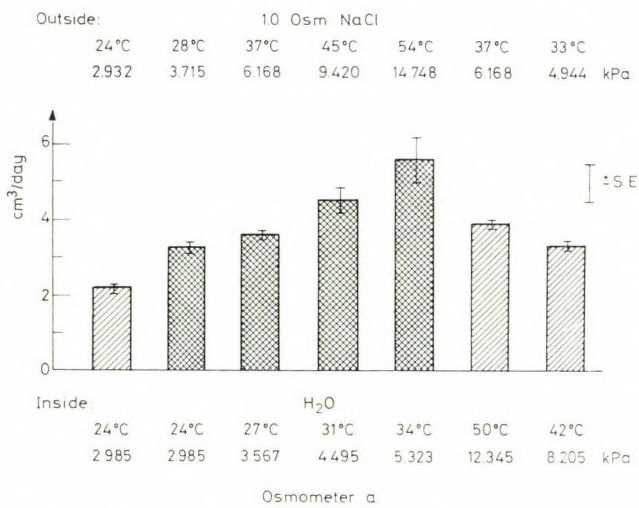

Fig. 7. Osmosis of H_2O into a warmer NaCl solution having greater vapour pressure

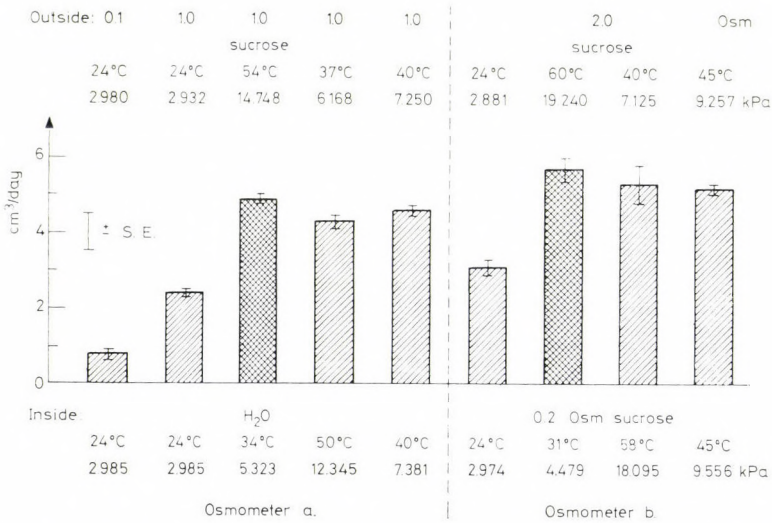

Fig. 8. Osmosis of H_2O into a solution of greater vapour pressure, and the independence of the velocity of osmosis of the difference of vapour pressure

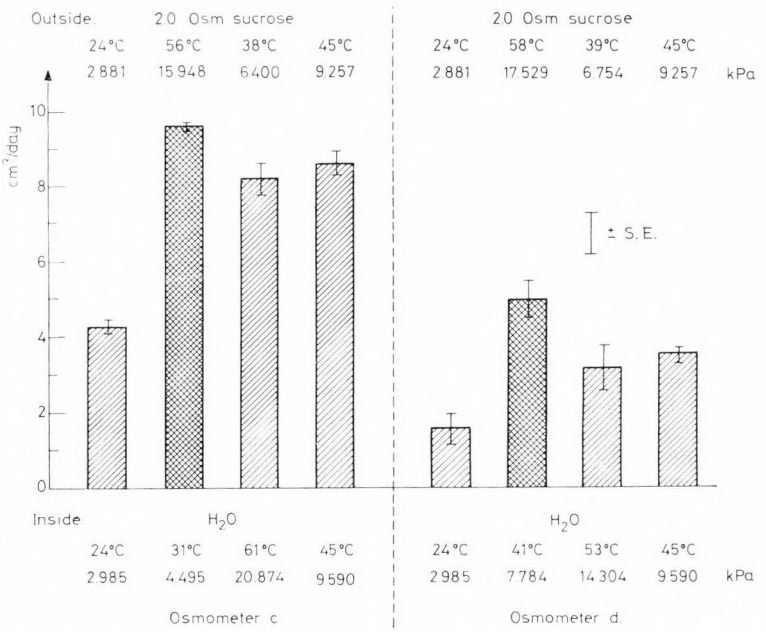

Fig. 9. Osmosis of H_2O into a solution of greater vapour pressure and the independence of the velocity of osmosis of the difference of vapour pressure

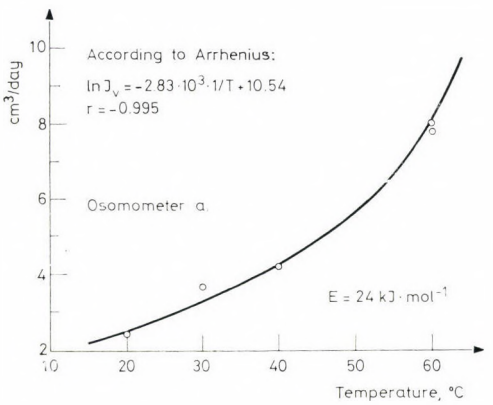

Fig. 10. Temperature dependence of the velocity of osmosis and the energy of activation of osmosis (E)

Discussion

On the basis of our data the answer to the question raised in the title is: no. The driving force of real osmotic water transport is not the difference of vapour pressure; if this presumption would be true, there would have occurred a water flow with a direction opposite to the direction measured really in several of our cases.

In reality the driving force of water flow beside the chemical potential difference of water is the hydrostatic pressure difference, the difference of temperature (Katchalsky, Curran, 1965), and in the case of electro-osmosis it can also be the difference of electric potential depending on the structure and composition of the system. Through dynamic interactions and cross-effects the water flow can also be influenced by the motion of other components being present.

The "water potential" difference used in plant physiology, and particularly its subdividing into various components cannot be a solution of general validity, and is not free from doubts, either (Dainty, 1976; Zimmerman, Steudle, 1978). The same refers also to the "tensile solvent" conception (Hammel, Scholander, 1976) (see the debate: Mysels, 1978; Hammel, 1979; Mauro, 1979; Hildebrand, 1979; Soodak, Iberall, 1979). In any case, it is an aim that after a comprehensive collection of data a new theory be constructed which, by employing a minimum number of parameters, describes at least the "passive" transport processes as exactly and "predictably" as possible. This is one of the indispensable pre-requisites of understanding biological transport processes. Efforts in this direction are well illustrated by the reviving activity in literature about osmosis (e.g. Sha'afi, 1977; Del Castillo, 1979; Halary et al. 1980) and about water in general (e.g. Ernst, Hazlewood, 1978; Lobishev, Kalinichenko, 1978; Kjellander, 1978; Drost-Hansen, Clegg, 1979). However, for the time being, the best way of describing these transport processes is apparently the phenomenological nonequilibrium thermodynamical formalism (even this only within limits). We can agree with the opinion of Steudle, Lüttge, Zimmermann (1975): "Our knowledge is very limited and not sufficient to develop a coherent physical model of water relations which is generally applicable." To recognize this is the condition of stepping forward.

This work is dedicated to my master, academician Ernst, following his teaching: "... facts are stronger than formulations" (E. Ernst. Acta Biochim. Biophys. Acad. Sci. Hung. *8*, 177, 1973).

