are not so marked as those occurring in young and foetal liver. This is to be expected by analogy with thymine catabolism.

The effect of X-radiation on thymine catabolism in regenerating liver shows that, under conditions in which the formation of thymidine kinase is delayed, a delay in the depression of thymine catabolism also occurs. It is possible that the depression in thymine catabolism may be influenced by the appearance of thymidine kinase which occurs at about 18 hr. after partial hepatectomy (Bollum & Potter, 1959). When the formation of thymidine kinase is inhibited by X-radiation, the depression in thymine catabolism is delayed.

SUMMARY

1. The activities of enzymes involved in nucleic acid metabolism in foetal, young and regenerating rat liver were compared with those in normal rat liver.

2. Thymidine phosphorylase and uridine phosphorylase were less active in foetal and young liver than in adult liver. Thymidine-phosphorylase activity was also reduced in regenerating liver.

3. Thymidine was incorporated into DNA *in vitro* more rapidly in the liver of foetal and young rats than in adult rats.

4. Dihydro-orotase activity was maximal in the liver at birth when it was twice as active as in the adult.

5. Total-body X-radiation (400 r.), when given to rats 6 hr. after partial hepatectomy, delayed the decrease in thymine catabolism.

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The Association of Protein Synthesis with the Formation of Pigments in some Photosynthetic Bacteria

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The formation of the photosynthetic pigments, bacteriochlorophyll and carotenoids, by *Rhodospirillum rubrum* and related organisms is closely associated with protein synthesis under a variety of conditions (Lascelles, 1959; Cohen-Bazire & Kunisawa, 1960; Gibson, Neuberger & Tait, 1962a; Sistrom, 1962). This association also occurs in suspensions of non-pigmented organisms which form bacteriochlorophyll and carotenoids when incubated at a low pressure of oxygen. The

inhibition of protein synthesis by the addition of chloramphenicol, 8-azaguanine or *p*-fluorophenylalanine, or by omitting a nitrogen source, also inhibits pigment synthesis (Lascelles, 1959). During incubation of the organisms under these conditions there is a rise in the activity of enzymes required in the early stages of bacteriochlorophyll synthesis, namely δ -aminolaevulinate synthetase and δ -aminolaevulinate dehydratase; this increase precedes bacteriochlorophyll formation, suggesting

that synthesis of these enzymes is necessary before pigment synthesis can occur.

The pigments of photosynthetic bacteria are associated with subcellular particles, the chromatophores, which can be isolated by differential centrifuging from cell-free extracts as particles sedimenting at 153s (Schachman, Pardee & Stanier, 1952); these correspond to structures seen in electron micrographs of sections of intact organisms (Vatter & Wolfe, 1958; Hickman & Frenkel, 1959). They are rich in lipoprotein (Newton & Newton, 1957; Bergeron, 1959; Gibson *et al.* 1962a; Lascelles, 1962). When grown aerobically, organisms of the family Athiorhodaceae do not form the photosynthetic pigments (van Niel, 1944; Cohen-Bazire, Sistrom & Stanier, 1957), and chromatophores cannot be demonstrated either by electron microscopy (Vatter & Wolfe, 1958; Hickman & Frenkel, 1959) or by ultracentrifuging (Schachman *et al.* 1952). However, aerobically grown organisms do contain particles sedimenting at 55s which are not present in those grown in the light (Schachman *et al.* 1952).

Synthesis of the pigments might be obligatorily linked to the formation of part or all of the chromatophore protein, possibly by a modification of the 55s particle or by complete synthesis of the 153s particle. Alternatively there might be a link between the synthesis of the pigments and of enzymes needed for their formation. Linkage of either, or both, of these types could account for the observations of a relation between the synthesis of protein and that of pigment.

In the present work these possibilities have been considered in examining the relation between the synthesis of protein and that of pigment in growing cultures and suspensions of some members of the Athiorhodaceae. The effect of inhibitors of protein synthesis on the differential rate of formation of bacteriochlorophyll, and of various enzymes including aminolaevulinate synthetase, has been examined in cultures growing anaerobically in the light. In addition, the synthesis of chromatophore protein by suspensions of non-pigmented organisms, adapting to form pigments, was investigated by measuring the incorporation of [1-¹⁴C]phenylalanine. The rate of incorporation into chromatophore protein was compared with that into the soluble protein fraction under various conditions to see if there was synthesis of chromatophore protein specifically associated with the formation of the photosynthetic pigments.

MATERIALS AND METHODS

Chemicals. 8-Azaguanine, DL-5-methyltryptophan and DL-β-thien-2-ylalanine were obtained from the Sigma Chemical Co. (St Louis, Mo., U.S.A.) and DL-ethionine from

Roche Products Ltd. (Welwyn Garden City, Herts). DL-*p*-Fluorophenylalanine was from L. Light and Co. (Colnbrook, Bucks), chloramphenicol from Parke, Davis and Co. Ltd. (Hounslow, Middlesex) and 5-fluorouracil was a gift from Dr Morrison of Roche Products Ltd. (Welwyn Garden City, Herts).

DL-[1-¹⁴C]Phenylalanine and [2-¹⁴C]uracil were purchased from The Radiochemical Centre (Amersham, Bucks.).

Organisms. For most of the experiments and unless stated otherwise the strain of *Rhodospseudomonas spheroides* used, N.C.I.B. 8253, was that described by Lascelles (1956). The mutant 6A5 requiring histidine and adenine was isolated from this strain after ultraviolet irradiation. The white mutant L56, which formed no photosynthetic pigments, was obtained by repeated subculture of *Rps. spheroides* 8253 under highly aerobic conditions as described by Griffiths & Stanier (1956). *Rps. spheroides* strain 2.4.1, from the collection of Professor C. B. van Niel, was used in one experiment. A pigment mutant from this parent strain, the 'tan' mutant M5 which synthesized carotenoids but no bacteriochlorophyll, was obtained from Professor R. Y. Stanier (Stanier & Smith, 1959). *Rhodospseudomonas capsulata* strain 2.3.11 came from the collection of Professor C. B. van Niel.

Stock cultures of all strains except L56 and M5 were maintained as reported by Lascelles (1956). Mutants L56 and M5 were maintained aerobically on agar slopes of the yeast-extract-malate-glutamate medium.

Growth and preparation of suspensions. The growth medium used for all experiments with *Rps. spheroides* 8253, L56, 2.4.1 and M5 was that given by Lascelles (1959). *Rps. spheroides* mutant 6A5 was grown in the same medium as the parent strain but supplemented with adenine (0.2 mM) and L-histidine (1 mM). *Rps. capsulata* was grown in the same medium as *Rps. spheroides* but with succinate in place of malate (Carr & Lascelles, 1961). All media contained ferric citrate (10 μM). The methods used to grow the organisms anaerobically in the light and aerobically in the dark, together with details of harvesting, were as described by Lascelles (1956, 1959).

