TABLE II

INVOLVEMENT OF PRPP AND ATP IN THE SYNTHESIS OF GAR

Basic system: 5 μM C^{14}-glycine, 5 μM L-glutamine, 7 μM $NaHCO_3$, 2 μM L-azaserine, 0.3 ml. 0.03 *M* phosphate buffer, *p*H 7.4, containing 0.13 *M* KCl and 0.01 *M* $MgCl_2$, and 10 mg. of dialyzed, Norite-treated 15–45% ethanol fraction of pigeon liver extract; incubated 45 minutes at 38°.

Addition to basic system	μM. GAR synthesized
None	0.00
Ribose-5-phosphate, 2 μM.	0.02
ATP, 2 μM.	0.04
Ribose-5-phosphate + ATP, 2 μM. each	0.07
PRPP, 2 μM.	0.13
PRPP + ATP, 2 μM. each	0.43

It is seen in Table II that PRPP is the active ribose phosphate moiety in this ribotidation reaction but that maximal synthesis is realized only when PRPP and ATP are present together. These experiments demonstrate the requirement for ATP in a step in the formation of GAR other than that concerned with pyrophosphorylation of ribose-5-phosphate.

DIVISION OF BIOCHEMISTRY
DEPARTMENT OF BIOLOGY STANDISH C. HARTMAN
MASSACHUSETTS INSTITUTE OF TECHNOLOGY BRUCE LEVENBERG[12]
CAMBRIDGE 39, MASSACHUSETTS JOHN M. BUCHANAN

RECEIVED DECEMBER 2, 1954

(12) Public Health Service Research Fellow of National Institute of Neurological Diseases and Blindness. Work supported by Grants from the Damon Runyon Memorial Fund for Cancer Research and the National Cancer Institute, National Institutes of Health, United States Public Health Service.

A NEW CRITICAL VOLUME EFFECT IN HIGH POLYMER SOLUTIONS

Sir:

Staudinger[1] and several other authors[2] have predicted from the length of the macromolecules that these would be separated from each other in very dilute solution, but on the contrary would form a continuous network in concentrated solution. The transition from a discontinuous to a continuous solution would be expected to take place at a critical value of the concentration.

Streeter and Boyer[3] by viscosity measurements of very dilute solutions of polystyrene in toluene, have observed a phenomenon which they have attributed to this critical concentration. Daoust and Rinfret,[4] studying the heats of mixing of polyvinyl acetate in S-dichloroethane and S-tetrachloroethane, discovered at high dilution the existence of a concentration at which the graph of heat of mixing *vs.* volume fraction shows an inflection point. Parent and Rinfret[5] have subsequently shown that the value of these critical concentrations varied inversely as the molecular weight of the P.V.A.

We have thought here that it was possible to determine those critical concentrations by very precise measurements of the specific partial volume at high dilution. Using a magnetically controlled float similar to that of Lamb and Lee,[6] we have attained a precision of $\pm 1 \times 10^{-6}$ g. ml.$^{-1}$. Five series of density measurements of carefully fractionated P.V.A. in dilute chlorobenzene solutions were made; Fig. 1 shows that at concentrations lower than one gram per hundred grams of solution, each molecular weight gives rise to a different inflection point in the graph of density *vs.* concentration.

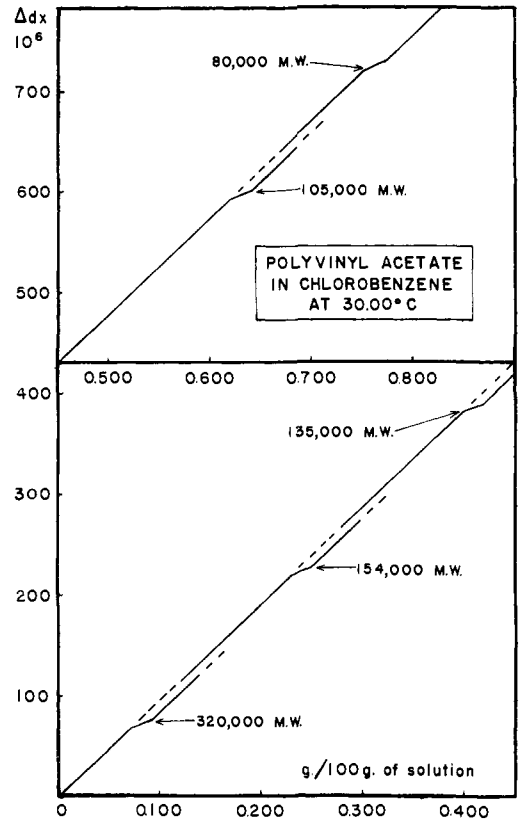

Fig. 1.

