Brescia, V. T. Tymsine transport in Neurospora.

Uptake of tyrosine by Neurospora conidia was studied using ^{14}C tyrosine in the manner described by DeBusk and DeBusk (1965 Biochim. Biophys. Acta 104: 139) for phenylalanine. Conidiol suspensions which consistently gave 0.19 - 0.28 mg dry weight of conidia per 5 ml sample were prepared by adjusting OD_{397} to 0.9-0.95 (B and L Spectronic 20). The usual conditions were a temperature of 30°C and tyrosine concentration of 1 µmole per 25 ml (4×10^{-5} M) reaction mixture (Vogel's minimal + cells). The optimum temperature was later found to be between 31-33°C and the pH optimum 5.8.

Incubation at 45°C for 20 minutes did not inactivate the transport system - as little as 2 minutes at 50" did temporarily inactivate (uptake less than 70% of control at 20 minutes). Recovery occurred in cells held at 30° for 30 minutes following 50" heat inactivation. Concentrations from 0.2 µmole/25ml to 3.2µmole/25 ml gave increasing initial rates of uptake; no increase was observed above 5 µmole/25 ml. A reciprocal plot of initial uptake vs tymsine concentration (Lineweaver-Burke) gave a straight line. By extrapolation, the Km was estimated at 1.2-1 .8 $\times 10^{-4}$ M in three experiments. After 50 minutes uptake, the amount of label chromatographically identical with tyrosine that can be extracted with 5% TCA at room temperature in IO minutes is at least 30 x the external concentration.

Glucose (final conc. 1%) added to an actively transporting system will inhibit further transport within 6 minutes and will continue inhibiting for at least 15 minutes, after which transport is resumed, apparently at the same rate. Sodium azide and 2,4-dinitrophenol at 10^{-3} M restrict transport to about 10% of the control. With azide, at least the inhibition is almost instantaneous. A variety of compounds were tested at concentrations 25 x that of tymsine for their effects on uptake of ^{14}C L-tyrosine at a concentration of 4×10^{-5} M. Shikimic acid and para-hydroxyphenylpyruvate, among others, had no effect whereas L-tryptophon and L-phenylalanine reduced uptake to 20% or less of control. Since all of the &we-mentioned compounds con supplement appropriate mutants, they must be capable of entering the cell. Therefore, the lack of effect of shikimic acid and para-hydroxyphenylpyruvate must reflect a stereospecificity of the tyrosine transport system. This is further demonstrated by the fact that D-tyrosine reduces uptake to 87% of control, whereas an equivalent amount of ^{12}C L-tyrosine reduces it to 25% of control. - - - Deportment of Biological Science, Florida State University, Tallahassee, Florida 32306.