JOURNAL OF BACTERIOLOGY, Feb. 1981, p. 889-895
0021-9193/81/020889-07$02.00/0 Vol. 145, No. 2

Lipid Metabolism During Encystment of *Azotobacter vinelandii*†

R. N. REUSCH AND H. L. SADOFF*
Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824

The formation of cysts by *Azotobacter vinelandii* involves the synthesis of lipids as major metabolic products. Cells which encyst at low levels in aging glucose cultures undergo the same pattern of lipid synthesis as cells which undergo reasonably synchronous encystment in β-hydroxybutyrate or *n*-butanol. The accumulation of poly-β-hydroxybutyrate (PHB) precedes the synthesis of 5-*n*-heneicosylresorcinol and 5-*n*-tricosylresorcinol (AR1), which is then followed in about 6 h by the synthesis of the 5-*n*-alkylresorcinol galactosides (AR2). In the mature cyst, PHB, AR1, and AR2 account for 8, 5.6, and 4.5%, respectively, of the dry weight. Phospholipid formation levels off 4 h postinduction, which coincides with the final cell division, but fatty acid synthesis continues at a very low level throughout encystment, suggesting some turnover of fatty acid. Distribution studies show that AR1 and AR2 are found in roughly equal amounts in the exine and central body of the cysts, with only trace amounts recovered from the intine. Studies of cysts labeled during encystment with [^{14}C]β-hydroxybutyrate or during vegetative growth with [^{14}C]glucose suggest that the exine structure is synthesized during encystment, but that the intine is composed largely of vegetative cell components.

Azotobacter vinelandii is a gram-negative, nitrogen-fixing soil organism which undergoes differentiation to form metabolically dormant cysts. These resting forms occur in late-stationary-phase cultures when growth is on glucose and other carbohydrates, but yields of cysts under these conditions are usually less than 1% (11). Reasonably synchronous and complete encystment can be induced by culturing exponential-phase cells on agar plates containing *n*-butanol or in liquid medium containing β-hydroxybutyrate (BHB) (10). We have reported that encystment induced by BHB is accompanied by the formation of novel lipid substances (14). The most abundant of these were characterized by us as the homologs 5-*n*-heneicosylresorcinol and 5-*n*-tricosylresorcinol in a ratio of 7:1 (AR1) and their galactoside derivatives in the same ratio (AR2).

We have questioned whether these phenolic lipids are cyst-specific compounds with specific functions or if they merely accumulate as end products of a unique secondary metabolism. The basis of these questions was the following. BHB is probably present at low levels in encysting cells as a metabolic intermediate since intracellular accumulation of poly-β-hydroxybutyrate (PHB) and its subsequent depolymerization are necessary prerequisites for encystment (10) when cells are grown on carbohydrates. However, the "flooding" of cells with BHB during the induction of encystment could modify their metabolic and synthetic patterns and lead to the formation of resorcinols in artificially large amounts. It was therefore necessary to examine a variety of encysting *A. vinelandii* cultures, both natural and induced, for the presence of resorcinols in order to establish whether they are essential components of cysts.

Encystment is a relatively long process involving a sequence of events, many of which have been characterized enzymatically and microscopically (7). To establish a possible role for the resorcinolic substances in the formation of mature cysts, we investigated the time course for the synthesis of AR1 and AR2 relative to that of the major storage lipid, PHB, and the membrane phospholipids. We also examined cyst components, using radioactive labels, to determine the distribution of AR1 and AR2 and to elucidate the origin of the exine and intine structures.

MATERIALS AND METHODS

Organism and culture. The organism used in these studies was *A. vinelandii* ATCC 12837. Cells were grown vegetatively in Burk nitrogen-free buffer at pH 7.4 as previously described (14) with either 1% glucose or 0.5% sodium acetate as the carbon source. Cells were encysted in Burk nitrogen-free buffer containing half the usual concentration of phosphate at

† Journal article 9614 of the Michigan Agricultural Experiment Station.

pH 7.0 (12) with 0.2% BHB or on Burk nitrogen-free agar with 0.2% *n*-butanol.

