progeny, but 5 out of 72 isolates took 12-13 days to grow the length of the tube. Kekaha-I yielded stop-start progeny either as a male parent (1 out of 86) or as a female parent (2 out of 53). Thus in these strains, the abnormal phenotype is evidently inherited in a non-Mendelian manner. The other three variants have not yet been analysed.

Hanalei-I was investigated for the presence of virus-like particles using electron microscopy, but none were found. Preliminary biochemical studies of these two strains revealed some similarities to the group I mitochondrial mutants of *N. crassa* (e.g., *poky*) and also some differences. *Botany Department, University of British Columbia, Vancouver, B.C. V6T 1Z1.*


Cellobiose-induced β-galactosidase and β-glucosidase activities of *Neurospora crassa*.

β-glucosidases, "cellobiase," has optimum activity at pH 6 (Eberhart and Beck 1970 J. Bacterial. 101: 408), we investigated whether or not the pH 6 form of β-galactosidase has any β-glucosidase activity.

Beta-galactosidase and β-glucosidase activities were determined by use of the chromogenic substrates, o-nitrophenyl-β-D-galactopyranoside (ONPG) and o-nitrophenyl-β-D-glucopyranoside (PNPG). These and the other procedures used in this study (e.g. growth conditions, medium, cellobiose concentration, etc.), were described by Perry and Lester (1973 Biochem. Biophys. Res. Commun. 54: 1476). A unit of enzyme activity is that amount which releases 1.0 μmole of ONP or PNP per hour at 37°C under the assay conditions. For ion exchange chromatography, diethylaminoethyl (DEAE) cellulose (Cellex D), was equilibrated with 0.01 M potassium phosphate, pH 6.8 containing 0.001 M EDTA, and poured into a 2.2 x 15-cm column (bed volume, 50 ml). Ammonium sulfate (AS) fractions were applied in volume of less than 5 ml and elution was carried out with a linear NaCl gradient (0.025 to 0.25 M) made in 0.01 M potassium phosphate buffer.

![Figure 1](image-url)

*Figure 1.* DEAE-cellulose chromatography of a resuspended 50-to-75% AS fraction from cellobiose-grown mycelia. The sample applied contained 101 units of β-galactosidase activity and 135 units of β-glucosidase activity. (o) β-galactosidase activity; (x) β-glucosidase activity; (---) NaCl concentration.
When extracts of cellobiose-grown mycelia were assayed at pH 6, both β-galactosidase and β-glucosidase activities were detected. The extracts were then subjected to AS fractionation. Very little of the enzyme activities (1% of the β-galactosidase and 4% of the β-glucosidase present in the crude extract) was precipitated by 0.50% AS; whereas, much more (75% of the β-galactosidase and 40% of the β-glucosidase) was precipitated by 50-75% AS. The 50% AS-fraction was then separated by chromatography on DEAE-cellulose. The fractions obtained were assayed for both activities (see Fig. 1). β-glucosidase activity appeared in two well-defined peaks (I and II), while β-galactosidase activity appeared in only one peak, which closely coincided with peak I of the β-glucosidase activity. There was, essentially, total recovery of both activities applied to the column. When activities were normalized to the peak maxima, the β-galactosidase peak and the β-glucosidase peak I were superimposable.

Fractions corresponding to the two peaks were used to characterize further the β-galactosidase and β-glucosidase enzyme activities. Effects of pH on these activities are shown in Fig. 2. The pH optima of 5 and 6 for peak-I and peak-II β-glucosidase activities are similar to the optima reported for aryl-β-glucosidase (Mahadevan and Eberhart 1964 Arch. Biochem. Biophys. 108:22) and celllobiose (Eberhart and Beck 1970), respectively. The peak-1 β-galactosidase and β-glucosidase cannot be distinguished by the effect of pH upon their activities nor by the use of inhibitors. D-cellobiose or mercuric chloride. Finally, the peak-1 activities were tested for their thermal stabilities. At 50°C, β-glucosidase and β-glucosidase activities showed very similar kinetics of inactivation, with half-lives of 3 to 4 min.

In summary, these data show that the cellobiose-induced, pH 6-β-galactosidase of Neurospora is associated with a β-glucosidase activity, possibly celllobiose. It is not known at this time whether this is because Neurospora possesses a single enzyme activity that has dual specificity for β-galactosidase and β-glucosidase substrates, or whether there are two separate enzymes that copurify in the procedures used.

Figure 2. Effect of pH on DEAE-cellulose peak-1 β-galactosidase activity (•), peak-1 β-glucosidase activity (○), and peak-II β-glucosidase activity (x). The number of units used were: peak-1 β-galactosidase, 1.30; peak-1 β-glucosidase, 1.30; and peak-II β-glucosidase, 1.84. Activities were measured at pH optima.

We reported earlier (Schablik et al. 1977 Acta biol. Acad. Sci. hung. 28:273) that the Neurospora crassa ragged mutant strain R2506-5-101 incorporates a substantial amount of 3H-labelled DNA under optimal experimental conditions.

Since DNA accumulation was found to be inversely proportional to the age of the culture, we suspected that in older cultures the thickening of the cell wall might interfere with the attachment of DNA molecules to cell membrane receptors.

Schablik, M., B. Kocsar and G. Szabo.

Factor(s) in the culture medium of a slime strain which stimulate DNA uptake.

We report here studies of DNA uptake by slime cells, and present results which suggest: (1) enhanced DNA accumulation at the early stationary phase of growth; and (2) the presence of heat-sensitive factor(s) in the culture medium of 48 h slime cells which stimulates DNA uptake.

The slime strain (FGSC 111118) was obtained from the Fungal Genetics Stock Center. DNA was extracted from wild type strain (RL-3-8 A) obtained from Rockefeller University, New York, utilizing a modified Marmur's method (Aradi et al. 1978 Acta biol. Acad. Sci. Hung. 13: 259).

Slime cells were maintained and grown on Nelson B medium (Nelson et al. 1975 Neurospora News1. 22: 15) containing 1.5% saccharose, 7.5% L-sorbose, 0.8% Vogel's salts with or without 1.5% agar. Liquid medium (80 ml) in 100 ml flasks was inoculated with 2 x 105 cells from 6 to 7 day old agar slants and cultures were grown in a New Brunswick incubator at 27°C with shaking (100 rev/min). The isolation of 3H-labelled, high molecular weight...