Some Effects of Cocaine on the Metabolism of *Saccharomyces cerevisiae*

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Since the classical work of Dienert (1900) the phenomenon of enzyme adaptation in micro-organisms has provided material for much research and controversy and several reviews have been written on the subject (Dubos, 1940; Gale, 1943; Spiegelman, 1948, 1950; Hinshelwood, 1950). Enzymic adaptations of a purely quantitative nature have also been demonstrated in mammalian tissues (Cruz, 1946; Davies & Yudkin, 1951).

In recent years much progress in pharmacology and chemotherapy has resulted from the study of the interaction of drugs and enzyme systems. It is possible that the habit-forming properties of certain drugs might be due to their ability to induce adaptive changes in enzyme systems, whereby the cells or tissues become dependent on the presence of the drug for their continued respiratory and metabolic balance. In the present work we have begun our investigations into the validity of this hypothesis by making comparative studies of the effects of habit-forming drugs on the respiration and fermentation of pure strains of yeasts, previously grown in the presence and in the absence of the drug under consideration. We have chosen yeast cells for our preliminary experiments in order to avoid the use of animals for work of an exploratory nature.

In a preliminary report of part of this work (Ryman & Walsh, 1951) evidence was presented that the effect of added vitamin B₁ on the fermentation rate of washed yeast cells was considerably modified, even reversed, with cells which had been grown in the presence of cocaine. The details of these experiments and of investigations into the action of cocaine on the carboxylase activity of *Saccharomyces cerevisiae* are here presented.

MATERIALS AND METHODS

Yeast. *Saccharomyces cerevisiae* (National Collection of Type Cultures, no. 815) was used throughout this work.

Media. Media having the following composition were employed. (A) Malt extract, 20 g., KH₂PO₄, 4 g., K₂HPO₄, 1 g. and glucose 30 g./l., pH 7.0. (B) Medium A containing 0.004M-cocaine and the pH adjusted to 7.0. Plugged tubes, each containing 8 ml., were sterilized at 100° for 30 min.

Buffer. Clark and Lub's standard buffer, 0.05M-phosphate.

Yeast suspension. Tubes of media A and B respectively, were inoculated with *S. cerevisiae* and incubated at 25°. After a suitable period of growth the cultures were centri-

fuged, the cells were shaken with buffer, pH 6.0 (5 ml.), and again centrifuged after allowing them to stand for 15 min. This procedure was repeated and followed by a brief washing (approx. 2 min.) with buffer, pH 7.0. The cells from A and B were suspended finally in equal volumes of distilled water.

Dried yeast suspension. Cells from cultures A and B, after washing as above, were shaken with acetone (5 ml.), centrifuged at once and dried as rapidly as possible by connecting the centrifuge tubes in parallel to a suction pump.

Sodium pyruvate. Sodium pyruvate was prepared from commercial pyruvic acid by the method of Robertson (1942).

Determination of CO₂. All measurements were made in the Warburg apparatus at 30°. For CO₂ produced at pH 7.0, the contents of the flasks were acidified by the addition of 0.1 ml. 30% (v/v) H₂SO₄ from their side arms, at zero time for the controls and after a suitable interval for the experimental vessels. From the data so obtained, the volumes of CO₂ produced during the experimental period were calculated. The oxygen uptake under the experimental conditions was negligible.

RESULTS

Effects of cocaine on glucose fermentation

The effects of cocaine (0.004M) on the production of carbon dioxide at pH 7.0 with glucose as substrate by *S. cerevisiae*, previously grown in the presence (B) and in the absence (A) of cocaine, are compared in Table 1. Higher concentrations of cocaine were found to be unsuitable for these experiments since fermentation rates became somewhat erratic and death of the cells frequently resulted. From preliminary experiments we found that small changes in pH had a considerable effect on the behaviour of the yeast towards cocaine. In each experiment, therefore, the buffer-substrate solution which contained cocaine was carefully adjusted to the same pH as the solution without cocaine.

