

Distribution of Cellular Carbohydrates during Development of the Mycelium and Fruitbodies of *Flammulina velutipes*¹

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ABSTRACT

Flammulina velutipes (Curt. ex Fr.) Sing. was grown on potato-glucose solution freed of most starch. Glucose uptake and dry weight changes in the colony indicated that the large fruitbodies derived their substrates partly from glucose remaining in the medium and partly from cellular constituents stored in the mycelium and small fruitbodies. Changes in the amounts of low molecular weight carbohydrates, glycogen, and four cell wall polysaccharide fractions were followed in the mycelium and fruitbodies. Trehalose, arabitol, and smaller amounts of mannitol were the main stored low molecular weight carbohydrates. A large net loss of these compounds occurred in the mycelium and small fruitbodies after their growth ceased. The carbohydrates accumulated in the large fruitbodies, but were also partly metabolized in the colony. Reducing sugars were minor components, and included about 30 to 50% glucose and a small undetermined quantity of fructose. Glycogen was the main storage carbohydrate in the mycelium, and was also stored in the small fruitbodies. It was broken down in both structures during growth of the large fruitbodies which accumulated only small amounts. During the same period, almost 45% of the maximum amount of cell wall polysaccharides were degraded in the small fruitbodies, but not in the mycelium.

By feeding ¹⁴C-glucose in replacement medium, incorporation of radioactivity into carbohydrates was followed in the colony during fruitbody development. Total incorporation was highest in trehalose, next highest in glycogen, and the rest was found in polyols and cell wall polysaccharides except for a few per cent which remained in endogenous glucose. In the large fruitbodies, specific radioactivity in glucose was much lower than in trehalose and mannitol. The labeling patterns in the mycelium and large fruitbodies suggested that trehalose, mannitol, and possibly arabitol were translocated into the stipes and pilei.

Stipe elongation in the fruitbodies of *Flammulina velutipes* (Agaricales) requires a supply of water and nutrients from the vegetative mycelium during most of the growth period (9, 10). The identity of the translocated nutrients was not determined, but the elongation of isolated whole fruitbodies is promoted by glucose and other low mol wt carbohydrates (10). Stipe elongation also depends on the lamellae in the pileus (7), and excised lamellae release a diffusate into agar which promotes stipe growth. Production of this diffusate is stimulated by glucose (8). These results indicated that a knowledge of the nature and distribution of cellular carbohydrates would contribute to a better understanding of the relationship between fruitbody growth and the mycelium.

Few reports have been published on the quantitative distribution of cellular carbohydrates during the development of the vegetative mycelium and fruitbodies of Hymenomycetes. Most of the available information has been obtained for *Schizophyllum commune* (Aphyllophorales) where changes in total alcohol-soluble carbohydrates and especially in several polysaccharide fractions were followed in the mycelium and fruitbodies together with glucose uptake from the medium (15, 20). Some data have also been provided on polyols in the mycelium and fruitbodies of this species (3, 14). Less is known concerning changes in cellular carbohydrates during the fruitbody development in Agaricales which differs from that of *Schizophyllum*. Concentration levels of trehalose and mannitol were determined in primordia and at three later stages of growth in the stipe, pileus, and lamellae of *Agaricus bisporus* (16). In *F. velutipes*, it was found that trehalose disappeared from the mycelium during fruitbody growth (19).

In the work described below, glucose uptake, dry weight changes, and changes in the distribution of several low mol wt carbohydrates and polysaccharides were followed quantitatively in the mycelium and fruitbodies of *F. velutipes* until cessation of growth in the colony. The incorporation of ¹⁴C into the various carbohydrates was also followed to obtain information on their translocation into the fruitbodies.

MATERIALS AND METHODS

Culture Conditions and Preparation of Fungus Material. *F. velutipes* (Curt. ex Fr.) Sing., strain 3-6, was used for all experiments. Inoculum for the cultures on liquid medium was obtained from mycelium grown on PDA³ prepared from Norland potatoes as described previously (7). The liquid medium used for all experimental cultures contained in 1 liter a decoction from 400 g peeled, diced potatoes which were freed of most of the starch by filtering once through a coarse sintered glass filter and once through three sheets of Whatman No. 2 filter paper. This procedure reduced the final starch concentration in the medium to 0.1% (w/v) from close to 2% in unfiltered medium. Glucose in the amount of 2% was added to the potato solution. The total concentration of reducing sugars in the autoclaved medium was 23 to 26 g/l, and the pH was 5.8.

Crystallizing dishes 12.5 cm in diameter and 6.5 cm in height, closed with large Petri dish halves, were each filled with 50 ml of the medium, and were sterilized by autoclaving for 5 min. Thiamine hydrochloride was dissolved in 0.1 N acetic acid, sterilized by filtration through a Millipore filter, and 10 μg were added aseptically to each dish. Each dish was inoculated with a piece of mycelium measuring close to 3.5 × 3.5 mm² which was cut from near the edge of a colony grown on PDA for 10 to 12 days at 25 C in the dark. The experimental cultures were main-

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³ Abbreviations: PDA: potato-dextrose agar; LFB: large fruitbodies; SFB: small fruitbodies; VM: vegetative mycelium.

tained at 20 to 21 C until fruitbodies had formed. During the first 2 weeks, the dishes were kept wrapped in newspaper to prevent contamination, and to reduce the light intensity because the mycelium grew somewhat faster in dim light than under the unshielded lights of the culture room. After 2 weeks, the dishes were exposed to continuous illumination of 500 lux from white fluorescent lamps. When primordia had formed, the cultures were placed under continuous illumination at 15 to 17 C. When the fruitbodies elongated, the covers were removed, and each dish was enclosed in a small polyethylene bag to allow the stipes to grow to their full length.

