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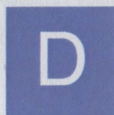

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Call #: **QH301 .F37 v.34 no.???? 1975**

Location: **SCI-ENG JRNLS**

Book/Journal Title:

**FEDERATION PROCEEDINGS**

Book Author:

**Other Info:**

Year: **1975**

Pages: **684-684**

Article Author: **HODGINS, MG**

Article Title: **SUPERVISCOUS EFFECTS AT LOW SHEAR-STRESS DURING EXTRUSION OF RNA FROM VIRUS**

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## Physical Methods in Biochemistry

2642 BIOCHEMISTRY

FLUORESCENCE OF A MONOLAYER OF GLYCERYL 3-[4(3-PYRENYL) BUTYRYL]-1-OLEYL ETHER AT AN AIR-WATER INTERFACE. Louis C. Smith and Kong-Yi Hong*. Baylor Col. Med., Houston, Tx. 77025

An apparatus for measuring the relative fluorescence intensity of monomolecular films of glyceryl 3-[4(3-pyrenyl) butyryl]-1-oleyl ether has been constructed. Excitation light, 365 nm, from a high pressure mercury lamp was passed through a 0.25 meter monochromator. In either side of a Teflon monolayer tray beneath the surface film was a quartz window inclined $60^o$ with respect to the surface. The light beam entered through one window, was totally reflected from the air-water interface, and exited through the other window. The fluorescent light emitted by the lipid film was analyzed at 475 nm with a monochromator placed above and parallel to the surface. Emission intensity was measured by single photon counting. Background count rate was 300 ± 50 counts per second. At the collapse pressure the count rate increased to 1200 ± 50 counts per second. Changes in surface fluorescence intensity corresponded to changes in surface pressure. Fluorescence properties of this ester and other lipids containing fluorescent moieties can be determined in surface monolayers and in aqueous dispersions. It should be possible to correlate the physical state of lipids in the more well defined monolayer systems with the properties of these lipids in bilayer membrane systems. (Supported by The Robert A. Welch Fdn., Q-343; Am. Heart Assoc., 73-778; and USPHS HL-15648, 14194 and 71-2156.)

2643 BIOCHEMISTRY

A COMPARISON OF NANOSECOND TIME-DEPENDENT SPECTRAL SHIFTS OF A FLUORESCENCE PROBE ADSORBED TO EGG LECITHIN VESICLES AND DISSOLVED IN GLYCEROL. Robert P. DeToma*, J. Hamilton Easter* and Ludwig Brand. Johns Hopkins Univ., Baltimore, MD 21218.

The monophoton counting technique was used to obtain nanosecond time-resolved fluorescence spectra of 2-p-toluidinonaphthalene-6-sulfonate (2,6 p-TNS) adsorbed to lecithin vesicles and dissolved in glycerol. It was shown that the spectra obtained are distorted by convolution errors. These errors can be corrected for by multi-exponential deconvolution. Analysis of the fluorescence decay at various wavelengths indicates that the spectral shifts observed both with the vesicles and the dye in glycerol are due to excited state reactions. Negative amplitudes are observed with the decay curves obtained at the low energy side of the emission band. A two-state relaxation model predicts a double exponential decay with lifetimes independent of emission wavelength. This behavior was not observed either with the dye adsorbed to vesicles or dissolved in glycerol. The decay kinetics are complex and do not approximate a simple two-state model. Preliminary data supports a non-specific dipolar interaction model according to the operational definition of Bakhshiev [Opt. Spectrosc. 28, 490 (1970)]. The potential of time-resolved emission spectroscopy for biochemical investigations will be discussed. (Supported by NIH grant No. GM11632.)

2644 BIOCHEMISTRY

DIFFUSION OF PHOSPHOLIPID VESICLES MEASURED BY PULSED-GRADIENT FOURIER TRANSFORM NUCLEAR MAGNETIC RESONANCE (NMR). George G. McDonald* and J.M. Vanderkooi*. (SPON: M. Pring). University of Pennsylvania, Philadelphia, Pa. 19174.

Pulsed gradient Fourier transform nuclear magnetic resonance (NMR) can be used to measure the diffusion coefficient of individual moieties in multicomponent systems (T.L. James & G.G. McDonald, J. Mag. Res 11, 581-601, 1973). This technique has been used to measure the diffusion coefficient of dimyristoyl lecithin (DML) liposomes prepared by sonication of DML in a $D_2O$-phosphate buffer. The effects of micro and macroscopic viscosity were determined by measuring the diffusion rate of DML in a DNA solution and a Sepharose suspension. The diffusion coefficient of DML at 37°C is $D = 1.9 \times 10^{-6}$ $cm^2/sec$ in buffer and $3.5 \times 10^{-7}$ $cm^2/sec$ in DNA solution. The diffusion rate of DML was not significantly slower in Sepharose than in buffer. These data suggest that DML diffusion is controlled primarily by whole liposome migration as opposed to the movement of individual DML molecules within the liposome. Furthermore these data compare favorably with the diffusion rate for whole liposomes as determined by ultracentrifugation and by Stokes law calculation. (Supported in part by USPHS NIH Grant RR 542-4).

