# THE ISOLATION AND ABSORPTION SPECTRUM MAXIMA OF BACTERIAL CAROTENOID PIGMENTS

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The production of pigments by bacteria has attracted attention from the beginning of the study of bacteriology, possibly because the presence of pigment is one of the characteristics of microorganisms which is most readily observed. In recent years special emphasis has been placed on the chemical nature of the pigments and on their probable rôles in the metabolism of bacterial cells.

The bacterial pigments whose chemical structures have been determined fall into four main groups (White, 1939). Prodigiosin, the red pigment of *Serratia marcescens*, is a pyrrole derivative. Pyocyanin, the dark blue pigment of *Pseudomonas aeruginosa*, was the first phenazine derivative to be found in nature. Phthiocol, isolated from the tubercle bacillus, is a naphthaquinone derivative. However, the largest group of bacterial pigments belongs to the class of compounds known as carotenoids. Most yellow, orange, and red pigmented bacteria contain carotenoids. This interesting group of compounds occurs extensively throughout both the plant and the animal kingdoms. In the plant world the carotenoids are present in forms ranging from bacteria to the highest plants. Similarly, in the animal kingdom we find these compounds in many forms of both invertebrates and vertebrates.

Research on the carotenoids in plants and animals has resulted in the development of techniques whereby these compounds can be separated and identified. Because of the ease of obtaining large amounts of material, considerable knowledge of the carotenoids in plants has been gained as evidenced by such monographs as those of Palmer (1922) and Strain (1938). The investigation of the carotenoids present in bacteria has lagged, possibly, because of the greater difficulties of obtaining sufficient material.

The presence of carotenoids in bacteria was demonstrated by Zopf (1889) who designated this group of bacterial pigments, lipochromes, because of their solubility in the fat solvents. Later, Kligler (1914) and Krainsky (1914) demonstrated the widespread presence of carotenoid pigments in different species of bacteria.

Only a few of the large number of bacteria known to contain carotenoids have been subjected to pigment identification. Reader (1925) found beta-carotene and lycopene in *Sarcina aurantiaca* and a hitherto unreported pigment, which she named coralin, in *Streptothrix corallinus*. Chargaff and Dieryck (1932) reported the presence of a xanthophyll and a hydrocarbon, which they called sarcinene, in *Sarcina lutea;* Nakamura (1936) found a xanthophyll ester in this bacterium. Chargaff (1933) found zeaxanthin to be the only pigment in *Staphylococcus aureus* and differed with Reader (1925) in reporting beta-carotene and

zeaxanthin in *Sarcina aurantiaca*. Chargaff (1933) isolated beta- and gamma-carotenes and Ingraham and Steenbock (1935) isolated alpha- and beta-carotenes, cryptoxanthin, and esters of lutein, zeaxanthin, and azafrin from *Mycobacterium phlei*. Two additional acid-fast bacteria were studied by Chargaff and Lederer (1935). *Bacillus lombardo-pelligrini* contained beta- and gamma-carotenes and the bacillus of Grassberger was found to contain beta- and gamma-carotenes, and lycopene.

The carotenoid pigments in some purple sulphur bacteria were investigated by Karrer and Solmssen (1936). Rhodoviolascin, rhodopin, rhodopurpurin, flavorhodin, and rhodovibrin were isolated and described briefly. Probably most of these pigments are limited in their occurrence to the sulphur bacteria.

A carotenoid pigment named spirillo-xanthin was isolated from *Spirillum rubrum* by van Niel and Smith (1935). Leprotin, a carotinoid hydrocarbon, was found by Grundmann and Takeda (1937) in an acid-fast bacterium isolated from a leprous lesion.

The carotenoid pigments most commonly found in bacteria belong to the following groups: (1) hydrocarbons, such as beta-carotene; (2) alcohols, such as xanthophyll; (3) esters; and (4) carotenoid acids.