The cultures which were used for each series of experiments on changes in dry weight and carbohydrate levels were inoculated simultaneously from one colony on PDA. Only those cultures were used as source of fruitbodies which produced clearly detectable primordia at 16 to 19 days after inoculation. Numerous primordia usually formed in one cluster in each culture. Weighings and extractions were begun when some primordia had reached 2 to 4 mm in length. At each time interval, the four longest fruitbodies per culture were removed for separate treatment, and are called LFB. During the early phase of fruitbody growth, until 22 days after inoculation, differences in length were often difficult to establish, and four individuals with the largest pilei were arbitrarily chosen as LFB. From the 24th day onward, only those fruitbodies were included among the four largest which had a pileus diameter of at least 5 mm. The mean length of the LFB was over 3 cm at that time. Stipes and pilei of the LFB were first separated for weight determinations at 21.5 days (length over 1 cm), and for extractions from the 24th day onward. All other fruitbodies in the clusters are designated as SFB. The whole cluster was cut from the mycelial mats, and both the mycelium and the SFB were washed three times with distilled H₂O. The hyphae beneath the excised mass of SFB were scraped off as much as possible and were added to the mycelium. All colony portions were dried to constant weight by lyophilization at 10⁻⁵ mg Hg for 2 days before the weights were determined. At each time interval, the mean weights of five cultures were obtained for the study of dry weight changes, and carbohydrate analyses were performed on material from five or more cultures.

Fractionation and Identification of Carbohydrates. The lyophilized samples of fungus material were homogenized with 80% (v/v) ethanol first in a glass homogenizer and then again in a Teflon-glass homogenizer. The homogenate was centrifuged, and the supernatant solution was used for analysis of low mol wt carbohydrates, including polyols. Polysaccharides in the residue were fractionated according to the procedure used for *S. commune* by Wessels (20). After extracting glycogen with 0.5 N acetic acid at 75 C, the residue was suspended in 5% (w/v) KOH for 18 hr at 25 C. The alkali-soluble, acid-insoluble fraction (S-glucan) was precipitated from the supernatant with 22.5% (v/v) acetic acid and washed with 0.5 N acetic acid. The alkali-soluble, acid-soluble fraction remained in the supernatant. The residue from the 5% KOH treatment was first extracted with 10% KOH at 100 C to obtain the hot alkali-soluble fraction, and then with 90% (v/v) formic acid to obtain R-glucan in the supernatant. The same extraction procedures were used for the radioisotope experiments. Wessel's designation of two polysaccharide fractions as R- and S-glucans was used for the *Flammulina* fractions with the same solubilities without necessarily implying that they had identical structures in the two species.

Total carbohydrates were determined in each fraction by the anthrone method (11). The reducing sugars were measured by the Somogyi-Nelson method (13). The low mol wt carbohydrates in the 80% ethanol-soluble fraction were further analyzed by descending chromatography on Whatman No. 1 paper with methyl ethyl ketone-acetic acid-water saturated with boric acid (9:1:1 v/v/v) as the solvent system according to the method of

Rees and Reynolds (17). Trehalose remained at the origin and the R-glucose values were 1.8 for fructose, 2.5 for mannitol, and 4.1 for arabinol. Co-chromatography with known standards and visualization of spots in duplicate runs of extracts served to locate the chromatogram regions which were eluted with 80% ethanol at 25 C for 1 day. The eluates were concentrated and dissolved in 5 ml of distilled H₂O for colorimetric analysis. Arabinol and mannitol were determined by the chromotropic acid procedure of West and Rapoport (21).

Radioisotope Experiments. Colonies which had been grown on potato-glucose medium were removed at different times after inoculation, and were washed three times with 0.05 M K phosphate (pH 7.3). After absorbing the remaining buffer with filter paper, the colonies were floated on 50 ml of replacement medium in Petri dishes. This medium was the same as the one used for *Psilocybe panaeoliformis* (Kitamoto, unpublished), and consisted of the replacement medium used for *Favolus arcularius* (12) and several additions. It contained the following per liter of distilled H₂O: glucose, 5 g; casamino acids, 1 g; KH₂PO₄, 3 g; MgSO₄·7H₂O, 0.1 g; CaCl₂·2H₂O, 0.1 g; ZnSO₄·7H₂O, 0.3 mg; FeSO₄·7H₂O, 0.15 mg; CuSO₄·5H₂O, 0.1 mg; MnSO₄·5H₂O, 0.1 mg; H₃BO₃, 0.3 mg; sodium pyruvate, 11 mg; sodium citrate, 29.4 mg; adenine hydrochloride, 5 mg; orotic acid, 0.5 mg; nicotinamide, 0.3 mg; sodium pantothenate, 0.3 mg; pyridoxine hydrochloride, 0.2 mg; folic acid, 0.03 mg; thiamine hydrochloride, 0.01 mg; cyanocobalamin, 0.01 mg. The pH was adjusted to 7.5 with 1 N NaOH.

