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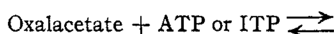
(10) H. J. Koepsell and H. M. Tsuchiya, *J. Bacteriology*, **63**, 293 (1952).

(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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