References

Alexander, K. F., Wirtz, K. (1950) Zeitschrift für Phys. Chem. *195* 165–174
Dainty, J. (1976) in: Transport in Plants II. Part A. (Eds.: Lüttge, U., Pitman, M. G.) Springer, Berlin
Dariel, M. S., Kedem, O. (1975) J. Phys. Chem. *79* 336–342
Del Castillo, L. F., Mason, E. A., Revercomb, H. E. (1979) Biophys. Chem. *10* 191–201
D'Ilario, L., Canella, M. (1977) Polymer *18* 206–207

Drost-Hansen, W., Clegg, J. Eds. (1979) Cell-Associated Water. Acad. Press, New York
Ernst, E., Homola, L. (1952) Acta Physiol. Acad. Sci. Hung. *3* 487—505
Ernst, E. (1975) Acta Biochim. Biophys. Acad. Sci. Hung. *10* 243—249
Ernst, E., Hazlewood, C. F. (1978) Inorganic Persp. Biol. Med. *2* 27—49
Haase, R., de Greiff, H. J. (1965) Zeitschrift für Phys. Chem. NF. *44* 301—313
Haase, R., de Greiff, H. J. Buchner, H. J. (1970) Zeitschrift Naturforschung *25a* 1080—1085
Halary, J. L., Noël, C., Monnerie, L. (1980) Polymer Bull. *2*, 89—96
Hammel, H. T., Scholander, P. F. (1976) Osmosis and Tensile Solvent. Springer, Berlin
Hammel, H. T. (1979) Am. J. Physiol. *237* R95—R107
Hammel, H. T. (1979) Am. J. Physiol. *237* R123—R125
Hildebrand, J. H. (1979) Am. J. Physiol. *237* R108—R109
House, C. R. (1974) Water Transport in Cells and Tissues. Arnold, London
Katchalsky, A., Curran, P. F. (1965) Nonequilibrium Thermodynamics in Biophysics. Harvard Univ. Press, Cambridge, Mass.
Kjellander, R. (1978) J. Coll. Interface Sci. *66* 303—312
Kohn, P. G. (1965) in: State and Movement of Water in Living Organisms. Cambridge, Univ. Press
Lobishev, V. I., Kalinichenko, L. P. (1978) Isotopnie effekty D_2O v biologicheskikh systemakh. Nauka, Moscow (in Russian)
Mauro, A. (1979) Am. J. Physiol. *237* R110—R113
Mysels, K. J. (1978) J. Chem. Education *55* 21—22
Sha'afi, R. I. (1977) in: Membrane Transport in Red Cells (Eds.: Ellory, J., Lew, V. L.) Acad. Press, London
Soodak, H., Iberall, A. (1979) Am. J. Physiol. *237* R114—R122
Spanner, D. C. (1954) Symposia of the Soc. for Exp. Biol. *8* 76—93
Steudle, E., Lüttge, J., Zimmermann, U. (1975) Planta Berl. *126* 229—246
Vető, F. (1974a) Acta Biochim. Biophys. Acad. Sci. Hung. *9* 141—149
Vető, F. (1974b) Acta Biochim. Biophys. Acad. Sci. Hung. *9* 359—366
Wexler, A. (1976) J. Res. National Bureau of Standards *80A* 775—785
Zimmermann, U., Steudle, E. (1978) Adv. Bot. Res. *6* 45—117

Correspondence: F. VETŐ

Biophysical Institute, Medical University,
H-7624, Pécs, Szigeti út 12.

Book Reviews

Fibrinolysis by Neville A. Marsh, John Wiley and Sons, Chichester—New York—Brisbane—Toronto. 1981, 254 pp. with 16 tables and 27 figures

It has long been known that the fibrinolytic enzyme system which removes blood clots after completion of wound healing also protects the organism against occlusive vascular disease. The realization that thrombosis may be successfully treated by enhancing fibrinolysis gave a new impetus to research efforts aimed at understanding the role, mechanism and regulation of fibrinolysis. This book surveys the vast literature accumulated on fibrinolysis in the last decade and sums up current concepts about the function of the fibrinolytic enzyme system and the local and central control mechanisms responsible for regulating fibrinolytic activity. Being a single-authored book, it provides a personal overview of the subject rather than an exhaustive review of the literature.

The first three chapters of the monograph give a concise summary of the molecular biology of plasminogen and plasminogen activators, fibrinogen and fibrin degradation, physiological and synthetic inhibitors of fibrinolysis. Subsequent chapters deal with physiological factors influencing normal fibrinolysis (effect of excercise, age, diet, pregnancy, mental stress etc. on fibrinolysis), survey congenital disorders of fibrinolysis, occlusive vascular diseases as well as derangement of fibrinolysis as a result of liver disease, renal disease, obstetric complications, inflammation and malignant diseases. One of the chapters reviews the different therapeutic procedures used for enhancement of fibrinolysis and evaluates the merits of steroids, antidiabetic drugs, streptokinase- and urokinase-based therapeutic regimens. The Appendix of the book is devoted to laboratory methods useful to clinical technicians or researchers entering the field.

The book is written in a concise, readable style and should be valuable for clinical scientists and researchers as an introduction to the field of fibrinolysis.

L. PATTHY

Molecular Biology Biochemistry and Biophysics 31 Membrane Spectroscopy by Ernst Grell (ed.) Springer Verlag, Berlin—Heidelberg—New York, 1981.

This new book in the series of Molecular Biology, Biochemistry and Biophysics, "Membrane Spectroscopy", fills a long-existing gap between membrane biochemistry and biophysics. According to the editor, Ernst Grell, the principal aim of the book was to introduce the readers to the application of spectroscopic techniques to study biological membranes. This aim was excellently carried through by the 18 authors. The editor selected the 9 most sought-after spectroscopic methods widely applied by both membrane biophysicists and biochemists.

The first two chapters deal with nuclear magnetic resonance studies of the phospholipid bilayer membrane and with spin-label techniques in membrane biophysics as studied by electronspin resonance spectroscopy. In the first chapter Dr. Chan and his co-workers assumed that the reader is fully aware of the basic principles of NMR spectroscopy

and they give only a more advanced introduction, however, to people who have ever used NMR methods their chapter is easily readable and instructive.

The second chapter written by Derek Marsh has a different aspect and is more understandable even to beginners without being elementary or neglecting newly developed methods like saturation transfer EPR.

The third, fourth, fifth, sixth and eighth chapters offer optical spectroscopic methods to the readers. The absorption and circular dichroism spectroscopies (third chapter) are shortly but well treated and are intended to both beginners and more advanced readers.

The next chapter concentrates on monolayers as well as multilayer assemblies and model membranes, studied by different indicator probes. Here the emphasis is put on data gained with a wide variety of membranes. Beside the second chapter, the fifth is perhaps the best elaborated one of deals with fluorescence spectroscopy of biological membranes, and is a concise and well written summary of the most recent developments in this powerful technology. Great emphasis is put on the important lateral diffusion studies. Since the book was published in 1981, this chapter should contain the room temperature time dependent phosphorescence studies introduced mostly in 1979. The lack of these types of references puts the otherwise excellently written chapter second to that of the one by Dr. Marsh.

The sixth and eighth chapters dealing with infrared membrane and Raman spectroscopies are professionally summarized but are not better than many of such introductions available elsewhere. The number and quality of references give some additional value to the chapters.

The seventh chapter, interestingly enough, introduces the chemical relaxation spectroscopy as a part of the optical methods. Since the authors were among the scientists who helped to develop Eigen's original ideas, the chapter has a freshness and a very up-to-date review of the applications.

The last chapter introduces the reader into the short theory of low-angle X-ray diffraction and its application in studying different membranes like protein and lipid model systems, myelin, the membrane structure of the visual system, sarcoplasmic reticulum, erythrocyte membranes, gap junction, the purple membrane of Halobacterium halobium.

The sum up, this book is an excellent collection of methods and most recent data written by outstanding scientists who not only contributed to these fields of science but actively participated in developing the methods written by them.

S. DAMJANOVICH

Manual of Pharmacologic Calculations with Computer Programs by Ronald J. Tallarida and Rodney B. Murray. Springer-Verlag, New York—Heidelberg—Berlin, 1981. 150 pages, 26 figures, 26 tables, 93 equations, 21 references

The manual is divided into two parts. In the first, the pharmacologic basis for the calculation is stated. Then the appropriate equations are given and an example of the respective calculation is provided. For each of the 33 procedures the calculations can be done without reference to any other source.

One-half of the 33 computational procedures is commonly used in pharmacology practice: how much drug and saline is to be dissolved to make a given volume of specified molarity and osmolarity; how large is the standard error in a set of data; which is the best-fitting straight line to a set of data; estimation of ED16, ED50 and ED84 from a dose-response curve; dissociation constants for agonists and partial agonists; enzyme kinetic and inhibition parameters from double reciprocal plots; single exponential regressions for drug-receptor reactions, drug decomposition or decay curves; evaluation of Scatchard plots; calculation of the ratio of the concentrations of ionized to non-ionized forms of a drug from the Henderson—Hasselbalch equation: numerical integration of a curve.