The synthesis of the photosynthetic pigments and of various specific protein fractions was measured under three sets of conditions: (a) cultures growing exponentially were incubated anaerobically in the light as described by Lascelles (1960); (b) suspensions of about 2.5 mg. dry wt. of organisms/ml. were incubated with low aeration, under the conditions of Lascelles (1959), in their respective growth media, unless otherwise stated; (c) suspensions of about 2.5 mg. dry wt. of organism/ml. were incubated with high aeration, which was achieved by shaking 50 ml. of suspension in a 2 l. Erlenmeyer flask (i.e. a small volume in a large vessel) on a horizontal reciprocating shaker.

In experiments on the incorporation of [1-¹⁴C]phenylalanine, 2.5 μC of DL-[1-¹⁴C]phenylalanine and 25 μmoles of carrier DL-phenylalanine were added to each incubation mixture (200 ml. in experiments with growing cultures and 50 ml. in those with suspensions).

Preparation and fractionation of cell-free extract. Organisms (about 10 mg. dry wt./ml.) were disrupted by ultrasonic vibration as described by Lascelles (1959). The crude extract was then fractionated by a process combining the techniques of differential and density-gradient centrifuging (Scheme 1). The bulk of the cell debris was removed by

centrifuging at 25 000g for 10 min. at 0°. The clear supernatant fluid, designated the 'cell-free extract', was spun for a further 60 min. at 105 000g in a Spinco model L ultracentrifuge. The pigment-free supernatant is designated the 'soluble cell-protein fraction'; the pellet, which contained all the pigment when extracts from light-grown cells were used, is referred to as the 'crude particulate fraction'. The latter was resuspended with a hypodermic syringe in 0.5–1 ml. of 40 mM-tris buffer, pH 7.5. The suspension was layered on the top of a discontinuous sucrose gradient in a 5 ml. centrifuge tube. The gradient consisted of 1.5 ml. portions of 0.29 M-, 0.59 M- and 1.17 M-sucrose in 40 mM-tris buffer, pH 7.5. The tubes were spun for 60 min. at 125 000g in the S.W. 39 swinging-bucket rotor of the Spinco ultracentrifuge. Three fractions were obtained: (1) A pellet which consisted of cell debris and a few unbroken organisms. (2) A band at the junction of the 0.59 M- and 1.17 M-sucrose layers; this fraction contained the bulk of the pigment when extracts from light-grown organisms were used and was essentially free of RNA, as shown by both chemical analysis and tracer experiments with [2-¹⁴C]-uracil. Electron micrographs revealed an essentially homogeneous preparation contaminated with a few smaller particles, probably ribosomes. This fraction was called the 'purified chromatophore preparation'. The same term has been used in referring to the corresponding fraction from aerobically grown organisms, although it contained no pigment, and no particles could be seen in electron micrographs. Of the total particulate fraction from both pigmented and non-pigmented organisms, 40% of the protein was recovered in the 0.59 M-sucrose layer. (3) The third fraction was in the 0.29 M-sucrose layer and the upper part of the 0.59 M-sucrose layer; it contained the bulk of the particulate RNA and was designated the 'ribosome fraction'. Fractions (1) and (3) were discarded.

Measurement of the incorporation of [1-¹⁴C]phenylalanine into the soluble cell and chromatophore proteins. The soluble cell-protein and the purified chromatophore preparations were each fractionated by the method of Roberts, Abelson, Cowie, Bolton & Britten (1957). The preparation counted

was that remaining after the precipitation of each fraction with trichloroacetic acid followed by extraction of the precipitate with ethanol, ether and hot trichloroacetic acid. The protein precipitates were homogenized in 2–5 ml. of water with a glass homogenizer. Portions containing 0.2–1.2 mg. of protein and 100–2000 counts/min. were pipetted on to aluminium planchets (3 cm.²) containing a disk of lens tissue; samples were counted with a Geiger-Müller tube fitted with a thin mica end-window. The count obtained was corrected both for background count and for self-absorption, by using a curve obtained with radioactive protein from *Rps. spheroides* grown on generally labelled acetate. The efficiency of the counting system was about 4.5%. The protein in samples was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951). The specific activities obtained by this method were reproducible to within $\pm 5\%$.

Determination of phenylalanine in whole organisms and in the chromatophore-protein fraction. Organisms (10 mg. dry wt.) were harvested from a sample of a culture and washed twice. After being resuspended in 1 ml. of water and boiled to extract free amino acids (Mandelstam, 1958a; Hancock, 1958), organisms were washed twice in water, and finally suspended in 0.5 ml. of 6N-hydrochloric acid. A purified preparation of chromatophores was likewise suspended in 0.5 ml. of 6N-hydrochloric acid. Both samples were then heated in sealed ampoules for 18 hr. at 105° and the hydrochloric acid was subsequently removed *in vacuo*. The residues were dissolved in 5 ml. of water. The total amino acid composition was determined by the method of Cocking & Yemm (1954) as modified by Hancock (1960), with glutamic acid as a standard. The phenylalanine content of the hydrolysate was estimated by bioassay, with *Leuconostoc mesenteroides* (P 60) in the medium described by Lascelles, Cross & Woods (1954), with phenylalanine replaced by 3 mM-DL-serine; DL-phenylalanine (0 to 30 μ moles/ml.) was the standard, results being calculated in terms of the L-isomer. The whole-cell protein and the chromatophore protein contain respectively 2.45 ± 0.05 and 2.50 ± 0.30 μ moles of phenylalanine/100 μ moles of amino

Scheme 1. Preparation of cell fractions of *Rps. spheroides* and of *Rps. capsulata*. Full experimental details are given in the text.

acid. Since there is essentially the same proportion of phenylalanine in total cell protein as in chromatophore protein, it was concluded that the soluble cell protein, which makes up 80% of the total cell protein, must also have the same proportion of phenylalanine as the chromatophores.

Two-dimensional paper chromatography of hydrolysates of ^{14}C -labelled protein from incubations with ^{14}C -phenylalanine showed that the radioactivity was confined to this amino acid. (We are indebted to Dr G. Coleman for these analyses.)

Determinations. The extinction of cultures was measured at 680 m μ and the dry weight obtained by reference to a calibration curve (Cohen-Bazire *et al.* 1957).

Bacteriochlorophyll and carotenoids were extracted from the organisms with an acetone-methanol (7:2, v/v) mixture. The bacteriochlorophyll and carotenoids in these extracts were determined spectrophotometrically by the method of Cohen-Bazire *et al.* (1957).

Protein was determined by the method of Lowry *et al.* (1951), with crystalline bovine plasma albumin as standard. At the concentrations involved, the presence of the photosynthetic pigments in crude cell extracts of chromatophores did not interfere with the determination (Cohen-Bazire & Kunisawa, 1960).

Enzyme assays. Succinate dehydrogenase was estimated in cell-free extracts by the spectrophotometric method of Singer & Kearney (1957). The unit of activity was taken as that amount reducing 1 μmole of dichlorophenol-indophenol/min.

