SPECTROPHOTOMETRIC CHARACTERIZATION OF THE CAROTENOID PIGMENTS ISOLATED FROM *MICROCOCCUS RADIODURANS*

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

SPECTROPHOTOMETRIC CHARACTERIZATION OF THE CAROTENOID PIGMENTS ISOLATED FROM *MICROCOCCUS RADIODURANS*

INTRODUCTION

The pink pigmentation of the radiation resistant micrococcus, isolated during the course of studies on food sterilization by ionizing radiation in 1954 (1, p. 576) was the most readily recognized property.

During the following years, the mode of radiation resistance, (6, p. 377-382) its metabolic properties (21, p. 291-298) and detailed description have been investigated. Therefore, it was felt necessary to identify the pigment in order to obtain a more complete description.

The pigment was soluble only in organic fat solvents and gave stable blue color reaction with concentrated sulfuric acid. These are considered to be the properties of carotenoid pigments. The subsequent studies were carried out in accordance with the techniques developed by the various workers for the characterization of carotenoids.

An attempt was also made to study the role of pigment production and the relationships between the radiation resistant property and the pigment.

HISTORICAL REVIEW

Pigmentation is a property easily recognized and it is not surprising to find the description of bacterial pigment appearing as early as 1873. Lankester (17, p. 408-425) described the photosynthetic bacterium, Rhodospillirum rubrum isolated from the river water. Although he did not recognize the photosynthetic characteristics of the organism, he noted its slow growth and phototactic behavior. The pigment which he could not dissolve in various organic solvents, water, acids or bases was named bacteriopurpurin. Van Niel et al. (26, p. 219-229) studied this organism and its pigment very extensively. The presence of this pigment is still being uncovered in various organisms. As late as 1960 Baxter, (2, p. 418-424) demonstrated its presence in halophilic bacteria.

Another group of pigments which have been extensively studied is that of the Sarcina. Reader (22, p. 1039-1046) studied the pigment from Sarcina aurantica and discovered that the exposure to light during growth, incubation temperature and the variation of pH near neutrality did not affect the pigment production, although growth had been affected. Ingraham and her collaborators (14, p. 2553-2562)

claimed as many as ten carotenoids in Mycobacterium phlei including alpha-carotene, beta-carotene, cryptoxanthin, zeaxanthin, azafrin and esters of lutein by the spectrophotometric method. They found that the pigment production was affected by the mineral elements in the media. Ferric salts, cupric salts, potassium and phosphate ions all inhibited the carotenoid production while the growth of the cell was unaffected. The lipogenic substances, glycols, glycerols and simple alcohols enhanced the pigmentation noticeably without growth stimulation.

Sobin and Stahly's (24, p. 265-276) study revealed that various carotenogenic bacteria were characterized by different carotenoids, particularly by the xanthophylls or their combinations, while showing that an identical carotenoid pigment could be found from the various sources. It is also important to note that beta-carotene was not as commonly found among bacteria as it was found in higher plants. They described carotenoids from various bacteria sources. Sarcina lutea and Sarcina flava contained sarcinine while Sarcina aurantica produced zeaxanthin. Micrococcus flavus yielded same pigment found in S. flava but Micrococcus luteus contained a xanthophyll, sarcinoxanthin. Their description included an unidentified new hydrocarbon commonly found in Flavobacterium esteroaromaticum, Flavobacterium suaveolens and Flavobacterium fecale,

Flavobacterium sulphrum, on the other hand, produced sarcinene. Flavobacterium arboresens contained five hydrocarbons including sarcenene and alpha-bacteriopurpurin. Their study showed that there was a similarity in pigment content among closely related species, as well as other commonly shared properties; however, exception to this generalization was also very frequent.

The isolation and identification of carotenoid pigments from bacterial sources have been extended to numerous organisms. Nevertheless, the complete characterization was hindered by the limited amount of pigment available. The optical property of the carotenoids in connection with the pigment structures as pioneered by Zechmeister and his co-workers (18, p. 1647-1653 and 28, p. 1930-1947) provided invaluable methods for the study of bacterial carotenoids. By understanding these optical properties, it was possible to use a formula to correlate the longest absorption maximum, the total numbers of double bonds, and the numbers of [=C(C)-] groups with a carotenoid structure (20, p. 291-300).

A gradual shift of interest from the isolation and identification of carotenoids to the formulation of biological functions and biosynthesis has become noticeable in recent years. As briefly mentioned before, (15, p. 18-19) the pigment formation is influenced by the growth

conditions and the availability of lipogenic substances which were believed to be the precursers for the biosynthesis of carotenoids. The findings indicated that the pigment formation by the cell is, to a degree, influenced by the growth conditions, accompanied with the physiological changes. Therefore, the functions of the carotenoids could not have been a vital one among organisms. Such a view is further supported by the fact that optimal growth and optimal pigmentation conditions are not identical.

