reported from several laboratories.[1] These "oxygenases"[2] are generally concerned with the transformation (hydroxylation, ring cleavage, cyclization) of various ring compounds such as aromatic amino acids, phenols and steroids. In this communication we wish to report a novel type of decarboxylation reaction in which atmospheric oxygen is incorporated into a simple aliphatic compound.

L-Lactate was incubated with a crystalline lactic oxidative decarboxylase prepared from *Mycobacterium phlei*[3] in an atmosphere of O_2^{18} and with H_2O^{16} as a medium. Potassium acetate isolated from the incubation mixture was found to have incorporated approximately one atom of atmospheric oxygen (Table I). The O^{18} enrichment of the incorporated oxygen atom corresponded to approximately 82% of that of the atmospheric oxygen used in this experiment, assuming only one atom was inserted into the product. On the other hand, when the reaction was carried out in the medium of H_2O^{18} and with O_2^{16} as a gas phase, potassium acetate isolated did not contain appreciable O^{18}. Carbon dioxide did not contain O^{18} in the first case, but was highly enriched in the second experiment. This incorporation of O^{18} from H_2O^{18} into carbon dioxide probably was caused by a non-enzymatic exchange reaction as reported by Cohn and Urey[4] and more recently by Rothberg and Steinberg in their studies of various microbial decarboxylases.[5]

TABLE I

ENZYMATIC INCORPORATION OF O^{18} INTO ACETATE

Experiment I: Crystalline lactic oxidative decarboxylase[3] (3,350 units), 2 millimoles of DL-lithium lactate and 2 millimoles of potassium phosphate buffer (*p*H 6.0) were incubated in a total volume of 50 ml. of water in a special flask designed for this type of experiment.[a] An O_2^{18}-helium gas mixture (2:3) was used as a gas phase.[b] Experiment II: The same reaction components as in Experiment I were employed except that H_2O^{18} was used as a solvent and O_2^{16}-helium gas mixture (2:3) was used as a gas phase. The incubation was carried out at 37° for 30 minutes with vigorous shaking. After the oxygen and carbon dioxide were removed for mass spectrometric analyses, the incubation mixture was immediately chilled at 4° and *p*H was adjusted to 3.5 with several drops of 2*N* H_2SO_4. The solution was then frozen and water and acetic acid were distilled from the frozen state. The distillate was neutralized with 1*N* KOH to *p*H 6.5 and water was removed by lyophilization. The residue dried over P_2O_5 was pyrolyzed[c] and the O^{18} content determined with a mass spectrometer.[d]

Expt.	Type	Atom % excess	Cpd. analyzed	Atom % excess found
I	O_2^{18}	6.31868	KOAc	2.59578
			CO_2	0.00452
II	H_2O^{18}	1.3380	KOAc	0.01772
			CO_2	1.32422

[a] Details will be published elsewhere. See also 1b. [b] Highly enriched O_2^{18} gas was prepared by electrolysis of approximately 33% enriched H_2O^{18} purchased from the Weizmann Institute of Science, Israel. We are indebted to Dr. Y. Saito and Mr. L. Wartofsky for the preparation of O_2^{18} gas. [c] The mass spectrometric analyses were carried out in collaboration with Mr. W. E. Comstock of this Institute. [d] Pyrolysis was carried out at 400° for 1 hour with $HgCl_2$ as a catalyst according to D. Rittenberg and L. Ponticorvo (*Intern. J. Appl. Radiation and Isotopes*, **1**, 208 (1956)).

The enzyme which catalyzes "oxidative" decarboxylation of L-lactate has been described from various microörganisms.[6] The over-all reaction involves oxidation and decarboxylation of L-lactate to form stoichiometric quantities of acetate and carbon dioxide, but the mechanism of this unique enzymatic reaction has not yet been completely understood.

The available evidence suggests that at least one atom of atmospheric oxygen was incorporated into acetate when the C_1–C_2 bond of lactate was cleaved and that the other atom of the oxygen molecule probably was reduced to water

$$CH_3—CHOH—COOH + O_2^{18} \longrightarrow CH_3COO^{18}H + CO_2 + H_2O^{18}$$

The mechanism of this reaction may be analogous to that of the so-called "mixed function oxygenase"[1c,f] except that instead of reduced pyridine nucleotides, the substrate itself is providing electrons to reduce one atom of oxygen.

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POLYNUCLEOTIDE PHOSPHORYLASE IN LIVER NUCLEI

Sir:

Polynucleotide phosphorylase has been described in bacteria.[1] It catalyzes the reversible synthesis of polynucleotides from nucleoside diphosphates as well as the phosphorolysis of isolated ribonucleic acids. Thus, adenylic polynucleotide (Poly A) reacts with orthophosphate (P_i) to form adenosine diphosphate (ADP)

$$\text{Poly A} + n\text{P}_i \rightleftharpoons n\text{ADP}$$

An enzyme has now been fractionated from guinea pig liver nuclei which converted up to 30% of the adenylic acid units of Poly A to ADP (Table I). Part of the ADP then formed ATP and AMP due to the presence of adenylate kinase. The rest of the polymer was utilized by a competing hydrolytic enzyme, shown to occur in liver nuclei.[2] ADP was identified by its R_f in four solvent systems, electro-

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phoretic mobility[3] and by enzymatic assay with the pyruvic kinase system.[4] Both ADP and ATP had the expected specific activity, based on that of P^{32}-labeled P_i used in the incubation. No ADP was formed in the absence of P_i. Inorganic pyrophosphate was not required, and in fact was rapidly hydrolyzed to P_i by the liver fraction.

