EFFECTS OF SODIUM CAPRYLATE ON CANDIDA ALBICANS

II. INFLUENCE OF VARIOUS CONCENTRATIONS ON BIOCHEMICAL CHANGES

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Abstract

Payne, W. J. (University of Georgia, Athens) and E. R. Bannister. Effects of sodium caprylate on Candida albicans. II. Influence of various concentrations on biochemical changes. J. Bacteriol. 86:558–562. 1963.—Suspending resting cells of Candida albicans in buffered solutions of 0.1 m sodium caprylate resulted in leakage of K+, protein, ribonucleic acid (RNA), and carbohydrate (including glucose) but not of deoxyribonucleic acid (DNA) or sterol. In 0.025 m caprylate, the yeast leaked carbohydrate but not any of the other metabolites, whereas at 2.5 \( \times 10^{-3} \) m or in buffer alone there was no significant leakage. Inclusion of 2.5 \( \times 10^{-3} \) m sodium caprylate in Sabouraud dextrose broth cultures, however, suppressed growth greatly. Assays of relative protein, RNA, and DNA contents of dried cells taken from the suppressed cultures revealed changes in the rates of production of each from those of untreated cultures.

Previously, we observed that sodium caprylate at a concentration of 0.1 m inhibited aerobic and anaerobic growth, as well as respiration and fermentation in resting cells, of Candida albicans (Watt, Adams, and Payne, 1962). A much lower concentration of 2.5 \( \times 10^{-3} \) m, however, was barely tolerated by the yeasts, which could produce a few nonmaturing buds when exposed to this concentration in the culture medium. Respiration of resting cells was greatly elevated in the presence of this lower concentration of sodium caprylate.

More recently, thin-section studies by Adams, Painter, and Payne (1963) have shown that exposure of C. albicans to varying concentrations of the inhibitor in the range between 2.5 \( \times 10^{-3} \) and 0.1 m resulted in progressive loss of organization of the cytoplasm and its organelles. At the higher concentration, the cytoplasm was seen largely to have leaked out of the cells. This paper represents a study done simultaneously, in which we observed biochemical changes and now correlate these observations with those obtained cytologically.

Materials and Methods

The strain of C. albicans used in this study was employed previously (Watt et al., 1962; Adams et al., 1963). Cells were cultured on Sabouraud dextrose broth at 37 C with constant agitation for 24 hr. In certain experiments, enough sodium caprylate to make the medium 2.5 \( \times 10^{-3} \) m with respect to this compound was filter-sterilized and added aseptically to the broth before inoculation.

Analysis for leakage products. Yeast cells were harvested by centrifugation, washed twice with 0.067 m sodium phosphate buffer (pH 7.0), and suspended in fresh buffer in a concentration which gave 5% light transmission at 420 m\( \mu \) (8.1 mg dry weight/ml). Enough sodium caprylate to make the suspending medium either 2.5 \( \times 10^{-3} \), 0.025, or 0.1 m was added, and 10-ml samples were taken at zero time and then periodically during 3-hr incubations at 30 C. Control suspensions without added sodium caprylate were assayed as well. At the appropriate times, the tubes containing the suspensions were placed in an ice bath and chilled to 0 C. The yeasts were centrifuged off at 0 C in a refrigerated centrifuge (Servall RC-2), and the supernatant fluids were collected for analysis.

The K+ content of test and control systems was analyzed by flame photometry. Protein was determined by the method of Lowry et al. (1951), ribonucleic acid (RNA) by the orcinol technique, deoxyribonucleic acid (DNA) by the

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1 A preliminary account of this work was presented at the 63rd Annual Meeting of the American Society for Microbiology, Cleveland, Ohio, 5 to 9 May 1963.
diphenylamine technique (Dische, 1955), and carbohydrate (after extraction of nucleic acids) with the anthrone reagent as described by Pelezar, Hansen, and Konetzka (1955). These latter, extracted supernatant fluids were then analyzed chromatographically as well for mono- or oligosaccharides. We assayed saponified samples of the test supernatant fluids for sterols by the chromatographic method of Lampen et al. (1962).

