Biochem. J. (1980) **186**, 13–19
Printed in Great Britain

Microbial Metabolism of Amino Alcohols

BIOSYNTHETIC UTILIZATION OF ETHANOLAMINE FOR LIPID SYNTHESIS BY BACTERIA

Shivendra D. SHUKLA* and John M. TURNER
Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

(*Received* 1 *June* 1979)

1. Ten bacteria utilizing [2-^{14}C]ethanol-2-amine as the sole or major source of nitrogen for growth on glycerol+salts medium incorporated radioactivity into a variety of bacterial substances. A high proportion was commonly found in lipid fractions, particularly in the case of *Erwinia carotovora*. 2. Detailed studies of [^{14}C]ethanolamine incorporation into lipids by five bacteria, including *E. carotovora*, showed that all detectable lipids were labelled. Even where phosphatidylethanolamine was the major lipid labelled, radioactivity was predominantly in the fatty acid rather than the base moiety. The labelled fatty acids were identified in each case. 3. The addition of acetate to growth media decreased the incorporation of radioactivity from ethanolamine into both fatty acid and phosphatidyl-base fragments of lipids from all the bacteria except *Mycobacterium smegmatis*. Experiments with [^{3}H]ethanolamine and [^{14}C]acetate confirmed that unlabelled acetate decreased the incorporation of both radioactive isotopes into lipids, except in the case of *M. smegmatis*. 4. Enzyme studies suggested one of two metabolic routes between ethanolamine and acetyl-CoA for each of four bacteria. A role for ethanolamine *O*-phosphate was not obligatory for the incorporation of [^{14}C]ethanolamine into phospholipids, but correlated with CoA-independent aldehyde dehydrogenase activity.

Many bacteria are capable of growth on media in which ethanolamine serves as a nitrogen source (Blackwell *et al.*, 1976). Initial studies showed that *Erwinia carotovora* incorporated radioactivity from [^{14}C]ethanolamine into cell substance, probably phospholipid, although most was excreted as acetaldehyde (Jones & Turner, 1971). The deamination of ethanolamine by species of *Erwinia*, *Flavobacterium*, *Pseudomonas* and *Achromobacter* is known to involve biodegradative kinase and phospho-lyase enzymes with ethanolamine *O*-phosphate as the intermediate (Jones & Turner, 1971, 1973; Jones *et al.*, 1973; Faulkner & Turner, 1974*a*). In contrast a coenzyme B_{12}-dependent ethanolamine ammonia-lyase forming acetaldehyde directly is responsible in *Escherichia coli*, *Klebsiella aerogenes* (Scarlett & Turner, 1976; Blackwell *et al.*, 1977; Blackwell & Turner, 1978) and a species of *Pseudomonas* (Faulkner & Turner, 1974*b*).

Although bacteria are believed to synthesize phosphatidylethanolamine via phosphatidylserine, by the CDP-diacylglycerol route (Kanfer & Kennedy, 1964), a role for ethanolamine *O*-phosphate and CDP-ethanolamine in phospholipid biosynthesis by a species of *Mycobacterium* has been proposed (Nandedkar, 1974, 1975). The possibility that this route, characteristic of eukaryotes (Thompson, 1973), may provide the base fragment of phosphatidylethanolamine in this and other bacteria prompted a comparative study. Bacteria known to metabolize ethanolamine via its *O*-phosphate, or otherwise, were included.

* Present address: Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

Materials and Methods

Micro-organisms and media

E. carotovora (N.C.P.P.B. 1280) was obtained from the National Collection of Plant Pathogenic Bacteria, Ministry of Agriculture, Fisheries and Food, Harpenden, Herts., U.K. *M. smegmatis* ('Mycobacterium 607' of Nandedkar, 1974, 1975) was obtained as N.C.T.C. 7017 (A.T.C.C. 607) from the National Collection of Type Cultures, Central Public Health Laboratory, London NW9 5HY, U.K. All other bacteria were obtained from the National Collection of Industrial Bacteria, Aberdeen AB9 8DG, Scotland, U.K., and are designated by their N.C.I.B. catalogue numbers in Table 1.