The correlation between pigment composition and other property of a strain of bacteria was shown by Reimann et al. (23, p. 605-614). They found Micrococcus tetragenus to produce delta-carotene, lycopene, rubixanthin, lutein and rhodoxanthin. The kinds of pigments found in a given strain could be predicted from its colony morphology, i.e., lutein was the predominant pigment in yellow rhodoxanthin and pink to yellow ones were dominated by gamma carotene and rubixanthin. The function of carotenoid in above organisms serve only as a key for the differentiation and it was obvious that the pigments, which varied widely among the different strains of the same species, could not have been a physiologically fundamental one.

It was reported that a more pigmented Mycobacterium phlei had an increased acid-fastness over a less pigmented one (11, p. 128). It was later found, however, that this

was due to the elevated melting point of the lipoidal substance of the cellular periphery in which the pigment was dissolved (11, p. 128).

Among many postulated roles of carotenoids, the role in photosynthetic bacterium is better known. Volk et al. (27, p. 169-170) separated bacterial chlorophyll with methanol and the remaining pigment with chloroform from Rhodospillirum vannili and noted that the absorption maxima for the chlorophyll fraction in methanol were at 590, 805 and 880 mu while the carotenoids, beta-carotene, rhodopin and various isomeric forms of rhodovibrin had their absorption maxima between 350 to 550 mu. Further study by Thomas (25, p. 186-196) revealed the most efficient wave lengths for the bacterial photosynthesis were 460, 490, 525 and 590 mu which corresponded closely to the range of carotenoid absorption maxima. A similar study by French (21, p. 21-87 and 22, p. 469-481) indicated that the carotenoid pigments of Rhodospillirum rubrum were present in the cell as the protein conjugate which had absorption maxima at 590 and 900 mu. It was more than a coinciden that he found the cells using monochromatic lights of wave lengths, 590 and 900 mu, had incorporated carbon dioxide most effectively.

The actual demonstration that the light energy absorbed by carotenoids could be transfered to chlorophyll was not shown until Duysen (7, p. 548-550) measured the fluorescence of a chlorophyll fraction induced by the absorption of shorter wave length. For without carotenoid, the chlorophyll fraction did not show fluorescence when exposed to the shorter wave length. Therefore, the light energy absorbed by carotenoid must have been made available to chlorophyll. The chlorophyll fraction obtained by methanol extraction of R. rubrum had absorption maxima at 800, 850, and 890 mu. The 890 mu portion showed fluorescence. When the fluorescence action spectrum, which is proportional to the light energy absorbed, was studied the monochromatic rays corresponding to the absorption maxima of the carotenoid pigment produced fluorescence at 890 mu with about fifty per cent efficiency. This indicated beyond doubt that the light energy was absorbed by the carotenoid and the absorbed energy had been transfered to chlorophyll.

The biosynthesis of carotenoids is closely associated with the synthetic processes of non-saponifiable hydrocarbons such as terpenes and sterols. The isoprenoids arise from acetyl-Co A by condensation and conjugation (10, p. 664-666). The relationship between acetate and carotenoid has been shown by Guirard et al. (13, p. 361-379). They noted that lycopene could replace the sodium

acetate requirement of Lactobacillus arabinosus and postulated that sodium acetate and carotenoid were interconvertible, possibly acetate being the precurser of carotenoid synthesis. Millerd and Bonner (19, 1954 p. 343-355) studied enzymes from spinach leaf which catalyzed the conversion of acetyl-Co A to beta-hydroxy methyl crotonate involving the intermediates of acetoacetate, beta-hydroxy glutarate and beta-hydroxy isovalerate. Chichester et al. (5, 1955 p. 515-517) provided more direct evidence that acetyl-Co A was actually the precurser for carotenoid biosynthesis. They labeled pyruvate and noted that methyl carbon and carbonyl carbon were recovered in the beta-carotene of a carotenogenic mold of Phycomyces species. The labeled carboxyl carbon of pyruvate was lost presumably as carbon dioxide.

The indication that carotenoid biosynthesis might involve same intermediates for sterols synthesis was shown later by Braithwaite and Goodwin (3, 1957 p. 31p-32p). When unlabeled mavelonic acid was added to carotenogenic mold culture which contained labeled acetate, the radioactivity recoverable in beta-carotene was considerably reduced. Another intermediate for sterols synthesis, squalene, also diluted the activity of acetate recoverable in beta-carotene when they examined the same system later (4, 1957 p. 13p -14p).

EXPERIMENTAL METHODS AND MATERIALS

Growth of Cells

Cells of Micrococcus radiodurans were grown with vigorous aeration in trypton-glucose-yeast extract (TGY) broth fortified with 0.03 per cent by weight of DL-methionine. The cells were grown for 74 hours at 30°C and harvested with a Sharples steam turbine centrifuge. They were then washed three times with cold distilled water then frozen immediately unless used. For inoculum, cells kept growing continuously in the synthetic medium were used (21, p. 292). A 50 ml quantity of 48 hour old cells were used as inoculum for 7 liters of medium.

