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Trehalose as an Endogenous Reserve in Spores of the Fungus *Myrothecium verrucaria*

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ABSTRACT

MANDELS, G. R. (U.S. Army Natick Laboratories, Natick, Mass.), RASMA VITOLS, AND FREDERICK W. PARRISH. Trehalose as an endogenous reserve in spores of the fungus *Myrothecium verrucaria*. J. Bacteriol. **90**:1589–1598. 1965.—Gross analysis of *Myrothecium verrucaria* spores showed approximately 3% fat, 33% carbohydrate, and 9.5% nitrogen. The water-soluble carbohydrates were trehalose, glucose, mannitol, and an unidentified phosphorylated compound. Water-soluble amino acids include leucine or norleucine (or both), valine, γ-amino-*n*-butyric acid, β-amino-*n*-butyric acid, ergothionine, glutamic acid, glutamine, glycine, aspartic acid, asparagine, cystine, and cystathionine. Ergosterol was also present. αα-Trehalose is the major reserve (20% of the dry weight), although approximately 30% of it appeared to be at the spore surface and was released by nonlethal treatment with 0.1 N HCl. Treatment with toluene or exposure to heat sufficient to kill the spores (20 min at 60 C) caused rapid liberation of all of the trehalose. Although spores could utilize exogenous trehalose with no appreciable lag, some stimulus, such as exposure to heat (10 min at 55 C), incubation with azide, or germination on exogenous substrates, was necessary to effect utilization of trehalose reserves. Spores have trehalase, but it is apparently at the spore surface, since it is inactivated by acid treatment which does not kill the spores. The metabolic pathway for utilization of trehalose is not known, but presumably it is not mediated by trehalase. The involvement of mannitol is indicated, since it tends to increase as trehalose decreases, although the changes are not quantitatively equivalent.

The mechanisms controlling endogenous activity of a spore are fundamental to its survival, dormancy, longevity, and germination. The general subject of endogenous metabolism, particularly as related to microorganisms, has been reviewed recently (Lamanna, 1963). Although spores should be uniquely suited for such studies, scant attention has been devoted to this aspect of their metabolism.

Previous investigations with spores of *Myrothecium verrucaria* showed that a variety of treatments can stimulate their endogenous respiration. In particular, azide at 10^{-3} M and heat treatment at 50 C cause increases by factors of 10 and 5, respectively (Mandels, 1963). The present studies were undertaken to determine the endogenous reserves of spores of this fungus and to investigate the utilization of these reserves under conditions affecting their mobilization.

MATERIALS AND METHODS

Spores of *M. verrucaria* QM 460 were harvested from agar cultures grown at 29 C with filter paper as carbon source and were washed before use, as described previously (Mandels, 1951). Since sporulation is complete in about 5 days, spore age is equal to culture age minus 5 days.

Respiration was determined by use of standard Warburg techniques at 30 C. All enzyme assays and metabolic studies were by incubation at 29 C on a reciprocal shaker, unless noted to the contrary. Viability was determined by counting the percentage of spores germinating on agar containing 1% sucrose and 1% yeast extract.

Water-soluble nitrogen compounds were determined by extracting spores in hot water (100 C for 1 hr), concentrating the centrifuged supernatant fluid under vacuum at ca. 35 C, and chromatographing on paper with three solvent systems. Sulfanilic acid, sodium azide-iodine, ferricyanide-nitroprusside, and *p*-dimethylaminobenzaldehyde were used in addition to ninhydrin for spraying the chromatograms (Block, Durrum, and Zweig, 1958).

Total carbohydrate (soluble), exclusive of hexitols, was determined by the orcinol reagent (Rimington, 1940) and reducing sugar by the dinitrosalicylic acid method (Sumner and Somers, 1944). Since the data showed most of the hot water-soluble orcinol-positive material to be

trehalose, the total carbohydrate value determined by orcinol was used to express the trehalose content. Chromatographic separation and determination of carbohydrates were effected on Whatman no. 3 filter paper with isopropanol-glacial acetic acid-water (27:4:9) or pyridine-ethyl acetate-water (4:10:3) and $AgNO_3$ or benzidine sprays (Block et al., 1958).

Mannitol was determined quantitatively by Parrish's (1958) modification of the method of MacFadyen (1945).

Acid treatment was with 0.1 N HCl at 30 C.

To acid-treat spores, samples of an aqueous suspension were combined with an equal volume of 0.2 N HCl. After incubation at 30 C for appropriate times, the samples were brought to approximately *p*H 5.8 with two volumes of phosphate or with appropriate smaller volumes of 2 N NaOH.

RESULTS

Spore composition. Gross analyses to determine the major types of compounds present showed, on a dry weight basis, ca. 3% fat (ether extraction), ca. 9.5% total nitrogen (Dumas), and ca. 33% total carbohydrate (digestion with 1.5 N H_2SO_4 at 100 C for 2 hr).

