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THE UNIVERSITY OF MICHIGAN
COLLEGE OF LITERATURE, SCIENCE, AND THE ARTS
Department of Botany

First Annual Report

METABOLISM AND ULTRASTRUCTURE OF UREDOSPORES OF Puccinia graminis tritici

(Period: February 28, 1967 - March 31, 1968)

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ORA Project 08737

under contract with:

FORT DETRICK
CONTRACT NO. DAAA 13-67-C-0122
FREDERICK, MARYLAND

administered through:

OFFICE OF RESEARCH ADMINISTRATION ANN ARBOR

April 1968

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ABSTRACT

The ultrastructure and metabolism of cold-dormant uredospores of Puccinia graminis tritici were examined in comparison to these properties of untreated and germinating cells. There is no obvious change in the intracellular organelles of uredospores as a result of the induction of cold-dormancy although an increase in the amount of endoplasmic reticulum is a possibility. The differences between ungerminated and germinating uredospores include a great increase in the endoplasmic reticulum as germination proceeds. In addition, the mitochondria elongate markedly during germination and lose their spherical to ellipsoid shape. At least three types of vesicles are present, two of which may be seen in both aldehyde- and permanganate-fixed uredospores. The third type is seen only in those fixed in permanganate. Membranous inclusions in the mitochondria and free in the cytoplasm have been found, along with organized arrays of ribosomes or microtubules which surround some mitochondria and lipid droplets.

Trehalose, which is ubiquitous in the fungi, has been found in P. graminis tritici to be present on the outside of uredospores as well as inside. Although organic substances have been localized on the outside before, this is the first report of a fungal product being found at this site. A comparison has been made of the destination of C^{14} from various sugars in the presence and absence of fatty acids. Label enters carbohydrates more quickly than lipids and glucose appears to be most quickly metabolized. Cold-dormant uredospores, even after heat-activation release much less labeled CO_2 than do untreated cells, even 22 hr after incubation commenced. Carbohydrases like invertase and trehalase have been studied and their functions analyzed.

ULTRASTRUCTURAL STUDIES OF UREDOSPORES OF Puccinia graminis tritici

When uredospores of Puccinia graminis tritici are stored at temperatures above freezing they germinate rapidly between 20-30°C. However, if exposed to temperatures from -1.1 to -196°C germination under the above conditions is markedly reduced, so that "cold-dormancy" is induced (Bromfield, 1964). Reversal of cold-dormancy can be effected by treatment at 40°C for 5 min or by hydrating spores for 16-24 hr at 20°C, prior to seeding. As Bromfield points out, cold-dormancy in uredospores differs from the dormant state of Neurospora ascospores in that the respiratory rate of the former is not appreciably lower than that in normal uredospores, nor are there chemical means of breaking dormancy. However, the mechanism through which dormancy is imposed on uredospores is still not known.

Therefore, we have investigated the ultrastructure of uredospores in various conditions, including cold-dormant ones, in order to determine whether there are any differences. Although Williams and Ledingham (1964) have studied the ultrastructural changes in uredospores of P. graminis tritici during germination they did not use cold-dormant ones. Nor did they succeed in using glutaraldehyde-osmium fixation effectively with spores so that important intracellular organelles like ribosomes were not found. Similarly, Manocha and Shaw (1967), who worked with uredospores of Melampsora lini, used only permanganate to fix spores so that their work is limited in the same way. Consequently, we have examined cold-dormant and untreated uredospores, along with stages in the germination process, and have used both glutaraldehyde-osmium and permanganate for fixation, applying a technique we developed for use with ascospores of Neurospora (Lowry and Sussman, 1968).

MATERIALS AND METHODS

Permanganate Fixation

Uredospores were fixed in 1% KMnO_4 for 1/2 to 2 hr. However, the longer periods of fixation caused the spore wall to swell and disintegrate while shorter times appeared to give adequate fixation. Spores were then washed and embedded in agar in order that they could be transferred more conveniently.

Glutaraldehyde-Osmium Fixation

Uredospores were fixed in varying concentrations of formalin and glutaraldehyde (1.5% formalin plus 1.5% glutaraldehyde) for periods of several hours to one day. The treatment was varied because we did not obtain good

results with intact ungerminated spores. However, the problem seems to be inadequate infiltration of the embedding medium, not poor fixation, because uredospores were fixed even in the lower concentrations of aldehydes. Therefore, spores were broken a few minutes after immersion in the fixative, or several hours later, no differences being apparent between the two methods. After fixation, the broken spores were centrifuged and an agar pellet prepared. Post-fixation was accomplished in OsO_4 for 1 hr and in uranyl acetate for 1 hr to overnight.

Both permanganate and aldehyde fixed spores were dehydrated in an ethanol series and transferred to propylene oxide. After several changes of the latter, epon was added in a ratio of approximately 1:3. After several hours, the container was uncapped and the propylene oxide was allowed to evaporate. The uredospores were transferred through 2 changes of epon over a 24-hr period at room temperature before being polymerized at 60°C . To enhance infiltration a variety of regimes were used, including epon without accelerator and infiltration for long periods in the cold and at room temperature. However, none of these gave better results than the standard infiltration technique described above and in some the spores were disrupted.

RESULTS

Dormant Uredospores (Aldehyde-Osmium-Fixed)

The notorious difficulty of fixing and embedding fungus spores is well illustrated in the case of uredospores of *P. graminis*. Thus, attempts to fix intact untreated and cold-dormant spores with osmium and glutaraldehyde produced the results seen in Figs. 1 and 5. Both pictures show numerous mitochondria, many of which have inclusions. In addition, numerous vesicles can be seen in Fig. 1, along with osmiophilic granules. Two nuclei also are visible in Fig. 5 and a membrane-bound inclusion can be seen in both. However, the definition of these organelles is poor so improved techniques were sought.

Our previous experience (Lowry and Sussman, 1968) suggested that fixatives and embedding media penetrate ascospores of *Neurospora* only with difficulty. Penetration was markedly improved in this case by breaking the wall of the spore in order to disrupt the permeability barrier so a similar technique was adopted with uredospores. The improved definition which is obtained when spores are broken is demonstrated in Fig. 2 wherein intact and broken untreated ones are compared. In this illustration the mitochondrial cristae illustrate how much better definition is obtained with broken spores. Figure 3 is a broken spore in which mitochondria can be seen along with vesicles. At increased magnification, as in Fig. 4, at least two types of vesicle may be seen in untreated uredospores, and the details of mitochondrial membranes in Fig. 13. Moreover, endoplasmic reticulum is present along with ribosomes which, on occasion, cluster around a vesicle, as at the arrow in Fig. 4.