Radioactive studies. Cells were labeled in vegetative growth with 17 nCi of D-[6-^{14}C]glucose per ml (New England Nuclear Corp.) or during encystment with 14 nCi of D-(−)-3-hydroxy[3-^{14}C]butyric acid, sodium salt (New England Nuclear Corp.).

Separation of cyst components. Cysts were collected by centrifugation, washed with Burk buffer, and suspended in 0.05 M Tris, pH 8.0, to one-third of their original culture volume. Disruption was achieved by adding 2 mg of trisodium citrate per ml and gently stirring overnight at 4°C. Separation of exines and intines was by the method of Lin and Sadoff (11). Central bodies were purified by filtration through microfiber glass prefilters as described by Parker and Socolofsky (13).

Extraction of lipids. Cells were collected by centrifugation and washed once with distilled water. Lipids were extracted by the one-phase method of Bligh and Dyer (1) as previously described (14).

Separation of lipids. Preliminary separation of large samples of AR1 and AR2 was by column chromatography on Florisil (Matheson, Coleman, and Bell; 60 to 200 mesh) in a 1.2 by 25-cm column (1 g of Florisil/10 mg of lipid). AR1 was eluted with chloroform-methanol (95:5, vol/vol), and AR2 was eluted with 100% methanol.

Final purification of AR1 and AR2 was by thin-layer chromatography (TLC) on precoated Silica Gel G glass plates (Analtech Laboratories). Precoated TLC Silica Gel 60 aluminum sheets (EM Laboratories) were used for analytical separation of AR1, AR2, and phospholipids. Development was in solvent system 1 (chloroform-methanol, 85:15, vol/vol) for AR1 and AR2 and solvent system 2 (chloroform-methanol-water, 65:25:4, vol/vol/vol) for phospholipids.

Total lipids were detected by exposure to iodine vapor, phospholipids were detected with mercury-molybdate reagent (19), and phosphatidylethanolamine was detected with 0.1% ninhydrin in acetone.

Lipids of interest were scraped off glass plates or cut from aluminum plates, and the samples were placed in scintillation vials. Ethanol was added (0.2 ml) followed by 10 ml of a 9:1 mixture of PCS (Amersham Corp.) scintillation fluid and water. Samples were counted in a Packard model 3320 Tri-Carb scintillation spectrometer. All counts were corrected for quenching by addition of 100 μl of ^{14}C-labeled lipid (extracted from cysts of *A. vinelandii*) as an internal standard.

Determination of AR1 with the vanillin reagent. AR1 in lipid extracts of the cyst components was determined by assaying for *meta*-hydroxyl aromatic compounds with the vanillin reagent. Samples of the lipids containing between 10 and 100 μg of resorcinols were added to test tubes (16 by 120 mm) in an ice bath. Then 0.5 ml of chloroform and 1 ml of 4% vanillin in methanol were added, and the tubes were blended until the sample was in solution. One milliliter of concentrated sulfuric acid was added slowly, maintaining the temperature below 35°C. The tubes were blended for 30 s and incubated at room temperature for 15 min. The top layer was removed with a Pasteur pipette, and its optical density was read at 520 nm. A standard curve was prepared with 10 to 100 μg of orsellinic acid.

Determination of PHB. PHB was determined by the disk assay of Ward and Dawes (17). Samples (0.2 ml) of cell culture were collected on prewashed glass fiber disks (Whatman GF/A, 2.5 cm), digested with sodium hypochlorite, washed, and dried as described. The content of PHB in the sample was determined by spectrophotometric assay as described. For radioactive counting, disks were placed in scintillation vials with 10 ml of PCS scintillation fluid. A solution of D-(−)-3-hydroxy[3-^{14}C]butyric acid, sodium salt in water, was used as an internal standard.

