mice was found to contain insufficient progesterone to give a positive response by the sensitive McGinty technic. Lipid extracts of the AP which stimulate duct growth in the male mouse were also found to be negative for progesterone. These observations are taken to indicate that neither the mammogenic duct nor lobule-alveolar effects of the AP are due to the presence of progesterone.

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Effect of Selective Poisons on Utilization of Glucose by Yeast.

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Well washed suspensions of *Saccharomyces cerivisiae* (American Type Culture Collection No. 575) may utilize glucose by oxidizing it to carbon dioxide and water, by fermenting it to carbon dioxide and alcohol, and by synthesizing it to cell material. With well aerated suspensions the oxygen consumption, as determined at 37°C by the Warburg technic, is approximately one-third of the theoretical amount required for complete combustion, less than 5% of the sugar is fermented and the remainder appears to be synthesized to cellular material.

The possibility of so altering the course of glucose metabolism by yeast that the substrate will be entirely oxidized has been suggested by studies with a number of microörganisms. Clifton and Logan,[1] using resting suspensions of *Escherichia coli*, showed that glucose is oxidized to completion in the presence of sodium azide or 2:4-dinitrophenol (DNP). Similarly, Winzler[2] showed that M/1250 azide or M/2000 DNP blocks synthesis from acetate by yeast suspensions, the oxidation going to completion in the presence of these poisons.

The following results indicate that sodium azide and DNP block synthesis from glucose by yeast. However, in the presence of these poisons the oxidation does not go to completion but instead fermentation accounts for the bulk of the substrate disappearing from the medium. Typical results are reported in Table I.

In the presence of M/1000 DNP the assimilatory process is in-

[1] Clifton, C. E., and Logan, W. A., *J. Bact.*, 1939, **37**, 523.

[2] Winzler, R. J., *J. Cell. and Comp. Physiol.*, 1940, **15**, 343.

TABLE I.

Effect of Sodium Azide and of Dinitrophenol on Aerobic Utilization of Glucose by Yeast. Each Warburg Vessel Contained 0.1 ml of M/10 Glucose, 0.1 ml of Poison (or H_2O) and 1.8 ml of a M/15 Phosphate Buffer (pH 6.0) Suspension of Yeast.

Poison	$\mu l\ O_2$	$\mu l\ CO_2$	R.Q.	%substrate oxidized	%substrate fermented	Total
—	466	481	1.03	34.7	3.1	37.8
M/1000 DNP	495	779	1.57	36.8	63.4	100.2
M/10,000 NaN_3	543	691	1.27	40.4	30.3	70.7
M/2000 NaN_3	104	520	5.00	7.7	85.3	93.0

hibited and fermentation now accounts for two-thirds of the added substrate. The oxidative system appears to be unaffected by this concentration of DNP. Both respiration and synthesis, however, are inhibited by sodium azide, the substrate being almost entirely fermented in the presence of M/2000 azide. Although the total oxygen consumption was greater in the presence of M/10,000 NaN_3 than in its absence, the Q_{O_2} was significantly lower (Q_{O_2} normal = 90; with M/10,000 azide = 50; with M/2000 azide = 8) and there was an appreciable lag period before the rate of oxygen consumption reached a constant level. The oxygen consumption observed in the presence of azide may be attributed to oxidation of the alcohol which appears as an end product of fermentation. The rates of glucose and of alcohol oxidation in the absence of a poison are approximately equal. Oxidation of the latter compound proceeds at about 60% of the normal rate in the presence of M/10,000 azide but is almost completely inhibited by M/2,000 azide. These results suggest that fermentation may be the normal mode of glucose utilization by this strain of yeast, the alcohol produced being immediately oxidized to carbon dioxide and water. This hypothesis is difficult to reconcile with Lundsgaard's[3] report that fermentation of glucose by yeast may be blocked by monoiodoacetic acid, respiration being maintained near its normal intensity. It must be borne in mind, however, that Kluyver[4] has reported slightly higher concentrations of this poison also block respiration.

In order to confirm the manometric results a number of Warburg experiments were carried out on a semi-macro scale, subsequent analyses being made for alcohol, glycerol, glucose and reducing sugar content of the yeast cells following acid hydrolysis. The data from 2 such experiments are summarized in Table II. These results show that alcohol, only a trace of which appears as an end product

[3] Lundsgaard, E., *Bioch. Z.*, 1930, **220**, 1, 8.

[4] Kluyver, A. J., and Hoogerheide, J. C., *Proc. Akad. Wetensch. Amsterdam*, 1933, **36**, 596.

TABLE II.

Recovery of Initial and End Products from Yeast Suspension. Warburg Experiment Terminated as Soon as Rate of Metabolism Fell to Endogenous Level, and Cells Immediately Separated from Medium by Centrifugation. 10 ml Yeast Suspension in M/15 Phosphate Buffer at pH 6.0, glucose M/50, 37°C; Oxygen Atmosphere.

Medium	Glucose only	Glucose + M/10,000 NaN_3	
Mg carbon added as glucose	14.40	14.40	14.40
Mg carbon recovered as:			
CO_2	6.00	4.42	5.38
Alcohol	0.18	5.91	5.34
Glycerol	0.36	0.58	0.51
Glucose in medium	1.32	0.43	0.18
Synthesized carbohydrate	3.88	1.08	trace
Total	11.74	12.42	11.41
% carbon recovered	81	86	79

in the absence of azide, accounts for 30-40% of the added glucose when the cells are poisoned with M/10,000 azide. At the same time the synthesis of carbohydrate is markedly inhibited. A trace of glycerol is formed both in the presence and in the absence of azide.

It is also shown in Table II that, even in the presence of azide, 15-20% of the added glucose is unaccounted for as end products. A similar discrepancy was observed when the amount of synthesis was determined by increase in dry weight of the cells. Since tests for acetyl-methyl-carbinol and volatile, pyruvic, and lactic acids were consistently negative or showed only traces of these compounds, it has been tentatively assumed that there may be an accumulation of non-reducing phosphate esters in the experimental medium. Further evidence supporting this hypothesis will be reported at a later date.

Summary and Conclusions. The synthesis of carbohydrate from glucose by resting yeast is completely inhibited by M/1000 2:4-dinitrophenol. Fermentation, however, is markedly stimulated, while respiration appears to be unaffected. Both synthesis and respiration are inhibited by M/10,000 sodium azide, but a portion of the alcohol formed by fermentation of the glucose is later oxidized. Higher concentrations of azide also inhibit the oxidation of alcohol, thus permitting the accumulation of alcohol as an end product of the utilization of glucose.