The Production of Psilocybin in Submerged Culture by *Psilocybe cubensis*

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Psychotomimetic mushrooms have long been employed as intoxicants by various native peoples. Schultes (10) describes their employment in magico-religious ceremonies by the Indians of Mexico and Central America, and extensive documentation of such usage has been provided by the Wassons (14, 15) and by Heim and Wasson (6).

Especially noteworthy is the occurrence of certain indole derivatives in many of the basidiomycetes to which psychotomimetic properties have been ascribed. Several species of *Panaeolus* contain serotonin (5–hydroxytryptamine), first demonstrated in a fungus, *Panaeolus campanulatus* (Fr.) Quel., by Tyler (13). Bufotenine (5–hydroxy–*N*,*N*–dimethyltryptamine) was shown to exist in *Amanita citrina* (Shaeff.) S. F. Gray [*Amanita mappa* (Lasch) Quel.] by Wieland and Motzel (16). Psilocybin (4–phosphoryl–*N*,*N*–dimethyltryptamine), together with the closely related psilocin (4–hydroxy–*N*,*N*–dimethyltryptamine), was first found in *Psilocybe* species by Hofmann, *et al.* (8). The latter two principles are the most recently discovered hallucinogens in basidiomycetes and are of particular interest since they are the only principles isolated from mushrooms which are capable of inducing psychotomimetic effects in human beings following oral ingestion.

Heim, *et al.* (7), established conditions favoring the formation of both sclerotia and sporocarps from mycelial growth obtained upon subculture of the spores and tissue of the naturally occurring Mexican *Psilocybe* species. In this way they obtained ample amounts of materials for isolation and structural studies of the active principles. The psychotomimetic effects on human beings following ingestion of either the wild-grown sporocarps or sporocarps, sclerotia, and mycelium produced in laboratory culture were identical (9). Singer (11) and Stein (12) obtained similar results, but neither they, Heim, *et al.* (7), nor Hofmann, *et al.* (8), studied the production of psilocybin or psilocin in submerged culture fermentations. This consistuted a major objective, and data presented herein were derived from submerged culture fermentations of *Psilocybe* species.

Experimental evidence regarding the biosynthesis of psilocybin or psilocin is quite limited. The structural similarity between these compounds and tryptophan suggested they might derive from that common amino acid. Part of the psilocybin isolated from the surface-cultured mycelium of *Psilocybe semperviva* Heim & Cailleux by Brack, *et al.* (2), was derived from the isotopically labeled tryptophan added to the nutrient medium. An additional objective of the present study concerned the fate of tryptophan added to replacement culture media containing washed mycelial pellets from submerged culture fermentations.

MATERIAL AND METHODS

Culture techniques.—Mycelial cultures of the organisms investigated were maintained on potato-dextrose-yeast agar slants (1). The compositions of the nutrient medium (medium no. 1) and the replacement medium (medium no. 2)

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are given in table 1. Various modifications of these media will be described subsequently.

For shaken-flask studies, transfers were made from agar slants to 125-ml Erlenmeyer flasks containing 30 ml of the desired medium. After four to seven days on a shaker at 25 ± 1°C, the mycelial growth (pellet form) was homogenized in a sterile semimicro Waring Blendor for fifteen seconds and 5 ml per 100 ml added as inocula to all experimental flasks. Unless otherwise noted, flasks were then shaken at 25°C for periods of five to fifteen days.

TABLE 1. *Nutrient media.*

Basic Nutrient Medium		
Medium no. 1		
Ammonium succinate	1.0	Gm
Glycine	9.0	Gm
Glucose	5.0	Gm
Yeast extract	0.5	Gm
KH_2PO_4	0.1	Gm
Thiamine hydrochloride	0.003	Gm
$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$	0.05	mg
$ZnSO_4\cdot 7H_2O$	0.3	mg
$MnCl_2\cdot 4H_2O$	0.35	mg
$FeSO_4\cdot 7H_2O$	2.5	mg
$CuSO_4\cdot 5H_2O$	0.5	mg
$MgSO_4\cdot 7H_2O$	0.5	Gm
Distilled water, to make	1.0	liter
Adust to *p*H 5.5 with hydrochloric acid.		