Determination of the carbohydrates in hot-water extracts by paper chromatography showed the presence of trehalose, mannitol, glucose, and an unidentified nonreducing compound, "X." Average values from a number of quantitative determinations show trehalose to account for 18.6% of the dry weight; mannitol, 2.0%; glucose, 0.3%; and the unidentified component, probably less than 0.3%.

The trahalose content of spores did not change significantly as they aged in cultures up to 50 days. Since the total dry weight per spore would have decreased considerably by 50 days (Mandels, 1963), we must assume that there is a nonselective loss of trehalose during aging. Until additional analytical data are available, we cannot comment on the compositional changes which probably occur as spores age.

The spores used in these studies have all been grown on a cellulose substrate. Growing them on trehalose has no significant effect on their trehalose content.

The identity of trehalose was confirmed by preparation of the octaacetate from an aqueous extract (100 C for 1 hr) of spores. The crystalline product had a melting point and optical rotation in agreement with published values (Hudson and Johnson, 1915) for $\alpha\alpha$-trehalose octaacetate. Carbon and hydrogen analyses and nuclear magnetic resonance spectra support the identification, and the infrared spectrum is identical with that published by Isbell et al. (1957). Furthermore, extensive tests by paper chromatography with a variety of spray reagents, column chromatography (Nuchar), and gas chromatography (Sweeley et al., 1963) confirmed trehalose as the main water-soluble carbohydrate of the spores.

The identity of D-mannitol was confirmed as follows, by use of 95% ethyl alcohol extracts (Scharoun, *unpublished data*). After gradient elution on an alumina column, the fractions eluting below 65% ethyl alcohol were further chromatographed on a Darco-Celite column. The compound crystallizing out upon cooling was identified as D-mannitol by comparison of melting points, infrared spectra, and X-ray diffraction patterns. The characteristics of the hexa-*o*-acetyl derivative also agreed with that prepared from pure D-mannitol.

The unidentified carbohydrate component, "X," appears to be a nonreducing sugar phosphate. As separated by paper chromatography, it reacts with the orcinol reagent and with silver nitrate-sodium hydroxide spray. Its reaction with Amidol reagent (Bartlett, 1959) and with ferric chloride-sulfosalycylic acid spray indicate it to be phosphorylated. It is not glucose-1-phosphate, glucose-6-phosphate, fructose-1-phosphate, fructose 1,6-diphosphate, trehalose-6-phosphate, trehalose-6,6-diphosphate, ribose-1-phosphate, ribose-5-phosphate, or glycerol-1-phosphate. Since "X" is in aqueous spore extracts and reacts with orcinol, it is included in analytical values for trehalose. In no case was it found in high enough concentrations to affect interpretation of data regarding trehalose changes.

One constituent of the fat fraction is ergosterol or a closely related compound (Scharoun, *unpublished data*). Ethyl alcohol (95%) extracts were placed on an alumina column. The first fractions obtained by gradient elution at about 95 to 90% ethyl alcohol showed the characteristic absorption spectrum of ergosterol, with maxima at 2,935, 2,815, 2,710, and 2,610 A (Gillam and Stern, 1954).

Qualitative determination of amino acids and amides in hot-water extracts showed the presence of leucine or norleucine (or both), valine, γ-amino-*n*-butyric acid, β-amino-*n*-butyric acid, ergothionine, glutamic acid, glutamine, glycine, aspartic acid, asparagine, cystine, cystathionine, and an unidentified component.

Trehalase activity. Viable spores placed in buffer at *p*H 6 or in mineral salts medium did not hydrolyze added trehalose, at least not in excess of metabolic utilization; i.e., no reducing sugars appeared in the medium. Hydrolysis of exogenous trehalose was observed, however, if metabolism was blocked by addition of inhibitors (azide) or removal of oxygen, or if the cells were killed by agents such as toluene or iodoacetate (Table 1). During autolysis under toluene, the trehalose

reserves were rapidly released from the cells and then more slowly hydrolyzed to glucose (Fig. 1). Although not shown here, the final quantity of glucose formed during autolysis is equivalent to the trehalose content. Similarly, heating spores to temperatures above lethal exposures resulted in rapid release of trehalose followed by gradual hydrolysis to glucose, provided the heat exposure did not inactivate the trehalase. Identification of the glucose in these experiments was by paper chromatography or by use of glucose oxidase.

The optima for trehalase activity of spores under toluene were about *p*H 3.7 (Fig. 2) and 40 C (Fig. 3). The temperature optimum for hydrolysis of endogenous trehalose was also 40 C.

TABLE 1. *Hydrolysis of trehalose by spores*

System*	Trehalase activity†
Spores + trehalose	0
Spores + trehalose + azide (5×10^{-3} M)	15
Spores + trehalose + iodoacetate (5×10^{-3} M)	17
Spores + trehalose + toluene	19
Spores + trehalose in N_2 atmosphere	10

* Contained 0.05 M $KHPO_4$ buffer, *p*H 6.25; 0.5% trehalose.

† Expressed as micrograms of reducing sugar formed per milligram (dry weight) of spores per hour.

