Physiology and Nutrition of *Lampropedia hyalina*

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A detailed study of the physiology and nutrition of *Lampropedia hyalina* revealed that it is an aerobic, cytochrome-containing chemoheterotroph which is limited in its energy sources to a few intermediates (and close derivatives) of the Krebs cycle. Reducing compounds at low levels are potent growth inhibitors. The microbe has no photosynthetic ability (despite its previous taxonomic position with the sulfur purple bacteria). The results of a general investigation of its physiology are reported. Added biotin and thiamine are needed for growth in defined media; pantothenate is strongly stimulatory. Alanine, arginine, and tyrosine, as well as NH_4Cl , serve as sole nitrogen sources. A unique motion exhibited by cells of a rapidly growing culture is described. Aspects of its metabolism of poly- β -hydroxybutyrate and limiting aspects of its physiology as related to its ecology are discussed.

In recent years, the morphology and cytology of *Lampropedia hyalina* have been the subjects of several studies (1, 2, 11-13). Although it is a relatively obscure microorganism, its intimate structure has been documented in considerable detail, which may be attributed to the interest in several of its striking features. For example, *Lampropedia* forms rectangular or square cell masses, one cell thick, and sometimes containing many dozens of cells. Groups of cells in the cell mass are surrounded by one or more membranes. Intracellular deposits, identified as poly- β -hydroxybutyric acid (D. A. Kuhn, M. P. Starr, and J. Pangborn, *Bacteriol. Proc.*, p. 44, 1963) appear prominently in microscopic preparations. The references cited above contain excellent illustrations and descriptions of these various structures.

Among observations of *L. hyalina* under natural conditions are those of Pringsheim (13), who isolated a strain from a farmyard puddle. Eadie (5) and a number of other workers observed the "window-pane sarcinae" in the rumen of sheep and cattle. Hungate (6) noted that *Lampropedia* comprised one of a group of bacteria found in the rumen which could be identified readily under the microscope by its unique appearance, and he expressed the view that "*Lampropedia* may be ingested and not grow in the rumen." Schad, Knowles, and Meerovitch (14) found *Lampropedia* as part of the gut flora of herbivorous reptiles and also as inhabitants of nematodes found in the gut of a number of such reptiles.

Lampropedia has been taxonomically associated by various investigators with a number of different microbial groups, including algae, but principally with the photosynthetic sulfur bacteria. In *Bergey's Manual of Determinative Bacteriology*, 6th ed., the genus is tentatively included in the appendix with the sulfur purple bacteria. The lack of comprehensive information about *Lampropedia* accounts for its uncertain status, as well as the fact that it is not described in the seventh edition of *Bergey's Manual*. Studies of its physiology and nutrition have been somewhat neglected, and the present work was undertaken to supply additional information about this microorganism.

MATERIALS AND METHODS

Cultures and media. Three cultures of *L. hyalina* were studied, two of which were derived from the original culture isolated by Pringsheim (13). Strain 11041 was deposited in the American Type Culture Collection, and strain 1441-1 has been carried in the Pflanzenphysiologisches Institut, Göttingen, Germany, and was obtained by the authors from a subculture carried in the laboratory of R. G. E. Murray. These strains, propagated for a number of years in different laboratories, were known to exhibit certain cytological and cultural differences (11). A third culture, strain Mac 583, obtained from R. Knowles late in this work, was also examined. Although groups of cells which are in all likelihood *lampropedia* are seen frequently in rumen content samples, attempts to isolate additional strains during this study were unsuccessful, chiefly because of overgrowth by competing, rapidly growing forms.

In most experiments, Difco dehydrated culture media were used; all chemicals were of reagent or chromatographically pure grade.

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Conditions of growth. Stock cultures of *L. hyalina* were maintained in nutrient broth or on nutrient agar slants and were transferred weekly. Reference cultures of the microorganism were maintained successfully in the lyophilized state over a period of 18 months. For the study of colony formation, cells from 2-day-old broth cultures were streaked on nutrient agar plates and incubated for 3 to 4 days. An incubation temperature of 30 C was usually used throughout the work, although most nutritional work was done at 28.5 C.