It is clear that more information is desirable concerning the chemical nature of the bacterial carotenoid pigments. Such knowledge would be an aid in the solution of fundamental problems, such as the role of these pigments in bacterial metabolism. Although the structure and certain other characteristics of some of these pigments have been established, data on other pigments are lacking. The isolation and spectrometric analysis of the carotenoids are basic preliminary steps in their investigation. An aspect of the present study has been the adaptation of the methods used for the separation and identification of the carotenoid pigments of higher plants to the bacterial carotenoids. Absorption spectrum maxima have been determined and compared with those recorded in the literature for similar pigments. Complete identification of the pigments which were isolated is not claimed but pertinent data are presented.

## MATERIALS AND METHODS

*Cultivation of the bacteria.* The pigmented bacteria were grown on nutrient agar containing two per cent glycerol and adjusted to pH 7.2. Inoculations were made by spraying the surface of the medium with a twenty-four-hour broth culture, using an atomizer through which sterile air was passed. Sixteen-ounce French square bottles were used for culturing the bacteria. After incubation for one week at room temperature, the cells were removed by the addition of a 70 per cent aqueous solution of acetone and the scraping of the surface with a glass rod bent at a right angle. Water was not used because it frequently forms a hydrophilic suspension with the cells from which the latter are removed with difficulty. The pigments were not extracted by the aqueous acetone and the bacterial cells were separated by centrifugation.

*Extraction of pigments.* Kuhn and Brockmann (1932) employed absolute methanol for extracting the carotenoid pigments from finely divided plant

material. The present investigators found that cold methanol was effective in extracting the carotenoids from moist bacterial cells when the mixture was ground with an abrasive such as alundum. A convenient method of extraction involved the addition of 50 ml. of methanol to approximately one gram of moist bacterial cells and the placing of the container in hot water to bring the methanol to the boiling point quickly. The pigments were extracted in a few minutes. The solution was then cooled and the cells removed by centrifugation. The short exposure to an increased temperature was found to produce no injurious effects on the pigments.

*Separation of types of pigments.* The methanol extract then was subjected to partition between immiscible solvents to separate the various types of carotenoid pigment. A modification of the method of Kuhn and Brockmann (1932) is represented schematically in figure 1.

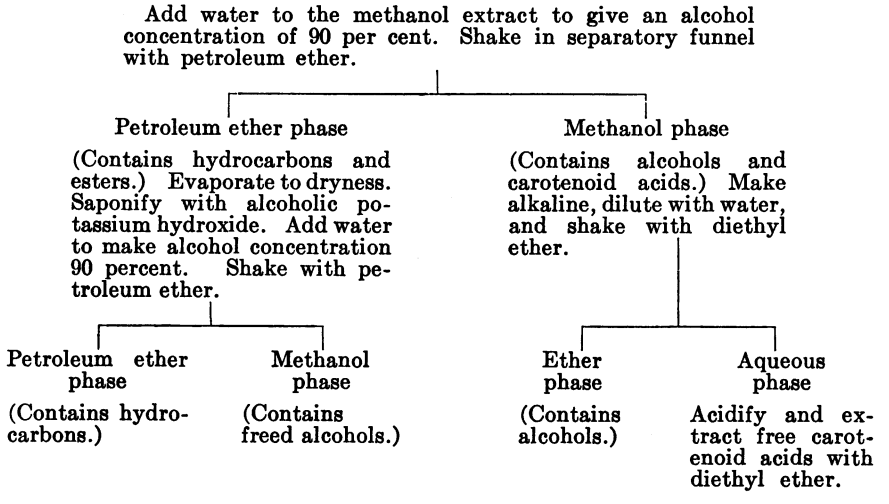


FIG. 1. SCHEME OF SEPARATION OF BACTERIAL CAROTENOID PIGMENTS

The methanol extract was diluted with water to give an alcohol concentration of 90 per cent and then was shaken in a small separatory funnel with an equal volume of petroleum ether. The petroleum ether layer was drawn off and shaken several times with fresh portions of ninety per cent methanol. Similarly, the alcohol layer was shaken repeatedly with petroleum ether in order to insure a complete separation of the pigments.