It can be seen from the table that yeast grown in medium B is rather more sensitive towards cocaine than is that grown in medium A and that, within the limits of the experiments, this effect is independent of the age of the cultures.

The average ratio of the inhibitions (b/a) is 1.3 (1.2–1.5). In these and in all experiments where the behaviour of yeast grown in the presence of cocaine is compared with that of yeast grown in the absence of cocaine, the suspensions A and B were freshly and simultaneously prepared from twin cultures. That is, tubes of media A and B respectively were inoculated at the same time with similar quantities of yeast from the same parent culture. They were incu-

Table 1: *Effect of cocaine on the fermentation of Saccharomyces cerevisiae*

(Each flask contained 3% (w/v) glucose in 0.05M-phosphate buffer, pH 7.0, with or without 0.006M-cocaine (2.0 ml.) and yeast suspension (1.0 ml.). For each experiment the yeast suspensions, *A* and *B*, were freshly and simultaneously prepared from twin cultures which had been incubated together. In each experiment suspensions *A* and *B* contained an approximately equal number of cells.)

Duration of experiment (min.)	Age of culture (hr.)	<i>A</i>			<i>B</i>		
		Yeast grown in nutrient medium (no cocaine)			Yeast grown in nutrient medium (0.004M-cocaine)		
		CO ₂ (μl./hr.)		(a)	CO ₂ (μl./hr.)		(b)
		Cocaine (0.004M)	Inhibition (%)		Cocaine (0.004M)	Inhibition (%)	
45	48	127	97	24	123	80	35
30	48	302	234	23	248	172	31
60	24	209	140	33	146	89	39
60	24	129	97	25	121	82	32
60	48	129	90	30	107	70	35
45	4	143	112	22	153	108	29

Table 2: *Effect of vitamin B₁ on fermentation of glucose by Saccharomyces cerevisiae*

(Each flask contained 3% (w/v) glucose in 0.05M-phosphate buffer, pH 7.0, ±0.006M-vitamin B₁ (2.0 ml.) and yeast suspension (1.0 ml.). For each experiment the yeast suspensions, *A* and *B*, were freshly and simultaneously prepared from twin cultures which had been incubated together. In each experiment the suspensions, *A* and *B* contained an approximately equal number of cells. In experiments where the cells were to be in contact with cocaine for several days the twin cultures were subcultured into corresponding media and the subcultures were used.)

Duration of experiment (min.)	Age of culture (days)	Total time of <i>B</i> on cocaine (days)	<i>A</i>			<i>B</i>		
			Yeast grown in nutrient medium (no cocaine)			Yeast grown in nutrient medium (0.004M-cocaine)		
			CO ₂ (μl./hr.)		Activation (%)	CO ₂ (μl./hr.)		Activation (%)
			Aneurin (0.004M)	Inhibition (%)		Aneurin (0.004M)	Inhibition (%)	
60	2	2	247	274	+ 11	267	194	- 27
60	2	4	202	201	0	206	169	- 18
60	2	6	208	218	+ 5	205	176	- 14
30	2	4	168	172	+ 2	202	194	- 4
30	2	6	232	248	+ 6	190	164	- 14
60	2	3	183	183	0	163	135	- 17
60	2	4	111	129	+ 16	101	96	- 5
60	1	1	140	148	+ 6	162	128	- 21
60	2	5	137	140	+ 2	153	139	- 9
60	3	4	150	204	+ 36	162	158	- 2
60	1	1	178	222	+ 24	172	163	- 5
60	2	2	129	135	+ 5	129	116	- 10

bated and harvested together, and all subsequent procedures were conducted in parallel.

The results of cell counts, made with quantitative dilutions in 1% phenol, indicated that growth occurred at closely similar rates in the twin cultures and, in so far as we were able to judge, the cultures showed no difference in microscopical appearance.

Effects of vitamin B₁ on glucose fermentation

The effects of vitamin B₁ (0.004M) on the production of carbon dioxide at pH 7.0 with glucose as substrate by *S. cerevisiae* grown in media *A* and *B*

are compared in Table 2. The effect in these experiments of the added vitamin B₁ is to stimulate the normal yeast and to depress the cocaine-grown yeast.