Alkaline phosphatase was assayed by a modification of the method of Bessey, Lowry & Brock (1946), described by Torriani (1960). Suspensions of organism (1 mg./ml.) in 40 mM-tris buffer, pH 7.5, were shaken with 2 $\mu\text{l.}$ of toluene/mg. of organisms. To 1 ml. of this toluene-treated suspension was added 0.5 ml. of M-tris buffer, pH 9.0, and 0.5 ml. of 0.04 M-*p*-nitrophenyl phosphate. The mixture was incubated at 34° for 15 min., and the reaction stopped by the addition of 1 ml. of M-sodium carbonate. Cells were removed by centrifuging and the *p*-nitrophenol released was determined spectrophotometrically at 420 m μ . The enzyme was assayed immediately after the addition of

toluene, as the activity declined with the length of incubation with toluene before the assay. On the other hand the treatment with toluene was absolutely necessary as untreated organisms showed almost no activity (Table 1). Fractionation of a cell-free extract showed that 96% of the total alkaline-phosphatase activity was located in the soluble protein fraction. This enzyme is also confined to the soluble fraction in *Escherichia coli* (Torriani, 1960; Malamy & Horecker, 1961). The unit of enzyme was taken as that amount catalysing the hydrolysis of 1 μmole of *p*-nitrophenyl phosphate/hr.

Succinate thiokinase was assayed as described by Lascelles (1959).

δ -Aminolaevulic synthetase was determined in cell-free extracts either by the method of Lascelles (1960) or by that of Burnham (1962). A unit of enzyme was defined as that amount which catalyses the formation of 1 μmole of δ -aminolaevulinic acid/1 hr. under the conditions of the assay.

In experiments with growing cultures the increase in units of enzyme/ml. of culture or of μmoles of pigment/ml. is plotted against the dry wt. of organisms/ml.; the slopes from such plots represent the differential rate of synthesis of enzyme or pigment.

RESULTS

Effect of antimetabolites on the synthesis of pigments and enzymes in growing cultures of Rhodospseudomonas spheroides

The synthesis of pigments and enzymes was measured in cultures of *Rps. spheroides* growing anaerobically in the light, with and without analogues of purines, pyrimidines and amino acids. The chosen concentration of each antimetabolite permitted growth, but in most cases at a reduced rate.

Samples (200 ml.) were removed at intervals of 2 hr., and the pigment content and dry weight of the organisms in 5 ml. portions were determined.

Table 1. *Effect of toluene treatment on the alkaline-phosphatase activity of Rhodospseudomonas spheroides*

Suspensions of light-grown cells of *Rps. spheroides* 8253 (1 mg./ml.) were incubated with toluene (2 $\mu\text{l.}$ /mg. of cells) at 34° for the periods indicated. The suspensions were then assayed for alkaline-phosphatase activity. Experimental details are given in the text.

Time between additions of toluene and assay (min.)	<i>p</i> -Nitrophenyl phosphate hydrolysed ($\mu\text{m-moles/hr./mg. of cells}$)
0	582
15	426
30	288
45	204
60	138
No toluene treatment	42

Fig. 1. Effect of various antimetabolites on the synthesis of (a) bacteriochlorophyll and (b) carotenoids by exponentially growing cultures of *Rps. spheroides*. The antimetabolites were added at points indicated by the arrows. The curves were taken from two experiments. O, No addition; ●, 8-azaguanine (2 mM); ▲, DL-ethionine (0.1 mM); △, 5-fluorouracil (0.1 mM).

Table 2. *Effect of antimetabolites on the bacteriochlorophyll content of whole cells and particulate fraction of Rhodospseudomonas spheroides*

Cultures (200 ml.) were grown anaerobically in the light to an extinction at 680 m μ of 0.75 (0.5 cm. cell) with and without 8-azaguanine or ethionine. The bacteriochlorophyll content/mg. of cells was determined on 5 ml. portions, the remainder of the cells were harvested and disrupted ultrasonically, the crude particulate preparation was obtained from the cell-free extract, and the bacteriochlorophyll and protein contents were determined. Experimental details are given in the text.

Antimetabolite	Bacteriochlorophyll content of whole cells		Bacteriochlorophyll content of crude particulate preparation	
	(μ m-moles/mg. dry wt. of cells)	(Percentage of control)	(μ m-moles/mg. of protein)	(Percentage of control)
None	14.0	100	82	100
8-Azaguanine (2 mM)	10.0	71	54	66
DL-Ethionine (0.2 mM)	9.2	66	64	78

Alkaline phosphatase was determined in the organisms from a 10 ml. portion, and the remainder of the sample was made into a cell-free extract for the assay of aminolaevulate-synthetase and succinate-dehydrogenase activities.

Pigment synthesis. In the control cultures this followed the pattern found by Cohen-Bazire *et al.* (1957). It began at an initially low rate compared with growth, and became relatively faster as the culture became more dense (Fig. 1). With 8-azaguanine (2 mM) the growth rate was reduced to about 80% of the control value and pigment synthesis to about 25%. With 5-fluorouracil (0.1 mM) the growth rate was reduced to about 60% of the control and the synthesis of pigment to about 5%. With DL-ethionine (0.1 mM) growth was reduced to about 85% of the control and pigment synthesis to about 7%. The synthesis of bacteriochlorophyll was inhibited rather more than that of carotenoid by each of the antimetabolites (Fig. 1). The decrease in bacteriochlorophyll synthesis brought about by ethionine was accompanied by increased excretion of porphyrin, as observed by Gibson, Neuberger & Tait (1962b).

The other antimetabolites tested, DL-*p*-fluorophenylalanine (0.2, 1 or 2 mM), DL- β -thien-2-ylalanine (0.3 mM), DL-5-methyltryptophan (1 mM) and mixtures of *p*-fluorophenylalanine (1 mM) with β -thien-2-ylalanine (0.3 mM) or with 5-methyltryptophan (1 mM), inhibited growth and pigment synthesis to the same extent.

A crude particulate preparation was made from extracts of organisms grown photosynthetically in the presence and absence of 8-azaguanine or ethionine. The ratio of bacteriochlorophyll to protein in such preparations was decreased when compared with preparations from organisms grown in the absence of the analogues. This decrease was about the same as that observed in the bacteriochlorophyll content of intact organisms (Table 2).

Enzyme synthesis. In an attempt to localize the site of action of the active antimetabolites on pigment synthesis, their effect on the formation of

three enzymes was studied. The enzymes were (a) δ -aminolaevulate synthetase, which is required for the first step in bacteriochlorophyll synthesis from glycine and succinyl-CoA (Gibson, 1958; Kikuchi, Kumar, Talmage & Shemin, 1958; Kikuchi, Kumar & Shemin, 1959) and which possibly has a controlling role in the formation of this pigment (Lascelles, 1959, 1960), (b) succinate dehydrogenase, an enzyme confined to the particulate protein fraction in the Athiorhodaceae (Woody & Lindstrom, 1955; Cohen-Bazire & Kunisawa, 1960), and (c) alkaline phosphatase.