The petroleum ether layer was freed from water by shaking with saturated salt solution and standing over anhydrous sodium sulphate for half an hour, and then was evaporated to dryness under partial vacuum. The dried pigment may contain hydrocarbons and esters. Fifty milliliters of a two-and-one-half-per cent solution of potassium hydroxide in methanol was added and the solution was kept at 40°C. for three hours. Water was then added to give an alcohol concentration of 90 per cent and the solution was shaken in a separatory funnel with petroleum ether.

The petroleum ether layer was drawn off, washed repeatedly, freed from water, and evaporated to dryness under reduced pressure. The residue was taken up in petroleum ether and the individual hydrocarbons were then separated by chromatographic analysis.

The freed carotenols in the alkaline methanol layer were forced into diethyl ether by the addition of half-saturated salt solution. The ether layer was washed with water until the washings were no longer alkaline to phenolphthalein, freed from water, and then evaporated to dryness under vacuum.

The original methanol phase containing carotenols and carotenoid acids was made alkaline to litmus, diluted with water, and shaken with diethyl ether. The ether portion was washed, freed from water, and evaporated to dryness.

If there are any carotenoid acids present, they remain in the aqueous phase in the form of their salts. The acids may be recovered by the addition of dilute hydrochloric acid and extraction with diethyl ether.

The procedures just described cover all four of the main types of carotenoid pigments; certain steps are eliminated if partition tests reveal the absence of any of these groups.

*Chromatographic adsorption.* After separation of the pigments into groups, the individual components in each group were separated by the chromatographic adsorption technic devised by Tswett (1906) and excellently described by Cook (1936). Zechmeister and Cholnoky (1937) have also presented a thorough description of chromatographic adsorption.

Successful separation of the carotenoids is dependent on the selection of the proper adsorbent and solvent and on the careful preparation of the adsorption column.

The choice of adsorbent and solvent depends largely upon the particular group of carotenoids to be separated. The hydrocarbons have a weak affinity for adsorbing substances; hence, a strongly adsorbing material must be employed. Strain (1938) recommended for the separation of leaf xanthophylls a mixture of a specially prepared magnesia[1] with an equal amount of heat-treated siliceous earth[2]. Bacterial carotenols were too strongly adsorbed on this mixture but it proved to be entirely satisfactory for the separation of the carotenoid hydrocarbons, when either petroleum ether or ethylene chloride was employed as the solvent.

Calcium carbonate, activated by heating at 150°C. for five hours, followed by cooling in a vacuum desiccator, served well for the separation of the carotenoid alcohols. Petroleum ether and carbon disulphide are the solvents of choice for the carotenols.

Calcium hydroxide and a mixture of aluminum oxide and siliceous earth were tested as absorbents but were found to be less satisfactory than those described.

The adsorption column must be prepared carefully in order to insure satisfactory separation of the pigments. The adsorption device used in this investi-

[1] Micron Brand, magnesium oxide No. 2641, California Chemical Company, Newark, California.

[2] Hyflo super cel F.A. 501, Johns-Manville Co., New York.

gation consisted of a glass tube, 15 cm. long and 15 mm. in diameter, sealed at one end to a tube of 6 mm. bore and approximately 8 cm. in length. The tube was supported in a vertical position by attachment to a vacuum flask. A wad of cotton was placed just above the constricted portion of the tube. The adsorbent was added in small portions, each of which was packed with a rod to which a metal disk, slightly smaller than the diameter of the tube, was attached. One of the solvents was poured onto the column and suction was used to test for the presence of cracks in the column.

Certain precautionary measures must be observed in chromatographic adsorption. The pigments must be free of water before they are dissolved in the adsorbing solvent. Small amounts of moisture may be removed by adding benzene and evaporating *in vacuo* at 50°C. The presence of a trace of ethanol also interferes markedly with the adsorption.