Effect of cocaine on carboxylase activity

Carbon dioxide was not produced by the yeast suspension with added pyruvate as substrate. This is in agreement with others who have shown that pyruvate is not decarboxylated by the intact yeast cell (Stoppani, 1951). Dried yeast was used therefore for experiments with pyruvate.

Cocaine (0.004M) inhibited the carboxylase activity (pH 7.0) of either acetone-dried *S. cere-*

visiae or commercial dried baker's yeast by 80–90%. In a typical experiment with the washed, commercial yeast, inhibitions of 50 and 83% respectively were obtained with 0.002 and 0.004M cocaine. A concentration of 0.005M cocaine completely inhibited the carboxylase system. The carboxylase activity was only slightly inhibited by cocaine at pH 6.0.

A comparison of the inhibition by cocaine of carbon dioxide production by yeast grown in medium A, with that by yeast grown in medium B, was made with washed, acetone-dried *S. cerevisiae*. Pyruvate in optimum concentration, 0.162M (Green, Herbert & Subrahmanyam, 1941) at pH 7.0 was used as substrate. The effect of cocaine (0.001M) was to inhibit both the yeast grown in the presence and that grown in the absence of cocaine to a similar extent (45%).

Table 3. *Effect of growth in cocaine on carboxylase activity of Saccharomyces cerevisiae*

(Each flask contained 0.333M-pyruvate in 0.05M-phosphate buffer, pH 6.0 (1.0 ml.) and suspension of dried yeast (1.0 ml. $\equiv$ 3–4 mg. dry wt.). For each experiment the yeast suspensions, A and B, were freshly and simultaneously prepared from twin cultures which had been incubated, harvested and dried together.)

Age of culture (days)	CO ₂ μ l./mg. dried yeast/hr.	
	A Yeast grown in nutrient medium (no cocaine)	B Yeast grown in nutrient medium (0.004M-cocaine)
2	71	74
2	72	73
3	51	48
3	62	67
3	50	46

In view of the high inhibitory effect of cocaine on the carboxylase system, the possibility was investigated that some compensatory adaptation of a quantitative nature might have occurred in the yeast during growth in medium containing cocaine. The carboxylase activity per mg. dry weight of yeast was determined and the results are summarized in Table 3.

DISCUSSION

In spite of the inhibitory effect of cocaine on fermentation, *S. cerevisiae* grows quite well in its presence, provided that the concentration be not greater than 0.004M (0.005M inhibits growth completely), and in this respect the organism appears to acquire a tolerance for the drug. Similarly, the yeast which has been grown in the presence of cocaine grows quite well in its absence. On the other hand, the experiments with vitamin B₁ indicate that changes have occurred in the cells as

a result of growth in the presence of cocaine and it is quite conceivable that if similar changes occurred in the tissues of a higher animal with a nervous system, they might well contribute towards the establishment of a state of addiction.

The apparent increase in sensitivity of the cocaine-grown yeast towards the drug, as evidenced by the experiments on glucose fermentation, may be a cumulative effect or it may be the result of a change in the permeability of the cell membrane. Danielli (1950) has pointed out that evidence has been accumulating that cocaine exerts its action primarily on the cell surface.

The increase in enzymic toxicity which cocaine exhibits with rise in pH has been observed with other alkaloids; nicotine and morphine, for example, both inhibit cellular respiration at pH 7, but neither exhibit more than negligible activity at pH 6. These observations are in agreement with Rona and others (Rona & Bloch, 1921; Rona & Grassheim, 1923) who concluded from their studies with quinine that the free, un-ionized base was the inhibiting substance. Travell (1940) has demonstrated that the absorption of certain alkaloids including cocaine is dependent on the pH, and she concluded that the rate of absorption is closely related to the concentration of the free, undissociated base. It is interesting to compare the results of these observations with the behaviour of acids, such as hydrazoic and malonic, which inhibit more strongly as the pH falls (Beever & Simon, 1949).

