The Effect of Adenosine Triphosphate on Porphyrin Excretion and on Glycine Metabolism in *Rhodopseudomonas spheroides*

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1. ATP, GTP, CTP and UTP at concentrations of 1 mM markedly decrease the amount of coproporphyrin excreted by *Rhodopseudomonas spheroides* illuminated in a medium containing glycine, succinate and fumarate. 2. The effect of ATP is decreased if ethionine is also added to the medium. 3. Evidence is presented showing that ATP is taken up by the organisms from the medium. 4. ATP is shown to have a marked effect on the utilization of glycine. In the presence of ATP the incorporation of the methylene carbon atom of glycine into the fatty acid moieties of the phospholipids is greatly increased, and more of the carboxyl carbon atom is lost, probably as carbon dioxide. 5. ATP has little effect on the utilization of succinate or fumarate. 6. The possible significance of these results with regard to the control by ATP of porphyrin synthesis and excretion and glycine metabolism is discussed.

Gajdos & Gajdos-Török (1963a) reported a decrease in porphyrin excretion after administration of purine nucleosides and nucleotides to rats with experimental porphyrias induced by a variety of compounds. *In vitro* these nucleosides and nucleotides were found to inhibit porphyrin synthesis when added to systems such as liver homogenates and reticulocytes incubated in the presence of glycine (Gajdos & Gajdos-Török, 1963a). These workers also observed that the excretion of coproporphyrin, which occurs when *Rhodopseudomonas spheroides* is illuminated in a medium containing glycine, fumarate and succinate (or α -oxoglutarate), was inhibited by AMP, ADP and ATP and by UTP (Gajdos & Gajdos-Török, 1963b, 1965).

It has now been found that GTP and CTP are as effective in reducing porphyrin excretion as the two triphosphates tested previously. Unlike AMP and ADP, mono- and di-phosphates of uridine, cytidine and guanosine have no effect on porphyrin excretion. Ethionine, previously found to stimulate porphyrin excretion under these illumination conditions (Gibson, Neuberger & Tait, 1962b), reverses the effect of ATP.

Evidence is presented that the intact ATP molecule enters the cells. The rate of incorporation of glycine into cellular material is increased by ATP and is decreased by ethionine. In the presence of ATP the methylene carbon atom of glycine is

incorporated to a greater extent into the fatty acid moieties of phospholipid. ATP does not have any effect on the incorporation of succinate or fumarate into cellular material. The significance of these observations with regard to the control of porphyrin synthesis and excretion and to the pathways of utilization of glycine is discussed.

MATERIALS AND METHODS

Organisms. *Rhodopseudomonas spheroides* (N.C.I.B. 8253) was maintained and grown semi-anaerobically in the light as described by Gibson, Neuberger & Tait (1962a).

Illumination of cell suspensions. Illuminations were carried out in mixture IS (Gibson *et al.* 1962a), which contains glycine, fumarate, succinate and salts.

Chemicals. AMP, ADP and ATP were purchased from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany, from Pabst Laboratories, Milwaukee, Wis., U.S.A., or from British Drug Houses Ltd., Poole, Dorset. All other nucleotides were from Pabst Laboratories. L-Ethionine was obtained from the California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.

¹⁴C-labelled compounds were obtained from The Radiochemical Centre, Amersham, Bucks., and [8-¹⁴C]ATP and [³²P]ATP from C.E.A., Saclay, France.

Estimations. The dry weight of organisms, their bacteriochlorophyll content and the amount of porphyrin formed were estimated as described by Gibson *et al.* (1962a). Lipid phosphorus was estimated by the method of Bartlett (1959). Protein was estimated by the method of Lowry, Rosebrough,

Farr & Randall (1951) after the samples had been boiled in NaOH (Sistrom, 1962).

Radioactivity. Samples were plated on 6.25 cm.² aluminium planchets, dried and counted at infinite thinness in a Nuclear-Chicago gas-flow counter fitted with a Micromil end-window and operated at the centre of the plateau. For differential counting of ¹⁴C and ³²P, samples were counted in the presence and absence of an aluminium screen, which was found to reduce to zero the counts from ¹⁴C and to reduce by 18% those from ³²P.

Fractionation of cells after illumination in radioactive medium. Samples taken during the course of the illuminations were centrifuged and the supernatants removed. Cells were washed with water by suspending and centrifuging. They were then suspended in water to a volume equal to that of the original sample taken. The cells were fractionated in the following manner.