The second half of the computational procedures in this manual is perhaps much more useful, because their practical and concise presentations can hardly be found in common textbooks. The following problems are solved computationally: how to estimate standard errors of slope, y-intercept and x-intercept of a regression line; how to test

parallelism of two regression lines in order to estimate the relative potencies of a number of drugs; how to determine standard error of the affinity of a competitive antagonist for its receptor; what is the mean level of an eliminating drug administered intravenously in repeated doses and the number of doses in order to achieve a prescribed peak level; how to test whether treatment with different drugs are equally effective or not (analysis of variance, t-test for grouped data, t-test for paired data, chi-square test, Dunnett's test, Mann-Whitney test); confidence limits of ED50.

The second part of the book contains the computer programs written in standard BASIC. Only minor modifications are required to run them on desktop computers with other versions of BASIC language. Programs are easy to use and understand because their documentation are excellent.

F. BARTHA

Lipids in Evolution by W. R. Nes—W. D. Nes. Monographs in Lipid Research, by D. Kritchevsky (ed.) Plenum Press, New York—London, 1980.

W. R. Nes and W. D. Nes, the authors of this book somewhat unusually, put into the focus of their evolutionary studies the lipids, this profoundly important, albeit extremely heterogenous group of compounds. On formulating the conception of this work the authors raise the question whether lipids can be considered as phylogenetic markers, and that their structure and function have played some role in the course of evolution, with special regard to steroids. They attempt to demonstrate the occurrence and role of lipids during the history of evolution of the living world. The examples are taken from both the prehistoric and modern ages, from paleontological findings, from meteorites, at different levels of differentation from the prokaryotes to the eukaryotes. Thus, they examine the age, origin, function and environment of the cells as determinants of their biochemical character and, based on various biological considerations, compare these with the similarities and differences of the lipids they contain. The authors give a critical evaluation of the information available to them and compare these with other well-known parameters of evolution. Among the several factors influencing evolution, the role of age as the principal determinant of the way of cellular evolution, is discussed. Accordingly following the chapter on the general characterization of lipids, a further chapter deals with the ages of the Universe, and of the Earth, and with the chronology of phylogenesis as a means of labeling biological events. Data concerning lipids detected in fossils found on the Earth, and those of extraterritoreal origin, are integrated into this picture. The chapter, which deals with the origin of oxygen, and in which geological and astrophysical proofs are deployed, also illustrates the authors' principal intentions. In further chapters the roles of pH and temperature in the evolution of thermophylic bacteria are discussed.

From a biological point of view, one of the most intriguing chapters of the book is the one which analyzes the correlations between the occurrence of lipids and phylogenesis. Similarities and differences among different individual organisms, species, and families are presented on the ground of lipid research. A similar approach is used with respect to the phylogenesis and biosynthesis of steroids, fatty acids and carbohydrates.

The book by W. R. Nes and W. D. Nes is not the first attempt to place a molecular structure or a group of compounds into the focus of the origin and evolution of life. Preferably, the authors try to critically avoid this one-sidedness of approach and help orientate the reader to judge the several incoherent and vague data. The book, being beyond doubt new and interesting to experts of biology, biochemistry and physiology, is supplemented with abundant references, author index and a well-compiled subject index.

T. KREMMER

Biochemische Grundlagen der Zahnmedicin, by E. Buddecke 90 figures, 19 tables Walter de Gruyter, Berlin, New York, 1981

Professor Buddecke, the excellent author of several medical textbooks widely popular

in the German linguistic area (Grundriss der Biochemie, Pathobiochemie) now presents another textbook intended to meet special demands. This volume, the structure of which is rather individual and slightly unusual for the biochemist, was written for use in dental training, nearly everywhere separated from medical training.

The composition certainly seems unusual to specialists working on classical biochemical problems; however, for dental students a knowledge of the problems described in the book is really useful and important. In this regard it is evident that the chemical composition of the tooth or the characteristics and composition of saliva are closer to what a dentist needs than would be different views on oxidative phosphorylation. In fact I would rather call this book "oral biology" or "oral biological chemistry". This is not dictated by my own views but by the intention and content of the book. The role and significance of various inorganic substances (phosphate, calcium, fluorine) are discussed in detail more thoroughly than in the usual biochemical textbooks that seldom assign a separate chapter to inorganic components.

The book is made up of 14 parts of moderate length. The introductory part discusses the significance of teeth and of the oral cavity. The two subsequent chapters summarize information on the inorganic and organic composition and the metabolism of the area. The subject of the three following chapters is mineralization, the metabolism of solid tissue and the development of teeth. The role of fluorine is discussed on about 10 pages. The problems of salivary glands and saliva and of the oral cavity are then presented in two chapters, including the processes brought about by microorganisms in the oral cavity. Two chapters discuss the emergence and prevention of the most common disease, caries, followed by a chapter on parodontopathies. Oral hygiene and the characteristics and effects of cleaning agents are described in a separate chapter.

From this short summary of the content of this volume it is evident that the author kept practical aspects very much in view and essentially summarized those problems the knowledge of which is indispensable for dentists. He took great care not to dwell on any subject of "theoretical" or "scientific" character, since the overwhelming majority of dentists pursue "practical" activity and are interested only in what they can make use of in their practice.

The content of this book is also thought-provoking as regards other fields of medical training. New information is being gathered at such a speed in every field of science, including also medical sciences, that the instructor feels an ever increasing uncertainty as to what to teach and what to omit. In the field of dentistry, professor Buddecke's book stands up for the instruction of strictly practical material and gives an example for the way this should be done. This example is certainly thought-provoking and may also be worth following in many respects.

P. ELŐDI

Adrenergic Activators and Inhibitors by L. Szekeres, Handbook of Experimental Pharmacology Vol. 54 (Continuation I and II of Handbuch der experimentellen Pharmakologie, Springer-Verlag, Berlin–Heidelberg–New York 1980

This volume was written under the surveillance of the Editorial Board (G. V. Born, A. Farah, H. Herken and A. D. Welch) of the "Handbook of Experimental Pharmacology", a continuation of the "Handbuch der experimentellen Pharmakologie". The editor of this volume, L. Szekeres, states his object to be to produce a "systemic approach to the various effects of adrenergic activators and inhibitors" starting with the chemistry and structure-activity relationship studies followed by the evaluation of adrenergic activators and inhibitors and discussing their mode of action and analyzing their effects on the central nervous system, the autonomic nervous system, on the cardiovascular, the respiratory, the digestive and the endocrine systems. His attempt at this enormous task amounts to more than 2000 pages and the result is almost unique. He found excellent contributors to this work and the authors could collectively outline the most important aspects of the activation and inhibition of the adrenergic systems. Each chapter provides not only stimulating review material but, most significantly, analysis

of conceptful issues which are relevant to different sites of action.

Two excellent physiological reviews on the catecholamine receptors are in this volume. The contribution of Rand, McCulloch and Story (Department of Pharmacology, University of Melbourne) gives a fascinating account of the prejunctional alpha- and beta-adrenoreceptors and dopamine-receptors. This review provides a general view on the different mechanisms involved in the control of peripheral noradrenergic transmission. The authors convince us that prejunctional alpha-adrenoreceptor- and dopamine-receptor-activation inhibits, whereas beta-receptor activation enhances noradrenaline release and this statement throws a new light on the transmission process.

Anden (Department of Medical Pharmacology, Biomedicum Uppsala) covers the functional studies of post-synaptic noradrenalin- and dopamine receptors in the central nervous system. In the concluding remarks he states that autoreceptors on the nerve terminale should be activated by the transmitter released by nerve impulses in the same way as found for the postganglionic noradrenaline neurons in the periphery. Unfortunately he does not describe the molecular mechanism by which receptors regulate the synthesis and utilization of monoamines.

There are many other stimulating contributions in both parts of this volume. The metabolic consequences of the actions of catecholamines on the heart and the molecular nature of these processes have been excellently reviewed by Will-Sahab and Krause (Akademie der Wissenschaften der DDR, Zentralinstitut für Herz- und Kreislauf-Regulationforschung, Berlin). They outline the specific role of cyclic AMP in mediating the metabolic and physiological effects of catecholamines and point to a series of intracellular reactions followed the interaction of catecholamines with the receptor-site. Philippu (Institut für Pharmakologie und Toxikologie der Universität Würzburg) provides a relatively small but excellent chapter on the central actions of adrenergic activators and inhibitors. He summarizes the effects of alpha- and beta-adrenoreceptor blocking drugs and different antihypertensive drugs on the central nervous system, and gives a fascinating explanation of the mechanism of action of catecholamines in experimental hypertension. The closing contribution is written by Prichard, Owens and Tuckman on the clinical features of adrenergic agonists and antagonists and this is the main message of the book.