Where large quantities of cells were required, *L. hyalina* was grown in 250-ml portions in 2-liter flasks on a New Brunswick gyratory shaker for 3 days. For a comparison of growth in various media, cells were grown in 100-ml portions of media dispensed in 250-ml Erlenmeyer flasks on a gyratory shaker. Optical densities were determined at 660 nm on a Kromatrol photometer or a Bausch & Lomb Spectronic-20 colorimeter.

For amino acid deaminase determination, cells were grown in 40 ml of nutrient broth supplemented with 2 ml of 10% neutralized acid-hydrolyzed casein and adjusted to pH 7.4. After 3 days of growth on a shaker at 30 C, the cells were centrifuged, washed twice with phosphate buffer at pH 7.4, and suspended in 10 ml of the same buffer. Amino acids were dissolved in 25 ml of buffer, at pH 7.4, to produce a 0.05 M solution. A 1-ml amount of cell suspension was added to 10 ml of amino acid substrate and incubated at 30 C; portions were tested at hourly intervals for NH_3 production with Nessler's reagent on a spot plate. For manometric determination of amino acid decarboxylase activity, *L. hyalina* was grown in 100-ml portions of nutrient broth enriched with various 0.05 M substrates on a gyratory shaker at 30 C. The cells were harvested after 24-, 48-, and 72-hr intervals and washed twice with phosphate buffer, pH 7.0. Substrates were employed at 0.05 M concentrations; the pH of the reaction mixture was 6.5; the evolution of CO_2 was measured on a precision Warburg apparatus by conventional manometric techniques.

Compounds used by *L. hyalina* as energy sources were determined in two ways. In the first, a minimal defined medium, complete in other respects, with the test compound as the only source of energy, was employed. A description of the detailed techniques used in the nutritional study follows. A second approach involved the manometric determination of the oxidation of various substrates by preparations of *L. hyalina*. Cells for this purpose were grown in nutrient broth supplemented with the substrate in question to promote enzyme induction. Various quantities of media were used during the course of these experiments as conditions dictated; the cells were centrifuged, washed, and suspended in phosphate buffer at pH 7, and adjusted to an optical density of 0.550, corresponding to a cell count of about 2×10^8 cells per ml. Oxygen uptake was measured by using 0.1 M substrates and KOH in the center well of single side-arm cups. Considerable difficulty was encountered with a high endogenous respiration resulting from the ability of young cells of

L. hyalina to metabolize stored poly- β -hydroxybutyrate at a rapid rate under the conditions of these tests. The results are corrected for endogenous activity.

Microscopic work was done with a Leitz Ortholux microscope equipped with various phase objectives. Physiological reactions were measured by methods outlined in *Manual of Microbiological Methods* (15) or by other commonly accepted procedures.

Cytochrome determinations were made on cells of *L. hyalina* strain 11041 grown in nutrient broth for 5 days at 30 C on a rotary shaker. Cells were centrifuged at 5,000 rev/min for 15 min, washed twice, and suspended in phosphate buffer (pH 7.0) at 10 times the original concentration of cells. Spectra were measured in the range from 300 to 650 nm on a Cary 14 recording spectrophotometer equipped with a scattered transmission accessory. Potassium ferricyanide was added to the cuvette containing the cell suspension (and to a water blank control) to a final concentration of 0.001 M. A few grains of sodium hydrosulfite were added as a reducing agent to the oxidized cells and to the blank.

Glassware used in nutritional studies was washed with detergent and water, rinsed, and soaked overnight in acid dichromate cleaning solution. It was then rinsed exhaustively with tap water and 10 times with distilled water. Plastic test-tube caps were cleaned by soaking in detergent and water, followed by careful rinsing with tap and distilled water. Optical density was determined on a Kromatrol photometer set at a wavelength of 650 nm; increased sensitivity was obtained by the attachment of an external galvanometer. Cell growth was measured in standardized Pyrex tubes (16 $\times$ 100 mm). Biocytin was a gift of Donald Wolf, Merck Research Div., Rahway, N.J. Stock solutions were stored at 4 C.