These particular enzymes were chosen in the hope that it might be possible to distinguish between an effect on particulate protein synthesis, which might affect the succinate dehydrogenase, and an effect on the enzymic mechanism of pigment synthesis, which might be shown by an effect on the aminolaevulate synthetase. The alkaline phosphatase was included as a control by which to compare the effects on the other two enzymes, since it is not directly involved in pigment synthesis and is confined to the soluble fraction of the cell protein.

The assay of alkaline-phosphatase activity in samples from cultures growing exponentially in the light established a direct relationship between the synthesis of this enzyme and growth of the organism; this relationship was not disturbed by any of the three antimetabolites tested. Similar results were obtained with succinate thiokinase, another 'soluble' enzyme. The differential rate of synthesis of succinate dehydrogenase, which is a particulate enzyme, was also unaffected (Fig. 2a).

In contrast, the formation of aminolaevulate synthetase was markedly affected by the antimetabolites. Immediately after their addition, synthesis of this enzyme diminished and finally ceased (Fig. 2b).

The inhibitory effects of 8-azaguanine, 5-fluorouracil and ethionine were completely relieved by 0.6 mM-guanine, 1 mM-uracil and 0.1 mM-methionine respectively.

Effects of various inhibitors on the synthesis of pigments and enzymes by suspensions of Rhodospseudomonas spheroides

When organisms grown under high oxygen partial pressure (and therefore non-pigmented) are

Fig. 2. Effect of antimetabolites on the differential rate of synthesis of enzymes by exponential cultures of *Rps. spheroides* growing anaerobically in the light. The antimetabolites were added at zero time, and samples were removed at intervals for the determination of growth and for the assay of enzymic activity. O, No addition; ●, 8-azaguanine (2 mM); △, 5-fluorouracil (0.1 mM); ▲, DL-ethionine (0.1 mM). In Fig. 2 (a) the continuous line represents succinate-dehydrogenase, the broken line alkaline-phosphatase, activity. In Fig. 2 (b) δ -aminolaevulate synthetase is shown (assayed by the method of Lascelles, 1960).

incubated under low oxygen there is little net synthesis of protein (about a 20% increase). There is a four- to five-fold increase in aminolaevulate-synthetase activity, and the carotenoids and bacteriochlorophyll increase by about 16- and 80-fold respectively. Enzymic activity becomes maximal after incubation for about 1 hr. under the low oxygen pressure, but at this time little more than 25% of the final pigment content has been reached (Lascelles, 1959) (Fig. 3).

8-Azaguanine, 5-fluorouracil, *p*-fluorophenylalanine, DL-ethionine and chloramphenicol, when added at the beginning of the incubation under low aeration, each greatly decreased the formation of both bacteriochlorophyll and aminolaevulate synthetase (Table 3).

The inhibition of bacteriochlorophyll synthesis by chloramphenicol and by the various antimetabolites could be interpreted as being due to their action in preventing the formation of aminolaevulate synthetase. In another series of experiments therefore, the inhibitors were added after the suspensions had been incubated for 60–90 min. to attain a high content of the enzyme. Bacteriochlorophyll synthesis was again inhibited, but, since the aminolaevulate-synthetase activity de-

Fig. 3. Increase in δ -aminolaevulate-synthetase activity during adaptation of *Rps. spheroides* to form the photosynthetic pigments. Aerobically grown organisms (2.5 mg. dry wt./ml.) were suspended in 50 ml. of the 'standard' mixture containing glycine, fumarate and oxoglutarate (Lascelles, 1959), and were incubated in large T-tubes aerobically in the dark. Tubes were removed at intervals, and samples were taken for the determination of bacteriochlorophyll (●) and of carotenoids (▲); the rest of the organisms were centrifuged and washed, and extracts were prepared and assayed for aminolaevulate-synthetase activity (O) by the method of Burnham (1962).

clined to 25 to 50 % of the initial value during the incubation with the antimetabolites, a clear-cut differentiation could not be made between an effect on enzyme content and an effect on some other factor controlling pigment formation.

Need for a nitrogen source for bacteriochlorophyll synthesis by suspensions of Rhodospseudomonas spheroides

Requirement for ammonium ions. For maximum synthesis of bacteriochlorophyll by suspensions incubated with low aeration, a nitrogen source (added as NH_4^+ ions) is required in addition to

glycine (Lascelles, 1959). To determine whether this might be accounted for solely in terms of synthesis and maintenance of a high concentration of aminolaevulate synthetase, experiments were done of a similar type to those with the analogues.

When non-pigmented organisms were incubated in a medium containing glycine as sole nitrogen source, bacteriochlorophyll synthesis was lowered to about 40 % of that in the control containing NH_4^+ ions, but there was only a 10 % decrease in the formation of aminolaevulate synthetase (Table 4). Ammonium ions were still required for maximum pigment formation after the organisms had first been incubated in complete medium to allow maximal synthesis of aminolaevulate synthetase. During the second incubation there was no decline in aminolaevulate synthetase, indicating that maintenance of the concentration of this enzyme did not account for the requirement for NH_4^+ ions for bacteriochlorophyll synthesis (Table 4).

Requirement for adenine and histidine for bacteriochlorophyll synthesis by a mutant strain. *Rps. spheroides* 6A5 requires L-histidine and adenine for growth. The effect of their omission from the medium on the formation of bacteriochlorophyll and of aminolaevulate synthetase was examined by the same experimental techniques as those described above. The omission of either from the start completely prevented synthesis of aminolaevulate synthetase and there was only a fractional increase in bacteriochlorophyll (Table 5). Organisms in which the concentration of aminolaevulate synthetase had been raised by a preliminary incubation in a medium supplemented with adenine and histidine still required both nutrients for the formation of bacteriochlorophyll and for maintaining the concentration of aminolaevulate synthetase (Table 5).

Table 3. *Effect of inhibitors on the formation of bacteriochlorophyll and of aminolaevulate synthetase by Rhodospseudomonas spheroides*

Cells were grown aerobically and transferred to low oxygen pressure as described in Fig. 3. The various inhibitors were added at the start of the incubation under low aeration. The tubes were incubated for 2-2.5 hr., samples were then taken for the determination of bacteriochlorophyll and extracts were prepared for assay of aminolaevulate-synthetase activity by the method of Burnham (1962). Experimental details are given in the text. The initial bacteriochlorophyll concentration was $0.3 \mu\text{m-mole/ml.}$, and the initial activity of aminolaevulate synthetase was 93 units/mg. of protein.

Inhibitor	Concn. of bacteriochlorophyll ($\mu\text{m-moles/ml.}$)	Aminolaevulate-synthetase activity (units/mg. of protein)
None	8.0	350
5-Fluorouracil (0.1 mM)	5.1	215
8-Azaguanine (2.0 mM)	1.4	195
DL-p-Fluorophenylalanine (0.4 mM)	0.6	115
DL-Ethionine (0.3 mM)	0.3	33
Chloramphenicol (0.01 mM)	0.3	45

Table 4. *Requirement for ammonium ions for bacteriochlorophyll synthesis by suspensions of Rhodospseudomonas spheroides*

In Expt. 1 aerobically grown cells were incubated for 2.5 hr. with low aeration as in Fig. 3 with omission of ammonium phosphate as shown. Aminolaevulate-synthetase activity was assayed by the method of Burnham (1962). In Expt. 2, aerobically grown cells were first incubated for 1 hr. under low aeration in the complete 'standard' mixture. After being harvested and washed they were incubated for a further 2 hr. with low aeration in the standard mixture with and without ammonium phosphate before the determination of bacteriochlorophyll and of aminolaevulate synthetase. Experimental details are given in the text.