Time course study. Cells were grown at 30°C on a rotary shaker in 1-liter Erlenmeyer flasks containing 300 ml of Burk nitrogen-free buffer (pH 7.4) with 1% glucose. Cells were collected at late exponential growth phase (optical density at 620 nm = 0.8), washed with the same buffer, and suspended in 300 ml of prewarmed Burk nitrogen-free buffer (pH 7.0) to an optical density at 620 nm of 0.5. Encystment was initiated by the addition of BHB and [^{14}C]BHB to a final concentration of 0.2% and 14 nCi/ml. Samples (10 ml) were removed at various time intervals throughout encystment, chilled rapidly in dry ice-acetone to stop metabolic activity, and then refrigerated. One milliliter of culture sample was used for the determination of PHB by disk assay. The remainder was centrifuged, washed with distilled water, and extracted with methanol-chloroform (2:1, vol/vol), and the extract was chromatographed on TLC plates with appropriate solvent systems to determine AR1, AR2, and phospholipid.

RESULTS

Occurrence. Resorcinols were not found in vegetative cells of *A. vinelandii* during exponential growth on glucose or acetate. However, small quantities of resorcinols could be detected in acetate-grown cells in their early stationary phase and in glucose-grown cells in their late stationary phase. Microscopic examination of these resorcinol-containing cultures showed that a small percentage of cells (3 to 10%) appeared to be encysting; i.e., they were rounded and phase bright with accumulations of PHB, though little exine was visible. The formation of cyclopropane fatty acids which occurs upon induced encystment (16) also took place in aging glucose-grown cultures (Table 1). It has been reported that less than 1% of the cells in these kinds of cultures form mature, desiccation-resistant cysts (10).

Cells cultured in encystment medium synthesized resorcinols whether they were in liquid culture with BHB or on solid medium with *n*-butanol. The addition of glucose or ammonium ion to encysting cultures prevented the formation of mature, desiccation-resistant cysts but did not inhibit the formation of resorcinols. When the nonencapsulated strain, *A. vinelandii* OP, was cultured in BHB, the cells rounded up

TABLE 1. *Fatty acid composition of aging glucose-grown cells of A. vinelandii*[a]

Culture age (h)	% of total[b]					
	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17\Delta}$	$C_{18:1}$	$C_{19\Delta}$
24	3	40	40	0.5	16	0.5
48	4	42	43	2	7	2
72	8	42	27	10	8	5
120	6	39	32	7	10	6
168	5	41	27	9	8	10

[a] Total lipid extract was subjected to mild alkaline methanolysis (19).

[b] Determined by gas chromatography as previously described (16).

and accumulated PHB but did not form exines. Resorcinols (AR1) were formed, but resorcinol galactosides (AR2) were not.

Cultures of *A. vinelandii* which have been grown in glucose and are in late stationary phase (72 h from the time of inoculation) contain about 3 μg of AR1 per ml. Since vegetative (elongated) cells of *A. vinelandii* do not contain resorcinols, it was assumed that the phenolic lipids were associated with the encysting subpopulation. If this were the case, we would expect to find that the early-stage cysts had resorcinol contents about equal to those of central bodies prepared from mature cysts.

To test this assumption, a 300-ml culture of *A. vinelandii* was grown at 30°C in Burk buffer-1% glucose medium for 6 days. Then 20-ml samples were taken at 24-h intervals for determination of the percentage of rounded cells (observed microscopically), dry weight of cells, PHB content, and AR1 content. The data from one such set of observations are shown in Table 2, where the average AR1 content of encysting (rounded) cells in 2- to 5-day postexponential cultures was 5.5%.

