appears reasonable that the mechanism whereby pyridine prevents complete substitution of $POCl_3$ to R_3PO involves an effective reduction in Grignard reagent concentration, probably by association with RMgX. The suggestion that phosphonic acids may be produced in better yield by using larger proportions of pyridine at lower temperatures[6] may be valid, but not because of blocking Cl atoms in $POCl_3$. Work has been started in this Laboratory to study quantitatively the effect of bases on Grignard reactions involving $POCl_3$ and related compounds.

The work with pyridine and α-picoline indicates that $POCl_3$ probably does not form quaternary salts with any pyridine bases. Actually, compounds have been isolated between $POCl_3$ and acids such as SO_3[17] and $SnCl_4$.[18] In light of the results obtained with pyridine bases, formula I, may be considered a more likely structure than formula II. An incidental result of the present work is that doubt is cast on the postulated reaction mechanism in a recent paper,[19] which requires quaternary compound formation between $POCl_3$ and a pyrazine derivative.

(17) G. Oddo, *Gazz. chim. ital.*, **57**, 29 (1927).
(18) S. Sugden and H. Wilkens *J. Chem. Soc.*, 1291 (1929).
(19) G. Karmas and P. E. Spoerri, THIS JOURNAL, **74**, 1580 (1952).

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COMMUNICATIONS TO THE EDITOR

THE NATURE OF THE XANTHINE OXIDASE FACTOR

Sir:

A factor(s) in liver residue and soy flour which increases the level of liver and intestinal xanthine oxidase when fed to weanling rats has been described by Westerfeld and Richert.[1,2,3,4] An excellent assay for this factor utilizing rat intestine also has been described by these authors.[5] However, the nature and mode of action of this substance(s) is unknown.

During the course of fractionation studies on liver residue it was found that the xanthine oxidase factor(s) could be partially liberated by autoclaving in water. The activity of the extracts so obtained was found to be dialyzable and stable to severe acid or alkaline treatment.

When liver residue or extracts from liver residue were ashed, the activity, surprisingly enough, was found to be unaltered. The inorganic material so obtained, when assayed spectrographically,[6] was found to contain many elements among which were Al, Sb, Ba, B, Cr, Co, Pb, Mo, Ni, Ag, Sn, Ti, V and Zn. The more "common" elements such as K, P, Na, Cu, Fe, Si, Mg and Mn were also present. In addition, the activity of liver residue or its ash could be replaced by including a supplement of Hoagland's A-Z solution[7] in the diet of rats. Further investigation with single salt supplements revealed that the ingestion of molybdate ion is responsible for the increased xanthine oxidase levels.

The addition of as little as 1 mg. of sodium molybdate/kg. diet or the injection of 10γ subcutaneously gave values for xanthine oxidase equal to that obtained when 10% liver residue was fed. Table I shows some typical data. Preliminary studies indicate that no other element is able to replace molybdenum and the highly specific nature of this effect is therefore apparent.

TABLE I

EFFECT OF LIVER RESIDUE, LIVER RESIDUE FRACTIONS AND MOLYBDATE ION ON RAT INTESTINAL XANTHINE OXIDASE VALUES

Supplement added to basal diet/kg.	Average X. O. value, c.mm.O_2 uptake/unit time/unit wgt. of intestine
None	4.4
10% liver residue (LR)	25.6
Liver residue extract (LRE) ≏16% LR	32.0
Ash of LR ≏ 10% LR	28.2
Ash of LRE ≏ 20% LR	26.9
Dialyzed LRE ≏ 20% LR	5.1
Sodium molybdate, 1 mg.	23.5

To our knowledge, this represents the first report suggesting an *in vivo* role for molybdate in an animal enzyme system. The possible importance of this finding on the role of molybdenum in animal nutrition is of course obvious. Studies are now in progress to elucidate the precise role of molybdenum on the activity of xanthine oxidase.