Replacement Medium		
Medium no. 2		
Citric acid (0.1M)	307.0	ml
$Na_2HPO_4\cdot 7H_2O$ (0.2M)	193.0	ml
Distilled water, to make	1.0	liter
To each replacement culture flask containing 30 ml of this medium (*p*H 4.2) add 2 mg tryptophan.		

Replacement culture inocula were obtained in a similar manner, but the homogenizing step was extended to one minute. The homogenate from one 125-ml flask was quantitatively transferred to a 2800-ml Fernbach flask containing 300 ml of medium, and mycelium was allowed to develop on a rotary shaker. Mycelial pellets produced in this manner were carefully washed with 500 ml of sterile distilled water; portions were transferred to 125-ml flasks containing 2 mg of tryptophan in 30 ml of medium no. 2 and shaken for the specified period of time.

The shaking machines routinely employed in these studies were a New Brunswick rotary shaker, model V, and a Gump rotary shaker, model M, which were operated at 240 and 185 rpm, respectively.

Analytical methods.—The mycelial pellets, separated from the culture or replacement media by suction filtration on a Büchner funnel, were dried in a forced-air drying oven at 48°C for a minimum period of forty-eight hours and subsequently stored in a desiccator over anhydrous calcium chloride. After determining the *p*H values of the filtered culture media, the filtrates were evaporated to dryness in a flash evaporator at 40°C under reduced pressure. The residues were carefully extracted with 2-ml portions of absolute methanol to dissolve the psilocybin and psilocin, and the solutions were stored in a refrigerator until subjected to chromatographic analysis.

After drying, the mycelial pellets from the individual culture flasks were weighed and then reduced to a no. 60 powder in a Wiley laboratory mill. Ten ml of methanol was added to each sample (ca. 25–100 mg) of powdered material in a 25-ml flask. After shaking for eight hours, the resulting slurry was centrifuged, and the supernatant liquids decanted into a 50-ml round bottom flask. The marc was treated in an identical manner two additional times, after which the combined methanol extracts were evaporated to a small volume (1–2 ml) in a rotary film evaporator at 40°C under reduced pressure. The concentrated extract was transferred to a 5-ml volumetric flask with a microdropper. Residual material in the evaporation flask was removed by a methanol wash, added to the volumetric flask, and the final volume adjusted to 5 ml. Extracts prepared in this manner were refrigerated until used for assay.

An ascending two-dimensional chromatographic procedure was employed to establish the presence or absence of psilocybin and psilocin. Extracts (50–200 μl) were spotted on sheets of Whatman no. 1 filter paper (8×8 in.) and formed with *n*–butanol–acetic acid–water (4:1:5) in the first direction and *n*–propanol–1N ammonium hydroxide (5:1) in the second direction. Location of the indole derivatives was accomplished by spraying the sheets with a solution of 2 percent *p*–dimethylaminobenzaldehyde in 1N hydrochloric acid (PDAB) and drying the sheets with a laboratory heat gun.

Assay procedure.—A paper partition chromatographic assay for psilocybin was developed based on the serial dilution procedure employed by Brown, *et al.* (4), for the assay of muscarine in *Inocybe* species. The technique involved visual observation of the minimum detectable quantity of psilocybin following reaction with PDAB on paper chromatograms. It was determined that 1.0 μg of psilocybin could be detected with certainty on chromatograms developed in *n*–butanol–acetic acid–water (4:1:5), sprayed with PDAB, and permitted to dry spontaneously at room temperature for thirty minutes. Volumes of mycelial extracts corresponding to this amount of the compound were determined experimentally, and the quantities of psilocybin in the extracts and the mycelium were calculated (dry-weight basis).

EXPERIMENTAL AND RESULTS

Growth, pH and psilocybin production.—Cultures employed in the investigation included *Psilocybe cubensis* (Earle) Singer obtained from the Northern Regional Research Laboratory, Peoria, Illinois (NRRL A–9109), *Psilocybe cyanescens* Wakefield and *Psilocybe pelliculosa* (Smith) Singer & Smith, isolated from sporocarp tissue of mushrooms collected in the Seattle area. Mycelium was grown in submerged culture in various media under different laboratory conditions to determine if psilocybin and/or psilocin would be produced. Mycelial pellets of *Psilocybe cubensis* grown in medium no. 1 contained psilocybin, although the culture medium did not; psilocin could not be detected in either the medium or mycelium. The other two species failed to produce detectable amounts of either of these compounds in any of the media tested. It was concluded that the fungi differed in their metabolic capabilities, and, of the three, only *Psilocybe cubensis* was subjected to further investigation.