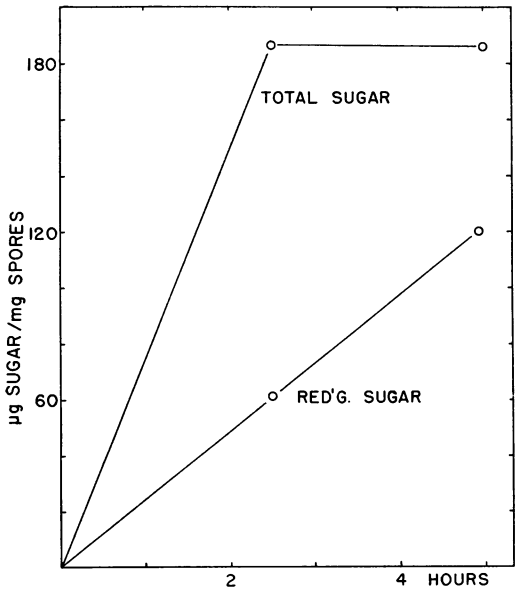

FIG. 1. *Release of trehalose and hydrolysis to glucose during autolysis of spores under toluene [0.05 M citrate buffer (pH 3.6); spore age, 25 days].*

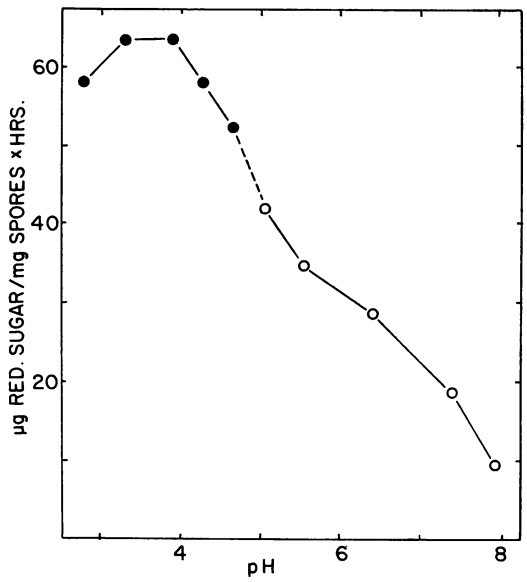

FIG. 2. *Effect of pH on trehalase activity of spores under toluene (6-hr incubation at 29 C; 0.5% trehalose). Symbols: ● = 0.05 M citrate buffer; spore age, 28 days; ○ = 0.05 M phosphate buffer; spore age, 18 days.*

The enzyme in the spores withstood 5 min at 70 C, but was completely inactivated in 10 min at 70 C.

Trehalase was readily extracted by rupturing spores. As with invertase (Mandels, 1951), the enzyme was not associated with wall fragments or particulates, but was in the supernatant fraction, the relative values for trehalase and invertase extraction being approximately equal (Table 2). Incomplete extraction of enzyme was probably due to incomplete rupture of all spores.

Utilization of endogenous trehalose. Spores suspended in water, buffer, or nutrient salts solution did not germinate, and their endogenous respiration was very low, the Q_{O_2} being about 3 to 4 and the trehalose losses about 5 μg per mg of spores per hr. A Q_{O_2} of 3.5 is equivalent to a rate of disaccharide oxidation of about 4.5 μg per mg of spores per hr, assuming complete oxidation to CO_2 and water.

More rapid utilization of trehalose occurred, however, during germination or if the spores were activated. Despite the presence of relatively large carbohydrate reserves, these spores require extracellular metabolites for normal germination. Rapid changes in trehalose content of spores occurred during early stages of germination (prior to emergence of a germ tube) in a glucose-yeast extract medium (Fig. 4). After an initial decrease at a rate of about 40 μg per mg per hr, there was a rapid increase in trehalose due to assimilation

of added substrates. It is noteworthy that mannitol increased gradually while trehalose was decreasing and then increased more rapidly. No reducing sugars accumulated, nor could any new carbohydrates be detected by paper chromatography. Analysis of supernatant fluids showed no increase in carbohydrate, so the decrease in trehalose could not have been due to leakage from the cells.

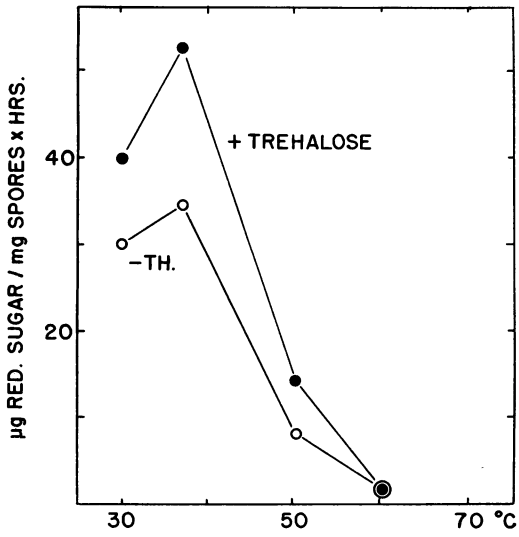

FIG. 3. *Effect of temperature on trehalase activity (+ trehalose) and autolysis (− trehalose) of spores under toluene [6-hr incubation; 0.5% trehalose where added; 0.05 M citrate buffer (pH 3.4); spore age, 8 days].*