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RECEIVED JANUARY 21, 1953

(1) W. W. Westerfeld and D. A. Richert, *J. Biol. Chem.*, **184**, 163 (1950).
(2) W. W. Westerfeld and D. A. Richert, *Science*, **109**, 68 (1949).
(3) W. W. Westerfeld and D. A. Richert, *Proc. Soc. Exp. Biol. Med.*, **71**, 181 (1949).
(4) W. W. Westerfeld and D. A. Richert, *J. Biol. Chem.*, **192**, 35 (1951).
(5) D. A. Richert and W. W. Westerfeld, *ibid.*, **192**, 49 (1951).
(6) We are indebted to Mr. W. L. Dutton and his staff of the Stamford Research Laboratories, American Cyanamid Co., for the spectrographic analysis.
(7) D. R. Hoagland and W. C. Snyder, *Proc. Amer. Soc. Hort. Sci.*, **30**, 288 (1933).

MOLECULAR DIMENSIONS OF CELLULOSE DERIVATIVES[1]

Sir:

Although it has been widely believed that the molecules of cellulose derivatives are considerably more extended than typical vinyl polymers, the direct evidence supporting this is principally that of early light scattering measurements,[2] the accuracy of which might be in doubt due to the colloidal contaminants which are difficult to remove. We report here current work on the angular distribution of scattered light from sodium carboxymethyl cellulose (unfractionated, 1.15 acid groups per glucose), NaCMC, and cellulose trinitrate (fractionated), CN, which define more precisely the extent of coiling in these derivatives and demonstrate the inapplicability of the Flory–Fox[3] relation to cellulose derivatives of low and moderate molecular weight.

The customary interpretation of Zimm plots of the scattering data for NaCMC showed its weight average molecular weight to be 173,000 and its z-average end-to-end length to be 2410, 2590 and 3100 Å. in 0.5, 0.05 and 0.01 N NaCl solutions respectively. The corresponding intrinsic viscosities were 7.03, 10.1 and 15.9. These sizes are to be compared with the contour length which is estimated to be 5280 Å. on the assumption that the z-average molecular weight is 1.5 times the weight average one. Hence the mean length of these chains is about half their contour length. Since the Gaussian distribution of segments is no longer a good approximation for chains with valence angle near 110° when the mean length is more than one-third the contour length,[4] we are clearly dealing with non-Gaussian chains.

This fact has implications in both light scattering and intrinsic viscosity interpretations. In the former it means that the radius of gyration and not the mean length should be evaluated from the data since it is only the radius that can unambiguously be determined. The mean length is equal to $\sqrt{6}$ times the radius for Gaussian coils but the proportionality constant increases to $\sqrt{12}$ as a rod-like configuration is approached. Consequently, the sizes listed above are the radii of gyration divided by $\sqrt{6}$. It is of interest to note that the reciprocal intensity *versus* $\sin^2 \theta/2$ plot has a pronounced downward curvature as would be expected for chains deviating from Gaussian behavior. Consequently, the use of dissymmetry measurements leads to dimensions that are too small.

With respect to intrinsic viscosity, it is clear that non-Gaussian chains of this type cannot possibly satisfy the premise of the Flory–Fox relation, that is that the effective hydrodynamic volume of the polymer molecule is spherical and can be characterized by a radius that varies directly with a linear parameter of the Gaussian distribution. This is borne out by the constant Φ of the Flory–Fox relation calculated from the mean lengths and intrinsic viscosities given above and the z-average molecular weight. It is found to have values of about 0.15×10^{21} in contrast to 2.1×10^{21} for Gaussian coiled polymers. The use of the latter value implies that the unusually large temperature dependence of the viscosity observed in cellulose derivatives results solely from changes in dimensions. When the chains are non-Gaussian, however, the temperature dependence of Φ tends to mask these dimensional changes. Consequently, the dimensions of cellulose derivatives cannot be determined from viscosity measurements except within the region of high molecular weight or high temperature where the configurations are Gaussian. The neglect of these considerations has led to the erroneous conclusion that cellulose derivatives at ordinary temperatures are as flexible as typical vinyl polymers.[5]

As an example of Gaussian behavior possible in cellulose derivatives of high molecular weight, we report measurements on one CN fraction in acetone which was found to have a molecular weight of 3.9 million and a mean length of 4070 Å. The ratio of this to the contour length is 1:16.7. Assuming that the mean end-to-end length is proportional to the molecular weight, we find that the transition from Gaussian to non-Gaussian character occurs at a molecular weight of about 150,000 for CN. Of course, the transition occurs at a higher molecular weight for NaCMC since it is somewhat more extended. In the CN fraction mentioned the intrinsic viscosity calculated from the Flory–Fox relation is 35.5, which compares favorably with 31.5 obtained from extrapolating measured values to zero gradient.

