

kinase (prepared according to Berger *et al.*, "Step 5"), 0.1 ml; alcohol dehydrogenase, 60 γ . Additions after 5 min. of preincubation with or without amytal: ethanol, 30 μ moles; DPN, 0.67 μ moles. pH 7.5. Final volume, 2.0 ml. Temp. 30° C. Gas phase, air. Time of incubation, 26 min.

1. The alcohol dehydrogenase was generously supplied by Dr. A. P. Nygaard, Biochemical Dept., Medical Nobel Institute, Stockholm.
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Trehalosemonophosphoric Acid, a Probable Intermediate in the Formation of Trehalose in Yeast

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In a recent note it has been shown by Leloir and Cabib¹ that trehalosemonophosphoric acid (THP) is formed from UDPG and G-6-P in extracts from yeast. Earlier Robison and Morgan² had found that this ester is normally produced during fermentation of glucose and fructose with dried yeast and zym. Some years later Veibel³ investigated the formation of THP during the fermentation of glucose with fresh brewers' yeast, incubated with toluene. He found that such a considerable amount of THP was formed that it could neither have been performed in the yeast nor could it have arisen through phosphorylation of trehalose in the yeast itself. Considerably later it was shown that even formation of free trehalose occurs during fermentation of glucose with mazeration juice from brewers' yeast^{4,5}. Elander and Myrbäck assumed that the disaccharide was formed through dephosphorylation of THP. Later the author has tried in vain to demonstrate THP in the same samples where trehalose was present, when the fermenting system was acetone dried bakers' yeast. The preformed trehalose of the yeast was removed by washing with phosphate buffer, pH 6.3. Coenzymes necessary for fermentation and probably also for the formation of trehalose were removed hereby, too, but were supplied through addition of boiled extract from air dried brewers' yeast which usually contains only small amounts of trehalose.

The reason of the failure to demonstrate THP in the presence of trehalose was not elucidated until close analysis of the different fractions of phosphate esters had been performed. The results will be published later in detail. Thus directly reducing esters, fructose, organic phosphate and the changes of these components at different times of hydrolysis have been determined. The optical rotations of the fractions were measured, and they were also subjected to paperchromatographic analysis. The analysis showed that one have to count with at least two hexosemonophosphoric esters which were unknown when Robison and Morgan studied the formation of THP. At this time they only had to deal with F-1,6-P, F-6-P and G-6-P, and the properties of these esters were well known. One of the new esters was G-1-P which has been shown by Cori, Colowick and Cori⁶ and by Kiessling⁷ to be an intermediate in the formation and degradation of glycogen in yeast. The other one has, according to the analysis, turned out to be F-1-P. Its occurrence in yeast is so far not known. The amount of the two phosphoric acids is quite considerable and they interfere with such determinations of THP as the optical rotation and the reducing power after hydrolysis. G-1-P as well as THP shows a high positive rotation. The rotation of F-1-P is negative. After acid hydrolysis G-1-P as well as THP becomes reducing. F-1-P, F-6-P and free fructose lose about 50 % of their reducing power when hydrolysed for 5 hours in 1 N H₂SO₄ at 100°. As the fructose esters formed a considerable part of the total esters, their decrease in reducing power will counteract the increase from THP and G-1-P.

After these investigations it was clear that a reliable determination of THP could only be performed if the concentration of G-1-P and F-1-P were known or if these esters could be removed from the fractions containing THP.

In a new series of experiments it was shown that G-1-P and F-1-P could be completely removed by precipitation with BaAc₂ and alcohol while the main part of THP was left in the trehalose fraction which only contained difficultly hydrolyzable organic phosphate. In fermenting mixtures THP is the only known ester with this property which is not reducing. It could thus be determined as the difference between glucose equivalent of organic phosphate and the glucose equivalent of directly reducing substances. During the time of the experiments, 24 hours, the concentration of THP was rather constant while the concentration of trehalose after 4 hours reached a maximum. After that it was slowly broken

down. When the concentration of trehalose was highest THP formed 21 % of trehalose. Details of the experiments will be published later.

The investigation is to be continued with paperchromatographic analysis and with attempts to isolate THP and F-1-P.

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On the Intestinal Absorption of Phospholipids in the Rat

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Earlier works on phospholipids by Artom and Swanson¹ and Bloom *et al.*² have been extended.

The mechanism concerned with the absorption of phospholipids has been studied on the rat using phospholipids, labelled in the glycerol, fatty acid or phosphate portions of the molecule and the distribution of radioactivity in the

collected lymph from the thoracic duct has been studied.

The experimental data demonstrate that phospholipids are hydrolyzed to a considerable extent during their absorption. Glyceride glycerol is utilized for the synthesis of lymph phospholipids and phospholipid glycerol for glycerides indicating an interconversion of these lipids during absorption.

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On the Chemical Nature of the FMN-binding Groups in the Old Yellow Enzyme

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Kinetics of Alcohol Dehydrogenases, Studied with the Aid of a Fluorescence Recorder

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