TABLE 2. *Extraction of trehalase and invertase from homogenized spores**

System	Activity†		Relative activity	
	Trehalase	Invertase	Trehalase	Invertase
1. Intact spores	31.4	465	100	100
2. Homogenized spores	36.2	562	115	122
3. Supernatant fluid	24.1	321	77	69
4. Residue	7.8	168	25	36

* Spores were homogenized in refrigerated Nossal disintegrator; centrifugation was at 30,000 × *g*; trehalase measured in 0.025 M sodium citrate buffer at *p*H 3.5, invertase was in 0.025 M potassium phosphate buffer at *p*H 6.25; systems 1, 2, and 4 were incubated with toluene for assay; they were heated at 30 C for 1.5 hr; substrates were at 0.5%.

† Expressed as micrograms of reducing sugars per milligram (dry weight) of spores per hour.

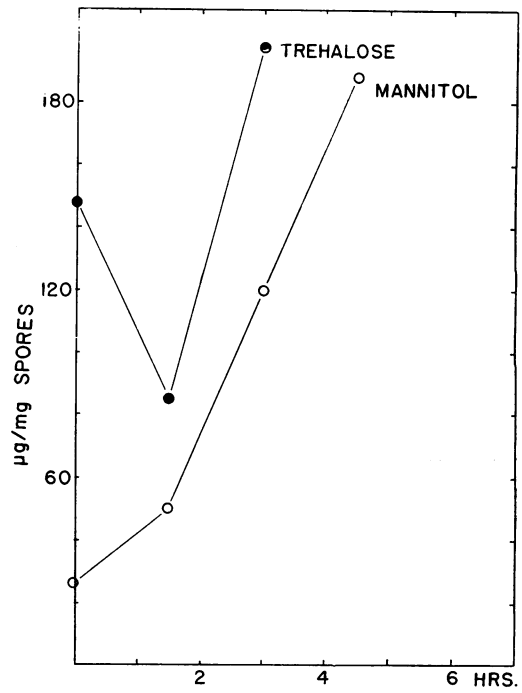

FIG. 4. *Changes in trehalose and mannitol content of spores during the early stages of germination in glucose-yeast extract medium (2% glucose + 1% yeast extract + nutrient salts; spore age, 27 days).*

Azide (ca. 10^{-3} M) stimulates endogenous respiration of *Myrothecium* spores very markedly—about 10-fold (Mandels, 1963). Measurements of carbohydrate changes during incubation with azide showed that trehalose decreases more or less linearly at a rate of about 17 µg per mg per hr (Fig. 5). Concurrently, mannitol increased, although at a slower rate. No reducing sugars could be detected in the spores, nor were any significant amounts of carbohydrate released into the medium.

Endogenous respiration was also stimulated by exposure to heat, 50 C for about 20 min increasing the endogenous Q_{O_2} about fourfold (Mandels, 1963). Measurements of carbohydrate changes during incubation at 30 C after heat treatment showed decreases in trehalose and increases in mannitol (Fig. 6), comparable to those induced by azide. The decrease in trehalose content of the spores was not due to release into the medium. Varying the duration of heat treatment (Fig. 7) showed a pronounced maximum in trehalose utilization between 5- and 10-min exposure.

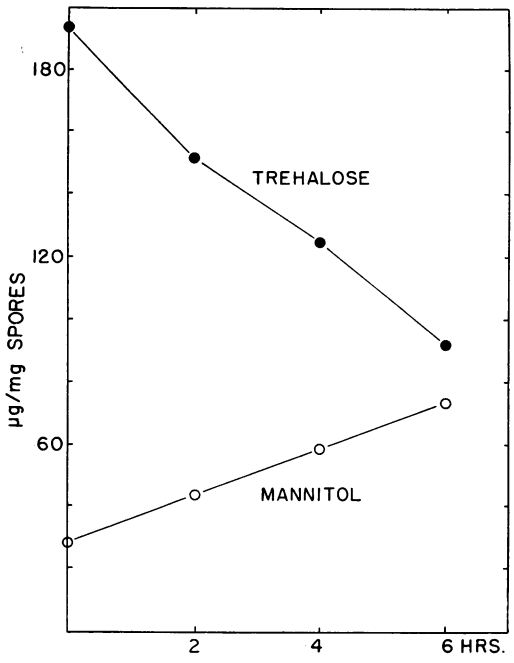

FIG. 5. *Effect of incubation with azide on trehalose and mannitol content of spores [29 C; 1.5 × 10⁻³ M azide; 0.025 M phosphate buffer (pH 6.25); spore age, 7 days].*

