is located predominantly at the α-carbon atoms of the glyceride. The greater loss of the acid with increasing dilution is undoubtedly a reflection of the inhibition of the exchange reaction between long-chain glyceride fatty acids and the free acids of the medium[5].

In further experiments the glyceride fraction isolated after lipase action was fractionated into mono-, di- and triglycerides by the procedure of QUINLIN AND WEISER[7] and the butyric acid content was determined in each species. The data obtained in one experiment are presented in Table II. Each of the experiments with varying incubation periods and enzyme and substrate concentrations yielded monoglycerides with no detectable butyric acid.

These results show that the butyric acid in the bovine milk fat is attached exclusively at the α-carbon atoms of the glyceride. This and the negligible exchange of the lower fatty acids between the different species of glycerides in the presence of lipase would suggest that they are incorporated into α, β-diglycerides, most probably by the mechanism suggested by WEISS, KENNEDY AND KIYASU[8].

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L-Xylulokinase and L-xylulose 5-phosphate–L-ribulose 5-phosphate 3-epimerase in *Aerobacter aerogenes*

Growth of *Aerobacter aerogenes* on L-xylose evokes the formation of a cobalt-activated isomerase which produces L-Xu fom L-xylose[1], and two additional new enzymes, a kinase and a 3-epimerase, which effect the further metabolism of L-Xu. The L-xylulokinase has been purified 500-fold from cell extracts by protamine, ammonium sulfate, alumina Cγ, and DEAE-cellulose fractionations. Of the four ketopentoses, only L-Xu was phosphorylated. The product of L-Xu phosphorylation was prepared by incubating 1 mmole L-Xu, 1 mmole ATP, 100 μmoles NaGSH, 80 μmoles ethylenediaminetetraacetate, and 2 mmoles $MgCl_2$ with 633 units kinase* (before DEAE-

Abbreviations: Xu, xylulose; Ru, ribulose; -P, phosphate; NaGSH, sodium glutathione; DEAE-cellulose, diethylaminoethylcellulose; ATP, adenosine triphosphate; TPN, triphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide.

* The pH was maintained at 6.8 to 7.5 with 0.1 N NaOH. 1 unit = 1 μmole phosphorylated/h.

cellulose). After 2 h, the phosphorylation product was isolated as the barium salt (143 mg) by standard methods[2]. The kinase product, with a pentose to phosphate ratio of 0.94, acted as substrate for L-Ru-5-P 4-epimerase[3], but not for a mixture of phosphoriboisomerase, D-Ru-5-P 3-epimerase, and D-Xu-5-P phosphoketolase*. After incubation with purified acid phosphatase and chromatography on Dowex-1-borate, two peaks were eluted. Peak 1 (70 % of the ketose) was identified as Xu and Peak 2 behaved identically with authentic Ru (Table I).

TABLE I

IDENTIFICATION OF FREE KETOSE FROM L-XYLULOKINASE PRODUCT

Tests	*Peak 1*	*Xylulose*	*Peak 2*	*Ribulose*
Yield, μmoles	101	—	42	—
Dowex-1-borate, elution concn. (M)	0.02	0.02	0.03	0.03
Orcinol, 540/670 mμ	0.40	0.42–0.48	0.87	0.79–0.99
Cysteine-carbazole, min for maximum color	>60	>60	<15	<145
Chromatography*				
R_F	0.57	0.57	0.54	0.54
Dimethylphenaline**	Gray	Gray	Gray-Brown	Gray-Brown
Orcinol-trichloroacetic acid	Rose***	Rose***	Pink§	Pink§

* butanol-pyridine-water (6:4:3)[4].
** N,N-Dimethyl-*p*-phenylenediamine.
*** Rose fluorescence.
§ Orange fluorescence.

Peak 1 acted as substrate for TPN-specific xylitol (L-Xu) dehydrogenase[4], but not for a specific D-arabitol (D-Xu) dehydrogenase[5], or for a purified ribitol (D-Ru) dehydrogenase[5,6]. Peak 2 reacted with L-arabinose (L-Ru) isomerase[7], but not with D-arabinose (D-Ru) isomerase, or the pentitol dehydrogenases. Therefore, an enzymic conversion of L-Xu-P to L-Ru-5-P was indicated.

