The result of one experiment is shown in Fig. 1. Following treatment with butyl alcohol, the leucocytes maintain their normal volumes for a long time in a mixture of 1 part isosmotic sucrose and 5 parts isosmotic saline; but the cells swell rapidly in isosmotic NaCl solution alone, shrink rapidly in isosmotic sucrose solution alone, and shrink with rapidity and then less rapidly recover, or slightly surpass, their original volume when a little concentrated NaCl is added to the surrounding medium. Shrinkage of cells in isosmotic sucrose is due to the diffusion of salt out of the cells followed by a movement of water. Such cells have enormously increased permeability to anions, as well as to cations, but retain their normal impermeability to sucrose and protein. These results illustrate an essential agreement in the behavior of leucocytes and erythrocytes.

It should be remembered that, although an active transport of cations is essential to the continued existence of the erythrocyte, its absence would be expected to produce swelling only very slowly. It is now known that the physical permeability of this cell to cations is so low that, despite its extremely favorable surface-volume relationship, rates of exchange of potassium across the surface of the human erythrocyte at body temperature are less than 2 percent of the cell potassium per hour (7, 8). By way of contrast, Davies and Galston (9) have reported for kidney cells exchanges of the order of 15 percent per minute. It follows, therefore, that, while a considerable degree of surface injury by butyl alcohol or other agents is needed to produce rapid swelling of the erythrocyte, a mere cessation of the normal ionic transport process might soon bring about the same result in the kidney cells.

Perhaps the strongest reason at present for believing that ionic movements may be involved in the volume changes of tissue cells outside the body is the recent observation by Deyrup (10) that, while kidney slices swell in solutions of NaCl or monosaccharides isosmotic with blood, they shrink in similar solutions of disaccharides. This behavior, which strongly resembles that of the erythrocyte and the leucocyte after treatment with butyl alcohol, cannot be explained by a simple initial hypertonicity of the cells themselves. Preliminary evidence has also been obtained that the mammalian intestine behaves in the same way. In six experiments, rat intestine increased in water content 17 percent over the control when placed in 0.15M NaCl at 0°C, increased 7.6 percent in a solution composed of 1 part 0.3M lactose and 5 parts 0.15N NaCl, and decreased 13.2 percent in a 0.3M lactose solution.

The data presented are consistent with the hypothesis that the tendency of salts and water to enter cells owing to the intracellular colloid osmotic pressure is, under normal conditions of oxygenation, temperature, and so forth, exactly balanced by the active transport of ions and passive movement of water out of the cell.

The valuable advice and criticism of M. H. Jacobs throughout the course of this work is gratefully acknowledged.

References

- 1. J. R. Robinson, Proc. Roy. Soc. (London) B137, 378 (1950).
- E. Huf, Arch. ges. Physiol. (Pflügers) 235, 655 (1935). W. Wilbrandt, Arch. ges. Physiol. (Pflügers) 245, 22 3.
- (1941). 4. M. H. Jacobs and D. R. Stewart, J. Cellular Comp.
- M. H. Jacobs and M. Willis, Biol. Bull. 93, 223 (1947).
 M. H. Jacobs and M. Willis, Biol. Bull. 93, 223 (1947).
 M. G. Netsky and M. H. Jacobs, Biol. Bull. 77, 319 6.
- (1939)
- J. W. Raker et al., J. Gen. Physiol. 33, 691 (1950). C. W. Sheppard and W. R. Martin, J. Gen. Physiol. 33,
- 703 (1950). R. E. Davies and A. W. Galston, Nature 168, 700 (1951).
 I. Deyrup, J. Gen. Physiol. 36, 739 (1953).
- 12 February 1954.

Ascomycete Spore Mutants and Their Use in Genetic Studies

George Bistis and L. S. Olive

Department of Botany, Columbia University, New York

Ascobolus stercorarius is a heterothallic ascomycete which in its life-cycle resembles in many ways the eight-spored species of Neurospora. In wild-type crosses of the two mating types, every fruiting body produces hundreds of asci, each of which contains eight haploid, uninucleate ascospores. These spores become purple, then brown, as they mature.

In the course of our genetic studies, two interesting cultures have been obtained, both of which appear to be single-gene mutants. The first of these carries a factor for ascospore abortion. Thus, in a cross between this mutant and a wild-type isolate, the resultant asci contain four viable, brown-colored spores and four abortive, colorless spores (Fig. 1). This particular mutation apparently occurs quite frequently in this species, and it has been found by at least two previous workers. Dowding (1) reported that in wildtype crosses many of the asci contained these two types of spores. Ingold (2) pictures a fruiting body of A. stercorarius showing an ascus with these two spore types.