(1) This work was carried out under ONR Contract N5ori-07654, T. O. 54, NR-330-025.

(2) (a) R. S. Stein and P. M. Doty, THIS JOURNAL, **68**, 159 (1946); (b) R. M. Badger and R. H. Blaker, *J. Phys. Colloid Chem.*, **53**, 1051 (1949).

(3) P. J. Flory and T. G. Fox, THIS JOURNAL, **73**, 1904 (1951).

(4) L. R. G. Treloar, *Proc. Phys. Soc.*, **55**, 345 (1943).

(5) L. Mandelkern and P. J. Flory, THIS JOURNAL, **74**, 2522 (1952); see also, S. Neuman and P. J. Flory, *J. Polymer Sci.*, **10**, 121 (1953).

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RECEIVED DECEMBER 31, 1952

TRACER STUDIES ON THE FORMATION OF SULFUR FROM HYDROGEN SULFIDE AND SULFUR DIOXIDE IN AQUEOUS SOLUTIONS

Sir:

The reaction of hydrogen sulfide and sulfur dioxide in water leads almost quantitatively to sulfur if H_2S is present in sufficient excess[1,2]

$$2H_2S + SO_2 \longrightarrow 3S + 2H_2O$$

If sulfur dioxide is used in excess elementary sulfur is formed together with a number of sulfur compounds in solution, mainly polythionic acids (Wackenroder liquid). The composition of this mixture varies strongly with the experimental procedure.[3]

We have investigated the origin of the elementary sulfur using hydrogen sulfide and sulfur dioxide, alternatively labeled with S^{35}, and comparing the specific activity of the sulfur obtained with that

(1) E. H. Riesenfeld and G. W. Feld, *Z. anorg. allgem. Chem.*, **119**, 225 (1921).

(2) O. von Deines and H. Grassmann, *ibid.*, **220**, 337 (1934).

(3) For a recent review, see: Margot Goehring, "Fortschritte der Chemischen Forschung," Band 2, Heft 3, Springer Verlag, Heidelberg, 1952.

of the labeled reaction partner. These activities were measured on samples of barium sulfate, prepared from S, H_2S and SO_2 by oxidation, and were corrected for self-absorption.[4] The results are given in Table I.

TABLE I

Reactant labeled with S^{35}	Reactant used in excess	Ratio (as %) of specific activities of elementary sulfur and of labeled react. partn.
H_2S	H_2S	61, 64
SO_2	H_2S	34, 32, 34
SO_2	SO_2	31, 28
H_2S	SO_2	71, 70, 67, 66 68, 74, 74, 70

The conditions in each series of experiments have been varied widely; reactants were sometimes added as solutions, sometimes in gaseous form, either undiluted, or diluted with nitrogen. Occasionally an excess of 0.1 *N* HCl was present, in which case the reactants were added as solutions of their sodium salts.

Lanthanum nitrate was added, sometimes before, sometimes after the reactants were mixed, to precipitate the sulfur. In most cases the elementary sulfur was filtered off within two hours but occasionally it was left in the solution overnight. In all experiments the mixing of the reactants was performed in less than one hour, in order to reduce the effect of reactions between the added compound and the reaction products, like polythionic acids.

Our results indicate that under all circumstances the main portion of the elementary sulfur originates from H_2S and SO_2 in the ratio 2:1. This conclusion is in agreement with all theories on the subject, in which the partial reaction responsible for the sulfur formation does not simultaneously produce other sulfur compounds.[1,5,6,9]

There is also formal agreement with the mechanism proposed by Zil'berman,[7] but only if the primary reaction is supposed to be really termolecular—as it is written by him—which is highly improbable. The reaction-mechanism of von Deines and Grassmann[2] could be made to agree with our results by introduction of several suppositions not explicitly made by these authors. Our observations exclude the mechanism proposed by Heinze.[8]

Another conclusion which may be derived from our results is that no appreciable exchange of sulfur between H_2S and SO_2 occurs within the time required for the formation of sulfur. The absence of such an exchange rules out the rapid formation of an intermediate compound in equilibrium with both reactants, unless the former compound contains sulfur atoms in positions which are not equivalent.