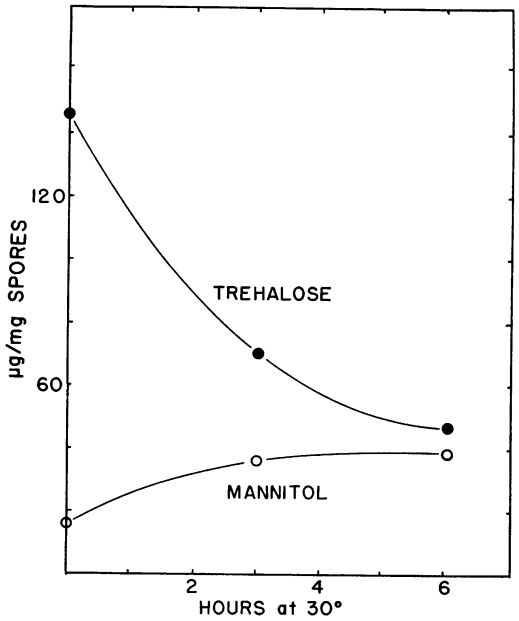

FIG. 6. *Effects of heat exposure on trehalose and mannitol content of spores during subsequent incubation at 30 C. (Spores, 35 days old, heated in 0.05 M phosphate buffer, pH 6, at 50 C for 25 min.)*

Viability tests showed no loss after 5- but complete loss after 10-min exposure. No significant carbohydrate release occurred into the medium, even by the dead spores.

Exposure to azide or to heat at levels stimulating utilization of endogenous trehalose does not affect trehalase activity as measured by determination of reducing sugars after incubating the treated spores with trehalose and toluene.

Effects of heat treatment on release of trehalose. Trehalose did not leak out of spores exposed to moderate, but lethal, heat treatment. More severe exposures were used, therefore, to see if the data would shed any light on the manner in which trehalose was bound within the cells. Spores were heated at 55 or 60 C for different times and then incubated at 30 C. Samples were removed for analysis of the supernatant fraction during heat treatment and during subsequent incubation (Fig. 8). Viability tests showed that spores were killed at all treatments. At 55 C, appreciable trehalose was released only after 25-min exposure, and no more leaked out during incubation. Spores heated 10 min at 60 C lost some trehalose, and slow leakage continued after cooling. At longer exposures, the release occurred rapidly and only during heating. The carbohydrate released during

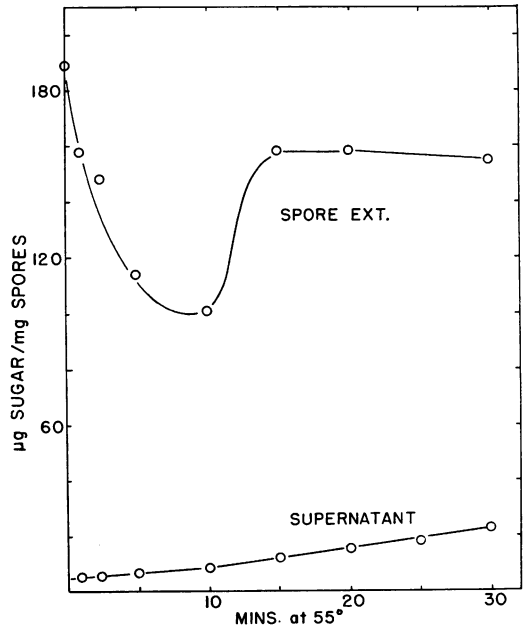

FIG. 7. *Effect of time of heat exposure on trehalose content of spores and on leakage to the medium after incubation for 2.5 hr at 29 C. Spores heated 10 min were dead. (Spores, 14 days old, heated in water at 55 C and then incubated for 2.5 hr at 29 C.)*

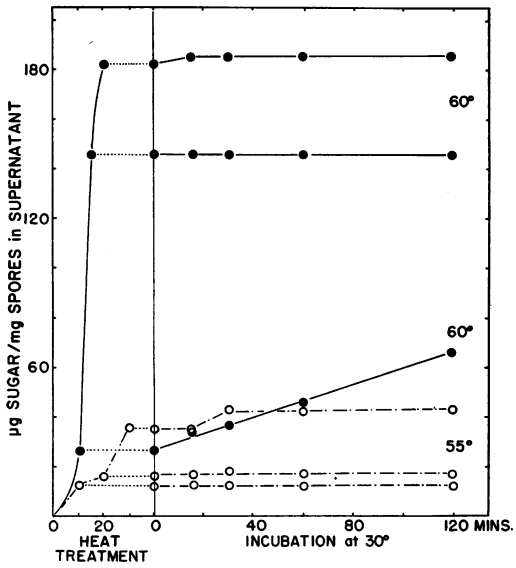

FIG. 8. *Release of trehalose from spores during heat treatment at 55 C (○) or 60 C (●) and during subsequent incubation at 30 C. (Spore age, 11 days; suspended in water.)*