The inorganic salts solution contained 0.10 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.20 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.20 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.25 g of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ per liter. The growth medium contained an appropriate amount of a 1:10 dilution of the stock solution.

Vitamin solutions were filtered through 0.1- μm filters (Millipore Corp., Bedford, Mass.). Stock concentrations of 100-ml quantities included calcium pantothenate, nicotinic acid, pyridoxamine hydrochloride, riboflavin, and thiamine hydrochloride at 10-mg levels, and biotin at 0.01-mg and folic acid at 0.1-mg levels. Stock preparations were usually diluted 1:100 in final media. Thiamine was prepared in acetate buffer at pH 4.5. Riboflavin and folic acid were protected against light with dark paper and aluminum foil.

The phosphate buffer contained 0.7 g of KH_2PO_4 and 1.3 g of K_2HPO_4 in 100 ml of distilled water. Unless otherwise specified, the buffer was diluted 1:20 in final media. Amino acids were of the L configuration, except leucine and isoleucine, which were DL. Stock solutions of amino acids for growth experiments were prepared at concentrations of 10 mg/ml, except for cysteine and phenylalanine which were prepared at 1 mg/ml, and aspartic acid, asparagine, glutamic acid, and glutamine which were prepared at 500 mg/ml. Adenine sulfate, guanine hydrochloride, thymine, and uracil were prepared at

concentrations of 0.50 mg/ml and xanthine at 0.25 mg/ml. A small amount of 0.1 N HCl was added to facilitate solution of bases where necessary.

The following procedure served to minimize carry-over from inoculation. Standing cultures were incubated at 30 C in nutrient broth for 2 days. Five loopfuls of surface growth were transferred aseptically to 10 ml of sterile, neutral phosphate buffer in a test tube. After thorough mixing, one loopful was transferred to the experimental medium. The suitability of defined medium for supporting continued growth was tested by passing the culture through 18 to 20 serial transfers.

Table 1 shows the composition of the medium used to determine the B vitamin requirements of *Lampropedia*. Variations involved the omission of selected vitamins. When the vitamin requirements of *L. hyalina* were known, amino acids were substituted for casein hydrolyzate. This medium is shown in Table 2. Variations of this medium involved the omission of selected amino acids in order to examine for inhibitory or stimulatory effects. The composition of a final medium, using NH_4Cl as a nitrogen source and giving growth comparable to media using organic nitrogen, is shown in Table 3.

Several compounds were substituted for pyruvate in the final defined medium to test for their utilization as energy sources. These were usually added at levels of 0.3 and 0.15%. The effect of sodium thioglycolate in the defined medium was measured at levels of 0.01 to 0.1%. The stimulatory effect of B vitamins was measured by using a range of concentrations up to 10 $\mu\text{g}/\text{ml}$. Several compounds were substituted for biotin (which was found to be an absolute requirement) in the minimal medium. Aspartic acid at a concentration of 1 mg/10 ml of medium was added in the presence and absence of biotin. Pimelic acid and DL- α -amino pimelic acid were added at 10-, 20-, and 30- μg levels. Oleic acid was added in 0.3-, 0.5-, and 1.0-mg amounts. Some

media contained oleic acid plus 1 g of Tween 80 per liter. In others, biotin was replaced by Tween 80. Biocytin was tested for biotin activity within the range of 0.01 to 0.1 μg of biotin per 10 ml of medium.