Pigment Extraction

The harvested cells were dispersed in ten times their volume of a half and half mixture of acetone and petroleum ether*. These were disrupted by sonic oscillation. The freeze stored cells were thawed and the pigment was extracted as follows: Three times the cell volume of absolute methanol was added to the disrupted cell suspension and filtered through Watman #1 filter paper. The above solvent mixture extracted most of the pigments from the cell and in addition coagulated the cellular debri to

*Petroleum ether refers to the fractions of boiling range between 30 to 75°C throughout this paper.

a sufficient degree that it enabled the recovery of pigment in clear filtrate. The usual amount of wet cells from 7 liters of 3 day old culture was about 40 grams. About 50 ml of solvent was necessary for the extraction.

The extract was dried free of solvent under reduced pressure and subsequently taken up in 250 ml of 0.5 molar methanolic potassium hydroxide and subjected to a temperature of 35°C for 16 hours to saponify the fats and esters.

The methanol-KOH solution was made to 300 ml with saturated aqueous sodium chloride upon the completion of saponification. This gave about an 80 per cent methanolic concentration and enough salt to force the hypophasic pigments into the petroleum ether phase during subsequent partition. A 100 ml portion each of above methanolic solution was shaken with equal volume of petroleum ether in separatory funnel. The petroleum ether phases and the methanol phases were pooled separately. They were repeatedly washed with fresh portions of each solvent for three times. The petroleum ether phases were combined and washed with distilled water by shaking in the separatory flask until the effluent water was no longer alkaline to Hydrion paper.* The washed petroleum ether layer was freed from water by drying over anhydrous sodium sulfate for 30

*Micro Essential Laboratory, Brooklyn 10, New York

minutes. Upon filtration through Whatman #1 filter paper, the water free petroleum ether phase was obtained. This fraction was dried under reduced pressure to obtain a pigment extract free of solvent. The anhydrous sodium sulfate adsorbed a portion of the pigment and an additional quantity of acetone was necessary to free the adsorbed pigment. On some occasions the drying process was omitted in order to eliminate this difficulty; however, the evaporation of solvent under such condition was very slow. The dried pigment preparation was stored at -25°C before fractionating with the column.

Column Separation of Pigment

The dried pigment preparation was taken up in 5 ml of petroleum ether and poured onto aluminum oxide.* This column was 10 cm in height and 2 cm in diameter. The aluminum oxide column gave the best separation for both epiphasic and hypophasic pigments. Magnesium oxide which was very effective for the separation of carotenoids of various bacterial origin as described by Sobin and Stahly (24, p. 268) adsorbed the pigments too strongly. Another common adsorbent, calcium oxide, was satisfactory for the separation of epiphasic pigments only.

The pigment preparation was adsorbed on top of the

*Baker analyzed reagent grade.

column, which had been flushed with petroleum ether previously. The solvent was prepared by mixing 5 parts of acetone with 95 parts of petroleum ether. As soon as the pigment layer came in contact with the solvent it started to migrate down the column and separated into two rapidly moving yellow bands and three slower moving pink bands.

Growth and Pigment Production of the Cell

In order to follow the pigment production and cell mass increase, a viable cell count and the pigment changes were studied and their inter-relationships observed.

The age of cell and pigment production. Two groups of 100 ml trypton-glucose-yeast extract culture flasks were prepared. A group of flasks contained no added methionine while the other was fortified with 0.03 gram of DL-methionine. The methionine added medium was the exact replica of the medium used to grow the cells for the pigment extraction in large quantity. Although the later medium paralleled closely the cultural conditions used for the extraction of pigments, it was not suited for study of the growth of cells by the turbidometry due to the darkening of such medium when cells were grown for longer periods (3 days or longer). These sets of cultures were compared for any difference of growth rate to insure the accuracy of the comparison with pigment production.

One ml of a cell suspension from a 48 hours old growth in synthetic medium was innoculated into all the flasks and incubated on 30°C shaker. A flask from each set was removed at 2 hours interval over a period of 5 days. Cells were harvested from each flask by centrifugation, washed three times with distilled water and suspended in 20 ml of petroleum ether-acetone-methanol mixture (10:10:3). The cell suspensions were left over night in the refrigerator (4°C) and then filtered through Whatman #1 filter paper. The pigment extraction was complete by this process. The extract was freed from water and solvents by drying under reduced pressure. The dried pigment was taken up with 10 ml of absolute methanol. The optical density of this solution was measured by Beckman spectrophotometer model DU against methanol blank at wave length of 475 mu.