We thank the Foundation for Fundamental Research of Matter (F.O.M.) and the Netherlands Organization for Pure Research (Z.W.O.) for their support.

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RECEIVED JANUARY 5, 1953

(4) A. H. W. Aten, Jr., *Nucleonics*, **6**, No. 1, 68 (1950).
(5) F. Foerster and A. Hornig, *Z. anorg. allgem. Chem.*, **125**, 86 (1922).
(6) H. Stamm and M. Goehring, *cf.* the review by the latter, *loc. cit.*
(7) Ya. I. Zil'berman, *J. Gen. Chem.* (*U.S.S.R.*), **10**, 1257 (1940).
(8) E. Heinze, *J. Prakt. Chem.*, **99**, 109 (1919).
(9) H. Basset and H. G. Durrant, *J. Chem. Soc.* (London), 1401 (1927).

β-RAY INITIATION OF POLYMERIZATION OF STYRENE AND METHYL METHACRYLATE

Sir:

The effect of β-radiation as an initiator of polymerization of pure styrene, pure methyl methacrylate and an equimolar mixture of these monomers has been studied.

The source of β-particles was an equilibrium mixture of anhydrous $Sr^{90}Cl_2$ and $Y^{90}Cl_3$ (half life of 19.9 years) giving a spectrum of electrons with a maximum energy of 2.24 Mev. The source was placed in a small glass bulb of about 2 mm. diameter. It was held by a rigid stem at the center of the spherical end of a small distilling flask which had a capacity of 8 cc. and which was filled with the monomer to be studied. The total energy of the particles entering the monomers was $5.0 \times 10^{-6} \pm 0.5 \times 10^{-6}$ cal./sec. and was completely absorbed by the surrounding monomer. The range of the 2.24 Mev. β-particles is *ca.* 11.5 mm. (depending upon the density of the material); the inner radius of our monomer-containing bulb was 12.4 mm.

The rates of β-ray induced polymerization of styrene, methyl methacrylate and the equimolar mixture were all measured at 30.5° in the vessels described above. Control experiments were made under identical conditions to measure the purely thermal rate. The results are shown in Table I.

TABLE I

POLYMERIZATION AT 30.5°
% CONVERSION PER HOUR

β-Ray induced	Monomer	Thermal
0.019	Styrene (S)	0.007
.26	Methyl methacrylate (MMA)	.11[a]
.039	Equimolar mixture (S) and (MMA)	.016

[a] This rate is somewhat high due probably to adventitious peroxides remaining after our purification procedure.

Each result given in Table I is the average of at least three runs—the maximum deviation being approximately 10% in the case of the β-ray induced polymerization. Air was found to be a powerful inhibitor of the β-ray induced polymerization and the thermal polymerization.

The composition of the copolymers was determined by C–H analysis and calculation of the % methyl methacrylate. The result of the determination[1] gave a methyl methacrylate content in the copolymer of 50.2% ± 4.0.

Walling, *et al.*,[2] have shown that an equimolar solution of styrene and methyl methacrylate gives a copolymer containing 49% methyl methacrylate

(1) Clark Microanalytical Laboratory, Urbana, Ill., and Joseph F. Alicino, Metuchen, N. J.
(2) C. Walling, E. R. Briggs, W. Cummings and F. R. Mayo, THIS JOURNAL, **72**, 48 (1950).

and 51% styrene when initiated by typical free radical catalysts such as benzoyl peroxide at 60°; for typical cationic initiators such as $SnCl_4$ a copolymer containing more than 99% styrene is obtained; for typical anionic initiators the copolymer contains more than 99% methyl methacrylate. Thus, the composition of the copolymer formed from β-ray initiation indicates that a free radical mechanism is operative.

By polymerizing pure styrene at 30° with various concentrations of 2-azobisisobutyronitrile, we have shown that the monoradical line[3] at 30° is given by: $1/D.P. = 2.0 \times 10^{-5} + 69.2\ R_p$ (R_p = rate of polymerization in moles liters^{-1} sec.$^{-1}$). For the β-ray induced polymerization the value of R_p is 4.16×10^{-7} (see Table I) and the $D.P.$ is 1.07×10^4. This point falls on the monoradical line, which constitutes another proof of the free radical mechanism.

