Sebo, T. J. and J. C. Schmit. **A convenient method for the storage and cultivation of slime.**

The cell wallless strain of Neurospora crassa, slime (Emerson 1963 Genetica 34:162) is a convenient source of subcellular organelles (Woodward and Woodward 1968 Neurospora Newsl. 13:18; Hsiang and Cole 1973 3. Biol. Chem. 248:2007; Judewicz and Torres 1979 FEBS Letters 107:160). We have used slime (fz; sg; os-1, arg-1, cr-1, aur) to isolate nuclei for studying RNA processing in Neurospora. However, the convenience of cell component isolation is, to some degree, offset by the difficulty in maintaining uniform liquid shake cultures of slime for extended periods of time.

We have found that small aliquots of slime can be stored frozen in growth medium at -80°C for more than a year with little or no alternation in subsequent growth properties. The procedures described below are similar to those of Creighton and Trevithick (1973 Neurospora Newsletter 20:32), but do not require dimethyl sulfoxide. The frozen slime inocula yield cultures minimally contaminated with cellular debris and "soap bubble" cells, and give very reproducible growth rates.

Slime was recovered from the heterocaryon {(fz; sg; arg-1, cr-1, aur, os-1) + (al-2, nic-1, lys-3, os-1)} (FGSC 1121) using the procedures described by the Fungal Genetics Stock Center. This slime strain was grown in 50 ml of liquid medium containing Vogel's salt solution, 2% sucrose, 10% sorbitol, 0.05% L-arginine, and 50 units each of penicillin and streptomycin. The culture was incubated at room temperature on a rotary shaker at 100 rpm. Vegetative growth was maintained by transferring two ml of two day old culture to 48 ml of fresh medium When slime became the predominant component, the culture was grown to a density of 2 x 10^7 cells/ml, and two-ml aliquots were immediately frozen in a -80°C freezer. To begin a new culture, an aliquot was thawed at room temperature, filtered through sterile aquarium filter material (spun polypropylene) and added to 49 ml of fresh medium. The culture was incubated at 30°C without shaking for 24 hours, followed by rotary shaking at 100 rpm.

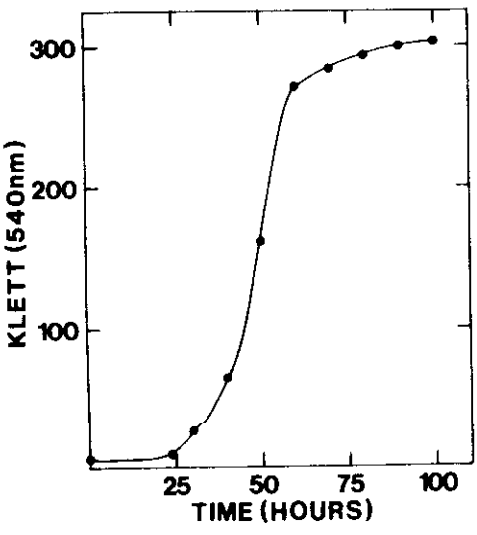

Figure 1. -- Growth of slime in liquid shake cultures. Growth conditions are described in the text.

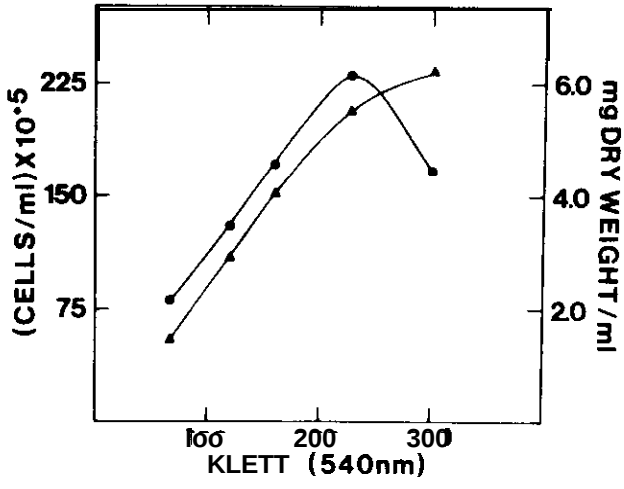

Figure 2. -- Number of slime cells and dry weight per milliliter of culture as a function of Klett units (540 nm). Growth conditions are described in the text. Cell number was determined using a hemacytometer (only cells at least three microns in diameter were counted). Dry weight was determined by lyoohilizing cells that had been harvested by centrifugation. Symbols: (●), cells per ml of culture; (A), mg dry weight per ml of culture.

Figure 1 presents a typical growth curve for slime. Growth was monitored with a Klett-Summerson colorimeter using a 540 nm filter. Klett values of 150, representing the mid-point of logarithmic growth, were attained after 50 ± 2 hours of incubation with cultures inoculated with cells that had been stored frozen for one week to one year. A Klett-Summerson colorimeter is convenient for monitoring slime growth since both dry weight and cell number are linearly related to Klett values throughout most of the growth curve (Figure 2). However, as Klett value exceeded ZOO, the general morphology of slime cells deteriorated, and cell lysis began occurring at a rate faster than growth. As a result, the number of cells began to decrease, although the culture's Klett value continued to rise due to an increase in total mass (Figure 2). In all of our experiments, cells were always harvested before the cultures reached a Klett value of 200. (This work was supported in part by NSF grant PCM77-12551 to JCS.) • • School of Medicine and Department of Chemistry and Biochemistry, Southern Illinois University at Carbondale, Carbondale, Illinois 62901.