4. In an operant situation, r is equal to N/T, where N is the number of responses and T is the time available for responding. Here, N equals the number of responses in the light and T the sum of all latencies. The mean latency is thus T/N, and its reciprocal is r.
5. A more sensitive, and therefore preferable, way of treating latency (L) data is in terms of the ratios of the frequency of any L to the number of opportunities for that L to occur. The limitations of the present experiment, however, were such that the difficulties inherent in the use of this ratio could not be overcome. [See D. Anger, *J. Exptl. Psychol.* **52**, 145 (1956)]. The e^{-rt} function is here taken as a fairly acceptable first approximation to the data.
6. P. L. Carlton, *U.S. Army Med. Research Lab. Rept. No. 371* (Fort Knox, Ky., 1958).
7. In the study discussed here the latencies between successive responses made during the preliminary sessions, when each lever depression was reinforced, were not recorded. Data from another, unpublished, study suggest, however, that the e^{-rt} function, when allowance is made for the time the animal spends consuming the water (reinforcement), provides an adequate description of the distributions obtained under these conditions.

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Role of Trehalose in Ascospores of Neurospora Tetrasperma

Abstract. The anthrone-positive material extractable in 80 percent alcohol, whose disappearance is correlated with the breaking of dormancy, has been found to be a non-reducing sugar which yields only glucose upon hydrolysis. On the basis of its crystal structure, infrared spectrum, melting point, specific rotation, and chromatographic properties, this material has been identified as trehalose.

Ascospores of *Neurospora* remain dormant unless provided a heat-shock (*1*), furfural or furfuryl alcohol (*2*), other furans and heterocyclic compounds (*3*), or certain organic solvents (*4*). Activation of these cells is accompanied by a 20-fold increase in respiratory rate (*5*), the origin of which is still uncertain. Our recent data have revealed that whereas the dormant ascospore utilizes endogenously contained lipids as the respiratory substrate, activated ones consume an endogenous carbohydrate fraction which is extractable in 80 percent ethanol (*6*). Thus, within a few minutes after exposure to temperatures which break dormancy, this material begins to disappear, and it is almost completely exhausted by the time the germ tube is protruded. The present report concerns the analysis of the carbohydrates in the 80-percent-ethanol-soluble fraction and the identification of the principal component as the nonreducing disaccharide trehalose.

Weighed aliquots of ascospores were killed by boiling in 80 percent ethanol for 5 minutes and then were centrifuged free of the supernatant fluid. The spores were broken in a Nossal disintegrator (*7*) and defatted by extraction in ethyl ether for 24 hours. The defatted cells were extracted in 80 percent ethanol at 60°C until no more anthrone-positive material could be removed, after which the supernatant was clarified by centrifugation. The extract was decolorized by mixing with Norit (1 percent by weight) at 60°C; this process was followed by boiling for 15 minutes. After removal of the Norit, the extract was concentrated under a vacuum until a thin syrup was obtained, which was added to a mixed-bed resin containing Dowex 50 (H^+) and Dowex 1 (CO_3^-). Approximately 95 percent of the anthrone-positive materials were recovered by washing the resin with deionized water, and the clear solution was concentrated into a thick syrup, in a vacuum. The syrup was put in a beaker, and an equal volume of hot 80 percent ethanol was added; the beaker was placed in a desiccator at 4°C, and crystals formed within 24 hours. When left undisturbed in the cold for 3 weeks, most of the liquid evaporated and large numbers of clear orthorhombic crystals formed, some as large as 4 by 2 mm. These crystals were redissolved in hot 80 percent ethanol, recrystallized, washed again in cold ethanol, and dried under a vacuum. The yield, on the basis of dry weight of ascospores, was 10.2 percent; this represented approximately 78 percent of the anthrone-positive materials in the 80-percent-alcohol extract.

Various analyses of the crystalline material were carried out, including chromatography with N-butanol, acetic acid, and water (4:1:5) as a solvent system, and a single spot was found which corresponded to trehalose. The periodate-permanganate reagent of Lemieux and Bauer (*8*) was used, and the length of time required for the development of the spot was found to be identical for the material obtained from the ascospores and for an authentic sample of trehalose. Therefore, the characteristics of the crystals obtained from the ascospores were compared with those of authentic trehalose; the data are summarized in Table 1. In addition, the infrared spectra of these two samples were found to be identical when Nujol mulls were observed in a Perkin-Elmer model 21 spectrograph (*9*). Finally, the crystalline material was found to be nonreducing and liberated only glucose upon hydrolysis in 1*M* H_2SO_4, as revealed by analysis with the glucose oxidase system (*10*).

Extracts that had not been passed through the resins yielded another spot upon being chromatographed. Such material, when concentrated, formed not only the rhomboidal crystals of trehalose but a small number of needle-shaped crystals as well. This second substance was found also to be nonreducing, but it did not give the blue-green color with anthrone. For these reasons, and because its R_f in the solvent system described above was identical with that of mannitol, it was tentatively identified as that sugar-alcohol.

Table 1. Comparison of properties of extract from ascospores of *Neurospora tetrasperma* and trehalose.

Property	Ascospore extract	Trehalose
Melting point	97°–99° and 205°–210°	96.5°–97.5° and 203°*
Mixed melting point	97°–99° and 203°–206°	
$[\alpha]_D^{20}$	+176	+176
R_f in butanol, acetic acid, and H_2O	0.06	0.06

* Data from (*12*).

These data suggest that trehalose is probably the substrate, soluble in 80 percent ethanol, whose utilization is correlated with the activation of ascospores of *Neurospora tetrasperma.* Investigations are now under way to determine the locus of the metabolic block which prevents the consumption of trehalose, thereby restraining the development of the dormant ascospores (*11*).

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References and Notes

1. C. L. Shear and B. O. Dodge, *J. Agr. Research* **34**, 1019 (1927).
2. M. Emerson, *J. Bacteriol.* **55**, 327 (1948).
3. A. S. Sussman, *J. Gen. Microbiol.* **8**, 211 (1954); M. Emerson, *Plant Physiol.* **29**, 418 (1955).
4. A. S. Sussman, R. J. Lowry, E. Tyrell, *Mycologia,* in press.
5. D. R. Goddard, *J. Gen. Physiol.* **19**, 45 (1935); ——— and P. E. Smith, *Plant Physiol.* **13**, 241 (1938).
6. B. T. Lingappa and A. S. Sussman, *Plant Physiol.,* in press.
7. P. M. Nossal, *Australian J. Exptl. Biol. Med. Sci.* **31**, 583 (1953).
8. R. E. Lemieux and H. F. Bauer, *Anal. Chem.* **26**, 920 (1954).
9. We are indebted to Venkoba Rao, of the Randall Laboratory, University of Michigan, for having obtained the infrared spectra.
10. The assay for glucose was based upon the "Glucostat" reagent, which was obtained from the Worthington Biochemical Corp., Freehold, N.J.
11. This work was supported by grants from the National Science Foundation (G-5901) and the Phoenix Project of the University of Michigan.
12. F. Shafizadeh and M. L. Wolfrom, in *Encyclopedia of Plant Physiology,* A. Arnold, Ed. (Springer, Berlin, 1958), vol. 6, pp. 63-86.

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