RESULTS

Growth characteristics. Previous reports (11, 13) described in detail the formation of sheets of cells characteristic of *L. hyalina* when grown in undisturbed surface culture. In stationary liquid medium, the culture developed in thin patches of

TABLE 2. *Composition of medium used to determine the amino acid requirement, stimulation, or repression of Lampropedia hyalina*^a

Contents	Concn per 10 ml of medium
Sodium pyruvate.....	30 mg
Thiamine hydrochloride.....	10.0 μg
Biotin.....	0.01 μg
Threonine.....	1.0 mg
Methionine.....	1.0 mg
Valine.....	1.0 mg
Leucine.....	1.0 mg
Isoleucine.....	1.0 mg
Lysine.....	1.0 mg
Histidine.....	1.0 mg
Phenylalanine.....	1.0 mg
Tyrosine.....	1.0 mg
Proline.....	1.0 mg
Glycine.....	1.0 mg
Alanine.....	1.0 mg
Arginine.....	1.0 mg
Serine.....	1.0 mg
Glutamic acid.....	0.5 mg
Aspartic acid.....	1.0 mg
Cysteine.....	1.0 mg
Tryptophan.....	1.0 mg
Hydroxyproline.....	1.0 mg
Asparagine.....	1.0 mg
Basic salts.....	1:10 dilution of stock
Buffer.....	1:20 dilution of stock

^a Initial pH, 7.0.

TABLE 3. *Chemically defined medium for growth of Lampropedia hyalina*

Contents	Concn per 10 ml of medium
Basic salts.....	(1:10 dilution of stock)
3% Sodium pyruvate.....	30 mg
3% Ammonium chloride.....	30 mg
Phosphate buffer.....	(1:20 dilution of stock)
Thiamine hydrochloride.....	10.0 μg
Biotin.....	0.01 μg
NaOH.....	0.7 mg

TABLE 1. *Composition of medium used for the determination of B vitamin requirements for the growth of Lampropedia hyalina*^a

Contents	Concn per 10 ml of medium
5% Casein (hydrolyzed).....	50 mg
Tryptophan.....	10 mg
Cysteine.....	1 mg
Biotin.....	0.01 μg
Thiamine hydrochloride.....	10.0 μg
Calcium pantothenate.....	10.0 μg
Pyridoxamine hydrochloride...	10.0 μg
Folic acid.....	0.1 μg
Riboflavin.....	10.0 μg
Nicotinic acid.....	10.0 μg
Basic salts.....	(1:10 dilution of stock)
Buffer.....	(1:20 dilution of stock)

^a Initial pH of medium, 7.0.

surface film which finally united and moved up the sides of the container 2 to 3 mm above the surface of the liquid, exhibiting a strongly hydrophobic nature. When the entire surface was overgrown, the cells settled as a sediment to the bottom of the flask and a new thin layer began to form. Fluid medium increased in viscosity after 3 to 4 days and the surface growth took on a stringy quality. Shaker-grown cultures in a complex medium were densely turbid at 48 hr. Shaking conditions disturbed the sheet formation, although the strain designated as Mac 583 continued to form sizable sheets of cells even when shaker-grown. On nutrient agar, *L. hyalina* formed whitish, more-or-less circular, rough colonies with undulate edges. Smooth glistening colonies occurred among the predominant rough colonies; factors controlling the development of smooth colonies are unknown.

L. hyalina grew nearly as well in nutrient broth as in yeast extract-tryptone broth. Growth was characteristically accompanied by a rise from an initial pH of 7.0 to a final pH of 8.4 to 8.6, an effect resulting from the oxidation of various organic constituents of the medium and the accumulation of alkaline salts. A similar rise in pH occurred in a minimal defined medium. No growth occurred in APT (Difco) medium, presumably because of its content of reducing compounds which were found to be markedly inhibitory to this microorganism. This was also true of defined media, where cysteine or sodium thioglycolate is inhibitory even at low levels.

Microscopic observations. *Lamprospedia* cells stained readily with common bacterial stains. They are gram-negative, do not form spores (as determined by Dorner's and the malachite green stains), and prominent inclusions of poly- β -hydroxybutyrate were usually seen in 2- to 3-day-old cells. The polymer granules disappeared as the cells aged and were nearly or entirely absent in 5-day-old cells grown in media with a limiting energy source. The depolymerase responsible for the disappearance of the storage product is the subject of a separate investigation. Breakage of *L. hyalina* cells by sonic treatment occurred in 2 to 3 min in a 9-kc oscillator. The resulting aggregates of poly- β -hydroxybutyrate globules were readily sedimented by centrifuging at 12,000 rev/min for 10 min.

