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.

(12) N. Semenoff, "Chemical Kinetics and Chain Reactions," Oxford University Press, London, 1935.

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RECEIVED MAY 31, 1957

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

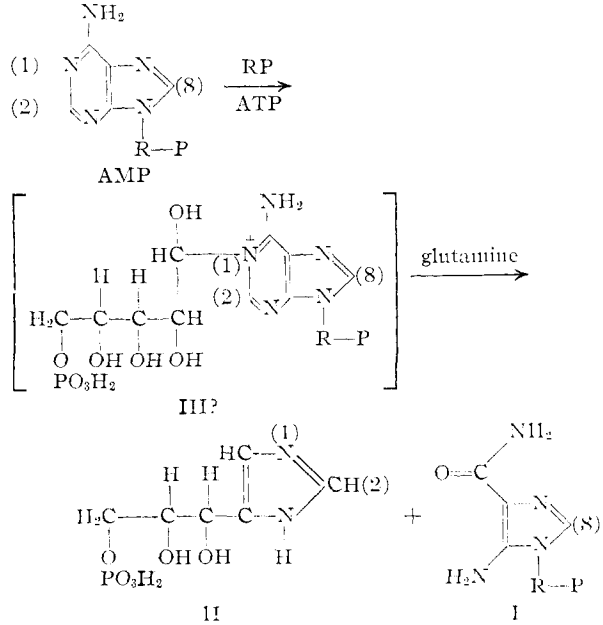
ENZYMATIC FORMATION OF AICAR AND IGP

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.

(3) S. H. Love, *J. Bact.*, **72**, 628 (1956); J. S. Gots and E. G. Gollub, *Bact. Proc.*, 122 (1956).

(4) B. N. Ames and H. K. Mitchell, *J. Biol. Chem.*, **212**, 687 (1955); B. N. Ames, *ibid.*, in press.

(5) C. Mitoma and E. E. Snell, *Proc. Nat. Acad. Sci.*, **41**, 891 (1955); B. Magasanik, H. S. Moyed, and D. Karibian, THIS JOURNAL, **78**, 1510 (1956); B. Magasanik, *ibid.*, **78**, 5449 (1956); A. Neidle and H. Waelsch, *Fed. Proc.*, **16**, 225 (1957).

mutants of *S. typhimurium*[6] blocked before IGP are either unable to form compound III (strain hi-F-41) or are unable to convert it to IGP (strain hi-A-5).

According to these results purine nucleotides play a catalytic role in the biosynthesis of histidine: AMP donates an N-C fragment to RP for the formation of the imidazole ring of histidine and is concomitantly converted to AICAR; this compound in turn reacts with a single carbon unit to regenerate the purine ring.

(6) Kindly supplied by Dr. M. Demerec.

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A CADMIUM PROTEIN FROM EQUINE KIDNEY CORTEX

Sir:

Cadmium never has been demonstrated to be an integral part of a natural product although present in various species.[1,2,3] Physiological function has not been shown.

Colorimetric analyses[4] of human, horse, cow, hog, and sheep kidney for cadmium led to the choice of horse kidney cortex for fractionation.

Fractionation of horse kidney cortex with ethanol and ammonium sulfate gave a product containing 20 to 25 *milligrams* of cadmium per gram dry weight of trichloroacetic acid precipitable material in successive fractionations. Ultracentrifugation[5] in a synthetic boundary cell showed the final products of four successive fractionations to be monodisperse with a sedimentation constant (uncorrected for viscosity and diffusion) varying from 0.94 to 1.22×10^{-13}. Paper electrophoresis at *p*H 8.5 of the product of one fractionation showed three components moving toward the cathode, the slowest comprising about 70% of the total material.

The fractions were analyzed colorimetrically[4] and by emission spectrography[6] (Table I). The cadmium content rises throughout the fractionation, a 30-fold increase from the first extract to the product. Cadmium is not removed by dialysis at *p*H 7, but is by treatment with hot trichloroacetic acid. With the exception of zinc, the other metals present initially and introduced during fractionation are removed and are low in concentration in the final material. Isomorphism cannot be excluded as an explanation of the substantial, although lesser, increase of zinc content as fractionation proceeds, nor can it be ruled out that cadmium is associated with one and zinc with another of the three electrophoretic fractions.

The product contains 14% nitrogen, measured on the material precipitated by trichloroacetic acid. It reacts positively to the biuret and ninhydrin tests. Hydrolysis and paper chromatography showed serine, glycine, aspartic, and glutamic acids, among other amino acids not identified. The last fraction (Table I, Fraction VII) contains about 1% of hexoseamine.[7] A carbazole test for uronides[8] was negative.

There is no ultraviolet absorption maximum near 280 mμ at *p*H 7 or *p*H 12, indicating a low content of aromatic groups. Absorption bands have not been found in the visible region. The infrared spectrum of a potassium bromide pellet of the lyophilized product closely resembles those obtained for several proteins.[9]

The low sedimentation constant and high metal content of this material are indicative of a low molecular weight protein, probably containing a small number of cadmium atoms per molecule. Characterization of this unusual natural product is in progress.

TABLE I

EMISSION SPECTROGRAPHIC AND COLORIMETRIC ANALYSES OF HORSE KIDNEY CORTEX FRACTIONS

Cadmium determined by spectrography[5] and by colorimetry[4]; all other metals determined spectrographically. Protein measured by dry weight of material precipitated by trichloroacetic acid. Data expressed as μg./g. wet weight of cortex, and as μg./g. protein for the fractions.

	Cortex	Fraction I	Fraction II	Fraction III	Fraction IV	Fraction V	Fraction VI	Fraction VII
Preparation E: colorimetric cadmium	82.7	1130	3050	17,100	14,300	*a*	*b*	24,500
Preparation C: colorimetric cadmium	83.7	754	3440	8,900	9,990	17,100	21,600	22,400
Spectrographic cadmium	137	687	2630	9,730	*c*	*c*	20,700	24,200
Spectrographic zinc	91	340	1440	3,600	3,330	*c*	4,910	5,880
Other metals (spectrographic)[e]	829	2410	2200	4,910	3,190	45,200[d]	15,400[d]	2,470

[a] Protein concentration too small to measure by trichloroacetic acid precipitation. [b] Sample lost. [c] Sample size inadequate. [d] Contamination with Mg and Ca introduced with ammonium sulfate. [e] Mg, Ca, Ba, Sr, Al, Fe, Mn, Cr, Pb; Na, K, Cu not determined.

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