mild acid hydrolysis precludes any form of phenolic glucuronide formation. Bushby & Woiwod (1955) assumed from analogy with 4:4'-diaminodiphenyl sulphone *NN'*-didextrose sulphonate (Promin) that the linkage was through an anil bond. Such a linkage might explain the sugar-containing metabolite of 3:5-dibromosulphanilamide isolated from human urine by McChesney (1947), and also the so-called labile glucuronides found by Smith & Williams (1949*a, b*) in the urine of rabbits fed on aniline and phenetidine. However, Boyland & Manson (1955) have found in the urine of rabbits given naphthylamine a conjugate of naphthylamine with glucuronic acid which resembles our conjugate in its lability to acid and to which they ascribe an *N*-glucosiduronic acid structure. This they suggested could also be the structure of the labile glucosiduronates reported by Smith & Williams (1949*a, b*).

Although this report is concerned with the metabolism of DADPS in the rabbit and the monoconjugate of DADPS and glucuronic acid has so far been identified only in this animal, preliminary experiments (Bushby & Woiwod, 1955) show that the drug is excreted by man as a monoconjugate with glucuronic acid, though the linkage may possibly be that of a phenolic glucuronide rather than that of an anil. Monosubstituted derivatives of DADPS are known to have antileprotic and antitubercular activity (Cochrane, 1952; Smith, Jackson & Bauer, 1949). Therefore, in view of the high proportion of DADPS present in the body in a monoconjugated form, these observations may be of chemotherapeutic importance.

SUMMARY

1. The major diazotizable metabolite of 4:4'-diaminodiphenyl sulphone (DADPS) has been isolated from rabbit urine and identified as a glucuronic acid conjugate.

2. One molecule of DADPS is combined with one of the acid, and ionophoresis shows that the carboxyl group of the acid is free. The metabolite is acid-labile and breaks down to glucuronic acid and DADPS. The conjugation is therefore probably between the aldehydic carbon and the nitrogen of one of the amino groups, and the linkage may be either that of an anil or an *N*-glucosiduronic structure.

3. The metabolite has been synthesized.

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Studies in Carotenogenesis

21. THE NATURE OF THE CHANGES IN CAROTENOID SYNTHESIS IN *RHODOSPIRILLUM RUBRUM* DURING GROWTH*

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The studies of Engelmann (1883, 1888), later confirmed by Molisch (1907) and Buder (1919), had established that purple bacteria can respond by phototaxis, reversing the direction of their motion when experiencing a sudden drop in light intensity. Hence the bacteria tend to accumulate in areas where the light intensity is perceived as highest. In a spectrum they aggregate in bands whose position

indicates which wavelengths are most effectively absorbed. Pertinent experiments had indicated that the accumulation bands coincide with regions of maximum absorption by both the green (chlorophyllous) and the red (carotenoid) pigments of the organisms. Thus a simple and satisfactory picture had emerged. However, as our knowledge of the carotenoids of the purple bacteria developed, some curious inconsistencies became apparent.

* Part 20: Goodwin & Land (1956*a*).

It has been shown that *Rhodospirillum rubrum* contains spirilloxanthin as its main if not only carotenoid (van Niel & Smith, 1935; Polgár, van Neil & Zechmeister, 1944; Goodwin & Osman, 1953). To this pigment are attributable the absorption maxima of the bacteria at 550, 510 and 480 m μ . Both Molisch (1907) and Buder (1919) detected accumulation by phototaxis of red spirilla, not at 550, but at 530 m μ , where absorption by spirilloxanthin is at a minimum. This discrepancy was not commented on till 1944. Based on the finding that some types of photosynthetic spirilla do not produce spirilloxanthin but, instead, other carotenoids with absorption maxima around 530 m μ , the suggestion was then made (van Niel, 1944, pp. 55, 66-68) that Molisch and Buder might have used such brownish rather than red spirilla for their phototaxis experiments. From the study of Giesberger (1947), it is evident that the brown spirilla are quite as common in nature as are the red ones.