The pigment mixture was dissolved in about 10 ml. of solvent and the solution then was poured onto the adsorption column so that the latter was covered quickly. As the solution passed through the column the pigments formed a narrow band near the top. The column then was washed with fresh portions of pure solvent which caused the adsorbed pigments to move slowly through the adsorbent. If filtration is slow, gentle suction may be applied. The pigments are separated gradually into a series of bands each of which represents a distinct pigment. When several zones were present, continued washing with the solvent washed through those which were adsorbed weakly. The latter were collected separately in the suction flask. The zones remaining in the column were separated mechanically and eluted with ethanol and then filtered from the adsorbent, using a mat of siliceous earth.

In addition to its use for the separation of similar pigments, chromatographic adsorption is useful for pigment identification. If a solution of an unknown pigment is mixed with that of a known pigment and adsorbed on a column, the formation of a single colored zone indicates that the two pigments are very similar or identical. The formation of two zones proves that the two pigments are not identical. Similarly, if two pigments found to possess identical or nearly identical absorption maxima by spectrometric analysis are suspected of being the same pigment, they can be tested by mixing and adsorbing them on a column. The formation of a single band indicates their unity; the formation of two zones proves their dissimilarity.

*Spectrometric analysis.* The carotenoid pigments are distinguished conveniently by their absorption spectra. There are several instruments available for obtaining such spectra. In this investigation a Bausch and Lomb spectrometer, equipped with a constant deviation prism of the Pellin-Broca type, was used. At a sufficiently low concentration of pigment, the bands of maximum absorption are symmetrical enough to allow one to determine the mean value of their boundaries by placing the crossed hairs of the instrument over the darkest portion of the band. The value so obtained was recorded as the absorption maximum for each band.

The absorption spectrum maxima of the carotenoids were determined in 95 per cent ethanol, in carbon disulphide, and in chloroform.

A Baly tube was used for varying the amount of solution through which the light passed. A photographic flood lamp was used as the light source. The spectrometer was calibrated with the sodium D line and checked periodically with this standard. The absorption maxima obtained were compared with those recorded in the literature for various carotenoids dissolved in the same solvents.

TABLE 1
*Bacterial cultures used*

| NAME | CULTURE NUMBER | SOURCE |
|---|---|---|
| *Flavobacterium arborescens* | 435 | American Type Culture Collection |
| *F. suaveolens* | 958 | American Type Culture Collection |
| *F. esteroaromaticum* | | Cornell University |
| *F. sulphureum* | 42.70 | Ohio State University |
| *F. fecale* | | Cornell University |
| *Sarcina lutea* | 8.40 | Ohio State University |
| *S. flava* | 147 | American Type Culture Collection |
| *S. aurantiaca* | 146 | American Type Culture Collection |
| *Micrococcus luteus* | 379 | American Type Culture Collection |
| *M. flavus* | 400 | American Type Culture Collection |
| *Erwinia lathyri* | | National Type Culture Collection, England |
| *E. ananas* | | Malaya—Dr. Perry Elrod |
| *Bacterium mycoides* | 35.10 | Ohio State University |
| *Cellulomonas flavigena* | 482 | American Type Culture Collection |
| *Staphylococcus aureus* | 209 | U.S.D.A. Phenol Coefficient Test Strain |
| *Staphylococcus aureus* | 610 | Food poisoning—Ohio State University |
| *Staphylococcus aureus* | 614 | |
| *Staphylococcus aureus* | 615 | |
| *Staphylococcus aureus* | 616 | |
| *Staphylococcus aureus* | 617 | |
| *Staphylococcus aureus* | 620 | |
| *Staphylococcus aureus* | 626 | |
| *Staphylococcus aureus* | 628 | Throat infection—Ohio State University |
| *Staphylococcus aureus* | 628a | Animal infection—Ohio State University |
| *Staphylococcus aureus* | 628b | Furuncle—Ohio State University |
| *Staphylococcus aureus* | 628c | |

*Bacterial cultures.* The pigmented bacteria used in this study are listed in table 1.