'Acid-soluble' compounds. To the washed suspension at 0° was added an equal volume of cold 10% (w/v) trichloroacetic acid. After being mixed and standing at 0° for 15 min. the suspension was centrifuged. The supernatant was extracted three times with ether to remove the trichloroacetic acid, and samples were taken for determining radioactivity.

Lipids. To the precipitate after the above treatment, CHCl₃-methanol (1:1, v/v) was added. After the suspension had been mixed and centrifuged, the supernatant, which contained lipids and pigments, was removed and washed with 0.2 vol. of 0.1% KCl. Samples were taken for the determination of lipid phosphorus and of radioactivity.

Nucleic acids. To the residue after removal of lipids and pigments there was added 5% (w/v) trichloroacetic acid, and the suspension was heated at 90° for 15 min. The suspension was cooled and centrifuged, and the supernatant was extracted with ether to remove the trichloroacetic acid.

Protein. The precipitate, which consisted mainly of protein, was suspended in water at alkaline pH. Samples were taken for the determination of protein and of radioactivity.

Fractionation of lipids. The washed lipid extract prepared as described above was evaporated to dryness in a stream of N₂, and the lipid was dissolved in a small volume of CHCl₃. Chromatography was performed on columns of silicic acid (100 mesh; Mallinckrodt Chemical Works, St Louis, Mo., U.S.A.) activated at 100° overnight before use. Elution of the pigments was effected with CHCl₃ followed by a small volume of acetone. Phospholipids were then eluted with methanol.

Deacylation of the phospholipids. The methanol eluates were evaporated to dryness in a stream of N₂, the residues were dissolved in ethanol containing 5% CCl₄ and treated with 0.2 N-KOH in methanol at 37° for 20 min. (Benson & Maruo, 1958). Ethyl formate was then added to neutralize the alkali and incubation was continued at 37° for a further 5 min. The reaction mixture was evaporated to dryness in a stream of N₂, and partition was effected between 1 vol. of the upper phase and 2 vol. of the lower phase of water-2-methylpropan-1-ol-CHCl₃ (9:6:12, by vol.; Rhodes & Dawson, 1960). The organic phase contained the fatty acids and any non-saponifiable material; the aqueous phase contained the phosphate diesters. Portions of both phases were taken for the determination of radioactivity. Phosphorus was estimated in the aqueous phase. Specific radioactivities are reported as counts/min./μg. of phosphorus.

RESULTS

Inhibition of porphyrin excretion by nucleotides. Gajdos & Gajdos-Török (1965) reported that ATP and UTP inhibited porphyrin excretion by *R. spheroides* illuminated in mixture IS. Inhibition, which increased with the concentration of nucleotide added, was almost complete at 3 mm. At equivalent concentrations AMP was less effective than ADP, which in turn was less effective than ATP. It has now been found that CTP and GTP produce about the same degree of inhibition of porphyrin excretion as ATP and UTP (Table 1). Unlike ADP and AMP, none of the other nucleoside di- and mono-phosphates tested (UDP, CDP, TDP, UMP, CMP and TMP) caused more than 15% inhibition when used at a concentration of 3 mm.

In the course of the illuminations, which were usually carried out for 17-24 hr., the dry weight and the bacteriochlorophyll increased about two-fold. ATP had no effect on these increases.

ATP was effective in stopping further excretion of porphyrin at whatever time after the start of the illumination it was added.

Porphyrin excretion in the presence of ATP and ethionine. In contrast with the effect of ATP shown above, ethionine is known to increase porphyrin excretion under these conditions (Gibson *et al.* 1962b). The effects on porphyrin excretion of adding both ATP and ethionine at different concentrations are shown in Table 2. ATP at 1 mm and 2 mm greatly decreased porphyrin excretion in the absence of ethionine but had a less marked effect in

Table 1. *Effect of nucleotides on porphyrin excretion by R. spheroides*

R. spheroides (1.1 mg. dry wt./ml.) was illuminated in mixture IS at 32-34° for 17 hr. in the presence of the concentrations of nucleotides shown. At the end of illumination the organisms were removed by centrifuging and the coproporphyrin in the supernatant was estimated.