Radiochemically pure D-glucose-¹⁴C (lot No. 646171; specific radioactivity 11.1 mCi/mmol) was purchased from ICN Pharmaceuticals. For uptake work, 0.1 ml of ¹⁴C-glucose (100 mCi/ml, dissolved in water) was added to each culture on replacement medium. Following incubation at 15 C for 4 hr in the light, the colonies were placed on a sheet of filter paper and were washed extensively by suction with 200 ml of 0.05 M phosphate buffer. The stipes and pilei of the four largest fruitbodies, the remaining SFB, and the mycelium were homogenized separately as described above. Following fractionation and chromatographic separation of the carbohydrates (see above), a 1-ml sample of each solution was placed into a scintillation vial containing 14 ml of scintillation fluid (PCS solubilizer, Amersham/Searle, Arlington Heights, Ill.). Radioactivity was determined with a liquid scintillation spectrometer (Nuclear-Chicago, model Unilux II).

RESULTS

Fruiting and Glucose Consumption in the Medium. Fruitbodies were initiated at 16 to 19 days after inoculation in about 90% of the cultures on potato-glucose solution. Commonly, the mycelial mat did not completely cover the surface of the medium even after fruitbodies had been initiated. Numerous primordia appeared at approximately the same time and usually formed one compact cluster or sometimes two or three per culture. Occasionally, a few scattered primordia were also produced. Most of the primordia aborted at an early stage of development or grew only a few millimeters in length. Only three to five primordia in each dish (average 3.9) developed into fruitbodies of over 5 cm in length. They had pilei 5 mm or more in diameter when the stipes measured about 3 cm in length, whereas fruitbodies of less than 5 cm final length had smaller pilei. The quantity of nutrients used in these experiments seems to limit the number of large fruitbodies to at most five. The four largest fruitbodies in several dishes attained a mean final length of 9 cm at 30 days after inoculation and their mean final pileus diameter was 1.3 cm.

Figure 1 shows the changes in dry weight of the VM, the stipes, and pilei of the four largest fruitbodies and of the remaining SFB during the colony development. The weight increase of

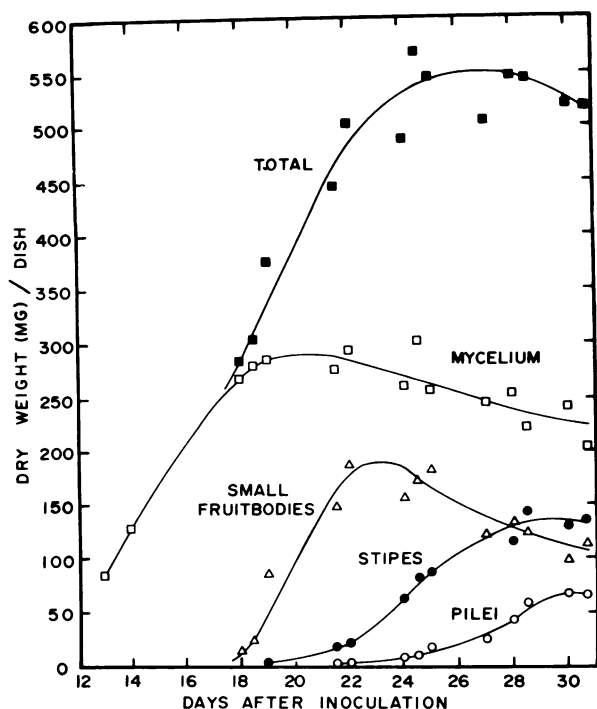


Fig. 1. Changes in dry weight of the vegetative mycelium, the small fruitbodies, and the stipes and pilei of the four largest fruitbodies during growth of the *Flammulina velutipes* colony on potato-glucose medium. The change in total colony weight after onset of fruiting is also given.

the VM was slow at first, but became rapid 1 week after inoculation. Its maximum weight of close to 290 mg/dish was attained between 19 and 21 days after inoculation when numerous fruitbody primordia were growing actively. Subsequently, the weight of the VM decreased to about 220 mg/dish at the termination of growth of the LFB. The maximum dry weight of the SFB portion was reached at close to 23 days after inoculation, shortly after the stipes of the LFB began to grow rapidly. While the LFB grew to their final size at 30 days, the weight of the SFB decreased by 90 mg/dish to almost half of its highest value. The weight loss by the VM and SFB suggests that some of their cell constituents may serve as translocated substrate for the growth of the LFB. However, the total loss of about 160 mg/dish by the VM and SFB was insufficient to account completely for the final combined weight of 200 mg/dish for the stipes and pilei of the LFB. This suggests that at least part of the nutrient supply for growth of the LFB derives from the culture medium where glucose was the main carbon source.

The change in concentration of reducing sugars calculated as glucose was determined in the medium at different stages of colony development. The results in Figure 2 compared to those in Figure 1 show that slightly more than half of the glucose (close to 700 mg/dish) was consumed during the first 18 days of mycelial growth. A further 350 mg were consumed during the following 5 days while the SFB grew to their maximum weight. The remaining 130 mg of glucose disappeared between the 23rd day and the cessation of growth of the LFB. These results agree with the above suggestion that the substrates for growth of LFB are supplied partly by glucose uptake from the medium and partly by substances stored in the mycelium and SFB before the onset of rapid elongation in the LFB. However, during this last stage, the dry weight loss by the VM and SFB together with the quantity of sugar taken up from the medium amount to about 90 mg more/dish than the total weight attained by the LFB. The main loss in dry matter was probably caused by respiration, but there may also have been some excretion of metabolites into the medium, and some losses must have occurred through spore discharge.