The standard growth curve. Cell growth was followed by examining 10 ml of culture turbidometrically from each set of flasks at the same time the cultures were removed for pigment extraction. The methionine less cultures were diluted with media and optical densities determined against the media blank. The methionine cultures were centrifuged and the cells were resuspended in distilled water in proper dilutions to read the optical densities against water blank. Both determinations were made at wave length of

680 mu. The viable cell counts were also determined at the same time in order to relate the total cell mass gain with the numbers of viable cells.

Determinations of wave lengths. The wave length of 475 mu used for the determination of pigment concentration was derived from the observation that the absorption maximum of the crude pigment extract was always near 475 mu in methanol when checked with the spectrophotometer (Cary model 11). The wave length of 680 mu used to determine the cell mass concentration was selected because it was beyond the absorption range of carotenoid pigments and gave the least deviation from the growth curve as determined by a viable count during the logarithmic growth phase.

RESULTS AND DISCUSSION

The Pigments of M. radiodurans

When the saponified pigment extract was passed through the aluminum oxide column with petroleum ether-acetone solvent, a total of six recognizable bands were obtained repeatedly. The yellow fraction, constituting about 20 per cent of the total pigment, separated into two bands. The first yellow band (band #1) was recovered in the effluent solvent within forty minutes after the pigment extract came in contact with the solvent on top of the column. The entire column passage was carried out with a slight suction at room temperature (25°C).

The second yellow band (band #2) followed in the effluent solvent in next ten minutes. Both yellow bands were collected in separate containers and were freed from the solvents under reduced pressure. In partition tests, both bands #1 and #2 showed epiphasic behaviours. The yellow bands were dissolved in various solvents and the absorption maxima were determined by a recording spectrophotometer (Cary model 11) against each solvent blank. Band #1 gave absorption maxima at 420, 395 and 374 mu in petroleum ether, in carbon disulfide, the absorption maxima were at 498, 450, and 423 mu and in methanol they were at 420, 397 and 372 mu (Fig. 1).

Band #2 seemed to be a mixture of band #1 and band #3 because it showed absorption maxima corresponding to both bands in less definite peaks. The repeated column passage of this fraction through the aluminum oxide, magnesium oxide and calcium carbonate columns with various solvents did not result in the satisfactory separation of band #2. This might indicate the close chemical relationship between band #1 and band #3 .

Fraction #3 was eluded from the column approximately twenty minutes following the removal of band #2. It was orange in color and consisted of 10 to 15 per cent of the total extractable pigment from the cell. This fraction did not vary in its concentration to any appreciable degree throughout the age of the cell (Table 1). It showed the most definite absorption spectra, as determined by Cary recording spectrophotometer. The absorption peaks in petroleum ether were at 483.5, 451 and 426 mu; in methanol they were at 481, 451 and 426 mu; and in carbon disulfide at 518, 484 and 458 mu, respectively (Fig. 1). On partition between petroleum ether and 90 per cent methanol this fraction showed a complete hypophasic behaviour, indicating the presence of at least two hydroxy groups in the pigment molecule (16, p. 21).

The fourth red band was eluded from the column ten minutes after band #3 and showed a single absorption peak

Table I

Cartenoids found from M. radiodurans

Band number	Elusion time from column in minutes[1]	Partition behavior between pet. ether and 90% methanol	Absorption maxima in mu			Per cent conc. of pigment fraction[2]	Remarks
			pet. ether	CS_2	CH_3OH		
1	40	Epiphasic	420 395 374	498 450 423	420 397 372	10-20	It may be the shorter than 40 carbon chain pigment derivative
2	50	Epiphasic	-	-	-	0-10	Mixture of 1 and 3
3	70	Hypophasic	483.5 451 426	518 484 458	481 451 426	10	Probably zeaxanthin
4	80	Hypophasic	473	515	475	5-70[3]	Non-acidic keto form of either or both pigments #3 and #6
5	85-115	Hypophasic	-	-	-	10-20	Mixture of #4 and #6
6	120	Hypophasic	498 470 447	541 508 475	500 475 447	10-60	Resembles lycophyll

Table 1 - continued

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[1]10 cm of aluminum oxide (Baker analyzed reagent) of 2 cm inner diameter with 1:1 mixture of acetone-petroleum ether solvent at 25°C with slight suction.

[2]The comparison of the absorption peak heights of each fraction obtained during numerous repetition of the pigment separation.

[3]The quantitative conversion of fraction 6 into fraction 4 was observed during the prolonged alkali saponification.

at 475 mu in methanol, 585 mu in carbon disulfide and 473 mu in petroleum ether (Fig. 1). It was hypophasic.

The fifth band did not show a sharp bend but rather diffused between bands #4 and #6. As was the case with band #2, it seemed to be a mixture of band #4 and band #6.