The rate of initiation of polymer chains for the β-ray induced polymerization can be computed from R_p and the slope of the monoradical line.[2] It is equal to 2.40×10^{-11} mole liter^{-1} sec.$^{-1}$ A similar calculation was made for methyl methacrylate.

If the β-ray induced polymerization, which we have proved to proceed via radicals, occurs homogeneously throughout the medium, and if the energy to produce the initiating radicals be estimated at 50 kcal./mole, we compute that 0.19% of the absorbed radiant energy is effective in producing initiating radicals in the case of styrene and 2.3% of the energy is effective in producing initiating radicals in methyl methacrylate.

(3) D. H. Johnson and A. V. Tobolsky, THIS JOURNAL, **74**, 938 (1952).

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RECEIVED DECEMBER 18, 1952

THE REACTION OF HYDRAZINE AND SYMMETRICAL DIMETHYLHYDRAZINE WITH DIBORANE

Sirs:

The reaction of diborane with hydrazine and with symmetrical dimethylhydrazine in ethereal solution at −80° produces white, crystalline, somewhat ether-soluble 1:1 adducts of the formula $H_2BNHRNHRBH_3$ in which R is either hydrogen or a methyl radical.[1] These formulas are based on the stoichiometry of the reagents and on the fact that hydrolysis of the adducts regenerates the original hydrazine. Furthermore, pyrolysis of these compounds generates one mole of hydrogen per gram atomic weight of nitrogen, in analogy with the pyrolysis of dimethylamine borine, $(CH_3)_2HNBH_3$. Both hydrazine adducts continue to generate hydrogen at a greatly reduced rate after the initial loss. The solid product of pyrolysis of the unsubstituted hydrazine-diborane adduct at 130° was not isolated; the pyrolysis of the dimethylhydrazine adduct at 69° yielded hydrogen, a slightly volatile liquid, discussed below, and small amounts of free dimethylhydrazine and diborane. These facts suggest that decomposition of the adduct is accompanied by slight dissociation.

The hydrazine–diborane adduct was analyzed by hydrolysis, measurement of the evolved hydrogen and subsequent titration of boric acid and hydrazine.[2] *Anal.* Sample weight 0.0780. Calcd. for $N_2H_4 \cdot B_2H_6$: B, 36.15; N_2H_4, 53.59; H, 10.12. Found: B, 36.28; N_2H_4, 53.59; H, 10.12. Similar analysis of the symmetrical dimethylhydrazine adduct gave a B:H ratio of 1:3, and a qualitative identification of symmetrical dimethylhydrazine as its hydrochloride. Quantitative determination of symmetrical dimethylhydrazine awaits development of a satisfactory method of analysis.

Trimethylamine displaces hydrazine from an ethereal solution of its diborane adduct forming trimethylamine borine, $(CH_3)_3NBH_3$. The adduct does not react with either excess diborane or excess hydrazine but is slightly soluble in the latter reagent.

A purified sample of the less volatile liquid obtained in the pyrolysis of the symmetrical dimethylhydrazine–diborane adduct had a melting point of about 0.4° and a molecular weight of 82 (83.8 calculated for $N_2(CH_3)_2 \cdot 2BH_3$) as determined by vapor density measurements at 38.4° and 45.9°. Vapor tensions observed at various temperatures and those calculated by the equation $\log_{10} P_{mm} = 7.8005 - (2027/T)$ were as follows:

T, °K.	273.2	282.5	286.0	291.5	303.6
P_{obs}	2.35	4.25	5.05	7.55	12.75
P_{calc}	2.44	4.22	5.16	7.03	13.30

The preceding facts suggest the structural formula $H_2BN(CH_3)N(CH_3)BH_2$ for the liquid decomposition product. At approximately 60° the liquid slowly produces what appears to be a solid polymer, since the change occurs without the generation of hydrogen, nitrogen or methane.

Data obtained from the reactions of hydrazine and its derivatives with trimethyl boron and boron trichloride as well as the reaction of ethylene diamine with diborane will be presented at a later date.

(1) The present investigation was begun before the article by H. J. Emeléus and F. G. A. Stone (*J. Chem. Soc.*, 840 (1951)) on the reaction of diborane with hydrazine had appeared. Their failure to obtain definite results was probably due to the facts that they used no solvent, and did not recrystallise their product.

