It is generally recognized that biochemical information is of great importance to microbial taxonomy. This is particularly true in the case of catabolic behavior of microorganisms in utilizing glucose and allied compounds. Once the pathways operating in a species are identified, and the relative participation of these pathways is determined, then differences in catabolic patterns among organisms or groups of organisms become taxonomically significant. There is evidence that microorganisms in a given genus may rely on a given set of primary pathways for glucose utilization, and that closely related genera will also display similar patterns of catabolic pathways. Thus, phylogenetic models may be constructed for whole groups of microorganisms by correlating catabolic information with other morphological and physiological data.

In the present work, pathway identification and estimation has been made in species of *Chromobacterium* using a radiorespirometric
method which provides yield data of respiratory $^{14}$CO$_2$ derived from carbohydrate substrates specifically labeled with $^{14}$C.

Five representative species of the genus Chromobacterium were selected for the present study. Results from radiorespirometric experiments indicate that Ch. lividum catabolizes 86% of administered glucose via the Entner-Doudoroff (ED) pathway and the remaining 14% via the pentose phosphate (PP) pathway. Ch. amethystinum catabolizes 87% of administered glucose via the ED pathway and the remaining 13% via the PP pathway. Ch. violaceum routes 80% of catabolized glucose through the ED pathway and 20% through the PP pathway. Ch. maris-mortui, on the other hand, relies heavily on the PP and PC pathways (100%) for glucose catabolism, while Ch. viscosum utilizes the Embden-Meyerhof-Parnas (EMP) pathway for 84% of the catabolized glucose and the PP pathway for the remaining 16%.

On the basis of the present understanding of carbohydrate catabolism, and other morphological and physiological data, it is recommended that 1) Ch. lividum and Ch. violaceum be retained as type species of the psychrophilic and mesophilic groups, respectively; 2) Ch. amethystinum be designated as a strain within the species Ch. lividum; 3) the halophile Ch. maris-mortui be excluded from the genus Chromobacterium; and 4) the organism Ch. viscosum be excluded from the genus Chromobacterium.
Comparative Carbohydrate Catabolism in Chromobacterium Species

by

Carol Meyer Wehr

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INTRODUCTION

Phylogenetic relationships are of great importance to taxonomy. It has been comparatively easy to classify plants and animals, because anatomical characteristics are numerous and grossly visible. It is more difficult to classify bacteria. Morphology, nutritional requirements, the ability to utilize certain carbon and nitrogen sources, and the ability to reduce or oxidize inorganic compounds have all served as the basis for bacterial classification.

With the vast broadening in scope of biochemistry over the past several decades, whole new sets of criteria for taxonomy have emerged. These include information on the molecular characteristics of structural and functional components and on mechanisms for biosynthesis or metabolism.

Among the metabolic manifestations, carbohydrate catabolism may be the most useful for recognition of phylogenetic relationships. As an example one can cite the Entner-Doudoroff (ED) pathway, discovered in 1952, which appears to occur only in certain bacteria, such as species of *Pseudomonas* and several closely related genera.

One of the best schemes of phylogenetic taxonomy for microorganisms was devised by De Ley in 1962. He tabulated data on
biochemistry, morphology, physiology, and intermediary metabolism for different microorganisms, organizing the material so that phylogenetic relationships could be recognized. This scheme was admittedly primitive since it could only include the fragmentary information available. Similar approaches have been used by many investigators; to cite a few, Sneath, 1957 a, b; Sneath and Cowen, 1958; Lysenko and Sneath, 1959; Hill, 1959. All have recognized that a complete unbiased biochemical taxonomy, analyzed by computer methods, is necessary.

There are several main pathways by which carbohydrates can be catabolized in microorganisms. These pathways often operate concurrently, and the extent to which each plays a role may be a useful key to classification. De Ley (1962) and others (Hugh and Leifson, 1953; Kitos, et al., 1958; Zagallo and Wang, 1962a, b; Wang, 1964) held the concept that closely related organisms will share similar or identical pathways of carbohydrate utilization. Thus, De Ley has recognized close phylogenetic relationships among Pseudomonas, Zymomonas, Xanthomonas, Agrobacterium, Azotobacter, and Rhizobium on the basis of the occurrence of the ED pathway. In his tentative classification model, De Ley (1962) has suggested that several species related to pseudomonads may also rely on the ED pathway for glucose catabolism, such as Protaminobacter, Vibrio,
Flavobacterium, Achromobacter, and Chromobacterium. Among these genera, Chromobacterium appears to be the most interesting case. This is due in part to the extensive but controversial work which has been done with Chromobacterium species.

In reviewing the historical development of the genus Chromobacterium, one finds that the first published report of a violet pigmented bacterium resembling the genus was in 1872 by Schroeter; he termed the organism Bacteridium violaceum. Another strain was described by Bergonzini in 1880 under the name of Cromobacterium [sic] violaceum, and this is generally considered to be the type species of the present genus. Several other strains were described in the next few years by various workers (Zopf, 1883; Hueppe, 1884; Eisenberg, 1886, 1891; Plagge and Proskauer, 1887). Plagge and Proskauer (1887) first distinguished between the groups now termed mesophilic and psychrophilic. Other early work was done by Sternberg, 1892; Wright, 1895; Lehmann and Neumann, 1899; Beijerinck, 1900; Bampton, 1913; Buchanan, 1918; Winslow, et al., 1920.

Most early workers were content to leave this chromogenic group of bacteria in the same genus as most of the other gram negative organisms, usually Bacterium or Bacillus. Buchanan (1918) attempted to establish the genus Chromobacterium, with the type species given as Chromobacterium violaceum Bergonzini. Since that time this generic name has been almost universally used, and
although various red, yellow, and blue chromogenic bacteria have
at times been classified within the genus (e.g., Serratia, Flavobac-
terium, Pseudomonas, and other non-related organisms), this ar-
rangement stood much as it was in the first edition of Bergey's
Manual of Determinative Bacteriology (1923) until revision in the
fifth edition (1939).

In 1938, Conn established the family Rhizobiaceae to cover
the genus Rhizobium and other bacteria such as Chromobacterium
which were nonsporulating gram negative rods, often pleomorphic,
with a few flagella on each rod. Most of those he placed in this
family were soil or water forms or plant pathogens. They seldom
produced much acid from carbohydrates since they generally oxidized
glucose completely to carbon dioxide and water (see Sguros and
Hartsell, 1952). In the fifth, sixth, and seventh editions of Bergey's
Manual (1939, 1948, 1957), the genus Chromobacterium has been
placed in the family Rhizobiaceae, along with Achromobacter, Agro-
bacterium, and Alkaligenes.

Although the placement of the genus Chromobacterium within
this family is probably a natural one, there remains much confusion
about the species within the genus. The organism is easily isolated
from soil and water (Corpe, 1951, 1953, 1954), and appears to con-
sist of many strains, most of which have been given species status
by various authors (Calderini, 1925; Cruess-Callaghan and Gorman,
1935; Waeldele, 1938; Leifson, 1956; Eltinge, 1957), with little evidence of differentiation between these and existing species or strains. Sneath (1960) has suggested that only two species should be recognized, i.e., Chromobacterium violaceum and Ch. lividum, with some 20 strains within each species.

Previous attempts to separate the species of Chromobacterium are based on a number of characteristics, including pigmentation, flagellation, temperature dependence, pathogenicity, serology, and fermentations. Although pigmentation has in practice been the recognizable feature of this genus, color is not a reliable characteristic for several reasons. Chromogenesis is quite variable, is easily lost upon continued transfer, and is less defined or not present in certain media. Oxygen is necessary for the pigmentation of this genus, hence embedded colonies or stabs are often white or colorless. A lower than optimum temperature stimulates chromogenesis; thus cultures grown at 30°C often show color upon storage at 4-10°C. In addition, until recently any bacterium with purple or violet pigmentation was placed in this genus, and many other colors were also included. Most recent authors would exclude not only all colors except purple as the characteristic of the genus, but would also include only those purples which are chemically based on violacein, which is now recognized as the pigment produced by Chromobacterium species.
The chemistry of violacein has been extensively studied (Schneider, 1894; Lehmann and Neumann, 1899; Bampton, 1913; von Eisler and von Portheim, 1914; Calderini, 1925; Reilly and Pyne, 1927; Friedheim, 1932; Kögl, 1932; Tobie, 1935, 1936, 1938, 1939; Ehrismann and Noethling, 1936; Waeldele, 1938; Sartory, Meyer, and Waeldele, 1938; Strong, 1944; Beer, et al., 1948, 1949; Gilman, 1953; Audebaud, et al., 1954; Sneath, 1956a; Barrett, et al., 1957; Ballantine, et al., 1957a, b, 1958; DeMoss and Evans, 1958. The first study was made by Schneider in 1894. Violacein has a complex indole-pyrrole structure and is found within the cells (Sneath, 1960); it is water- and chloroform-insoluble (Schneider, 1894) but is soluble in ethanol (Reilly and Pyne, 1927), methanol (Friedheim, 1932), and acetone (Calderini, 1925). In ethanol, the absorption spectrum exhibits a maximum at 580 μm and a minimum at 430 μm (Sneath, 1960). The structure suggested by Beer, et al. (1949) and others (see review article by Sneath, 1960) but confirmed by Ballantine, et al. (1958) is:

\[
\text{HO} \quad \text{VIOLACEIN: C}_{20}\text{H}_{13}\text{O}_3\text{N}_3 \\
\text{M. wt.} = 343.33
\]
The pigments of *Ch. violaceum* and *Ch. lividum* are identical and are indeed violacein (Sneath, 1960). The pigments of *Ch. viscosum*, *Ch. iodinum*, and other red, yellow, orange, brown, and blue organisms have different absorption spectra and are clearly not violacein (Gilman, 1953). Gilman suggests on the basis of his spectrophotometric data that *Ch. viscosum* and *Ch. iodinum* should be reassigned to the genus *Pseudomonas* since the pigments appear to be water-soluble phenazine derivatives like other pigments in that genus. In fact, Clemo and McIlwain in 1938 demonstrated the pigment of *Ch. iodinum* to be 1,2-dihydroxyphenazine-9,10-di-N-oxide. The orange and brown pigments analyzed by Gilman (*Ch. orangium* and *Ch. chocolatum*) were similar to that of *Sarcina lutea* and seemed to be carotenoids. These two organisms have since been removed from the genus *Chromobacterium* (Bergey's Manual of Determinative Bacteriology, 7th edition, 1957). Many of the greenish-blue pigmented organisms have also been transferred from the genus, among these being both *Ch. viscosum* and *Ch. iodinum*, according to Bergey's Manual (1957).

Most studies have shown the genus *Chromobacterium* to be motile, and nonmotile strains seem to be rare. In fact, only Eisenberg (1891) and van der Sleen (1894) report nonmotile strains. Flagellation of these bacteria has caused a great deal of confusion. Both single and multiple polar flagella have been reported (Wright,
1895; Waeldele, 1938; Corpe, 1953; Sippel, Medina and Atwood, 1954), as have peritrichous flagella (Mehta, 1925). Other authors have reported that various mixed forms of flagellation seem to be present (Peppler, 1901; Bampton, 1913). Leifson (1956) and Sneath (1956b) have shown the flagellation to be of a heretofore unreported type: the cells possess both polar and peritrichous flagella.

Lehmann and Neumann observed two kinds of flagella in 1899 and noted that since some strains had only one type and others had both, this characteristic would prove of little taxonomic value. It is probable that the characteristic flagellation of both varieties is found on most strains, and thus could be used in defining the genus but not in distinguishing between strains, since the differences in flagellation do not appear to correlate with other features dividing the strains.

Range of temperature for growth has been widely used to define species within the genus. Many of the strains will grow at 2-4°C but show no growth at 37°C; others grow well at 37°C but will not grow at 4°C. Both groups grow well at 20-25°C (Sneath, 1960, 1966) but none will grow at the widest extremes. Insofar as growth temperature is concerned, it is doubtful that there can be a more accurate classification than that of Sneath's which recognizes a mesophilic and a psychrophilic group. However, it also seems doubtful whether this characteristic alone is sufficiently specific
to serve as the basis for an entirely separate species.

Pathogenicity has been one of the chief grounds for separating the genus into more than two species. Several strains of the mesophilic group definitely are pathogenic to man and animals (Ward, 1898; Bertarelli, 1903; Hanssen, 1912; Bampton, 1913; Soule, 1939; Schattenberg, 1940; Christopher and McCleskey, 1941; Dodd, 1941; Schattenberg and Brown, 1941; Schattenberg and Harris, 1941, 1942; Sneath, et al., 1953; Sippel, Medina, and Atwood, 1954; Sneath and Buckland, 1959), causing urinary or bowel infections which often give rise to general septicemias and hence liver infections. All known cases of septicemias have been fatal in humans (Sneath, 1960). The organisms are resistant to penicillin, due to their production of penicillinase (Czekalowski, 1950). The mesophilic group often produce α or β hemolysis (Black and Shahan, 1938; Dodd, 1941; Sneath, et al., 1953; Audebaud, et al., 1954; Sippel, Medina, and Atwood, 1954) but the psychrophiles have never exhibited any hemolysis (Bampton, 1913). Although it would be desirable to have some method of identifying the strains which are pathogenic, no useful criteria have been established to correlate classification with pathogenicity. It seems doubtful that a separate species should be created to contain pathogenic chromobacteria if there are no other identifying or differentiating characteristics. These few strains are best retained within the mesophilic grouping.

Serology in this genus has been studied extensively (Bampton,
1913; Ramchandani, 1930; Soule, 1939; Dodd, 1941; Sneath, et al., 1953; Davies, 1955; Davies, et al., 1958) and when it became apparent that the organisms possess two distinct flagellar antigens (Sneath, 1956a, 1960) corresponding to the polar and lateral flagella, much of the earlier confusion was clarified. Sneath (1960) gave an extensive review of the findings; here it can be noted 1) that infectious strains formed no homologous group, 2) that within the mesophilic and psychrophilic groups there was much cross agglutination while between these two groups there was little, and 3) that there were no agglutinations or reactions between true chromobacteria and other organisms, including Ch. viscosum, Ch. iodinum, Ch. janthinum, six Serratia species, two Pseudomonas species, and others. This supports previous arguments that the above named organisms do not belong in the genus Chromobacterium.

Sneath (1960) found that there are nine carbohydrate fermentation tests which can be used to differentiate between mesophilic (m) and psychrophilic (p) groups. The first appears to give the most consistent results, which involve: trehalose (m+, p-); arabinose, xylose, lactose, m-inositol, mannitol, cellubiose, glycerol, and galactose (m-, p+). Several other differentiating characteristics Sneath (1966) noted are HCN production (m+, p-), aesculin hydrolysis (m-, p+), and casein hydrolysis (m+, p-).

The cell wall of Ch. violaceum has been studied extensively,
and mucoprotein fractions, a new amino sugar, muramic acid, and glucosamine were isolated and characterized (Wheat and Rollins, 1962a, b; Wheat, et al., 1963; Wheat, 1966). Liposaccharide and mucoprotein fractions were extracted with phenol from the cell walls of whole Ch. violaceum cells (Wheat, et al., 1963) and a deoxyaldohexose was characterized (Wheat, Rollins and Leatherwood, 1962). Studies were also made on the nature of bonding of cell wall polysaccharides (Wheat, 1964). Ginsberg, O'Brien and Hall (1962) isolated guanosine-2, 3-cyclic phosphate from this organism, and Corpe (1953, 1960, 1964; Corpe and Salton, 1966) studied the extracellular polysaccharides in gelatinous strains of the organism. Tryptophan and cyanide formation were studied in Ch. violaceum (Michaels and Corpe, 1965; Michaels, Hankes and Corpe, 1965) and Corpe has also studied indole compounds which accumulate in cultures (1961) and the formation of pyrrole (1962) in cultures of Ch. violaceum. It has been found that cyanide forms during log phase growth and accumulates in the media (Michaels, Hankes, and Corpe, 1965). By utilizing $^{14}$C-labeled glycine, it was shown by these authors that the cyanide carbon is derived from the amino carbon of glycine, although not all of the $^{14}$C was recovered in cyanide: some was found in the protein fraction of the cells.

At the present time, Bergey's Manual of Determinative Bacteriology (1957) lists four species of Chromobacterium, giving the
type species as *Ch. violaceum* (Schroeter) Bergonzini, a psychrophile able to grow at 4°C but not at 37°C. Also included within the genus is an obligate halophile, *Ch. maris-mortui*, which produces a blackish pigment upon certain media. Found in the Dead Sea, it grows in media containing from 3% to 30% NaCl, with an optimum concentration of 12% NaCl (*Bergey's Manual of Determinative Bacteriology*, 1957). Sneath (1960) suggested that this organism be excluded from the genus. Although all strains of *Chromobacterium* he tested would grow on 3.5% NaCl, none would grow at 6.5% NaCl, and all grow better without salt. *Bergey's Manual* (1957) also included *Ch. amethystinum* within the genus; after intensive studies with over 40 strains, Sneath (1960) concluded that this is merely one of about 20 strains of the psychrophilic group and does not deserve species status. *Ch. janthinum* is the fourth species listed in *Bergey's Manual* (1957). Sneath (1960) concluded that this is one of about 20 strains within the mesophilic group, and in fact he felt that the original species so named is no longer identifiable.

The Judicial Commission in 1958 (Opinion 16) declared that *Chromobacterium violaceum* Bergonzini (ATCC 12472) is the type species of the mesophilic group in accordance with Sneath's suggestion (1956c) despite the fact that *Bergey's Manual* (1957) listed this organism as a psychrophile. They have not as yet acted upon his accompanying proposal that *Ch. lividum* (ATCC 12473) be the type
species of the psychrophilic group.

