

Scott, W. A. and R. L. Metzenberg. Location

of aryl sulfatase in *Neurospora conidio*.

grown for five days at 24°C on Fries' minimal medium supplemented with 5mM methionine. Under these conditions this strain is derepressed for aryl sulfatase synthesis (Metzenberg and Parson 1966 Proc. Natl. Acad. Sci. U.S. 55: 629).

A suspension of intact conidia was capable of hydrolysing p-nitrophenyl sulfate. Such an assay detected about 65% of the total aryl sulfatase activity that could be demonstrated in cell free extracts. Washing with various buffers removed about 30% of the total activity; this fraction may be considered an exoenzyme. A portion of the activity which could not be washed away, even with repeated washings, could be inactivated by a short treatment with 0.05 N HCl at 0°C - a treatment which does not reduce conidial viability. After acid treatments for up to one hour, about 10% of the total enzyme activity remained.

Brief treatment of the conidia with cold chloroform or acetone (B. M. Eberhart, personal communication) revealed a "cryptic" compartment of enzyme amounting to about 35% of the total activity seen in extracts of unwashed conidia. A "cryptic" fraction of this size was also observed after disruption of conidio with an X-Press (Edebo 1960 J. Biochem. Microbiol. Technol. Eng. 2: 453). heating for 5 minutes at 60°C, or incubation with 100 µg/ml of nystatin or ascocin for 30 minutes at 0°C. Each of these treatments, excepting the antibiotic treatment, rendered all of the enzyme activity acid-sensitive, presumably by rendering the cell membrane permeable to H<sup>+</sup> ions. Thus it appears that there are at least three distinguishable compartments of aryl sulfatase in conidia: a "washable" fraction outside the cell wall, a cell-bound fraction outside the cell membrane, and an internal fraction.

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As part of our studies of sulfur metabolism in *Neurospora crassa*, experiments were carried out to determine the location of aryl sulfatase in conidia. The strain eth-1<sup>-</sup>, cys-5 (formerly called r-eth-1, cys-5) was used. Conidia were collected from plates