Additions	Concn. (mm)	Coproporphyrin (μmoles/ml.)
None	—	68.3
ATP	0.5	58.8
	1.0	46.0
	1.5	26.4
	3.0	1.4
GTP	0.5	45.8
	1.0	31.0
	1.5	19.0
UTP	0.67	49.6
	1.17	45.6
	3.0	1.5
CTP	0.67	40.6
	1.17	35.5

Table 2. *Effect of ATP and ethionine on porphyrin excretion*

R. spheroides (1.1mg. dry wt./ml.) was illuminated in mixture IS at 32–34° for 17hr. in the presence of the concentrations of ATP and ethionine stated. At the end of illumination the organisms were removed by centrifuging and the coproporphyrin in the supernatant was estimated.

Expt. no.	ATP (mM)	L-Ethionine (mM)	Coproporphyrin (mμmoles/ml.)
1	—	—	27.4
	—	2.0	77.4
	1.0	—	11.6
	1.0	2.0	70.8
	2.0	—	2.0
	2.0	2.0	60.8
2	—	—	39.0
	2.0	—	3.8
	—	0.1	53.8
	2.0	0.1	19.2
	—	0.2	65.0
	2.0	0.2	33.0

Table 3. *Effect of time of addition of ethionine on porphyrin excretion in the presence of ATP*

R. spheroides (1.2mg. dry wt./ml.) was illuminated in the presence of ATP (3mM). At various times after the start of illumination DL-ethionine (2mM) was added. Illumination was for a total of 18hr. Coproporphyrin was then estimated in the supernatants.

Time of addition of ethionine (hr.)	Coproporphyrin (mμmoles/ml.)
0*	46.5
0	17.2
0.5	19.9
1.0	16.4
2.0	12.6
3.0	10.5
4.0	0.8

* No ATP.

the presence of 0.1 mM- and 0.2 mM-ethionine, and its effect was virtually abolished by 2mM-ethionine. The inhibition of growth and of the production of bacteriochlorophyll caused by ethionine was not relieved by ATP.

When ATP (3mM) was present at the start of the illumination and ethionine (2mM) was added up to 3hr. later, porphyrin excretion was observed at the end of illumination (Table 3). However, if the addition of ethionine was delayed for 4hr., porphyrin excretion did not occur. In contrast, when ethionine was present at the start of illumination the addition of ATP up to 8hr. later immediately stopped porphyrin excretion.

Table 4. *Uptake of ATP by R. spheroides*

R. spheroides (50mg. dry wt.) was illuminated at 32–34° for 24hr. in 50ml. of mixture IS containing ATP (3mM). ATP labelled with ^{32}P (2μC) in the terminal phosphate group and 2μC of [^{14}C]ATP were added. After incubation cells were harvested and washed with buffer until the washings were free of radioactivity. The radioactivity of samples was determined as described in the Materials and Methods section.

	Time of incubation (hr.)	Radioactivity (counts/min./0.1 ml.)		Ratio $^{32}\text{P}/^{14}\text{C}$
		^{32}P	^{14}C	
Whole suspension	0	2197	1075	2.04
Whole suspension	24	2302	1125	2.05
Supernatant	24	2137	1125	1.90
Cells	24	36	21	1.71

Uptake of ATP. The marked effect of ATP on porphyrin excretion by *R. spheroides* together with the fact that it was more effective than equivalent concentrations of ADP or AMP suggests that ATP as such is taken up by the cells. Measurements of intracellular amounts of ATP support this suggestion. When illuminations were performed with ATP labelled both with ^{32}P (in the terminal phosphate) and ^{14}C (in C-8 of the adenine ring), the ratio $^{32}\text{P}/^{14}\text{C}$ in the cells was approximately the same as that of the ATP added (Table 4). Direct measurement of ATP in the cells by the method of Strehler & Totter (1954), after illumination for short periods in mixture IS containing ATP (3mM), showed an increase in intracellular ATP amounts that was markedly greater than the increase observed under the same conditions but without addition of ATP. It was found that with different batches of cells there was rather a wide variation in the ATP content before illuminations were started. The possible reasons for this variation are being investigated.

Effect of ATP and ethionine on the utilization of glycine, succinate and fumarate. The utilization of the organic constituents of mixture IS in the presence and absence of ATP and ethionine was investigated to see whether there were any alterations that might lead to information on the mechanisms by which ATP and ethionine were producing the marked changes in porphyrin excretion.

