ously reported,[6,7] provides a new mechanism for the biological transformation of sugars. The formation of glucose from galactose[8] or ribose phosphate from xylose[9] may occur by such transfer or exchange reactions.

(6) B. L. Horecker and P. Z. Smyrniotis, THIS JOURNAL, **75**, 1009 (1953).
(7) E. Racker, G. de la Haba and I. G. Leder, *ibid.*, **75**, 1010 (1953).
(8) R. H. Caputto, L. F. Leloir, C. E. Cardini and A. C. Paladini, *J. Biol. Chem.*, **184**, 333 (1950).
(9) J. O. Lampen, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," Vol. II, The Johns Hopkins Press, Baltimore, Md., 1952, p. 375.

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTE OF HEALTH
PUBLIC HEALTH SERVICE
FEDERAL SECURITY AGENCY
BETHESDA 14, MD.
B. L. HORECKER
P. Z. SMYRNIOTIS

RECEIVED MARCH 14, 1953

THE ENZYMATIC SYNTHESIS OF N-ACETYLIMIDAZOLE

Sir:

Extracts of *Clostridium kluyveri* oxidize butyrate to acetyl phosphate (acetyl-P) and acetate in orthophosphate buffer[1] and to acetoacetate in the absence of orthophosphate (Pi).[2] In the presence of imidazole (Pi absent) a labile acetyl compound is formed. This compound has been tentatively identified as N-acetylimidazole (AcI) on the basis of comparative studies with the synthetic compound.[3] AcI and the enzymatic product are readily hydrolyzed at *p*H 7.0 (30°), but in aqueous solution they react preferentially with amino acids, alcohols, Pi and sulfhydryl compounds to give the corresponding acetyl derivatives and with neutral hydroxylamine to give acethydroxamic acid.

The acetylation of Pi (reaction 1) is of particular interest since it establishes the energy-rich nature of AcI.

$$\text{AcI} + \text{Pi} \rightleftharpoons \text{acetyl-P} + \text{Imidazole} \qquad (1)$$

Equimolar amounts of AcI and Pi (0.1 *M*, *p*H 7.0) react to give a 50% yield of acetyl-P (20 min., 30°). The free energy of hydrolysis of AcI is therefore at least as great as that of acetyl-P (*i.e.*, 12,000–15,000 cal.[4]).

Advantage has been taken of the strong absorption band of AcI at 235–255 mμ to demonstrate reversibility of reaction 1. Thus an increase in optical density at 245 mμ is observed when acetyl-P is incubated with imidazole (*p*H 7.0, 25°). The non-enzymatic reaction does not occur readily at low acetyl-P concentrations (0.01 *M*); however, in the presence of dialyzed extracts of *C. kluyveri* a rapid enzymatic acetylation of imidazole occurs. The enzymatic reaction may be followed spectrophotometrically, as above, or indirectly by measuring the decrease in acetyl-P when incubated with imidazole in the presence of enzyme.

A partially purified imidazole acetylase (IA) obtained by fractionation of the bacterial extracts will not catalyze the acetylation of imidazole with acetyl-P unless Coenzyme A (CoA) and phosphotransacetylase (PTA)[5] are added (Table I).

(1) E. R. Stadtman and H. A. Barker, *J. Biol. Chem.*, **180**, 1095 (1949).
(2) E. P. Kennedy and H. A. Barker, *ibid.*, **191**, 419 (1951).
(3) J. H. Boyer, THIS JOURNAL, **74**, 6274 (1952).
(4) F. Lipmann, *Advances in Enzymol.*, **6**, 231 (1946).

TABLE I

REQUIREMENTS FOR THE ENZYMATIC ACETYLATION OF IMIDAZOLE

The complete system contained acetyl-P, 5 micromoles; cysteine, 50 micromoles; imidazole, 100 micromoles; triethanolamine·HCl buffer, 100 micromoles; CoA, 0.05 micromole; PTA, 9 units; IA, 1.5 mg. protein. The final volume was 0.5 ml. (*p*H 7.0). Samples were incubated at 30° for 20 min.

	Δ Acetyl-P[a]
Complete system	2.6
Complete system − Imidazole	0
Complete system − CoA	0.7
Complete system − PTA	.3
Complete system − IA	.2
Complete system − CoA + PTA	0

[a] Amounts in micromoles.

The requirements for CoA and PTA in addition to IA suggest that the following reactions are involved.

$$\text{Acetyl-P} + \text{CoA} \overset{\text{PTA}}{\rightleftharpoons} \text{Acetyl CoA} + \text{Pi} \qquad (2)$$

$$\text{Acetyl-CoA} + \text{Imidazole} \overset{\text{IA}}{\rightleftharpoons} \text{AcI} + \text{CoA} \qquad (3)$$

$$\text{AcI} + \text{Cysteine} \xrightarrow{\text{Non-enzymatic}} \text{Imidazole} + \text{N-acetylcysteine (and/or 2-methyl-4-carboxythiazoline)} \qquad (4)$$

$$\text{Acetyl-P} + \text{cysteine} \longrightarrow \text{N-acetylcysteine (thiazoline ?)} \qquad (5)$$

Cysteine is used in the test system as the ultimate acetyl acceptor since the N-acetylcysteine (or the thiazoline derivative which may be formed by ring closure) produced does not form a hydroxamic acid under the conditions used.[6] Thus the reaction can be followed by measuring the decrease in acetyl-P by the hydroxamic acid method.[7] The relatively slow direct non-enzymatic reaction between acetyl CoA and cysteine[6] does not occur to a significant extent under these experimental conditions (*p*H 7.0, low CoA concentration). Substitution of glutathione for cysteine in the test system leads to the accumulation of S-acetyl glutathione which was identified as previously described.[6]

The enzymatic formation of AcI appears significant for a theory of acetyl transfer at the high energy level in which imidazole may serve as a model compound in reactions that normally involve a naturally occurring imidazole derivative or related compound (possibly a coenzyme). In terms of the mechanism of enzyme-action it is suggested that the imidazole moieties of the histidine components of proteins may be implicated as acyl carriers in acyl-transfer reactions.

NATIONAL HEART INSTITUTE
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
FEDERAL SECURITY AGENCY
BETHESDA 14 MARYLAND
E. R. STADTMAN
F. H. WHITE, JR.

RECEIVED FEBRUARY 26, 1953

(5) E. Stadtman, *J. Biol. Chem.*, **196**, 527 (1952).
(6) E. Stadtman, *ibid.*, **196**, 535 (1952).
(7) F. Lipmann and L. C. Tuttle, *ibid.*, **158**, 505 (1945).