Initial experiments were conducted to determine the optimal growth period and its relationship to psilocybin production. Cultures of the organism in medium no. 1 were havested on alternate days, five to fifteen days after inoculation. Replicates of four cultures were examined at each growth period. The *p*H of the medium at the termination of the experiment, the dry weight of the mycelium, and the amount of psilocybin produced were determined. The results are summarized in table 2 and figure 1.

These data revealed that the *p*H of the medium remained acid until the ninth day and then rapidly increased until a plateau was reached near *p*H 8. Maximum

production of psilocybin (percentage basis) occurred on the seventh day, whereas mycelium production reached its peak on the ninth day. Psilocybin was not detected in the medium. Maximum production of both mycelium (dry weight) and psilocybin (percentage basis) occurred in acid medium, and both underwent a decline as the *p*H of the medium began to rise after the ninth day of fermentation. It was noted that psilocybin was produced during the most active growth period of the organism.

Although psilocybin was initially identified by chromatography, additional evidence was obtained from ultraviolet spectral data. Methanol extracts of mycelium were chromatographed in *n*–butanol saturated with water, the zone corresponding to psilocybin eluted with methanol, and the eluate rechromatographed

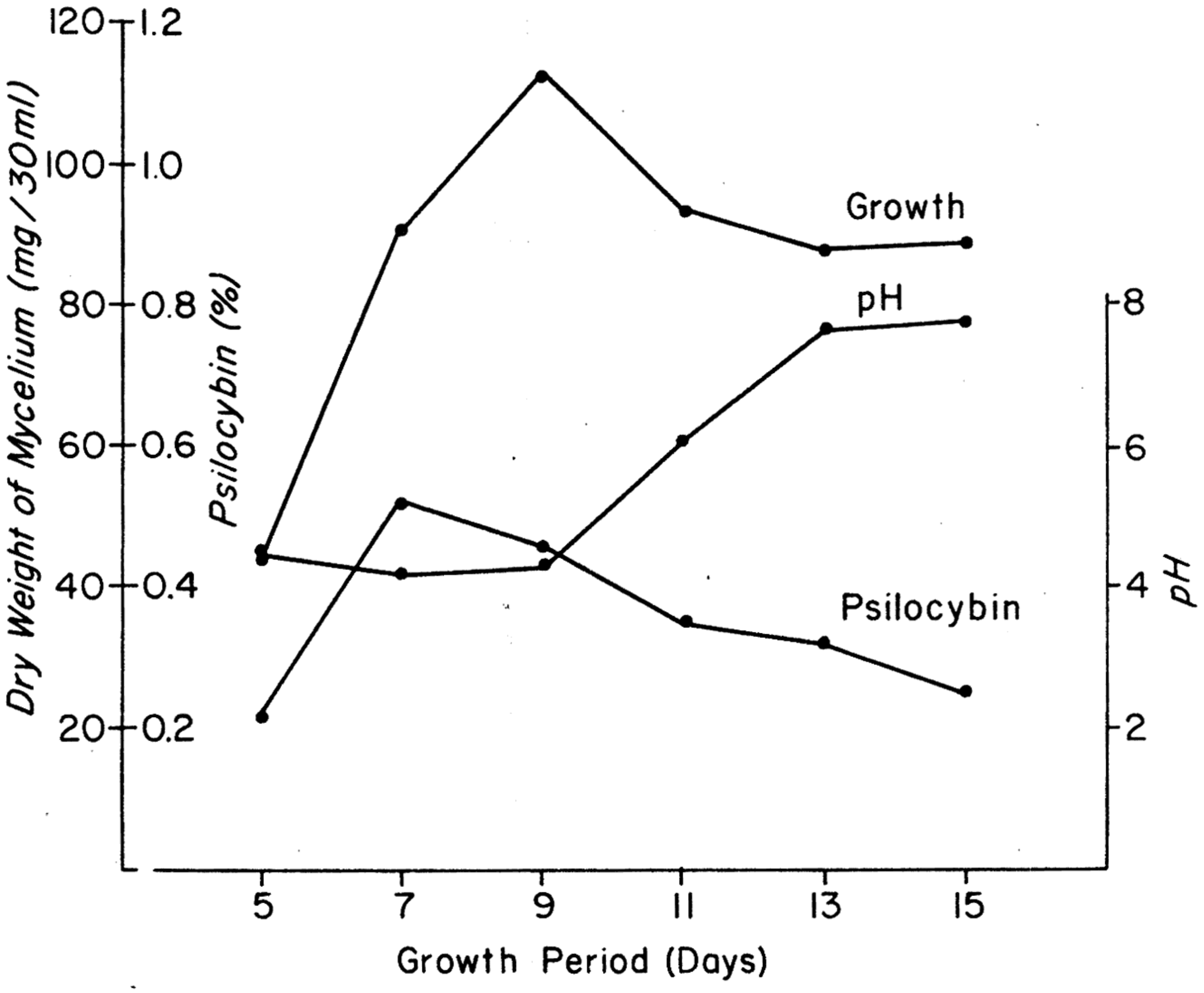

FIG. 1. *Growth, pH of medium, and psilocybin production.*

as before. The ultraviolet absorption spectrum obtained from the final eluate in a Cary spectrophotometer, model 14, was found to coincide with that of known psilocybin (maxima 268 mμ and 290 mμ).

Influence of the omission of certain nutrients.—Subsequent studies were conducted to determine the influence on growth and psilocybin production when each of the following components was omitted singly from medium no. 1: glucose, ammonium succinate, yeast extract, thiamine, and glycine. In a further study, yeast extract and thiamine were concurrently omitted from the medium. Replicates of four cultures for each of the modified combinations, plus control groups, were harvested on the fifth, seventh, and eleventh days after inoculation. Controls were grown in the unmodified medium no. 1. The results are summarized in table 3.