Cell motion. Neither the Bailey and Leifson stains nor electron micrographs revealed flagella on *Lamprospedia*. The motion of the cells, described below, differed from that normally associated with flagella. When wet preparations of a young culture were examined, small groups of cells were seen to break free from larger aggregates, move for short distances (a few mi-

crometers), and become attached to other aggregates. This does not occur with single cells, but only with small groups of cells which change position in both vertical and horizontal planes. Both the detachment of cells from aggregates and the attachment to new aggregates take place in a decisive manner, and cells seem to "click" into place.

The motion is characteristic of young, rapidly growing cultures from either liquid or solid media, and becomes weaker with age. In a preparation of actively growing cells, motion was evident to the extent that a constant flickering effect was produced in the microscopic field as small groups of moving cells entered and left the plane of focus. Chilling at 4 C stopped the motion, but it resumed when the cells were warmed to room temperature. Heating the cells to 45 C stopped the cell motion irreversibly. When cells from 6-day-old cultures with a limiting source of energy, which were almost devoid of poly- β -hydroxybutyrate granules and which did not show motion, were transferred to a fresh medium at 30 C, weak motion and newly dividing cells appeared in about 4 hr, and the culture showed typical motion at 8 hr.

In citrate-phosphate buffer, the cells did not show motion in the pH range from 2.8 to 4.8, but did show motion from 4.9 to 7.6. In acetate buffer, there were no signs of motion below pH 5.0. In citrate buffer, the cells did not exhibit motion at pH 2.6 to 4.9, but did from pH 5.0 to 5.8. Cells suspended in distilled water continued to show motion. Young cells were not lysed by lysozyme, ethylenediaminetetraacetic acid, Tween 80, or distilled water. At concentrations of NaCl above 5.0%, the cells were markedly shrunken and distorted, although they still showed signs of motion. Tween 80 caused the breaking up of large aggregates of cells, but motion of the smaller groups remained unaffected in concentrations of ethylenediaminetetraacetic acid up to 7.5%.

General physiology. Reactions of two strains of *L. hyalina*, 11041 and 1441-1, are given in Table 4. The chief difference noted between the strains is the longer lag period and slower response of strain 1441-1. The number of substrates found to be available to *L. hyalina* as sole sources of energy was rather sharply limited—fumarate, malate, β -hydroxybutyrate, lactate, pyruvate, and succinate. In addition, acetate served as an energy source in the presence of catalytic levels of pyruvate. The organism appeared not to utilize carbohydrates, alcohols, glucosides, fatty acids, a variety of organic acids, and many related compounds (Table 5). Other substrates not attacked were several proteins, fats, and polysaccharides. Ammonia is produced from

TABLE 4. General reactions of *Lampropedia hyalina*

Characteristic	Reaction
Hydrolysis	
Arginine.....	+
Blood-agar.....	— (Hemolysis)
Casein.....	—
Deoxyribonucleic acid.....	—
Fats.....	—
Gelatin.....	—
Sodium hippurate.....	—
Starch.....	—
Urea.....	—
Reduction	
Nitrate.....	—
Nitrite.....	—
Nitrification.....	—
Coagulase.....	—
Litmus milk.....	No reaction
NaCl tolerance	
1.0%.....	+
1.5%.....	—
Sucrose tolerance	
2%.....	+
4%.....	—
Bile tolerance (0.5%)	—
Solubility in bile (5–40%)	—
Catalase	+
Acetylmethylcarbinol production	—
Indole	—
Benzidine test (for heme)	+
Indophenol oxidase (about 3.5 min)	+
Final pH in medium	8.4–8.6
Temp limits of growth	About 10–35 C
Optimal temp	About 30 C
Oxygen relationship	Strict aerobe

arginine (the only deaminase activity detected), and arginine serves as a sole nitrogen source in a defined medium. Of 20 amino acids tested, decarboxylase activity could not be shown by the manometric method.