In view of the confusion regarding the taxonomy of various species within the genus *Chromobacterium*, it is the objective of this study to examine carbohydrate metabolism in *Chromobacterium* species, a useful biochemical criterion for microbial taxonomy. No work has been done in this genus on carbohydrate metabolism, although Sneath (1960) speculated that the fermentation of *Ch. violaceum* resembles the "mixed acid fermentation" of the coliform bacteria. This tentative suggestion was based on end-product analysis (James and Webb, described in Sneath, 1956a). Acetic, n-butyric, succinic, and lactic acids were found, plus a large amount of an unidentified nonvolatile acid. Some gluconic acid was found, but no significance was attached to this by Sneath (1960). It is hoped that understanding of catabolic mechanisms in various *Chromobacterium* species will provide useful grounds for proper classification of these bacteria.

Several representative species of the genus were included in the present study. The type species of the mesophilic group, *Ch. violaceum* (ATCC 12472), and the suggested type species for the psychrophilic group, *Ch. lividum* (ATCC 12473), were initially chosen for study. The halophile *Ch. maris-mortui* (ATCC 17056) was also selected in view of its unique physiological behavior. As a representative of those organisms Sneath felt were strains rather than separate species, *Ch. amethystinum* (ATCC 6915) was selected.
In addition, to verify the validity for excluding the species from the genus on the grounds of pigmentation, *Ch. viscosum* (ATCC 6918) was included in the present study.
MATERIALS AND METHODS

Culture Conditions

All organisms used in this study were obtained from American Type Culture Collection (ATCC). Except in the case of the halophilic organism, *Ch. maris-mortui*, they were maintained with weekly transfers in a broth medium consisting of glycerol, 5%; peptone, 1%; yeast extract, 0.1%; buffered with 0.01 M KH$_2$PO$_4$ and Na$_2$HPO$_4$ to a pH of 6.8. The halophile was maintained on the experimental media described below with the addition of 3% NaCl. All cultures were grown at 30°C under aerobic conditions in a rotary shaker (New Brunswick Scientific Model G25). After routine transfer, cultures were stored at 4°C. Although cultures of *Chromobacterium* are often difficult to maintain in culture collections (Sneath, 1955; Bergey's Manual of Determinative Bacteriology, 1957), preservation in glycerol-peptone broth with frequent transfer alleviates this problem. However, maintenance is difficult or impossible on solid media without excessive transfer.

Experimental cultures were inoculated aseptically from maintenance cultures into 250 ml Erlenmeyer flasks containing 100 ml of the experimental medium, consisting of glucose, 2%; yeast extract, 0.1%; NH$_4$Cl, 0.1%; and buffered with 0.01 M KH$_2$PO$_4$ and Na$_2$HPO$_4$ to a pH of 6.8.
Growth of each organism was followed turbidometrically in the experimental medium employing a Klett-Summerson colorimeter (filter number 66; 640-700 m\(\mu\)). The relationship between dry cell weight and optical density was determined by periodic sampling of the cell suspension, collection of cells on Millipore filters (HAWP 047 00, 0.45 \(\mu\)), and determination of cell weight (Mettler Analytical Balance Type H5) after drying overnight in a desiccator.

Organic constituents of media were obtained from Difco Laboratories Incorporated. Reagent grade chemicals were utilized for medium preparation, liquid scintillation counting, and other related experiments.

**Radiochemical Substrates**

Specifically labeled substrates used in this study were:

- D-glucose labeled with \(^{14}\text{C}\) at the C-1; C-2; C-3; C-3, 4; or C-6 position;
- D, L-glutamate labeled with \(^{14}\text{C}\) at the C-1; C-2; C-3, 4; or C-5 position;
- D-glutamate and L-glutamate labeled at the C-1 position, all obtained from New England Nuclear Corporation.
- D-gluconate labeled with \(^{14}\text{C}\) at the C-1 or C-6 position was obtained from Nuclear Chicago Corporation, while D-gluconate labeled with \(^{14}\text{C}\) at the C-2, C-3, or C-3, 4 position was prepared from correspondingly labeled glucose in our laboratory by Dr. George Ikeda following the method of Moore and Link (1940).
Radiorespirometric Experiments

Radiorespirometric studies involving single dose substrate administration were carried out according to the methods described by Wang and Krackov (1962) and Wang (1967, 1969). In essence, $^{14}$C substrate was administered to a given microorganism under proliferating conditions, respiratory $^{14}$CO$_2$ was continuously collected and its radioactivity was periodically determined. When evolution of $^{14}$CO$_2$ subsided to an insignificant level, the experiment was terminated. The cell suspension was then processed to separate the medium from the cells, and the radioactivity in the medium and the cells was determined.

In each experiment, 10 ml of a cell suspension in experimental medium without the carbon source under examination were introduced into a radiorespirometric flask, 50 ml in size. A separate portion of the cell suspension was processed to determine the dry cell weight. The flasks were placed in a Warburg water bath maintained at 30°C, aerated at the rate of 40 ml/min, and the shaking mechanism was set in operation. After a brief incubation period lasting 10-20 min, the specifically labeled substrate was tipped into the cell suspension from a sidearm, thus marking the beginning of the experiment.

At the end of the experiment, the cell suspension was quantitatively transferred into a centrifuge tube and centrifuged at 7000 rpm.
for 10 min in a refrigerated Sorvall centrifuge (Model RC2B). The cells were resuspended in experimental medium without the carbon source under examination and recentrifuged, then harvested. Radioactivity of cells and medium was assayed by liquid scintillation counting in a Packard Tricarb Spectrometer (Model 314-EX 2) using the thixotropic gel techniques described by White and Helf (1956) for sample preparation. Radioactivity of the \(^{14}\)CO\(_2\) was assayed by liquid scintillation counting procedures as described by Wang (1967) and Wang and Willis (1965). The efficiency of counting was determined by use of internal standards (Wang and Willis, 1965). All countings were carried out over a sufficient period of time so that the relative standard deviation of the counting data was less than one percent except in the case of glucose-3-\(^{14}\)C, gluconate-3-\(^{14}\)C, and gluconate-3,4-\(^{14}\)C. Because of limited supply, these substrates were used with a lower specific activity; hence countings were carried out over a sufficient period of time so that the relative standard deviation of the counting data was less than two percent.

At least two replicate experiments were performed with each organism and each substrate. Deviations of experimental results were found not to exceed five percent among replicate experiments.
RESULTS AND DISCUSSION

Radiorespirometric data obtained with microorganisms can be used to identify the nature of glucose catabolism or allied substrates in a given microorganism. Conclusions on catabolic behavior can be drawn from the relative rates and extents for the conversion of individual carbon atoms of a given substrate to respiratory CO₂. Moreover, since the amounts of cells and substrate level are constant in a given set of experiments with a given substrate, the ¹⁴CO₂ yields observed at the end of an experiment, when the administered substrate has been completely utilized, can be readily cross compared to provide information on the relative participation of concurrent pathways. This is true since any complications derived from substrate permeability and differences in metabolic rate of individual pathways are self-compensating and hence can be ignored.

It may be desirable to review briefly the major catabolic mechanisms for assimilation and utilization of carbon sources known to function in microorganisms. In essence, these catabolic pathways can be classified in two categories. The primary pathways are responsible for the degradation of glucose, the most common carbon source for biological systems. The key product of primary pathways is generally recognized as pyruvate. Secondary pathways (indicating order of events, not reflecting importance) are responsible for the
catabolism of pyruvate and other intermediates, giving rise to various carbon skeletons and eventually to respiratory $\text{CO}_2$.

Primary glucose pathways known to function in microorganisms include the Embden-Meyerhof-Parnas (EMP) pathway or glycolysis, the Entner-Doudoroff (ED) pathway, the pentose phosphate (PP) pathway, and the closely related pentose cycle (PC) pathway. It is generally recognized that either the EMP or the ED pathway operates concurrently with the PP pathway in microorganisms. Evidence for concurrent function of the EMP and ED pathways has not been reported. This is understandable since both pathways assume essentially the same function: cleavage of glucose into two $\text{C}_3$ units.

Figure 1 illustrates schematically three primary pathways for glucose utilization, showing sequences for the conversion of individual carbon atoms of glucose to respiratory $\text{CO}_2$. The major steps involved are presented from left to right (without indicating enzymes, coenzymes, cofactors, etc.); the time sequence of $\text{CO}_2$ formation is implied as one traces the substrates from left to right. The numerals designate the original numbering of the carbon atoms of the glucose administered.

A variation of the typical ED pathway was first recognized by Hochster and Katznelson (1958) and termed the hexose cycle.
Figure 1. Relative rates of carbon atoms of glucose appearing in respiratory CO$_2$ via known catabolic pathways in microorganisms. EMP = Embden-Meyerhof-Parnas pathway; ED = Entner-Doudoroff pathway; PP = pentose phosphate pathway; PC = pentose cycle; TCA = tricarboxylic acid cycle; F-6-P = fructose-6-phosphate; G-6-P = glucose-6-phosphate.
Conversion of glucose to pyruvate proceeds as in the ED pathway. However, if the organism is genetically deprived of the ability, partially or completely, to transform glyceraldehyde-3-phosphate into pyruvate, two trioses can be condensed to yield fructose-1,6-diphosphate. This in turn is converted to glucose-6-phosphate and follows the fate of substrate glucose.