(2) I. M. Kolthoff, THIS JOURNAL, **46**, 2009 (1924).

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RECEIVED JANUARY 21, 1953

L-HISTIDINE CONVERSION TO A URINARY GLUTAMIC ACID DERIVATIVE IN FOLIC-DEFICIENT RATS

Sir:

The previously reported[1] derivative of glutamic acid, excreted in the urine of folic-deficient rats, has been crystallized as a barium salt, containing one equivalent of glutamic and formic acids and ammonia.[2] A similar barium salt has been crys-

(1) (a) H. A. Bakerman, M. Silverman and F. S. Daft, *J. Biol. Chem.*, **188**, 117 (1950); (b) M. Silverman, R. C. Gardiner and H. A. Bakerman, *ibid.*, **194**, 815 (1952).

(2) M. Silverman, unpublished data.

tallized after the degradation of histidine by liver extracts[3]; both salts are converted to L-glutamic acid by *Pseudomonas fluorescens* extracts, which degrade L-histidine to L-glutamic and formic acids and ammonia.[4] The relationship of the excreted glutamic derivative to L-histidine metabolism was strengthened by the observation of a large increase in the excretion of bound (heat-labile) glutamic acid when histidine was added to the diet of folic-deficient rats.[5] The assay used[1,5] usually failed to detect any glutamic derivative in the urine of normal rats, even when histidine was added to the diet.[5]

To establish the origin of the glutamic acid derivative, L-histidine, labeled with N^{15} in the γ position,[6] was fed to five folic-deficient rats.[7] The barium salt of the glutamic derivative was crystallized from the pooled urines after chromatography on Dowex 50 and Dowex 1.

TABLE I

	Millimoles	Atom % excess N^{15}[a]
L-Histidine fed[b]	10.6	1.61 (in 3 N atoms)
Glutamic derivative excreted	4.6	1.37 (in 2 N atoms)

[a] We are indebted to Dr. Julius White for the N^{15} analyses. [b] Including the histidine of the dietary casein.

If the dietary N^{15} histidine were not diluted by body histidine, the N^{15} content of the glutamic derivative would have been 2.4 atom per cent. excess. The observed value of 1.37 therefore indicates that approximately 55% of this glutamic derivative excreted was derived from the dietary N^{15}-histidine. Crystalline L-glutamic acid ($[\alpha]^{20}D$ = 30.4° in 6 *N* HCl), isolated after hydrolysis of the barium salt with *Pseudomonas* extract, was found to contain essentially all of its N^{15} (2.5 atom % excess).

The major pathway of histidine degradation in both liver homogenates[8,9,10] and *Pseudomonas* extracts[11] has been shown to proceed via urocanic acid, rather than by a primary rupture of the imidazole ring[12]; the γ (rather than the α) nitrogen of the histidine persists in the glutamic acid ultimately found. The use of folic deficient rats has permitted demonstration of this pathway *in vivo*, as the glutamic acid moiety of the compound excreted contains the isotope of the nitrogen of the administered histidine. Although glutamic derivatives, obtained by incubating histidine with liver preparations, have been assigned various structures by other workers,[9,10,12,13] our synthetic and degradative studies do not yet permit an unequivocal structure to be written for our barium salts. The role of folic acid in the metabolism of histidine, and the relationship of the intermediates of histidine metabolism to one-carbon metabolism[14] remain to be determined.

(3) A. Mehler and H. Tabor, unpublished.

(4) H. Tabor and O. Hayaishi, *J. Biol. Chem.*, **194**, 171 (1952).

(5) F. S. Daft, M. Silverman, H. Tabor and A. Mehler, in preparation.

(6) Synthesized essentially as described by C. Tesar and D. Rittenberg, *J. Biol. Chem.*, **170**, 35 (1947).

(7) The folic-deficient (succinylsulfathiazole-containing) diet was that previously used (1, 2, 5).

(8) A. Mehler and H. Tabor, *J. Biol. Chem.*, in press.

(9) K. Sera and D. Aihara, *J. Osaka Med. Soc.*, **41**, 745 (1942).

(10) Y. Oyamada, *J. Biochem. (Japan)*, **36**, 227 (1944).