Expt. no.	$(\text{NH}_4)_2\text{HPO}_4$ (3 mM)	Final incubation time (hr.)	Concn. of bacteriochlorophyll ($\mu\text{m-moles/ml.}$)	Aminolaevulate-synthetase activity (units/mg. of protein)
1 (no preincubation)	—	0	0.2	91
	—	2.5	3.1	278
	+	2.5	8.1	310
2 (preincubated for 1 hr. in complete mixture)	—	0	2.4	310
	—	2	5.5	308
	+	2	10.8	340

Table 5. Requirement for adenine and histidine for bacteriochlorophyll synthesis by suspensions of *Rhodospseudomonas spheroides* mutant 6A5

Cultures of *Rps. spheroides* mutant 6A5 were grown aerobically in growth medium containing adenine and L-histidine. In Expt. 1 these cells were incubated for 5.25 hr. with low aeration as in Fig. 3, with additions of adenine and histidine as indicated. Aminolaevulate-synthetase activity was assayed by the method of Burnham (1962). In Expt. 2 the cells were first incubated for 3 hr. in the 'standard' mixture supplemented with adenine (0.2 mM) and L-histidine (1 mM). After being harvested and washed they were incubated for a further 3 hr. with low aeration in the standard mixture, with and without adenine and L-histidine, before the determination of bacteriochlorophyll and of aminolaevulate synthetase. Experimental details are given in the text.

Expt. no.	Adenine (0.2 mM)	L-Histidine (1 mM)	Final incubation time (hr.)	Concn. of bacterio- chlorophyll (μ m-moles/ml.)	Aminolaevulate- synthetase activity (units/mg. of protein)
1 (no preincubation)	-	-	0	0.11	42
	+	-	5.25	0.28	20
	-	+	5.25	0.28	39
	+	+	5.25	19.0	274
2 (preincubated for 3 hr. in supplemented mixture)	-	-	0	2.6	258
	+	-	3	3.3	67
	-	+	3	4.0	136
	+	+	3	16.0	319

Composition of chromatophore preparations from *Rhodospseudomonas spheroides*

In the experiments which follow, the incorporation of [1^{14} C]phenylalanine into the chromatophore protein was compared with that into the soluble protein of several strains of *Rps. spheroides* and one of *Rps. capsulata*, under a variety of conditions. The chromatophore fraction studied was the purified chromatophore preparation (Scheme 1). With preparations from non-pigmented organisms the same fraction from the sucrose gradient was used. The most important difference between the purified chromatophores and the crude particulate fraction was the great decrease in the nucleic acid content (Table 6).

Incorporation of [1^{14} C]phenylalanine by growing cultures of *Rhodospseudomonas spheroides* and of *Rhodospseudomonas capsulata*

When cultures of *Rps. spheroides* or *Rps. capsulata* were grown in the presence of [1^{14} C]phenylalanine, either aerobically in the dark or anaerobically in the light, there was essentially the same percentage incorporation of 14 C into the 'chromatophore' and soluble protein fractions.

Incorporation of [1^{14} C]phenylalanine by suspensions of *Rhodospseudomonas spheroides* and of *Rhodospseudomonas capsulata*

Effect of incubation conditions. When suspensions of non-pigmented *Rps. spheroides* were incubated under the conditions of low aeration which promote pigment synthesis there was an almost steady incorporation of [1^{14} C]phenylalanine into the chro-

matophore and soluble protein fractions. The rate of incorporation into the chromatophore fraction was almost twice that into the soluble fraction. The incorporation of 14 C into both protein fractions started from zero time, whereas the formation of bacteriochlorophyll was detectable only after a lag of about 30 min. (Fig. 4).

Similar results were obtained with *Rps. spheroides* strain 2.4.1, *Rps. spheroides* mutant 6A5 and *Rps. capsulata* incubated in their appropriate growth media, and also with *Rps. spheroides* incubated in the 'standard' mixture of Lascelles (1959) (Table 7).

Preferential incorporation into the chromatophore protein also occurred with suspensions forming pigment when incubated anaerobically and with illumination. Suspensions of non-pigmented cells of *Rps. spheroides* were incubated for 1 hr. with low aeration and were then harvested and resuspended in fresh growth medium containing [1^{14} C]phenylalanine, and incubated anaerobically in the light. The synthesis of bacteriochlorophyll and the relative incorporation of 14 C into the chromatophore and soluble fractions was similar to that by suspensions incubated continuously under low oxygen partial pressure (Table 7).

Association of incorporation with protein synthesis. That incorporation of [1^{14} C]phenylalanine represented, at least in part, the synthesis of new protein was shown by its dependence on a nitrogen source with *Rps. spheroides*. In the absence of a nitrogen source the incorporation into both cell fractions was diminished to the same extent (Table 8). With the mutant 6A5 the incorporation was greatly diminished by the omission of histidine (Table 8).

Like pigment synthesis, the incorporation of [$1\text{-}^{14}\text{C}$]phenylalanine was inhibited by 8-azaguanine and chloramphenicol (Table 8).

During the incubation under low aeration there was a net increase in protein of about 20% as measured by the Folin-Lowry method. The relative amount of protein in the crude particulate fraction (19%) remained constant under these conditions.

Table 6. *Composition of a purified chromatophore preparation from photosynthetically grown cells of Rhodospseudomonas spheroides*

(a) A culture of *Rps. spheroides* grown photosynthetically was harvested, washed and made into a cell-free extract as described in the Materials and Methods section. The extract was spun for 60 min. at 105 000g and the pellet from this spin, the crude particulate fraction, was further purified by sucrose-gradient centrifuging (see the Materials and Methods section). The soluble protein fraction was taken as the protein remaining in the buffer on top of the gradient. The percentage distribution of material on the gradient (other than the debris fraction) is given below. RNA was determined by the distribution of [$2\text{-}^{14}\text{C}$]uracil.

	Chromatophore fraction (0.59 M-sucrose)	Ribosome fraction (0.29 M-sucrose)	Soluble protein fraction
Protein	44	44	12
Pigment	85	15	0
RNA	10	83	7

(b) The chemical analysis of a purified chromatophore preparation was compared with a crude particulate preparation. The determinations were carried out as follows: protein, by the method of Lowry *et al.* (1951); lipid, extracted and determined by the method of Few (1955) after refluxing with methanol to break lipoprotein complexes (Reichert, 1944); total water-soluble carbohydrate, by the method of Loewus (1952); bacteriochlorophyll and carotenoids, by the method of Cohen-Bazire *et al.* (1957); total nucleic acid, by the method of Gale & Folkes (1954); total P and lipid P, by the method of Sumner (1944) after digestion of whole chromatophore or lipid preparations in the conc. sulphuric acid-60% (v/v) perchloric acid (3:2, v/v) mixture of Hanes & Isherwood (1949). All values except those for phosphorus are expressed as percentages of the dry weight.

