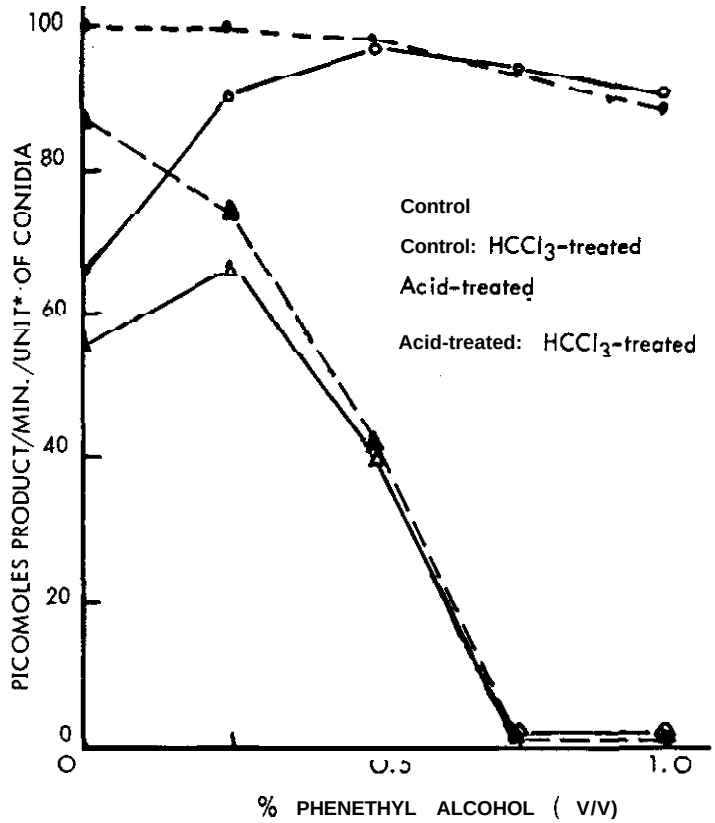
Scott, W.A. and R.L. Metzenberg. Permeabilization of Neurospora conidia with phenethyl alcohol.

It was previously reported (Scott and Metzenberg 1967 Neurospora Newsl. 11: 8) that Neurospora conidia contain a cryptic compartment of aryl sulfatase which can not be detected in intact conidia but can be observed after the conidia have been permeabilized by treatment with one of several agents. In addition, part of the enzyme which con be assayed in intact conidia is insensitive to acid inactivation unless the conidia have been previously permeabilized. Although direct evidence is lacking, it is useful to think of these compartments as follows: The enzyme accessible to both substrate and acid represents aryl sulfatase in the periplasmic space. The acid-inaccessible, substrate-accessible compartment represents enzyme imbedded in the plasma membrane. The enzyme inaccessible to both substrate and acid (cryptic compartment) corresponds to truly intracellular aryl sulfatase.

This communication describes conditions under which phenethyl alcohol will reveal the cryptic compartment and will render all of the enzyme susceptible to acid inactivation. Phenethyl alcohol has been previously shown (Lester 1965 J. Bacteriol. 90: 29) to inhibit uptake of various amino acids and glucose in germinated conidie. In addition, 0.3% phenethyl alcohol prevented germination of Neurospora conidio for 8.5 hrs. at 30°C without loss of viability.

Conidio from the strain eth-1 (r), cys-5 (85518) A were grown under conditions of derepression for aryl sulfatase synthesis as previously described (Scott and Metzenberg 1967 Neurospora Newsl. 11: 8). Conidia were harvested, filtered twice through gloss wool, washed twice with 0.1 M Na-acetate/acetic acid buffer, pH 5.0 and treated with HCl at a pH of 1.3 at 4°C for 15 minutes; then the pH was readjusted to 4.8 with NaOH. Conidia so treated were centrifuged and resuspended in 0.1 M Na-acetate/acetic acid buffer, pH 5.0, containing 0. 1 mM cycloheximide and were incubated with various concentrations of phenethyl alcohol at 37°C for 30 minutes. Conidio were kept in suspension by adding a gloss bead and agitating on a shaker. During the incubation, the conidial concentration, measured by turbidity of a suitably diluted sample, was $OD^{1.9cm}_{420m\mu}$ = 21.2. This corresponds to 2.8 mg protein per ml by the method of Lowry et al. (1951 J. Biol. Chem. 193: 265), modified by incubating the sample of conidio in the Lowry alkali reagent at least 12 hours before adding the copper reagent. This modification gives reproducible values for protein but probably does not measure all of the protein present in the conidio.



• One unit of conidio is 1 ml at $O^{1.9\ cm}_{420\ m\mu}$ = 1

At the end of the thirty minute incubation, on aliquot from the incubation mixture was treated with HCl at pH 1.3 and 4°C for 2 minutes; then the mixture was readjusted to pH 4.8 with NaOH. Another aliquot was diluted with NaCl as control. Samples from the acid-treated ond control tubes were collected by vacuum filtration onto filter paper discs and were washed on the paper with cold 0. 1 M Na-acetate/acetic acid buffer, pH 5.0. Additional samples were collected ond washed as above and further washed with ice-cold chloroform. Eberhart and Tatum (1961 Am. J. Botany 48: 702) reported on analogous technique using acetone. All of these samples were assayed for aryl sulfatase by shaking the filter disc and conidio under the previously established assay conditions (Metzenberg and Parson 1966 Proc. Natl. Acad. Sci. U.S. 55: 629) with the addition of 0. 1 mM cycloheximide. The results ore shown in the occomponying figure.

It con be seen that, after incubation in the absence of phenethyl alcohol, 34% of the enzyme is not detected in the assay unless the conidio hove been treated with chloroform (cryptic compartment). In addition, about 12% of the enzyme has become vulnerable to acid inactivation during the incubation. Low concentrations (0.25% and 0.50%) of phenethyl alcohol reveal the cryptic comportment almost completely, but a large port of the enzyme is still protected from acid inactivation. After incubation with 0.75% or 1. 00% phenethyl alcohol, all of the cryptic compartment her become accessible to substrate and all of the enzyme hos become susceptible to acid inactivation. The conidio ore still able to retain enzyme molecules, however. Even after incubation of the conidio at the highest phenethyl alcohol concentration, washing the conidia removed no more than 10% of the enzyme.

Repitition of this experiment with the some conidial suspension on the some day gave results differing, at most, by 10%. If conidia collected on different doys were used, qualitatively the same results were obtained, but there was some variation in the concentration of phenethyl alcohol required to permeabilize the conidia in a fixed period of time. ▪ ▪ ▪ Department of Physiological Chemistry, University of Wisconsin, School of Medicine, Madison, Wisconsin 53706.