Nevertheless, this suggestion fails to explain some recent results, notably the action spectrum for phototaxis of *Rsp. rubrum* determined by Manten (1948) and Milatz & Manten (1953), which, too, has maxima at 530 and 490, and a minimum at 550 m μ . Manten concluded that spirilloxanthin is not involved in inducing phototaxis, which is actually associated with a minor carotenoid with absorption maxima at 530 and 490 m μ , and stated that 'the hypothesis of a complete identity of the bands of accumulation in a spectrum and the location of the absorption bands of a suspension of living cells in the green and blue regions, as generally accepted since Engelmann, however, must be refuted' (Manten, 1948, p. 47, footnote).

Manten further deduced that the response by phototaxis is the result of a sudden drop in the photosynthetic rate. This deduction has been supported by later studies (Thomas & Nijenhuis, 1950; Clayton, 1953*a, b, c*, 1955*a, b, c*). Especially significant are the photosynthetic action spectra (Thomas, 1950; Thomas & Goedheer, 1953) which again show maxima at 530 and 490, and a minimum at 550 m μ . Hence Thomas inferred that the major pigment of *Rsp. rubrum* is ineffective as a collector of radiant energy for photosynthesis, in contrast to a minor one with the requisite absorption characteristics.

The inner consistency of these results and conclusions is, however, marred by the action spectrum for phototaxis of *Rsp. rubrum* determined by Clayton (1953*a*); it shows maxima at positions coinciding with the absorption maxima of both spirilloxanthin and another carotenoid, presumably the one to which Manten ascribed all activity in the green region. Furthermore, preliminary experiments with L. R. Blinks (unpublished work; see van Niel, 1954) have indicated that *Rsp. rubrum* can

carry out a normal photosynthesis with radiant energy primarily absorbed by spirilloxanthin. To be sure, the differences in action spectra for phototaxis found by Manten and Clayton respectively may be tentatively ascribed to differences in the strains of *Rsp. rubrum* used in the investigations (Clayton, 1953*a*). But such an explanation is not wholly satisfactory, because it introduces a new difficulty, namely, the need to understand why the major carotenoid, spirilloxanthin, sometimes does and sometimes does not function as appropriate photosensitizer for phototaxis and photosynthesis.

Another interpretation has now been found which can account for the observed facts in a simpler manner. This is based upon the report of Duysens (1952) that the absorption spectrum of *Rsp. rubrum* changes with the age of the culture. Fully corroborated by systematic measurements (van Niel & Airth, unpublished work; see van Niel, 1954), it now appears that the absorption maxima of spirilloxanthin are undetectable in cultures of *Rsp. rubrum* growing exponentially; such cultures contain, instead, considerable amounts of pigments with absorption maxima at about 530, 490 and 460 m μ , whereas in old cultures these carotenoids are almost completely absent and spirilloxanthin becomes predominant. Thus the alleged inactivity of spirilloxanthin, deduced from the lack of an action maximum at 550 m μ , may be due merely to the fact that spirilloxanthin was not (yet) present in sufficient quantity to effect an observable action maximum at this wavelength. Confirmation of this view is supplied by the absorption spectra published by Thomas & Goedheer (1953, Fig. 3).

Since, for the isolation of carotenoid pigments from bacteria, one is likely to use cultures that have reached their maximum density, the previous experience that spirilloxanthin is the main carotenoid of *Rsp. rubrum*, based as it is on the actual isolation of this substance, is fully understandable. Conversely, the predilection for physiologically young cultures in studies on phototaxis and photosynthesis implies that the organisms used are apt not yet to contain this pigment to any appreciable extent, so that its physiological role cannot make itself felt.

The unexpected differences in carotenoid composition of young and old cultures of *Rsp. rubrum* have raised the problem of interpreting this change in detail. The following study presents the results of an attempt in this direction. A brief preliminary report has already been given (Goodwin & Sissins, 1955).

EXPERIMENTAL

Culture. The *Rsp. rubrum* strain used was that normally in use in this Laboratory (Goodwin & Osman, 1953, 1954).

Cultural conditions. The bacteria were grown in the light at 30° under the conditions previously described in detail (Goodwin & Osman, 1953, 1954).

Extraction, chromatography and identification of pigments. The pigments were extracted by Goodwin & Osman's (1953) slight modification of the original method of Polgár *et al.* (1944) and chromatographed on alumina (Goodwin, 1952*a*). Iodine isomerization of the pigments was carried out by the method of Zechmeister (1944).