The last pigment eluded from the column about forty minutes after band #4. This hypophasic pigment showed less definite peaks at 500, 475, and 447 mu in methanol; in carbon disulfide they were at 541, 508, and 475 mu, and were at 498, 470, and 447 mu in petroleum ether (Fig. 1). The shift of absorption peaks toward the longer wave length indicated the presence of more conjugated double bonds than in band #3 (16, p. 54).

The sharpness of peaks and the repeated isolation of band #1 from the pigment extract indicated that it was a distinct and stable chemical entity produced by M. radiodurans. The stable blue color reaction with sulfuric acid suggested that it was a carotenoid despite the fact that the absorption range of this pigment was at the shorter wave length. This description of a pigment corresponding to band #1 was lacking in the literature but the above information suggested that it could have been a carotenoid of less than forty carbon skeleton. From the data at hand one could postulate the following: (1) The pigment #1

could have been the biosynthetic intermediary of other pigments of the organism; (2) The degradative product of other pigments or, (3) an independent pigment. If it were either a biosynthetic intermediary or a degradative product one could have expected the concentration to fluctuate in relation to other pigments during growth. The cultures were examined for the pigment changes during growth but no evidence was present to indicate whether fraction #1 had increased or decreased during growth. When the same experiment was carried out with 7 liter cultures, band #1 fraction had increased at 120 hours of growth over that of 24 hours. This would have seemed to favor the possibility (2) but the change of concentration was very slight and other pigment fractions which were expected to decrease with the increase of fraction #1 also increased. Besides, it is not known whether the carotenoid biosynthesis is a de novo phenomenon in that a pigment is produced individually without any accumulation of detectable intermediates (10, p. 666) or via the condensation of preformed isoprene units involving recognizable steps (10, p. 664-666). The possibility (3) was the simplest and most attractive explanation; however, such conclusion must be reserved until more physico-chemical data become available.

The absorption spectra of band #3 agreed closely with that of zeaxanthin which is 3, 3' dihydroxy beta-carotene (16, p. 180-185). The spectra also indicated the pigment contained 11 conjugated double bonds, for the spectra would have shifted toward shorter wave length if it had contained less than 11 double bonds and if it had more than 11 double bonds, the shift would have resulted toward longer wave length (16, p. 54). The absorption spectra and the partition test did not rule out the possibilities that the pigment was either one of the following carotenoids such as xanthophyll ($C_{40}H_{56}O_2$), or pectanoxanthin ($C_{40}H_{56}\pm 2O_3$) besides zeaxanthin ($C_{40}H_{56}O_2$). The solubilities and color reactions with concentrated hydrochloric acid of zeaxanthin and xanthophyll are similar. They are fairly soluble in carbon disulfide and chloroform and produce stable blue colors in sulfuric acid. Therefore, the solubility test and the color reaction of fraction #3, which confirmed above properties, did not permit the distinction to be either one of the two pigments. Pectenoxanthin is a pigment isolated from sex organ of St. Jaques shell (16, p. 326) and the description of this pigment was too scanty to allow adequate comparison with pigment #3. Since, with such small amounts, no chemical test was available for identification of this pigment, it was suggested that this pigment could be zeaxanthin.

The single absorption peak of band #4 suggested the presence of a ketonic carotenoid (16, p. 56). The ketonic carotenoids having absorption range similar to fraction #4 were astacene, astaxanthin and chrysophlein (16, p. 58-59, 229, 231). The alkali saponification process and the atmospheric oxygen were found to convert astaxanthin to its oxidized form (16, p. 235-237) and it was possible that this fraction was the oxidized form of pigment #6 which constituted the major pigment portion. The prolonged alkali saponification resulted in the quantitative increase of band #4 while band #5 and band #6 decreased proportionary. The complete conversion of pigment #6 into #4 was observed at the end of 24 hours incubation at 35°C in an extract of 1.5 molar methanolic potassium hydroxide. The chemical composition of this ketonic carotenoid is not known. All the ketonic carotenoids so far reported were acidic while this pigment did not show acidic property. The only suggestion that could be made regarding this pigment was that it could have been the ketolated form of pigment #6.

The absorption spectra of pigment #6 were related to that of lycophyll which has a structure of 3,3' dihydroxy lycopene. The structure is closely related to zeaxanthin. By simply opening the closed rings of zeaxanthin between carbons 1 and 6 and 1' and 6', it assumes the structure