Omission of glucose prevented any appreciable growth of the organism, and

detectable quantities of psilocybin were not produced. The absence of yeast extract impaired initial growth; however, the organism adapted to this deficiency and subsequently underwent vigorous growth and comparable psilocybin production. The yield of mycelium in ammonium succinate-free medium was depressed and seven-day-old pellets showed the lowest levels of psilocybin. When glycine was omitted, the amount of psilocybin on a dry weight basis was slightly greater. When yeast extract and thiamine were concurrently omitted, the results paralleled those obtained when yeast extract alone was not included, except that psilocybin accumulation was substantially lower.

TABLE 2. *Growth, pH of medium, and psilocybin production.*

Growth period (days)		*p*H of medium	Dry wt of mycelium mg	Psilocybin %
5		4.4	40.5	0.23
		4.2	53.9	0.20
		4.3	50.0	0.21
		4.8	29.0	0.25
	ave.	4.4	43.4	0.22
7		4.2	72.0	0.51
		4.0	110.6	0.51
		4.6	114.1	0.54
		4.2	67.3	0.54
	ave.	4.2	91.0	0.52
9		4.1	114.9	0.46
		4.4	114.6	0.50
		4.5	106.1	0.44
		4.1	114.8	0.46
	ave.	4.3	112.6	0.46
11		4.6	101.0	0.36
		4.6	109.4	0.34
		7.8	81.7	0.27
		7.7	82.9	0.37
	ave.	6.1	93.2	0.35
13		7.6	81.9	0.32
		7.6	88.1	0.33
		7.8	102.3	0.31
		7.8	80.2	0.31
	ave.	7.7	88.1	0.32
15		8.0	82.6	0.28
		7.9	81.5	0.27
		7.6	104.6	0.20
	ave.	7.8	89.5	0.25

Influence of the concentration of certain nutrients.—Experiments were designed to determine the influence of different concentrations of glucose, potassium acid phosphate, and ammonium succinate on the growth and metabolism of the organism. Duplicate cultures containing the following concentrations (percentage basis) of the three individual constituents with normal concentrations of the

other ingredients in medium no. 1 were prepared: glucose—1.0, 0.5, 0.25; potassium acid phosphate—0.02, 0.01, 0.005; ammonium succinate—0.2, 0.1, 0.05 These cultures were prepared and processed as previously described. The results are summarized in table 4.