Cook & McDevitt (1945) have shown that yeast respiration is depressed by cocaine and other local anaesthetics. They observed that this effect was not offset by addition of yeast extracts, but temporary protection was afforded if the extract was added before the drug. These observations are of particular interest in view of our experiments in which we find the fermentation of glucose by cocaine-grown yeast is inhibited by added vitamin B₁, whereas the normal yeast is slightly activated. This effect is small when compared with the results obtained with vitamin B₁ on certain alkali-washed, dried, baker's yeasts in the presence of added cocarboxylase (Ochoa & Peters, 1938; Atkin, Schultz & Frey, 1939; Lipton & Elvehjem, 1940). We have avoided washing with alkali, which dissociates carboxylase (Green *et al.* 1941), in view of the possibility that some modification in the coenzyme might have occurred under the influence of cocaine. The washing procedure adopted was aimed at removing free cocaine.

This modification in the normal function of the vitamin led us to investigate the effects of cocaine on pyruvate metabolism, and it has been shown that the alkaloid is a powerful inhibitor of the carboxylase system. So far, however, we have been unable to demonstrate any direct competition between cocaine and vitamin B₁.

Determinations of the carboxylase activity of the yeast indicate that no quantitative adaptation had occurred as a result of growth in the presence of cocaine.

SUMMARY

1. The effects of cocaine and of added vitamin B₁ on the glucose fermentation of *Saccharomyces cerevisiae* at pH 7, previously grown in the presence and in the absence of cocaine, have been compared. There is a difference in behaviour towards added

vitamin B₁ between the normal yeast and the cocaine-grown yeast.

2. Cocaine in concentration sufficient to arrest growth (0.005M) at pH 7 inhibits completely the carboxylase system of *S. cerevisiae*.

3. The carboxylase systems of yeast grown in the presence and in the absence of cocaine are inhibited by cocaine to a similar extent.

4. No quantitative adaptation of the carboxylase system of *S. cerevisiae* has been found as a result of growth in the presence of cocaine at pH 7.

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Fat Utilization by Muscle

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It has generally been stated that the primary function of the liver in the catabolism of the fatty acids is to convert them into ketone bodies. Only the further oxidation of these intermediate substances was presumed to represent an extra-hepatic function. On the other hand, it has long been questioned whether muscle can also oxidize higher fatty acids directly. Gemmill (1942) found no experimental evidence to indicate the direct utilization of fat by muscle, but this view was not shared by Stadie in his review of this subject in 1945. Cruickshank & Kosterlitz (1941) were able to demonstrate that when cardiac glycogen was low the heart was able to utilize stored fatty acids for its metabolic needs. Lehninger (1946) showed that a rat-heart muscle suspension oxidizes octanoate, laurate and palmitate. Geyer, Matthews & Stare (1949) demonstrated the oxidation of octanoate and tri-laurin by various extra-hepatic tissue slices; and Grafflin & Green (1948) had previously shown that

kidney enzyme preparations rapidly oxidize all of the saturated monocarboxylic fatty acids. In a recent investigation Goldmann *et al.* (1950) undertook the study of the extent to which the extra-hepatic tissues of the intact animal participate in the oxidation of physiologically occurring fatty acids. Palmitic acid labelled with ¹⁴C at the carboxyl position was used, and its conversion to ¹⁴CO₂ was compared in normal and in hepatectomized dogs. Evidence was presented for the view that less than 40 % of the ¹⁴CO₂ exhaled by the normal dog could have resulted from direct oxidation of the labelled palmitic acid by all the extra-hepatic tissues of the animal. At the same time a study of the oxidation of isotopic palmitic acid by various animal tissues was published by Weinhouse, Millington & Volk (1950). Palmitic acid was found to be oxidized *in vitro* by a variety of tissues besides liver; for example, by pigeon-breast muscle, but not by rat skeletal muscle or by rat brain.