Similar ultrastructural components are present in cold-dormant spores, as can be seen in Figs. 6-8. Thus, two kinds of vesicles, nuclei and numerous mitochondria are present, as well as abundant ribosomes. That breakage of spores may disrupt the internal organization of cells is suggested in Fig. 8 wherein mitochondrial membranes and the ground cytoplasm seem abnormal.

Dominant Uredospores (Permanganate-fixed)

In contrast to aldehyde and osmium fixatives, permanganate fixes intact cold-dormant spores and permits their subsequent embedding. Such spores may be seen in Figs. 9 and 10 which reveal several differences in comparison to aldehyde-osmium-fixed uredospores. One difference concerns the nucleus in which a fibrous electron-transparent region is distinguished from the rest of the nucleus. Also, an inclusion appears in the nucleus (Fig. 9) although similar objects may be present in aldehyde-osmium-fixed material (Figs. 5 and 17) as well. Another difference is in the presence of electron-transparent bodies of small (sl in Fig. 9) and large (ll in Fig. 9) size which may be related to the osmiophilic bodies to be seen in Fig. 1. These appear to be lipid drops in varying degrees of aggregation. Although vesicles of the type found in aldehyde-osmium-fixed spores are present (cf. vl in Figs. 2, 3, and 9) in permanganate-fixed ones, a different type also is found (v3 in Figs. 9 and 10). These vesicles are smaller than the other two types and are distributed throughout the cell in dormant uredospores.

A germ pore is visible in Fig. 10 as an area where the wall is either absent or composed of a different material than that around the rest of the spore. The latter is the more likely because of the appearance of the germ pores in Fig. 12 in which the wall is continuous but consists of fibrous and amorphous elements of different electron density. Proof that the germ tube actually exits from this region is furnished by Fig. 23. As in the case of aldehyde-osmium-fixed uredospores, endoplasmic reticulum and numerous mitochondria may be found when permanganate is used.

Germinating Uredospores (Aldehyde-Osmium-Fixed)

The mitochondria of germinating uredospores are particularly well shown in Figs. 14-16. One hour after germination is induced they still retain the inclusions which are characteristic of dormant uredospores (Fig. 14). These inclusions appear to consist of concentric membranes (Figs. 14 and 16). But as soon as germination has been extended for two hours, the inclusions decrease in number although occasional ones can still be found (Fig. 16). As germination proceeds the mitochondria elongate (Figs. 16-18) so that few remain round or ellipsoid as are those in dormant spores (Figs. 3 and 6), or those germinated for only an hour (Fig. 14). Ribosomes are abundant and often appear to be aggregated around mitochondria (Fig. 14). Type 1 vesicles are still seen in germinating spores but they, like mitochondria, are transformed from circular

to elongate in shape (Figs. 16 and 18) and they increase in size. Type 2 vesicles and endoplasmic reticulum are commonly found and seem not to change as a result of germination. A nucleus is visible in Fig. 17 in which nuclear pores and inclusions are present. Details of the wall echinulations which were mentioned above are found in Fig. 19 in which the base is particularly well illustrated.

A variety of structures which are osmiophilic are found within uredospores of P. graminis tritici. For example, the type found in Figs. 18 and 20 occur in both dormant and germinating spores and consists of closely wound concentric membranes. A second type is represented by the one found in Fig. 19 in which the membranes are more irregularly arranged than in the previous kind described. The second type usually is found inside an area of the cytoplasm that is not surrounded by a membrane and it appears to be an artifact of fixation by aldehydes.

Germinating Uredospores (Perurauganate-fixed)

Early stages of germinating uredospores fixed with permanganate show features in common with those fixed with aldehydes and osmium. Thus, the mitochondria are larger and more elongated than in dormant cells (Figs. 11 and 22), endoplasmic reticulum is present and the nuclei often contain inclusions (Fig. 21). The pattern of electron-dense and electron-transparent materials in the nucleus differs from that in dormant spores (cf. Figs. 9 and 21) in that the latter materials are more dispersed and of smaller size in germinating spores. Types 1 and 3 vesicles are present in large numbers (Figs. 11, 12, 21, and 22) but, in addition, a fourth type appears which is more elongated than the type 1 vesicle and less electron-dense (Fig. 12).

Later stages in the germination of uredospores are illustrated in Figs. 23 and 24. At the stage shown in Fig. 23, in which the germ tube is extended, some disorganization of its internal contents is visible, as though the cytoplasm has flowed toward the apex of the germ tube. However, several intracellular organelles are visible as in the wall around the germ pore which seems to have been digested away during germination (Fig. 23). By contrast, the tip of the germ tube, which is illustrated in Fig. 24, has many large mitochondria and extensive vesicular endoplasmic reticulum. Irregular electron-transparent patches occur which have fibrous contents. However, few if any of the numerous vesicles which characterize dormant and early germinating stages of uredospores are present at the apex of the germ tube.

DISCUSSION

No difference in the ultrastructure of cold-dormant and untreated uredospores of Puccinia graminis tritici have been found in these studies. As far

as can be determined there is no obvious change in the intracellular organelles which accompanies the induction of cold-dormancy. One possibility is that there is less endoplasmic reticulum in the dormant cells but not enough data are available to authenticate this conclusion.

These results reveal the presence of some endoplasmic reticulum in untreated and, perhaps, in cold-dormant uredospores with little increase during the early stages of germination. However, when the germ tube has been developed as in Fig. 24, a great increase in the vesicular form of the endoplasmic reticulum occurs so that there is a shift in the type to be found before and after germination. That endoplasmic reticulum is more plentiful in the germ tube of uredospores of P. graminis tritici than in ungerminated spores was suggested by Williams and Ledingham (1964) and little endoplasmic reticulum was found in ungerminated uredospores of Melampsora lini (Manocha and Shaw, 1967). An increase in endoplasmic reticulum is a common feature of germination in a variety of fungus spores, including sporangiospores of Rhizopus (Hawker and Abbott, 1963; Buckley, Sjeholm, and Sommer, 1966), conidia of Botrytis (Hawker and Hendy, 1963) and spores of Blastocladiella emersonii (Cantino et al., 1963) but the increase in the vesicular type observed in these studies does not seem to have been noted previously.