When *R. spheroides* was illuminated in mixture IS containing [^{14}C]glycine there was a slightly more rapid incorporation of radioactivity into cellular material when ATP (3mM) was also present (Fig. 1a) and a markedly decreased incorporation when ethionine (1mM) was present (Fig. 1b). At 6hr. 48% and 58% of the radioactivity was in the cells in the absence and presence of ATP respectively.

Fig. 1. Effect of ATP and ethionine on the rate of uptake of [2-¹⁴C]glycine. *R. spheroides* (1.2mg. dry wt./ml.) was illuminated at 32–34° in mixture IS [total volume 10ml. in (a) and 12ml. in (b)] containing [2-¹⁴C]glycine (1μC). At the times stated, 1ml. samples were removed and centrifuged immediately at 0° for 10min. at 23 000g. All the supernatant was removed and the cells were suspended to 1ml. in water. Portions (0.1ml.) of those suspensions were plated, dried and counted. Additions: ●, ATP (3mM); ▲, L-ethionine (1mM); ○, none. Whole suspensions at zero time in Expt. (a) gave 3800 counts/min./0.1ml., and in Expt. (b) 2700 counts/min./0.1ml.

Samples of cells taken at various times during the experiments described in Fig. 1 were fractionated as described in the Materials and Methods section. ATP greatly increased the rate of incorporation of label from [2-¹⁴C]glycine into the lipid fraction (Fig. 2a) and ethionine greatly lowered incorporation (Fig. 2b). ATP had no marked effect on the net amount of phospholipid synthesized, into which most of the radioactivity was incorporated (see below), but ethionine did lower it. The rate of incorporation of radioactivity into protein was slightly increased by ATP but was decreased by ethionine. Both ATP and ethionine lowered the rate of incorporation into the nucleic acids.

Organisms were illuminated for 22hr. with [1-¹⁴C]glycine and [2-¹⁴C]glycine in the presence and absence of ATP (3mM), and the radioactivity incorporated into various cellular components was determined (Table 5). All the radioactivity from [2-¹⁴C]glycine was present either in the cells or in the supernatant at the end of illumination, whereas

Fig. 2. Effect of ATP and ethionine on the rate of incorporation of radioactivity from [2-¹⁴C]glycine into lipids. The suspensions of cells in water from the experiments described in Figs. 1(a) and 1(b) were fractionated as described in the Materials and Methods section. The lipid fraction from each was dissolved in CHCl₃ to give a volume equal to that of the suspension extracted. Portions of these solutions were plated, dried and counted. Additions: ●, ATP (3mM); ▲, L-ethionine (1mM); ○, none.

more than half of the radioactivity from [1-¹⁴C]glycine was lost. This loss is unlikely to be due to loss of volatile organic acids, since the samples of cells and supernatants were dried at neutral pH before being counted. It is probable that it was lost as ¹⁴CO₂, although this was not measured.

It can be seen that, although both carbon atoms of glycine were incorporated into protein, essentially only the methylene carbon atom was incorporated into lipid. At 22hr., with [2-¹⁴C]glycine and no ATP, 64% of the radioactivity was in the cells; of this, 24% was in the lipid and 69% in the protein. In the presence of ATP, 78% of the radioactivity was in the cells; of this, 38% was in the lipid and 58% in the protein.

Samples of protein were hydrolysed, the amino acids were separated by two-dimensional paper chromatography and the chromatograms were

Table 5. *Illumination in the presence of [1-¹⁴C]glycine and [2-¹⁴C]glycine*

R. spheroides (1.25 mg. dry wt./ml.) was illuminated in mixture IS (total vol. 10 ml.) at 32–34° for 21 hr. in the presence and absence of ATP (3 mM) with the addition of 0.5 μ C of [1-¹⁴C]- or [2-¹⁴C]-glycine. After illumination the cells and supernatant were separated by centrifuging. Portions of each were counted. The cells were then fractionated as described in the Materials and Methods section. Radioactivities are expressed per ml. of original suspension.