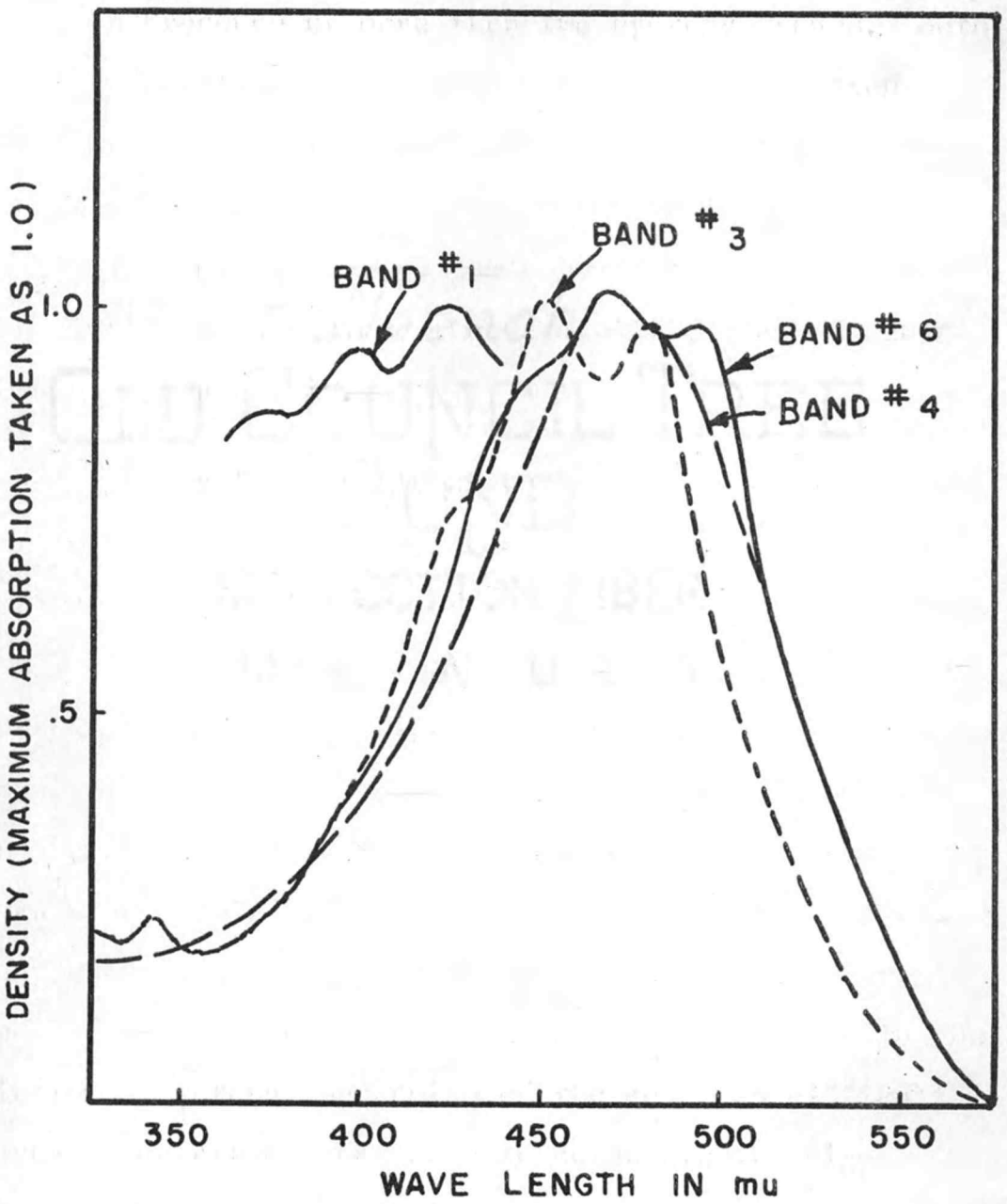

Figure 1. Absorption spectra of pigments in methanol.

of lycophyll. The absorption spectra also indicated the presence of 13 double bonds while the partition test suggested the presence of more than two hydroxy groups. Both bits of information strongly supported the suggestion that pigment #6 was lycophyll. If we assume that all of the pigments of M. radiodurans were structurally related, the possibility that pigment #6 could be lycophyll also furnished indirect support to the possibility that pigment #3 could be zeaxanthin.

Role of Pigment in M. radiodurans

It has been observed by the previous workers that the gamma radiation resistance of M. radiodurans was dependant on the age of the cell (1, p. 577 and 6, p. 382). The older the culture became, the higher the resistance up to about five days. One of the possible explanations given for this phenomenon was that during the cell growth there was an accumulation of protective material being formed. The pigments of this organism were suspected; however, direct demonstration that the pigments participated in the radiation resistance mechanism of the cell was difficult to prove. An attempt was made to correlate the pigment production with radiation resistance in order to demonstrate any possible relationships which might exist.

The viable count of the cells was less reliable than the turbidometric determination of growth, because there was no assurance that a colony originated from a single cell or from the multiples of it.

The pigment increase, when followed by the optical density, showed the same exponential increase as that of the cell mass as illustrated in Fig. 2. However, the pigment production when plotted simultaneously with cell mass did not coincide with each other. It lagged approximately 10 hours behind. The younger cells contained less pigment per cell. Since the duration of the exponential phases of both growth and pigment production were similar, the post exponential cells had higher pigment contents. The maximum pigmentation was attained between 40 to 60 hours of growth. Beyond that period the decrease of pigment was rather rapid (Fig. 2). Figure 3 gives Optical Densities at 475 mu divided by the corresponding O.D. at 680 mu plotted against time in hours. A comparison with the rate of cell mass increase, radiation resistance, adapted from a previous work, illustrates their relationships.