The presence of spherical to ellipsoid mitochondria in ungerminated uredospores and their subsequent elongation during germination is a new observation for Williams and Ledingham could observe these organelles in uredospores only with difficulty. However, the behavior of mitochondria of aeciospores of Cronartium fusiforme (Walkinshaw et al., 1967) resembles that of uredospores of P. graminis tritici. By contrast, the mitochondria of ungerminated spores of other fungi are larger and more numerous than those of germinating stages, as in ascospores of Neurospora (Lowry and Sussman, 1968), sporangiospores of Rhizopus (Hawker and Abbott, 1963) and spores of Blastocladiella emersonii (Cantino et al., 1963). In several of these cases, as in uredospores of P. graminis tritici (Williams and Ledingham, 1964) changes in the orientation of cristae also occur.

It was also noted by Williams and Ledingham (1964) that small vesicles are formed in uredospores of P. graminis tritici and that these might be vestiges of the ectoplast whose structure was altered as a result of permanganate fixation. Similar vesicles have been described by us (type 2 vesicles) but these are distributed throughout uredospores so that their origin from the ectoplast is unlikely. Whether they are artifacts of permanganate fixation is uncertain but they do not appear in glutaraldehyde-osmium-fixed material so they may be disaggregated elements of type one vesicles which they resemble in electron density. Type 3 vesicles appear only after germination commences and seems not to be found in the germ tube.

Among the unique, but as yet unexplained, features of uredospores that have been observed by us are the membranous inclusions of mitochondria and cytoplasm. The latter may be an artifact of glutaraldehyde-osmium fixation and

related to one of the types of intravacuolar bodies described by Thomas and Isaac (1967). Another interesting ultrastructural element of uredospores is the organized array of ribosomes or microtubules which surround lipid droplets (Fig. 4) and mitochondria (Fig. 14). Mitochondria have been observed in association with ribosomes or microtubules in aeciospores of Cronartium fusiforme (Walkinshaw et al., 1967). As the latter authors point out there often is a close association between mitochondria and cisternae of the endoplasmic reticulum in higher organisms (Fawcett, 1966) so the phenomenon probably is of wide distribution.

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EXPLANATION OF PLATES

Electron Micrographs of Uredospores of Puccinia graminis tritici

Abbreviations used

B - broken uredospore	n - nucleus
er - endoplasmic reticulum	ni - nuclear inclusion
gp - germ pore	s - spine
gt - germ tube	sb - base of spine
I - intact uredospore	sl - small lipid droplet
ll - large lipid droplet	v1 - type 1 vesicle
m - mitochondrion	v2 - type 2 vesicle
mb - membrane body	v3 - type 3 vesicle
mi - mitochondrial inclusion	

Fig. 1. Glutaraldehyde-osmium-fixed untreated intact uredospore. Note numerous mitochondria and inclusions within them. Osmiophilic bodies that are probably lipid droplets also are visible (sl). mag. X. 8,700

Fig. 2. A comparison of an intact (I) and broken (B) untreated uredospore fixed with glutaraldehyde-osmium. The better definition of mitochondria and cristae is apparent. mag. X. 13,000

Fig. 3. Glutaraldehyde-osmium-fixed untreated broken uredospore. Type 1 vesicles (v1) are visible. mag. X. 8,700

Fig. 4. Glutaraldehyde-osmium-fixed untreated broken uredospore. Type 1 vesicles and an array of ribosomes or microtubules around a lipid droplet may be seen at the arrow. mag. X. 17,000

Fig. 5. Glutaraldehyde-osmium-fixed intact cold-dormant uredospore. Mitochondrial and nuclear inclusions are visible along with lipid droplets. mag. X. 8,700

Fig. 6. Glutaraldehyde-osmium-fixed broken cold-dormant uredospore. Compare definition of mitochondria and vesicles of this figure and Fig. 7 with that in Fig. 5. mag. X. 8,700

Fig. 7. Glutaraldehyde-osmium-fixed broken cold-dormant uredospore. Note lipid droplets, vesicles (v1), and nucleus (n) with pores. mag. x. 8,700

Fig. 8. Glutaraldehyde-osmium-fixed broken cold-dormant uredospore. The internal organization of this spore is disrupted, probably by the breakage of the spore during fixation, so that tears appear in the cytoplasm (arrows). mag. X. 8,700

Fig. 9. Permanganate-fixed untreated uredospore. Large (l1) and small (s1) lipid droplets are visible along with types 1 and 2 vesicles (v1 and v2). The nucleus contains an inclusion and electron- and dense-transparent areas are well delineated. A spine (s) may be seen at the surface of the spore at the top of the figure. mag. X. 13,000

Fig. 10. Permanganate-fixed untreated uredospore. The wide distribution of lipid droplets is apparent as is the large number of type 2 vesicles. Some endoplasmic reticulum (er) and a germ pore (gp) may also be seen. mag. X. 3,100

Fig. 11. Permanganate-fixed uredospores after one hour of incubation during germination. Many type 2 vesicles are present along with extensive endoplasmic reticulum. mag. X. 4,400

Fig. 12. Permanganate-fixed uredospore after two hours' incubation during germination. The wall and spines are well illustrated along with 2 germ pores. Swelling of the wall may be seen around the germ pores. Lipid droplets and type 3 vesicles (v3) also are present. mag. X. 4,400

Fig. 13. Glutaraldehyde-osmium-fixed untreated broken uredospore showing mitochondrial structure including cristae (c). mag. X. 34,000

Fig. 14. Glutaraldehyde-osmium-fixed uredospore after one hour incubation during germination. Ribosomes (r), mitochondrial inclusions and type 1 vesicles are visible. mag. X. 34,000

Fig. 15. Glutaraldehyde-osmium-fixed uredospore after two hours' incubation during germination. Membrane bodies (mb), endoplasmic reticulum and type 1 vesicles are illustrated. mag. X. 25,000

Fig. 16. Glutaraldehyde-osmium-fixed uredospore after two hours' incubation during germination. Elongating mitochondria, a mitochondrial inclusion, and type 1 vesicles are visible. mag. X. 25,000

Fig. 17. Glutaraldehyde-osmium-fixed uredospore after two hours' incubation during germination. A nucleus with inclusions and membrane pores is illustrated. mag. X. 17,000

Fig. 18. Glutaraldehyde-osmium-fixed uredospore after two hours' incubation during germination. A membrane body (mb) and elongating mitochondria, and type 1 vesicles are visible. mag. X. 13,000