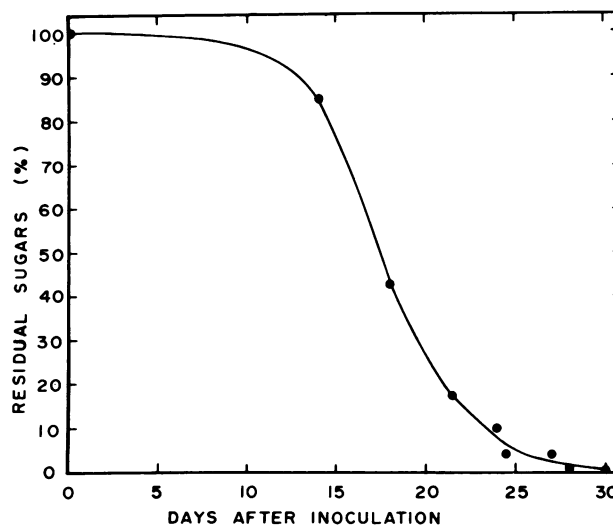


Fig. 2. Consumption of reducing sugars in the medium during fruitbody development. The residual sugar was determined as glucose at successive time intervals, and was calculated as percentage of the initial concentration (23.5 g/l).

Changes in Cellular Low Molecular Weight Carbohydrates during Development.

Changes in the amounts of ethanol-soluble carbohydrates were followed in the four portions of the developing colonies. The major low mol wt carbohydrates were trehalose, and the polyols arabitol and mannitol. Reducing sugars contributed only 1 to 1.5% of the dry weight of the fungus. Glucose constituted about 30 to 50% of the endogenous reducing sugars. Fructose in small amounts was also detected on chromatograms of all colony portions, but was not studied quantitatively. Limited accumulation of reducing sugars (Fig. 3, A and B) accompanied the weight increases in the VM and SFB (Fig. 1), and after the LFB began to grow rapidly, the sugar content decreased slightly, first in the SFB and somewhat later in the VM. In the LFB, the small increase in reducing sugars continued in the pilei until their growth stopped (Fig. 3D), but terminated 3 days earlier in the stipes (Fig. 3C).

Accumulation of trehalose in the VM attained its maximum at 22 days after inoculation, but decreased by about 50% during fruitbody development (Fig. 3A). Trehalose in the SFB (Fig. 3B) followed a similar trend as in the mycelium, but decreased to an even greater extent during growth of the LFB. Trehalose accumulated in the LFB, but during the final 3 days of growth, the amount decreased in the stipes (Fig. 3C) and continued to increase in the pilei (Fig. 3D).

Arabitol was more abundant than mannitol in all portions of the fungus (Fig. 3). The amounts in the VM and SFB increased to maximum levels when the dry weights also reached their highest values (Fig. 1). As the LFB developed, the polyols disappeared almost completely from the VM and SFB (Fig. 3, A and B). Mannitol continued to increase in the stipes and pilei of the LFB until the 27th and 28th day, respectively, and then decreased in both portions. In contrast, arabitol accumulated in the stipes until the end of the growth period (Fig. 3C), but behaved in a similar manner in the pilei as did mannitol (Fig. 3D).

Changes in Cellular Polysaccharides during Development.

Quantitative changes in five polysaccharide fractions were followed during colony development. Rapid accumulation of the acid-soluble fraction (glycogen) began in the VM 2 weeks after inoculation, and continued to the 24th day (Fig. 4A) despite the decrease in dry weight after 21 days (Fig. 1). Thus, glycogen accumulated in the VM throughout the growth period of the SFB and during early growth of the LFB. At its maximum level, glycogen accounted for about 25% of the mycelial dry weight.

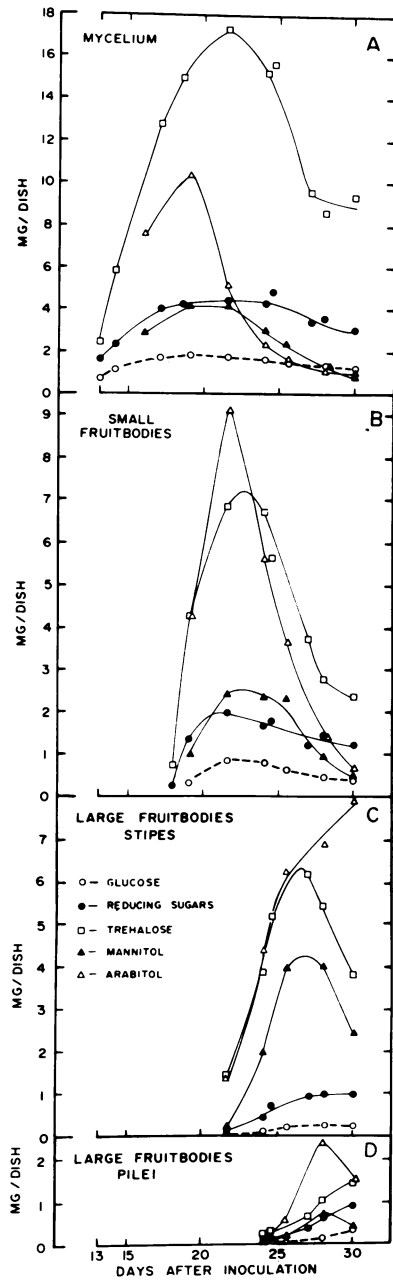


FIG. 3. Changes in the amounts of low mol wt carbohydrates in the colony during fruitbody development. The amounts in mg/dish of glucose, total reducing sugars, trehalose, mannitol, and arabitol were determined at successive time intervals for the vegetative mycelium (A), the small fruitbodies (B), and the stipes (C) and pilei (D) of the four largest fruitbodies. Symbols identifying the individual carbohydrates are given in the graph.