It should be noted that, as indicated in Figure 1, although the PP and PC pathways share the initial reactions, there are important differences. Pentose phosphate formed via the PP pathway can give rise to fructose-6-phosphate which in turn can be metabolized via the EMP pathway (see Figure 1). Alternatively, pentose phosphate formed via the PP pathway can be converted to fructose-6-phosphate which in turn can be isomerized to glucose-6-phosphate, thereby completing the pentose cycle process.

In the present work, the pathways functioning in microorganisms are defined as follows:

1) The EMP pathway: 1 glucose-6-P \rightarrow fructose-1,6-diP \rightarrow x \text{ triose-3-P} \rightarrow y \text{ pyruvate}

in which $x$ and $y$ are actual yields of the respective compounds, having a limiting magnitude of 2.

2) The PP pathway:

$$3 \text{-glucose-6-P} \rightarrow 3 \text{ CO}_2$$

$$\rightarrow 3 \text{ pentose-P} \rightarrow z \text{ fructose-6-P} + q \text{ glyceraldehyde-3-P}$$
in which \( z \) and \( q \) represent the actual yields of fructose-6-phosphate and glyceraldehyde-3-phosphate, respectively. In the uppermost limiting case, \( z = 2 \) and \( q = 1 \). On the other hand, if pentose phosphate is used completely for biosynthetic purposes (an unlikely case), \( z = 0 \) and \( q = 0 \).

3) The PC pathway (Wood and Katz, 1958):

\[
\begin{align*}
3 \text{ glucose-6-P} & \rightarrow 3 \text{ CO}_2 \\
-3 \text{ pentose-P} & \rightarrow 2 \text{ fructose-6-P} + 1 \text{ glyc-3-P} \\
 & \rightarrow 2 \text{ glucose-6-P} \rightarrow \text{etc.}
\end{align*}
\]

Net reaction: 1 glucose-6-P \( \rightarrow 3 \text{ CO}_2 + 1 \) glyceraldehyde-3-P

4) The ED pathway: 1 glucose-6-P \( \rightarrow \) 6-phosphogluconate \( \rightarrow \)

\[
v \text{ pyruvate} + w \text{ glyceraldehyde-3-P} \\
u \text{ pyruvate}
\]

in which \( u \), \( v \), and \( w \) represent actual yields of the respective compounds, each having a limiting magnitude of 2.

In order to obtain information on glucose catabolism, several methods can be used. Among the most important ones have been the identification of specific enzymes and the identification of product accumulation resulting from inhibition of certain steps within the system. With the advent of radiotracer methods, isotope distribution patterns of key intermediates derived from glucose or information on \( ^{14}\text{CO}_2 \) production can be correlated with the original \( ^{14}\text{C} \) labeling of glucose to assess the roles played by individual pathways.
In studies with microorganisms, the $^{14}\text{CO}_2$ yield method (Stern, Wang, and Gilmour, 1960; Wang and Krackov, 1962) has been extensively used for estimation of concurrent glucose pathways. Equations have been derived by these authors for pathway estimation under the following assumptions:

a--Glucose is catabolized primarily via two concurrent catabolic sequences, namely, either the EMP and PP pathways or the ED and PP pathways. Concurrent operation of EMP and ED pathways have not been observed.

b--The conversion of the C-1 of glucose to $\text{CO}_2$ by phosphogluconate decarboxylation is virtually irreversible.

c--The trioses formed in the EMP pathway are equivalent to each other with respect to further metabolic reactions.

d--$\text{CO}_2$ fixation of the type $\text{C}_3 + \text{CO}_2$ does not operate to any significant extent.

e--Recombination of trioses to form fructose-1,6-diphosphate is considered to be insignificant. Consequently, whenever the operation of the hexose cycle is demonstrated by experimental evidence, there will be some inherent error associated with the resulting pathway estimation.

f--In the ED pathway, the two $\text{C}_3$ products are equivalent at the pyruvate level.

g--Pyruvate derived from glucose is decarboxylated promptly
and extensively.

h--Exogenous gluconate is utilized in a manner identical with the phosphogluconate formed \textit{in vivo}.

i--Decarboxylation of gluconate, as derived from glucose, is extensive and rapid.

Justification for these assumptions is based on current understanding of glucose catabolism. When the microorganism relies on the concurrent operation of the EMP and PP pathways, the following equation, derived by Wang and Krackov (1962) applies:

\begin{equation}
G_p = \frac{G_1 - (G_6 - A_6 \cdot G_p)}{G_T - G_{T'}}
\end{equation}

where:

\begin{align*}
G_p &= \text{fraction of glucose catabolized via the PP pathway} \\
G_e &= \text{fraction of glucose catabolized via the EMP pathway} \\
G_1 &= \text{cumulative yield of respiratory }^{14}\text{CO}_2 \text{ at end of time course for complete substrate utilization, as fraction of administered glucose-1-}^{14}\text{C} \\
G_6 &= \text{cumulative yield of respiratory }^{14}\text{CO}_2 \text{ at end of time course for complete substrate utilization, as fraction of administered glucose-6-}^{14}\text{C} \\
A_6 &= \text{cumulative yield of respiratory }^{14}\text{CO}_2 \text{ at end of time course for complete substrate utilization, as fraction}
\end{align*}
of administered gluconate-6-\(^{14}\)C

\[ G_T = \text{total activity of labeled substrate, taken as unity} \]

\[ G_{T'} = \text{fraction of administered substrate which undergoes anabolism} \]

It is generally recognized that with most microorganisms little substrate glucose is routed into anabolic functions. Hence when the magnitude of \( G_{T'} \) is small, the term \( G_T - G_{T'} \) approaches unity. Equation 1 can then be expressed as:

\[ G_p = G_1 - G_6 + A_6 \cdot G_p \]  \hspace{1cm} (2)

and with simplification,

\[ G_p = \frac{G_1 - G_6}{1 - A_6} \]  \hspace{1cm} (3)

If one assumes that the triose phosphate derived from glucose via the PP pathway behaves catabolically identical to that derived from administered gluconate via the PP pathway, Equation 3 can be expressed as:

\[ G_p = \frac{G_1 - G_6}{1 - G_6} \]  \hspace{1cm} (4)

Since it is assumed that the EMP and PP pathways constitute the major routes for glucose catabolism in this case, the participation of the EMP pathway can be estimated by difference:

\[ Ge = 1 - G_p \]  \hspace{1cm} (5)
When the microorganism relies on the concurrent operation
of the ED and PP pathways, the following equation applies:

\[
G_p = \frac{G_1 - G_4}{G_T - G_T'}
\]  

(6)

where:

\( G_p \) = fraction of glucose catabolized via the PP pathway

\( G_{ed} \) = fraction of glucose catabolized via the ED pathway

\( G_1, G_4 \) = cumulative yield of respiratory \(^{14}\)CO\(_2\) at end of time
course for complete substrate utilization, as fraction
of administered glucose-1-\(^{14}\)C or glucose-4-\(^{14}\)C.

As in Equation 1, the term \( G_T - G_T' \) in Equation 6 approaches unity
when the magnitude of \( G_T' \) is small; Equation 6 can then be simplified
to:

\[
G_p = G_1 - G_4
\]  

(7)

Since it has been assumed that the ED and PP pathways constitute
the major routes for catabolism in this case, the participation of
the ED pathway can be estimated (Stern, Wang, and Gilmour, 1960)
by difference:

\[
G_{ed} = 1 - G_p
\]  

(8)

As stated previously, pyruvate is generally recognized as the
key intermediate of all known primary pathways for glucose catab-
olism. Further catabolism of pyruvate relies on the operation of
secondary pathways. Major secondary pathways known to function
in microorganisms include the tricarboxylic acid (TCA) cycle in particular, and the glyoxylic acid cycle. The operation of the TCA cycle in biological systems can be demonstrated by enzyme studies or more conveniently by examination of the catabolism of $^{14}\text{C}$ specifically labeled D, L-glutamic acid. This is true since glutamate can be readily converted biologically to $\alpha$-ketoglutarate which in turn can be catabolized to $\text{CO}_2$ following the sequence depicted in Figure 2. The numerals designate the position of carbon atoms in glutamate, and the time sequence of $\text{CO}_2$ production is indicated as one traces from left to right.

It is generally recognized that biochemical information is of great importance to microbial taxonomy. This is particularly true in the case of catabolic behavior of microorganisms in utilizing glucose or allied substrates. Once the relative participation of the pathways in a species of microorganism is determined, differences in catabolic patterns among organisms or groups of organisms become taxonomically significant. There is evidence that microorganisms in a given genus may rely on a given set of primary pathways for glucose utilization, although they may differ with respect to the relative participation of these pathways.

In the present work, the following approaches have been used in classifying the selected species of *Chromobacterium*:

1) Identification of the nature of catabolic pathways functioning
Figure 2. Relative rates of carbon atoms of glutamic acid appearing in respiratory CO₂ via the intermediary formation of α-ketoglutarate and the TCA cycle.
in glucose utilization by radiorespirometric methodology;

2) Determination of the relative participation of the identified pathways;

3) Identification of the nature of the secondary pathways, including capability for utilization of the optical isomers of glutamic acid.