Fig. 19. Glutaraldehyde-osmium-fixed uredospore after two hours' incubation during germination. A membrane body (mb) or intravacuolar body, is visible along with spines and their bases (sb). mag. X. 25,000

Fig. 20. Glutaraldehyde-osmium-fixed uredospore after two hours' incubation during germination. Details of membrane bodies are presented. mag. X. 50,000

Fig. 21. Permanganate-fixed uredospore after two hours' incubation during germination. Two nuclei are visible in which the pattern of electron- and dense-transparent materials should be compared with that in Fig. 9. Nuclear inclusions and pores also are visible. In addition, types 1, 2, and 3 vesicles may be seen. mag. X. 6,300

Fig. 22. Permanganate-fixed uredospore after two hours' incubation during germination. Elongating mitochondria and types 1 and 2 vesicles are present. mag. X. 8,700

Fig. 23. Permanganate-fixed uredospore with germ tube. Note swollen wall and surrounding germ pore. mag. X. 4,400

Fig. 24. Permanganate-fixed germ tube. Endoplasmic reticulum of the vesicular and membranous types may be seen. Note absence of vesicles and presence of electron transparent areas in which fibrous elements may be detected. mag. X. 13,000



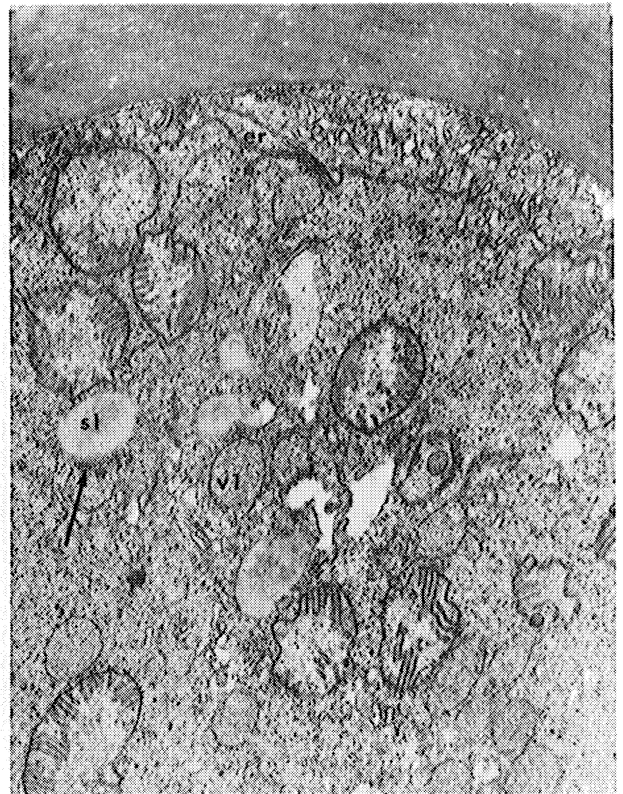
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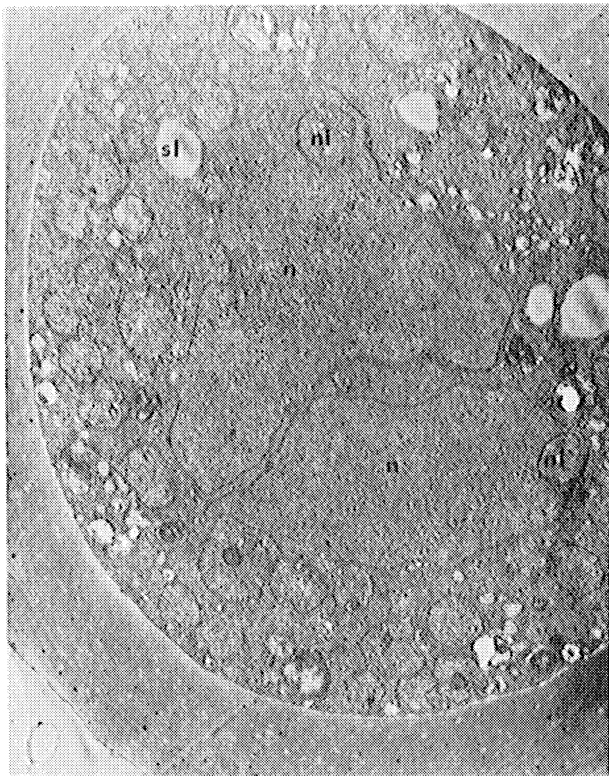
