Work is now in progress to identify the volatile material in cockroach grease, and to examine the possibility of wax solvents being concerned in the secretion of waterproofing layers in other insects.

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A Depressant Effect of Adrenaline on the Rat Diaphragm

It is well known that adrenaline usually augments the contractions of striated and cardiac muscle; this is also true of the isolated phrenic nerve diaphragm preparation of the rat stimulated rhythmically, especially if it is depressed by excess potassium.

Goffart and Brown¹, in 1947, reported a depressant action of adrenaline correlated with a low intramuscular potassium, and it has now been found that when the Krebs-Ringer solution in which the preparation is placed is modified to contain less than 2.5 mgm. per cent calcium, adrenaline at a concentration of $1/10^7$ – $1/10^8$ produces a marked and rapid depression of the responses to electrical stimulation. The depression is preceded by a brief potentiation and is followed by a slower spontaneous recovery, the whole response taking 15–30 min. The extent of the depression produced varies somewhat as the initial contraction height, but its duration is never proportionately increased. From similar experiments in Tyrode's solution, potassium ions appear to be essential for the recovery.

The depression is not present in denervated muscle, but occurs in neuromuscular block brought about by curare and by complete absence of calcium.

This observation is in accord with the observation of McDowall and McWhan² that blood vessels perfused with Ringer's solution deficient in calcium are dilated by adrenaline, and with those of many observers who have found that adrenaline may depress the perfused heart under similar conditions.

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Pyruvate Metabolism in *Saccharomyces cerevisiae*

It is generally assumed that *Saccharomyces cerevisiae* (baker's yeast) can metabolize pyruvate: (a) by simple decarboxylation, a reaction not requiring oxygen, catalysed by carboxylase¹, and (b) by oxidation, as described by Lieben² and Meyerhof³, an oxygen-requiring reaction the precise mechanism of which is not known. In this communication

experiments are briefly described which provide a general explanation of the mechanism of pyruvate aerobic oxidation by yeast and determine the role of carboxylase.

Pure suspensions of starved, pressed baker's yeast have been used for the experiments. General methods are the same as in a previous communication⁴. The oxygen uptake and carbon dioxide evolution were measured by the Warburg direct method⁵. Pyruvate was estimated with the Friedemann and Haugen⁶ or the Straub⁷ method, with consistent results. All experiments have been carried out at pH 4.5, corresponding to the physiological hydrogen ion concentration of the medium for the growth of *S. cerevisiae*⁸.

TABLE 1. EFFECT OF DICARBOXYLIC ACIDS AND CARBON DIOXIDE ON PYRUVATE CONSUMPTION.

5.7 mgm. yeast (weighed after drying at 100°) suspended in 2.0 ml. 0.05 M buffer, in Warburg flasks with air, or air and 5 per cent carbon dioxide, in the gas space. Potassium hydroxide in the central well of the flasks filled with air. 60 μ M pyruvate added after 30 min. equil. Final pH, 4.5. The figures represent μ M pyruvate consumed after 90 min. incubation, at 30°, and allow for the respective controls.

Medium	Atmosphere	
	Air	Air and 5% carbon dioxide
Phthalate	10.4	17.0
Succinate	17.0	16.4
Fumarate	18.7	17.9
L-Malate	18.0	18.3

Our results can be summarized as follow. (1) At pH 4.5, living yeast cells consume pyruvate only in the presence of oxygen, as earlier reported by Smythe⁹, notwithstanding the significant amount of carboxylase present in the cells, as proved by (a) the ability to ferment glucose, evolving carbon dioxide under strictly anaerobic conditions, and (b) the intense carboxylatic activity of the saline extracts obtained from the cells after drying. (2) C_4 -dicarboxylic acids stimulate the aerobic pyruvate consumption by the yeast cells (Table 1). It may be admitted that the effect of dicarboxylic acids is produced with about the same concentration activating pyruvate oxidation by the brain pyruvate oxidase¹⁰ as the concentration of the acids inside the yeast cells is 5–10 times smaller than in the suspension medium¹¹. Dicarboxylic acids replace the stimulating effect of carbon dioxide on pyruvate consumption, earlier described by Brandt¹², and confirmed by our experiments. This implies that the effect of carbon dioxide may be mediated through synthesis of a dicarboxylic acid (Wood and Werkman reaction¹³). (3) Dinitrophenols, some of which (for example, the 2,4-compound) dissociate oxidation from phosphorylation and interfere with oxidations where the substrate activation is apparently dependent on a phosphate exchange¹⁴, inhibit intensely pyruvate oxidation and the corresponding carbon dioxide formation (Table 2). At pH 4.5 the pI_{50} ($-\log_{10}$ molarity inhibiting 50 per cent pyruvate consumption) is 4.50, 3.50 and 3.72 for 2,4-, 2,5- and 2,6-dinitro-

TABLE 2. EFFECT OF 2,4-DINITROPHENOL ON PYRUVATE OXIDATIVE DECARBOXYLATION

12.1 mgm. (dry wt.) yeast, suspended in 0.05 M phosphate with 52.0 μ M pyruvate, pH 4.5. Air in the gas space. The figures represent μ M oxygen or pyruvate consumed and carbon dioxide evolved after 90 min. incubation, at 30°, and allow for the respective controls. Relative inhibition in parentheses.

Inhibitor	CO ₂	O ₂	Pyruvate
None	12.5	10.3	10.8
10^{-4} M 2,4-dinitrophenol	1.2 (90.4)	1.3 (87.4)	0.8 (92.6)