Table 1. The action of different agents on the P/O ratio in the succinic oxidase system.

Agent blocking fumarate oxidation	Additions after 5 min of pre-incubation with blocking agent	Respiration (μatoms oxygen)	Phosphorylation (μmoles phosphate)	P/O
—	succinate	16.6	27.6	1.66
amytal	succinate	16.2	23.0	1.42
Ca^{++}	succinate, ATP, Mn^{++}	16.9	19.0	1.12
amytal + Ca^{++}	succinate, ATP, Mn^{++}	16.8	15.3	0.91

Each Warburg vessel contained: rat liver mitochondria (prepared in 0.25 *M* sucrose — 0.01 *M* versene), 1/12 liver; adenylic acid, 4.3 μmoles; orthophosphate, 40 μmoles; glucose, 47 μmoles; KCl, 270 μmoles; Mg^{++}, 7.5 μmoles; yeast hexokinase (prepared according to Berger *et al.*, "Step 5"), 0.1 ml. Additions (where indicated): succinate, 30 μmoles; amytal, 3.6 μmoles; Ca^{++}, 1.15 μmoles; ATP, 1.5 μmoles; Mn^{++}, 1.5 μmoles. pH 7.5. Final volume, 2.0 ml. Temp 30° C. Gas phase, air. Time of incubation, 20 min.

1. Ernster, L., Lindberg, O. and Löw, H. *Nature (In press)*.
2. Eiler, J. J. and McEwen, W. K. *Arch. Biochem.* **20** (1949) 163.

The Action of 5-Ethyl-5-*iso*amylbarbiturate (Amytal) on the Oxidation of Reduced Diphosphopyridine Nucleotide (DPNH) in Rat Liver Mitochondria

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Previously (Jalling *et al.*, *cf.* p. 198) it has been shown that $1.8 \cdot 10^{-3}$ *M* amytal completely blocks those mitochondrial oxidations which proceed *via* pyridine nucleotides. To study further the localization of this effect within the respiratory chain, DPNH, as generated by added alcohol dehydrogenase [1] and ethanol was used as a substrate for mitochondria. As seen in Table 1, the respiration in this system is, in contrast to the case of DPNH generated by the mitochondrial dehydrogenases, but partially — about 33 % — inhibited by $1.8 \cdot 10^{-3}$ *M* amytal. The accompanying phosphorylation is, on the other hand, almost completely inhibited.

This indicates that DPNH generated by "external" dehydrogenases is probably oxidized by two pathways, only one of which is sensitive to amytal. Both pathways differ markedly with respect to the extent of phosphorylation coupled to them. In the system studied, the amytal-insensitive pathway seems to be inaccessible to DPNH generated by mitochondrial dehydrogenases.

These findings suggest a possible similarity between the action of amytal and that of antimycin A on mitochondrial DPNH- and TPNH-cytochrome *c* reductase systems, as recently studied by Reif and Potter [2] and by Pressman and de Duve [3].

Table 1. The effect of amytal on the oxidation of DPNH in rat liver mitochondria.

	Without amytal	*With* 1.8 m*M* amytal
Respiration (μatoms oxygen)	9.6	6.4
Phosphorylation (μmoles phosphate)	13.1	2.2
P / O	1.37	0.34

Each Warburg vessel contained: rat liver mitochondria (prepared in 0.25 *M* sucrose — 0.01 *M* versene), 1/12 liver; adenylic acid, 4.3 μmoles; orthophosphate, 40 μmoles; glucose, 47 μmoles; cytochrome *c*, 0.0002 μmoles; KCl, 270 μmoles; Mg^{++}, 7.5 μmoles; yeast hexo-

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 μmoles; DPN, 0.67 μmoles. 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.
2. Reif, A. E. and Potter, V. R. *Arch. Biochem. Biophys.* **48** (1954) 1.
3. Pressman, B. C. and de Duve, C. *Arch. intern. physiol.* **62** (1954) 306.

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 Cabib [1] that trehalosemonophosphoric acid (THP) is formed from UDPG and G-6-P in extracts from yeast. Earlier Robison and Morgan [2] had found that this ester is normally produced during fermentation of glucose and fructose with dried yeast and zymin. Some years later Veibel [3] 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 preformed 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 Cori [6] and by Kiessling [7] 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* H_2SO_4 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 $BaAc_2$ 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