(11) H. Tabor, A. H. Mehler, O. Hayaishi and J. White, *J. Biol. Chem.*, **196**, 121 (1952).

(12) S. Edlbacher, *Ergeb. Enzymforsch.*, **9**, 131 (1943).

(13) A. Abrams and H. Borsook, *J. Biol. Chem.*, **198**, 205 (1952).

(14) The metabolism of the 2C of histidine has been reviewed recently by M. Toporek, L. Miller and W. F. Bale, *ibid.*, **198**, 839 (1952).

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RECEIVED NOVEMBER 22, 1952

FACTORS AFFECTING MOLECULAR WEIGHT OF ENZYMATICALLY SYNTHESIZED DEXTRAN

Sir:

Dextrans, glucose polymers in which the α-1,6-glucopyranosidic linkage predominates[1,2] generally have molecular weights of 6 to 100 million when produced by conventional fermentation procedures.[3] Polymers in the same molecular weight range are produced in reaction mixtures containing initially 10% sucrose and dextransucrase,[3,4,5] the dextran-synthesizing enzyme. To be suitable as a blood plasma substitute, such dextran must be degraded to a molecular weight of *ca.* 75,000.[6,7] By variation of reaction conditions, part of the enzymatically synthesized dextran was obtained having a molecular weight of 400,000 or less. Dextransucrase used in our investigations was derived from *Leuconostoc mesenteroides* NRRL B-512.[8] Average molecular weights were determined either by ultracentrifugal or light scattering measurements.

As stated above, dextran with a high molecular weight is synthesized in reaction mixtures containing initially 10% sucrose. However, low molecular weight polysaccharide of *ca.* 8000 was synthesized in 70% sucrose reaction mixtures. The molecular weight distribution at intermediate sucrose levels was bimodal, a portion distributed about 40 million and the other about a varying molecular weight below 30,000. This effect was due at least partially to the influence of accumulated fructose on the course of the polymerization.

Reaction mixtures containing enzyme, sucrose, and certain glucosyl acceptors such as fructose or maltose yield oligosaccharides and low molecular weight dextran, as well as high molecular weight polymer.[9] The average molecular weight of the former was raised to its maximum of 35,000 by the

(1) T. H. Evans and H. Hibbert, "Advances in Carbohydrate Chem.," Academic Press, Inc., New York, N. Y., **2**, 203 (1946).

(2) Allene Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950).

(3) F. R. Senti and N. N. Hellman, Amer. Chem. Soc., Abstract of Papers for 121st Meeting, 8O, (1952).

(4) E. J. Hehre, *Science*, **93**, 237 (1941).

(5) E. J. Hehre, "Advances in Enzymology," Interscience Publishers, Inc., New York, N. Y., **11**, 297 (1951).

(6) Military Medical Purchase Description, No. 1, May 24, 1951, Armed Services Medical Procurement Agency, Brooklyn 1, New York.

(7) E. J. Pulaski, *Chem. Eng. News*, **30**, 2187 (1952).

(8) H. M. Tsuchiya, H. J. Koepsell, J. Corman, G. Bryant, M. O. Bogard, V. H. Feger and R. W. Jackson, *J. Bacteriology*, **64**, 521 (1952).

(9) H. J. Koepsell, H. M. Tsuchiya, N. N. Hellman, A. Kazenko, C. A. Hoffman, E. S. Sharpe and R. W. Jackson, *J. Biol. Chem.*, in press.

slow addition of sucrose to mixtures of enzyme and maltose, under the experimental conditions employed. The glucosyl acceptors affect molecular weight of the product presumably by either initiating or terminating chains.

When solutions containing dextransucrase and dextran with molecular weight of *ca.* 5000, as glucosyl acceptor, were placed in dialysis membrane sacks and the sacks immersed in sucrose solutions, the resulting polymers displayed a bimodal distribution of molecular weights. The low molecular weight polysaccharides synthesized ranged from 39,000 to 325,000, the lower molecular weights occurring with higher amounts of added low molecular weight polymer. From a reaction mixture containing 40,000 dextransucrase units,[10] 2000 mg. of added low molecular weight dextran, and 40 g. of sucrose, dextran (32.8% of theoretical), molecular weight 81,400, was obtained. A comparison of ultracentrifugal sedimentation diagrams of this sample with a commercial sample of clinical dextran revealed that the former had the narrower molecular weight distribution. The latter sample, prepared by degradation of high molecular weight polymer, had been fractionated so as to meet stringent clinical specifications. The direct synthesis of dextran with molecular weight in this range is significant because of its possible utility in production of a blood plasma substitute.