The amount decreased sharply in the VM during the later phase of growth of the LFB. This suggests that a considerable amount of glycogen was utilized during this period as the main substrate for the LFB. In the SFB, glycogen decreased after the 21st day (Fig. 4B), about 2 days before the dry weight began to decline. Accumulation of glycogen in the stipes of LFB continued to about the 26th day, and was followed by a slight decrease (Fig. 4C). In the pilei, the increase continued until growth stopped (Fig. 4D).

The four other polysaccharide fractions are cell wall constituents (20). The formic acid-soluble fraction (R-glucan) predominated, and the alkali-soluble, acid-soluble fraction was next highest in amount. The alkali-soluble, acid-insoluble fraction (S-

glucan) and the hot alkali-soluble fraction were minor constituents in this fungus.

The amounts of the four polysaccharide fractions increased in the VM until the onset of fruiting and then remained almost constant (Fig. 4A). In the SFB, the amounts reached maximum levels between 22 and 25 days after inoculation, and three of the fractions decreased thereafter while the S-glucan level remained almost unchanged (Fig. 4B). Accumulation of cell wall polysaccharides in the LFB continued until the termination of growth, except for the alkali-soluble, acid-soluble fraction in the stipes (Fig. 4, C and D).

Incorporation of ^{14}C into Carbohydrates. The results in Figures 3 and 4 suggest that the glucose taken up from the medium

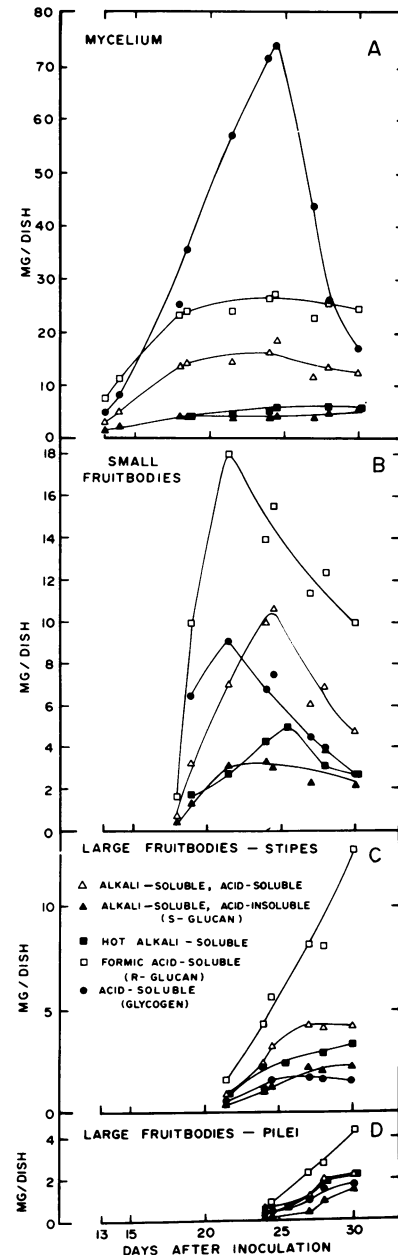


FIG. 4. Changes in the amounts of polysaccharides in the colony during fruitbody development. The amounts in mg/dish of acid-soluble polysaccharides (glycogen) and of four cell wall fractions were determined at successive time intervals for the vegetative mycelium (A), the small fruitbodies (B), and the stipes (C) and pilei (D) of the four largest fruitbodies. Symbols identifying the individual polysaccharide fractions are given in the graph.

was metabolized to translocatable forms of carbohydrates in the mycelium, and that carbohydrates present in the VM and SFB were eventually transported into the LFB either directly or after enzymic conversion. To test this hypothesis, the fate of ^{14}C -glucose was followed by determining the incorporation of the label into low mol wt carbohydrates and polysaccharides in the different colony portions during fruitbody development. Colonies of different age were transferred to replacement medium, and were incubated for 4 hr with ^{14}C -glucose. The label was incorporated into all endogenous carbohydrates. Trehalose contributed 40 to 50% of the radioactivity in all carbohydrates of the whole colony. The next highest degree of labeling was found in glycogen with about 20 to 30% of the total activity. Almost all of the remaining radioactivity was found in polyols and cell wall polysaccharides.

Distribution of Radioactivity in Low Molecular Weight Carbohydrates. Labeling of low mol wt carbohydrates is shown in Table I. The maximum radioactivity in the endogenous glucose pool of the VM accounted for less than 6% of the radioactivity in all carbohydrates. The ^{14}C incorporation into mycelial glucose decreased after the 24th day during the period of rapid growth of the LFB, but increased again at the end of the growth period. The specific radioactivity showed a similar trend. Since a large amount of glycogen disappeared from the VM between the 24th and 30th days (Fig. 4A), it appears that the radioactivity in the endogenous glucose pool was diluted by glucose derived from reserve glycogen in the vegetative hyphae. Labeling of glucose in the SFB was about 10 times less than in the VM, decreased further in the stipes of the LFB, and reached very low levels in the pilei.