Additions	Radioactivity (counts/min./ml.)				
	Suspension (0 hr.)	Cells (21 hr.)	Supernatant (21 hr.)	Lipid	Protein
[1- ¹⁴ C]Glycine	31 100	6650	7380	353	5298
[1- ¹⁴ C]Glycine + ATP	31 850	7025	4180	793	5117
[2- ¹⁴ C]Glycine	29 625	18 913	137 40	4527	13 162
[2- ¹⁴ C]Glycine + ATP	29 250	22 688	6600	8640	13 246

Table 6. *Incorporation of radioactivity from [2-¹⁴C]glycine into phospholipid*

R. spheroides (1.5 mg. dry wt./ml.) was illuminated in mixture IS (total vol. 200 ml.) at 32–34° for 30 hr. in the presence and absence of ATP (3 mM) and DL-ethionine (1 mM). [2-¹⁴C]Glycine (10 μ C) was also added. After illumination, coproporphyrin was determined in the supernatants. Phospholipid was isolated from cells and degraded as described in the Materials and Methods section. The values given for the specific radioactivities/newly synthesized phospholipid were calculated assuming that there was no turnover of any portions of the phospholipid molecules.

Additions	Coproporphyrin (m μ moles/ml.)	Lipid phosphorus (μ g./ml.)	Sp. radioactivity (counts/min./ μ g. of phosphorus)			
			Total phospholipid		Newly synthesized phospholipid	
			Phosphate diesters	Fatty acids	Phosphate diesters	Fatty acids
None	93.5	6.9	88	138	223	300
Ethionine	132	5.5	66	113	316	534
ATP	5.0	6.4	132	652	424	1799
ATP + ethionine	43.5	4.9	87	287	720	2330
Not illuminated		4.1				

radioautographed. In samples from experiments with [1-¹⁴C]glycine radioactivity was confined to the glycine and serine spots. In contrast, in experiments with [2-¹⁴C]glycine most of the amino acids were markedly radioactive. Those most highly labelled, apart from glycine, were leucine, isoleucine and phenylalanine. The extent of incorporation into these amino acids was increased by ATP, as judged from the radioautograms.

The effect of ethionine, ATP and ethionine plus ATP on the incorporation of the methylene carbon atom of glycine into lipid was investigated further. In the experiment described in Table 6, porphyrin excretion was increased by ethionine, almost completely abolished by ATP and markedly decreased by ethionine plus ATP. The amount of phospholipid synthesized, measured as lipid phosphorus, was slightly decreased by ATP, halved by ethionine and even more markedly decreased by ethionine plus ATP.

About 75% of the radioactivity incorporated into the lipid was found in the fraction eluted from silicic acid with methanol. This fraction, which consisted mainly of phospholipids, was deacylated and the

fatty acids and phosphate diesters were separated. The incorporation of radioactivity into these constituents is shown in Table 6. The results are given both as radioactivity/total phospholipid and as radioactivity/phospholipid synthesized during the illumination on the assumption that there was no turnover of any portion of the phospholipid molecule. In the presence of ethionine, which markedly decreased the synthesis of fatty acids, the specific radioactivity of the total fraction was lower than in its absence, but the specific radioactivity of the newly synthesized fatty acids was greater. ATP, which did not markedly decrease synthesis, produced a very much greater increase in specific radioactivity. This was also shown by ATP plus ethionine. The specific radioactivity of the newly synthesized phosphate diesters was also increased by the various compounds added to the medium, but not to such a marked extent. The phosphate diester fraction was hydrolysed further and ethanolamine and choline were separated (Fig. 3). In the absence of ATP or ethionine (sample 1) both ethanolamine and choline were labelled. In the presence of ATP (sample 3) they were more highly labelled.

Ethionine did not affect the labelling of ethanolamine (sample 2), but that of choline was decreased. With ethionine plus ATP (sample 4), incorporation into choline was virtually abolished, suggesting that very little net synthesis of phosphatidylcholine occurred.

In contrast with the marked effect of ATP on the utilization of glycine, it has very little effect on the rate or extent of incorporation of succinate or fumarate. Experiments were done as described above with [1,4- $^{14}\text{C}_2$]- and [2,3- $^{14}\text{C}_2$]-fumarate and -succinate and it was found that the incorporation of radioactivity into both the phosphate diester portion and the fatty acids of the phospholipids was unaffected by ATP.