## EXPERIMENTAL

The absorption maxima, as determined by spectrometric analysis, for 12 different pigments are recorded in table 2. The pigments were isolated from 14 different species of bacteria and consist of 7 carotenols and 5 hydrocarbons. No esters or carotenoid acids were isolated from these bacteria. The number of the pigments, as referred to in table 2, is arbitrary and is used to identify the several pigments.

*Flavobacterium.* Inspection of table 2 shows that the single carotenol pigments isolated from *F. esteroaromaticum*, *F. suaveolens*, and *F. fecale* had the same absorption spectrum maxima. Additional evidence for their identity was obtained by subjecting a mixture of the three pigments to chromatographic adsorption on a column of calcium carbonate. A single pigmented zone was obtained. A search of the literature failed to reveal any carotenol possessing the same absorption maxima as the pigment isolated from these three species of *Flavobacterium.*

TABLE 2.

*Absorption maxima in different solvents of pigments isolated from various bacteria.*

| BACTERIUM | TYPE OF CAROTENOID | NO. OF PIGMENT | COLOR AND ZONE IN ADSORPTION COLUMN | ABSORPTION MAXIMA (MU) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $CS_2$ | | | $CHCl_3$ | | | $C_2H_5OH$ | | |
| *Flavobacterium esteroaromaticum* | Alcohol | 1 | Orange | 453 | 482 | 513 | 460 | | 488 | 452 | | 482 |
| *F. suaveolens* | Alcohol | 1 | Orange | 453 | 483 | 514 | 459 | | 488 | 451 | | 482 |
| *F. fecale* | Alcohol | 1 | Orange | 453 | 482 | 513 | 459 | | 488 | 451 | | 483 |
| *F. sulphureum* | Hydrocarbon | 2 | Yellow | 437 | 466 | 499 | 451 | | 481 | 440 | | 470 |
| *F. arborescens* | Hydrocarbon | 3 | Zone 1—orange | 465 | 498 | 536 | 450 | 480 | 516 | 440 | 470 | 503 |
| | Hydrocarbon | 4 | Zone 2—red | 495 | 530 | 572 | 477 | 508 | 546 | 463 | 494 | 530 |
| | Hydrocarbon | 5 | Zone 3—orange | 465 | 500 | 540 | 450 | 480 | 515 | 439 | 469 | 503 |
| | Hydrocarbon | 2 | Zone 4—yellow | 435 | 465 | 499 | 450 | | 480 | 439 | | 469 |
| | Hydrocarbon | 6 | Zone 5—orange | 465 | 500 | 540 | 450 | 480 | 515 | 439 | 469 | 503 |
| *Sarcina lutea* | Alcohol | 7 | Zone 1—yellow | 460 | | 496 | 451 | | 480 | 439 | | 470 |
| | Alcohol | 8 | Zone 2—yellow | 464 | | 499 | 450 | | 480 | 440 | | 470 |
| *S. flava* | Alcohol | 7 | Yellow | 460 | | 495 | 450 | | 480 | 440 | | 470 |
| *S. aurantiaca* | Alcohol | 9 | Orange | 479 | | 514 | 463 | | 498 | 456 | | 487 |
| *Micrococcus luteus* | Alcohol | 7 | Zone 1—yellow | 460 | | 495 | 451 | | 480 | 440 | | 470 |
| | Alcohol | 8 | Zone 2—yellow | 465 | | 499 | 450 | | 480 | 440 | | 470 |
| *M. flavus* | Alcohol | 7 | Yellow | 459 | | 494 | 449 | | 480 | 439 | | 469 |
| *Erwinia lathyri* | Alcohol | 10 | Yellow | 478 | | 513 | 458 | | 485 | 452 | | 483 |
| *E. ananas* | Alcohol | 11 | Yellow | 474 | | 508 | 460 | | 493 | 450 | | 480 |
| *Bacterium mycoides* | Alcohol | 12 | Red | 477 | 508 | 548 | 454 | 487 | 521 | 446 | 474 | 506 |
| *Cellulomonas flavigena* | Alcohol | 7 | Zone 1—yellow | 460 | | 496 | 451 | | 480 | 440 | | 470 |
| | Alcohol | 8 | Zone 2—yellow | 464 | | 499 | 450 | | 480 | 440 | | 470 |