As the position of these inflection points shifts downward with increasing molecular weight, it would seem that the phenomenon observed confirms the prediction of Staudinger, *et al.*, about the change of structure of the polymer in solution with concentration.

(1) H. Staudinger, "Die hochmolekularen organischen Verbindungen," Springer, Berlin, 1932, p. 128.
(2) R. F. Boyer and R. S. Spencer, *J. Polymer Sci.*, **5**, 375 (1950).
(3) D. J. Streeter and R. F. Boyer, *ibid.*, **14**, 5 (1954).
(4) H. Daoust and M. Rinfret, *Can. J. Chem.*, **32**, 492 (1954).
(5) M. Parent, Ph.D. Thesis, Université de Montréal, 1954.
(6) A. B. Lamb and R. E. Lee, THIS JOURNAL, **35**, 1668 (1913).

DÉPARTMENT DE CHIMIE
UNIVERSITÉ DE MONTRÉAL ALCIDE HORTH
MONTREAL, CANADA MARCEL RINFRET

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PHOSPHORYLATION COUPLED WITH ELECTRON TRANSPORT TO CYTOCHROME C BY SOLUBLE ANIMAL ENZYMES

Sir:

Intramitochondrial diaphorase is inhibited by hydroxylamine. Soluble diaphorase is not inhibited. A factor necessary for this inhibition was isolated from guinea pig liver.[1] We observed that

(1) I. Raw, *Science*, **118**, 159 (1953).

cytochrome *c* reductase, prepared from guinea pig by a method similar to that of Edelhoch, *et al.*,[2] with this hydroxylamine inhibiting factor, promotes an exchange reaction between P_{32} and ATP,[3] in a system containing ADP, DPNH and Mg^{++}. This exchange does not require cytochrome *c* or oxygen, and DPNH can be substituted by DPN and, furthermore is not inhibited by 2–4 dinitrophenol or fluoride. As this exchange depends on the presence of reductase and DPN or DPNH we are dealing with oxidative phosphorylation.

Experiments were performed with a digitonin extract, prepared by extracting rat liver mitochondria with 2% digitonin in 0.55 *M* KCl–0.1 *M* TRIS *p*H 7.5–0.1 *M* reduced glutathione. This extract together with cytochrome *c* reductase and hydroxylamine inhibiting factor was tested for oxidative phosphorylation in a system containing: (a) alcohol and alcohol dehydrogenase as electron source, (b) cytochrome *c* as electron acceptor, (c) glucose plus hexokinase to trap the ATP formed, (d) ITP which is necessary in this system. Glucose 6-phosphate is not metabolized in this system, and was determined by the increase of absorption at 340 mμ with TPN and glucose 6-phosphate dehydrogenase.[4]

TABLE I

TRIS buffer *p*H 7.5, 80 μM.; ADP 2.4 μM; KCl 36 μM.; $MgCl_2$ 4 μM.; $MnCl_2$ 1 μM.; glucose 6 μM.; ITP 0.3 μM; cytochrome *c* 0.33 μM.; DPN 1 μM; ethanol 200 μM.; orthophosphate 5 μM.; alcohol dehydrogenase 220 units; hexokinase 70 units; cytochrome *c* reductase approx. 1 mg.; hydroxylamine inhibiting factor approx. 1 mg.; mitochondria extract approx. 3 mg.; total volume 0.9 ml., incubated during 15 min., at 20°, under nitrogen.

	μM., glucose 6-phosphate
Complete	0.400
Without cytochrome *c* and DPN	0.015
Without mitochondria extract	0.115
Without hydroxylamine inhibiting factor	0.057
Without mitochondria extract and reductase	0.115
Complete	0.576
Without ITP	0.144
With $10^{-4}M$ dinitrophenol	0.150
With $10^{-4}M$ 8-hydroxyquinoline	0.132
Complete	0.298
With 0.04 *M* hydroxylamine	0.087
Without ITP	0.168
Complete, with 0.03 *M* fluoride	0.360
Same, with 10^{-4} *M* dinitrophenol	0.194

The present data show that phosphorylation depends on cytochrome *c* and ITP, and is inhibited as *in situ* by 2,4-dinitrophenol. Inhibition by 8-hydroxyquinoline and hydroxylamine relates our system to a metallo-flavoprotein.[5]

Although no measurements were made on cytochrome *c* reduction, present data suggest an ATP/-cytochrome *c* reduction ratio with a value above 1. The present enzymes seem to be responsible for two of the three phosphorylations occurring during DPNH oxidation in mitochondria.