Cultures of *L. hyalina*, grown in the presence of tyrosine, produced an intense darkening within 4 days; carotenoid or photosynthetic pigments were not formed. Attempts to grow the microorganism under anaerobic conditions with prolonged exposure to red light (range ca. 700 to 990 nm) and with increased CO₂ tension failed. Reducing compounds such as cysteine and thioglycolate inhibited growth even at exceedingly low levels. *L. hyalina* is an obligate aerobe and both its growth rate and the final cell crop are increased by vigorous aeration. The organism failed to grow in media of initial pH values below 6.0 and above 9.0. It is relatively heat-sensitive and failed to develop after incubation at 45 C for 25

min. There was no evidence of growth in media designed to test for nonsymbiotic nitrogen fixation but to which lactate was added to satisfy the energy requirement of this heterotroph.

When 3-day shaker-grown cells of *L. hyalina* were examined manometrically for the ability to oxidize various substrates, the results correlated well with those of growth experiments in which substrates were tested for their ability to serve as energy sources. Rapid oxygen uptake occurred with lactate, sodium β -hydroxybutyrate, succinate, fumarate, pyruvate, glutamine, and an unidentified component in beef extract. There were two differences in the results, depending upon the experimental approach. (i) Butyrate was oxidized by nutrient broth-grown resting-cell preparations, but did not serve as a sole source of energy in a chemically defined medium; (ii)

TABLE 5. Compounds not metabolized by *Lampropedia hyalina*

Substrates not supporting growth ^a	Substrates not fermented
Fats	Polyhydric alcohols
Tri- <i>n</i> -butyric	Arabitol
Trilaurin	Dulcitol
Fatty acids	Erythritol
Butyric	Glycerol
Crotonic	Mannitol
Lauric	Ribitol
Myristic	Sorbitol
Oleic	Xylitol
Palmitic	Deoxysugars
Propionic	Fucose
Stearic	Rhamnose
Miscellaneous acids	Disaccharides
DL-Amino- <i>n</i> -caproic	Cellobiose
α -Aminoisobutyric	Lactose
DL- α -Amino- <i>n</i> -valeric	Maltose
DL- α -Amino- <i>n</i> -butyric	Sucrose
Benzoic	Trehalose
Citric	Glucosides
γ -Amino- <i>n</i> -valeric	Esculin
Glycolic	Salicin
Isovaleric	Hexonic acid
Malonic	Sodium gluconate
Phenylacetic	Hexoses
Sorbic	Fructose
Amino acids	Galactose
Alanine	Glucose
Arginine	Mannose
Serine	Sorbose
Tyrosine	Pentoses
Miscellaneous compounds	D-Arabinose
Cycloheximide	L-Arabinose
1,3-Butanediol	Ribose
2,3-Butanediol	Xylose
<i>n</i> -Butyramide	Xylulose
Chlorophyll	Polysaccharides
Phenol	Inulin, pectin, starch
1,2-Propanediol	Tetrose: erythrose
Cholesterol	Trisaccharide: raffinose

^a When supplied as sole sources of carbon in a chemically defined medium.

malate, which was not oxidized by the resting-cell preparation, served as an energy source in the defined medium.

Absorption peaks obtained in the Cary spectrophotometer with reduced cytochrome preparations were found in the Soret region at 426 nm and at 530, 560, and 590 nm.

Nutrition. In media containing hydrolyzed casein (enzymatic or acid-hydrolyzed), good growth occurred, except when thiamine or biotin was omitted. Subsequent work confirmed the fact that these vitamins are essential for growth of *L. hyalina*. Although pantothenate is not an absolute requirement, it shortens the lag period considerably and increases the average size of the cells and the storage of poly- β -hydroxybutyrate. The presence of riboflavin at levels of 7 to 10 μ g/10 ml of medium results in a lag in growth for 24 hr.

