Pittenger, T.H. Special growth tubes for the study of transport of growth factors and other phenomena.

When a auxotrophic mutant such as nic-2 is used in certain incompatible heterokaryotic combinations, the nic-2 strain may grow for long distances in the growth tube before ceasing to grow. This is not observed with nic-2 homokaryons under similar conditions. This growth, except at the very proximal end of the tube where a mixture of conidia has been placed, appears from certain criteria, except growth on minimal, to be homokaryotic. This is assumed not only because of the dark brown accumulation in the media characteristic of this mutant when grown on submaximal amounts of the growth factor but also because conidial plating fails to disclose the presence of anything except the nic-2 strain. Such observations prompted a reexamination of the studies of Ryan et al., Am. J. Bot. 30: 784, 1943 concerning the extent of the translocation of various compounds in the mycelium of Neurospora. For this purpose a special type of growth tube, an improved design of one originally used by Pittenger and Atwood, was constructed; subsequently such tubes have been found to be equally useful in other experiments. With the hope that others may find it adaptable to other problems, a brief description of the growth tube is presented, along with some preliminary results illustrating its use.

This growth tube was originally designed to help determine indirectly whether a mycelium of an auxotrophic mutant growing on supplemented medium could translocate enough of the essential growth factor from such a medium to enable the organism to continue to grow for an extended time, on minimal medium. The growth tube consists of two 20 mm. diameter pyrex glass tubes united by a 24/40 standard taper ground glass interchangeable joint (Corning No. 6540). The distal end of the inter portion of the ground glass joint is partially sealed to provide a partition between the two sections of the tube when the joints are united. The glass partition prevents contact of the media present in the proximal and distal ends of the growth tube, but the small horizontal opening in the upper portion of the partition permits the mycelium to pass readily from one section of the tube to the other. To facilitate sampling of conidia and for aeration, the tubes are equipped with sampling ports along the upper surface. These are made of 10 mm. diameter tubing, 30 mm. high and are spaced at 4-6 inch intervals along the length of the tube. The proximal portion of the tube is approximately nine inches long and the distal portion, 20 inches long, but length may vary with different types of experiments. The opposite ends of the finished tube are bent at a 45° angle.

Observations relating to the efficiency of transport to growth factors essential to auxotrophic mutant strains can be made in such tubes. The growth factor required by the mutant strain is added to the proximal section of the tube, along with 3% agar solidified Fries medium and 1.5% sucrose. To the distal portion of the tube is added the same medium without the growth factor. The glass partition prevents the

diffusion of any portion of the media from the proximal to the distal section of the tube so the only way the test compound can pass from one section of the tube to the other is *via* the mycelium which connects the two sections. Obviously the prolonged growth of the mutant strain on the minimal medium does not in itself prove an effective transport system, but rapid cessation of growth indicates a limited system of translocation, as the work of Ryan *et al.* suggested. For example, when *nic-2* mutants were used in such experiments and the proximal portion of the tube supplemented with nicotinamide, it was found that such a strain could occasionally grow as far as 600 mm. on the minimal medium before stopping. These initial observations were originally interpreted to suggest a highly efficient translocation system in the mycelium and they agreed with our early observations of the prolonged growth of *nic-2* strains as homokaryons in certain incompatible heterokaryotic combinations. This interpretation seemed unlikely, however, on the basis of subsequent results using other nutritional mutants. When separate mutant strains with individual requirements for lysine, pantothenate, arginine, riboflavin, inositol and nicotinamide were inoculated in the growth tubes with excess amounts of the requirements added to the proximal ends of the tubes, the respective distance in millimeters that each grew on minimal medium were as follows: 14, 77, 95, 150, 331, and 487. Thus it is apparent that the distance a strain is capable of growing varies with different mutants. Further experiments with the *nic-2* strain revealed that the distance that this strain grew on minimal medium was independent of the concentration of nicotinamide present in the proximal end of the growth tube, although sublimiting amounts were not used. In other experiments identical tubes were inoculated with the *nic-2* strain and the mycelium was then allowed to pass from the proximal to the distal portion of the tube. Once the mycelium had passed over the partition and reached the minimal medium, one of the tubes was disjoined, severing the mycelium, and the open end was then sealed with a plug made from a ground glass joint. The second tube was left intact. It was found that the mycelium was able to grow just as far on minimal medium whether or not it was still in contact with the supplemented media in the proximal end of the tube. Thus, it is clear that the growth on minimal was not due to the continued presence of nicotinamide being translocated from the proximal section via the mycelium. Rather it appears that only the growth factor originally present in the cytoplasm, and transported mechanically within the mycelium as it reached the minimal medium, would be available for growth on the minimal medium present in the distal portion of the growth tube. In the case of the *nic-2* mutant, one would assume that the absolute requirement of the growth factor is so low that growth can continue for some time on the nicotinamide present in the mycelium as it reaches the minimal medium.

Obviously crude experiments of this type in which only growth is studied do not permit one to make definitive statements about translocation of nutrients, but by using labeled compounds one should be able to carry out much more precise and meaningful experiments with growth tubes of this kind.

In view of the above results we were somewhat surprised in a study of the translocation of sugars in such tubes to find that Neurospora was apparently able to grow indefinitely in the absence of sucrose. This was true even when the agar was thoroughly washed to remove any contaminating sugars. Growth on minimal medium soon became very thin and only at half maximal rates, but growth continued for over 1,500 mm. at which time the experiment was terminated. Neurospora was also able to grow in conventional growth tubes in the absence of sugar. Since Neurospora cannot grow on liquid minimal medium in the absence of sugar, it must be able to utilize the agar as a carbon source. Neurospora shows limited growth on galactose, and agar is known to be a sulfuric acid ester of a linear polygalactose.

In addition to the above experiments, we have found the growth tubes to be especially useful in experiments with balanced heterokaryons. With such tubes it is possible to start growth on minimal medium, to determine the nuclear ratios, and then to allow the heterokaryons to proceed on various types of supplemented media. A study can then be made of changes in nuclear proportions that may have taken place under the influence of various types of supplemented media. Various modifications of the above procedure might be useful with various intraallelic heterokaryons. ---Department of Agronomy, Kansas State University, Manhatten, Kansas.