Most bacteria were cultivated at 28–30°C on either a simple synthetic liquid medium containing (per litre) 3g of glycerol, 0.15g of ethanolamine and mineral salts (Jones *et al.*, 1973; Clough *et al.*, 1975), or on the same medium supplemented with 0.1g of yeast extract or 40μg of vitamin B_{12} (Blackwell *et al.*,

0306-3283/80/010013-07 $1.50/1

1976) as indicated in Table 1. Growth in liquid cultures was followed spectrophotometrically (A_{540}^{1cm}) and bacteria were harvested by centrifuging as described previously (Jones *et al.*, 1973). In the case of *M. smegmatis* only, growth was on the unsupplemented medium solidified with 2% (w/v) agar at 37°C (Blackwell *et al.*, 1976). Surface cultures, grown in Roux bottles, were harvested by suspending in water and centrifuging at 50000*g* for 30min.

Incorporation of radioactivity from [^{14}C]ethanolamine into bacterial substances

Growth media contained (per litre) 5μCi (35mg of N) of [2-^{14}C]ethanol-2-amine. The fate of ^{14}C assimilated was followed by fractionating bacteria by the extraction procedure of Roberts *et al.* (1957) and measuring the radioactivity of each fraction. Radioactive lipids were obtained by solvent extraction of ultrasonically disrupted bacteria. Freshly harvested bacteria were washed, resuspended in water and treated for approx. 4min at full power in an MSE 100W ultrasonic disintegrator. The disrupted suspension was centrifuged for 1h at 300000*g* and the pellet was washed once by recentrifugation. Lipids were extracted from the pellet, essentially by the method of Folch *et al.* (1957), by treatment with chloroform/methanol (2:1, v/v) for 30min at room temperature. Extracts were washed twice with 0.2vol. of choloroform/methanol/water (3:48:47, by vol.) containing 0.1M-KCl. The washed 'total lipid' extract was dried under N_2 and stored at 0°C.

Analysis of radioactive lipid

T.l.c. of lipid samples was done on silica gel G (Merck) with chloroform/methanol/1M-NH_3 (80:36:5, by vol.) as the solvent system. Lipids were detected on plates with ninhydrin, acid molybdate or vanillin reagents, and with I_2, by standard procedures. Pure lipids were used as reference markers. In quantitative experiments, spots or bands of lipid on the absorbent, revealed by I_2, were removed and eluted with portions of chloroform/methanol (2:1, v/v), chromatography solvent and methanol, used successively. Eluates were combined and dried under N_2.

Analysis of lipids by saponification

Saponification. Lipid samples (approx. 10μmol of lipid phosphate) were saponified by incubation with 1–2ml of 5M-KOH in aq. 50% (v/v) methanol for 2h at 100°C. To this, 1ml of water was added and extracted three times with 3ml of pentane. The pentane layer contained the non-saponifiable fraction (neutral lipids). The hydrolysate was then acidified with HCl to pH2.0 and extracted three times with 3ml of pentane. This pentane layer was washed twice with 2ml portions of water and contained the saponifiable fraction (fatty acids). The residual aqueous fraction, containing glycerophosphoryl base, was also retained for analysis.

Analysis of aqueous fraction by t.l.c. 'Aqueous fraction' obtained from saponification of lipids (see above) from *E. carotovora* was hydrolysed in 6M-HCl or 5M-NaOH in a boiling water bath for 1h. The hydrolysates were run on cellulose (Whatman CC41) plates (0.25mm) in the solvent system methanol/formic acid/water (80:13:7, by vol.). The spots were identified by spraying with ninhydrin for amino groups, acid molybdate for phosphate and vanillin for sugar alcohols, by using standard reagents and procedures. Radioactive spots were detected by using a Panax t.l.c. scanner.

