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.

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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

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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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(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

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

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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