	Crude particulate fraction	Purified chromato- phore preparation
Protein	69.5	58.4
Lipid	20.7	24.6
Carbohydrate	6.2	4.2*
Bacteriochlorophyll	4.5	4.5-5.5
Carotenoid	~1.0	~1.0
Nucleic acid	3.4	0.3-0.9
Total P ($\mu\text{mole/mg. dry wt.}$)	0.35	0.22
Lipid P ($\mu\text{mole/mg. dry wt.}$)	0.18	0.16

* This value may be low owing to the dialysis necessary to remove the traces of sucrose in which the chromatophores were purified.

Similar observations were made by Cohen-Bazire & Kunisawa (1960) with *Rhodospirillum rubrum*. The amount of new protein formed, as determined by the incorporation of [$1\text{-}^{14}\text{C}$]phenylalanine and by using the value of $2.50 \mu\text{moles}$ of phenylalanine/100 μmoles of amino acid obtained by analysis of *Rps. spheroides* protein, is about 37% in the soluble fraction and 64% in the particulate fraction. The difference between this increase and the amount of synthesis estimated by the Folin-Lowry method could be due to turnover. When non-pigmented cells were incubated with low aeration in a nitrogen-free medium, the incorporation was reduced to about 50%. The remaining incorporation was probably mainly due to turnover, as only a 1% increase in total protein could be detected by the method of Lowry *et al.* (1951).

Preferential incorporation into chromatophore protein associated with pigment synthesis. The preferential incorporation of labelled amino acid into the chromatophore fraction was shown to be associated with pigment synthesis under the following conditions.

(1) Suspensions of aerobically grown *Rps. spheroides* were incubated for 1 hr. with low aeration, and were then transferred to high aeration for the next hour; they were finally returned to

Fig. 4. Preferential incorporation of [$1\text{-}^{14}\text{C}$]phenylalanine into chromatophore protein. Aerobically grown cells of *Rps. spheroides* were harvested, washed and resuspended (2.5 mg./ml.) in normal growth medium containing DL-[$1\text{-}^{14}\text{C}$]phenylalanine ($2.5 \mu\text{C}$, $25 \mu\text{moles/50 ml.}$) in large T-tubes. These were incubated with low aeration in the dark (Lascelles, 1959). Tubes were removed at intervals and further incorporation was prevented by the addition of chloramphenicol (0.1 mM). Samples were removed for the determination of pigment; the rest of the cells were fractionated, plated and counted as described in the Materials and Methods section. ●, Bacteriochlorophyll; ▲, carotenoids; ○, specific activity of chromatophore protein; △, specific activity of soluble protein.

Table 7. Association between pigment synthesis and incorporation of [1-¹⁴C]phenylalanine into chromatophore protein by *Rhodopseudomonas spheroides* and by *Rhodopseudomonas capsulata*

Suspensions of cells (2–2.5 mg. dry wt./ml.) were incubated under various conditions in 50 ml. volumes in big T-tubes. The appropriate growth medium was used except in the instances marked with an asterisk (*), when the standard mixture of Lascelles (1959) was used. In all experiments the medium was supplemented with [1-¹⁴C]phenylalanine (2.5 μ C, 25 μ moles/50 ml.). Pigment synthesis and incorporation of ¹⁴C into the chromatophore and soluble protein fractions were determined as described in the Materials and Methods section. N.D., Not determined.

Organism	Growth conditions	Incubation conditions	Pigment synthesized (μ m-moles/ml.)		Specific-activity ratio (chromatophore protein to soluble protein)
			Bacterio-chlorophyll	Carotenoid	
<i>Rps. spheroides</i> 8253	Aerobic, in the dark	Low aeration for 3 hr.	18.0	5.5	1.75
* <i>Rps. spheroides</i> 8253	Aerobic, in the dark	Low aeration for 3 hr.	13.5	4.5	1.85
<i>Rps. spheroides</i> 2.4.1	Aerobic, in the dark	Low aeration for 3 hr.	2.9	5.0	1.75
<i>Rps. capsulata</i> 2.3.11	Aerobic, in the dark	Low aeration for 3 hr.	17.0	5.0	2.80
<i>Rps. spheroides</i> 6A5	Aerobic, in the dark	Low aeration for 3 hr.	9.7	N.D.	1.70
<i>Rps. spheroides</i> mutant M5	Aerobic, in the dark	Low aeration for 3 hr.	0	1.0	1.32
<i>Rps. spheroides</i> mutant L56	Aerobic, in the dark	Low aeration for 3 hr.	0	0	1.08
* <i>Rps. spheroides</i> 8253	Aerobic, in the dark	Low aeration for 1 hr. followed by anaerobic-light conditions for 3 hr.	10.5	N.D.	1.72
<i>Rps. spheroides</i> 8253	Aerobic, in the dark	High aeration for 1.5 hr.	0.17	N.D.	1.16
<i>Rps. spheroides</i> 8253	Anaerobic, in the light	Low aeration for 3 hr.	1.5	N.D.	1.22

Table 8. Incorporation into chromatophore protein associated with protein synthesis by *Rhodopseudomonas spheroides* and by *Rhodopseudomonas capsulata*

Aerobically grown cells were incubated under low oxygen pressure in the appropriate growth medium (see the Materials and Methods section) with various additions and omissions as indicated in the Table. [1-¹⁴C]-Phenylalanine was added in all experiments (2.5 μ C, 25 μ moles/50 ml.). Incubation was for 3 hr.

Organism	Incubation medium	Bacterio-chlorophyll synthesized (μ m-moles/ml.)	Specific activity of protein (counts/min./mg.)	
			Chromatophores	Soluble
<i>Rps. spheroides</i> 6A5	Normal	9.8	1140	660
	Without histidine	0.5	300	180
<i>Rps. spheroides</i>	Normal	16.0	1200	690
	Without NH ₄ ⁺ ions and glutamate	4.6	610	290
	With 8-azaguanine (2 mM) added after 1 hr.	10.3	840	540
	With chloramphenicol (0.1 mM) added at zero time	0	110	17
	With 8-azaguanine (2 mM) added after 1 hr.	6.0	720	360
<i>Rps. capsulata</i>	Normal	17.0	1500	540
	With 8-azaguanine (2 mM) added after 1 hr.	6.0	720	360

low aeration for a further hour. An abrupt cessation of bacteriochlorophyll synthesis occurred on transfer to high oxygen tension, but synthesis resumed on return to the low oxygen pressure. The incorporation of [1-¹⁴C]phenylalanine into the chromatophore protein showed a decrease on transfer to the high oxygen pressure, becoming parallel to the incorporation by the soluble fraction. On returning to the low oxygen pressure, the incorporation into the chromatophore fraction again became greater than incorporation into the soluble fraction (Fig. 5).