In tackling such a broad subject, this volume inevitably has its shortcomings. For example, little attempt has been made to discuss the biochemical characterization of different adrenoreceptors as the main sites of action of adrenergic activators and inhibitors. Similarly, the editor neglected to discuss the effects of enzyme inhibitors such as those involved in neuronal and vesicular uptake processes. The subdivision of the classical receptors seems also to be incomplete, especially in the case of dopamine receptors. Here it does not reflect recent findings. I would also have preferred to see a more detailed section on effects on the central nervous system.

But these observations apart, "Adrenergic Activators and Inhibitors" is an excellent handbook especially for pharmacologists. The contributors did a good job and the editor collected these authoritative and thoughtful contributions very carefully giving a complete textbook for those who are interested in the recent state of this very important subject.

S. Husti

Calcium and cAMP as synarchic messengers by Howard Rasmussen, John Wiley and Sons, New York, Toronto, 1981. 370 pages

This monograph surveys data of the last twenty years concerning stimulus-response coupling in both neural and endocrine systems with calcium ion and cAMP as the coupling factors. The data show that calcium and cAMP are interrelated intracellular messengers. The author introduces the term "synarchy" to indicate that calcium and cAMP nearly always act in concert to elicit the specialized response of cells to the particular extracellular messengers, but the relationship between the two mediator systems differ from cell type to cell type.

In the first chapter a brief review is given on the historical development of our present-day knowledge on the stimulus-response

coupling in excitable and non-excitable cells where the stimuli alter the cellular function by acting on a surface membrane receptor. The second chapter deals with the cellular calcium metabolism. The steps of the process of information flow in the cAMP- and calcium-employing systems and the molecular basis of each step are discussed in the third chapter. Chapter 4 summarizes the known molecular mechanisms of the various interrelationships in detail, in order to demonstrate that minor variations in the interaction of different components of the two systems cause marked changes in the behavior of the overall regulatory systems in different cells. The different types of overall interrelation between calcium and cAMP are characterized as coordinate, hierarchical, redundant, antagonistic and sequential patterns of interaction and each is discussed in separate chapters.

The greatest value of this monograph is that the author summarizes in a conceptional frame all the recent important data concerning the regulatory action of cAMP and calcium in different cells and tissues. Therefore, this book is very useful for the cell biologists, biochemists, physiologists and pharmacologists interested in this field.

A. FARAGÓ

Elektronenmikroskopische Laborpraxis by Walter Nagl, Springer Verlag, Berlin—Heidelberg—New York, 1981

This book is a methodological collection written mainly for beginners, research workers as well as students. The well-illustrated volume containing 28 figures, mainly electron micrographs and 42 tables aims at encouraging pedagogical and research work in the field of medical sciences and cell biology.

Its author, professor at the University of Kaiserslautern, made use of his own long pedagogical and research experience in the preparation of this work. The significance of the book is pointed out on the one hand by electron microscopy becoming a routine method in the above-mentioned fields and, on the other hand, by teaching this particular subject as special courses at more and more universities. All this means that the number of beginners interested steadily increases.

Although non-beginners may also gain by reading this book (e.g. in systematizing their knowledge), their benefit is seriously limited by the author's deliberately aiming at the construction of a methodological collection of a not very wide range; the book sometimes is more laconic than necessary and therefore there is no way for a detailed discussion of some problems.

This concision, however, is an advantage for the beginner, since it spares him the trouble of reading an enormous bulk of literature to start with. The book is written in German and this is a special advantage for the German reader, as most of the special literature is in English, complicated by many technical terms. In accordance with this helpful intention, the author enlists the most important English words of the subject and their German equivalents at the beginning of the book. (Most of these do not present a serious problem, as they are commonly used in labor slang.) The list of the most commonly used abbreviations and their meaning, found also at the beginning of the book, is of great practical value.

An important merit of this book is that in all respects it is very matter-of-fact and functional: a real practical handbook. By this the detailed descriptions are meant, due to which the book alone is adequate for organizing practical work.

This matter-of-factness in a good sense is also shown by the fact that, along with the types of smaller instruments necessary for the work, the possible sources of supply are also listed. Pedagogical work is helped by suggestions for the most suitable objects to be used in practical courses on electron microscopy.

The work of M. A. Hayat (USA) is a good example of a functional electron microscopy handbook, and is excellent at the scientific level of the early seventies. No book of a similar level has ever been published in Europe, and therefore Nagl's book is of special significance in the eighties.

The citation index contains the more important handbooks, monographs and atlases; however, it should cover a wider range and be at the same time more informative: it should be divided into "compulsory" and

recommended literature. The references of the methods are good and detailed; in each case the original publication is cited.

It must be stressed that the Praktikum covers essentially only transmission electron microscopy and related fields. Although scanning and transmission scanning electron microscopic techniques are briefly mentioned, no methodical descriptions or references are included, in agreement with the original aim of the book. By now, however, scanning techniques have become so important that the necessity of a second volume on this subject may soon arise.

In contrast, transmission electron microscopy is described in a methodically comprehensive way. Very correctly, light microscopy is chosen as a starting point, without which electron microscopy cannot be properly understood and practised. Light microscopic pictures of semithin sections are also presented.

The outline of the book is the following: after a short introduction, the theoretical bases of electron microscopic image formation, fixation and embedding are briefly summarized.

The second chapter, the methodological collection, is more detailed. Ultrathin sectioning techniques, including pretreatment, fixation, embedding, blade preparation, sectioning, contrast development and special thin layer techniques are presented. We must note that nowadays cryomicrotomy would be worthy of a separate if short discussion. In the part on special procedures, oriented embedding as well as serial sectioning, autoradiography, the study of isolated organelles and the freeze-etching method are discussed.

The third chapter discusses the increasingly important fields of exposition and image processing, unfortunately in a very short-spoken manner. Several important branches of so-called quantitative electron microscopy are not even mentioned.

In the fourth chapter, further methods other than transmission electron microscopy are briefly summarized (scanning, scanning-transmission, X-ray analysis.) It is in this chapter that the replica technique is touched upon, which would perhaps be more adequately placed in the methodological chapter. Its significance is now certainly greater than judged by the author.

The aforementioned citation index, the list of sources of supply and of the recommended experimental objects and the subject index are followed by the illustrations. The first 11 tables demonstrate the methods presented. The ultrastructure of the cell and that of different tissues are illustrated by a similar number of pictures; the last and smallest group of illustration cover various further objects (plant cell wall, giant chromosomes etc.).

Owing to the choice of field, little plant material is shown, although some are included in the section on the ultrastructure of the cell.

The technical level of the pictures is high: they are printed on paper of the required quality. The majority of the pictures chosen is scientifically good and at the level of the present state of science. The author and the original publication of all the pictures are indicated, i.e. they are published with permission. From this it follows that the scientific responsibility is always taken by the original author, and the writer of the book may not feel his duty to make those critical remarks he most probably does not omit in his courses.

I. VERES

Technique for the Analysis and Modelling of Enzyme Kinetic Mechanisms by Chan F. Lam in Medical Computing Series 4. (Series editor: D. W. Hill), Research Studies Press, a Division of John Wiley and Sons Ltd, Chichester—New York—Brisbane—Toronto 1981. 396 pages, 60 figures, 289 equations, 249 references.

The aim of this book is to present to the specialist in the field of enzyme kinetics a systematic method for analyzing kinetic data, deriving the rate equation, estimating its parameters and testing the best-fitting models. Many topics covered in this book can hardly be found in others, and some have not yet been published. In contrast to traditional books, the technique covered herein are designed for mechanisms with nonlinear double-reciprocal plots.

The first step in enzyme kinetic data analysis is the derivation of the rate equation for a proposed model. Chapter 1 describes

the manual and computer methods currently available for the generation of steady-state rate equations. (Computer programs are written in PL/1 listed in Appendices.)

Chapter 2 (Principles of detailed balance and constraint equation) presents algorithms for identifying independent cycles and constraining rate constants in accordance with thermodynamic principles.