Analysis of fatty acids by radio-g.l.c. Samples of fatty acids (taken in Teflon-capped sample tubes) were dissolved in 0.2ml of pentane and 0.5ml of methanolic 14% (w/v) BF_3 was added. The mixture was heated at 100°C in a boiling-water bath for 10min and then 0.3ml of water was added. The methyl esters of fatty acids thus formed were extracted three times with 0.5ml portions of pentane and were pooled together. This pentane layer was concentrated by evaporation under N_2 and then injected directly into Pye–Unicam radio-gas–liquid chromatography (Pye series 104) fitted with automatic radioactivity counting and print-out system. A 3% SILAR column was used in all cases and operated at an oven temperature of 175°C. Authentic fatty acid methyl esters were run as markers and sample peaks were identified by comparing their relative retention times.

Radioactive counting

Aqueous samples (1ml) were mixed with 10ml of Aquasol Universal liquid-scintillation-counting 'cocktail' (NEN Chemicals G.m.b.H., Dreieichenhain, Germany). For non-aqueous samples 10ml of 5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole/toluene liquid scintillator [7g of 5-(biphenyl-4-yl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole/litre of toluene] was used. Spots from thin-layer plates were directly taken into radioactive vials and 10ml of Aquasol was added. All these samples were counted for radioactivity in an Intertechnique ABAC SL 30 liquid-scintillation spectrometer. By using appropriate programmes this machine was capable of background subtraction and calculating quenching and efficiency.

Enzyme extracts and assays

Suspensions of bacteria, harvested when the A_{540}^{1cm} was 0.9, were disrupted in an Aminco–French pressure cell (American Instrument Co. Inc., Silver Spring, MD, U.S.A.), cell debris was removed by centrifuging and the protein content of extracts

measured as previously described (Turner, 1966). Enzyme assays were done at 37°C. No attempts were made to optimize the conditions of assay for the enzymes in extracts of each micro-organism.

Ethanolamine ammonia-lyase. Activity was assayed at pH 7.5 by a method involving the colorimetric measurement of acetaldehyde (Scarlett & Turner, 1976; Blackwell & Turner, 1978).

Ethanolamine O-phosphate phospho-lyase. Assays were done at pH 8 by the colorimetric measurement of acetaldehyde formation (Jones & Turner, 1973; Jones *et al.*, 1973; Faulkner & Turner, 1974*b*).

Acetaldehyde dehydrogenase. CoA-dependent activity was measured spectrophotometrically, by following NAD^+ reduction at pH 7, by the procedure of Rudolph *et al.* (1968). CoA-independent enzyme activity was also measured spectrophotometrically, at pH 9, by the procedure of Jakoby (1958).

Results

Assimilation of ethanolamine by bacteria during growth

Of 24 strains of bacteria capable of growth with ethanolamine as the sole nitrogen source, ten taxonomically diverse species were examined in more detail. Cultures grown on glycerol+salts medium supplemented with a growth-limiting concentration of [^{14}C]ethanolamine were harvested in the late-exponential-growth phase and fractionated by the differential extraction procedure of Roberts *et al.* (1957). The results of a typical experiment (Table 1) showed that in most bacteria a high proportion of the radioactivity was incorporated into the alcohol-soluble fraction containing lipids. Significant radioactivity was also found in the residue fraction consisting of polymeric materials including protein. *E. carotovora* had the largest proportion of radioactivity in the lipid-containing fraction and *M. smegmatis* was unusual in that this fraction possessed little radioactivity.

[^{14}C]*Ethanolamine incorporation into bacterial lipids*

Four bacteria found to incorporate ethanolamine preferentially into the lipid-containing fraction, together with the anomalous *Mycobacterium* sp., were studied further. Bacteria harvested after growth on nitrogen-limited [^{14}C]ethanolamine medium were disrupted and extracted with chloroform/methanol as described in the Materials and Methods section. *E. carotovora* incorporated up to 3% of the radioactivity supplied into lipids, whereas the other bacteria incorporated somewhat less.

