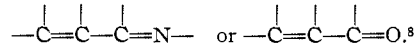
rence of reaction 4. As expected, the conversion of C-AIR, isolated as described below, to SAICAR (and AICAR) does not require bicarbonate, whereas bicarbonate is required for the conversion of AIR to SAICAR. Excess enzyme II was added so that any SAICAR formed was converted to AICAR according to reaction (2). The AICAR formed was measured by the Bratton–Marshall procedure after quantitatively destroying AIR and C-AIR by heating in 1 $N$ $H_2SO_4$ for 15 minutes at 100°.

C-AIR is rapidly decarboxylated by heat or acid to yield AIR. Its isolation was therefore performed at 3° and exposure to acid was kept at a minimum. The deproteinized reaction mixture at *p*H 9 was placed on a Dowex 1 acetate column from which the residual AIR was rapidly eluted under pressure with 0.04 $M$ ammonium acetate buffer, *p*H 5.1. A solution of 0.01 $M$ Tris bromide *p*H 9 was then passed through the column with pressure until the eluate had reached a *p*H of 8 to 9. C-AIR was obtained by a gradient elution in which a solution containing 0.2 $M$ potassium acetate and 0.01 $M$ Tris bromide, *p*H 9, dropped into a mixing vessel containing 400 ml. of 0.01 $M$ Tris bromide, *p*H 9. The ribotide, after concentration, was precipitated from an 85% ethanol solution as the barium salt.

The structure of C-AIR was first suggested by the finding that only two substrates, AIR and bicarbonate, were required for its formation. The conversion of C-AIR to SAICAR by reaction 4, and the known incorporation of bicarbonate into position 6 of inosinic acid, made highly unlikely any position of attachment of the bicarbonate to the imidazole nucleus other than that shown. In addition, the possession of a specific ultraviolet absorption band by C-AIR with $\lambda_{max}$ at 249 m$\mu$ at *p*H 8.5, provides physical evidence for the proposed structure of C-AIR, since it has been shown that the ultraviolet absorption of purines and related compounds is due to chromophoric systems of the type



Further evidence of the structure of C-AIR comes from the report of Rabinowitz[9] that the aglycone, 5-amino-4-imidazolecarboxylic acid, decomposes similarly in acid solution to form 5-aminoimidazole.

(8) L. F. Cavalieri, A. Bendich, J. F. Tinker and G. B. Brown, THIS JOURNAL, **70**, 3875 (1948).

(9) J. C. Rabinowitz, *J. Biol. Chem.*, **218**, 175 (1956).

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## THE ENZYMATIC CLEAVAGE OF 5-AMINO-4-IMIDAZOLE-N-SUCCINOCARBOXAMIDE RIBOTIDE[1]

*Sir:*

The enzymatic conversion of 5-amino-4-imidazole-N-succinocarboxamide ribotide (SAICAR) to 5-amino-4-imidazolecarboxamide ribotide (AICAR) and to a mixture of fumaric and malic acids previously has been reported as a step in the *de novo* synthesis of inosinic acid.[2] It has now been found through purification of this enzyme that fumaric acid is the initial product of the cleavage of SAICAR and that this enzyme is in all probability identical with adenylosuccinase described by Carter and Cohen.[3]

The enzyme was obtained free from fumarase from extracts of pigeon or chicken liver by a procedure including ethanol precipitation, negative gel absorption on Alumina C$\gamma$, and differential heat inactivation. Other sources of the enzyme are baker's yeast, *Escherichia coli*, *Salmonella typhimurium* and *Neurospora crassa*. The equilibrium constant of the splitting reaction determined with an enzyme purified 35-fold from baker's yeast[3] is approximately $7 \times 10^{-3}$ mole per liter. The Michaelis–Menten constant for SAICAR is $1.9 \times 10^{-4}$ mole per liter.

Because of the similarity of this reaction to the cleavage of adenylosuccinic acid[4] (AMP-S) to adenylic acid and fumaric acid and to the cleavage of argininosuccinic acid[4] to arginine and fumaric acid, an investigation of the specificity of the SAICAR splitting enzyme was undertaken. The splitting of SAICAR was inhibited 50% in the presence of an equimolar concentration of AMP-S but was not inhibited by a 6-fold excess of argininosuccinic acid. While preparations of the SAICAR splitting enzyme split AMP-S, no evidence could be obtained for the cleavage of argininosuccinic acid. During purification from yeast, the ratio of SAICAR splitting activity to adenylosuccinase activity remained constant within 5%. Both AMP-S and SAICAR were found to protect the SAICAR splitting enzyme from denaturation during heating steps in the purification procedure.