Koser, Wright, and Dorfman (7) demonstrated that the requirement of yeasts for biotin is greatly reduced in the presence of sufficient aspartate. Stokes, Larsen, and Gunness (16) noted that many lactic acid bacteria required higher concentrations of biotin in the absence of aspartate, suggesting a role for biotin in aspartate synthesis. Lardy, Potter, and Burris (8) demonstrated that *Lactobacillus arabinosus* requires biotin in order to fix CO₂ for aspartic acid synthesis, and that this reaction ceases when ample aspartate is supplied in the medium.

In our work, aspartate did not substitute for biotin. Also, since aspartic acid is inhibitory for *L. hyalina*, the role played by presupplied aspartic acid in decreasing the biotin requirement could not be readily determined.

Desthiobiotin, a normal intermediate in biotin synthesis, serves as a substitute for biotin with equal activity. Pimelic acid, which is known to be a specific precursor of biotin (4), and DL- α -amino pimelic acid did not satisfy the biotin requirement of *L. hyalina*. Biocytin, which will substitute for biotin with equal activity in some microorganisms (17), also did not substitute for biotin. *L. hyalina* did not grow when oleate, Tween 80, or oleate plus Tween 80 was substituted for biotin in the complete defined medium.

Good growth of *L. hyalina* was obtained in the minimal defined medium with ammonium chloride as the only nitrogen source. Several amino acids, including alanine, arginine, and tyrosine, also served as the sole nitrogen sources.

The same general growth characteristics were apparent in the defined medium as in complex media, except that cells and poly- β -hydroxybutyrate granules tended to be somewhat smaller. With continued transfer in defined medium, both the size of the cells and the quantity of intracellu-

lar deposits increased. The slight viscosity which was apparent in nutrient broth was more pronounced in defined medium.

Lampropedia is independent of supplied purine and pyrimidine bases and grows equally well in their presence or absence.

DISCUSSION

The inability of *L. hyalina* to metabolize commonly used substrates suggests that in natural conditions its carbon and energy sources are obtained from matter decomposed by other microorganisms. *L. hyalina* reveals no photosynthetic properties in either autotrophic or heterotrophic environments and there is no basis for continuing to relate *L. hyalina* taxonomically to the photosynthetic sulfur bacteria. Its metabolism differs widely from that of *Leucothrix*, which resembles the blue-green algae in some respects but which is able to use a large variety of carbohydrates and other simple inorganic compounds as sources of carbon and energy. If the cultures used in the present investigation correspond to the *L. hyalina* of early reports, it may be speculated that the error of associating it with the photosynthetic group in the past may have stemmed from one of several sources. *L. hyalina* bears a grossly superficial resemblance to the photosynthetic form, *Merismopedia*, as Murray (11) and others have pointed out, although cells of the latter are several times larger than *L. hyalina* cells. Also, the poly- β -hydroxybutyrate inclusions, which are prominent in microscopic preparations of *L. hyalina*, bear a resemblance to intracellular sulfur globules stored by the photosynthetic sulfur purple bacterium, *Thiopedia*, and might easily have been mistaken for such. In media of relatively high tyrosine content, *L. hyalina* forms a brownish extracellular pigment which in turbid preparations gives a reddish cast to the culture, a superficial appearance which might be mistakenly ascribed to a photosynthetic pigment.

Recent work (J. A. Lauritis, Ph.D. Thesis, Iowa State Univ., Ames, 1967) has revealed that *Rhodotherce* (query *Thiopedia*), a nonsulfur purple bacterium, has complex superficial structures closely resembling those of *L. hyalina*. Although the physiology of these two microbes is vastly different, their morphological similarities could have contributed to a confusion of identity.

The matter of acetate metabolism by *L. hyalina* has been open to some question. Pringsheim (13) recommended an acetate-containing medium for *L. hyalina*, and Murray (11) noted that the omission of acetate from a yeast extract-peptone medium results in slower growth of the cells. Puttlitz and Seeley (Bacteriol. Proc., p. 22, 1965) reported that *L. hyalina* will not initiate growth in

a defined medium containing acetate as the only source of energy unless low levels of pyruvate or other carbon sources are also supplied in the medium. These relationships would be expected to prevail if acetate were added to a complex medium and would explain the stimulatory effect of acetate noted by other investigators.