Analysis of the extracted lipids by t.l.c. (see the Materials and Methods section) showed that all those detectable were radioactive. In the cases of *E. carotovora* and *Escherichia coli* phosphatidylethanolamine accounted for more than 80% of the radioactivity found and in *Flavobacterium rhenanum* it represented the most radioactive single lipid found (see Table 2). In *M. smegmatis* radioactive phosphatidylethanolamine was only a minor product of ethanolamine assimilation. In *C. aquaticum* radioactive phosphatidylethanolamine was not detectable. In both these bacteria diphosphatidylglycerol,

Table 1. *Fractionation of bacteria grown on* [^{14}C]*ethanolamine*

Liquid cultures of bacteria were grown on nitrogen-limiting media containing glycerol+ethanolamine (35 mg of N/litre) and [^{14}C]ethanolamine (1 μCi/100ml) at 30°C except *M. smegmatis*, which was grown as surface cultures in Roux bottles at 37°C (see the Materials and Methods section). Bacteria were collected and washed by centrifuging (see the Materials and Methods section) and then fractionated by the method of Roberts *et al.* (1957). The values represent the percentages of the total radioactivity recovered. Bacterial strains are identified by their National Collection of Industrial Bacteria (N.C.I.B.) catalogue numbers unless otherwise indicated (see the Materials and Methods section). Abbreviation: TCA, trichloroacetic acid.

	Radioactivity (% of total)			
Bacterium	Cold TCA treatment	Alcohol-soluble	Hot TCA treatment	Residue (protein)
Arthrobacter globiformis (8605)*	13	18	7	62
Corynebacterium aquaticum (9460)*	36	30	10	24
E. carotovora (N.C.P.P.B. 1280)	14	58	8	20
Escherichia coli (8114)†	15	55	6	24
Flavobacterium rhenanum (9157)	11	49	8	32
Klebsiella aerogenes (418)	47	25	9	19
M. smegmatis (N.C.T.C. 7017)	2	9	15	74
Proteus mirabilis (6389)*	45	23	10	22
Pseudomonas putida (9304)	6	15	7	72
Staphylococcus aureus (6571)*	10	28	8	54

* Medium supplemented with 0.01% (w/v) yeast extract.
† Medium supplemented with vitamin B_{12} (40 μg/litre).

Table 2. *T.l.c. of lipids extracted from bacteria grown on [^{14}C]ethanolamine as the nitrogen source*
Bacteria were grown on media containing nitrogen-limiting [^{14}C]ethanolamine (5 μCi/litre), harvested in the late-exponential-growth phase, disrupted ultrasonically and centrifuged to obtain the pellet (see the Materials and Methods section). Lipids from these pellets were extracted by the method of Folch *et al.* (1957), separated by t.l.c. and detected with I_2. Each lipid was identified by comparison with authentic markers and transferred to a vial. To this vial 10ml of Aquasol was added and the radioactivity was measured (see the Materials and Methods section). Several experiments showed that 85–95% of the radioactivity of lipid extracts was recovered after separation by t.l.c. Values represent the percentage of total radioactivity recovered.

	Radioactivity (% of total)				
Bacterium ... / Phospholipid	*E. carotovora*	*Escherichia coli*	*F. rhenanum*	*M. smegmatis*	*C. aquaticum*
Phosphatidylethanolamine	95	83	34	5	0
Phosphatidylglycerol	0	8	21	<2	0
Phosphatidylinositol	3	0	0	4	18
Phosphatidylserine	<1	<1	16	3	0
Diphosphatidylglycerol	<1	6	18	12	10
Lysophosphatidylethanolamine	<1	<1	<2	3	0
Phosphatidic acid	<1	0	<2	12	12
Origin	<1	<2	<2	6	14
Solvent front	<1	<1	7	54	41
Unknown	0	0	0	0	4

phosphatidic acid and a variety of lipids running at the solvent front were major products of ethanolamine assimilation.

