

phoretic mobility measurements. I am indebted to Dr. M. Dixon for suggesting the problem, and for advice and encouragement.

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Reversion of the Effect of Vitamin B₁ on Yeast Fermentation as a Result of Growth in the Presence of Cocaine

IN order to test the hypothesis (unpublished) that the habit-forming properties of certain drugs might be due to their ability to induce adaptive changes in the enzyme systems of cells and tissues, we have made comparative studies of the effects of cocaine on the respiration and fermentation of pure strains of yeasts, previously grown in the presence and in the absence of cocaine. A detailed report of this work will be published elsewhere. In brief, we find that cocaine in 0.004 M concentration depresses the respiration (*Torulopsis utilis*, var. *major*, type No. 6593) and the fermentation (*Saccharomyces cerevisiae*, type No. 815) of the washed cells in glucose and phosphate buffer at pH 7.0, but is without effect at pH 6.0. Some degree of tolerance towards the drug appears to be established, as the depressant effect is less with cells that had been grown in the presence of cocaine.

It has been shown¹ that addition of vitamin B₁ to alkali-washed yeast in the presence of cocarboxylase stimulates the carboxylase activity. We have investigated the action of vitamin B₁ on the fermentation-rate of *S. cerevisiae*, and we find that whereas the acid-washed yeast in 2 per cent glucose and M/20 phosphate at pH 7.0 is stimulated by 0.004 M thiamin, that which had been grown in the presence of 0.004 M cocaine is actually depressed by 0.004 M thiamin. In all these experiments, one- or two-day old cultures were used. They were grown at 25° C. in nutrient medium (20 gm. malt extract, 4 gm. K₂HPO₄, 1 gm. KH₂PO₄, and 30 gm. glucose per litre; pH 7.0) with and without cocaine (0.004 M).

Tubes of nutrient medium (a) with and (b) without cocaine (0.004 M) were inoculated with *S. cerevisiae* and incubated at 25° C. After one or two days growth, the cells were separated by centrifuging, washed twice with M/20 phosphate buffer at pH 6.0, once at pH 7.0 and suspended in M/20 phosphate buffer containing 2 per cent glucose. 1.0 ml. of the yeast suspension, either (a) or (b), was placed in each fermentation vessel, together with 2.5 ml. of the buffer-substrate solution, with and without thiamin (0.0056 M). Production of carbon dioxide was measured in the Warburg apparatus at 30° C. The contents of the flasks were acidified by the addition of acid from their side-arms, at zero time for the controls, and one hour later for the experimental vessels. From the data so obtained the volumes of carbon dioxide produced in one hour were calculated.

In a typical experiment the yeast, previously grown in the absence of cocaine, produced in one hour 208 μl. carbon dioxide per ml. yeast suspension and 218 μl. carbon dioxide in the presence of added vitamin B₁; whereas that previously grown in the

presence of cocaine produced 205 μl. carbon dioxide, but only 176 μl. carbon dioxide in the presence of vitamin B₁. The effect in this experiment of the added vitamin B₁ is therefore to stimulate the normal yeast (5 per cent activation) and to depress the cocaine-grown yeast (14 per cent inhibition).

Our experiments suggest that small quantities of cocaine can induce in living cells a change of marked physiological significance, and that the normal function of vitamin B₁ suffers some modification as a result. Further investigations are in progress and it would be premature at this stage to indulge in speculation as to the possible mechanisms involved. Similar studies with morphine and with nicotine are also in the course of investigation. We would like to emphasize that the medium we used is favourable to the healthy growth of the yeasts, and in this respect the conditions of our experiments are quite different from those usually employed in the study of enzyme adaptation, where the organisms are partly starved in order to force them to utilize some 'unnatural substrate' as a food.

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Existence of an Enzyme catalysing the Hydrogenation of Oximes in Silkworms

RECENTLY¹, I found that, in silkworms, nitrites, nitrates and ammonium salts could be converted into oximes, and suggested that this insect might utilize inorganic nitrogenous salts as nutritive substances. I have now been able to demonstrate that in the silkworm body there exists an enzyme which catalyses the transformation of oximes to corresponding amino compounds.

A typical experiment was as follows. Fifth instar worms from which the digestive organs were carefully removed were thoroughly washed with sterilized water. 3 gm. of washed bodies was mixed with 0.5 gm. of quartz sand, completely ground and diluted with water to 100 ml. The enzyme solution obtained contained no intact tissue cells or micro-organisms. For the determination of enzymatic activity, 5 ml. of this enzyme solution was mixed with a solution of pyruvic acid oxime or acetoxime and kept at 30° C. for 1 hr. Then the mixture was heated at 100° C. for 5 min., centrifuged after adding 0.05 ml. of saturated lead acetate solution, and the oxime content in the supernatant fluid was estimated. The total volume of reaction mixture was 10 ml., and in the control the enzyme solution was used after heating at 100° C. for 5 min.

Experiments showed that the enzyme activity was reduced to half by heating at 60° C. for 5 min. The optimum pH of enzyme action was about 6 and the optimum temperature 40° C. Under the experimental conditions mentioned above, half the oxime in 0.001 M solution was decomposed, and, even in the case of 0.1 M solution, about 20 per cent of oxime added was split. In the test solutions, free hydroxylamine was not detected. It was further observed that the amount of amino-nitrogen, which was determined according to Van Slyke's method,