AMP was not an intermediate in the formation of ADP from Poly A. Thus, with 50 μg. of enzyme and conditions similar to those of Table I, these quantities of P^{32}-labeled P_i were esterified (in μmoles): (1) 0.035 with Poly A equivalent to 0.4 μmole of adenine, in 3 hours; (2) 0.000 with 0.5 μmole of AMP, in 3 hours; (3) 0.000 with 0.1 μmole of AMP and 0.02 μmole of ADP, in 11 hours.

TABLE I

PHOSPHOROLYSIS OF ADENYLIC POLYNUCLEOTIDE

A mixture of 1.0 mg. of liver fraction, 19.3 μmoles of PO_4^{---} buffer (*p*H 7.2, 95,000 c.p.m. of P^{32} per μmole), Poly A[a] equivalent to 6.8 μmoles adenine and 9 μmoles of $MgCl_2$, all in 0.9 ml., was incubated at 37° for 12 hours. Nucleotides were adsorbed by norite in the presence of perchloric acid and the washed suspension was eluted with an ethanol–NH_4OH–water mixture. Aliquots of the eluate were assayed enzymatically for ADP, counted for P^{32}, and chromatographed on paper for quantitative separation of AMP, ADP and ATP.

Reaction product	Amount μmoles	Specific activity c.p.m. per μmole
AMP[b]	0.80	110
ADP	0.85[c]	80,000
ATP[b]	0.45	165,000
Total esterified P_i	1.75[d]	

[a] Synthesized from ADP, using polynucleotide phosphorylase from *A. vinelandii* (1a). [b] ATP and part of the AMP were formed by adenylate kinase. The remaining AMP was formed from Poly A by a nuclease. [c] From absorption at 260 mμ after chromatographic separation; 0.85 μmole of ADP was also found by enzymatic assay.[4] [d] From sum of ADP and 2 × ATP. Total esterified P_i determined by direct count of the washed norite suspension was 1.9 μmoles.

The liver enzyme had a *p*H optimum at about *p*H 7, required Mg^{++} and was inhibited completely by 0.06 *M* fluoride. K_m was $3 \times 10^{-3} M$ for P_i in the phosphorolysis reaction. The enzyme also catalyzed the exchange of 0.5 μmole of P^{32}-labeled P_i with ADP per hour per mg. of protein.[5] For this reaction K_m was $1.5 \times 10^{-3} M$ for Mg^{++}, $2 \times 10^{-4} M$ for ADP and $3 \times 10^{-3} M$ for P_i. An exchange of P_i with ATP was also noted, so far unexplained. Experiments with other nucleoside diphosphates must await removal of an interfering phosphatase (inactive with ADP).

Although phosphorolysis of adenylic polynucleotide to give ADP has been demonstrated here for the first time in animal tissues, net synthesis of polynucleotide could not be detected (even with C^{14}-ADP) because of contaminating nucleases. Previous results on the incorporation of C^{14}-labeled ATP[6,7] and UMP[8] into RNA of animal tissues suggest that nucleoside 5′-triphosphates are the substrates for polymerization. The exact function of the phosphorolysis reaction in RNA metabolism is under investigation.

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EXPONENTIAL KINETIC DEPENDENCIES IN INHIBITED AUTOXIDATIONS[1]

Sir:

The conventional treatment of the induction period, t_i, in inhibited autoxidations has been based upon the steady-state assumption with respect to chain carrier concentration,[2–5] an assumption which accounts well for the observed direct proportionality between inhibitor concentration and induction period.[5,6] Furthermore, the nonlinear relationships between t_i and inhibitor concentration, obtained for stabilized samples of cracked gasolines, have been fitted to an empirical equation[7] which is isomorphous with that deduced through a steady-state treatment of a simple chain-branching reaction scheme.[3]

There is, however, a significant body of uninterpreted observations of accelerating increases in t_i with increasing inhibitor concentrations[8] and of decelerating decreases in t_i with increasing concentrations of pro-oxidant catalysts.[9,10] These observations have not been explained in terms of steady-state kinetics.

We wish to report the obtainment of well-defined exponential dependencies of t_i upon both antioxidant and catalyst concentrations. The data shown in Table I were obtained with purified tetralin at 70° using a manometric apparatus.[11] These data conform to the relationship

$$t_i = t_i^0 \exp(kX) \qquad (I)$$

where X is the concentration variable. The least-squares values for k and the standard deviations are: set A, X = dibutylcresol concn., k = + 0.87 kg./millimole, s.d. ± 0.01; set B, X = cobaltous naphthenate concn., k = − 0.257 kg./micromole, s.d. ± 0.003; set *C*, X = cobaltous naphthenate concn., k = − 0.100 kg./micromole,

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