**Analyses of cellular components.** Yeasts in cultures incubated in Sabouraud dextrose broth containing 2.5 × 10⁻³ M sodium caprylate for various periods from 0 to 24 hr were harvested, washed, and dried over P₂O₅ in a vacuum desiccator. Dried samples from each incubation-time period were then powdered with mortar and pestle and extracted and defatted in turn with cold 10% trichloroacetic acid, 70% ethanol, and ethanol-ether (3:1). The residue was then extracted with 5% trichloroacetic acid for 15 min at 90 to 95°C. The RNA and DNA contents of this extract were determined, and the protein content of the hot trichloroacetic acid-insoluble residue, dissolved in 1 N NaOH, was then assayed.

**Results**

The results in Fig. 1 indicate that suspending *C. albicans* in the high concentration (0.1 M) of sodium caprylate resulted in a rapid loss of K⁺, followed by a much less rapid leakage. This slow leakage was no greater, in fact, than that observed to leak from cells suspended in buffer or in buffer containing the two lesser concentrations of sodium caprylate. The actual exposure time in these experiments is difficult to estimate, since there was an unavoidable time lapse while the cells were chilled and centrifuged out of each suspending medium after the specified period of incubation. Unavoidably, additional exposures of 10 to 15 min in the cold probably occurred while preparing for and using centrifugal harvesting. However, the data show clearly that there was a large differential in the amount of K⁺ lost very soon after the addition of sodium caprylate at the higher molarity.

Marini, Arnow, and Lampen (1961) found that added K⁺ protected the metabolism of yeasts exposed to nystatin during respiration and glycolysis. We found that inclusion of KCl in concentrations ranging from 0.0 to 0.1 M in Sabouraud dextrose broth containing 0.1 M caprylate did not reverse the inhibition of growth of *C. albicans*. Analyses for additional leakage products, however, indicate the reasons for this failure of K⁺ reversal in our experiments.

The curves in Fig. 2 reveal that leakage of RNA and protein was extensive. However, DNA

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**Fig. 1.** Leakage of K⁺ from resting *Candida albicans* cells suspended in solutions of sodium caprylate. No sodium caprylate, △; 2.5 × 10⁻³ or 0.085 M sodium caprylate, ○; 0.1 M sodium caprylate, ●. Corrected for K⁺ content of the suspending solutions which contained no more than 4 ppm.

**Fig. 2.** Leakage of RNA and protein from resting *Candida albicans* cells suspended in solutions of sodium caprylate. The lower curve represents the lack of these products in supernatant fluids taken from test systems containing 0.085 M sodium caprylate as well.
FIG. 3. Leakage of anthrone-reactive material from resting Candida albicans cells suspended in solutions of sodium caprylate.

TABLE 1. Chromatographic identification of a leakage product as glucose

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Rg with descending irrigation*</th>
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<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td>Butanol-acetic acid-water (4:1:5)</td>
<td>1.0</td>
</tr>
<tr>
<td>Butanol-pyridine-water (3:2:1.5)</td>
<td>1.0</td>
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</table>

* Solvents were permitted to run off the chromatograms, which were serrated to facilitate dripping. When the unknown was cochromatographed with each hexose separately, there was a single spot only with glucose. Rg is the migration distance of the test compounds as related to that of glucose.

was not discerned in any of the supernatant fluids, and neither RNA nor protein escaped from cells suspended in buffer or in buffer containing only $2.5 \times 10^{-3}$ or 0.025 M sodium caprylate.

Carbohydrates reactive with anthrone were additional components which leaked rapidly from cells suspended in the higher (but not the lower) concentration of the inhibitor (Fig. 3). The intermediate concentration of 0.025 M sodium caprylate did, in contrast to its inability to cause leakage of RNA and protein, induce the cells to release some polysaccharide. There were no oligo- or monosaccharides in the leakage products from the cells incubated in 0.025 M sodium caprylate or from the supernatant fluids from cells suspended in the lower concentration or in buffer. The only chromatographically mobile substance in the anthrone-reactive material from the cells treated with 0.1 M inhibitor was found to be a single reducing sugar, identified (Table 1) as glucose.

