Glycine Formation During Growth of *Pseudomonas* AM1 on Methanol and Succinate

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1. The mechanism of regeneration of glycine during the growth of *Pseudomonas* AM1 on C₁ compounds has been investigated by brief incubation of bacterial suspensions with [2,3-¹⁴C₂]succinate and observing the incorporation of radioactivity into various metabolites. 2. With the wild-type organism growing on methanol, radioactivity appeared rapidly in glycine and tricarboxylic acid-cycle intermediates, but there was a relatively slow labelling of serine and phosphorylated compounds. Serine became labelled predominantly in the C-2 position. 3. The proportion of radioactivity incorporated into glycine at earliest times was greatly diminished when succinate-grown cells were used. 4. Radioactivity was also incorporated from [2,3-¹⁴C₂]succinate into glycine and serine by methanol-grown mutant 20S, which lacks phosphoserine phosphohydrolase. Both the glycine and serine were labelled mainly in C-2. 5. The formation of predominantly [2-¹⁴C]serine from [2,3-¹⁴C₂]succinate in wild-type *Pseudomonas* AM1, and of [2-¹⁴C]serine and [2-¹⁴C]glycine in the mutant lacking the phosphorylated pathway from succinate to serine, is taken as strong evidence for a mechanism of glycine regeneration involving cleavage of a C₄ skeleton between C-2 and C-3, rather than by a direct combination of two C₁ units derived from the growth substrate. 6. The cleavage mechanism is quantitatively more significant during growth on methanol than on succinate.

Several lines of evidence (see Harder & Quayle, 1971*b*) have led to the suggestion that glycolytic intermediates are formed during growth of *Pseudomonas* AM1 on C₁ compounds by a pathway (the serine pathway) involving the following steps:

The serine pathway uses one molecule of glyoxylate to produce each molecule of phosphoglycerate. The mechanism of net synthesis of glyoxylate from C₁ units remains unknown. It is likely to happen by one of two general ways (Large *et al.*, 1962*a*): (a) direct

The serine pathway may be contrasted with the following sequence of reactions (the phosphorylated pathway) which accomplishes the synthesis of serine and glycine from phosphoglycerate during growth of *Pseudomonas* AM1 on compounds such as succinate (see Harder & Quayle, 1971*a*):

condensation of two C₁ units, which the radioisotopic data would suggest to be a reduced C₁ compound and carbon dioxide, or (b) extension of the carbon skeleton of phosphoglycerate by at least one carbon atom followed by cleavage to give glyoxylate (or a precursor) and a residual multicarbon molecule.

The multicarbon molecule would represent the net biosynthetic product of the overall cycle of C_1 fixation. An obvious possibility for alternative (b) is carboxylation of phosphoenolpyruvate via phosphoenolpyruvate carboxylase (Large *et al.*, 1962b) followed by cleavage of the resulting oxaloacetate, or a derivative, to two C_2 fragments.

All attempts so far to demonstrate the generation of glyoxylate or its precursor in cell-free extracts by either mechanism have been unsuccessful and, similarly, the results obtained from work with whole cells, including mutants, have not enabled a choice to be made between the two general types of mechanism. This paper records an approach to the problem through a study of the metabolism of [^{14}C]succinate by *Pseudomonas* AM1 and a mutant, 20S, lacking phosphoserine phosphohydrolase (reaction 8). The study was designed to test out three specific predictions, which may be made on the basis of regeneration of glyoxylate by cleavage of a C_4 compound identical with or derived from succinate.

The predictions, based on the assumption that glyoxylate leads directly to glycine (reaction 3), are: (a) exposure of cells growing on methanol to [^{14}C]succinate should result in early labelling of glycine, which should precede that of phosphorylated compounds and serine; (b) early labelling of glycine from [^{14}C]succinate should be more pronounced in cells growing on methanol than in cells growing on succinate; (c) [2,3- $^{14}C_2$]succinate administered to cells growing on methanol should lead to glycine and serine labelled predominantly in C-2.

Materials and Methods

Maintenance and growth of the organism

Pseudomonas AM1 was maintained in slope culture on an inorganic salts medium (Jayasuriya, 1955) containing methylamine hydrochloride (50mM) and agar (1.5%, w/v). The organism was subcultured monthly and stored at 4°C after incubation for 3–4 days at 30°C. Mutant 20S was maintained similarly on the above medium containing sodium succinate (50mM) and L-serine (1mM) in place of methylamine hydrochloride.