A low concentration of glucose was associated with a rapid rise in *p*H, whereas at the high glucose level an acid *p*H was maintained. Higher levels of glucose resulted in higher yields of psilocybin up to the seventh day, but this may have been due to the increased growth which occurred in this medium The rapid

TABLE 3. *Effect of modification of medium on pH, growth, and psilocybin production.*

Ingredients omitted	Growth Period (days)								
	5			7			11		
	*p*H	Dry wt mycelium mg	Psilocybin %	*p*H	Dry wt mycelium mg	Psilocybin %	*p*H	Dry wt mycelium mg	Psilocybin %
None	4.0	53.9	0.26	4.1	81.4	0.67	—	—	—
	4.0	54.6	0.25	3.8	115.0	0.57	7.1	110.0	0.32
	3.9	63.5	0.24	4.1	84.4	0.64	7.2	78.9	0.34
	4.2	45.2	0.25	3.9	—	—	6.9	106.4	0.34
ave.	4.1	54.3	0.25	3.9	93.6	0.62	7.1	97.5	0.33
Glucose	5.8	7.5	—	6.6	8.6	—	6.8	8.6	—
	5.7	9.4	—	6.4	7.9	—	6.9	9.1	—
	5.9	5.0	—	6.3	7.6	—	6.8	6.9	—
	5.8	6.1	—	6.3	5.5	—	6.9	7.6	—
ave.	5.8	7.0	—	6.4	7.4	—	6.8	8.0	—
Ammonium succinate	3.8	33.7	0.34	3.9	54.4	0.34	7.8	92.8	0.31
	3.8	37.3	0.30	4.0	61.1	0.29	7.8	86.2	0.30
	3.8	34.2	0.30	3.8	95.9	0.21	7.7	80.0	0.36
	3.9	23.0	0.31	3.8	109.3	0.18	7.6	99.2	0.29
ave.	3.8	32.0	0.31	3.9	80.1	0.25	7.7	89.5	0.31
Glycine	3.7	76.1	0.23	3.8	87.5	0.75	6.3	84.9	0.33
	3.7	89.8	0.38	3.5	100.0	0.75	7.4	88.8	0.33
	3.9	55.6	0.35	3.3	130.0	0.63	7.4	84.1	0.39
	3.9	50.1	0.35	3.3	105.4	0.71	7.2	88.4	0.41
ave.	3.8	67.6	0.33	3.5	98.9	0.71	7.0	86.5	0.36
Yeast extract	4.6	22.8	—	4.0	81.7	0.86	—	—	—
	4.4	31.7	0.08	4.2	56.8	0.64	3.9	102.6	0.28
	4.5	32.0	—	4.2	68.5	0.81	3.9	104.4	0.28
	4.4	31.1	—	4.3	55.9	0.68	3.9	100.2	0.35
ave.	4.4	31.0	—	4.2	65.7	0.74	3.9	102.4	0.30
Yeast extract & thiamine	4.2	23.3	0.30	4.2	45.4	0.30	3.7	91.0	0.24
	4.3	31.5	0.30	3.8	89.9	0.31	3.8	88.0	0.25
ave.	4.2	27.4	0.30	4.0	67.6	0.31	3.8	89.5	0.24

increase and subsequent decrease of the psilocybin content of mycelial pellets in a medium containing increased glucose is noteworthy. A low phosphate concentration resulted in a rapid decline of the psilocybin level. In this case also, the *p*H remained acidic through the eleventh day. Variations of the succinate concentration did not influence the organism markedly.