In Chapter 3 the statistical consideration in parameter estimation are explained. Nonlinear least-squares methods are presented in detail, with major emphasis on the gradient-free constrained and unconstrained optimization approach. General simplex optimization program is written in FORTRAN IV and listed in Appendix 4.

Chapter 4 provides calculus and graphical methods of determining the degree of polynomial of rate equation in order to explain nonlinear experimental data. The result is a systematic procedure for model building, which reduces computer time.

Chapter 5 is a very brief summary of analog and hybrid computer techniques in time course studies.

Literature references include all important papers up to 1976. Excellent discussions of practical difficulties in model building make this book indispensable for kinetic specialists.

F. BARTHA

Handbuch der Haut- und Geschlechtskrankheiten by J. Jadassohn Vol. 1, Part 4. B, *Normale und pathologische Physiologie der Haut III* by Forssman et al. (eds G. Süttgen, H. W. Spier and E. Schwartz), Springer Verlag, Berlin—Heidelberg—New York, 1981. 411 figs, 216 tab. 945 pages

This book discusses the water balance of the horn layer, the function of the perspiratory glands, the microcirculation and permeability of the skin and various drug effects.

The scanning electron micrographs of sudor excretion excellently illustrate the results of most recent research.

The authors point out the significance of water bound to proteins and polysaccharides in the skin.

In the microcirculation of the skin, the role of prostaglandins, among others, is important.

Regarding corticosteroids and microcirculation, the effect of glycocorticoids is also discussed, emphasizing that the effect was first recognized by dermatologists.

In percutaneous absorption the horn layer is a barrier of primary importance.

In the chapter entitled "The permeability of the skin" it is pointed out that the use of glucocorticoids has revolutionized dermatological therapy.

A very valuable chapter covers the pharmacology of locally administered drugs, discussing the effective agents, vehicles and their characteristics with special emphasis on penetration, as well as factors increasing or inhibiting the release of active agents. Absorption is different in various parts of the body. There is a difference between the absorption of drugs applied once a day at a high concentration and that administered several times a day in low doses, in favour of the former. Absorption in diseased skin areas is different from that in healthy skin. Sebum may alter the vehicle.

The principle of the choice of vehicle and the effect of active agents on the individual layers and formations of the skin are also discussed. The effect of corticosteroids, antihistamines and several other drugs is described in detail. For the practising dermatologist a knowledge of factors modifying the effects of local treatment is very important. Percutaneous treatment can be applied also in systematic diseases, if the systematically applied drug is quickly excreted or disintegrated in the liver. Here the evenness of absorption from the horn layer is made use of.

The permeability of the epidermal bloodvessels and penetration from these into the dermis raise interesting questions. Inverse penetration (from the dermis into the epithelium) is of importance in the case of certain systematically administered drugs (e.g. Griseofulvin). Particular attention is paid to the factors affecting penetration into oral mucous membranes, nails and cornea, furthermore into the skin. Among the toxicological aspects of skin permeability not only pharmaceutical agents but also insecticides, herbicides, heavy metals and decontaminants (radioactive and non-radioactive materials) are discussed. The latter are important from the point of view of the labour-safety regulations of certain enterprises.

The chapters on the penetration of gases and solid particles into electrolytes and on the movement of water in the skin contain useful information, just like those on glycocorticoids, mineralocorticoids, A-vitaminic acid, psoralenes and MTX.

The general laws of penetration are summarized in 13 entries.

This volume is mostly of theoretical character: however, a knowledge of its practical conclusions is indispensable for the practising dermatologist and the industrial physician. The valuable illustrations and tables are of excellent quality.

E. NAGY

Structure and Activity of Natural Peptides by W. Voelter and G. Weitzel (eds.), Walter de Gruyter, Berlin—New York, 1981. 634 pages

The book is the result of selected contributions presented at the Fall Meeting of the Gesellschaft für Biologische Chemie in Tübingen in 1979. The coverage of this volume consisting of five chapters ranges from traditional peptide chemistry to physically and biologically-oriented areas.

Chapter I contains the survey lectures. E. Wünsch, one of the best-known peptide chemists summarizes well the present state of research of the gastrointestinal hormones. The need for the unity of isolation, structure elucidation and synthesis proving the correct structure of peptides is critically stressed. T. Wieland's review on amatoxins and phallotoxins of Amanita verna and A. phalloides covers a great deal of work done in the field of structure determination, synthesis, biochemistry and biological effects of these sulphur-containing bicyclic peptides. Synthetic and semisynthetic work reviewed by R. Geiger has been a great challange for chemists, though at present neither (semi)-synthetic nor genetically engineered insulin can compete with that of natural origin. Recently the immunomodulating thymic hormones summarized by K. Folkers et al. have become one of the most promising fields of peptide research. Contributions by K. H. Voight et al. and G. Jung et al. concerning the immunocytochemical localization of ACTH and related peptides and properties of the membrane-modifying polypeptide antibiotics alamethicin and trichotoxin A-40, respectively, clearly delineate the exact state of affairs in these more specialized fields. Two excellent reviews on the application of field desorption mass spectrometry for structure elucidation of oligopeptides and the crystal structure analyses of polypeptides show the ever increasing significance of the most up-to-date physicochemical methods.

Chapter II describing some new peptides with interesting biological activities (high affinity to rabbit muscle actin; competitive inhibition of benzodiazepine binding; strong, opiate-specific analgetic effect; stimulation of ACTH and β-endorphin release; inhibition of prolactin, thyrotropin and growth hormones release; lipid-dependent antifungal activity, respectively), isolated from different natural sources (the mushroom Amanita virosa, porcine brain, casein peptone, the skin of the frog Phyllomedusa sauvagi and Enterobacteria) challenges the interests of synthetic chemists, biochemists, physiologists and even physicians.

Chapter III deals with some specific methods of purification, isolation and characterization of different peptides.

In Chapter IV syntheses of some biologically active peptides and their analogs are described. The well-established synthesis of the lymphocyte stimulating thymosin α_1 consisting of 28 amino acid residues affords a valuable substance for in vivo and in vitro biological studies.

A paper reviews the synthetic routes to Met- and Leu-enkephalins. Thyroliberin analogs with modified pyroglutamyl residue add some new conclusions to the known structure-activity relationships. Thyroliberin and somatostatin were synthesized by the application of a new, acid-labile amine protective group. Affinity-labelled vasopressin analogs were prepared for studies of binding to bovine kidney plasma membranes and frog skin. A very interesting paper deals with the planning of peptide syntheses with the aid of electronic data-processing.

The last chapter of the book describes the enzymatic degradation, CNS activity, sterical and electronic structure of thyroliberin analogs; clinical application of gonadotropin-

releasing hormone in delayed puberty; anti-inflammatory properties of the derivatives and fragments of the mast cell degranulating peptide; natural peptide lactones influencing ion-transport in membranes; different effects of thymic extracts on human fibroblasts and cancer cells in cultures; the changes in urinary hydroxyproline concentration in healthy subjects and in patients with prostatic carcinoma; physiological and clinical aspects of hypothalamic pituary-ovarian maturation.

This book cannot be missed from the libraries of institutes where at least one aspect of peptide research is studied.

I. SCHÖN

The Regulation and Control of Cell Proliferation (in Hungarian) by Lapis K. and Jeney A. (eds.) Akadémiai Kiadó, Budapest, 1981. 431 pages

Control of cell proliferation is a key issue of embryonic development, postembrional growth, physiological and reparative regeneration, radiobiology and cancer research. Exponentially growing experimental data and theoretical progress call for comprehensive reviews of this interdisciplinary field. This new book fulfils this requirement. General principles, including the cell cycle are outlined by K. Lapis. Up-to-date features of the nature and control of epithelial and hemopoietic stem cells are summarized by L. Lajtha from Manchester. The molecular and ultrastructural background of regulation is illustrated abundantly (A. Jeney, Zs. Schaff et al.).