The organisms were grown in liquid culture as described by Heptinstall & Quayle (1970) with methanol (0.5, v/v) as carbon source. The wild-type organism was also grown on sodium succinate (50mM). Growth was assessed by measuring the extinction at 650nm.

Incubation of cells with [^{14}C]succinate

Cultures of bacteria growing on methanol were harvested during exponential growth by centrifuging at room temperature and resuspended at a density of

6mg dry wt. of bacteria/ml in a solution containing 10mM-sodium phosphate buffer, pH7.0, 10mM- NH_4Cl and 100mM-methanol. For succinate-grown cells, methanol was replaced by sodium succinate (10mM) in the resuspension medium. The suspension was aerated vigorously in a cylindrical vessel (10cm × 4.9cm²) fitted with a sintered-glass base. After equilibration for 5 min at 30°C, 1ml of a solution of sodium [2,3- $^{14}C_2$]succinate (73μmol; 100μCi) was added and samples (approx. 1ml) were rapidly removed and transferred to boiling aq. 95% (v/v) ethanol (3ml) by means of a hypodermic syringe. After cooling, cell debris and precipitated protein were removed by centrifugation (5000g; 10min) and washed twice with 0.5ml of cold aq. 20% (v/v) ethanol. The combined supernatant fluids were evaporated to dryness under reduced pressure at 40°C and residues redissolved in 0.2ml of aq. 20% (v/v) ethanol for chromatography.

For each experiment, a corresponding control incubation was performed at one-fifth of the scale, with cells that had been previously heated at 100°C for 5 min.

Chromatography and identification of labelled compounds

Samples of the final ethanolic extracts were chromatographed in two dimensions on Whatman no. 4 paper (18½ × 22in) in descending fashion, with, as solvents, phenol–formic acid (90%, w/v)–water (500:13:167, w/v/v) (Kornberg, 1958) and butan-1-ol–propionic acid–water (47:22:31, by vol.) (Benson *et al.*, 1950). The radioactive compounds were located by radioautography (Large *et al.*, 1961) and the radioactivity was measured directly with a mica end-window Geiger–Müller tube (type 2B2) coupled with a scaler (type D657; Panax Equipment Ltd., Redhill, Surrey, U.K.). The observed values were corrected for background radioactivity and for counts appearing in corresponding positions in chromatograms from the control incubations with boiled cell suspensions.

After elution from the paper with water, radioactive materials were identified by co-chromatography or co-electrophoresis with authentic samples of suspected compounds. Carboxylic acids were identified chromatographically on Whatman no. 1 paper either with the original solvents or with ethanol–aq. NH_3 (sp. gr. 0.88)–water (80:4:16, by vol.; Long *et al.*, 1951). Alternatively, satisfactory separation of carboxylic acids was achieved by t.l.c. on cellulose (average thickness 100μm) as described by Myers & Huang (1969). Carrier acids were detected by spraying with a solution of Bromophenol Blue in ethanol (0.1%, w/v). Identification of amino acids was achieved by one-dimensional co-electrophoresis on Whatman no. 3MM paper with authentic samples at 40 V/cm on a flat-plate apparatus (Savant Instruments

Inc., Hicksville, New York, U.S.A.). Serine, glycine and glutamate were not resolved by chromatography of the original ethanolic extracts, but were resolved by co-electrophoresis with authentic compounds in the following buffer systems: A, formic acid (90%, w/v)–water (8:292, v/v), pH2; B, pyridine–acetic acid–water (20:0.8:279, by vol.), pH6.4. The latter buffer also permitted the resolution of aspartate and asparagine, which were not separated by chromatography of the original extracts. Alanine was identified by t.l.c. on cellulose with the authentic material and with the solvent systems described by Myers & Huang (1969). Carrier amino acids were detected with a solution of ninhydrin in acetone (0.25%, w/v) followed by brief heating at 80°C. Trehalose was co-chromatographed in the original solvents. Phosphorylated compounds were dephosphorylated by incubation of the eluted materials with a preparation of Polidase-S (Schwarz Laboratories, Inc., New York, U.S.A.) as described by Benson *et al.* (1951) and the resulting solutions were chromatographed two-dimensionally on Whatman no. 1 paper in water-saturated phenol and then butan-1-ol–propionic acid–water (47:22:31, by vol.) (Benson *et al.*, 1950).

