Table 1. Glucosamine and Interfering Substances Formation in Rabbit Cartilage

Incubated samples		Non-incubated samples		
Interfering substances	Glucosamine	Interfering substances	Glucosamine	Glucosamine synthetized
70	270	23·5	146·4	23·4

Average values of six determinations. Values expressed as γ-glucosamine/gm. fresh tissue. Experimental conditions were those proposed by Castellani and Zambotti (ref. 1).

Still lower values of hexosamine synthesis were obtained by this method, suggesting that the high values given by the Schloss method were due to interfering Ehrlich-positive substances formed or extracted during incubation (Table 1). Part of the interfering substances seems to be due to free glucose-6-phosphate (which gives Ehrlich-positive reaction) liberated from glucose-6-phosphate (which gives Ehrlich-negative reaction), accompanied by the increase of inorganic phosphorus of the incubated samples, as compared to the control samples (450γ free glucose/100 mgm. fresh tissue liberated during incubation with liver homogenates ; 150γ free glucose/100 mgm. fresh tissue liberated during incubation with cartilage homogenate). The incubation of cartilage or liver homogenates with glucose-6-phosphate only, in absence of glutamine, also leads to an apparent synthesis of glutamine.

My experiences suggest that, using the technique proposed by Castellani and Zambotti, in addition to the synthesis of glucosamine, Ehrlich-positive free glucose, liberated by a process of dephosphorylation of glucose-6-phosphate is measured.

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Glycolaldehyde Trapped from Aerobic Oxidation of D-Xylose by *Torulopsis utilis*

In previous experiments on the aerobic degradation of D-xylose by living cells of *Torulopsis utilis*, we succeeded in isolating the triose-phosphate, pyruvic acid and the acetyl groups using the phenylhydrazine trapping technique. Our results have been confirmed by Heath *et al.*[3], who purified from *Lactobacillus pentosus* an enzyme which phosphorolytically cleaves D-xylulose-5-phosphate into triose-phosphate and acetyl-phosphate. Schramm and Racker[4] have shown in a mutant of *Acetobacter xylinum* the presence of an enzyme which carries out the same phosphorolytic split of D-xylulose-5-phosphate and cleaves also the fructose-6-phosphate into erythrose-4-phosphate and acetyl-phosphate. From the results of our preceding experiments we supposed a split of an intermediate phosphorylated ketopentose into triose-phosphate and into an unknown C-2 intermediate, both originating the acetyl group according to the formulation given in ref. 2.

The unknown C-2 intermediate was glycolaldehyde, but we were unable to separate it at that time from the trapped intermediates. With chromatographic techniques we have now succeeded in isolating it together with the triose-phosphate, pyruvate and the acetyl group, in the same experimental conditions used in the preceding experiments[2]. Free glycolaldehyde was first isolated by Kaushal *et al.*[5] from the fermentation of pentoses by *Acetobacter acetigenum*. From our results it seems that D-xylulose-5-phosphate, probable intermediate of the fermentation of D-xylose[3], is enzymatically cleaved with the formation of triose-phosphate and glycolaldehyde : the so called 'active glycolaldehyde'. In our aerobic conditions, the acetyl group is formed from triose-phosphate by the way of the pyruvate and from the glycolaldehyde as suggested by us in a preceding paper[6] which deals with the oxidation of acetate to glycolate. In effect, this reaction appears to occur through the intermediate formation of an enolic form of acetyl-coenzyme *A*, which is transformed by hydration into glycolaldehyde, that is afterwards dehydrogenated to glycolic acid.

40 gm. wet weight of living cells of *T. utilis* (Windisch strain), grown on mineral solution at 1·5 per cent of raw saccharose, were washed three times and suspended in the following medium : distilled water 1,000 ml. ; D-xylose (Ciba) 5 gm. ; disodium hydrogen phosphate, 2 gm. ; potassium dihydrogen phosphate, 3 gm. ; ammonium sulphate, 2 gm. ; crystalline magnesium sulphate, 0·3 gm. The *p*H was adjusted to about 5·5, and the suspension aerated in a 1,500 ml. cylindrical glass flask through a sintered-glass disk at the base of the flask. Depending on the *p*H changes, three portions of 1 gm. of the phenylhydrazine oxalate were added within 2 hr. Each portion was dissolved in 50 ml. distilled water containing sodium hydroxide to *p*H 5·5. After 7–8 hr. incubation, the medium was centrifuged and the clear liquid analysed for fixed products[1,2]. For the separation of the glycolaldehyde, in the form of its 2,4-dinitrophenylosazone, the centrifuged medium was treated with an excess of benzaldehyde at 70°C. to free all the trapped intermediates from phenylhydrazine, except the osazone of the glycolaldehyde. The mixture was chilled and filtered to separate the precipitated phenylhydrazone of the benzaldehyde, after which the liquid was concentrated 3:1 and glycolaldehyde isolated by the chromatographic method previously reported[6]. The glycolaldehyde 2,4-dintrophenylosazone obtained melted at 325°C., and no depression was observed in the presence of the synthetic substance; found amounts, 10–70 mgm./l. Failure to trap larger amounts of glycolaldehyde is due to the fact that the aldehyde is degraded also in the presence of phenylhydrazine[6].

Glycolaldehyde was trapped even in experiments on fermentation of L-arabinose by a strain of *E. coli* and one of *Lactobacillus buchneri*. This work is continuing, and a detailed report will be published elsewhere.

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