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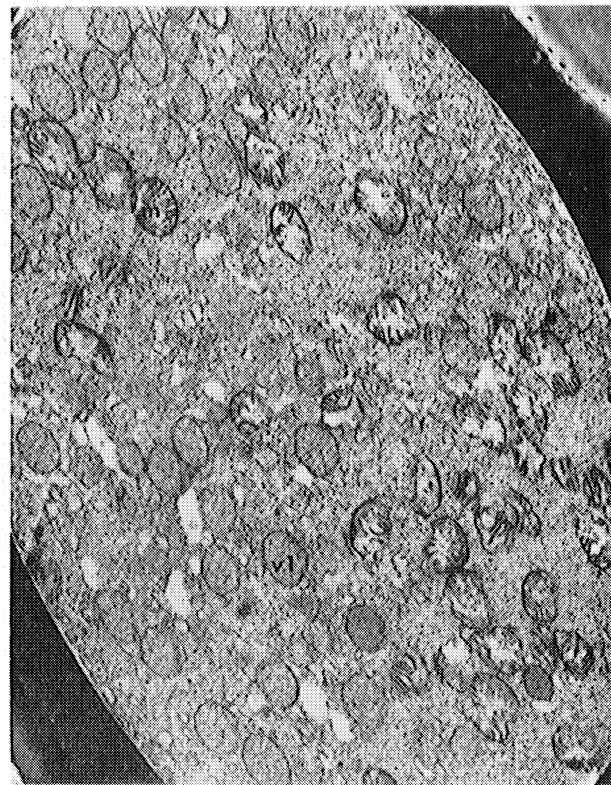
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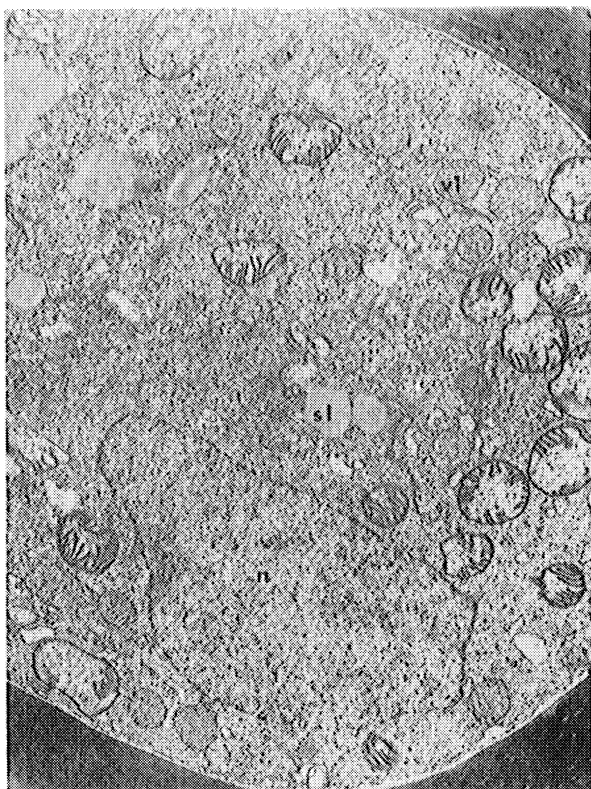
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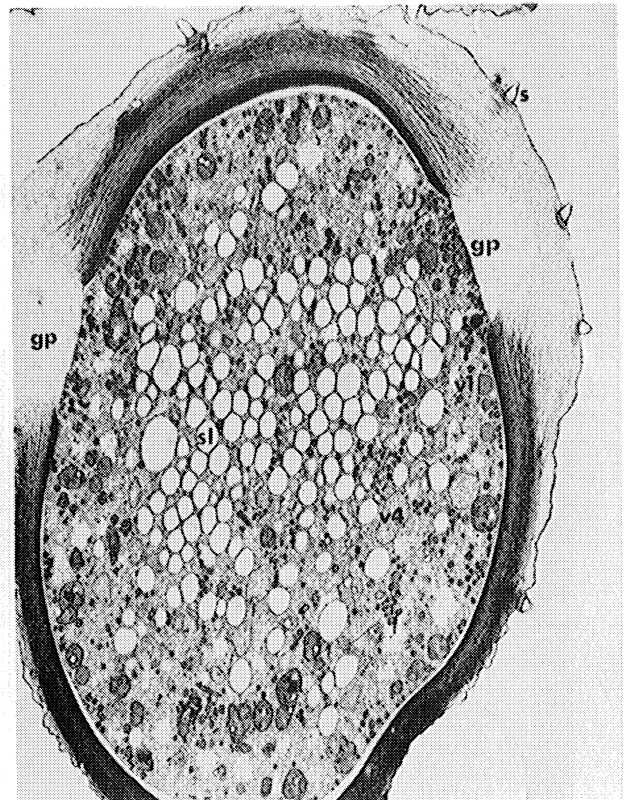
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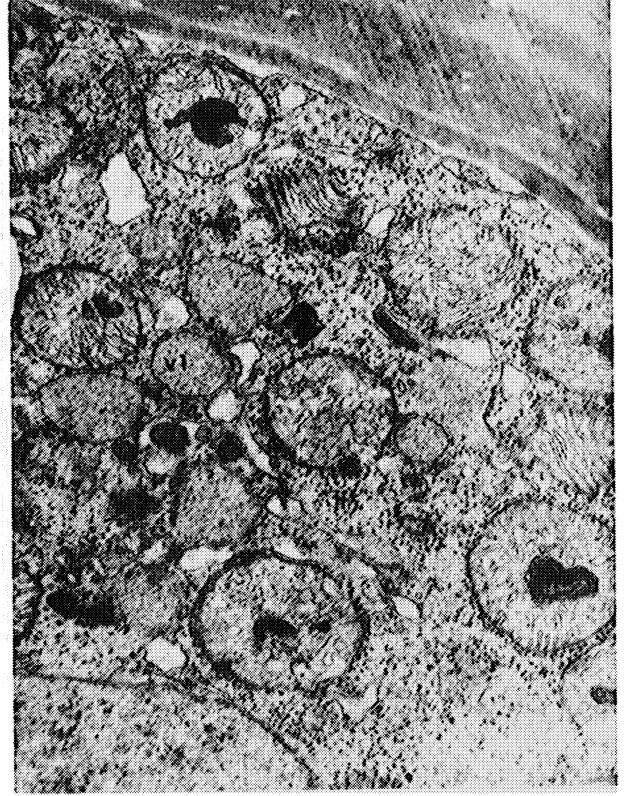
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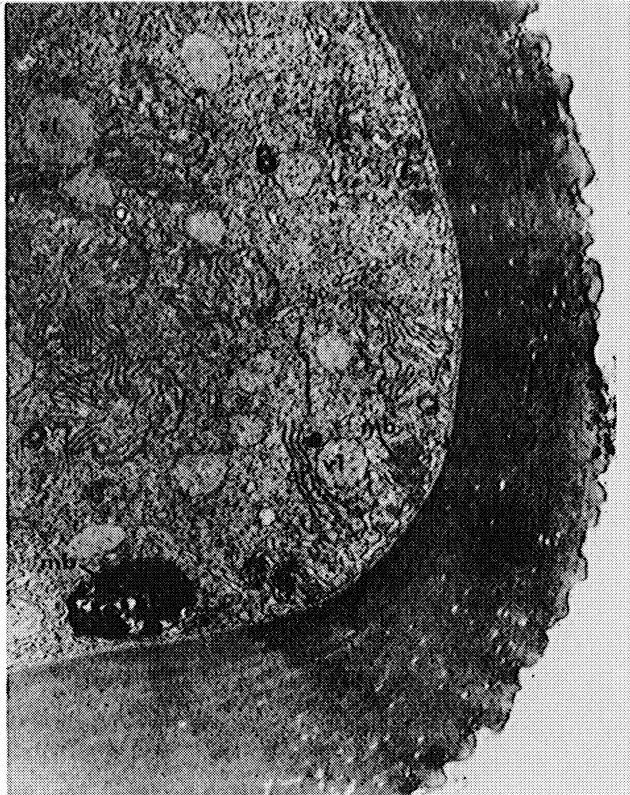
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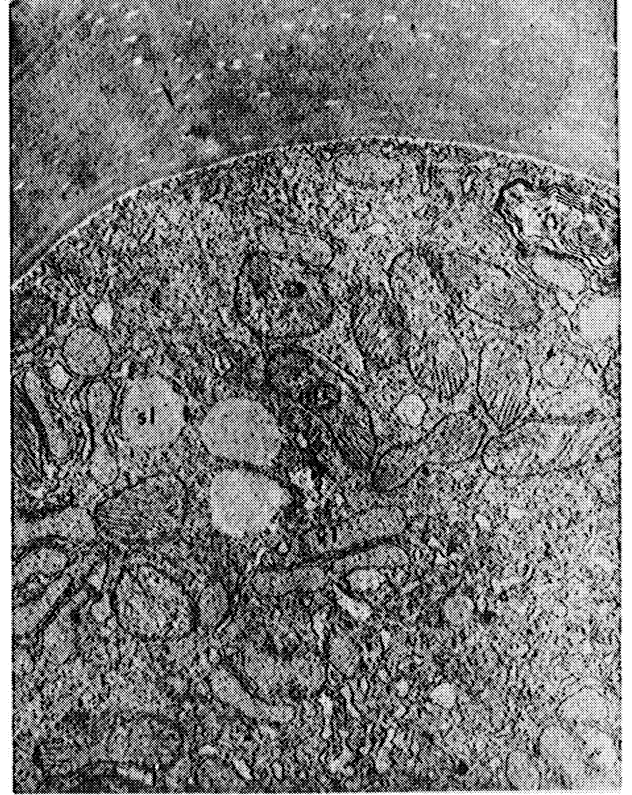
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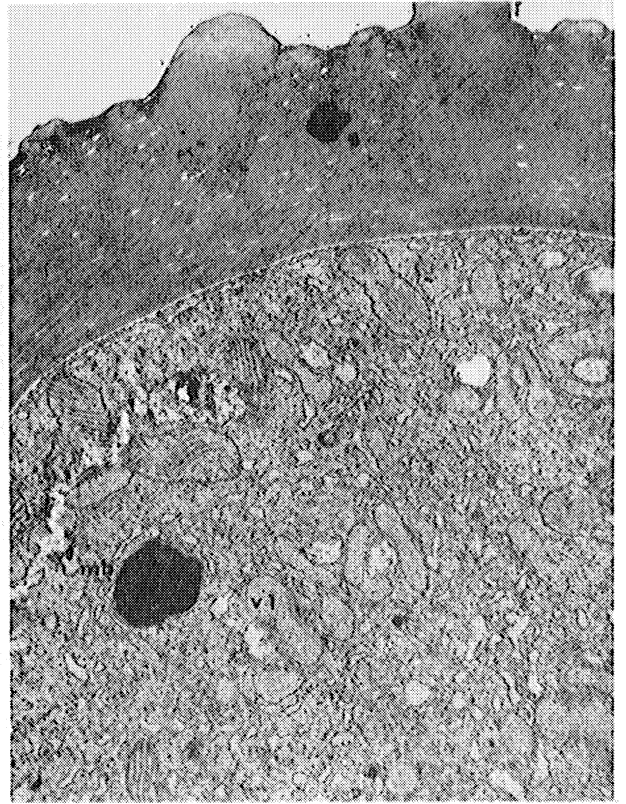