A single carotenoid hydrocarbon was obtained from *F. sulphureum.* A strongly adsorbed yellow zone was obtained with the specially prepared magnesium oxide mixture. Chargaff and Dieryck (1932) obtained a hydrocarbon from *Sarcina lutea* which had the same absorption maxima when dissolved in petroleum ether as has this pigment. They named their pigment sarcinene. Whether the two pigments are identical is not certain since none of Chargaff's pigment was available for chromatographic analysis.

The delicacy of chromatographic adsorption is exemplified by the separation of the pigments of *F. arborescens.* Partition experiments showed the presence of hydrocarbons only. Five distinct zones were obtained on the magnesium oxide mixture when ethylene chloride was used as the solvent. The third and fifth zones had identical absorption maxima in the three solvents. The orange

pigment from the first zone was also very similar. The three zones, however, were separated from each other on the adsorption column by distinctly different pigments. When these three pigments were mixed and adsorbed on a column of magnesium oxide, three zones were obtained, thus indicating their individuality. Molisch (1914) obtained a pigment from a sulphur bacterium which he called alpha-bacteriopurpurin. He determined its absorption maxima in carbon disulphide only; the values were the same as those obtained for the red pigment from zone 2. Perhaps, the yellow pigment from the fourth zone is the sarcinene of Chargaff and Dieryck.

When *F. arborescens* was grown on a medium containing glycerol or glucose, a red pigmentation was obtained; when grown on nutrient agar, an orange growth was produced. Pigment analysis, however, indicated only a quantitative difference; the same five hydrocarbons were identified. The amount of the red pigment was increased by growth on media containing glucose or glycerol.

*Sarcina.* Two carotenoid alcohols were obtained from *S. lutea.* As noted before, Chargaff and Dieryck reported a hydrocarbon which they called sarcinene in this species. A second strain of *S. lutea* was investigated in this laboratory and yielded the same two carotenols but no hydrocarbon. It is interesting to note (table 2) that the absorption maxima were almost identical in chloroform and in ethanol. When the two pigments were mixed and readsorbed on calcium carbonate, two zones were obtained again. Pigments with these absorption maxima have not been reported previously.

*S. flava* contained a single carotenol which apparently was identical with one of the pigments of *S. lutea* as indicated by absorption maxima and adsorption of the mixed pigments.

*S. aurantiaca* contained a single carotenol which was unreported previously.

*Micrococcus.* Two carotenols were found in *M. luteus.* Absorption maxima and chromatographic adsorption with mixed pigments indicated that these were the same as the alcohols obtained from *Sarcina lutea.* The two species of bacteria were differentiated, however, by their fermentation reactions.

A single carotenol was isolated from *M. flavus.* It apparently was the same pigment as one of those found in *S. lutea, S. flava,* and *M. luteus.*

*Erwinia.* Single and distinct carotenoid alcohols, both unreported previously, were isolated from *E. lathyri* and *E. ananas.*

*Bacterium mycoides.* This organism is not to be confused with *Bacillus mycoides.* An intense red pigmentation was obtained which was due to a single red carotenol. A pigment, isolated by Karrer and Solmssen (1935) from a purple sulphur bacterium and named by them rhodopin exhibited the same absorption maxima.