Quantitative determinations of the relative amounts of pigment present were made by dissolving the pigment fractions in a known volume of light petroleum (b.p. 40–60°) and measuring E values of the solutions at the wavelength of maximum absorption and comparing them with the $E_{1\%}^{1\text{cm}}$ values for the pure pigments (see Goodwin, 1952*b*; and Goodwin & Land (1956*b*)). When the values for $E_{1\%}^{1\text{cm}}$ were unknown they were assumed to be that of β -carotene (2500).

RESULTS AND DISCUSSION

A typical chromatographic separation of the pigment of 24–28 hr. *Rsp. rubrum* cultures is given in Table 1. Fraction *A* has the absorption spectrum and adsorptive properties of lycopene; this identification was confirmed (*a*) by the failure of a mixture of pigment *A* and an authentic specimen lycopene (from tomatoes) to separate on chromatography on alumina, and (*b*) by the observation that the change in the absorption spectrum of pigment *A* brought about by iodine isomerization was the same as that observed with authentic lycopene (Zechmeister, 1944; Goodwin & Land, 1956*b*). It is almost certain that rhodopurpurene isolated from *Rhodovibrio* and *Rhodobacillus* spp. (Karrer & Solmssen, 1936), and which 'showed great similarity with lycopene' (Karrer & Jucker, 1950), is lycopene (see also Goodwin & Land, 1956*b*).

Fraction *B* was identified by spectroscopic, chromatographic and iodine-isomerization tests with the carotenoid P481 first obtained from a *Chromatium* sp. by Goodwin & Land (1956*b*).

Fraction *C* was identified as spirilloxanthin by spectroscopic and chromatographic comparison with an authentic specimen obtained from *Rsp.*

rubrum (Goodwin & Osman, 1953). Traces of *cis*-spirilloxanthins were also always present in this fraction; it is probable that they were artifacts, for spirilloxanthin easily isomerizes under the mildest manipulative treatment.

Fraction *D* was similarly identified with lycopanthin (3-hydroxycopene) from berries of *Solanum dulcamara* by Zechmeister & Cholnoky (1936), and with rhodopin from *Rhodobacillus* and *Rhodovibrio* spp. (Karrer & Solmssen, 1936). Details of the proof of the identity of pigment *D*, which occurs in almost all Thio- and Athio-rhodaceae so far examined (Goodwin, 1956), with lycopanthin and rhodopin have recently been given by Goodwin & Land (1956*b*).

Fraction *E* was shown to be identical with hydroxy-P481, found in *Chromatium* spp. by Goodwin & Land (1956*b*). According to these latter investigations, it is also probably identical with rhodovibrin (Karrer & Solmssen, 1936).

Pigment *F*, which also occurs in *Rsp. rubrum* (Goodwin & Osman, 1953), has a chromophoric system identical with that of spirilloxanthin. Because of its increased adsorptive power, Goodwin & Osman (1953, 1954) suggested that it is either a monohydroxyspirilloxanthin or spirilloxanthin with one methyl group removed (i.e. demethylated spirilloxanthin).

Quantitative experiments

The absorption spectra of the total carotenoid extracts of *Rsp. rubrum* during growth (Fig. 1) show a progressive shift towards the red end of the spectrum; this indicates that the relative amounts of the constituent pigments are changing. Quantitative chromatography has confirmed this, and in Figs. 2 and 3 are recorded the results of a typical experiment. Fig. 2 gives the amounts of the pigments produced, and Fig. 3 the relative amounts of each pigment present (as percentages of the total pigments). Because the rate of growth in the early stages (24–36 hr.) can vary somewhat from experi-

Table 1. *Chromatographic separation of the carotenoid pigment in young (24 hr.) cultures of Rsp. rubrum*

Absorbent: deactivated alumina; developer: light petroleum containing increasing amounts of ethyl ether. Pigments listed in order of increasing adsorptive power.

Zone	Description	Absorption maxima (m μ) in light petroleum	Ethyl ether in light petroleum required for elution (%, v/v)	Identification
<i>A</i>	Yellowish	445, 472, 501	2–4	Lycopene (rhodopurpurene)
<i>B</i>	Pink	452, 480–481, 512	8	P481
<i>C</i>	Pinkish purple	465, 490, 524	18	Spirilloxanthin
<i>D</i>	Orange-pink	445, 472, 501	20–22	Lycopanthin (rhodopin)
<i>E</i>	Pink	452, 480–481, 512	25–28	Hydroxy-P481
<i>F</i>	Pinkish purple (traces only)	464, 490, 524	35–50	Demethylated spirilloxanthin

* Light petroleum (b.p. 40–60°) was used throughout this investigation.