The second mutant apparently carries a factor influencing only spore color. When it is crossed with a wild-type strain of appropriate mating type, the asci produced contain four wild-type, brown-colored spores and four mutant, tan-colored spores (Fig. 2). In this case, however, all eight spores are viable. In the case of both mutants, crosses with wild-type cultures always produce the six expected ascus segregation patterns, and a preliminary scoring of asci gives a second-division segregation frequency of approximately 20 percent for the spore abortion factor and about 62 percent for the tan-spored factor. Therefore, the two are not alleles, but whether they are linked has not yet been determined.

Figure 3 shows one type of ascus resulting from a cross between the two mutants. The spore abortion locus has segregated in the first division, while the tanspored locus has segregated in the second. All of the four expected ascospore phenotypes cannot be distinguished, since the four spores carrying the abor-



Fig. 1. Ascus showing first-division segregation of the spore abortion factor. Fig. 2. Ascus showing second-division segregation of the tan-spored factor. Fig. 3. Ascus resulting from a cross involving the two mutant strains. $(\times 500)$

tion factor are phenotypically alike regardless of whether they carry the wild-type or mutant factor at the tan-spored locus.

The use of this organism in genetic studies affords certain definite advantages. It is easily cultured in Petri dishes containing an agar medium of yeast extract and cellulose (3). The time required for one generation to mature is between 10 and 14 days. A single plate of paired cultures forms many fruiting bodies, each of which contains hundreds of asci. Since these asci can be scored merely by direct examination, the results of 500 meioses have been counted in one 3-hr period. Another advantage of this method is that the autonomous effects of single genes upon single haploid cells (the ascospores) may be observed without the complications of dominance and recessiveness. Furthermore, the ascospore pattern in each ascus is a visual and orderly replica of meiosis and, as such, is a valuable aid in the study of segregation and crossing over, eliminating the necessity for laborious ascus dissection. It is obvious from the evidence obtained with the use of these mutants that brachymeiosis does not occur in A. stercorarius.

A fuller presentation of these data will be published in the near future.

References

- 1. E. S. Dowding, Ann. Botany (London) 45, 621 (1931).
- 2. C. T. Ingold, Spore Discharge in Land Plants (Clarendon Press, Oxford, 1939).
- 3. Chuan-Chang Yu, Am. J. Botany 41, 21 (1954).

23 February 1954.

Oak Wilt Fungus Labeled with C14

Paul F. Hoffman and Bert M. Zuckerman Section of Applied Botany and Plant Pathology, Illinois State Natural History Survey, Urbana

Since the fungus Endoconidiophora fagacearum Bretz, causal agent of oak wilt, lives parasitically only in the woody conducting tissues under the bark of a diseased tree, its progress from a point of infection to distant parts of a tree can be traced only with laborious difficulty The fungus produces, when grown *in vitro*, a toxic substance that is capable of causing tomato and oak cuttings to wilt. Tagging either the fungus, as has been done by Wheeler (1, 2) with other fungi, or the toxic substance with a radioactive element, without altering the pathogenicity of the fungus or the nature of the toxin, would afford better opportunity to study the disease experimentally (3-5).

Uniformly C¹⁴-labeled sucrose (6) with a specific activity of 2.64 μ c/mg was used in the preparation of 1- and 2-ml lots of a sucrose-asparagine-yeast extract medium (7) having activities of 10, 20, and 30 μ c/ml, unlabeled sucrose being used to make up the 20-g/lit sucrose ratio in each lot. These lots, each in cotton-stopped, 10-ml Erlenmeyer-shaped flasks, were planted after sterilization with tiny wefts of mycelium and spores.

These cultures were then placed in moist chambers and incubated at 27° to 30° C. After having developed for periods of 7 to 56 days, the new mats of fungus were taken from the flasks, laid on filter paper, and washed with a stream of sterile water until no radioactivity (8) could be detected in the wash water. From the washed fungus, transplants were made to unlabeled agars in Petri dishes and allowed to develop into new colonies. Mycelium and spores from the peripheries of these colonies were used in subsequent tests.

Verification that the fungus had become labeled during its growth on the C14-containing media was obtained by autoradiography. Bits of fungus taken from colony peripheries were placed on glass microscope slides in drops of water, in drops of unlabeled liquid medium, or on thinly spread potato dextrose agar and allowed to grow for periods of 36 to 144 hr. Also, to obtain closely appressed growth, transplants were made to DuPont 600 P. T. cellophane spread on potato dextrose, chestnut meal, and water agars in Petri dishes. Before autoradiographs were made, the fungus was killed by heat at 80°C and the preparations were dried for several hours over P2O5. Autoradiographs of these preparations were made on Eastman No-Screen X-ray emulsion and on several Ilford nuclear-track emulsions (9)-NTB2, NTC2, NTD1, NTE1, and NTG5-ranging from 25 µ to 200 µ in thickness.

Well-defined autoradiographs of germinated spores were obtained on NTG5 emulsion 100 μ , and of mycelium and spores on Eastman No-Screen X-ray emulsion (Fig. 1). The two most satisfactory methods of