DISCUSSION

Effect of ATP on porphyrin excretion. The medium used here was designed specifically by Lascelles (1956) to produce porphyrin excretion by suspensions of *R. spheroides*; growth occurs only to a limited extent, dry weight and bacteriochlorophyll increasing about twofold over 20 hr. Although glycine, succinate and fumarate are precursors of the porphyrin molecule, they are poor substrates when used to grow a culture from a small inoculum (A. Gajdos, M. Gajdos-Török, A. Gorchein, A. Neuberger & G. H. Tait, unpublished work). Porphyrin excretion also occurs when cultures grown semi-anaerobically in the light in medium S have entered the stationary phase after consuming all the malate (Cohen-Bazire, Sistrom & Stanier, 1957).

It is probable that under the conditions described above the cells are not fully metabolically competent and that the normal control mechanisms for the biosynthetic pathways leading to haem and bacteriochlorophyll have broken down. Although a number of control mechanisms have been shown to exist, their detailed action and relative importance are not known. Haem has been shown to act as both a repressor and an inhibitor of δ -aminolaevulate synthetase (Lascelles, 1960; Gibson, Matthew, Neuberger & Tait, 1961) an intermediate in bacteriochlorophyll synthesis has been postulated to inhibit one of the early steps in porphyrin synthesis (Gibson *et al.* 1962b; Lessie & Sistrom, 1964) and other inhibitors, both of low and of high molecular weight, may also exist (cf. Kikuchi, Kumar, Talmage & Shemin, 1958).

In mixture IS, iron deficiency is certainly one important factor in giving rise to porphyrin excretion, since addition of even a small amount of Fe^{2+} markedly decreases porphyrin excretion (Lascelles, 1956; Gibson *et al.* 1962b). The presence of adequate amounts of iron is likely to result in the formation of haem. However, this is probably only

a partial explanation. Gajdos & Gajdos-Török (1965) have shown that both ATP and UTP diminish porphyrin excretion, and in the present paper a similar effect has been demonstrated for GTP and CTP. Since it is not easy to see how the addition of nucleoside triphosphates to the medium can promote haem formation from protoporphyrin, it is likely that they act in a manner different from Fe^{2+} . It seemed possible that control of porphyrin production might be maintained by ATP through a mechanism yet to be discovered, and that incubation in mixture IS might result in low levels of ATP. Gajdos, Gajdos-Török, Palma-Carlos & Palma-Carlos (1966) showed that the ATP concentration in livers of animals made porphyric by a number of different compounds, including ethionine, is very markedly lower than in normal liver. These workers also observed, in the rat, a significant negative correlation between the concentration of ATP in liver and that of porphyrins during Sedor mid-induced porphyria, as well as during its spontaneous remission. However, direct measurements of ATP in *R. spheroides*, although showing changes in the expected direction, were so variable that no definite conclusion could be drawn from the findings. It is not impossible that nucleotides other than ATP are involved in a control mechanism; their intracellular concentrations have not been measured.

Nandi & Waygood (1967) have reported that the δ -aminolaevulate dehydratase from wheat leaves is strongly inhibited by ATP; 14% inhibition at 0.16 mM and 100% inhibition at 3 mM were observed. Such an effect, as these workers point out, could control porphyrin synthesis in the whole cell. Work performed in this Laboratory showed that the δ -aminolaevulate dehydratase in extracts of *R. spheroides* was not inhibited at all by 3 mM-ATP or by other nucleoside mono-, di- or tri-phosphates at concentrations of 1 mM.

Combined effects of ethionine and ATP. The increase in porphyrin excretion brought about by ethionine can be largely prevented if ATP is also added to the medium (Tables 2 and 3). This effect of ATP is not found if the amount of ethionine is large. The effects of ethionine were attributed (Gibson *et al.* 1962b) to its interference, as *S*-adenosylethionine, with the methylation of magnesium protoporphyrin, and this was supported by the finding that methionine reversed these effects. Although this interpretation is not invalidated, the present results suggest that ethionine also acts in other ways. Thus ATP, unlike methionine, does not prevent the inhibition of bacteriochlorophyll synthesis caused by ethionine. In addition, if the sole factor controlling porphyrin excretion were the inhibition of methylation of magnesium protoporphyrin, ATP plus ethionine, which might be expected to yield more *S*-adenosylethionine and to

