lactic acid is stable throughout the manipulation. In both cases, the lactic acid isolated was found to contain less than 4% of the specific activity of the water. These results contra-indicate the mechanism of the ene-diol intermediate (II) as well as the proposition that the alpha-hydrogen of this ester is chemically labile*, at least under the conditions of these experiments.

Since pyridine nucleotide-coupled oxidation-reduction reactions are known to occur by direct "hydride shift"[6], it is of interest to examine the question of their involvement in the present reaction. The following evidence would seem to weigh against this: (1) the preparation of the partially purified enzyme has a 280 mμ: 260 mμ ratio of 1.55; (2) the small amount of material lost by treatment of the enzyme with charcoal of with Dowex-1-acetate likewise has a high ratio; (3) neither of these treatments of the enzyme caused any diminution of the glyoxalase activity.

The present data indicate therefore that a hydride shift occurs in the oxidation-reduction reaction catalyzed by glyoxalase I. In this respect the enzymic reaction is analogous to the alkaline-catalyzed Cannizzaro reaction in which methyl glyoxal is converted to lactic acid.

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After this paper had been submitted to the journal, the author learned of the prior publication by FRANZEN[7] of a study in which lactate formed from methyl glyoxal in D_2O by an acetone powder of yeast extract was found to be free of deuterium. We have thus confirmed this result and interpreted it in like manner.

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* In an experiment in which transacetylase from *E. coli* was used to catalyse the coenzyme A-dependent arsenolysis of acetyl-phosphate in TOH, the acetate formed was without radioactivity. From this it may be concluded that the α-hydrogens in acetyl-coenzyme A are also stable to spontaneous exchange at neutral pH.

Instability of ketopentose-5-phosphates in buffer solutions of varying pH*

The interconversion of glucose-6-phosphate and ribose-5-phosphate in living cells requires the presence in the cell of a mixture of ribose-5-phosphate and its two ketopentose-5-phosphate isomers. COHEN[1] reported in 1953 that ribulose-5-phosphate is destroyed in tris buffer of pH 8.3 or higher. As the stability of the ketopentose-5-phosphates, which can be assumed to be, if only in small concentration, permanently present in the cells, should be of importance for the cell economy, we investigated the effect of incubation of the two ketopentose-5-phosphates in various buffers of more physiological nature in the range between pH 5.3 and 9.5 and compared it with that in tris buffer. The results are presented in the table. The experiments were carried out with preparations of ribulose- and xylulose-5-phosphate, both of which were free of any significant contamination with the other ketopentose-5-phosphate. In addition, a series of experiments was carried out on an equilibrium mixture of ribose- and the two ketopentose-5-phosphates obtained by incubation of ribose-5-phosphate in a hemolysate for 10 min at 33°, coagulation of proteins at 100°, and separation of the esters from the coagulum by dialysis. The solutions were incubated for 2½ h at 33°, and the reaction was then stopped by adding trichloroacetic acid to a concentration of 2%. The disappearance of ketopentose was measured by comparing its

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TABLE I

EFFECT OF INCUBATION OF KETOPENTOSE-5-PHOSPHATES IN VARIOUS BUFFERS OF VARYING pH
Incubation 2½ h at 33° C

Substrate	*Buffer*	*pH*	*Decrease in % of original concentration*
I. Ribulose-5-phosphate 0.7 μmole/ml	0.1 *M* tris	7.0	3.8
	0.1 *M* tris	8.4	5.0
	0.1 *M* tris	9.0	3.5
	0.1 *M* phosphate	9.0	11.9
	0.1 *M* bicarbonate	9.5	12.6
II. Xylulose-5-phosphate 1 μmole/ml	0.1 *M* tris	7.0	10.5
	0.1 *M* tris	8.4	12.9
	0.1 *M* sodium citrate	8.4	6.3
	0.1 *M* phosphate	5.3	10.5
	0.1 *M* phosphate	7.0	13.7
III. Xylulose 1 μmole/ml	0.1 *M* tris	8.4	—
IV. Equilibrium mixture of ribose-5-phosphate and the two ketopentose-5-phosphate isomers 2.5 μmole/ml	0.1 *M* tris	7.0	8.4
	0.1 *M* tris	8.4	10.8
	0.1 *M* tris	9.0	8.4
	0.1 *M* tris	9.5	14.9
	0.1 *M* bicarbonate	9.5	40.6
	0.1 *M* phosphate	7.0	28.0
	0.1 *M* phosphate	8.4	28.0
	0.1 *M* phosphate	9.0	34.8
	0.01 *M* phosphate	7.0	15.8
	0.1 *M* glycyl glycine	8.0	10.9

concentration at the end of the incubation period with that at time 0. The determination of the petopentose was carried out by the cysteine-carbazole reaction[2]. That the disappearance of ketokentose was not due to an isomerization to aldopentose was shown by carrying out the phloroglucinol reaction for aldopentose[3] on all the solutions. As can be seen from the table, xylulose-5-phosphate and the isomer mixture were, in the range of pH 7.0–9.5, much more rapidly destroyed in various buffers than pure ribulose-5-phosphate. Changes in the pH had much less effect than differences in the nature of the buffer. Surprisingly, the rate of destruction was highest with bicarbonate and somewhat less with phosphate. Disappearance of ketopentose in phosphate was observed even at pH 5.3. In tris and glycyl glycine, on the other hand, the destruction of the ketopentose was much smaller, and still smaller in citrate buffer. As destruction of the esters was also observed in double glass distilled water at pH 7.0, these findings suggest a protective effect of these last three buffers, which at least in part might be due to chelating of heavy metals. All these findings about the instability of ketopentose-5-phosphates in solution under conditions approaching the intracellular environment, and the protective effect of certain ions, suggest the possibility that specific protective agents may be present in the living cells and prevent the non-enzymic wasteful destruction of ketopentose-5-phosphates. On the other hand, any balance experiments concerning the enzymic breakdown of pentose-5-phosphate must take into consideration the non-enzymic disappearance of certain amounts of pentose.

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