TABLE 4. *Effect of concentration on pH, growth, and psilocybin production.*

Concentration (%)	Growth Period (days)								
	5			7			11		
	*p*H	Dry wt mycelium (mg)	Psilocybin (%)	*p*H	Dry wt mycelium (mg)	Psilocybin (%)	*p*H	Dry wt mycelium (mg)	Psilocybin (%)
Glucose									
0.25	3.9	60.8	0.29	7.3	65.6	0.47	7.7	42.3	0.23
	4.2	51.7	0.28	6.8	97.8	0.45	7.6	44.1	0.25
ave.	4.1	56.2	0.28	7.0	81.7	0.46	7.6	43.2	0.24
0.50	4.7	27.2	0.32	4.0	72.4	0.64	7.3	100.5	0.22
	4.9	28.0	0.31	3.9	86.2	0.65	7.4	114.2	0.20
ave.	4.8	27.6	0.31	3.9	79.3	0.64	7.4	107.3	0.21
1.0	3.9	73.1	0.40	3.8	112.4	1.01	3.7	176.6	0.16
	3.9	72.0	0.41	3.9	108.4	1.06	3.9	181.9	0.15
ave.	3.9	72.5	0.40	3.8	110.4	1.03	3.8	179.2	0.15
Ammonium succinate									
0.05	4.3	28.0	0.30	3.7	89.6	0.62	7.9	88.4	0.32
	4.1	27.8	0.26	3.5	124.4	0.54	7.8	95.0	0.29
ave.	4.2	27.9	0.28	3.6	107.0	0.58	7.8	91.7	0.30
0.10	3.8	82.0	0.27	3.8	88.4	0.55	7.7	96.7	0.28
	3.9	61.7	0.30	3.8	96.3	0.58	7.6	91.5	0.24
ave.	3.8	71.8	0.29	3.8	92.3	0.56	7.6	94.1	0.26
0.20	4.7	40.3	0.29	4.0	95.3	0.45	7.7	104.2	0.26
	4.3	62.4	0.34	4.0	90.6	0.48	7.4	102.0	0.26
ave.	4.5	51.3	0.32	4.0	92.8	0.46	7.6	103.1	0.26
Phosphate									
0.005	4.4	31.2	0.40	3.9	80.1	0.57	3.9	102.2	0.17
	4.2	45.4	0.26	3.8	95.9	0.60	3.8	98.8	0.17
ave.	4.3	38.3	0.33	3.9	88.0	0.58	3.9	100.5	0.17
0.01	3.7	111.4	0.34	3.9	88.9	0.63	7.7	90.8	0.24
	3.7	102.3	0.35	3.8	100.8	0.55	7.1	104.1	0.27
ave.	3.7	106.8	0.34	3.9	94.8	0.59	7.4	97.4	0.25
0.02	4.1	43.4	0.25	4.0	88.4	0.41	7.4	87.1	0.25
	4.1	40.7	0.27	3.9	83.5	0.48	7.2	126.0	0.33
	4.1	42.0	0.26	3.9	85.9	0.44	7.3	106.5	0.29

TABLE 5. *Comparison between large-scale and small-scale culture of* ***Psilocybe cubensis.***

Culture flasks (designation)	Age of culture days	*p*H of medium	Wt of mycelium assayed mg	Psilocybin %
Fernbach.....	7	4.0	106.8	0.47
Erlenmeyer.....	7	4.2	43.5	0.46
Fernbach.....	9	7.1	133.8	0.04
Erlenmayer.....	9	4.2	83.1	0.23
Fernbach.....	11	8.0	159.4	0.03
Erlenmayer.....	11	3.8	95.0	0.12

Replacement culture studies —Since tryptophan had been shown to function as a biosynthetic precursor of psilocybin in surface cultures of *Psilocybe semperviva*, a replacement culture study was designed to determine if mycelium of *Psilocybe cubensis* previously grown in submerged culture could utilize exogenous tryptophan for increased synthesis of psilocybin. This investigation would also provide information regarding the relationship between psilocybin biosynthesis and the growth of the organism.

The replacement culture studies required large quantities of comparable mycelium, and preliminary studies indicated that the metabolism of the organism was markedly influenced by the size of the culture flasks. Mycelial inocula from the same source were shaken simultaneously in three Fernbach and three 125-ml

TABLE 6. *Effect of tryptophan on the biosynthesis of psilocybin in replacement cultures prepared from seven-day-old mycelium.*

Cultures	Incubation time hr	Dry wt mycelum mg	Psilocybin %
Replacement....	12	57.2	0.24
		42.1	0.29
			ave. 0.26
Control.....	12	28.1	0.33
		72.8	0.24
			ave. 0.28
Replacement....	24	65.4	0.18
		66.7	0.17
			ave. 0.17
Control.....	24	64.9	0.18
		58.6	0.14
			ave. 0.16
Replacement....	48	88.0	0.12
		91.4	0.12
			ave. 0.12
Control.....	48	69.1	0.11
		45.5	0.18
			ave. 0.14

Erlenmeyer flasks, and one culture in each size of flask was assayed after seven, nine, and eleven days of incubation, respectively. This comparison indicated that seven-day-old mycelium grown in a Fernbach flask contained an appreciable amount of psilocybin and that the *p*H of the nutrient broth in the Fernback flask was still acidic after seven days (table 5). Thus, the results revealed that seven-day-old mycelium grown in the Fernback flask had the potential to form psilocybin and was not physiologically old.