MacRae and Wilkinson (10), working with *Bacillus cereus* and *B. megaterium*, both of which form poly- β -hydroxybutyrate granules, found that acetate stimulates growth in the presence of certain carbon compounds, including pyruvate. It was postulated that the acetate stimulation effect might be explained by assuming that acetate unites with a two-carbon compound produced during the metabolism of other substrates and results in the formation of β -hydroxybutyrate or a derivative possibly formed through acetoacetate which, in turn, is polymerized. A pathway of poly- β -hydroxybutyrate synthesis similar to the *Bacillus* pathway may exist in *L. hyalina*. This postulation is supported by the demonstration that pantothenic acid which, although not an absolute nutritional requirement of the microbe, markedly stimulates the growth of *L. hyalina*, perhaps through its role in the formation of an acetyl coenzyme A complex which functions in the subsequent oxidation of acetate and its conversion to poly- β -hydroxybutyrate.

Although the unique motion of cell aggregates of *Lampropedia* is similar in the three different strains examined, its basis, whether physical or biological, cannot be explained at present. The failure to detect flagella either in stained preparations or electron micrographs, the unusual nature of the motion, and the hydrophobic nature of *L. hyalina* cell surfaces argue for a physical rather than biological explanation. The term "motion" rather than "motility" is used here with this reservation in mind. It is interesting to note that motion can be observed in the nonsheet-forming strain which Murray (11) described as being deficient in membranes outside the cell wall. This would indicate that the external layers incorporating masses of cells are not factors involved with the motion. Murray observed that his culture showed no motility and no flagella in electron micrographs. Pringsheim (13) reported that his original culture was motile when isolated but lost its motility upon laboratory cultivation.

A somewhat similar type of cellular motion observed in liquid cultures of the bacterial genus *Vitreoscilla* is described (3) as a "rolling and darting jerky movement" not resembling motility caused by flagella; it occurs when cells touch a surface. This property is presumably related to the normal gliding motility of the filamentous *Vitreoscilla* on solid surfaces and is lost upon mild

heat or chemical treatment of the cells, suggesting a biological nature for the phenomenon. An unusual method of surface translocation differing from gliding was reported (9) for a number of gram-negative species of eubacteria, including *Moraxella*, *Flavobacterium*, *Bacterium anitratum*, and nonflagellated variants of *Pseudomonas aeruginosa*. This is described as comprising small intermittent jerks covering distances of 0.5 to 1.0 μ m, the direction of movement being variable and independent of the cell axis.

L. hyalina strain Mac 583 (obtained from Knowles) gives the same physiological reactions as the other strains studied, but is outstanding in its sheeting ability. Intact sheets of 24 or more cells developed and remained intact in media vigorously agitated on the gyratory shaker. The tenacity with which cells are held together in regular units was pointed out by Chapman, Murray, and Salton (2), who concluded that the strong cohesion of cells was indicative of the presence of a cementing substance between cells.

Several physiological characteristics of *L. hyalina* do not favor the hypothesis that it is a part of the normal flora and fauna of the rumen and the gut of some animals. Since it is a strict aerobe, it would not be expected to grow in these anaerobic environments. The maximum temperature of growth of *L. hyalina* is 35 C and the limiting pH is 6, both limitations which are exceeded in the postulated habitats. Many intestinal inhabitants tolerate considerable concentrations of bile salts; *L. hyalina*, however, is completely inhibited at levels of 0.5% bile.

If *L. hyalina* is not a functional part of the normal flora and fauna of the rumen and gut of some animals, reports of its presence in these habitats may be explained as cases of mistaken identity, or more reasonably, since a number of descriptions and photomicrographs correspond to *L. hyalina*, that it must be present in these sources as a part of the transient microbial population arising through feeding. Such a view is consistent with observations by early workers who considered *L. hyalina* to be a widespread soil organism.

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