The usefulness of these approaches has been demonstrated with several genera of microorganisms. With *Xanthomonas*, there exists a close resemblance among all species examined with regard to the basic mechanisms for glucose utilization (Zagallo and Wang, 1967). It is practically impossible to distinguish one species from another on this basis. However, in the genus *Arthrobacter*, one finds two distinctly different patterns of glucose utilization: two species studied catabolize glucose via the EMP-TCA pathways, while three species studied rely on the ED-PP pathways (Zagallo and Wang, 1962a). Similar findings on glucose catabolism in *Corynebacterium* were reported by Zagallo and Wang (1962b). The five species studied were grouped into three categories: two species predominately utilize the EMP pathway, two species utilize both the EMP and PC pathways to an equal extent, and one relies solely on the PC pathway.

In the present work, three substrates were employed, each for a different purpose. Glucose was chosen since it is the most common carbon source. Gluconate is not a natural substrate; however,
understanding of gluconate catabolism often provides important supplementary information (if it can be utilized) to facilitate the analysis of the data obtained in the glucose experiments. Glutamate is probably the most useful substrate in detecting operation of the TCA cycle. In addition, it is also of interest to examine the capability of a microorganism relative to the utilization of the D and L isomers of glutamic acid. Understanding in this regard also facilitates taxonomic classification of microorganisms.

The radiorespirometric data for utilization of $^{14}$C specifically labeled D, L-glutamate by five species of Chromobacterium are given in Table 1. Radiorespirometric patterns for utilization of D, L-glutamate by Ch. violaceum are given in Figure 3, which constitutes a representative example for findings with Ch. lividum and Ch. amethystinum, as well.

With all five species of Chromobacterium studied, the rate and extent for the conversion of carbon atoms of glutamate to respiratory CO$_2$ follows the order C-1 > C-2 = C-5 > C-3, 4. Presumably, glutamate is utilized via the intermediary formation of α-ketoglutarate which is then catabolized via the TCA cycle as shown in Figure 2. These findings therefore can be interpreted to indicate that the TCA cycle is operative in all these organisms.

It should be noted that with three species, approximately 50% of the total administered radioactivity in D, L-glutamate is found in
Table 1. Utilization of $^{14}$CO$_2$ specifically labeled D, L-glutamate by Chromobacterium species.

<table>
<thead>
<tr>
<th>Organism</th>
<th>$^{14}$CO$_2$ position</th>
<th>$^{14}$CO$_2$ production</th>
<th>Substrate inventory: % of total substrate radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>label</td>
<td>Time, hours</td>
<td>Yield/1/2 hr, %</td>
</tr>
<tr>
<td>Ch. lividum</td>
<td>C-1</td>
<td>1.5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>1.5</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td>1.5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>C-5</td>
<td>1.5</td>
<td>14</td>
</tr>
<tr>
<td>Ch. amethystinum</td>
<td>C-1</td>
<td>1.5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>1.5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td>2.0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>C-5</td>
<td>1.5</td>
<td>6</td>
</tr>
<tr>
<td>Ch. violaceum</td>
<td>C-1</td>
<td>1.0</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>1.2</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td>1.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C-5</td>
<td>1.2</td>
<td>14</td>
</tr>
<tr>
<td>Ch. viscosum</td>
<td>C-1</td>
<td>2.3</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>2.3</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td>2.0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>C-5</td>
<td>2.3</td>
<td>8</td>
</tr>
<tr>
<td>Ch. maris-mortui</td>
<td>C-1</td>
<td>1.0</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>1.0</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td>1.0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>C-5</td>
<td>1.0</td>
<td>13</td>
</tr>
</tbody>
</table>
Figure 3. Radiorespirometric patterns for utilization of D, L-glutamate, administered as a single dose, by Ch. violaceum. Numerals refer to position of carbon atoms of D, L-glutamate labeled with $^{14}C$. 
the medium at the end of the radiorespirometric experiment. These findings suggest that, while L-glutamate can be promptly utilized, the D isomer of glutamate cannot be utilized by these three species. With Ch. viscosum, there are indications that not only the L-glutamate is readily utilized but that the D isomer can also be utilized although at a much slower rate.

In the case of Ch. maris-mortui, only a small fraction of the radioactivity of the administered D, L-glutamate is recovered in the medium, indicating that both the D and L isomers of glutamate can be utilized by this organism. It is noted that the radiorespirometric patterns for glutamate utilization, shown in Figure 4, display a maximum in the early phase of the experiment, followed by a slow leveling phase, indicating that a component of the substrate is being utilized at a significantly slower rate. These facts suggest again that D-glutamate and L-glutamate are not utilized at the same rate by this organism. To verify this, separate experiments employing D-glutamate-1-14C and L-glutamate-1-14C were performed; the results are shown in Figure 5 and Table 2. For ready comparison, the results of the experiment with 14C specifically labeled D, L-glutamate are also included in Table 2. The findings of these experiments provide direct evidence that the utilization rate for D-glutamate by this organism is indeed much slower than that of the L isomer.

Capability for a microorganism to utilize D or L isomers of
Figure 4. Radiorespirometric patterns for utilization of D, L-glutamate, administered as a single dose, by Ch. marismortui in culture medium containing 3% NaCl. Numerals refer to position of carbon atoms of D, L-glutamate labeled with $^{14}$C.
Figure 5. Radiorespirometric patterns for utilization of D-glutamate-1-14C or L-glutamate-1-14C, administered as a single dose, by *Ch. maris-mortui* in culture medium containing 3% NaCl.
Table 2. Utilization of $^{14}$C specifically labeled D-glutamate or L-glutamate by Chromobacterium maris-mortui.

<table>
<thead>
<tr>
<th>Organism</th>
<th>$^{14}$C-glutamate</th>
<th>$^{14}$CO$_2$ production</th>
<th>Substrate inventory: % of total substrate radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isomer</td>
<td>Label position</td>
<td>Time, hours</td>
</tr>
<tr>
<td>Ch. maris-mortui</td>
<td>D-</td>
<td>C-1</td>
<td>3.0</td>
</tr>
<tr>
<td>(cells: 10 mg)</td>
<td>substrate: 0.5 mg (NaCl: 3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ch. maris-mortui</td>
<td>L-</td>
<td>C-1</td>
<td>1.5</td>
</tr>
<tr>
<td>(cells: 10 mg)</td>
<td>substrate: 0.5 mg (NaCl: 3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ch. maris-mortui</td>
<td>D, L-</td>
<td>C-1</td>
<td>1.0</td>
</tr>
<tr>
<td>(cells: 10 mg)</td>
<td>substrate: 1.0 mg (NaCl: 3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td></td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>C-5</td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>
glutamic acid can be used as a basis for taxonomy. These capabilities are derived from the presence of enzymes in substrate transport and substrate metabolism. It is known that most transaminases are specific for either D or L amino acids but not both. It is interesting to note that transaminases often can catalyze reversible reactions between any number of α-keto acids and their α-amino acid analogs, yet still retain strict specificity with regard to the L or D isomers of amino acids. In higher animals, there is evidence (Meister, 1962) that no transaminases exist which are non-stereospecific or which are specific for only the D isomer of amino acids. However, in microorganisms, the presence of D-transaminase activity is consistent with the ability of certain microorganisms to utilize D amino acids (Meister, 1962). It is generally recognized that most microorganisms have the capacity to utilize the L forms of amino acids, but that relatively few will utilize the D isomers. This fact can be accounted for on the basis of enzyme specificity. Substrate utilization usually involves a permeation step which may also be stereospecific. Organisms which will not utilize the D isomer of, for example, glutamic acid, could either lack a permease, a transaminase, or a deaminase, any of which could be specific for L- but not D-glutamic acid.

It should be noted that taxonomic classification on the basis of capability in utilizing D or L amino acids provides one with only a
binary system. Such information is therefore at best an agent to other more reliable approaches. Thus, in the present study, five microorganisms are all similar in morphology, yet they display differing behavior with regard to utilization of D and L isomers of glutamate. As shown in Table 3, all of them can utilize L-glutamate but only two species can utilize the D isomer. Consequently more defined classification can be realized only with detailed studies on the catabolic behavior for carbohydrate utilization by these microorganisms.

It is generally recognized that glucose is the most common carbon source for microorganisms. Thus it is of great importance to examine the mechanisms for glucose utilization in these five species of Chromobacterium. Radiorespirometric data for utilization of $^{14}$C specifically labeled glucose substrates by five species of Chromobacterium are summarized in Table 4, and for gluconate substrates by four species of Chromobacterium in Table 5. Metabolic rates for utilization of substrates by five species of Chromobacterium are shown in Table 6. To facilitate the discussion of carbohydrate catabolism, data analysis will be presented individually for each organism studied.

In the case of Ch. lividum, the time course for glucose utilization observed in a set of radiorespirometric experiments are given in Figure 6. Prompt and extensive conversion of both C-1 and C-4 of
Table 3. Relative rate of D- or L-glutamate utilization by *Chromobacterium* species.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Relative rate of glutamate utilization</th>
<th>L isomer</th>
<th>D isomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch. lividum</td>
<td>+++++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ch. amethystinum</td>
<td>+++++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ch. violaceum</td>
<td>+++++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ch. viscosum</td>
<td>+++++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Ch. maris-mortui</td>
<td>+++++</td>
<td>+++++</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Utilization of $^{14}$C specifically labeled glucose by *Chromobacterium* species.