Trehalose contributed about 70% of the total radioactivity in the soluble carbohydrates of the VM and SFB, but the proportion decreased to about 50% in the LFB. The labeling of trehalose in the VM increased to a maximum on about the 22nd day,

and then decreased gradually as the LFB developed. About two-thirds of the maximum amount remained in the VM when growth ceased in the LFB. The high specific radioactivity of trehalose in the VM was comparable to that of glucose, and remained fairly constant during growth of the LFB. It can be concluded that the large trehalose pool (Fig. 3A) turned over rapidly, and was not much diluted by unlabeled trehalose which could have derived from degradation of polysaccharides stored in the VM or SFB. Moreover, the specific radioactivities of trehalose in the SFB and LFB were higher than those of glucose. These facts suggest that trehalose is a major translocated carbohydrate in this fungus.

In the VM, the radioactivity/dish in mannitol increased to a maximum on about the 22nd day, at the onset of rapid growth in the LFB, and decreased thereafter. The specific radioactivity remained fairly constant in the VM throughout the development of the LFB. The specific radioactivity in arabitol was low when fruitbodies were initiated, probably because of dilution by the large amount of unlabeled arabitol which was already present in the VM (Fig. 3A). However, during growth of the LFB, the activity increased more than 10-fold in the VM. In the LFB, the specific radioactivity was much higher in mannitol than in glucose. This suggests that the polyol was translocated from the mycelium.

Distribution of Radioactivity in Polysaccharides. Table II shows the incorporation of ^{14}C into the five polysaccharide fractions. In the VM, the highest proportion of ^{14}C was found in glycogen which contained more than 50% of the label found in all polysaccharides. Nevertheless, the specific radioactivity remained at the level of 0.5 to 1 nCi/mg carbohydrate which was only $1/10$ of that in glucose or trehalose. The radioactivity/dish in the VM increased slowly to a peak at 24 to 26 days after inoculation and then decreased gradually. The specific radioactivity increased only during the last days of growth of the LFB.

Table I. Changes in ^{14}C incorporation into low-molecular weight carbohydrates of different colony portions during fruitbody development of *Flammulina velutipes*.

Cultures at different stages of fruitbody development were incubated on replacement medium with ^{14}C -glucose for 4 hr in the light at 15 to 17 C. Incorporation of ^{14}C into the low-molecular weight carbohydrates was determined as described under Materials and Methods.

Colony age in days	^{14}C incorporation as nCi/dish and in parentheses as nCi/mg carbohydrates				
	Glucose	Trehalose	Mannitol	Arabitol	Total
	Vegetative mycelium				
19	7.6 (4.2)	43.8 (2.9)	9.1 (2.2)	3.5 (0.3)	64.0
21.5	8.3 (4.9)	66.0 (3.8)	12.0 (3.0)	3.3 (0.6)	89.6
24	8.0 (5.0)	63.0 (4.0)	9.5 (2.8)	9.9 (4.3)	90.4
26	4.0 (2.6)	57.4 (4.4)	6.6 (2.8)	11.0 (7.1)	79.0
28	4.9 (3.8)	41.4 (4.8)	4.0 (3.1)	5.4 (5.5)	55.4
30	8.0 (6.4)	43.0 (4.9)	4.1 (5.2)	4.2 (4.2)	59.3
	Small fruitbodies				
19	0.2 (0.6)	5.8 (1.8)	2.2 (2.2)	4.5 (1.1)	12.7
21.5	0.8 (0.9)	15.2 (2.3)	7.6 (3.1)	1.5 (0.2)	25.1
24	0.4 (0.5)	15.0 (2.4)	4.3 (1.8)	1.4 (0.3)	21.1
26	1.2 (1.8)	11.8 (2.3)	3.3 (1.4)	1.0 (0.3)	17.3
28	0.5 (0.9)	6.5 (2.2)	2.1 (2.4)	1.3 (0.9)	10.4
30	0.7 (1.6)	6.9 (2.9)	1.1 (2.4)	0.9 (1.4)	9.7
	Stipes of four largest fruitbodies				
21.5	<0.1 (0.2)	1.4 (0.9)	0.2 (0.2)	0.1 (0.1)	1.7
24	<0.1 (0.1)	5.3 (1.2)	1.7 (2.1)	2.5 (0.6)	9.5
26	0.1 (0.5)	6.9 (1.2)	3.3 (4.0)	4.7 (0.8)	15.0
28	0.4 (1.4)	12.2 (2.2)	2.3 (3.6)	0.7 (0.1)	15.6
30	0.1 (0.4)	4.2 (1.0)	1.0 (2.0)	0.9 (0.1)	6.1
	Pilei of four largest fruitbodies				
24	<0.01 (0.07)	0.1 (0.6)	0.07 (0.1)	0.04 (0.2)	0.25
26	<0.01 (0.09)	0.3 (0.7)	0.13 (0.2)	0.05 (0.9)	0.5
28	0.03 (0.2)	0.8 (0.7)	0.06 (0.6)	0.04 (<0.1)	0.9
30	<0.01 (<0.01)	0.3 (0.2)	0.03 (0.3)	0.02 (<0.1)	0.3

Table II. Changes in ^{14}C incorporation into polysaccharides of different colony portions during fruitbody development of *Flammulina velutipes*.

Cultures at different stages of fruitbody development were incubated on replacement medium with ^{14}C -glucose for 4 hr in the light at 15 to 17 C. Incorporation of ^{14}C into polysaccharide fractions was determined as described under Materials and Methods.