The Cell Age and Radio-resistance

This relationship is illustrated in Fig. 2 and 3. Although the radio-resistance increased with the age of cells, the cell mass increase and radio-resistance did not

follow an identical course. Since the growth curve and the radio-resistance were plotted on the different scales, no direct comparison was attempted. However, it was noteworthy that the exponential natures of growth and radio-resistance and their durations were similar. It was also noted that the cell mass decrease was rather pronounced during the advanced age.

Pigment Production and the Radio-resistance

The correlation between pigment production and the radiation resistance was less than that observed between the growth and resistance. The exponential increase of pigmentation started about 10 hours behind that of resistance. Furthermore, the pigmentation of the cell decreased rapidly at the age when the radio-resistance was still high. This appears not to be compatible with the view that the pigments are directly associated with the radiation resistant mechanism.

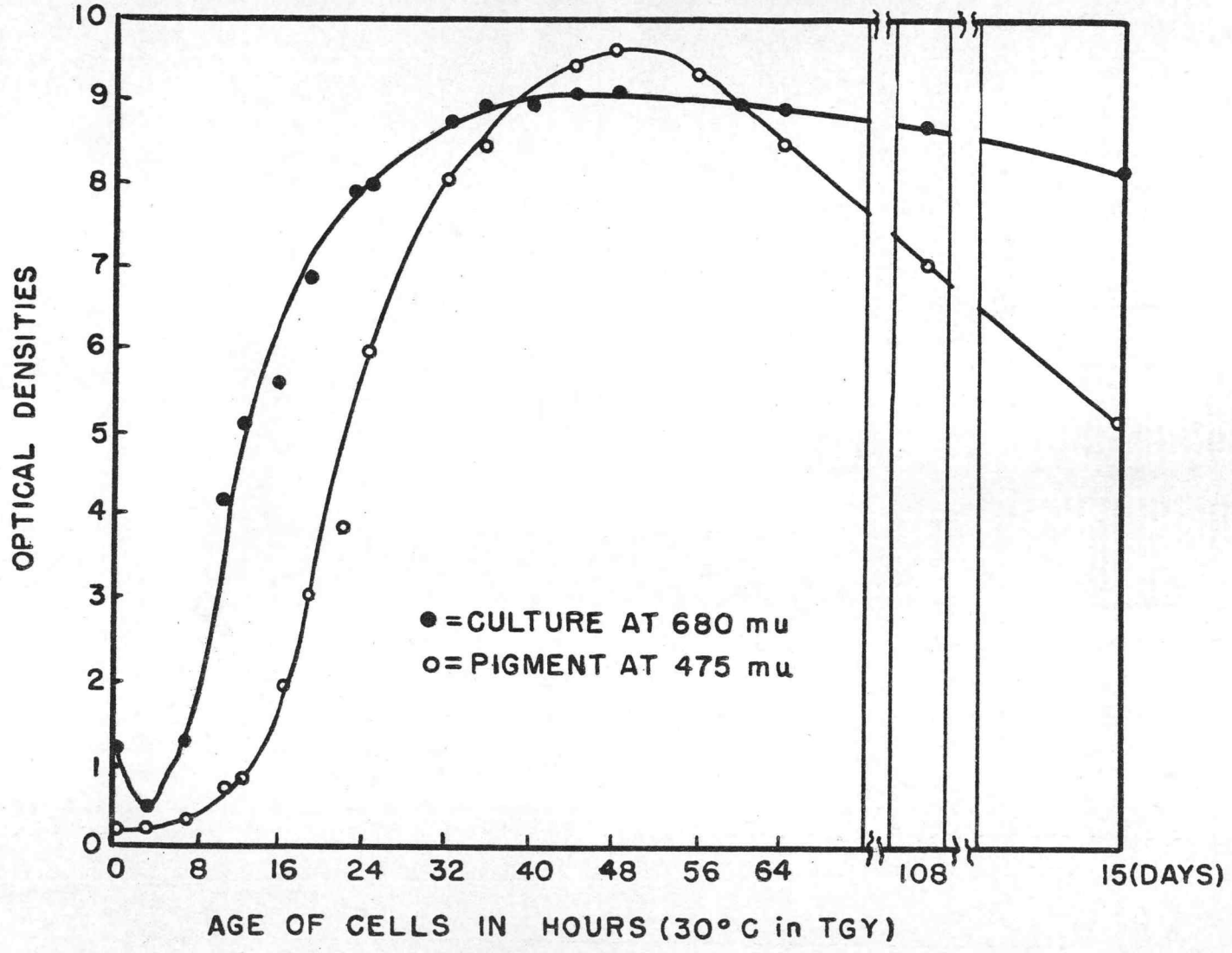

Figure 2. Cell mass increase compared with pigment production.