Intramolecular localization of radioactivity in phospholipids

Although [^{14}C]ethanolamine clearly gave rise to lipids other than phospholipids in some bacteria, it was possible that it could be utilized directly to provide the base moiety of phosphatidylethanolamine, as suggested by Nandedkar (1974, 1975), particularly in the case of *E. carotovora*. This bacterium metabolized ethanolamine to acetaldehyde, which was excreted and did not appear to be further metabolized (Jones & Turner, 1971; Jones *et al.*, 1973). Preliminary experiments showed that when radioactive phospholipid from *E. carotovora* was hydrolysed with phospholipase D and the products analysed by t.l.c., ethanolamine was a radioactive product (Clough *et al.*, 1975). In further experiments, lipids from all five bacteria were saponified and separated into saponifiable (fatty acid), aqueous (glycerophosphoryl base) and non-saponifiable (neutral lipid) fractions (see the Materials and Methods section). Radioactivity from [^{14}C]ethanolamine was found predominantly in the saponified fatty acid rather than the aqueous base-containing fraction in all cases (Table 3).

In *E. carotovora* the radioactivity in the base-containing fraction was relatively high. When treated with 6 M-HCl or 5 M-NaOH and the products separated by t.l.c. (see the Materials and Methods section), the hydrolysate consisted of glycerol, [^{14}C]ethanolamine *O*-phosphate and traces of [^{14}C]ethanolamine. Determination of the ratio of radioactivity/phosphorus in total lipid and phosphatidylethanolamine samples gave similar values confirming that the phospholipid was the major lipid present. The ratio for the aqueous fraction of the saponified lipid was much lower indicating that a significant amount of ^{14}C was present in the phosphatidyl as well as the ethanolamine fragment.

Table 3. *Radioactive ethanolamine incorporation into the saponified-lipid fractions of different bacteria*
The extraction of radioactive lipids from different bacteria grown with [^{14}C]ethanolamine as the nitrogen source was done as described in Table 2. Lipids were saponified as outlined in the Materials and Methods section. The amounts of radioactivity found in each fraction after saponification are represented as the percentage of the total recovered (recovery was greater than 95%).

	Radioactivity (% of total recovered)		
Bacterium	Non-saponified fraction	Saponified fraction	Aqueous fraction
E. carotovora	<2	78	21
Escherichia coli	<2	95	3
F. rhenanum	<1	98	<2
M. smegmatis	26	61	13
C. aquaticum	21	66	13

The nature of the fatty acids labelled during growth of the five bacteria with [^{14}C]ethanolamine was determined by lipid extraction, saponification, methylation and g.l.c., as described in the Materials and Methods section. The results showed that in each bacterium, [^{14}C]ethanolamine donated its

radioactivity indiscriminately to all of the fatty acids detected. The most radioactive were C_{16} and C_{18} fatty acids in all cases, with the *Mycobacterium* species characteristically possessing additional longer-chain fatty acids (see Table 4).

Effect of acetate on the assimilation of ethanolamine into phospholipids

Initial studies with *E. carotovora* showed that 8mM-acetate added to the growth medium decreased the incorporation of ^{14}C from ethanolamine into total lipids. Saponification showed that acetate decreased incorporation into both the fatty acids and glycerophosphoethanolamine fractions to a similar extent.

The effect of unlabelled acetate on the incorporation of [^{3}H]ethanolamine and [^{14}C]acetate into the lipids of each of the five bacteria was measured (see the Materials and Methods section). The radioactivities due to each radioactive isotope in the total lipid, fatty acid and glycerophosphoethanolamine fractions were progressively decreased by acetate concentrations up to the maximum (28mM) tested, in all cases except *M. smegmatis*. The results obtained with acetate at 20mM, measuring radioactive-isotope incorporation into total lipids, are shown in Table 5. Paradoxically, the inclusion of acetate in growth medium for *M. smegmatis* increased the incorporation of both ^3H and ^{14}C into each lipid fraction studied (Fig. 1).