The reverse reaction, L-Ru-5-P to L-Xu-P, was observed by incubating L-Ru-5-P (prepared with purified L-Ru kinase[2]) for 6 h with 43 units of the same L-Xu kinase fraction at pH 8.5. A disappearance of L-Ru-5-P was observed**, and D-ribose 5-phosphate, D-Ru-5-P and D-Xu-5-P were not produced. After phosphatase treatment, chromatography on Dowex-1-borate yielded two ketopentose peaks in elution positions for Xu (68 %) and Ru (32 %). As above, Peak 1 reacted in the chemical and enzymic tests as L-Xu, whereas Peak 2 was identified as L-Ru. D-Ru was not detected.

The conversion of L-Xu-P to L-Ru-5-P was also observed in a spectrophotometric assay for L-Ru-5-P[3,8]. With excess L-Ru-5-P 4-epimerase, phosphoketolase, triose phosphate isomerase and α-glycerophosphate dehydrogenase, the formation of L-Ru-5-P may be observed as DPNH oxidation. As shown in Fig. 1, DPNH was oxidized rapidly by the complete system, but was oxidized only slowly when either ATP, L-Xu, or 4-epimerase were omitted.

Because L-Ru-5-P is both formed from and converted to L-Xu-P, it is likely that L-Xu is phosphorylated at carbon 5. The conversion of L-Xu-5-P to L-Ru-5-P, thus, is analogous to the D-Ru-5-P 3-epimerase reaction. However, the L-Xu kinase fraction

* Under conditions in Fig. 1 except that L-Ru-5-P 4-epimerase was omitted.
** DPNH oxidation under conditions in Fig. 1.

does not contain D-Ru-5-P 3-epimerase, and purified D-Ru-5-P 3-epimerase from yeast does not interconvert the L-isomers. Therefore, it is concluded that a new L-Xu-5-P (or L-Ru-5-P) 3-epimerase catalyzes the interconversion of L-Xu-5-P and L-Ru-5-P. Since L-xylose-grown cells also contain L-Ru-5-P 4-epimerase, the metabolic sequence is considered to be: L-Xylose → L-Xu → L-Xu-5-P → L-Ru-5-P → D-Xu-5-P. This route is distinct from the mammalian system wherein two xylitol dehydrogenases mediate the conversion of L-Xu to D-Xu, which is then phosphorylated[9,10].

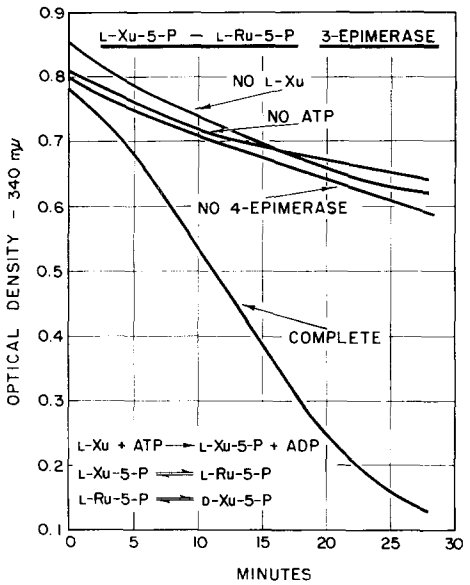

Fig. 1. Formation of L-Ru-5-P from L-Xu-5-P by L-Xu-5-P (L-Ru-5-P) 3-epimerase. The reaction mixture contained in 0.3 ml crystalline α-glycerophosphate dehydrogenase (containing triose phosphate isomerase), purified L-Ru-5-P 4-epimerase[7], purified D-Xu-5-P phosphoketolase[8] (each in excess), 8 units of kinase fraction containing L-Ru-5-P 3-epimerase, and the following in μmoles: glycylglycine, pH 7.5, 15; $MgCl_2$, 1; DPNH, 0.05; sodium arsenate, 2; NaGSH, 3; thiamine pyrophosphate chloride, 0.1; ATP, 3; and L-Xu, 2.9.

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