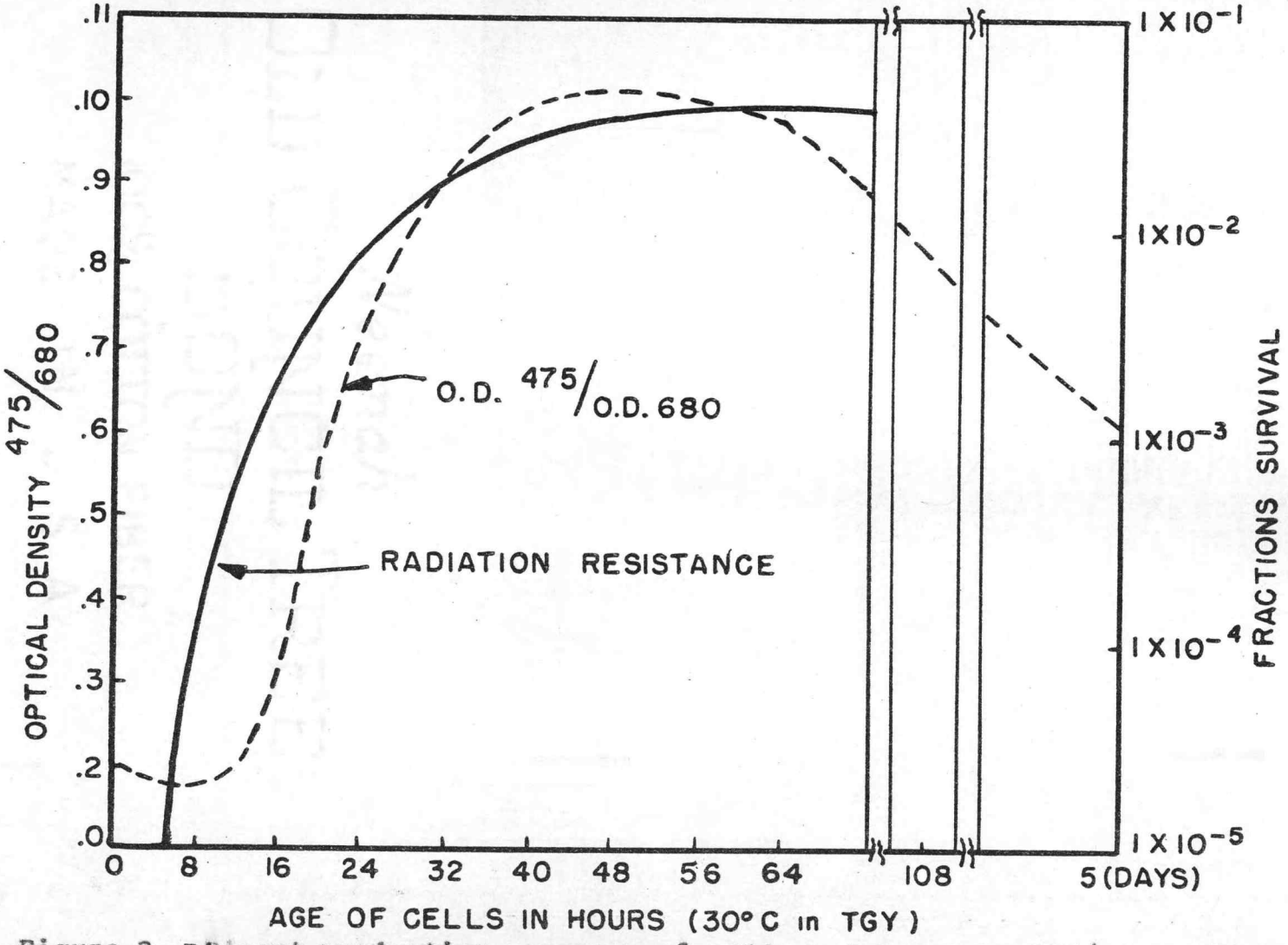

Figure 3. Pigment production per mass of cell as taken O.D. 475/ O.D. 680 and gamma radiation resistance under 1.85 megarad exposure adapted from Duggan et al. (6, p. 382)

SUMMARY

A system was developed to extract, purify and separate the carotenoid pigments of M. radiodurans. The pigments were:

1. A hydrocarbon probably having shorter than 40 carbon chains.

2. Two xanthophylls. One of them resembled zeaxanthin and the other, lycophyll.

3. A non-acidic ketonic xanthophyll. It appeared to be the oxidative product of either or both of zeaxanthin and lycophyll.

A study showed that the pigment production did not parallel the growth curve, rather it lagged about 10 hours behind.

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