Enzyme activities in bacterial extracts

The radioactive-isotope evidence suggesting that acetate or acetyl-CoA was an intermediate of ethanolamine metabolism in four of the bacteria studied

Table 5. *Effect of acetate on the incorporation of [^{3}H]ethanolamine and [^{14}C]acetate into lipids of bacteria*
Liquid cultures (100ml) growing on nitrogen-limiting media (see Table 2), containing various concentrations of unlabelled acetate (in the range 0–28mM) and 20μCi of [^{3}H]ethanolamine+0.5μCi of [^{14}C]acetate were used. Bacteria were harvested in the exponential phase and lipids were extracted as described in Table 2. The radioactivity of the lipids was measured (see the Materials and Methods section). Results obtained at 20mM-acetate only are shown together with the control values.

Bacterium	Acetate (mM)	Radioactivity (d.p.m.) ^{3}H	^{14}C
E. carotovora	0	637122	78737
	20	31426	11932
Escherichia coli	0	232819	54889
	20	143893	16359
F. rhenanum	0	30580	49739
	20	10853	12221
C. aquaticum	0	3460	20766
	20	1900	594

Table 4. *Analysis of fatty acids from bacteria grown on [^{14}C]ethanolamine*
Radioactive lipids were extracted from bacteria grown on [^{14}C]ethanolamine (see Table 2). The lipids were saponified and fatty acids converted into methyl esters (see the Materials and Methods section). A Pye–Unicam radio-gas–liquid chromatograph was used to separate the fatty acids, which were identified by comparison with authentic samples (see the Materials and Methods section). Values given represent the percentage of total radioactivity recovered in all fractions after chromatography.

Fatty acid	Radioactivity (% of total recovered) *E. carotovora*	*Escherichia coli*	*F. rhenanum*	*M. smegmatis*	*C. aquaticum*
C_{12}	1.4	2.4	1.7	Trace	6.4
C_{14}	Trace	8.2	6.9	4.5	2.4
$C_{14:1}$	Trace	2.8	0	0	0
C_{16}	27.3	24.7	28.7	27.2	35.1
$C_{16:1}$	38.9	0	9.1	11.3	19.4
$C_{16:2}$	0	0	Trace	0	0
C_{17}	0	13.7	9.7	Trace	18.9
C_{18}	0	20.0	15.2	14.1	7.1
$C_{18:1}$	24.2	11.9	10.8	13.5	3.8
$C_{18:2}$	Trace	0	12.1	0	0
$C_{18:3}$	0	0	0	2.9	0
C_{19}	Trace	8.8	5.7	0	0
C_{20}	0	4.5	0	6.1	0
$C_{20:1}$	0	0	0	2.4	0
C_{22}	0	0	0	5.5	0
$C_{22:1}$	0	0	0	8.3	0
C_{24}	0	0	0	3.8	0
Unidentified	0	2.8	0	0	6.8

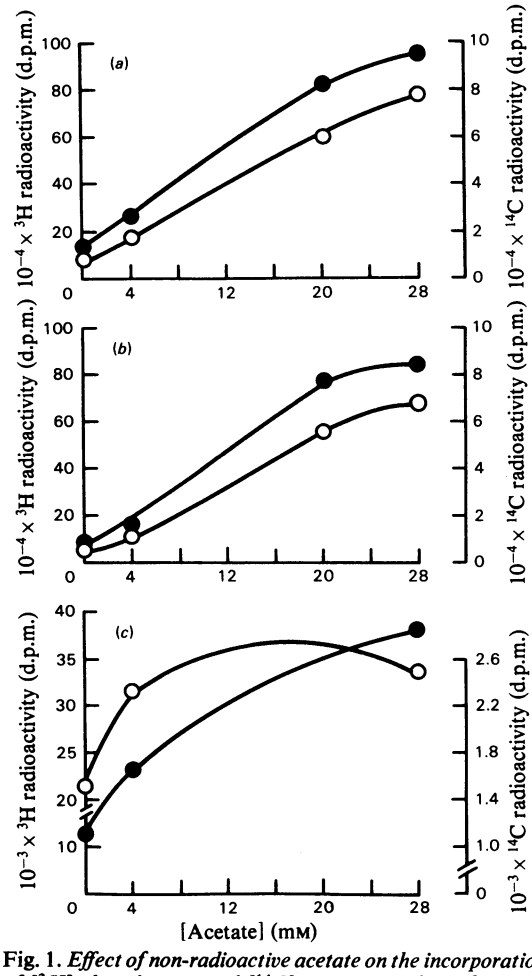

Fig. 1. *Effect of non-radioactive acetate on the incorporation of [^{3}H]ethanolamine and [^{14}C]acetate into lipid fractions of M. smegmatis*