2645 BIOCHEMISTRY

MICROSPECTROFLUOROMETRY FOR RAPID OPTIONAL ANALYSIS OF METABOLIC TOPOGRAPHY OR FLUORESCENCE SPECTRA IN SINGLE LIVING CELLS. Elli Kohen, Cahide Kohen* and Joseph Hirschberg*. Papanicolaou Cancer Research Institute, Miami, FL. 33136.

Multichannel microspectrofluorometry with low-light level camera tubes (BBA 263, 575, 1974), allows the study of metabolic transients (e.g., NAD(P) ⇌ NAD(P)H) due to intracellular microelectrophoretic addition of metabolites, e.g., glucose-6-P (G6P). Intracellular metabolic topography reveals discrete local differences in transient kinetics. Fluorescence spectra from EL2 cells grown with agents affecting the cell cycle or morphology (e.g., antibiotics, dibutyryl cyclic AMP) exhibit changes in the amplitude of the difference spectrum due to G6P (? of adaptive changes), but the analysis of associated small spectral shifts (? of changes in bound /free NADH) requires improved spectral resolution as provided by a more versatile instrument with adjustable spectral dispersion. In presence of exogenous fluorochromes with higher quantum yield (e.g., polycyclic hydrocarbons, 1-pyrene butyric acid, acridine orange) changes in NAD(P)H transient emission due to metabolites may be followed, as well as the metabolization of the exogenous probe or the appearance of new molecular species (e.g., hydrocarbon metabolites). The method exhibits unique capability at the unicellular level in the multisite detection of biochemical interactions, as well as possible metabolization or alterations of fluorescent chemicals associated with cell pathology or added as probes. (Supported by American Cancer Society grants BC-15 B & C and National Science Foundation GB-30224)

2646 BIOCHEMISTRY

DIHYDROFOLATE REDUCTASE FROM A METHOTREXATE-RESISTANT *ESCHERICHIA COLI*: PROTON MAGNETIC RESONANCE STUDIES OF COMPLEXES WITH FOLATE AND METHOTREXATE. Martin Poe, Myra N. Williams*, Norma J. Greenfield* and Karst Hoogsteen*, Merck Institute for Therapeutic Research, Rahway, N.J. 07065

Proton magnetic resonance studies of 1:1 complexes of dihydrofolate reductase from a methotrexate-resistant strain of *E. coli* with folate and methotrexate were done. Both folate and methotrexate when enzyme-bound appeared to be in slow exchange on the nmr time scale with the corresponding free ligand. A resonance at 1850 Hz in 1:1 enzyme-folate was assigned as the C-7 proton of bound folate by comparison with spectra of enzyme complexed with folate specifically deuterated at C-7. The first order rate constant for folate dissociation from the enzyme was calculated to be less than 110 $sec^{-1}$. The aromatic region of the pmr spectra of the two complexes were similar. In particular, four of the five histidine resonances exhibited C-2 proton pH titrations that were essentially superimposable for the two complexes. The pK values for the five histidine residues for enzyme-methotrexate and enzyme-folate, respectively, were 1, 8.0 ± 0.1, 8.0; 2 7.3, 7.35; 3 6.4, 6.55; 4, 6.25, 6.95; 5 5.15, 5.1. The differences in chemical shift and pK for histidine 4 in the two complexes were consistant with this residue being near the pteridine portion of the folate binding site on the enzyme.

2647 BIOCHEMISTRY

SUPERVISCOUS EFFECTS AT LOW SHEAR STRESS DURING EXTRUSION OF RNA FROM VIRUS. M. G. Hodgins*, O. C. Hodgins*, D. W. Kupke and J. W. Beams*. Dept. of Physics and Dept. of Biochemistry, University of Virginia, Charlottesville, Va. 22901.

The magnetic visco-densimeter, which can be used to determine both the viscosity and density on 0.2 ml solution, was employed to follow the time course of RNA extrusion (or capsid formation) from Turnip Yellow Mosaic virus after adjusting the pH to alkaline conditions. At certain virus concentrations (~0.5%, 20°C and shear stress ~$10^{-3}$ $dynes/cm^2$), the viscosity gyrated in a bizarre manner with time -- reaching values which were 10-30 times higher than the relative or zero-time viscosity. Below this concentration range the solutions behaved normally; above this range the viscosity rapidly approached a zero rate of shearing, after which elastic behavior could be observed. Upon disturbing the latter solutions, the viscosity was only fractionally higher than the zero-time values--and usually remained so. With small disturbances, however, the solutions simulated liquid crystal behavior. Density changes were not unusual. The separated RNA, capsids, capsid subunits and mixtures of these have not exhibited the superviscous character at the magnitude of stresses noted above. Instrumentation for the application of much smaller shear stresses is under development.

(Supported from grants from the National Science Foundation.)

# Federation of American Societies for Experimental Biology

## 59th Annual Meeting
## Atlantic City, New Jersey, April 13–18, 1975

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# Contents

## ABSTRACTS

Intersociety .......................... 219
Physiology .......................... 351
Biochemistry .......................... 483
Pharmacology .......................... 711
Pathology .......................... 821
Nutrition .......................... 879
Immunology .......................... 945
Additional Abstracts .................. 1049

INDEXES ...... 1051