Separate chapters deal with cell proliferation in different tissues, e.g. in the haemopoietic system, the intestinal epithelium and the liver (J. Gidáli, I. Fehér, L. Kovács, K. Lapis). Some general clinical considerations, both practical and theoretical, are dealt with by E. Kelemen and J. Sinkovics. Malignancy and cell division are described in detail in the following chapters: In Vitro and in Vivo Carcinogenesis (B. Szende, A. Jeney and K. Lapis), and Cell Kinetics of Experimental and Human Tumours (L. Kopper and K. Lapis). The influence of endogenous and exogenous regulatory molecules, ionizing radiation, cytostatics and the reactivity of proliferating and resting cells are summarized, too (J. Menyhárt, B. Szende, I. Fehér, J. Gidáli, O. I. Epifanova and A. Jeney et al.).

References and subject index make complete this excellent, up-to-date text-book.

A. BALÁZS

Stereospecificity in Organic Chemistry and Enzymology by J. Rétey and J. A. Robinson, Volume 13 of Monographs in Modern Chemistry, Series editor Hans F. Ebel, Verlag Chemia Weinheim, Deerfield Beach, Florida and Basel, 1982. 288 pages

This monograph deals with the mechanism of chemical reactions catalyzed by enzymes. The emphasis is on the steric course of transformation of the substrate. As to the role of the enzyme, participation of coenzymes has been examined in the first place. The involvement of the functional groups at the active site, which is, of course, frequently not known, is considered in a few cases.

The first chapter offers clear definitions, which help the reader clarify concepts related to stereochemistry. The second chapter deals with the mechanistic bases of stereospecificity. The following chapters are concerned with individual reaction types: $NAD(P)_+$-dependent oxidoreductases, flavin-dependent redox reactions, aldol and Claisen type condensations, biotin-dependent carboxylations and the enzymic carboxylation, pyridoxal phosphate-dependent enzymic reactions, the stereochemical course of the coenzyme B_{12}-dependent rearrangements, some aspects of terpenoid biosynthesis, addition-elimination reactions, and finally phosphoryl group transfer reactions.

The monograph highlights the most important achievements in the field of stereospecificity, among them the recent results obtained with isotope and NMR techniques, and is a useful book for organic chemists, biochemists, pharmaceutical chemists and for all interested in reaction mechanism and stereospecificity.

L. POLGÁR

Transport in Plants, by U. Lüttge and N. Higinbotham, Springer-Verlag, New-York, Heidelberg, Berlin, 1980. 468 pages with 180 figures

The book consists of 13 chapters. The first one is a theoretical, fundamental introduction, which contains two sub-chapters. In "The flow of matter and energy through a higher plant" the basic problems of the transport processes of substances and the transfer of energy are treated in the "Summarizing comparison" the authors express a dilemma in research about transport in plants by saying that "there is a hierarchy in complexity of models applicable to the problems attacked".

Part I, "Biophysical background and the substances subject to transport" consist of two chapters: "Biophysical relations" and "The materials of transport". The "Biophysical relations" starts from the simple elementary description of the transport phenomena and goes to more complex mathematical formulations. It makes clear — although very shortly — some basic concepts, the Ment equation, the membrane potential, Donnan potential, Goldman's voltage equation, the electrogen pumps, actions potentials, the Ussing-Theorell equation, the Onsager's reciprocity law, the osmoregulation, the criteria for active transport etc. The measurements of some important parameters are also treated. At the end of this chapter in "Problems and answers" the authors give some concrete examples, by which they help the reader understand the physical-chemical calculations.

Another chapter of this part, "Materials of transport" describes the roles as well as the cycling flows of various inorganic substances essential for life. The translocation of some organic compounds as sugars, amino acids and other N-compounds, organic acids, growth-regulating substances is also treated shortly.

Part II, "Complications of models by cellular structures" consists of four chapters. The first two detail the structures of the cell wall and the membranes across which the transport flows. The other two chapters describe some simplified cell models of transport physiology and various types of transport of substances, as well as the transport functions of cell organelles.

Part III, "Regulation and control of transport processes by cell metabolism" contains three chapters. One of them deals with respiration and photosynthesis, as the energy sources for active transport. Another chapter details the "signaling systems", phytochromes and phytohormones which affect the membran transport processes. The last chapter of Part III deals with coupling between energy transfer processes and transport mechanisms.

The last part, "Intercellular and interorgan transport" has three chapters. These describe the characteristics of the various types of transport in such complex living organisms as plants. Here interconnections as well as regulation are treated.

This book is an outstanding review which treats all important problems of the transport processes in general and their significance in plants. It is useful not only for students and postgraduates but also for biochemists and plant physiologists and for all biologists who want to know more details about transport processes.

L. Boross

From Genetic Experimentation to Biotechnology. The Critical Transition by W. J. Whelan and Sandra Black (editors). John Wiley and Sons. Chichester—New York—Brisbane - Toronto—Singapore. 1982. pp. 266

Seven years elapsed since the famous (or infamous?) Asilomar Conference, that strange and unprecedented event in the history of science took place, where eminent experimental scientists hotly debated the completely imaginary dangers of the new technique of "genetic engineering".

The sound and the fury of that meeting (or, for that matter, the whole age of the big debate before and after Asilomar) is conspicuously absent from this slender volume which presents the verbatim transcripts of another meeting held in Rome in 1981. The conference was organized by "Cogene", an international committee of ICSU (International Council of Scientific Unions). This is all the more remarkable, because "Cogene" was formed soon after the Asilomar conference in order to investigate and help to solve those problems raised by the new technique on which

the Asilomar discussions were focussed. Some of the contributors in this volume were active participants also in Asilomar.

The reader of this book will hardly find even a hint to these problems, i.e. the creation of new dangerous forms of life, meddling with natural evolution, biological warfare, fascistic interference with human genetics, etc.

In that remote pat (seven years ago) scientists were frequently compared to the folktale hero from 1001 Nights who let the evil spirit out of the bottle and could not control it. To the reader of this book another folktale hero springs to mind: the poor lad, who finds a treasure, which corrupts and finally destroys him.

The big issue of the Rome meeting was: Is the intrusion of industrial development money into the ivory tower of molecular biology good or bad? The pros and cons are numerous and both viewpoints are eloquently argued. Therefore the book is very amusing. Undoubtedly for historians and sociologists of science this is an important book of great documentary value. This can best be illustrated by quoting some characteristic titles from the table of contents: "Science in the marketplace. Historical precedents and problems", "From academic information to commercial products", "Faculty members as corporate officers: does cost outweights benefit?", "The roles of God and Mammon in molecular biology", "Communication or secrecy?", etc.

The potential reader must be warned, however, that the practicing scientist would find very little information of scientific value in this volume. Although some of the big names in the field are represented, their summaries are so sketchy that their information content is negligible even for the non-specialist.

In summary: the present state of the art cannot be learned from this book. It is an interesting document of the rapidly changing social environment of genetic engineering, and of the influence of the effects of this environment on the minds and moral value-systems of the practitioners of this exciting and rewarding (in both senses of the word) new technology.

P. VENETIANER

Electrophoresis in the Separation of Biological Macromolecules by Ö. Gaál, G. A. Medgyesi and L. Vereckey, Akadémiai Kiadó, Budapest 1980. 422 pages

The authors have compiled a manual which is an essential aid to researchers in the field of the separation of biological macromolecules. The book gives detailed information about the techniques of electrophoretic separation by summarizing and systematyzing the results of more than a thousand publications on the subject. The first chapter deals with the theoretical bases of the different electrophoretic methods: moving boundary electrophoresis, zone electrophoresis, filter paper electrophoresis, cellulose acetate electrophoresis, electrophoresis in agar and agarose gel, electrophoresis in starch gel, and electrophoresis in polyacrilamide gel. Isoelectric focusing and isotachophoresis are prescribed in particular detail. Detection, determination and characterization of macromolecules after electrophoresis are also given due consideration. The various methods are illustrated by informative figures. The second chapter would be regarded as the greatest merit of the book. It describes in a clear and intelligible manner those methods of electrophoresis of proteins which can actually be applied in any biochemical laboratory. Estimation of the molecular weight of proteins and two-dimensional electrophoretic techniques are also mentioned. Immunoelectrophoresis and related techniques are treated at length. Detailed information is given about the detection of enzymes grouped according to their function. At the end of the chapter the authors mention the electrophoretic separation of certain groups of proteins such as nuclear proteins, ribosomal proteins, membrane proteins. They also deal with the electrophoresis of the proteins of human haemoglobin, and blood plasma. The third chapter is about the electrophoresis of nucleic acids and nucleoproteins, and the fourth chapter describes methods for the electrophoretic separation of glycosaminoglycans. This manual meets a long-felt need in the literature of electrophoretic techniques, giving not only a simple description of the various techniques, but also presenting, on the basis of selected literature, recipes for the different methods. The book is supplemented with an index and list of references containing about

1500 publications. This manual can be of great interest to researchers of research institutes, university institutes, pharmaceutical factories, chemical industries and also to researchers and laboratory assistants in the field of general biochemistry and genetics.