The experimental details were as described for Table 5. Lipids were extracted, saponified and the radioactivity due to ^{3}H (●) and ^{14}C (○) measured in each fraction as described in the Materials and Methods section. (*a*) Total lipid fraction; (*b*) fatty acid fraction; (*c*) aqueous (glycerophosphoryl-base) fraction.

prompted a search for aldehyde dehydrogenase activities in cell-free extracts. Both CoA-dependent and -independent activities were assayed, together with ethanolamine ammonia-lyase and ethanolamine *O*-phosphate phospho-lyase activities (see the Materials and Methods section). The results are shown in Table 6. The presence of CoA-dependent acetaldehyde dehydrogenase in extracts correlated with the presence of the ethanolamine ammonia-lyase enzyme. Enzyme assays were also done with *Klebsiella aerogenes* for comparison purposes.

Discussion

The main conclusions to be drawn from the results described are that a wide variety of bacteria, growing on glycerol as the major carbon source, utilized both the nitrogen and carbon of ethanolamine biosynthetically and that acetate or acetyl-CoA was a key metabolite. Although lipids were important products of [^{14}C]ethanolamine assimilation, as in the rat (Sprinson & Coulon, 1954), a key role for acetyl-CoA was consistent with the finding that all fractions of the bacteria examined were radioactive.

The nature of the lipids formed from [^{14}C]ethanolamine was consistent with the known lipid composition of a wide variety of bacteria (see Asselineau, 1966; O'Leary, 1973). The observation that phosphatidylethanolamine was a major product of ethanolamine assimilation simply reflected the importance of this lipid in bacteria, i.e. as a membrane component (Lennarz, 1971; Ambron & Pieringer, 1973; Costerton *et al.*, 1974). The finding that phosphatidylethanolamine was a minor or undetectable lipid of the *Mycobacterium* and *Corynebacterium* species was consistent with the fact that members of these genera produce a variety of unusual non-membrane lipids including glycolipids and uncommon fatty acids, waxes etc. (O'Leary, 1973). The demonstration of enzymes involved in the metabolism of ethanolamine to acetate or acetyl-CoA, including NAD$^+$-dependent acetaldehyde dehydrogenase in extracts of *E. carotovora*, supported the radioactive-isotope evidence for lipid biosynthesis via acetyl-CoA.

Table 6. *Enzyme activity in extracts of bacteria grown on ethanolamine as the sole source of nitrogen*

The bacteria were grown on glycerol+ethanolamine+mineral salts medium, cell-free extracts were prepared and enzyme activities were measured as described in the Materials and Methods section.

	Enzyme activities (nmol/min per mg of protein)			
			Acetaldehyde dehydrogenase	
Bacterium	Ethanolamine ammonia-lyase	Ethanolamine *O*-phosphate phospho-lyase	CoA-dependent	CoA-independent
E. carotovora	0	114	0	12
Escherichia coli	88	0	64	0
F. rhenanum	0	184	0	19
C. aquaticum	28	0	21	0
K. aerogenes	106	0	82	0

No evidence supported the proposal that ethanolamine was incorporated directly into phosphatidylethanolamine via the CDP-ethanolamine pathway in the case of *Mycobacterium* 607 (Nandedkar, 1974, 1975) or any other bacterium. It seems certain that phosphatidylserine is an obligatory precursor of phosphatidylethanolamine in bacteria and that labelling of the phosphatidyl base from [^{14}C]-ethanolamine occurs by an indirect route. The possibility remains, however, that CDP-ethanolamine and CDP-choline are intermediates in the synthesis of cell-wall amino alcohols, as suggested for *Salmonella minnesota* (Lehmann *et al.*, 1971) and as recently shown for *Streptococcus pneumoniae* (Thomas *et al.*, 1978).