A detailed account of our experimental findings will appear later.

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(10) H. J. Koepsell and H. M. Tsuchiya, *J. Bacteriology*, **63**, 293 (1952).

(11) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

MECHANISM OF ACTION OF OXALACETIC CARBOXYLASE FROM LIVER[1]

Sir:

A study of partially purified oxalacetic carboxylase obtained from chicken liver leads us to propose the following mechanism of action for this enzyme

$$\text{Oxalacetate} + \text{ATP or ITP} \rightleftharpoons \text{Phospho-enol-pyruvate} + HCO_3^- + \text{ADP or IDP}$$

In studies to be published elsewhere it has been shown that oxalacetic carboxylase can be completely separated from "malic" enzyme[2] and the carboxylase used in the present experiments was entirely devoid of the "malic" carboxylase. The purified oxalacetic carboxylase catalyzes the decarboxylation of oxalacetate in a *p*H range of 5.8–7.8 in the presence of ATP or ITP. The enzyme catalyzes an exchange reaction between $NaHC^{14}O_3$ and oxalacetate[3] in the presence of the same cofactors and in the present experiments it also has been shown that a net synthesis of oxalacetate can be accomplished. Starting with 4 μM. of phosphopyruvate, 2.5 μM. of ADP, 50 μM. of $NaHCO_3$ and 2 μM. of $MnCl_2$ in a volume of 1.0 ml., 0.18 μM. of oxalacetate was formed in 5 minutes at 30° in an atmosphere of CO_2–N_2. When the reaction is displaced toward synthesis by the removal of ATP *via* the hexokinase reaction, 0.38 μM. of oxalacetate is formed. Replacement of the ADP by ITP in the presence of hexokinase increases the synthesis to 0.54 μM. By increasing the reaction time and the concentration of the reactants, 2–3 μM. of oxalacetate can be formed from 6 μM. of phosphopyruvate. The oxalacetate has been identified by its decarboxylative properties, by the chromatographic behavior of its 2,4-dinitrophenylhydrazone and by recrystallizing the hydrazone of oxalacetate formed from $NaHC^{14}O_3$ in the presence of a known amount of carrier hydrazone to constant specific activity.

The decarboxylation of oxalacetate in the presence of ATP or ITP leads to the formation of phosphopyruvate as shown in the following experiment in which 40 μM. oxalacetate, 2 μM. of ITP, and 2 μM. of $MnCl_2$, were incubated with the carboxylase at *p*H 6.0 in succinate buffer at 30° for 20 minutes.

	CO_2 produced (above control), μM.	9 min. acid-labile P decrease, μM.	Phosphopyruvate formed, μM.
No NaF	1.65	1.44	0.89
0.02 M NaF	1.51	1.42	1.13

The addition of NaF increases the amount of phosphopyruvate formed by inhibiting enolase which contaminates the carboxylase to some extent. Pyruvic acid cannot be substituted for oxalacetate in the formation of phosphopyruvate. The latter compound has been identified by its chromatographic behavior and by its reactivity with the purified enzyme, pyruvic phosphokinase.[4]

The formation of phosphopyruvate from a dicarboxylic acid may explain the results of Kalckar,[5] who reported the accumulation of this ester during the oxidation of malate by kidney preparation. Recent work by Shreeve[6] on the synthesis of glycogen from 2-C^{14}-pyruvate in liver slices also suggests that phosphopyruvate may be formed from a pathway involving dicarboxylic acids.

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(2) S. Ochoa, A. H. Mehler and A. Kornberg, *J. Biol. Chem.*, **174**, 979 (1948).

(3) M. F. Utter, *ibid.*, **188**, 847 (1951).

(4) A. Kornberg and W. E. Pricer, Jr., *ibid.*, **193**, 481 (1951).

(5) H. M. Kalckar, *Biochem. J.*, **33**, 631 (1939).

(6) W. W. Shreeve, *J. Biol. Chem.*, **195**, 1 (1952).