Fig. 3. Incorporation of radioactivity from $[2-^{14}\text{C}]$ glycine into ethanolamine and choline. The phosphate diesters, isolated from the experiment of Table 6, were hydrolysed *in vacuo* with 6*N*-HCl at 105° for 24 hr. After removal of the HCl, samples were chromatographed on a thin-layer plate of MN 300 cellulose (Macherey, Nagel and Co.), made up in *m*-KCl instead of in water, with the solvent system of Bremer & Greenberg (1959). The chromatogram was radioautographed with Kodirex X-ray film (Kodak Ltd., London, W.C. 2). The samples chromatographed were obtained from cells illuminated with the following additions: 1, none; 2, ethionine; 3, ATP; 4, ethionine and ATP. Spot A is ethanolamine; spot B is choline.

produce a greater inhibition of methylation, ought to stimulate porphyrin excretion more than ethionine alone. Ethionine and ATP inhibit the methylation of phosphatidylethanolamine to phosphatidylcholine more than does ethionine alone (Fig. 3). Thus the possibility exists, as is postulated in animals, that ethionine, in addition to inhibiting methylation, also acts by depleting the cell of nucleoside triphosphates or their precursors (Shull, 1962; Shull, McConomy, Vogt, Castille & Farber, 1966) or by interfering with oxidation and, especially, oxidative phosphorylation (Stekol, Mody, Bedrak, Keller & Perry, 1960).

Effects of ATP on glycine metabolism. It was found that ATP had a marked effect on the metabolism of glycine, but had no significant effect on that of fumarate or succinate. The rate of uptake of glycine into the cell was increased by ATP, as were the rate and extent of incorporation of the methylene carbon atom into lipids. The carboxyl carbon atom of glycine was incorporated into protein but not to a marked extent into lipid. The bulk of the radioactivity of $[2-^{14}\text{C}]$ glycine in the lipid was in the phospholipids. Although ATP slightly stimulated incorporation into the phosphate diester

portion of the phospholipids, it increased incorporation into the fatty acids about fivefold (cf. Table 6). The increase in phospholipids, measured as lipid phosphorus, was not significantly affected by ATP. It is known that the major fatty acid in the phospholipids of *R. spheroides* is the C_{18} acid vaccenic acid (Hands & Bartley, 1962). Under our conditions this was also the case; no unusual fatty acids were detected by gas chromatography. With this information and the data of Table 6, the average specific radioactivity of the carbon atoms of the newly synthesized fatty acids (assuming no turnover), and hence the contribution of the methylene carbon atom of glycine to these, was calculated. It was found that, whereas only about 10% of the carbon of the newly synthesized fatty acids came from the methylene carbon atom of glycine in the absence of ATP, about 50% came from this source in the presence of ATP. The methylene carbon atom of glycine comprises 7% of the carbon content of the medium. Thus the pathway by which the methylene carbon atom of glycine, but not the carboxyl carbon, is incorporated into acetate appears to be quantitatively important. The occurrence of such a pathway is also supported by the finding that the methylene carbon atom of glycine was incorporated into those amino acids whose biosynthesis is known to involve acetate. Such incorporation was also stimulated by ATP. One possible pathway that is consistent with the radioactivity data is glycine $\rightarrow$ serine $\rightarrow$ pyruvate $\rightarrow$ acetyl-CoA. Tsuiki & Kikuchi (1962) were unable to demonstrate the conversion of glycine into serine or serine into pyruvate in whole cells of *R. spheroides*, and concluded that these reactions did not occur. However, recent studies in this Laboratory have shown the presence of serine hydroxymethyltransferase and serine dehydratase in cell-free extracts.

Although ATP causes a marked increase in the incorporation of the methylene carbon atom of glycine into fatty acids it does not produce a concomitant decrease in the incorporation of radioactivity from the other carbon atoms of the medium, i.e. those of succinate and fumarate, nor does it significantly affect the increase in phospholipid occurring during illumination. These results can be explained in at least two ways. It is possible that ATP produces or stimulates turnover of fatty acids and that in its presence acetate derived from glycine is used preferentially for this synthesis. However, so far we have been unable to demonstrate turnover under these conditions. Alternatively, the production of acetate from endogenous precursors may be inhibited in the presence of ATP and the extra acetate required to maintain fatty acid synthesis may come from glycine. Although it is not known whether the effects of ATP

on porphyrin excretion and on glycine metabolism are related, it is possible that increased utilization of glycine for fatty acid production in the presence of ATP might bring about a fall in the intracellular glycine and thereby a decrease in porphyrin synthesis.

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