Initiation of resorcinol synthesis. Resorcinol (AR1) synthesis began in aging glucose-grown cultures between 24 and 48 h of incubation (Table 2). In *A. vinelandii* induced to encyst with BHB, the synthesis of AR1 either began immediately when cells were transferred to encystment medium or was delayed up to 15 h, but always preceded the synthesis of AR2 by several hours. The initiation of resorcinol synthesis was dependent on the carbon source used for vegetative growth, the stage of growth of the cells when they were transferred, and the presence of nitrogen in the encysting medium. Cells pregrown on acetate began to form AR1 within the first 2 h after the addition of BHB. On the other hand, cells pregrown in glucose and then induced with BHB began its synthesis after 6 to 10 h in nitrogen-free medium and after 12 h when nitrogen was present. Cells transferred to encystment medium in late exponential phase and which contained granules of PHB began to form resorcinols earlier than cells transferred in their mid-exponential phase.

Time course for lipid formation during encystment. Lipids are a major cell product of BHB metabolism during encystment and constitute 28.6% of the dry weight of the mature cyst (20.6% extractable lipid, 8% PHB). The time course for the synthesis of PHB, AR1, AR2, and the phospholipids was determined by measuring the incorporation of [^{14}C]BHB throughout the encystment process (Fig. 1). For this study, cells were grown in 1% glucose to late exponential phase and then transferred to Burk nitrogen-free medium with 0.2% BHB containing 14 nCi of [^{14}C]BHB per ml.

The major single lipid formed during encystment was the storage polymer PHB. It began to form immediately at an exponential rate and continued being produced about 54 h after the induction of encystment. It then appeared to be utilized.

AR1 synthesis began at about 6 h postinduction at a rapid rate and reached its peak level in cells at about 36 h. AR2 synthesis followed at a slightly lower rate, about 12 h postinduction, and peaked at 60 h.

Phospholipid synthesis plateaued at about 4 h, which coincided with the final cell division. Total phospholipid activity remained constant for 2 to 3 days and then decreased slowly. Pulse-labeling studies indicate that phospholipids continued to incorporate radioactive label at a low rate throughout encystment, suggesting that some turnover of fatty acids occurred. In aging glucose-grown cultures, a progressive formation of C_{17} and C_{19} cyclopropane fatty acids occurred

TABLE 2. *Analysis of PHB and AR1 content of a glucose-grown aging culture of A. vinelandii*[a]

Culture age (h)	Cell dry wt (mg)	PHB		Rounded cells		AR1	
		μg	%	mg[b]	%	μg	% in rounded cells
24[c]	6.3	60	1.0		<1	0	0
48	12.3	720	5.9	0.26	3	7	2.7
72	14.8	702	4.7	1.04	10	62	6.0
120	12.7	275	2.2	0.80	9	45	5.6
144	12.7	40	0.3	0.71	8	36	5.1

[a] Data calculated per 20 ml of culture. Uninduced glucose-grown cultures produced <1% desiccation-resistant cysts.

[b] Rounded, early-encysting cells are approximately 70% the volume of normal vegetative *A. vinelandii*. The weight of rounded cells/20 ml was equal to dry weight × percentage of rounded cells × 0.7.

[c] Exponential growth terminated by 24 h.

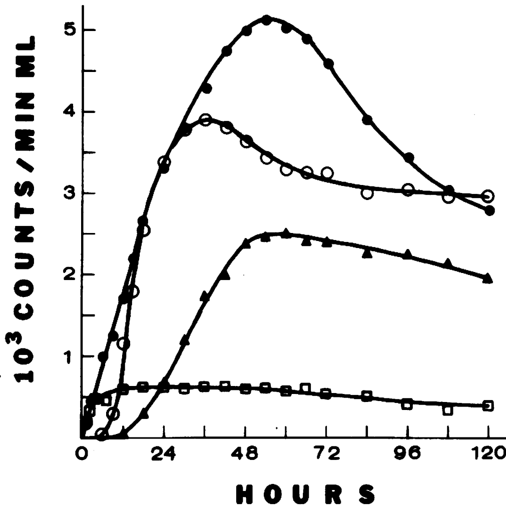

FIG. 1. *Time course for the formation of lipids in A. vinelandii after the initiation of encystment by 0.2% BHB containing 14 nCi of [¹⁴C]BHB per ml. Symbols: PHB (●); AR1 (○); AR2 (▲); phospholipids (□). Cells were pregrown to late exponential stage in Burk buffer plus 1% glucose.*

(Table 1). The identical process has been shown previously to occur in BHB-induced encystment of *A. vinelandii* (16).