FACULDADE DE MEDICINA DA UNIV. DE S. PAULO
DEPARTAMENTO DE QUIMICA FISIOLOGICA ISAIAS RAW
S. PAULO, BRAZIL

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(2) H. Edelhoch, O. Hayashi and L. O. Teply, *J. Biol. Chem.*, **197**, 97 (1952).

(3) ADP = adenosine diphosphate, ATP = adenosine triphosphate, DPN = diphosphopyridine nucleotide, DPNH = reduced diphosphopyridine nucleotide, ITP = inosine triphosphate, TRIS = tris (hydroxymethyl) aminomethane.

(4) A. Kornberg, *J. Biol. Chem.*, **167**, 805 (1950).

(5) D. E. Green, *Science*, **120**, 7 (1954).

THE PARTIAL CHARACTERIZATION OF A HISTAMINE METABOLITE FROM RAT AND MOUSE URINE

Sir:

Considerable work has been done during the past few years on the isolation and characterization of metabolites from rat and mouse urine after the injection of C^{14}-labeled histamine.[1]

The urine of rats which had been injected by Schayer with large amounts of low-isotopic histamine labeled with C^{14} in the 2-position of the imidazole ring was processed and several radioactive metabolites were isolated. One of the metabolites, isolated through the use of Dowex 50 columns with ammonium formate buffer, was crystalline in ethanol–ether mixtures, but on exposure to air it reverted to an oil. The hydrochloride was crystalline, m.p. 166–167°, $[\alpha]^{25}D$ −49.4° (methanol, c = 0.7%). Calcd. for $C_{10}H_{14}N_2O_6 \cdot HCl$: C, 40.75; H, 5.13; N, 9.51; Cl, 12.03. Found: C, 41.21; H. 5.46; N, 9.30; Cl, 11.99.

The analysis and hydrolytic data suggested a pentose-imidazoleacetic acid conjugate similar to that mentioned recently in unpublished work by Tabor.[2]

The conjugate is not hydrolyzed on heating at 100° for five hours in 12 *N* hydrochloric acid. It is readily hydrolyzed, however, in five hours in sealed tubes at 145° with 0.1 *N* acid. The hydrolyzed metabolite solution is applied directly to paper chromatograms.

Imidazoleacetic acid was shown to be present by comparative runs against a known sample, the developing spray being the Pauly reagent.[3]

In pyridine 65, water 35, the known and the hydrolysis solution gave spots with R_F values of 0.77 and 0.79, resp. In *n*-butanol 8, ethanol 2, water 2, both spots had an R_F of 0.18, and in *n*-butanol 4, ethanol 1,1, water 1.9 they both had an R_F of 0.39.

The sugar component was determined by spraying the paper chromatograms with 1% aniline oxalate in glacial acetic acid.[4]

The sugar was found to be ribose by R_F comparisons in three solvent mixtures: *n*-butanol 3, pyridine 2, water, 1.5 (R_F 0.57); ethyl acetate 2, pyridine 1, water 2 (upper phase), (R_F 0.49); and collidine-water (R_F 0.67).

The course of hydrolysis was followed by noting the increase in formation of imidazoleacetic acid and ribose on paper chromatograms, and by a decrease in concentration of the metabolite, the

(1) (a) R. W. Schayer, *J. Biol. Chem.*, **196**, 469 (1952); (b) A. H. Mehler, H. Tabor and H. Bauer, *ibid.*, **197**, 475 (1952); (c) H. Tabor, A. H. Mehler and R. W. Schayer, *ibid.*, **200**, 605 (1953); (d) L. P. Bouthillier and M. Goldner, *Arch. Biochem. Biophys.*, **44**, 251 (1953).

(2) H. Tabor, *Pharmacol. Rev.*, **6**, 299 (1954) (unpublished data of O. Hayaishi and H. Tabor).

(3) G. Hunter, *Biochem. J.*, **22**, 4 (1928).

(4) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).