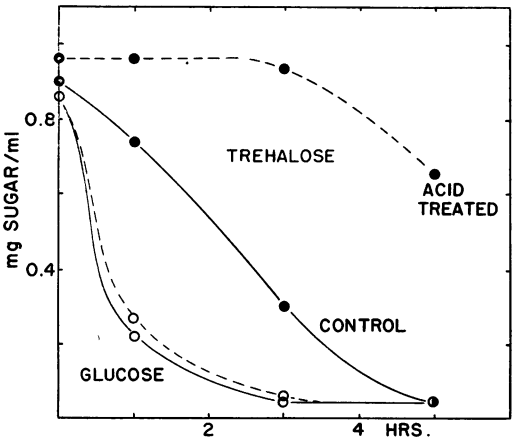

FIG. 9. *Effect of acid treatment on absorption of glucose and trehalose by spores. Acid treatment, 15 min, 0.1 N HCl; incubation in 0.05 M phosphate (pH 6.5) at 29 C; spore age, 24 days.*

all of these heat treatments was trehalose, as shown by paper chromatography. Hydrolysis to glucose occurred only during subsequent incubation at 30 C.

Killing cells by addition of toluene or iodoacetate caused trehalose to diffuse into the suspending medium, where it was gradually hydrolyzed by the trehalase remaining with the spores.

Metabolism of exogenous trehalose. Although endogenous trehalose reserves were utilized only after subjection of the spores to external stimuli, as discussed above, exogenous trehalose is readily metabolized. Thus, absorption of trehalose oc-

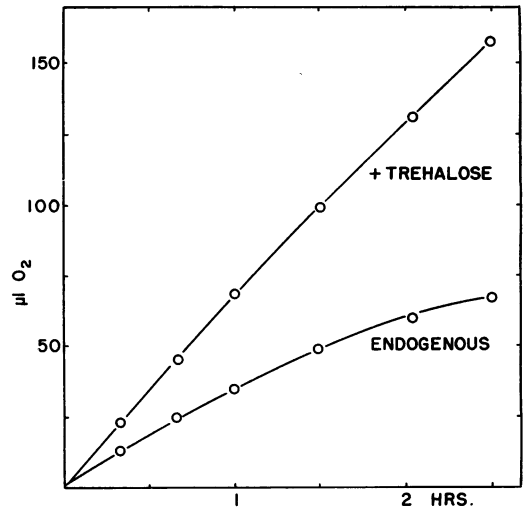

FIG. 10. *Respiration on exogenous trehalose [12.1 mg of spores, 32 days old, per vessel; 0.05 M potassium hydrogen phthalate buffer (pH 5.9); 20 mg of trehalose per ml].*

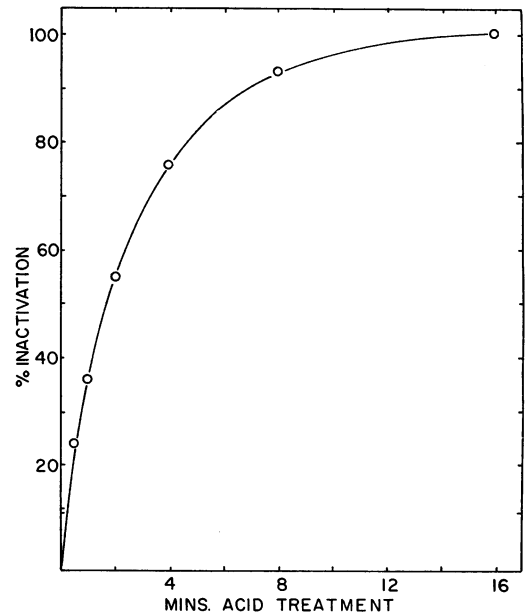

FIG. 11. *Inactivation of trehalase of spores by acid treatment (0.1 N HCl; trehalase assay: 0.5% trehalose in 0.05 M citrate at pH 3.6; spore age, 6 days; + toluene).*

curred with no significant lag, although not as rapidly as glucose (Fig. 9). Similarly, respiratory measurements showed no lag in oxygen uptake after addition of trehalose (Fig. 10).

Effects of acid treatment. The failure of spores to metabolize their endogenous trehalose reserves, despite the presence of adequate trehalase, could be due to physical separation or chemical blocking of enzyme and substrate. Previous studies (Mandels, 1953*b*) showed that brief exposure to 0.1 N HCl completely destroyed certain enzymatic activities of intact spores, whereas viability and respiratory activity were unaffected. Thus, a number of enzymes appear to be located at the spore surface, i.e., outside the osmotic barrier. As in these cases, acid treatment of spores inactivated the trehalase (Fig. 11), indicating surface localization of trehalase and implying that the initial step in metabolism of endogenous trehalose is not hydrolytic.

The effects of acid treatment were not confined to inactivation of surface enzymes. Absorption of exogenous trehalose was greatly decreased, whereas glucose absorption was unaffected (Fig. 9). Furthermore, considerable amounts of trehalose (Fig. 12) were released, as well as small amounts of phosphorus and nitrogen. The significance of the N and P released is not known. Presumably, at least part arose from the few cells killed by the acid. These data indicate that approximately 30% of the endogenous trehalose is not within the spore, but is bound in some unknown manner at its surface. It was not accessible to hydrolysis by an extracellular enzyme, as shown by incubation of spores with an enzyme preparation from culture filtrates of *Aspergillus niger* (QM 877) containing trehalase in addition to a number of other carbohydrases. Furthermore, it was not released by prolonged incubation in water or buffer, nor did heat treatment indicate some trehalose to be released more easily.