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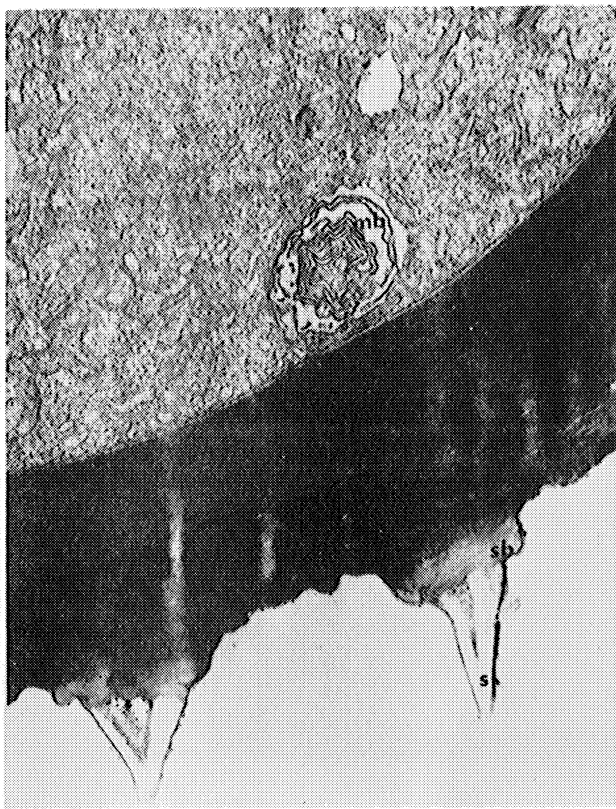
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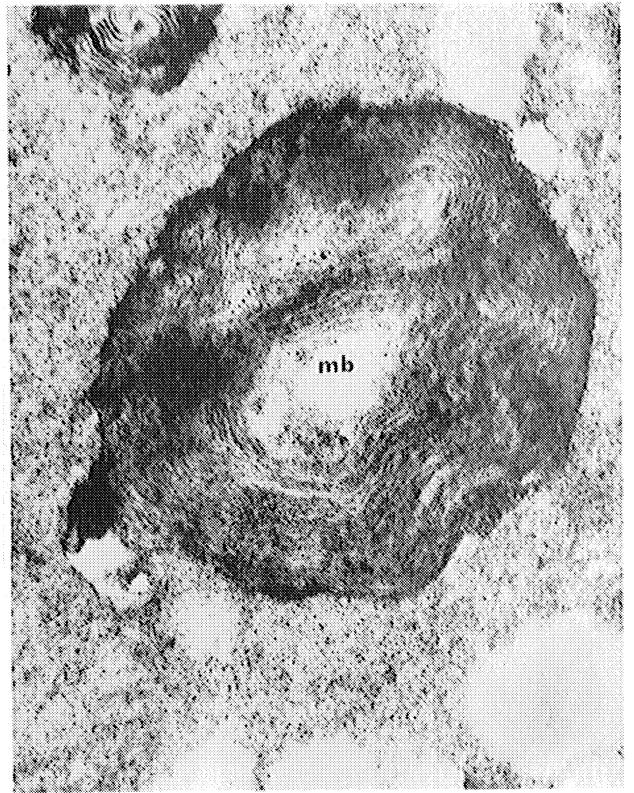
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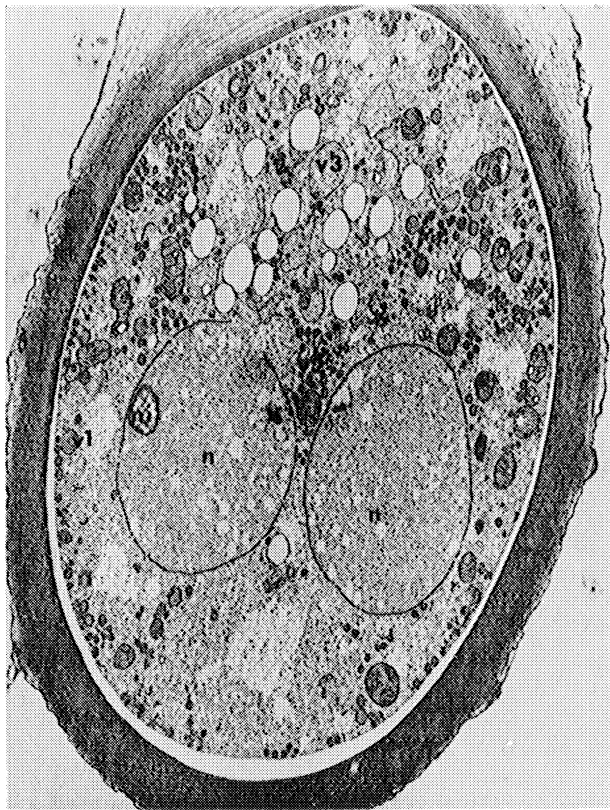
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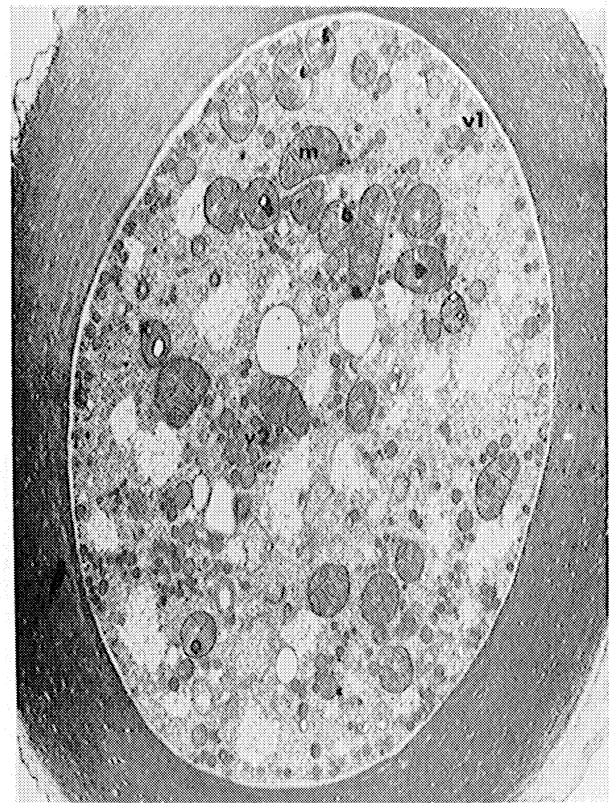
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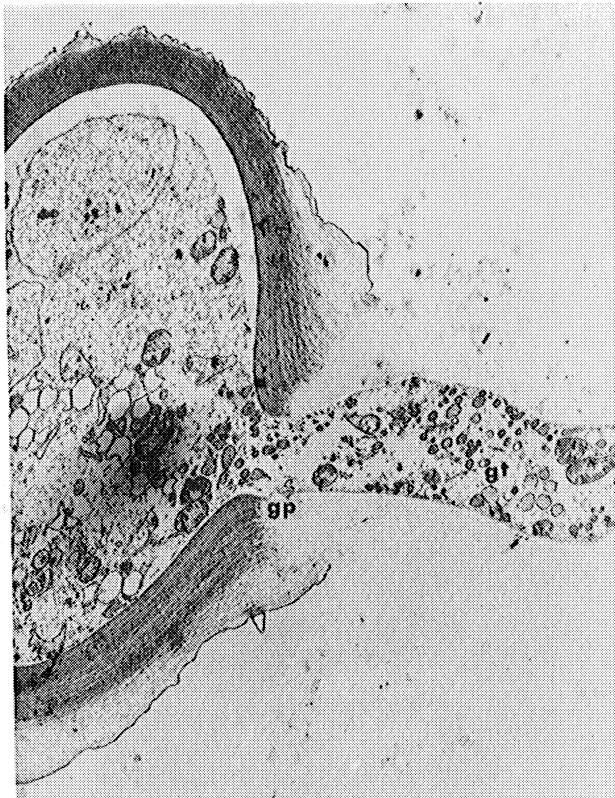
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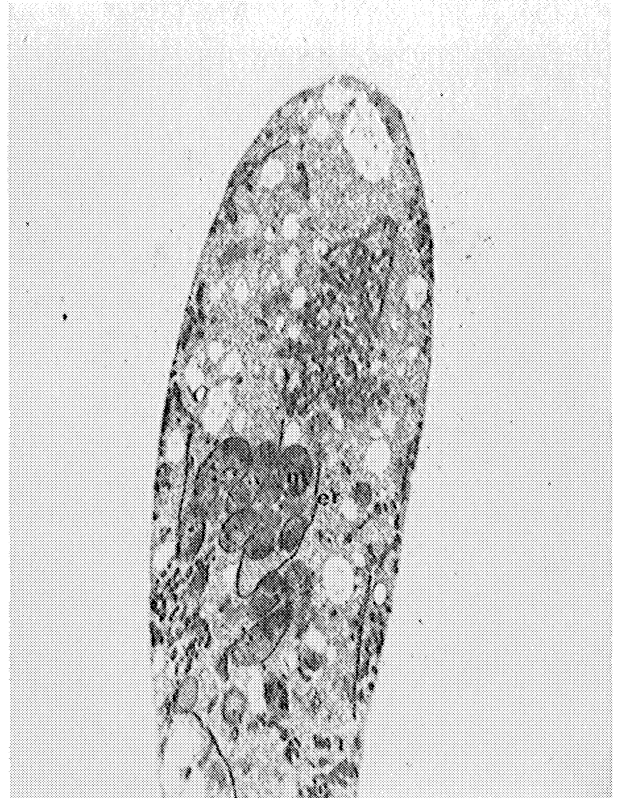
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METABOLIC STUDIES OF UREDOSPORES OF Puccinia graminis tritici