*Cellulomonas flavigena.* This bacterium produced two carotenols. Absorption maxima and mixed chromatographic adsorption indicated that these pigments were identical with those obtained from *S. lutea* and *M. luteus.*

*Staphylococcus aureus.* Twelve strains of *S. aureus* obtained from various sources and with different histories were subjected to pigment analysis. Table 3 presents pertinent data. All the strains examined were found to contain a

hydrocarbon whose adsorption maxima coincided with delta-carotene reported by Winterstein (1933) to occur in the fruit hulls of *Gonocaryum pyriforme*, and also a carotenoid alcohol whose absorption maxima were identical with rubixanthin isolated by Kuhn and Grundman (1934) from rose hips. In addition to these two pigments, four strains isolated from food poisoning outbreaks and four strains from staphylococcic infections contained an ester of rubixanthin.

The strain of *S. aureus* used by the Food and Drug Administration of the U. S. Department of Agriculture as the test organism for determining phenol coefficients contained, as a third pigment, a hydrocarbon with absorption maxima identical with those of sarcinene. Chargaff (1933) reported that zeaxanthin

TABLE 3

*Pigments isolated from various strains of Staphylococcus aureus*

| STRAIN | SOURCE | PIGMENTS | | |
|---|---|---|---|---|
| | | Hydrocarbon | Alcohol | Third pigment |
| 209 | Food and Drug Administration | *Delta-carotene | †Rubixanthin | ‡Hydrocarbon-sarcinene |
| 614 | Food poisoning | Delta-carotene | Rubixanthin | †Ester of rubixanthin |
| 615 | Food poisoning | Delta-carotene | Rubixanthin | Ester of rubixanthin |
| 616 | Food poisoning | Delta-carotene | Rubixanthin | |
| 617 | Food poisoning | Delta-carotene | Rubixanthin | |
| 620 | Food poisoning | Delta-carotene | Rubixanthin | |
| 626 | Food poisoning | Delta-carotene | Rubixanthin | †Ester of rubixanthin |
| 628 | Infection | Delta-carotene | Rubixanthin | Ester of rubixanthin |
| 628a | Infection | Delta-carotene | Rubixanthin | Ester of rubixanthin |
| 628b | Infection | Delta-carotene | Rubixanthin | Ester of rubixanthin |
| 628c | Infection | Delta-carotene | Rubixanthin | Ester of rubixanthin |

* This pigment had absorption maxima identical with those of delta-carotene.

† Same absorption maxima as for rubixanthin.

‡ Same absorption maxima as for Chargaff and Dieryck's sarcinene.

was the only pigment in *S. aureus*. In none of the twelve strains studied was this pigment isolated.

## DISCUSSION

It has been an important feature of this investigation to adapt some of the methods used for the separation and identification of the carotenoid pigments found in plant material to the separation and the determination of absorption spectrum maxima of the carotenoid pigments present in bacteria. It was found possible to make with ease a pigment analysis starting with approximately one gram of moist cells.

One of the problems encountered in this study was the extraction of the carotenoids from the bacteria. The extraction of carotenoids from plant material may be accomplished by the use of any one of many of the fat solvents. This was not true for the bacterial carotenoids. It was discovered that the

extraction of the carotenoids from the bacterial cells could be accomplished best in the presence of a small amount of water with a water-miscible fat solvent. Methyl alcohol proved to be well suited for this purpose.

Formerly, when carotenoid pigments were isolated they were given names, often without regard to their chemical structures. Sometimes, the name signified the source, e.g. sarcinene from *Sarcing lutea* and violacein from *Chromobacterium violaceum*. More recently, as new pigments have been isolated, names have commonly been withheld until the chemical structure of the pigments could be determined. This appears to be a rational policy, especially since source names lose their significance when the same pigment is found in different kinds of plants or animals.