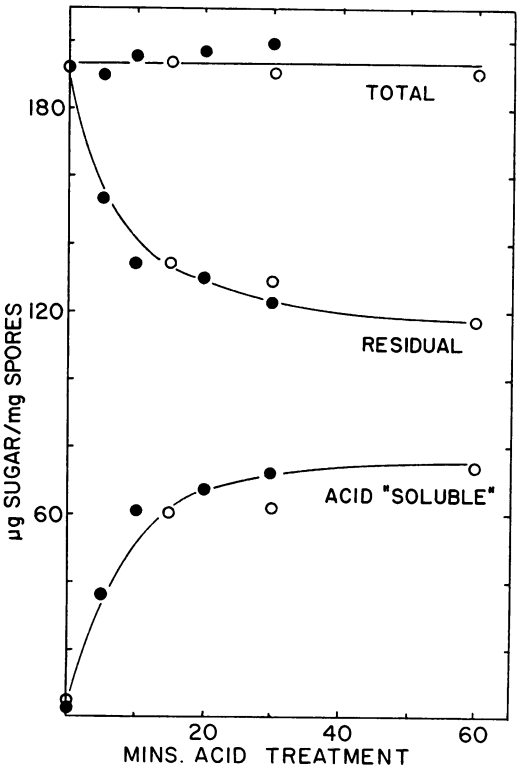

FIG. 12. *Changes in trehalose content of spores and accumulation in the medium during acid treatment [0.1 N HCl, 30 C; spore age, 8 days* (○) *or 9 days* (●)].

DISCUSSION

Trehalose appears to be the major endogenous reserve of *M. verrucaria* spores. This nonreducing disaccharide is apparently common in fungus spores, having been found in ascospores of *Neurospora tetrasperma* (Sussman and Lingappa, 1959), uredospores of *Puccinia graminis f. tritici* (Reisener et al., 1962), phaeodictyspores of *Pithomyces chartarum* (Andrew, 1964), and in conidia of *Penicillium chrysogenum* (Ballio, Divittorio, and Russi, 1964). We have also found it in conidia of *Aspergillus luchuensis* and *Memnoniella echinata*. Trehalose is also found in spores of the slime molds *Dictyostelium mucoroides* (Clegg and Filosa, 1961) and *D. discoideum* (Ceccarini and Filosa, 1964). The chemistry and biochemistry of trehalose, as well as its occurrence in nature, have been reviewed by Birch (1963).

Despite the presence of sufficient trehalose reserves to provide an adequate carbon source, germination does not occur even when spores are suspended in water containing all inorganic nutrients essential for growth and when conditions of temperature, *p*H, and oxygen are favorable. This failure to utilize trehalose does not appear to be due to a lack of appropriate enzymes, since exogenous trehalose is absorbed and respired by spores with essentially no lag. Furthermore, this fungus grows and sporulates well with trehalose as sole carbon source in either solid or liquid culture (Darby, *unpublished data*).

Under suitable conditions, however, the spores utilize their endogenous trehalose reserves readily. Such conditions include sublethal heat exposure or treatment with azide. Furthermore, spores germinating in the presence of exogenous substrate utilize their trehalose reserves, at least during the early spore swelling stages prior to emergence of a germ tube.

These observations indicate that all components of an endogenous metabolic system are present in resting spores, but that it is blocked in some way, possibly by an inhibitor or by separation of enzyme and substrate by chemical or physical means. Since no data indicating the involvement of an inhibitor are available, we will not consider this possibility further. It is also possible that de novo synthesis of some enzyme, or intermediate, is involved.

The mechanism of utilization of trehalose is not known. Although trehalase is present at quantitatively adequate levels, it appears to be located at the spore surface and is therefore not involved, except possibly in transport of exogenous trehalose into the cells. The surface location of trehalase is inferred from the results of treating spores with 0.1 N HCl for short periods, which completely inactivates the trehalase. Since this does not kill the spores, nor impair respiratory activity and absorption of sugars such as glucose, fructose, sucrose, and turanose, it is presumed that trehalase is at, or outside, the permeability barrier of the cells as has been shown for invertase, ascorbic acid oxidase, and a sulfhydryl oxidase, as well as in *Aspergillus luchuensis* spores for trehalase, invertase, maltase, and cellobiase (Mandels, 1951, 1953*a*, *b*, 1956).