TREHALOSE CONTENT OF UREDOSPORES

The presence of trehalose in uredospores of Puccinia graminis tritici has been detected qualitatively by analysis of extracts with paper chromatography in several solvent systems, periodate oxidation, and digestion by trehalase from *Neurospora*, an enzyme that is specific for this sugar. Further studies were conducted to learn the location of the sugar in the spores. 0.5 gm samples of uredospores (Race 56) were wash 3 times with 7.0 ml of 0.1% Tween 20 and harvested by centrifugation. The eluates were saved, taken to dryness and resuspended in 3.0 ml of water. After the washed in Tween 20, two tubes containing spore pellets were placed in an 80°C water bath for 30 min with 7.0 ml water, and were agitated to keep the spores in suspension. Two other tubes were suspended in 80% ethanol and extracted at 80°C for 30 min. The eluates were collected after centrifugation and the process repeated 2 more times after which they were taken to dryness and resuspended in water. Aliquots of each sample were mixed with buffer and trehalase and incubated 3 hr at 37°C and the glucose liberated determined by the Somogyi reaction with the results obtained in Table I.

TABLE I

LOCALIZATION OF TREHALOSE IN UREDOSPORES OF P. GRAMINIS TRITICI

Sample	No. of Samples	% Trehalose
Surface-localized	4	15.2
Interior		
hot-water extract	2	84.3
hot-ethanol extract	2	85.1
Protocol: 0.1 ml extract 1.7 ml 0.05 M NaPO ₄ , pH 5.6 0.2 ml trehalose 2.0 ml total		

These data reveal that a considerable portion of the trehalose of uredospores of P. graminis tritici is localized on the exterior of the cell. This is of significance because previous work that revealed the presence of organic

materials at this site led to the assumption that these substances originated in the host tissue surrounding the spores. However, the finding of trehalose, which is not found in grasses, suggests that at least this compound is produced by the fungus itself and is secreted to the outside.

INCORPORATION OF C¹⁴ INTO UREDOSPORES

Several sugars labeled with C¹⁴ were added to 50 mg uredospores according to the protocol provided in Table II. Then the uredospores were resuspended in 0.1% Tween-20 and transferred to 12 ml conical centrifuge tubes and centrifuged, followed by 2 more washes in Tween 20. The spores were extracted in 2.0 ml water at 80°C for 30 min and the eluate removed by centrifugation. This process was repeated and the pooled extracts brought to 5.0 ml. The uredospores were resuspended in 3.0 ml chloroform-methanol (2:1), shaken in a reciprocal shaker and left at 20°C for 24 hr, after which the extract was collected and counted. 0.2 ml of each extract was placed in 15.0 ml of scintillation fluid and counted with the results given in Table II.

TABLE II

INCORPORATION OF LABELED SUGARS INTO CARBOHYDRATE AND LIPID FRACTIONS OF UREDOSPORES OF P. GRAMINIS TRITICI

Sugar Added	Incorporation Into:	
	Carbohydrate, c.p.m.	Lipid, c.p.m.
Glucose (10 ⁶ c.p.m.)	35,287	26,932
Fructose (10 ⁶ c.p.m.)	86,850	22,342
Sucrose (10 ⁶ c.p.m.)	88,762	25,695
Trehalose (7 x 10 ³ c.p.m.)	5,437	1,820
Protocol: 50 mg spores (Races 56, w-143: received 6/13/67)		
20 ml dist. H ₂ O		
0.3 ml aureomycin		
0.1 to 0.5 ml C ¹⁴ -sugar		

It seems clear from these data that the principal destination of label is carbohydrate but appreciable amounts go into lipid. Inasmuch as the kinetics of incorporation are only now being investigated it is not possible to state

whether the glucose incorporation is lower because of faster dissemination of label but our latest data make this probable. The fact that sucrose and trehalose were used argues for the presence of invertase and trehalose.

Incorporation of label was studied further by exposing 15 mg of aged (harvested 6/23/67) and fresh uredospores (harvested 3/8/68) to glucose C¹⁴ (approx. 1 million c.p.m.) along with 0.1 ml 0.05 M unlabeled glucose, 0.3 ml aureomycin with distilled water to make 5.0 ml. The spores were incubated in 50 ml Erlenmeyer flasks with cork stoppers holding 1/2 x 1 inch pieces of Whatman 3M filter paper wetted with 0.1 ml 20% KOH. The papers were removed at the times indicated in Table III and replaced with fresh papers. The flasks were vigorously shaken in a reciprocal shaker at room temperature. At the conclusion of this part of the experiment the contents were transferred into 25 mm plastic Petri dishes and the percentage germination determined. Then they were reincubated at 22°C and germination determined again. The KOH papers were placed in 15 ml of scintillation fluid and counted as usual.

This experiment, whose results are given in Table III, revealed that cold-dormant spores, even after activation, released much less labeled CO₂ than did the untreated cells. Less labeled CO₂ was released even after 22 hr. It is also to be noted that the bulk of the CO₂ is released after 5 hr of incubation.

A series of other experiments have revealed that fatty acids like acetate, propionate, and valerate do not speed the rate of incorporation of glucose despite their ability to activate the uredospores. In fact, except in the case of fructose, the incorporation was reduced in the presence of the fatty acid.

Other experiments have revealed the presence of invertase and trehalose in smaller amounts than in other fungi we have studied. The role of these enzymes is currently under study in an attempt to correlate their presence with the utilization of endogenous and exogenous reserve sugars.

TABLE III

RELEASE OF C¹⁴O₂ BY UREDOSPORES OF P. GRAMINIS TRITICI

(Figures Represent the Average of Duplicate Samples)

Type of Spore	Treatment	Hours of Incubation	c.p.m. in CO ₂	Percent Germination
Old*	None	1	1,308	
		2	2,707	
		3	3,335	
		4	3,320	
		5	3,083	
		22	53,233	80
New**	None	1	3,673	
		2	8,197	
		3	5,989	
		4	5,300	
		5	4,279	
		22	28,541	Sparse
Old*	Cold-dorm.	1	371	
		2	71	
		3	33	
	Heated	4	26	
		5	20	
		22	16,906	80
New**	Cold-dorm.	1	2,570	
		2	1,371	
		3	1,205	
	Heated	4	1,035	
		5	944	
		22	13,388	65

*Uredospores harvested 6/23/67

**Uredospores harvested 3/8/68

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