Additional evidence concerning the identity of adenylosuccinase and the SAICAR splitting enzyme was obtained from three adenine-requiring (F group) mutants of *Neurospora*. Mycelial extracts of these mutants have been shown to lack adenylosuccinase.[5] We have now shown that these mutants lack the ability to split SAICAR, while the wild strain (74A) is active in splitting both of the succino compounds. Partridge and Giles[6] have recently demonstrated that two revertants of an "F" mutant regain the same per cent. of the lost enzymatic activity whether assayed for adenylosuccinase or the SAICAR splitting enzyme. These facts may indicate that the loss of both activities is the result of a single genetic event. The indication that a single mutation exerts the same effect on two enzymatic activities makes it probable that only one enzyme is involved.

(1) This work is supported by grants-in-aid from the National Cancer Institute, National Institutes of Health, United States Public Health Service and the National Science Foundation. For the proper systematic names of intermediates of purine biosynthesis see the accompanying communication, L. N. Lukens and J. M. Buchanan, THIS JOURNAL, **79**, 1512 (1957).

(2) L. N. Lukens and J. M. Buchanan, *Federation Proc.*, **15**, 305 (1956).

(3) C. E. Carter and L. H. Cohen, *J. Biol. Chem.*, **222**, 17 (1956). We wish to thank Dr. Carter for a sample of adenylosuccinate.

(4) S. Ratner and B. Petrack, *ibid.*, **200**, 175 (1953). We wish to thank Dr. Ratner for a sample of argininosuccinate.

(5) C. W. H. Partridge and N. H. Giles, *Arch. Biochem. and Biophys.*, in press.

(6) N. H. Giles and C. W. H. Partridge, personal communication. We wish to thank these investigators for samples of their mutant strains of *Neurospora*.

It is of interest that two distinct and metabolically separated steps of a biosynthetic sequence are catalyzed by one enzyme and are under the control of a common genetic unit.

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## SYNTHESIS OF DIPHOSPHOPYRIDINE NUCLEOTIDE FROM NICOTINIC ACID BY HUMAN ERYTHROCYTES *IN VITRO*[1]

*Sir:*

In 1943,[2] *in vitro* synthesis of pyridine nucleotides from nicotinic acid (NA)[3] by human erythrocytes was demonstrated under conditions wherein no comparable synthesis was obtained with nicotinamide (NAm). Erythrocytes were shown to be freely permeable to both compounds; however, the microbiological assay employed was non-specific and the synthesized material might equally well have been nicotinamide mononucleotide (NMN), DPN, TPN, or nicotinamide riboside. Later it was observed that, in the presence of very high concentrations of NAm, pyridine nucleotides were synthesized of which 75–95% was NMN and the remainder DPN.[4] Preiss and Handler[5] have shown NMN formation in this system to occur by condensation of NAm with 1-pyrophosphoryl ribose-5-phosphate. However, since extremely high and non-physiological concentrations of NAm were required for this reaction and since no DPN-pyrophosphorylase has been detected in human erythrocytes,[5,6] NMN may not be an intermediate in DPN synthesis in the human erythrocyte. In consequence, it appeared desirable to reinvestigate the reported synthesis of pyridine nucleotides from NA by erythrocytes and establish the nature of the synthesized material.

Table I shows that at low concentration of NA there was appreciable synthesis of pyridine nucleotide, all of which was accounted for as DPN by the alcohol dehydrogenase assay, whereas NAm at similar concentration did not elevate the pyridine nucleotide level significantly. Only at higher concentrations was NAm an effective precursor for DPN synthesis. At a concentration sufficiently great to permit significant DPN synthesis, NMN accumulated in almost equal quantity, while at still higher NAm concentration, NMN synthesis was dominant. In contrast, NMN synthesis has not been observed at any concentration of NA. Indeed, in most experiments in which DPN synthesis was observed from NA, the NMN of the erythrocyte, which usually accounts for about 50% of the total pyridine nucleotides, disappeared. With both substrates, virtually all of the total nucleotide synthesized, as measured by the fluorimetric assay, as accountable as NMN and/or DPN.