After a small sample of seven-day-old mycelial pellets was withdrawn from the Fernbach flask and tested for the presence of psilocybin, equal portions of the remaining pellets were transferred to twelve replacement culture flasks, each containing 30 ml of medium no 2. Tryptophan (2 mg/flask) was added to half of the replacement cultures, and the remainder served as tryptophan-free controls. Two control cultures and two tryptophan-containing cultures were removed from the shaker and analyzed at 12, 24, and 48 hours, respectively (table 6).

Addition of tryptophan to replacement cultures did not enhance psilocybin production However, visual comparison of chromatograms revealed that the psilocybin content decreased progressively and that kynurenine accumulation paralleled the decrease in tryptophan content over the forty-eight-hour period.

DISCUSSION

Pellets of *Psilocybe cubensis* produced in medium no. 1 accumulated psilocybin but not psilocin. Maximum production of the former compound occurred on the seventh day (0.52 per cent, dry weight of mycelium), whereas growth attained its maximum (average 112.6 mg, dry weight of mycelium per 30 ml of medium in a 125-ml flask) on the ninth day.

Maximum yields of both psilocybin and mycelium occurred in the acid *p*H range (4.0–4.6). However, the acidic nature of the medium does not preclude the possibility that the internal *p*H of the organism is maintained at a different level by an efficient buffering system Failure to find psilocybin in the medium may be attributed to its instability in the vigorously agitated acid medium, although permeability factors are probably also involved.

In the absence of a readily assimilable carbon source (glucose), detectable amounts of psilocybin did not accumulate. Omission of ammonium succinate did not significantly alter the *p*H of the medium but it did lower psilocybin yields. Without yeast extract, mycelial production was retarded at five days, and culture liquors remained acidic through the eleventh day. Adaption and/or synthesis of necessary precursors nevertheless permitted a continued increase in growth which was accompanied by a rapid increase in the yield of psilocybin by the seventh day. Similar results were obtained for media from which both yeast extract and thiamine had been omitted, except psilocybin levels remained lower.

According to Cochrane (5) most fungi have an absolute requirement for thiamine; however, where low levels are needed, synthesis takes place after initiation of growth. Under conditions of thiamine deficiency, glucose utilization is impaired, which may account for initial inhibition of growth when yeast extract or that ingredient and thiamine were omitted from the medium. Although glycine constituted more than one-half of the total weight of the dissolved solids in medium no. 1, the organism grew and metabolized efficiently in its absence.

The influence of different concentrations of glucose, ammonium succinate, and potassium acid phosphate in medium no. 1 was noted. A low level of glucose was associated with a rapid rise in extracellular *p*H, and lower levels of product accumulated. Doubling the normal amount of carbohydrate promoted psilocybin accumulation which reached a level of 1.03 percent (dry weight) by the seventh day After eleven days, this level dropped to 0.15 per cent; a similar sudden decline in psilocybin content was also noted in those flasks containing a reduced phosphate concentration. The significance of the low *p*H levels after

eleven days is unknown . In contrast, levels of ammonium succinate which were one-half or twice that of the normal medium had no significant effect upon *p*H or psilocybin production.

Extracellular tryptophan added to replacement flasks did not enhance psilocybin production, but, instead, underwent degradative reactions of the type reported to occur in *Neurospora* and other fungi (5). This conclusion is based on the observation that a progressive increase in kynurenine, a catabolic product of tryptophan metabolism, paralleled the disappearance of tryptophan. These results are not necessarily contradictory to those obtained by Brack, *et al.* (2), since they used a different organism and different experimental design which preclude a direct comparison. However, our results do establish the existence of a direct relationship between psilocybin production and mycelial growth. Utilization of the replacement culture technique served to separate growth and psilocybin production, whereas the method of Brack, *et al.*, did not afford this distinction. It was concluded that psilocbyin production is so intimately related to mycelial prolifieration that the nutrient-deficient replacement medium was of little value in the study of psilocybin biosynthesis. However, the technique had previously proven satisfactory for biosynthetic studies of other indole derivatives in fungi (3).

Increasing the scale of fermentation from 30 ml of medium in 125-ml flasks to 300 ml in 2800-ml flasks markedly affected psilocybin production. Significant differences in yields did not appear until after the seventh day when accumulation of product ceased, and a more rapid decline occurred in the large flasks as the pellets became physiologically older. Although the cause of this phenomenon was not established, it may be attributed, at least in part, to differences in the efficiency of aeration of the cultures.

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