S. D. S. is grateful for a Government of India National Scholarship. We thank Miss E. Duggan for technical assistance. The interest and support of Professor T. W. Goodwin, F.R.S., is also gratefully acknowledged. We thank Mr. T. T. Hansel, who did some preliminary experiments when an undergraduate in this Department.

References

Ambron, R. T. & Pieringer, R. A. (1973) in *Form and Function of Phospholipids* (Ansell, G. B., Hawthorne, J. N. & Dawson, R. M. C., eds.), pp. 289–327, Elsevier Scientific Publishers, Amsterdam, London and New York

Asselineau, J. (1966) *The Bacterial Lipids*, Holden-Day, San Francisco

Blackwell, C. M. & Turner, J. M. (1978) *Biochem. J.* **175**, 555–563

Blackwell, C. M., Scarlett, F. A. & Turner, J. M. (1976) *Biochem. Soc. Trans.* **4**, 495–497

Blackwell, C. M., Scarlett, F. A. & Turner, J. M. (1977) *J. Gen. Microbiol.* **98**, 133–139

Clough, H. B., Shukla, S. D. & Turner, J. M. (1975) *Biochem. Soc. Trans.* **3**, 769–772

Costerton, J. W., Ingram, J. M. & Cheng, K. J. (1974) *Bacteriol. Rev.* **38**, 87–110

Faulkner, A. & Turner, J. M. (1974*a*) *Biochem. Soc. Trans.* **2**, 133–136

Faulkner, A. & Turner, J. M. (1974*b*) *Biochem. J.* **138**, 263–276

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509

Jakoby, W. B. (1958) *J. Biol. Chem.* **232**, 75–87

Jones, A. & Turner, J. M. (1971) *J. Gen. Microbiol.* **67**, 379–381

Jones, A. & Turner, J. M. (1973) *Biochem. J.* **134**, 167–182

Jones, A., Faulkner, A. & Turner, J. M. (1973) *Biochem. J.* **134**, 959–968

Kanfer, J. & Kennedy, E. P. (1964) *J. Biol. Chem.* **239**, 1720–1726

Lehmann, V., Lüderitz, O. & Westphal, O. (1971) *Eur. J. Biochem.* **21**, 339–347

Lennarz, W. J. (1971) in *Lipid Metabolism* (Wakil, S. J., ed.), pp. 155–184, Academic Press, London and New York

Nandedkar, A. K. N. (1974) *Biochem. Med.* **11**, 67–70

Nandedkar, A. K. N. (1975) *Biochem. Med.* **12**, 116–122

O'Leary, W. M. (1973) in *Handbook of Microbiology* (Laskin, A. I. & Lechevalier, H. A., eds.), vol. 2, pp. 261–322, CRC Press, Cleveland

Roberts, R. B., Abelson, P. H., Cowie, D. B., Bolton, E. T. & Britten, R. J. (1957) *Studies of biosynthesis in Escherichia coli*, pp. 13–14, Carnegie Institute of Washington (Publication 607), Washington

Rudolph, F. B., Purich, D. L. & Fromm, H. J. (1968) *J. Biol. Chem.* **243**, 5539–5545

Scarlett, F. A. & Turner, J. M. (1976) *J. Gen. Microbiol.* **95**, 173–176

Sprinson, D. B. & Coulon, A. (1954) *J. Biol. Chem.* **207**, 585–592

Thomas, A. M., Lambert, P. A. & Poxton, I. R. (1978) *J. Gen. Microbiol.* **109**, 313–317

Thompson, G. A. Jr., (1973) in *Form and Function of Phospholipids* (Ansell, G. B., Hawthorne, J. N. & Dawson, R. M. C., eds.), pp. 71–72, Elsevier Scientific Publishers, Amsterdam, London and New York

Turner, J. M. (1966) *Biochem. J.* **99**, 427–433