Distribution of AR1 and AR2 in the cyst. The mature cyst consists of a highly vacuolated central body, with a volume approximately half that of a vegetative cell, surrounded by a water-soluble, electron-diffuse intine layer encapsulated by a lamellar exine (2, 6, 21). The distribution of AR1 and AR2 in the mature cyst was examined quantitatively by first labeling encysting cells with [^{14}C]BHB and fractionating them into exines, intines, and central bodies. The purified fractions were obtained with a 48% total yield. The distribution of [^{14}C]BHB in the cyst components is given in Table 3. Most of the radioactive label incorporated was distributed between the exine and central body, with only 9% in the intine. Since each component was purified by a separate procedure, it was not possible to determine whether the percentages of exine, intine, and central body obtained were the same as in the unbroken cysts. However, the weighted sum of the specific activity of the cyst components (81,900 cpm/mg) was approximately that of the specific activity of the cysts before fractionation (81,500 cpm/mg).

The individual cyst components were then extracted with a single-phase chloroform-methanol-water system to determine the extent of incorporation of [^{14}C]BHB in their lipids. PHB was not extracted by this procedure. After removal of insoluble material, the extracted substances were distributed between chloroform-methanol and water-methanol. Fifty-three percent of the [^{14}C]BHB incorporated into cysts was extracted by this procedure and, for the exine and central body, all radioactivity was in the lipid (chloroform-methanol) layer. Only the intine contained an appreciable (9.6%) level of BHB-derived label in the water-soluble fraction (Table 4). The distribution of extractable lipids in the cyst components and their incorporation of [^{14}C]BHB carbon is presented in Table 5. The data show that whereas exines and central bodies contained 1.6 and 1.8 times more lipid, respectively, than intines, these two cyst components contained about 10 times more BHB-derived carbon in their lipids than did the intines.

Only trace amounts of AR1 and AR2 were found in the intine. AR1 and AR2 were separated from the extractable lipids of exines and central bodies by column chromatography on Florisil followed by TLC on Silica Gel G. The distribution of AR1 and AR2 in the exine and central body and the degree of [^{14}C]BHB incorporation in each is shown in Table 6. The weights of the two phenolic lipids were determined from the total incorporation of [^{14}C]BHB in AR1 and AR2 and their specific activities. The validity of these measurements was established by an independent estimation of AR1 on a sample of the component lipid extracts, using the vanillin reagent (see Materials and Methods). Almost one-fifth the weight of the cyst exine was made up of AR1 and AR2.

Source of the intine. The specific activity of the intine from cysts produced in [^{14}C]BHB was less than half that of the exines and central bodies (Table 3). This indicates that they were synthesized to a large extent from cellular components or stored substances which were formed before the induction of encystment with [^{14}C]BHB. To evaluate the contribution of pre-encystment cellular material to the cyst components, cysts were formed with unlabeled BHB from cells labeled during vegetative growth with [^{14}C]glucose. Cyst components were separated and extracted as before. The distribution of the

TABLE 3. *Distribution of [¹⁴C]BHB carbon in cyst components per 100 ml of cyst culture*

Component	Wt (mg)	% by wt	[¹⁴C]BHB carbon incorporation		Sp act[a]
			cpm	%	cpm/mg
Exine	6.4	28	596,000	32	93,100
Intine	3.8	17	157,000	9	41,300
Central body	12.3	55	1,090,000	59	88,600

[a] Specific radioactivity of the intact cysts was 81,500 cpm/mg.

