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Call #: **QH301 .F37 v.35 no.5-9 Apr.-July 1976**

Location: **SCI-ENG JRNLS**

Book/Journal Title:
FEDERATION PROCEEDINGS

Book Author:

Other Info:

Volume: **35**

Issue: **7**

Year: **1976**

Pages: **1458-1458**

Article Author: **KUPKE, DW**

Article Title: **RIGIDITY OF VIRUS SOLUTIONS BY A THIN-FIBER TECHNIQUE**

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ELASTICITY MEASUREMENT - A USEFUL TOOL IN THE STUDY OF FIBRINOLYSIS AND FIBRINOGENOLYSIS. Linus L. Shen*
(SPON: C. Nolan). Abbott Labs, N. Chicago, IL 60064

A sensitive, easy-to-use Couette elastometer was constructed in our lab to measure the (static) elastic modulus of fibrin or plasma clots. It is demonstrated that measurement of this mechanical property is a sensitive method to detect minor structural changes in fibrin and in fibrinogen molecules. This technique is used to follow fibrinolysis by plasmin; results indicate that elasticity is a sensitive parameter and more reliable than other conventional parameters such as clotting time, turbidity and clottability. We also use this technique to study fibrinolysis initiated by the addition of urokinase and thrombin to solutions containing fibrinogen and plasminogen. The clot dissolution process consists of at least two steps: (1) a quick destruction of that part of the fibrin network which is functionally important to clot rigidity, as indicated by a decrease in elastic modulus to approximately 2% of the maximun, but accompanied by a small decrease in turbidity, (2) slow digestion of the remaining, functionless fibrin fibers accompanied by certain abnormal structural rearrangement indicated by a transient increase in turbidity during the terminal stage of disgestion. The lysis time, determined by extrapolating the linear portion of reaction (1) to zero elasticity, is a linear function of urokinase concentration in the range from 1 to 20 CTA units per mg fibrin in a log-log plot; this technique provides a reliable assay method for urokinase.

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SPECTROPHOTMETRIC ASSAY OF LOW LEVEL METABOLITES. Eva Christensen*, Joseph Higgins, and Richard L. Veech, M.D. Laboratory of Alcohol Research, NIAAA, St. Elizabeths Hosital Washington, D.C. 20032.

The assay of metabolites (e.g. GAP) at the tissue level of 1 to 10μM can not be achieved by conventional spectrophotometry. Flourometry is useful but unreliable due to numerous antifacts and background interference. Enzymatic cycling appears to be reliable but is time consuming. Using an Aminco DW-II in the split beam mode, we have developed a spectrophotometric assay for GAP which is chemically and optically balanced, thereby eliminating most artifacts and background problems. Since the method is insensitive to background absorption, it allows enzymatic coupling thru αGPdH rather than GAPdH. The method is accurate to 10% at assay levels of 0.2μM. Recovery experiments for GAP on freeze clamped livers gave results within 10% of the theoretical values as well as those obtained by enzymatic cycling. For meal-fed Wistar rats values of DHAP=21 and GAP=3.3 nmoles/gm. tissue were obtained.
Abreviations: GAP: glyceraldehyde-3-phosphate
DHAP: dihydroxyacetone phosphate
αGPDH: αglycerol phosphate dehydrogenase
GAPdH: glyceraldehyde-3-phosphate dehydrogenase.

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DETERMINATION OF INTRACELLULAR METABOLITE DISTRIBUTION BY CELL CAVITATION. Marc E. Tischler*. (Spon: J. Flaks). Dept. of Biochem. and Biophys., Univ. of Pa., Philadelphia, Pa. 19174

Measurement of metabolite distributions between the mitochondrial and cytosolic compartments in isolated rat liver cells may be achieved by forcing the cells at high velocity through a 25 gauge needle, followed by rapid centrifugation of the released mitochondria through silicone oil into acid. Monitoring the ejection velocity permits the Reynolds number to be calculated and maintained constant at 8000 to produce turbulent flow. Optimal conditions gave α-glycerolphosphate release of 88-92%, with 71-75% lactate dehydrogenase being released. Mitochondrial damage is low, however; the citrate synthase and glutamate dehydrogenase leak being 8% and 9% respectively, while 26% myokinase is released. Metabolic changes in the mitochondria are prevented by discharging the disrupted cells into a mixture of transport inhibitors. Their absence permits up to 50% changes of mitochondrial metabolites to occur over a 45 sec period. The cytosolic content is calculated by the difference between the whole cell and mitochondrial measurements. The above technique has been used to measure mitochondrial/cytosolic gradients for various metabolites using cells from fed rats with 8mM lactate and 0.8mM pyruvate as substrates. Preliminary values obtained were: malate,4; α-ketoglutarate,7; glutamate,5; aspartate,26; pyruvate,54; citrate,37 and isocitrate,295. The ATP/ADP ratios were 3.6 mitochondrial and 12.2 cyosolic. (Supported by grants AM-15120,AA-00292 and HL-14461).