Colony age in days	^{14}C incorporation as nCi/dish and in parentheses as nCi/mg carbohydrate					Total
	Acid soluble (Glycogen)	Alkali-sol., acid-soluble	Alkali-sol., acid-insol. (S-glucan)	Hot alkali-soluble	Formic acid-soluble (R-glucan)	
Vegetative mycelium						
19	25.0 (0.7)	12.1 (0.8)	4.6 (1.1)	3.3 (0.9)	7.2 (0.3)	52.2
21.5	35.6 (0.6)	11.8 (0.7)	4.2 (1.2)	3.2 (0.7)	7.8 (0.3)	62.6
24	39.0 (0.5)	9.8 (0.6)	2.6 (0.7)	3.0 (0.6)	5.4 (0.3)	59.7
26	39.4 (0.6)	12.0 (0.7)	2.4 (0.6)	3.2 (0.5)	5.3 (0.2)	62.3
28	21.6 (0.9)	10.6 (0.7)	2.8 (0.6)	2.9 (0.5)	6.0 (0.2)	43.9
30	17.2 (1.0)	7.9 (0.6)	1.6 (0.3)	1.6 (0.3)	5.1 (0.2)	33.3
Small fruitbodies						
19	4.0 (0.6)	1.8 (0.5)	0.8 (0.6)	0.5 (0.3)	1.7 (0.2)	8.8
21.5	3.9 (0.4)	3.4 (0.5)	1.0 (0.3)	0.8 (0.3)	2.4 (0.3)	11.6
24	3.3 (0.5)	3.2 (0.3)	1.1 (0.3)	0.7 (0.2)	1.5 (0.1)	9.8
26	3.6 (0.6)	2.4 (0.3)	0.6 (0.2)	0.5 (0.1)	0.7 (0.1)	7.8
28	4.1 (1.0)	1.5 (0.2)	0.5 (0.2)	0.4 (0.1)	0.6 (0.1)	7.0
30	4.3 (1.6)	1.3 (0.3)	0.1 (0.1)	0.2 (0.1)	0.4 (<0.1)	6.4
Stipes of four largest fruitbodies						
21.5	0.3 (0.5)	0.2 (0.2)	0.1 (0.2)	0.2 (0.2)	0.2 (0.1)	1.0
24	0.6 (0.5)	0.7 (0.2)	0.2 (0.2)	0.3 (0.1)	0.4 (0.1)	2.2
26	0.9 (0.5)	0.6 (0.2)	0.2 (0.1)	0.2 (0.1)	0.4 (0.1)	2.4
28	1.0 (0.6)	0.6 (0.1)	0.3 (0.1)	0.2 (0.1)	0.3 (<0.1)	2.4
30	1.1 (0.7)	0.2 (0.1)	0.1 (<0.1)	0.1 (<0.1)	0.2 (<0.1)	1.6
Pilei of four largest fruitbodies						
24	0.1 (0.2)	0.2 (0.2)	<0.1 (0.2)	0.1 (0.4)	0.1 (0.1)	0.4
26	0.1 (0.1)	0.2 (0.2)	0.1 (0.2)	0.1 (0.2)	0.1 (0.1)	0.4
28	0.1 (<0.1)	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)	0.1 (<0.1)	0.5
30	0.1 (<0.1)	0.1 (0.1)	<0.1 (0.1)	0.1 (<0.1)	<0.1 (<0.1)	0.3

Changes in the specific radioactivity of glycogen in the SFB and the stipes of LFB showed patterns similar to that of the mycelium, while activity in the pilei decreased during their growth.

The four fractions of cell wall polysaccharides in the VM incorporated the label at almost constant rate between the 19th and 28th day, although their amounts did not increase (Fig. 4A). Labeling of the four polysaccharides in the SFB showed similar trends as in the VM. In the LFB, less than 1% of the total activity was found in all carbohydrates of the colony.

DISCUSSION

F. velutipes formed numerous clustered primordia on potato-glucose solution, but only few developed into LFB. When these began to grow rapidly, growth had ceased in VM and almost ceased in the SFB. While the LFB completed their growth, the colony consumed the remaining glucose in the medium. Comparison of the dry weight gain by the LFB with the losses by other colony portions suggested that the substrates which were utilized during growth of the LFB derived partly from uptake of exogenous glucose and partly from cellular constituents stored in the rest of the colony.

During vegetative growth, part of the glucose in the medium was used to synthesize low mol wt carbohydrates and glycogen which accumulated in the hyphae and later also in the SFB. The VM lost only low mol wt carbohydrates and continued to accumulate glycogen until approximately the middle of the period of rapid growth of the LFB. The glycogen level began to decrease at that time, but the amounts of mycelial cell wall polysaccharides remained almost unchanged throughout fruitbody development. On the other hand, the SFB began to lose not only low mol wt carbohydrates, but also glycogen and R-glucan at the onset of rapid growth in the LFB. Two other cell wall fractions decreased only 3 to 4 days later. When growth had ceased in the

LFB, about 40 to 45% of the maximum amount of cell wall polysaccharides had been degraded in the SFB. Part of the breakdown products together with those of glycogen and with low mol wt carbohydrates from both the SFB and VM probably served as substrates for the LFB. Breakdown of cell wall polysaccharides was reported previously in the stroma and aborted primordia of *S. commune*, where the degradation products, especially R-glucan, were probably utilized for growth of the pilei (15, 20). The S-glucan to R-glucan ratio was much smaller in *Flammulina* than in *Schizophyllum*, and remained virtually constant (mean, 0.17) in the mycelium throughout fruitbody development. In the SFB, a small increase from 0.17 to 0.22 during cell wall degradation might reflect preferential R-glucan breakdown. The ratio remained constant in the stipes of the LFB (0.18–0.19), but increased in the pilei to 0.34. S-glucan from *Schizophyllum* has mainly α -1,3 linkages and R-glucan contains β -1,3 and β -1,6 bonds (15). S-glucan has also been identified in other Basidiomycetes (1).