The present investigation resulted in the isolation of several carotenoid pigments with absorption maxima previously unreported. Some bacteria were found to contain but one pigment while others contained several. With the exception of those in *S. aureus*, the carotenoid pigments in any one species of bacteria belonged to a single type, with the alcohols predominating. No carotenoid acids were found in any bacterium.

Several of the different species of bacteria contained identical pigments. An interesting problem arises as to whether pigment analysis should be included in securing characteristics for differentiation of bacteria. Consistency of pigment production is important in this respect. It has been found during the course of this study that each bacterium produced the same pigments repeatedly. When *F. arborescens* was grown on a medium containing glycerol or glucose, a red pigmentation resulted; on nutrient agar, an orange growth was obtained. However, upon pigment analysis the same five hydrocarbons were obtained in each instance with the amount of red pigment enhanced when the organism was grown on glycerol or glucose. Data are too few, however, to conclude that media have no effect on the kinds of pigment formed by a bacterium.

It is probable that different strains of a certain species of bacterium will be found to produce different pigments. Reader (1925), Chargaff (1933) and the present authors disagree on the pigment formed by *S. aurantiaca*. Likewise, Nakamura (1936), Chargaff and Dieryck (1932) and the present authors fail to agree with respect to the pigments of *S. lutea*. In order to test the hypothesis that different strains of one species produce different pigments when grown on a standard medium, the present investigators subjected twelve strains of *S. aureus* to pigment analysis. Two pigments were common to all, with some variation in regard to a third pigment. Again these results do not coincide with those of Chargaff (1933). Insufficient data on the subject are available to attempt a correlation of pigment type with other properties or with source.

There are interesting opportunities for research in determining the effects of environmental factors on the kinds and amounts of the carotenoid pigments produced by bacteria. Rapid growth makes possible a large number of studies in a short time, the environment may be controlled fairly well, and complicating factors are fewer, in general, than they are for the higher plants. For the above reasons, it appears also that bacteria offer definite possibilities in connection with

discovering the true function, if any, of the carotenoids in plants and animals. Two principal types of reactions have been attributed to the yellow pigments in leaves. One involves chemical reactions taking place in photosynthesis; the other is concerned with various oxidation-reduction reactions in which hydrogen is transferred, presumably by the pigments acting reversibly as hydrogen acceptors and donors. However, careful consideration of the evidence leads to the conclusion that the subject is still in need of much critical investigation.

It is known that some naturally occurring pigments, such as riboflavin, cytochrome, pyocyanine, phthiocol and toxoflavin function as hydrogen transporters in bacterial metabolism. It is evident that studies should be extended to investigations of the functional role of the carotenoids in bacteria with the ultimate purpose of discovering their role in plants and animals in general.

## SUMMARY

Methods for the extraction, isolation, and determination of absorption spectrum maxima of bacterial carotenoid pigments have been devised or adapted from those applicable to the higher plant pigments. The pigments were extracted from bacterial cells with hot methanol. They were separated by chromatographic adsorption and then subjection to spectrometric analysis.

Twelve carotenoid pigments were isolated from 14 different species of bacteria. These included five species of *Flavobacterium*, three of *Sarcina*, two of *Micrococcus*, two of *Erwinia*, one of *Bacterium*, and one of *Cellulomonas*. Seven of the carotenoids were alcohols and five were hydrocarbons. Some bacteria produced only one pigment while others produced several; *Flavobacterium arborescens* contained five distinct hydrocarbon carotenoids. Some species which can be separated by fermentation reactions produced identical pigment.

Twelve strains of *Staphylococcus aureus*, obtained from various sources, were subjected to pigment analysis. Pigments whose absorption maxima were identical with those of delta-carotene and rubixanthin were found in all the strains studied. The strain used as a test organism for determining phenol coefficients also contained another hydrocarbon. Four strains isolated from food poisoning cases and four from staphylococcic infections also contained an ester of a carotenol with absorption maxima the same as those of rubixanthin.

It is apparent that bacteria offer interesting possibilities for discovering the function of the carotenoid pigments in nature.

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