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RIGIDITY OF VIRUS SOLUTIONS BY A THIN-FIBER TECHNIQUE. D. W. Kupke and J. W. Beams*. University of Virginia, Charlottesville, Virginia 22901.

Solutions of Turnip Yellow Mosaic virus upon ejecting RNA exhibit solid-like mechanical properties when sufficiently small shearing forces are applied. At a critical applied force this property vanishes and the solutions behave as normal liquids having viscosities only a little higher than the intact virus controls. Initially, a magnetic suspension-rotation method was employed (Proc. Nat. Acad. Sci.72;3501, 1975). By a thin-fiber technique, the solid-like property is also observed in the absence of magnetic fields; in addition, the breaking strengths of the solutions are automatically measured. In this method, the restoring torque of a long fiber (~1 m) attached to a small, dense cylinder hanging and immersed in a slowly rotating solution (~0.03 rad/sec) is used to measure this breaking strength. Restoring constants of 10^{-4} to 10^{-5} dyne, cm/rad for the fiber assemblies have been employed. The resistance to stress generated in the solutions ranged from ~0.01 to ~1 dyne/cm^2, depending on the concentration of virus (0.1 to 2%); the breaking strength increased in a somewhat exponential manner with concentration over the studied range. Attempts to simulate some of the conditions at the target site (chloroplast) suggest that this resistance to shearing forces may be operative during the infection process.

(Supported by grants from NSF and USPHS.)

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DEVELOPMENT OF LOWRY PROTEIN COLOR BY SMALL PEPTIDES WITH AND WITHOUT TYROSINE. Harold Van Kley and Susan M. Hale*. Lab. for Biochemical Research, St. Mary's Health Ctr., St. Louis, MO 63117, and Dept. of Biochem., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Development of color in the Lowry protein assay (Lowry *et al.*, J. Biol. Chem., *191*, 265, 1951) is generally attributed to aromatic amino acid residues; some use tyrosine as a standard for the reaction. The original report showed color from gelatin was almost zero without copper and was greatly enhanced by alkaline copper. We have found that dipeptides containing tyrosine give approximately the same color with and without copper. LeuLeu gives some color with copper and none without. Copper gives a small increment of color with trityrosine. Triglycine is more chromogenic per mole than tetra- or pentaglycine and gives a greater color yield per μg than bovine serum albumin. Position of amino acids in a tripeptide is important - thus the order of color development was GlyGlyLeu < LeuGlyGly < GlyLeuGly. HisGlyGly was the most chromogenic tripeptide tested whereas GlyHisGly gave almost no color. It is concluded that color development from aromatic amino acid residues in the Lowry reaction does not depend on copper. The major chromogenic contribution is from copper chelates with peptide bonds with some contribution by amino acid side-chains. Competition for copper from chelating buffers or amino acid combinations reduces the color yield. (Supported by the Research Budget of St. Mary's Health Ctr.)

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FORMATION OF AMINO ACIDS AND RELATED OLIGOMERS FROM FORMALDEHYDE AND HYDROXYLAMINE IN MODIFIED SEA MEDIUMS. Fujio Egami and Hiroshi Hatanaka*. Mitsubishi-Kasei Institute of Life Sciences, Tokyo 194, JAPAN

Good correlation has been found between the biological behaviour of minor elements such as molybdenum and iron and their concentration in the present sea water (J.Mol.Evol.,4, 113). A hypothesis has been presented that the composition of the present sea water reflects that of the primeval sea water at the time of early evolution. Based on the above consideration, modified sea medium containing 10^{-4} M of several transition elements and clays was designed, in which various natural products are expected to be formed from simple starting materials. The reaction mixture was sealed under nitrogen and kept for 35 days at 105°C. After Biogel P-2 separation of the products, oligomerized mixtures were obtained which gave various amino acids by acid hydrolysis. The oligomer formation was supported by the evidence that the ratio of the content of primary amino groups after to before hydrolysis in column effluents increased by about 5-fold together with the molecular size. These results suggest the direct formation of oligopeptides from formaldehyde and hydroxylamine in the primeval aqueous mediums. The yield of amino acids was calculated as 5.24% from hydroxylamine (25 mmol). The amino acids consisted of about 40 spieces, including Gly (114 μmol), Ala (26 μmol), Ser (12 μmol), Thr, Ile, Asp, Glu, Met, Val, Leu, Arg, Lys and His, and non-protein amino acids such as β-Ala and homoserine.

American Society of Biological Chemists

67th Annual Meeting

Cosponsored by the
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Biochemical Societies

San Francisco, California
6–10 June 1976

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VOLUME 35, NUMBER 7 · MAY 15, 1976

Federation Proceedings

Official Publication of the
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY

FUTURE FASEB ANNUAL MEETINGS:

1977—Chicago, IL April 3–8
1978—Atlantic City, NJ April 9–14

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