(2) Fully pigmented *Rps. spheroides*, obtained by anaerobic growth in the light, showed only a very slight increase in pigment when incubated under low oxygen pressure, and the incorporation of [1-¹⁴C]phenylalanine into both fractions was almost equal (Table 7).

(3) Suspensions of non-pigmented aerobically grown *Rps. spheroides*, incubated with high aeration rather than with low aeration, synthesized only minute amounts of the photosynthetic pigments, and the rate of incorporation of ¹⁴C into each protein fraction was similar (Table 7).

Fig. 5. Association between the synthesis of chromatophore protein and pigments by *Rps. spheroides*. Suspensions of aerobically grown cells (2.5 mg./ml.) were incubated with low aeration in normal growth medium (containing [^{14}C]phenylalanine) in large T-tubes for 1 hr. The contents of the tubes were then transferred to 2 l. Erlenmeyer flasks and incubated with high aeration for 1 hr. on a horizontal reciprocating shaker. The suspensions were transferred back to large T-tubes and incubated with low aeration for a further hour. Tubes were removed at intervals and further incorporation was prevented by the addition of chloramphenicol (0.1 mM). Samples were removed for the determination of pigment; the rest of the cells were fractionated and the specific activities of their soluble and chromatophore-protein fractions were determined as described in the Materials and Methods section. ●, Bacteriochlorophyll; ▲, carotenoids; ○, specific activity of chromatophore protein; △, specific activity of soluble protein.

(4) The mutant *Rps. spheroides* L 56, which has lost the ability to synthesize the photosynthetic pigments, showed essentially the same percentage incorporation into each fraction when incubated under low oxygen partial pressure (Table 7).

(5) The mutant *Rps. spheroides* M 5, which synthesizes carotenoids but not bacteriochlorophyll, showed a rather greater incorporation into the chromatophore fraction than into the soluble fraction when aerobically grown organisms were incubated with low aeration. This differential incorporation was not, however, as great as in the parent strain (*Rps. spheroides* 2.4.1) which also synthesizes bacteriochlorophyll (Table 7).

DISCUSSION

The origin of the chromatophores in the *Athiorhodaceae* when adapting to form photosynthetic pigments has been frequently discussed (Cohen-Bazire *et al.* 1957; Cohen-Bazire & Kunisawa, 1960; Newton, 1960; Sistrom, 1962). The obligatory association of pigment formation with pro-

tein synthesis could suggest that chromatophore protein must be formed simultaneously with the pigments but it could also be due to the necessity to form enzymes concerned directly in pigment synthesis. The present work could support both possibilities.

Effect of antimetabolites. These substances, which showed a differential inhibition of pigment synthesis by photosynthetically grown cultures of *Rps. spheroides*, are all known to affect protein synthesis in other systems. These included 8-azaguanine (Chantrenne & Devreux, 1958, 1960a, b; Chantrenne, 1961), 5-fluorouracil (Naono & Gros, 1960) and DL-ethionine (Harris & Kohn, 1941; Loveless, Spoerl & Weisman, 1954). Aminolaevulate synthetase was the only enzyme of those assayed whose formation was preferentially inhibited, providing evidence for a link between the activity of this enzyme and the ability to form pigment.

The site of action of these antimetabolites may not be on protein synthesis. Ethionine also acts directly on the synthesis of bacteriochlorophyll by inhibiting the formation of the methyl ester group, which is derived from methionine (Tait & Gibson, 1961; Gibson *et al.* 1962b). The effectiveness of azaguanine and fluorouracil could suggest that a high rate of synthesis or turnover of RNA is specifically associated with the formation of pigments or maintenance of a high concentration of aminolaevulate synthetase or both.

There was no evidence for a preferential effect of the analogues on the formation of chromatophore protein. Succinate dehydrogenase, which is located in the chromatophore fraction, was formed at the same differential rate in the presence and absence of the analogues. In addition, the protein:pigment ratio of the chromatophore fraction from organisms grown with the analogues was higher than that for the controls; this ratio would have been equal to or less than that in normal organisms if the analogues were acting solely by preventing formation of the protein to which the pigment is bound.

Activity of aminolaevulate synthetase. The results of experiments designed to investigate the association of protein synthesis with bacteriochlorophyll formation in organisms initially containing a high concentration of aminolaevulate synthetase were obscured by the apparent lability of this enzyme under most conditions that prevented protein synthesis. Loss of enzyme activity was particularly marked in the presence of chloramphenicol and also during histidine deprivation of the mutant strain 6A 5. Aminolaevulate synthetase might be subject to rapid turnover in *Rps. spheroides*. Loss of this enzyme also occurs when organisms growing anaerobically in the light are transferred to aerobic conditions (Lascelles, 1960).

Purification of chromatophores. Cell-free extracts from photosynthetic bacteria contain at least three different types of particle, as judged by ultracentrifugal analysis (Schachman *et al.* 1952). Any study of the synthesis of protein raises the problem of obtaining a constant fraction that is essentially free of particles that are not chromatophores. Three types of purification procedure have been attempted: (a) partition between two immiscible aqueous phases (Albertsson, 1961); (b) electrophoresis in a starch slurry under the conditions of Cota-Robles, Marr & Nilson (1958); (c) density-gradient centrifuging with a sucrose-layer gradient. The last named gave the most reproducible results. This technique has been used to purify chromatophores from *Rsp. rubrum* (Frenkel & Hickman, 1959; Cohen-Bazire & Kunisawa, 1960) and for the similar problem of separating the ribosomes and 'oxidosomes' of *Gluconobacter liquefaciens* (De Ley, 1962).

The chromatophore preparation from pigmented *Rps. spheroides* appeared to be homogeneous by electron microscopy, and its composition was similar to other preparations from photosynthetic bacteria (Newton & Newton, 1957; Bergeron, 1959; Gibson *et al.* 1962a).

Synthesis of chromatophore protein. The preferential incorporation of [^{14}C]phenylalanine into chromatophore protein by organisms incubated under low aeration was apparently associated with pigment synthesis, since conditions which decreased pigment synthesis also decreased the preferential incorporation.

During adaptation to form the pigments the proportion of protein in the chromatophore fraction remained constant, despite the greater incorporation of phenylalanine into this fraction than into the soluble fraction. This suggests a preferential turnover of chromatophore protein under these particular experimental conditions. With growing cultures there was no evidence for such preferential turnover since phenylalanine was incorporated at equal rates into the chromatophore and soluble fractions. Sistrom (1962) found no turnover of total protein in growing cultures of *Rps. spheroides* that were forming bacteriochlorophyll in response to diminished light-intensity.

Incorporation by the cell suspensions was greatly reduced in a nitrogen-free medium. The incorporation that occurred under these conditions was equivalent to about 33 % of new chromatophore protein and 15 % of new soluble protein in 3 hr. Within this period the total protein increased by only 1 %. The balance would give a turnover rate of 4.7 % per hr. for the soluble protein and almost 11 % per hr. for the chromatophore protein. These values agree well with the estimated turnover in organisms incubated in complete medium,

where there was about a 20 % net increase in protein and an estimated turnover rate of about 5 % for the soluble fraction and of about 14.2 % for the chromatophore fraction.