TABLE I

PYRIDINE NUCLEOTIDE FORMATION FROM NICOTINIC ACID AND NICOTINAMIDE BY HUMAN ERYTHROCYTES

The reaction mixture contained: 50 μmoles phosphate *p*H 7.4, 22.5 mg. glucose, defibrinated whole blood 3.0 ml., NA, NAm, and glutamine in the amounts indicated. Total volume was 3.8 ml.; incubation time 22 hours.

| Additions μmoles | | μmoles | ΔTotal Pyridine nucleotide[a] μmoles | Δ*DPN*[b] μmoles | Δ*NMN*[c] μmoles |
|---|---|---|---|---|---|
| NA | 0.3 | | 0.045 | 0.041 | ... |
| NA | 0.3 | GAm 20 | .201 | .211 | ... |
| NA | 1.0 | | .060 | .049 | ... |
| NA | 1.0 | GAm 20 | .222 | .195 | ... |
| NA | 10.0 | | .061 | .059 | ... |
| NA | 10.0 | GAm 20 | .126 | .122 | ... |
| NA | 100.0 | | .093 | .139 | ... |
| NAm | 0.3 | | .016 | .000 | ... |
| NAm | 1.3 | GAm 20 | .015 | .000 | ... |
| NAm | 1.0 | | .019 | .000 | ... |
| NAm | 1.0 | GAm 20 | .000 | .000 | ... |
| NAm | 10.0 | | .111 | .048 | ... |
| NAm | 10.0 | GAm 20 | .068 | .036 | ... |
| NAm | 100.0 | GAm 20 | .587 | .323 | 0.208 |
| NAm | 300.0 | | 1.15 | .388 | .610 |

[a] Systems lacking NAm and NA contained 0.193 μmole. This value was subtracted from the observed value, yielding the increment shown. [b] Increment over the control value of 0.089 μmole. [c] Assayed with alcohol dehydrogenase after aliquot was treated with DPN pyrophosphorylase and ATP. NMN was calculated as the increment in DPN due to this treatment. The control contained 0.093 μmole NMN.

It is evident from these data that free NAm cannot be an intermediate in DPN synthesis from NA, suggesting that amidation may occur after nicotinic acid is converted to some presently unknown nucleotide derivative. Several explanations might be offered to account for DPN synthesis at high NAm concentration, but further work is necessary to establish the mechanism of this process.

Table II shows that DPN synthesis from NA is dependent on phosphate, glucose, and ammonia which may be supplied as glutamine. Asparagine

TABLE II

REQUIREMENTS FOR DPN SYNTHESIS BY ERYTHROCYTES

The complete reaction mixture contained 10 μmoles NA, 10 μmoles GAm, 50 μmoles phosphate *p*H 7.4, 22.5 mg. glucose, 20 μmoles $Mg^{++}$, defibrinated blood 3.0 ml., 0.9% NaCl to 4.74 ml., incubation time 21 hours.

| Omissions | Final DPN, μmole | ΔDPN, μmole |
|---|---|---|
| None | 0.285 | 0.148 |
| NA | .137 | .000 |
| TRIS instead of phosphate | .153 | .016 |
| Glucose | .180 | .043 |
| $Mg^{++}$ | .294 | .157 |
| GAm | .182 | .045 |
| $NH_4^+$ instead of GAm | .276 | .139 |
| Asparagine instead of GAm | .199 | .062 |

(1) These studies were supported in part by contract AT-(40-1)-289 between Duke University and the United States Atomic Energy Commission and by Grant RG-91 from the National Institutes of Health.

(2) P. Handler and H. I. Kohn, *J. Biol. Chem.*, **150**, 447 (1943).

(3) These abbreviations are used: NA, nicotinic acid; NAm, nicotinamide; GAm, glutamine; NMN, nicotinamide mononucleotide; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; TRIS, trishydroxymethylaminomethane.

(4) I. G. Leder and P. Handler, *J. Biol. Chem.*, **189**, 889 (1951).

(5) J. Preiss and P. Handler, Abstracts of the 130th National Meeting of the American Chemical Society (1956), p. 44c; *J. Biol. Chem.*, in press.

(6) A. Malkin and O. F. Denstedt, *Canadian J. Biochem. Physiol.*, **34**, 121 (1956).