<table>
<thead>
<tr>
<th>Organism</th>
<th>14 CO₂ position label</th>
<th>14 Peak rate of CO₂ production</th>
<th>Substrate inventory: % of total substrate radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time, hours</td>
<td>Yield/1/2 hr, %</td>
</tr>
<tr>
<td>Ch. lividum</td>
<td>C-1</td>
<td>1.0</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>1.0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>C-3</td>
<td>1.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C-4*</td>
<td>1.0</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>C-6</td>
<td>1.0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Ch. amethystinum</td>
<td>C-1</td>
<td>1.5</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>1.5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>C-3</td>
<td>1.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C-4*</td>
<td>1.5</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>C-6</td>
<td>1.5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Ch. violaceum</td>
<td>C-1</td>
<td>1.0</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>2.0</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>C-3</td>
<td>2.0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>C-4*</td>
<td>1.0</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>C-6</td>
<td>1.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Ch. viscosum</td>
<td>C-1</td>
<td>1.5</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>1.5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>C-3</td>
<td>1.5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>C-4*</td>
<td>1.5</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>C-6</td>
<td>1.5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Ch. maris-mortui</td>
<td>C-1</td>
<td>1.0</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>1.0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>C-3</td>
<td>1.0</td>
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<tr>
<td></td>
<td>C-6</td>
<td>1.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C-3, 4</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

*Data presented for substrate glucose-$^{14}$C are obtained by calculation from the observed data in glucose-3-$^{14}$C and glucose-3, 4-$^{14}$C experiments.
Table 5. Utilization of $^{14}$C specifically labeled gluconate by Chromobacterium species.

<table>
<thead>
<tr>
<th>Organism</th>
<th>$^{14}$CO$_2$ position label</th>
<th>14CO$_2$ position label</th>
<th>Peak rate of CO$_2$ production</th>
<th>Substrate inventory: % of total substrate radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time, hours</td>
<td>Yield/ 1/2 hr, %</td>
<td>Respiratory CO$_2$</td>
<td>Medium</td>
</tr>
<tr>
<td>Ch. lividum</td>
<td>C-1 3.0</td>
<td>39</td>
<td>82 5 12</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>C-2 3.0</td>
<td>30</td>
<td>59 5 32</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>C-3 3.0</td>
<td>23</td>
<td>49 7 41</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>C-4* 3.0</td>
<td>30</td>
<td>60 5 34</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>C-6 3.0</td>
<td>17</td>
<td>33 12 32</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>C-3,4 3.0</td>
<td>53</td>
<td>53 6 38</td>
<td>97</td>
</tr>
<tr>
<td>Ch. violaceum</td>
<td>C-1 1.5</td>
<td>36</td>
<td>82 6 15</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>C-2 1.5</td>
<td>24</td>
<td>56 7 38</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>C-3 1.5</td>
<td>17</td>
<td>40 8 49</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>C-4* 1.5</td>
<td>18</td>
<td>29 13 56</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>C-6 1.5</td>
<td>5</td>
<td>10 17 58</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>C-3,4 1.5</td>
<td>34</td>
<td>34 11 53</td>
<td>98</td>
</tr>
<tr>
<td>Ch. viscosum</td>
<td>C-1 1.0</td>
<td>55</td>
<td>90 4 2</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>C-2 1.0</td>
<td>10</td>
<td>16 6 61</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>C-3 1.0</td>
<td>12</td>
<td>18 6 72</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>C-4* 1.0</td>
<td>30</td>
<td>51 6 34</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>C-6 1.0</td>
<td>13</td>
<td>21 12 54</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>C-3,4 1.0</td>
<td>35</td>
<td>35 6 53</td>
<td>94</td>
</tr>
<tr>
<td>Ch. maris-mortui</td>
<td>C-1 1.0</td>
<td>15</td>
<td>77 11 9</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>C-2 1.0</td>
<td>7</td>
<td>40 20 34</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>C-3 1.5</td>
<td>5</td>
<td>33 27 40</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>C-4* 1.0</td>
<td>8</td>
<td>35 26 31</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>C-6 1.0</td>
<td>3</td>
<td>18 32 37</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>C-3,4 1.0</td>
<td>34</td>
<td>34 26 36</td>
<td>96</td>
</tr>
</tbody>
</table>

*Data presented for substrate gluconate-4-$^{14}$C are obtained by calculation from the observed data in gluconate-3-$^{14}$C and gluconate-3,4-$^{14}$C experiments.
Table 6. Metabolic rates of carbonaceous substrates in *Chromobacterium* species.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate and level</th>
<th>Cell weight (dry) in mg</th>
<th>Time required for complete utilization in hours</th>
<th>Metabolic rate: mg substrate/mg cell/hour</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ch. lividum</em></td>
<td>D-gluconate: 0.5 mg</td>
<td>12.0</td>
<td>5.0</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>D-glucose: 5 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D, L-glutamate: 1 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ch. amethystinum</em></td>
<td>D-glucose: 2.5 mg</td>
<td>13.5</td>
<td>3.5</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>D, L-glutamate: 1 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ch. violaceum</em></td>
<td>D-gluconate 1.5 mg</td>
<td>8.0</td>
<td>3.5</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>D-glucose: 10 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D, L-glutamate: 2 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ch. viscosum</em></td>
<td>D-gluconate 2.5 mg</td>
<td>8.0</td>
<td>3.0</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>D-glucose: 10 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D, L-glutamate: 1 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ch. maris-mortui</em></td>
<td>D-gluconate: 1 mg</td>
<td>8.0</td>
<td>5.0</td>
<td>0.03</td>
</tr>
<tr>
<td>(NaCl: 3%)</td>
<td>D-glucose: 0.71 mg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>D, L-glutamate: 1 mg</td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
</tbody>
</table>
Figure 6. Radiorespirometric patterns for utilization of D-glucose, administered as a single dose, by Ch. lividum. Numerals refer to position of carbon atoms of D-glucose labeled with $^{14}$C.
glucose to CO$_2$ provides evidence for the extensive operation of the ED pathway. However since the C-1 of glucose is converted to CO$_2$ at a significantly higher rate and extent in comparison to that of C-4, it is reasonable to believe that the PP pathway is also playing a role in the utilization of glucose by this organism. Inasmuch as the TCA cycle is known to be operative in this organism, presumably the pyruvate derived from substrate glucose via either the ED or PP pathways is further catabolized via the TCA cycle. According to such a scheme, one would expect that the extent and rate of CO$_2$ from various carbon atoms of glucose should follow the order of C-2 = C-5 > C-3 = C-6. The observation that the rate of conversion of C-6 to CO$_2$ is as extensive as that of C-2 and considerably greater than that of C-3 points to the involvement of other pathways. It is possible that glyceraldehyde-3-phosphate, equivalent to C-4, C-5, and C-6 of glucose, derived from substrate glucose via the ED pathway may have been converted to fructose-1, 6-diphosphate which is in turn transformed to glucose-6-phosphate via the intermediary formation of fructose-6-phosphate. The glucose-6-phosphate so formed, with C-6 of substrate glucose now in the C-1 position, can be readily catabolized via the ED pathway. Such a scheme, designated by Hochster and Katznelson (1958) as the hexose cycle, provides a mechanism in microorganisms equipped with the ED pathway for the preferential conversion of C-6 to CO$_2$ in comparison to C-2,
C-3, or C-5 of glucose. The operation of the hexose cycle has been demonstrated in microorganisms equipped with the ED pathway, such as *Xanthomonas phaseoli* (Hochster and Katzenelson, 1958), *Azotobacter vinelandii* (Still and Wang, 1964), *Acetobacter xylinum* (White and Wang, 1964a, b), etc.

The conclusions derived from the $^{14}\text{CO}_2$ data are in accordance with the findings on the incorporation of substrate radioactivity in cells and medium.

Gluconate is the key intermediate in either the ED or PP pathway. It is therefore of interest to examine the gluconate utilization by *Ch. lividum*. The radiorespirometric patterns for utilization of $^{14}\text{C}$ specifically labeled D-gluconate is shown in Figure 7. It is noted there exists a significant time lag for the organism to utilize this substrate. Conversion of carbon atoms of gluconate to CO$_2$ follows the order: C-1 > C-4 = C-2 > C-3 > C-6. This observation in general supports the conclusion drawn from the findings of the glucose experiments. However, the low yield of CO$_2$ from C-6 of gluconate suggests that the hexose cycle is not playing any significant role in gluconate utilization. This is understandable in view of the much lower rate of substrate utilization (Table 6) observed in the gluconate experiments in comparison to the glucose experiments. Evidently the hexose cycle can play a role in this organism only when glyceraldehyde-3-phosphate is produced at a high rate when
Figure 7. Radiorespirometric patterns for utilization of D-gluconate, administered as a single dose, by Ch. lividum. Numerals refer to position of carbon atoms of D-gluconate labeled with $^{14}$C.
substrate glucose is extensively utilized. In other words, the hexose cycle appears to be the mechanism for catabolizing excess amounts of glyceraldehyde-3-phosphate produced in the cells that cannot be readily converted to pyruvate due to insufficient capability of glyceraldehyde-3-phosphate dehydrogenase.

