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 (pH 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* (1[a]). [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 pH optimum at about pH 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 non-linear 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,

(1) Presented in part before the Division of Organic Chemistry at the 131st meeting of the American Chemical Society, Miami, Florida, April, 1957. This work was generously supported by a grant from The Research Corporation.

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TABLE I

VARIATION OF INDUCTION PERIOD (t_i) OF TETRALIN AT 70°

Antioxidant concn., millimoles/kg.	Cobaltous naphthenate concn., micromoles/kg.	Induction period, hours
Set A: 2,6-di-*tert*-butyl-4-methylphenol antioxidant		
5.488	25.0	0.60
6.644	25.0	1.63
7.888	25.0	4.66
8.450	25.0	7.93
Set B: 2,6-di-*tert*-butyl-4-methylphenol antioxidant		
7.50	10.0	107.7
7.50	20.0	8.19
7.50	25.0	2.29
7.50	35.0	0.18
7.50	50.0	<0.05
Set C: hydroquinone antioxidant		
1.250	nil	30.65
1.250	10.0	12.05
1.250	20.0	4.51
1.250	30.0	1.55
1.250	40.0	0.54
1.250	50.0	0.23

s.d. ± 0.0015. These data, it should be noted, were obtained in a system using a strong branching catalyst. In the absence of a branching catalyst simple power relationships obtain. The obtainment of both power and exponential dependencies appear contrary to the predictions of steady-state kinetics but are consistent with the concept of dynamic equilibria as developed in the branching-chain kinetics of Semenoff.[12] A general hypothesis, based upon non-steady-state equilibria, for the kinetics of inhibited autoxidations will be submitted following completion of analysis of further data.

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THE ROLE OF PURINES IN HISTIDINE BIOSYNTHESIS[1]

Sir:

The formation of AICAR[2] (I) in bacterial extracts incubated with ATP, an ATP-generating system, RP, and glutamine has been reported.[3] We have now found that IGP (II), an essential precursor of histidine,[4] is an additional product of this reaction. Furthermore, in the absence of glutamine a compound III accumulates which yields AICAR on mild acid hydrolysis. The experiments presented in Table I show that ATP does not merely supply energy, but is actually the source of the AICAR produced, and that RP is incorporated into IGP without dilution. According to previous observations, the N_1–C_2 portion of the imidazole ring of histidine can originate from carbon 2 and an attached nitrogen atom of guanine.[5] However, the actual donor of this N–C fragment appears to be an adenine derivative, as shown by our recent finding that a mutant of *S. typhimurium* blocked between IMP and AMP incorporates carbon 2 of adenine into histidine without dilution by added hypoxanthine, guanine or glycine.

TABLE I

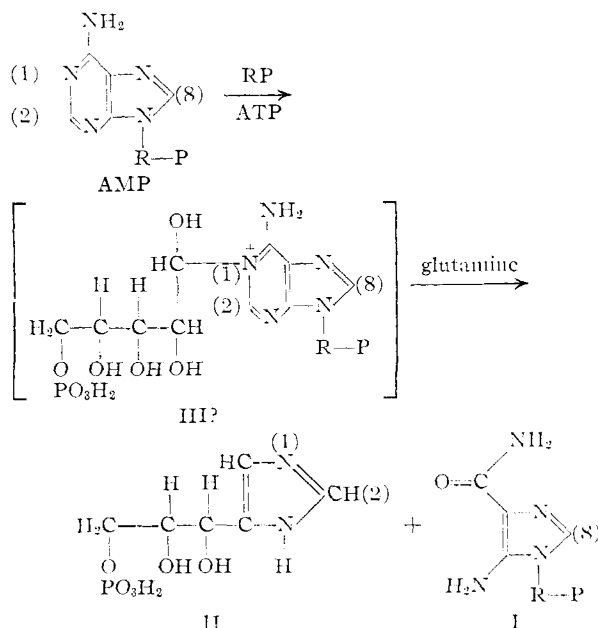
ENZYMATIC FORMATION OF AICAR AND IGP

The reaction mixture contained (per ml.): ATP (2.1 μmoles), RP (4.2 moles), acetylphosphate (16.8 μmoles), reduced glutathione (12.6 μmoles), tris-(hydroxymethyl)-aminomethane (84 μmoles) *p*H 8.06, $MgCl_2$ (16.8 μmoles), and a dialyzed, protamine-treated extract of *Salmonella typhimurium*, strain hi-B-12[a] containing 10 mg. of protein per ml. (0.33 ml.), and was incubated 190 min. at 37°. Expt. 1: total volume 24 ml., and RP-1-C^{14}[b] was used. Expt. 2: total volume 120 ml., and 9 μmoles of AMP-8-C^{14} was added.

Compound isolated	Expt. 1 (RP-1-C^{14}) μmoles	Expt. 1 (RP-1-C^{14}) RSA	Expt. 2 AMP-8-C^{14} RSA
IGP	4.9	104[c]	0
AICAR	5.2	17	102
AMP	6.1	19	100

[a] Requires histidine, excretes IGP. Kindly supplied by Dr. M. Demerec. [b] Kindly supplied by Dr. Bruce N. Ames. [c] IGP was oxidized with periodic acid to imidazoleformaldehyde of RSA 88.

The sequence of reactions shown would account for these observations



The reactions appear to be obligatory in histidine biosynthesis, since extracts of histidine requiring

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(2) Abbreviations: AICAR, 5-amino-1-D-(5'-phosphoribosyl)-4-imidazolecarboxamide; IGP, 4-(D-*erythro*-1',2'-dihydroxy-3'-phosphopropyl)-imidazole; ATP, adenosine-5'-triphosphate; AMP, adenosine-5'-monophosphate; IMP, inosine-5'-monophosphate; RP, ribose-5-phosphate.

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