TABLE I

PHOSPHORYLATION OF NUCLEOSIDES BY MALT ENZYME

The solutions containing, per ml. of 0.1 *M* acetate buffer of *p*H 5, 40 μ moles of nucleoside, 200 μ moles of phenylphosphate and 2 mg. of enzyme were incubated at 30° for 30 hours, when about 55% of the phosphorus has become inorganic

Nitrogenous constituent	Extent of phosphorylation, as % of nucleoside Riboside	Desoxyriboside
Hypoxanthine	5.0	8.9
Uracil	7.7	9.9
Cytosine	7.6	13
Thymine		9.4

The transphosphorylation system described here seems to be the first instance of a general enzymatic reaction leading to the formation of nucleotides from nucleosides. In some of its characteristics it resembles the transfer reactions studied by Axelrod[4] and Green and Meyerhof.[5]

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(4) B. Axelrod, *J. Biol. Chem.*, **172**, 1 (1948).
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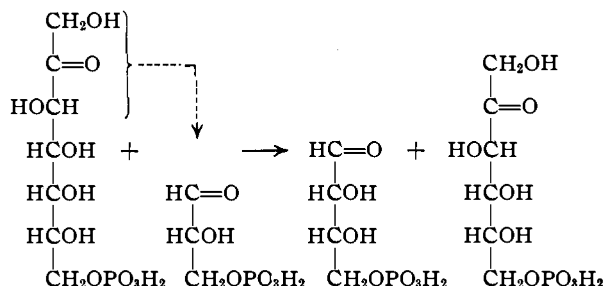
TRANSALDOLASE: THE FORMATION OF FRUCTOSE-6-PHOSPHATE FROM SEDOHEPTULOSE-7-PHOSPHATE

Sir:

Previous reports[1-3] have described the formation of ribulose-5-phosphate and sedoheptulose-7-phosphate as intermediates in the oxidation of hexosemonophosphate. This pathway is now established as a cyclic process by the finding that sedoheptulose-7-phosphate is converted to fructose-6-phosphate. Preparations from liver and yeast have been obtained which catalyze this reaction, but only in the presence of a source of triose phosphate (Table I). With a purified preparation from brewer's yeast the reaction is stoichiometric with respect to either sedoheptulose-7-phosphate or triose phosphate. The reaction mechanism has been clarified by the use of C^{14}-triose phosphate. Hexosemonophosphate produced from unlabeled sedoheptulose-7-phosphate and uniformly labeled triose phosphate (derived from fructose diphosphate) had a specific activity of 53,000 c.p.m. per micromole compared with 58,000 c.p.m. per micromole for the triose phosphate, indicating that three carbon atoms of the hexosemonophosphate were derived from triose phosphate. Degradation of the glucose obtained by hydrolysis of the hexosemonophosphate showed the label to be present only in carbon atoms 4, 5 and 6; carbon atoms 1, 2 and 3 which contained no radioactivity must have come from sedoheptulose-7-phosphate.[4] It is concluded from these results that the reaction mechanism involves a transfer of the dihydroxyacetone group from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate to form fructose-6-phosphate and erythrose-4-phosphate.



Since this enzyme catalyzes a transfer of aldol linkages, rather than their hydrolytic cleavage, it will be referred to as transaldolase.

The formation of fructose-6-phosphate as the initial reaction product is confirmed by the results in Table I, since little glucose-6-phosphate is formed in the absence of hexose phosphate isomerase.

TABLE I

THE FORMATION OF GLUCOSE-6-PHOSPHATE FROM SEDOHEPTULOSE-7-PHOSPHATE

The complete system contained 3.1 × 10^{-4} *M* sedoheptulose-7-phosphate, 3.3 × 10^{-4} *M* fructose diphosphate, 2.5 × 10^{-5} *M* triphosphopyridine nucleotide (TPN), 5.6 μg. of purified yeast transaldolase, 83 μg. of aldolase, 110 μg. of glucose-6-phosphate dehydrogenase and 340 micrograms of hexose phosphate isomerase, in 0.04 *M* triethanolamine buffer, *p*H 7.6. The total volume was 1.10 ml. and the temperature was 25°. The production of glucose-6-phosphate was followed spectrophotometrically by measuring the reduction of TPN in the presence of glucose-6-phosphate dehydrogenase. Hexose phosphate isomerase was a crude rabbit muscle ammonium sulfate fraction; all other enzymes were purified preparations.

System	Glucose-6-phosphate produced[a]
Complete system	4.7
Sedoheptulose-7-phosphate omitted	.0
Hexosediphosphate omitted	.0
Hexose phosphate isomerase omitted	.4
Transaldolase omitted	.4

[a] Millimicromoles per minute.

Preliminary evidence for a tetrose ester in the reaction mixture has been obtained with paper chromatography following hydrolysis with potato phosphatase. The chromatogram was developed with a 70–30 acetone–water mixture and sprayed with aniline phthalate. A fluorescent spot was observed with R_f = 0.65, identical with authentic erythrose.[5] However, since this solvent does not separate the aldo- and keto-tetroses, the nature of the tetrose remains to be determined.

The transfer of aldol linkages from one sugar to another, like the transfer of the ketol groups previ-

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(4) We are indebted to Dr. M. Gibbs of the Brookhaven National Laboratory for the degradation of the labeled glucose.
(5) Kindly furnished by Dr. H. S. Isbell of the National Bureau of Standards.

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.

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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.

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

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).

TABLE I

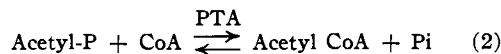
REQUIREMENTS FOR THE ENZYMATIC ACETYLATION OF IMIDAZOLE

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.

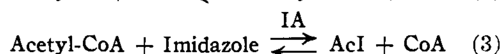
	Δ Acetyl-P[a]
Complete system	2.6
Complete system − Imidazole	0
Complete system − CoA	0.7
Complete system − PTA	.3
Complete system − IA	.2
Complete system − CoA + PTA	0

[a] Amounts in micromoles.

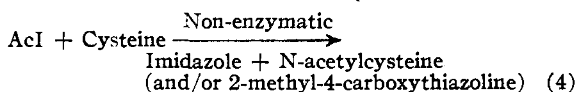
The requirements for CoA and PTA in addition to IA suggest that the following reactions are involved.



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



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



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

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

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.

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