E. LINK

Medizinische Radiographie mit schnellen Neutronen (Radiography with Fast Neutrons in Medicine) by Eckhardt Dühmke, Thiemig-Taschenbücher. Band 86, Karl Thiemig, München, 1980, 137 pages, 50 figures

The author treats the medical application of radiography with fast neutrons in four chapters.

After the neutrons have passed through a medium, their intensity changes similarly to that of the X-ray radiation. However, the change in intensity is independent of the charge of the nucleus. In the case of a given medium the change in intensity is the function of the effective cross section of the given neutron correlated with the energy. Thus a radiographic picture with thermic neutrons can be made only of thin biological medium. An appropriate converter medium is required to take radiographic pictures. The author deals with the above questions in the Introduction giving, at the same time, the effective cross section of the important elements, compiling biological tissues, as a function of the energy of neutron.

In the second chapter the author summarizes the methods of producing fast neutron, discusses the definition of the macroscopic effective cross section related to the neutrons brought about in a reactor. He gives a detailed analysis of the specificity of various converter media, as well as of the properties of the cellulose-nitrate foil as a replacer of films, the method of development. Development is carried out in a solution of 2N or 6N sodium-hydroxide. The author also gives an analysis of the properties of the developed photographic pictures related to the time of development, gives the result of measurements and defines the emerging artifacts.

The results gained by different methods are given in the figures.

The third chapter demonstrates medical applicability in the form of practical examples. Neutron radiographic pictures taken of cadaver tumourous vertebrate column are compared to X-ray, photographical and histological pictures after irradiation or different treatments. In neutron radiographic pictures lesions can be shown while they cannot be estimated in X-ray photos. In addition, histological pictures illustrate these lesions. In the case of neutron radiographic pictures radiation loading is extremely high, so this method is proposed for application if the sick person should get irradiation treatment as a result of his tumourous disease. With children this kind of treatment is contraindicated.

Radiation loading can be decreased with the further modification of the method, using better converter media, thus this method could be of favourable application. The energy of neutrons falls in the range of 1—14 MeV.

The book gives the summary of a new treatment, which, applied on cadaver, can serve as a source for gaining further valuable data in studying the properties of tumours.

Z. DÉZSI

Das peptiderge Neuron by M. Gersch and K. Richter (eds) with the contributions of Baumann, E., Berger, H., Bienert, M., Birkenbeil, H., Böhm, A., Bräuer, R., Eckert, M., Eibisch, H., Gersch, D., Gersch, M., Richter, K., Schulz, H., Schwarzberg, H., Ude, J. and Unger, H. VEB Gustav Fischer Verlag, Jena, 1981, 356 pages, 95 figures, 20 tables

Transmission and processing of information by neuropeptides as well as occurrence and importance of peptidergic neurons are especially fervent areas of biology and medicine. Authors and editors of this book, most of them from the Jena University, attempted to synthesize the immense amount of pertinent data in the literature and to arrive at general conclusions regarding the structure and function of peptidergic neurons. Their attempt was at least partly successful, and the resulting volume is a useful reference book that undoubtedly will serve the purposes of those who seek general orientation as well as of those who are interested in specific details. The number of references quoted is nearly 400.

The structure of the volume follows well-established lines of textbook organization.

First the morphology of peptidergic neurons is described and demonstrated at the level of light and electron microscopy; this is followed by the immune histochemistry of neurogenic peptides. The next chapter is devoted to the isolation and determination of neurosecretory peptides. Lexical enumeration of neurosecretory peptides, their biosynthesis and modification, are treated in Chapters 5, 6 and 7. This is followed by the electrophysiology and regulation of peptidergic neurons (Chapters 8 and 9). Mechanisms of effects (Chapter 10) as well as regulatory functions of neurosecretory peptides are discussed in detail (Chapter 11). General and phylogenetic aspects are the topics of Chapter 12.

The immense (and ever-increasing) number of peptide hormones and transmitters, or more properly, the neurosecretory products of peptidergic neurons, is excellently reflected by the Compilatory Table I (pp. 118—127). This compilation offers invaluable sources of information, regarding terminology, chemical composition, physiological action(s) and major references (literature data) related to neuropeptides. Some numerical data may illustrate the comprehensive character of this book; in vertebrates, 12 different hypothalamic neuropeptides (like thyroliberin, gonadoliberin, somatostatin etc.), 12 pituitary proteohormones of the CNS (like somatotropin, prolactin, corticotropin, endorphins etc.), 9 enteropeptides of the CNS (like cholecystokinine, gastrine, VIP etc.) and 10 miscellaneous neuropeptides (like substance P, neurotensine, angiotensine etc.) are characterized in detail. In another compilation (pp. 129—130) 6 neuropeptides of invertebrate origin (like proctoline, adipokinetic and egg-laying hormones etc.) are itemized; in addition (in Table 47, pp. 134—136), 27 neurogenic peptides of unknown structure (with molecular weights estimated in most cases), like various "neurotropic factors", "heart activators" etc. present in invertebrates are enumerated that makes a total of the presently known (identified and partly also chemically characterized) neuropeptides to 76. If nothing else, this dreadful number should warn scientists working in this field against far-reaching generalizations, not unfrequently drawn on the basis of studying only one or a few members of this immense population.

Another exceedingly important aspect of neuropeptides is synthesis and modification, i.e. differentiation and inactivation. This question is dealt with on the basis of the understanding that the synthesis basically proceeds like those of other peptides and the formation of a specific structure, responsible for specific function, is due to specific endopeptidases splitting of that very sequence of amino acids from the large polypeptide (or neuroprotein) molecule which is essential for specific action or which contains the high-impact portion of the molecule. It is, however, difficult to understand that the basic biochemical studies of fundamental importance related to this problem, by N. Marks and A. Lajtha, are omitted from the discussion.

Not less important is the question of specific receptors in the mechanism of effect of neurosecretory peptides. In this respect, the mobile receptor model (p. 195) and the role of cyclic nucleotides in the effect of peptides (p. 197) are of major interest. It is regrettable that, in striking contrast to the well-documented first part of the book ("Morphology of the peptidergic neuron") electron microscopic, histochemical and fluorescence microscopic illustrations are conspicuously failing from this chapter. The same applies also for the chapter dealing with immune histochemical demonstration of various neuropeptides; though described to some extent in the text, the localization of the major neuropeptides (except for neurophysin, insulin and proctolin) is not illustrated. Recent advances in the immune electron cytochemical localization of substance P, somatostatine and other similar peptides make imperative to include these important results, probably in the next edition of the book. FRAP (fluoride resistant acid phosphatase) characterizing primary nociceptive neurons, and closely related to neuropeptide metabolism is not mentioned at all.

In spite of its shortcomings it seems, however, clear that the "Gersch-Richter" will be one of those standard books one keeps on the shelf in the laboratory. The team of the Jena scientists, as well as Fischer Verlag, should be congratulated for the intrinsic and extrinsic values of this book.

B. CSILLIK

Biophysik — Praktikum by Wolf, H. and Lerche, D. VEB Gustav Fischer Verlag, Jena, 1980, 181 pages, 68 figures

In recent years it has become apparent that the necessity of quantitative description in biological and medical research work as well as the introduction of new techniques and sophisticated methods require, at the one hand, the acquisition of knowledge relating to this new concept that promotes and improves research work and, at the other hand, require an experimental readiness in the practical application. The book was written mainly for university students in order to get an introduction into the experimental technique and methodology of biophysics. It is a guide-book for exercises. The selection of the material to be treated is moderate and carefully composed with long university experience. It is readable for those who studied in other fields than biology, but are engaged or interested in medicine, agriculture, or in other branches of biology. It should be suitable for postgraduate students as well.