TABLE 4. *Extraction of cyst components per 100 ml of cyst culture*[a]

Component	[^{14}C]BHB labeled			[^{14}C]glucose labeled		
	Total extract[b] (cpm)	% of radioactive label		Total extract[b] (cpm)	% of radioactive label	
		Lipid layer	Aqueous layer		Lipid layer	Aqueous layer
Exine	447,000	99.8	0.2	25,700	28	72
Intine	50,900	90.4	9.6	551,000	3	97
Central body	461,000	99.4	0.6	232,000	28	72

[a] Bligh-Dyer (1) single-phase extraction, which excludes PHB. The extracts were then partitioned into $CHCl_3$-CH_3OH and CH_3OH-H_2O and their distribution was noted.
[b] Count obtained by summation of counts in lipid and aqueous layers.

TABLE 5. *Distribution of extractable lipids in cyst components per 100 ml of cyst culture*[a]

Component	Lipid wt (mg)	Lipid content[b] (%)	[^{14}C]BHB carbon incorporated into lipid		Sp act of lipid (cpm/mg)
			cpm	%	
Exine	1.8	28	446,000	75	248,000
Intine	1.1	29	46,000	29	42,000
Central body	2.0	16	458,000	42	228,000

[a] PHB is not included. In these experiments the average concentration of PHB in mature cysts was 8% of the dry weight.
[b] Extractable lipid content of the intact cysts was 20.6%.

radioactive label in the lipid and aqueous layers is shown in Table 4. For the intine, label was predominantly in the aqueous layers, which contained 97% of the radioactivity as compared with 9.6% when BHB was labeled. The intine had 68% of the total [^{14}C]glucose label incorporated, which demonstrates a major contribution by pre-encystment material, whereas the exine had only 3% of the label, which indicates it was synthesized almost entirely from BHB.

DISCUSSION

Encystment in *A. vinelandii* involves the formation of lipids as major metabolic products. This synthesis occurs in aging glucose-grown cells which encyst at low levels and in cells induced to encyst with BHB. The difference between the two kinds of cultures appears to be one of degree where the addition of BHB to a culture results in conditions approximating extensive PHB mobilization. Postexponential glucose-grown cells accumulate and then utilize PHB (Table 2), up to 10% of them undergo the early stages of encystment (rounded, phase-bright cells, or precysts), and they synthesize the phenolic lipids AR1 and AR2. We assumed that AR1 is associated with precysts and then calculated that they contain 5 to 6% of that lipid. It is significant that this is also the AR1 content of isolated central bodies prepared from cysts which have been induced in BHB.

A. vinelandii OP lacks a capsule and fails to encyst when induced with BHB. These cells enter into the precyst stage, synthesize PHB and AR1, but fail to make AR2. Thus it would appear that the syntheses of PHB and AR1 are necessary conditions for early stage encystment but an insufficient condition for cyst maturation.

Encystment in *A. vinelandii* can be dissected into three discrete stages based on lipid synthesis: preresorcinol, resorcinol, and postresorcinol periods. The events of the first phase, 4 to 6 h postinduction (termination of nitrogen fixation, final cell division, and loss of motility) are accompanied by rapid PHB accumulation and synthesis of phospholipid at approximately one-third the rate of that in exponentially growing vegetative cells (Fig. 1).

The next developmental phase, which extends from 6 to about 60 h, involves the synthesis of a family of encystment-specific lipids of which the resorcinols, AR1, and the resorcinol galactosides, AR2, form a major part. PHB accumulation continues throughout this period and terminates at approximately the same time. Total phospholipid remains constant, though pulse-labeling studies indicate that fatty acid synthesis continues at about 20% the rate of the first 4 h (unpublished data). This implies that turnover of fatty acids occurs during this period. Another event involving the fatty acids which begins at about 6 h postinduction is the formation of $C_{17\Delta}$ and $C_{19\Delta}$ fatty acids from the corresponding unsaturated acids $C_{16:1}$ and $C_{18:1}$ (16); possibly this occurs by the addition of a methylene group donated by *S*-adenosylmethionine to the double bond as it does in other organisms (3, 8, 9).