Once the nature of the catabolic pathways operative in Ch. lividum are identified, use can be made of Equations 7 and 8 to estimate the relative participation of concurrent glucose pathways. Calculations so performed reveal that glucose is catabolized 86% via the ED pathway and 14% via the PP pathway.

In similar fashion, the radiorespirometric patterns (Figure 8) obtained from $^{14}$C specifically labeled glucose utilized by Ch. amethystinum can be analyzed. The pattern is very similar to that of Ch. lividum: the rate and extent of CO$_2$ production is C-1 > C-4 > C-2 > C-6 > C-3. This indicates that the ED pathway, and to some extent, the PP pathway, are operative. Since the C-6 of glucose is converted to CO$_2$ more extensively than C-3, the hexose cycle appears to be functioning to a limited extent.

Pathway estimations made using Equations 7 and 8 reveal that 87% of administered glucose is catabolized via the ED pathway, while 13% is routed through the PP pathway. This is also confirmed by the observed findings on incorporation of glucose activity into the cells and in the fermentation media (Table 4). It should be noted that
Figure 8. Radiorespirometric patterns for utilization of D-glucose, administered as a single dose, by *Ch. amethystinum*. Numerals refer to position of carbon atoms of D-glucose labeled with $^{14}$C.
gluconate cannot be utilized by this organism as a substrate, even after a prolonged period.

In the case of Ch. violaceum, the radiorespirometric patterns (Figure 9) consist of two distinct phases. Evidently substrate glucose can be promptly utilized by the organism as indicated by the observed extensive conversion of C-1 and C-4 of glucose to respiratory CO₂. Meanwhile there appears to be a lag for the organism to catabolize pyruvate derived from glucose to CO₂ via the secondary pathway, presumably the TCA cycle. The accumulative yield for the conversion of individual carbon atoms of glucose to CO₂ follows the order of C-1 > C-4 > C-2 > C-3 > C-6, greatly resembling that observed with Ch. lividum and Ch. amethystinum. It can therefore be concluded that the ED pathway is playing a predominant role for glucose catabolism in this organism, with the PP pathway playing a minor role. The fact that the C-6 of glucose was promptly, although limited in extent, converted to respiratory CO₂ in the early phase of the radiorespirometric pattern (Figure 9) indicates that the hexose cycle is also operative in this organism to a limited extent. These findings are also confirmed by the observed data on incorporation of glucose activity into the cells and in the fermentation media (Table 4).

By use of Equations 7 and 8, it can be calculated that glucose is catabolized in this organism 80% by way of the ED pathway and
Figure 9. Radiorespirometric patterns for utilization of D-glucose, administered as a single dose, by Ch. violaceum. Numerals refer to position of carbon atoms of D-glucose labeled with $^{14}$C.
20% by way of the PP pathway.

The observed radiorespirometric patterns for utilization of gluconate by \textit{Ch. violaceum} (Figure 10) support the conclusions reached on catabolic mechanisms of glucose from the data in glucose experiments. Here again the hexose cycle does not appear to be operative to any great extent when gluconate is used as a substrate for \textit{Ch. violaceum}. The observed results on incorporation of gluconate radioactivity into cells and media support these conclusions.

In the case of the halophile \textit{Ch. maris-mortui}, the radiorespirometric patterns (Figures 11 and 12) observed with either glucose or gluconate as a substrate suggest that this organism relies heavily on the PP and PC pathways for glucose and gluconate utilization. This conclusion is drawn from the following considerations:

1) In contrast to the three \textit{Chromobacterium} species previously described, in \textit{Ch. maris-mortui} the extent of conversion of the C-4 to respiratory CO$_2$ is of the same magnitude as that of C-2 and C-3 (Tables 3, 4). It is therefore reasonable to assume that in this organism the major mechanism of conversion of C-4 to CO$_2$ involves the catabolism of glyceraldehyde-3-phosphate derived from the PP and PC pathways (Figure 1).

2) Other than the C-1, the remaining carbon atoms of substrate glucose and gluconate were converted to cellular constituents to approximately the same extent. Operation of the ED pathway will
Figure 10. Radiorespirometric patterns for utilization of D-gluconate, administered as a single dose, by Ch. violaceum. Numerals refer to position of carbon atoms of D-gluconate labeled with $^{14}$C.
Figure 11. Radiorespirometric patterns for utilization of D-glucose, administered as a single dose, by Ch. maris-mortui in culture medium containing 3% NaCl. Numerals refer to position of carbon atoms of D-glucose labeled with $^{14}\text{C}$. 
Figure 12. Radiorespirometric patterns for utilization of D-gluconate, administered in a single dose, by Ch. maris-mortui in culture medium containing 3% NaCl. Numerals refer to position of carbon atoms of D-gluconate labeled with $^{14}$C.
significantly reduce the incorporation of C-4 to cellular compounds.

3) The radiorespirometric patterns observed for glucose and gluconate utilization by Ch. maris-mortui resemble those observed in Acetobacter suboxydans. The predominate role played by the PC pathway in A. suboxydans has been well demonstrated by Kitos, et al. (1958).

In Bergey's Manual (1957) it is stated that the optimum salt concentration for growth of the halophile Ch. maris-mortui is 12%. However, repeated efforts in this laboratory lead one to conclude that this organism grows best in culture medium containing 3% NaCl. In order to detect any difference in glucose catabolism when the organism was grown in differing salt concentrations, radiorespirometric experiments were carried out with Ch. maris-mortui grown in medium containing 15% NaCl (Figure 13). Findings are given in Table 7 along with those obtained with the organism grown in 3% NaCl. It can readily be seen that there exists no noticeable difference insofar as the basic catabolic behavior is concerned. However, the rate for metabolism of glucose was found to be much slower at higher salt concentrations. These facts lead one to believe that the optimum salt concentration for growth of this organism is 3% rather than 12% NaCl.

The organism Ch. viscosum has been recently ruled out as one of the Chromobacterium species (Bergey's Manual, 1957).
Figure 13. Radiorespirometric patterns for utilization of D-glucose, administered as a single dose, by *Ch. maris-mortui* in culture medium containing 15% NaCl. Numerals refer to position of carbon atoms of D-glucose labeled with $^{14}$C.
Table 7. Utilization of $^{14}$C specifically labeled glucose by *Ch. maris-mortui* in proliferating medium containing 3% or 15% sodium chloride.

<table>
<thead>
<tr>
<th>Organism</th>
<th>$^{14}$CO$_2$ position label</th>
<th>14 Peak rate of $^{14}$CO$_2$ production</th>
<th>Substrate inventory: % of total substrate radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time, hours</td>
<td>Yield/1/2 hr, %</td>
</tr>
<tr>
<td><em>Ch. maris-mortui</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3% NaCl</td>
<td>C-1</td>
<td>1.0</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>1.0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>C-3</td>
<td>1.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>C-4*</td>
<td>1.0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>C-6</td>
<td>1.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C-3,4</td>
<td>1.0</td>
<td>34</td>
</tr>
<tr>
<td>15% NaCl</td>
<td>C-1</td>
<td>1.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>C-2</td>
<td>2.0</td>
<td>5</td>
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<tr>
<td></td>
<td>C-3</td>
<td>2.0</td>
<td>3</td>
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<tr>
<td></td>
<td>C-4*</td>
<td>2.0</td>
<td>5</td>
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<tr>
<td></td>
<td>C-6</td>
<td>2.0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>C-3,4</td>
<td>2.0</td>
<td>33</td>
</tr>
</tbody>
</table>

*Data presented for substrate glucose-4-$^{14}$C are obtained by calculation from the observed data in glucose-3-$^{14}$C and glucose-3,4-$^{14}$C experiments.

Experimental Conditions: When the concentration of NaCl was 3%, the weight of the cells was 7.0 mg, and the substrate level used was 0.71 mg. When the NaCl concentration was 15%, the substrate level used was 0.1 mg. The cell weight cannot be readily determined due to the high salt concentration.
Examination of the catabolic behavior of glucose and gluconate utilization in this organism has unveiled some interesting findings. The radiorespirometric patterns for utilization of glucose by this organism are given in Figure 14. It can be seen that the glucose is promptly utilized by *Ch. viscosum* and the $^{14}CO_2$ yield from the various carbon atoms of glucose follows the order $C-4 > C-1 > C-3 > C-2 \geq C-6$. The data on $^{14}CO_2$ yield as well as incorporation of substrate glucose into cellular constituents and fermentation products in the medium (Table 4) can be interpreted to mean that in *Ch. viscosum* glucose is catabolized in the following manner:
Figure 14. Radiorepirometric patterns for utilization of D-glucose, administered in a single dose, by Ch. viscosum. Numerals refer to position of carbon atoms of D-glucose labeled with $^{14}$C.
According to the foregoing scheme, the portion of the glucose that has been routed into the PP pathway gives rise to the formation of one mole of CO$_2$ and one mole of pentose-5-phosphate. The latter, by undergoing C$_2$-C$_3$ cleavage gives rise to one mole of acetate and one mole of pyruvate, with C-2 equivalent to the methyl carbon of the acetate and C-6 equivalent to the methyl carbon of pyruvate and hence to the methyl carbon of acetate upon oxidative decarboxylation. Such a mechanism therefore accounts for the observed catabolic equivalence of C-2 and C-6 of glucose and the prompt and extensive conversion of C-4 to respiratory CO$_2$ in comparison to C-3. The metabolism of pentose via C$_2$-C$_3$ cleavage has been previously demonstrated in *Leuconostoc* species (Altermatt, Blackwood and Neish, 1955), in *Lactobacillus* species (Gest and Lampen, 1952; Bernstein and Tiberio, 1953), in the mold *Fusarium lini* (Gibbs, et al., 1954), and in *Escherichia coli* (Cohen and Raff, 1951).