Turnover has not been proved definitely in these experiments as the degradation of labelled protein was not measured; it seems probable, however, that it was occurring. Turnover of protein has been observed in non-growing suspensions of other micro-organisms, including *Escherichia coli* (Mandelstam, 1957, 1958b), *Saccharomyces cerevisiae* (Halvorson, 1958) and *Bacillus cereus* (Urbá, 1959). The estimated rate of turnover of soluble protein in non-growing cultures of *Rps. spheroides* (about 5 % per hr.) is about the same as that reported for *E. coli* (3–6 % per hr.) and in *B. cereus* (7 % per hr.) (Mandelstam, 1960). In *E. coli* the particulate and soluble protein fractions turned over at about the same rate (Mandelstam & Halvorson, 1960). This contrasts with the observations with *Rps. spheroides*, if it is assumed that the preferential incorporation into chromatophore protein largely represents preferential turnover.

The formation of pigments by etiolated cells of *Euglena gracilis*, when illuminated in a medium that does not support growth, is a close parallel to the adaptation in the Athiorhodaceae. A very large turnover of protein occurs (about 90 %; see Mandelstam, 1960), but in contrast with the observations with *Rps. spheroides* there is a net transfer of about 17 % of the protein from the soluble to the chloroplast-protein fraction (Brawerman & Chargaff, 1959).

Though the preferential incorporation of phenylalanine by suspensions of *Rps. spheroides* occurred only under conditions that allowed pigment synthesis, the experiments do not prove that the two processes are linked obligatorily. The experiments also give no indication of the nature of the protein component that may be formed. It could be a structural lipoprotein which accommodates the pigment, an enzyme or enzymes concerned with the later stages in pigment formation, or a component of the photosynthetic phosphorylation system (see Cohen-Bazire & Kunisawa, 1960).

SUMMARY

1. The antimetabolites ethionine, 8-azaguanine and 5-fluorouracil preferentially inhibited the synthesis of bacteriochlorophyll and of carotenoids by cultures of *Rhodospseudomonas spheroides* growing photosynthetically. The chromatophores synthesized in the presence of these antimetabolites had a decreased content of the photosynthetic pigments.

2. These antimetabolites also preferentially inhibited the synthesis of δ -aminolaevulate synthe-

tase, but not that of succinate dehydrogenase, succinate thiokinase or alkaline phosphatase.

3. The synthesis of photosynthetic pigments and δ -aminolaevulinate synthetase by suspensions of organisms incubated with low aeration was inhibited by ethionine, *p*-fluorophenylalanine, 8-azaguanine, 5-fluorouracil and chloramphenicol. A nitrogen source (added as NH_4^+ ions) was necessary for the maximal synthesis of bacteriochlorophyll by such suspensions.

4. Suspensions of a mutant of *Rps. spheroides* requiring adenine and histidine for growth needed both of these nutrients for pigment synthesis when incubated with low aeration.

5. The phenylalanine content of soluble and chromatophore protein in *Rps. spheroides* was similar. The rate of incorporation of $[1-^{14}\text{C}]$ phenylalanine into these two protein fractions was similar in organisms growing either anaerobically in the light or aerobically in the dark.

6. Suspensions of organisms adapting to form photosynthetic pigments preferentially incorporated $[1-^{14}\text{C}]$ phenylalanine into the chromatophore protein. This preferential incorporation occurred only under conditions that permitted pigment synthesis. It ceased on transfer to conditions of high aeration which also prevented pigment formation.

7. Incorporation into both cell fractions was in part dependent on a nitrogen source and was inhibited by 8-azaguanine and chloramphenicol.

8. The net increase in protein in the chromatophore fraction of the organisms was insufficient to account for the amount of phenylalanine incorporated. This suggests that the preferential incorporation is largely due to preferential turnover of the chromatophore fraction during the synthesis of the photosynthetic pigments.

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Comparative Studies of 'Bile Salts'

17. A BILE ALCOHOL FROM *CHIMAERA MONSTROSA**

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The chimaeras (subclass Holocephali) are fishes that may be survivors of a group whose ancestors were early elasmobranchs (Moy-Thomas, 1939; Romer, 1945). The chemical nature of the bile alcohol, scymnol, apparently characteristic of living elasmobranchs has now been almost completely elucidated (Bridgwater, Briggs & Haslewood, 1962*a*), and we wished to find out whether the bile salts of chimaeras would show chemical characteristics compatible with the supposed origin of these fishes.

RESULTS

Alkaline hydrolysis of the bile salts of *Chimaera monstrosa* gave inorganic sulphate and neutral material; no bile acids were identified. The neutral 'bile alcohol' fraction so obtained is described below, and to investigate the 'native' bile alcohols we attempted cleavage of the sulphate esters present by the dioxan-trichloroacetic acid method that had previously yielded scymnol (Bridgwater *et al.* 1962*a*). This process gave, after purification, a crystalline alcohol, 'chimaerol', forming apparently hydrated crystals, m.p. about 180°, $[\alpha]_D$ in ethanol +41.5°. A small fraction with the chromatographic behaviour of scymnol (II) was also obtained.

Chimaerol ran at the same rate on paper chromatograms and showed the same infrared spectrum as the 3 α ,7 α ,12 α ,24 ξ ,26-pentahydroxy-25 ξ -copro-

stane (I) made by the method of Cross (1961) from anhydroscymnol, and also resembled a substance made by Bridgwater *et al.* (1962*a*) from cholic acid and thought to have formula (I). However, chimaerol is not, apparently, identical with Cross's substance ($[\alpha]_D +36^\circ$, in pyridine), with which it gave a small melting-point depression and which did not act as a 'seed' for the crystallization of chimaerol. We believe chimaerol to be 3 α ,7 α ,12 α ,24 ξ ,26-pentahydroxy-25 β -coprostane.

After alkaline hydrolysis at 120°, the main product was a substance with the mobility on paper, in the solvent systems used, of 'anhydrocypriol', a compound made in this Laboratory by a similar treatment of carp bile salts (see the Discussion section). Purification gave 'anhydrochimaerol' as solvated crystals, m.p. 141°, whose infrared spectrum and other properties suggest that it has structure (III).

EXPERIMENTAL

General

Methods and reagents were as described by Bridgwater *et al.* (1962*a*), and the remarks made by these authors about analytical figures apply. Bile of *C. monstrosa* was collected in ethanol. Evaporation of the filtered ethanol extract from about 100 gall-bladders (some very small) left bile salts (11 g.) as a dark-brown solid. Paper chromatography in 'solvent system T₂' (Bridgwater *et al.* 1962*b*) of this material showed a spot with R_f similar to that of taurocholate, and a second faster-running spot.

* Part 16: Bridgwater, Haslewood & Tammar (1962*b*).