PHB is the most abundant single lipid in encysting cells, and its accumulation precedes synthesis of resorcinols. Stevenson and Socolofsky (15) showed a direct relationship between the extent of PHB accumulation and the degree of encystment which indicates that PHB metab-

TABLE 6. *Distribution of AR1 and AR2 in cyst components per 100 ml of cyst culture*

Component	AR1				AR2			
	Wt (mg)	% by wt	% of total [^{14}C]BHB incorporated in component	Sp act (cpm/mg)	Wt (mg)	% by wt	% of total [^{14}C]BHB incorporated in component	Sp act (cpm/mg)
Exine	0.57	9.0	22	233,000	0.50	8.0	15	184,000
Central body	0.69	5.6	15	238,000	0.51	4.0	9	187,000

olism is involved in the encystment process.

Electron micrographs show that the secretion of membrane-like vesicles occurs from the surface of the cells, beginning at 6 h and reaching a maximal rate at about 30 h (2, 6). These vesicles break free, move through the capsular material, and form the layered sheets which comprise the exine. The formation of these vesicles corresponds to the time of maximal synthesis of the resorcinols which together with their galactoside derivatives form 17% of the exine by weight. The hydrophilic hydroxyl-substituted aromatic rings and long hydrophobic alkyl chains of AR1 and AR2 would promote the formation of membrane-like structures such as exines. At no time are *A. vinelandii* cells committed to encystment as are cells of *Bacillus* species to sporulation. Conditions which prevent the formation of desiccation-resistant cysts do not block resorcinol formation.

The final phase of encystment, which lasts about 3 days, does not involve appreciable lipid synthesis. There is some turnover of PHB and to a lesser extent of phospholipid. Resorcinols also seem to turn over, but this loss may be due to the sloughing off of layers of exine into the medium since both AR1 and AR2 can be extracted from the cell-free culture medium.

The studies on the distribution of lipids in the cyst components suggest that the exine and intine have diverse synthetic origins. The high degree of incorporation of [^{14}C]BHB into exine, in contrast to only trace incorporation of [^{14}C]-glucose in exine extracts, demonstrates that appreciable synthesis of the exine from BHB occurs during encystment. Examination of the results of radioactive incorporation in the intine leads to a different conclusion. Only 5% of the incorporated [^{14}C]BHB, but 68% of the incorporated [^{14}C]glucose, is found in the extract of the intine (Table 4). These results indicate that the intine is formed essentially from pre-encystment cellular material. The vegetative cell capsule appears to be a major contributor. This latter conclusion is supported by the following data. Page and Sadoff (12) found that the uronic acid polymer composition of the intine resembles that of the vegetative capsule. Both have mannuronic acid/guluronic acid ratios of 1.8, whereas the exine is unique in its high content of guluronic acid resulting in a mannuronic acid/guluronic acid ratio of 0.45. Eklund et al. (4) found that the presence of a capsule is a necessary prerequisite for the formation of mature cysts. Nonencapsulated mutants and cells in which capsule production was inhibited by the addition of nitrogen to the media produced rounded-up phase-bright precysts, but no desiccation-resistant mature cysts. When encystment was carried out with *n*-butanol on Burk buffer agar in the presence of the phage-induced polysaccharide depolymerase, which hydrolyzes the capsule of *A. vinelandii* (7), few cystlike bodies were produced, and in these the intine was reduced or almost absent.

The central body has a rich array of lipids which accounts for over half of the [^{14}C]BHB incorporated into cysts. AR1, AR2, and the other members of this family of lipids (C.-J. Su, R. Reusch, and H. L. Sadoff, unpublished data) represent about one-third of incorporated label, PHB represents approximately 15%, and the phospholipids represent only about 3%. The [^{14}C]glucose incorporated in the central body lipids (11%) is probably the result of PHB and phospholipid synthesis before the initiation of encystment with BHB.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant AI-01863 from the National Institute of Allergy and Infectious Diseases.

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