Fig. 1. Changes in the absorption spectrum of the carotenoids of *Rsp. rubrum* during growth. —, 24 hr. cultures; —●—, 36 hr. cultures; — — —, 48 hr. cultures. The $E_{\max}$ of each curve has been adjusted to the same value for ease of comparison.

Fig. 2. Synthesis of the component carotenoids by *Rsp. rubrum* during growth. —●●●—, Lycopene; —●—, P481; —●—, spirilloxanthin; —●—, lycopexanthin; —●—, hydroxy-P481.

Fig. 3. Relative amounts of the component carotenoids in *Rsp. rubrum* during growth. Values in percentage of the total pigment present. A, Lycopene; B, P481; C, lycopexanthin; D, hydroxy-P481; E, spirilloxanthin.

ment to experiment the following procedure was followed to obtain strictly comparable results: six 120 ml. cultures (24 hr.) were bulked and mixed thoroughly, and 200 ml. was taken for analysis; the remainder was redispersed in four 120 ml. bottles, two of which were examined after 36 hr., and two after 48 hr. Large cultures (300–1000 ml.) could not be used, as they did not produce appreciable growth, after 24 hr. under our conditions. The variations in cultures over 48 hr. old are slight (Goodwin & Osman, 1953), and separately incubated cultures were taken for analysis.

Figs. 2 and 3 show clearly that with the exception of spirilloxanthin the carotenoid components rise to their maximal levels about 24–48 hr. after inoculation and thereafter decrease, so that all except P481 and hydroxy-P481 have disappeared by the time the cultures are 72–96 hr. old. Spirilloxanthin, on the other hand, is synthesized relatively slowly during the early stages of growth, but continues to

increase rapidly after 36 hr. until growth is completed in 5–6 days (Goodwin & Osman, 1953). In 24–36 hr. cultures, spirilloxanthin represents only about 20–30 % of the total pigments present. Its increased rate of synthesis in older cultures, coupled with the gradual disappearance of the other pigments, results in it representing 92 % of the total pigments, in 5- to 6-day cultures. This agrees with previous observations (Polgár *et al.* 1944; Goodwin & Osman, 1953). The remaining 8 % is made up of P 481 and hydroxy-P 481, lycopene and lycoxanthin having disappeared completely after 72–96 hr. This differential rate of synthesis among the carotenoids parallels the situation in cultures of *Phycomyces blakesleeanus* inhibited by diphenylamine (Goodwin, Jamikorn & Willmer, 1953); in this fungus, the rate of synthesis of the phytofluene series is high in young cultures but falls off rapidly with age, whereas that of β -carotene reaches its maximum only in older cultures.

SUMMARY

1. The problem of carotenoids and phototaxis in *Rhodospirillum rubrum* is discussed.

2. Carotenoids present in young *Rsp. rubrum* cultures are lycopene (rhodopurpurene), spirilloxanthin, lycoxanthin (rhodopin), demethylated spirilloxanthin, pigments P 481 and hydroxy-P 481 (? rhodovibrin).

3. The relative amounts of the constituent pigments present in cultures of various ages are recorded. Spirilloxanthin gradually increases throughout the growing period. All the other components reach a maximum at about 24 hr. and thereafter decline, some being completely absent from 72–96 hr. cultures.

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On the Origin of the Multiple Forms of Rabbit γ -Globulin

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γ -Globulin is complex biologically in that it may contain many different antibodies; it is complex physically, as is shown, for example, during electrophoresis, and in certain species chemical complexity is apparent from the varying terminal amino acids. Most protein preparations show some degree of heterogeneity when examined with sufficient care (Colvin, Smith & Cook, 1954), but the complexity of

γ -globulin appears to be altogether greater than that found in well-characterized proteins.

It has been found that resolution of rabbit γ -globulin can be effected by partition chromatography and that the distribution of antibodies, among the fractions obtained, depends on the route and duration of the antigenic stimuli (Porter, 1955; Humphrey & Porter, 1956). It became apparent