The book contains four chapters. Chapter I (40 pages) deals with the experiments on thermodynamical phenomena in biological objects (viscosity, osmotic pressure, transport on biological membranes, temperature dependence of rate constants and energy of activation). Chapter II (50 pages) discusses the methods for measurements of electric phenomena on biological objects (impedance of biological tissue, electric conductivity of electrolytes and cell suspensions, equilibrium and non-equilibrium potentials, resting potential in cells, active transport, electrokinetic behavior of cells). Chapter III (35 pages) deals with the experiments for analysis of systems (compartment analysis, regulation, growing and proliferation) and Chapter IV (16 pages) contains some experiments on photobiology (photosynthesis, effect of radiation on biological objects). There is an Appendix attached to the text which briefly summarizes the elements of biological statistics, it contains tables and methods for preparation and the answers to the problems and self-control questions.

A basic theoretical survey is given in each chapter; followed by detailed description of the exercise, the instruments and the evaluation of the measurements. For self-control, questions and problems are attached to each task.

The book contains a list of references at the end of the exercises and there is a subject index as well, which facilitates the readers' orientation. I think this book is valuable both for teachers and for specialists. The clarity of the style, the subdivision of the main questions and the exemplary execution of the book makes it easy to handle.

J. BELÁGYI

Biomagnetism by S. N. Erné, H. D. Hahlbohm, and H. Lübbig (eds). Proceedings of Third International Workshop on Biomagnetism, Berlin (West), 1980, Walter de Gruyter, Berlin—New York, 1981

The volume, containing 558 pages, 19 tables and 207 figures, presents the full texts of papers submitted to the Workshop organized in parallel with the International Conference SQUID'80 (Superconducting Quantum Interference Devices and their Applications). The Workshop included 56 participants from 12 countries (a list of the names and addresses is given).

The 40 papers are organized according to their topics under the following headings: instrumentation, fundamentals, biomagnetic fields, susceptometry and miscellaneous (1 paper only). The chapter on instrumentation deals with the construction and performance of magnetically shielded rooms, condition of measurements using shields and with measurements in unshielded, normally noisy environments (11 papers). After discussing the generation and the medical significance of magnetic fields in the human body (3 papers) a detailed study of the various aspects of the biomagnetic field follows. 11 papers are devoted to cardiomagnetism from the description of a magnetocardiogram (MCG)-recording system to the theory and practice of magnetocardiography for clinical use and to the explanation of the structure of MCG. 8 papers are published on neuromagnetism, namely, on the magnetic field of the cerebral cortex, on various problems of the measurement and evaluation of magnetoencephalograms, retinograms and oculograms. In the chapter on susceptometry (6 papers) two main fields are discussed: magnetopneumography (the

study of accumulation and clearance of metal fume and dust particles in the lung) and thalassemia susceptometry (the diagnosis of liver iron overload and an apparatus for in vivo measurement of iron stored in human tissues).

The documentation (figures and tables) facilitates the understanding, while the author index and subject index promote the use of the volume. The comparatively large number of authors and the use of camera ready manuscripts lead to unavoidable effects on the uniformity of style, performance and appearence of the different papers. However, this does not diminish the information value of the material. The editors wish this volume to stimulate work for further and more refined volumes on biomagnetism. The reader believes that this volume certainly contributes to an arousal of interest in this field and will stimulate further research and practical application in biomedical topics. The volume is an interesting and important source for teachers and research workers, physicists, biologists, medical doctors interested in the relation of magnetism and the human body, in a rapidly growing field of promising new possibilities for the biomedical practice.

L. SZALAY

Das Stabilitätsverhalten von Osteosynthesen mit ausserer Knochenfixation und Kompression by G. Mayer, K. H. Haase (Ergebnisse der experimentellen und theoretischen Biophysik, Bd. 26.) VEB Georg Thieme, Leipzig 1981. 112 pages, 40 figures

It is astonishing that though examinations of stability by plate osteosynthesis with compression have a wide background in literature, precise measurements of the stabilizing effect of fixating fractures by external fixatives (fixateur extern) and the exact biomechanical influences of these fixations are little investigated fields of orthopaedics. This deficiency is remedied by this treatise of 112 pages published in the series "Fortschritte der experimentellen und theoretischen Biophysik". The two authors — an orthopaedic surgeon and a structural engineer — give an extremely thorough analysis of the biomechanical stabilizing effect of the five types of external fixatives used in orthopaedic surgery (AO, Hoffmann-Vidal, Wagner, Ilizarov and Hellinger-Hoffmann) on fractures brought about experimentally.

Transversal osteotomy of the middle third part of tibias from fresh corpses was used by the authors as model in their measurements. The bending and torsion stabilities of osteotomies fixed by five different devices were measured by up to date means, and with a high accuracy. Several tables, diagrams of the book contain the parameters of these measurements. To sum up, the conclusion has to be drawn from these measurements that the bending and torsion stability obtained by different fixatives is in direct proportion with the interfragmental compression reached by the apparatus.

The *in vitro* measurements described in the first part are supported by animal experiments described in the second part of the book. Here transversal osteotomies of sheep tibia were used as models by the authors, having fixed them with the same 5 types of fixatives. Their histomorphological examinations confirmed the fact — being not unknown in literature — that the great stability of fracture results in a primary "per primam intentionem" healing of fracture. The initial high interfragmental compression between the osteotomic bone ends ceases gradually by the rebuilding of the bone. At the same time the interfragmental pressure of osteotomies, stabilized less by slighter pressure, decreases quickly, and the healing of bone ensues with a mass callus-formation.

Though the book may be interesting for clinicians dealing with healing of fractures rather than for physicists, in my opinion it may be of interest also for those scientist involved in studies regarding the biomechanics of healing of fractures.

M. FORGON

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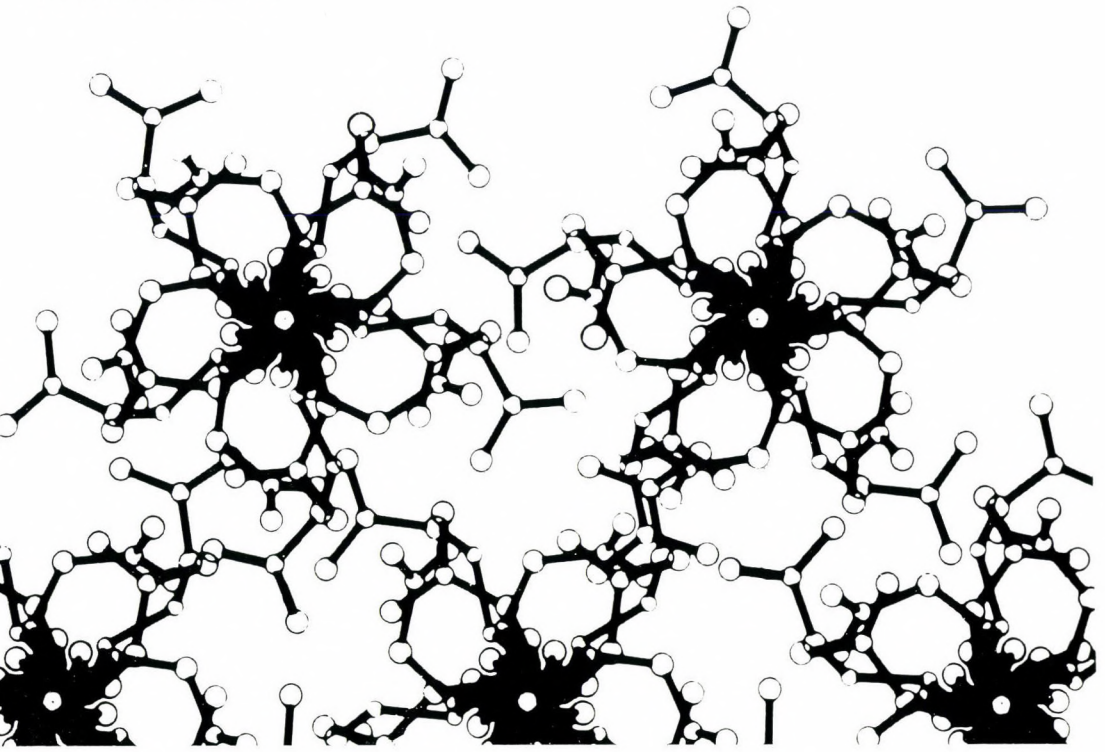

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