Assuming that trehalase is at the spore surface, it is noteworthy that no reducing sugars appear in the culture medium when intact spores are exposed to exogenous trehalose. The initial concentration of glucose is essentially zero in the spores and in the surrounding medium. One might assume, therefore, an equal probability for the hydrolytic product(s) to diffuse in either direction. Since it does not, we assume that some active transport mechanism effects translocation into the cells (see Mitchell, 1963, for a recent review). In this connection it is noteworthy that absorption of trehalose is suppressed by acid treatment, whereas no significant effects are observed on the absorption of glucose. Presumably, acid treatment destroys some mechanism governing the absorption of trehalose. Possibly this is the role of the surface-localized trehalase.

Attempts to establish how trehalose is metabolized have been unsuccessful. The only real clue is the increase in mannitol, as endogenous trehalose decreases when activated by heat treatment, incubation with azide, or during the early stages of germination. Whether mannitol is an intermediate in a metabolic sequence or a side product cannot be stated. Since trehalose is a D-glucosyl-D-glucoside, conversion to mannitol would require at least several steps, the configuration of the number 2 C atom being different in glucose and mannitol. The fact that fructose and mannitol are similar stereochemically (in the Fischer convention), and that enzymatic mechanisms have been established for the conversion of glucose to mannitol via phosphorylated intermediates of fructose and mannitol, suggests this as a possible pathway, although no changes or increases in phosphorylated carbohydrates could be detected.

We can conjecture that the enzyme involved in the initial attack is physically separated from the substrate. The substrate, trehalose, is apparently in a labile form, not rigidly bound within the cells. Indeed, some appears to be at the surface of the spores, i.e., outside the permeability barrier, since sublethal acid treatment releases in the order of 30% of the total trehalose. This surface trehalose is not susceptible to hydrolysis by added trehalase.

Trehalose is released quickly from cells killed by heat or toluene or by rupturing. In the case of heat-treated cells, the exposure must be appreciably greater than the minimum required for loss of germinability. When such conditions are met, nitrogen and phosphorus, as well as trehalose, diffuse from the cells. Although the ratios are not constant, their release is more or less parallel, implying a general breakdown in permeability barriers.

The effects of increasing exposure of these spores to heat are obviously complex and beyond the scope of this paper. Certain aspects are pertinent, however, and are summarized diagrammatically (Fig. 13). At sublethal exposures, the rate of germination decreases markedly (*unpublished data*). With increased exposure, the spores lose their ability to germinate, even on a complex sucrose-yeast extract medium. Leaching of soluble constituents does not yet occur, however, or at least only very slowly. At somewhat higher temperatures, osmotic barriers to the external environment are broken, and rapid leakage of trehalose occurs. Prior to this stage of extensive cell damage, more subtle internal effects must occur, since it is here that marked increases in endogenous respiration are observed, with attendant metabolism of trehalose and increases in mannitol.

The effects of azide treatment on stimulating endogenous respiration with attendant decreases in trehalose reserves and increases in mannitol are similar to those of heat treatment. Whether the activation mechanisms are similar is a matter of conjecture.

Sussman and Lingappa (1959) showed that trehalose is present in *Neurospora* ascospores, accounting for about 14% of the dry weight, and

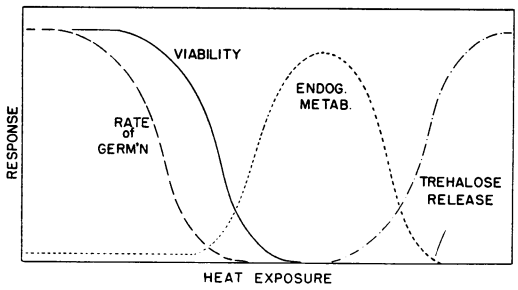

FIG. 13. *Diagrammatic summary of effects of heat exposure on rate of germination, viability, endogenous metabolism of trehalose, and trehalose release.*

that it is utilized after heat activation (Lingappa and Sussman, 1959). Calculations from their data show the rate of utilization to be about 26 μg per mg per hr, which is quite similar to that in *M. verrucaria* spores. Trehalase has also been demonstrated in these ascospores (Hill and Sussman, 1964), although the activity (4 μg per mg per hr) appears to be significantly less than required to account for trehalose utilization. In discussing the hypothesis that ascospore dormancy is due to inability to utilize trehalose, these authors proposed a number of possible activation mechanisms, including de novo enzyme synthesis through formation of an inducer or destruction of an inhibitor or breakdown of spatial barriers separating enzyme and substrate. They also raised the possibility that enzymes other than trehalase may be involved, although no evidence for this was found.

These two radically different types of spores thus have certain similarities. It is interesting to compare the effects of heat activation. The rate of germination of *M. verrucaria* spores is not significantly increased by heat treatment (Mandels, and Norton, 1948), whereas, in *Neurospora* ascospores, heat activation is an effective trigger for endogenous germination. We are thus faced with an interesting phenomenon. The ascospores have a much more effective dormancy, being much longer lived. Their dormancy is easily broken by heat activation, and germination occurs at the expense of endogenous reserves, presumably trehalose. In *M. verrucaria*, on the other hand, the stimulatory effects of heat on endogenous metabolism occur above lethal exposures, and the conidia are incapable of rapid endogenous germination, despite the presence of adequate trehalose reserves.

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