These results suggested that enzymes which hydrolyze glucose polymers may be among the proteins released by the 0.1 M sodium caprylate-treated cells. We confirmed this by incubating 1-ml samples of supernatant fluid from 0.0 and 0.1 M sodium caprylate treatments (harvested after 0- and 2-hr incubation periods at 30 C) with 1-ml portions of a 2% solution of glycogen. There was no increase in reducing sugar concentration in the mixture containing supernatant fluids from cells suspended for 2 hr in buffer or from either zero-hr suspension, whereas reducing sugar content increased by 450 mg/100 ml in the mixture with the supernatant fluids from cells suspended in 0.1 M sodium caprylate for 2 hr. Apparently, a glycogen-hydrolyzing enzyme did leak from the cells under the influence of 0.1 M sodium caprylate.

We did not detect sterols in any of the supernatant fluids in which yeast cells were suspended with or without sodium caprylate.

The data presented show that the inhibitory effect of 0.1 M sodium caprylate was clearly found to be owing to destruction of cellular integrity, resulting in loss of several essential metabolites and at least one enzyme. However, it is not clear that the leakage of anthrone-reactive material from the cells incubated in 0.025 M sodium caprylate was deleterious, since none of the other test metabolites leaked at this concentration. Inhibition by this lower concentration may be attributable to several factors, including leakage.

The inhibitory effect of $2.5 \times 10^{-3}$ M sodium caprylate, which we have seen does not induce leakage of any of several metabolites from resting cells, was nonetheless real for growing cells (Fig. 4A). Our analyses of certain of the biosynthetic processes indicated that the relative amounts of protein and RNA produced by growing cells subjected to this lower concentration of sodium caprylate increased, reached their peaks later, and remained more elevated longer than those in untreated cells (Fig. 4B and C). In contrast, the relative DNA content of the cells...
in the treated cultures did not become so elevated as in the untreated cultures. The production of DNA did, however, peak later than in untreated cells and returned more slowly to the original level (Fig. 4D).

**Discussion**

The destructive effect of 0.1 M sodium caprylate revealed in our thin-section studies (Adams et al., 1963) has been confirmed by these observations of leakage of essential metabolites from treated cells. Whereas Marinini et al. (1961) attributed the inhibitory action of nystatin to inducing K+ deficiency in *C. albicans*, we found this to be only one of several deprivations resulting from the action of sodium caprylate. Other notable differences in the effects of sodium caprylate from those of nystatin on yeasts are: (i) the effects of sodium caprylate were not stopped at 0°C, as are those of polyenes (Harsch and Lampen, Biochem. Pharmacol., *in press*); and (ii) cellular damage by sodium caprylate was extensive and easily seen in thin sections (Adams et al., 1963).

It is interesting that, destructive as the 0.1 M concentration is to the yeast cells, Keeney (1946) recommended use of more than ten times this concentration of the drug (20%) for topical application as an antifungal agent.

We find that the results presented here are consistent with our earliest observations (Watt et al., 1962) that resting cells exposed to 0.1 M sodium caprylate for periods varying from zero to 15 min were unable to respire or ferment endogenously after being retrieved and washed. Obviously, washing, which was designed in that previous study to remove the “bound” inhibitor, would not restore the lost metabolites, and actually removed any possibility of their reabsorption.

At the lower concentration of 2.5 × 10⁻³ M sodium caprylate, which diminished greatly but did not completely prevent growth of *C. albicans*, the suppressive mechanisms were not so clear. There were certain similarities in our findings with those involving the action of amphotericin B on *C. albicans* reported by Drouhet, Hirth, and Lebeurier (1960), who observed the polyene antibiotic to increase the endogenous respiration and alter the kinetics of synthesis of protein, RNA, and DNA in growing cells. However, in their studies, unlike in ours, the synthesis of RNA rather than DNA was more extremely diminished by the inhibitor.

Lampen and Weinstock (1962) suggested that several short-chain, saturated fatty acids may enter yeast cells and destroy the effectiveness of certain enzymes. We have yet to test this hypothesis with the eight-carbon sodium caprylate. The apparently destructive effect of sodium caprylate on the mitochondria revealed in thin sections (Adams et al., 1963) suggests that these may represent important organelle sites of impaired metabolism in the presence of the low concentration of inhibitor. We have begun, therefore, a study of the effects of sodium caprylate on the respiratory enzymes of *C. albicans*.

**Acknowledgment**

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LITERATURE CITED