Estimation of the concurrent pathways functioning in this organism using Equations 4 and 5 reveal that glucose is catabolized 84% via the EMP pathway and 16% by way of the PP pathway followed by the C$_2$-C$_3$ cleavage.

It is of great interest to find that the catabolism of gluconate by this organism follows an entirely different mechanism. As shown in Figure 15, the radiorespirometric patterns for utilization of D-gluconate provide convincing evidence that gluconate is catabolized
Figure 15. Radiorespirometric patterns for utilization of D-gluconate, administered in a single dose, by Ch. viscosum. Numerals refer to position of carbon atoms of D-gluconate labeled with $^{14}\text{C}$. 
via the concurrent operation of the ED and PP pathways, with the former probably playing a more important role. This conclusion is drawn not only from the extensive conversion of C-1 and C-4 of gluconate to respiratory CO₂ but also from the approximate catabolic equivalence between C-3 and C-6 of gluconate.

It appears from the foregoing discussions that understanding of catabolic behavior for glucose utilization and allied substrates is one of the important criteria for microbial taxonomy. In order to justify the classification of certain species under a given genus, the microorganism should have the same type of catabolic pathways for carbohydrate utilization as prevail in other species of the genus. In addition, one of the criteria for recognition of a given species is that relative participation of glucose pathways is different from other species under the same genus. The findings in the present work can therefore be analyzed in this light.

As indicated in Table 8, Ch. lividum, Ch. amethystinum, and Ch. violaceum all are found to utilize glucose predominately via the Entner-Doudoroff pathway, with the pentose phosphate pathway playing a minor role. It therefore seems justifiable to include these three species under the genus Chromobacterium with Ch. violaceum as the type species as ruled by the Judicial Commission (1958). However, in view of the fact that the relative participation of these two pathways in Ch. lividum is much the same as in Ch. amethystinum,
Table 8. Relative participation of primary glucose pathways in *Chromobacterium* species.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Relative participation of primary glucose pathways, % of administered glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EMP</td>
</tr>
<tr>
<td><strong>Ch. lividum</strong></td>
<td>86</td>
</tr>
<tr>
<td><strong>Ch. amethystinum</strong></td>
<td>87</td>
</tr>
<tr>
<td><strong>Ch. violaceum</strong></td>
<td>80</td>
</tr>
<tr>
<td><strong>Ch. maris-mortui</strong></td>
<td>100</td>
</tr>
<tr>
<td><strong>Ch. viscosum</strong></td>
<td>84</td>
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</tbody>
</table>
it is debatable whether these two organisms should be recognized as individual species. The findings from the glutamate data (Table 3) also support these conclusions.

On the basis of morphology and physiology, *Ch. lividum*, *Ch. amethystinum*, and *Ch. violaceum* are also strikingly similar. All three tend to form elongated rods when cultured in glucose medium, and do so to a lesser extent when cultured in glycerol-peptone medium. Of the three, *Ch. amethystinum* is most likely to form these string-like rods, and *Ch. violaceum* is least likely to do so. All three organisms form the pigment violacein easily in glycerol-peptone medium and to a lesser extent in glucose medium. Again, there is a variation between the organisms with regard to chromogenesis: *Ch. violaceum* produces pigment during log phase growth, *Ch. lividum* forms it after storage at 4°C, and *Ch. amethystinum* rarely exhibits pigmentation in broth, but forms it extensively on glycerol-peptone agar (as do the other organisms). *Ch. lividum* and *Ch. amethystinum* show similar growth and metabolic rates (Table 6) while those rates of *Ch. violaceum* are much faster. On the basis of the present catabolic work, observations regarding morphology and physiology, and in support of previous work, it is recommended that one of these two species be designated as the type species of the psychrophilic group and the other be recognized as a strain within that species. Earlier, Sneath (1960) suggested
that Ch. amethystinum be recognized as a strain within the species Ch. lividum, the proposed type species for the psychrophilic group. On the basis of the present work, it is also recommended that Ch. violaceum be retained as a separate species, the type species of the mesophilic group.

Analysis of data resulting from work with Ch. maris-mortui presents an entirely different picture. Carbohydrate catabolism in this organism is totally different from that in the other three Chromobacterium species described. As indicated in Table 8, the pentose cycle appears to be operative in this organism to the exclusion of other primary pathways.

Morphologically and physiologically, the halophile Ch. maris-mortui does not resemble other chromobacteria. Although Bergey's Manual (1957) states that long filamentous rods form in 30% saline solutions, this has not been observed in this laboratory. Rather the rods tend to be short and thickened with increasing salt concentrations; thread-like rods have not been observed. A blackish pigment has been observed rarely, and no purple coloration has been observed nor reported by others. This organism, an obligate halophile, grows better in glucose medium than in glycerol-peptone medium, in contrast to the other three organisms discussed. It is of interest to note that despite Sneath's findings (1960) that many strains of Ch. lividum and Ch. violaceum grow in 3.5% NaCl, the
three recognized Chromobacterium species failed to grow in NaCl-containing medium in this laboratory. In addition, as indicated in Table 3, in contrast to the three recognized Chromobacterium species, the halophilic organism is found to be capable of utilizing both D and L isomers of glutamic acid.

It is therefore recommended that Ch. maris-mortui be excluded from the genus Chromobacterium. The proper taxonomic classification of this halophilic organism can be made only when one learns more about the physiological behavior of this organism.

Ch. viscosum was selected for study as a representative species of those already removed from the genus Chromobacterium on the basis of pigmentation. Judging from the findings of the present work, there is little doubt that this organism was correctly excluded from the genus Chromobacterium in recent editions of Bergey's Manual. Thus, catabolic behavior for utilization of glucose, gluconate, and glutamate in Ch. viscosum is found to be distinctly different from the other recognized Chromobacterium species (Tables 3, 8).

On the basis of morphology and general physiology, this organism is quite different from the others, as well. It is gram negative in very young cultures but becomes gram positive by middle log phase. No pigmentation has been observed on the media used for growth in this laboratory, although Gilman (1953) observed and
studied the pigmentation from cultures in calcium lactate agar. This organism grows well at 4° and 37°C; it also grows easily on 6.5% NaCl medium. Both of these characteristics tend to separate it from other chromobacteria. Long thread-like rods have never been observed for this organism.

In many respects, Ch. viscosum resembles pseudomonads; however, it is questionable whether one should include this organism under the genus Pseudomonas in view of the fact that the EMP pathway is the predominant catabolic route in this organism, whereas the ED pathway is the major route for practically all Pseudomonas species (Stern, Wang and Gilmour, 1960).

With the exclusion of Ch. maris-mortui and Ch. viscosum from the genus Chromobacterium, the genus contains a rather well-unified group of organisms, resembling each other morphologically and physiologically. The most unique characteristic is the production of the chemical violacein, responsible for the purple coloration of the organisms. The genus as a whole relies predominantly on the Entner-Doudoroff pathway, with the pentose phosphate pathway playing a minor role. Since it may be assumed that closely related genera will display similar use of catabolic pathways, these data support De Ley's suggestion (1962) that the chromobacteria are related to the pseudomonads and to other genera utilizing the ED pathway.
The understanding of catabolism of carbohydrates and allied compounds in microorganisms is of great importance to microbial taxonomy, but it is not suggested that this should be the exclusive criterion used. However, as indicated by De Ley and others, and as findings of the present work have demonstrated, the biochemical behavior of microorganisms can be correlated with other data and can provide useful information to help devise a more reliable system for the establishment of phylogenetic relationships. The use of the radiorespirometric method has proven to be a useful and convenient way to collect information on catabolic behavior.
SUMMARY

Information on catabolism of glucose, gluconate, and glutamate in five species of *Chromobacterium* has been obtained by the use of radiorespirometry. Nature of the pathways has been identified, the relative participation of those pathways has been determined, and the resulting differences have been analyzed in terms of their taxonomic significance. On the basis of carbohydrate catabolism, and correlated with morphological and physiological data, the following recommendations have been made: 1) *Ch. lividum* and *Ch. violaceum* be retained as type species of the psychrophilic groups, respectively; 2) *Ch. amethystinum* be designated as a strain within the species *Ch. lividum*; 3) the halophile *Ch. maris-mortui* be excluded from the genus *Chromobacterium*; and 4) the organism *Ch. viscosum* be excluded from the genus *Chromobacterium*. 
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