Trehalose has been reported in fruitbodies of many Basidiomycetes (2, 18), including *F. velutipes* (18). Wakita (19) found that trehalose disappeared from the *Flammulina* mycelium during fruitbody growth. He postulated that part of the disaccharide was translocated into the fruitbodies while the remainder was consumed for respiration. In the present study, trehalose was partially lost from both the VM and the SFB, and accumulated in the LFB until 3 days before termination of growth. However, the net gain (5.6 mg/dish) by the LFB during the period of rapid stipe growth (21.5–27 days) was slightly less than half of the net loss by the SFB and VM during the same period. Thus, a considerable amount of trehalose was metabolized in the colony during growth of the LFB. The final decrease in the trehalose content of the stipe was probably caused in part by movement into the pileus and into spores which were discharged in large numbers at that time.

Polyols have been detected in fruitbodies of numerous Basidiomycetes (2, 18), including *F. velutipes* (18). Rast (16) concluded that mannitol is not used as reserve carbohydrate during normal growth of *A. bisporus* fruitbodies because they accumulate the polyol as they become older. Fruitbody sections synthesize mannitol from exogenous fructose, and to a much lesser extent, can also derive the polyol from glucose and other sugars (4). Dütsch and Rast (5, 6) suggested that oxidation of NADPH during mannitol synthesis, rather than the polyol itself, could play a role in regulating growth in *A. bisporus* by controlling glucose oxidation via the hexose monophosphate pathway. Arabinol is present in *S. commune* fruitbodies (3, 14), but has not been reported previously in those of other Hymenomycetes. *F. velutipes* mycelium and fruitbodies contain much more arabinol than mannitol. The mannitol content decreased in the VM and SFB only after the onset of rapid growth in the LFB, but arabinol began to decrease steeply in the VM shortly after growth began in the SFB, and then in the latter, at the onset of growth in the LFB. Both polyols accumulated in the LFB, but during the last 2 to 3 days of growth, mannitol decreased and arabinol accumulation became slower in the stipe. Small decreases also occurred in the pilei. The explanation for these final changes is probably the same as for trehalose (see above). Between 21.5 and 27 days after inoculation, the net increase in mannitol in the LFB was almost equal to the net loss by the rest of the colony. During the same interval, the net gain in arabinol by the LFB totaled close to 7.2 mg/dish, while the rest of the colony lost close to 11.1 mg. Moreover, the labeling data showed that both polyols continued to be synthesized in the mycelium until the end of the experimental period. Probably a substantial fraction of the arabinol, and possibly some mannitol, were metabolized in the colony during the development of the LFB. Isolated *Flammulina* fruitbodies can utilize mannitol as substrate for growth (10), but they were deprived of other exogenous nutrients for a relatively long period. Isolated *Agaricus* fruitbodies metabolized mannitol when it was provided as sole carbon source for 2 days. Mannitol oxidation apparently requires carbohydrate starvation in fruitbodies of this species, and does not seem to occur to a significant extent when they grow attached to mycelium (6).

The changes in carbohydrate levels during development of *Flammulina* evidently result from complicated metabolic interrelations among these cell constituents. This is also reflected in the data on ¹⁴C incorporation, and only tentative conclusions can be drawn regarding translocation of cellular carbohydrates. When labeled glucose was fed for a relatively short period only a few per cent of the incorporated ¹⁴C remained in endogenous glucose. The glucose pool changed very little with time, and was very small in all colony portions. Labeling of glucose was far weaker in the LFB than in the VM. If there was direct translocation of the hexose into the LFB, the amount could only have been very small. It is more likely that the radioactivity in glucose in the LFB derived from hydrolysis of trehalose in the stipes. Trehalose was synthesized at a higher rate than other carbohydrates in the mycelium and turned over rapidly. The specific radioactivity remained high until growth ceased in all fruitbodies. In the SFB and LFB, the specific radioactivity in trehalose was consistently higher than in glucose. Compared to the VM, the labeled trehalose was much less diluted than glucose by the respective unlabeled fractions which were present in the fruitbodies. The general labeling pattern for mannitol resembles that of trehalose, but the specific radioactivity was even slightly

higher in the rapidly growing stipes than in the VM. In the stipes, it was also higher than in trehalose and much higher than in glucose. The results of the labeling experiments favor the interpretation that at least a large part of the trehalose and probably most of the mannitol are translocated directly into the LFB from the VM and SFB. This view is also supported by the changes in the total amounts of these carbohydrates in the colony (Fig. 3).

Incorporation of radioactivity into arabinol reached a high rate in the VM after the onset of rapid growth in the LFB. Their stipes and pilei, but not the SFB, exhibited the same general labeling pattern in arabinol as the mycelium, with a peak at 26 days. The specific radioactivity in the LFB was quite low, but it exceeded that of glucose on the 24th to 26th day. This suggests that direct translocation of arabinol from the mycelium may have occurred, but synthesis of the polyol in the fruitbodies cannot be excluded, and additional studies are required to explain fully the results of the labeling experiments.

Note Added in Proof. M. Mikamo (J. Agric. Chem. Soc. Japan, 1974, 48: 69-71) reported that *Lentinus edodes* fruitbodies contain arabinol as well as mannitol and trehalose.

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