Preparation and degradation of labelled compounds

The ethanol-soluble extract after incubation of cell suspensions with [2,3-¹⁴C₂]succinate was evaporated to dryness at 40°C under reduced pressure. The residue was redissolved in 1 ml of 5M-HCl and the solution applied to a column (30mm×7mm²) of Dowex 50 (X8; H⁺ form; 100–200 mesh). After washing with 3 ml of water, the column was eluted with 4.5 ml of aq. 2M-NH₃ soln. The eluate, which contained amino acids, was evaporated to dryness at 40°C under reduced pressure and the residue dissolved in 0.1 ml of water. Samples (0.01 ml) of this solution were taken for two-dimensional t.l.c. on cellulose (average thickness 100 μm) with, as solvents: C, 2-methylpropan-2-ol–ethyl methyl ketone–water–aq. NH₃ (sp.gr. 0.88) (40:30:20:10, by vol.) and D, 3-methylbutan-1-ol–ethyl methyl ketone–formic acid (90%, w/v)–water (40:30:15:15, by vol.) (Kung & Wagner, 1969). Radioactive areas were located radioautographically and the cellulose was scraped from those which occupied positions corresponding to serine and glycine. The suspected compounds were eluted with water (3×0.75 ml) and their identity was confirmed by co-chromatography of samples with authentic serine and glycine in solvent D.

Each eluted radioactive compound was then mixed with about 300 mg of the authentic, unlabelled amino acid and recrystallized from aq. ethanol to constant specific radioactivity. The specific radioactivity was determined by total oxidation to CO₂ with Van Slyke–Folch reagent, as described by Sakami (1955). ¹⁴CO₂ was converted into barium [¹⁴C]carbonate by

Table 1. Distribution of ¹⁴C among the non-volatile labelled components of ethanol extracts of methanol-grown wild-type *Pseudomonas* AM1 incubated with [2,3-¹⁴C₂]succinate

Time (s)	Radioactivity (c.p.m.)									
	Malate	Fumarate	Glycine	Serine	Glutamate	Aspartate	Citrate	Trehalose	Alanine	Total
6	1635	632	493	107	156	768	567	0	0	5286
20	3843	128	1162	481	460	1443	706	180	1220	13409
37	4732	0	1251	514	1232	2459	1583	690	2012	22056
61	7174	0	1060	468	2608	2495	1879	1588	3192	31020
85	5009	0	1038	419	4560	2377	1900	3389	3975	37093
126	5095	0	1352	497	7320	2821	2992	7910	4993	52888
207	4219	0	1180	743	15930	2296	3587	16231	8655	82474
								Phosphorylated compounds	Other compounds	
								928	2355	
								1431	4526	
								4001	5077	
								6030	7101	
								9349	9623	
								12807		
								20010		

Sodium [2,3-¹⁴C₂]succinate (73 μmol; 100 μCi) was added at zero time to 5.5 ml of a suspension of bacteria (32 mg dry wt.) in 100 mm-methanol, 10 mm-sodium phosphate buffer, pH7.0, and 10 mm-NH₄Cl under vigorous aeration. Samples (approx. 1 ml) were withdrawn by hypodermic syringe at indicated times and transferred to 3 ml of aq. 95% (v/v) ethanol and treated as described in the Materials and Methods section.

Fig. 1. Variation with time of the percentage distribution of ^{14}C incorporated from [2,3- $^{14}\text{C}_2$]succinate into (a) amino acids and (b) other constituents of the ethanol-soluble fraction of wild-type *Pseudomonas* AM1 growing on methanol

○, Glutamate; ▼, alanine; ■, aspartate; ▽, glycine; ●, serine; □, malate; ▲, trehalose; ■, citrate; △, phosphorylated compounds.

Table 2. Distribution of ^{14}C in serine derived from wild-type *Pseudomonas* AM1 growing on methanol in the presence of sodium [2,3- $^{14}\text{C}_2$]succinate

Sodium [2,3- $^{14}\text{C}_2$]succinate (73 μmol ; 100 μCi) was added at zero time to an aerated suspension of bacteria (60mg dry wt.) in 9ml of 10mM-sodium phosphate buffer, pH 7.0, containing 10mM- NH_4Cl and 100mM-methanol. After incubation for 90s, the suspension was poured into 40ml of boiling aq. 95% (v/v) ethanol. The isolation and degradation of radioactive serine are described in the Materials and Methods section.

	Carbon atom no.	Percentage yield of CO_2 obtained from oxidation of each C atom	Sp. radioactivity of barium [^{14}C]carbonate derived from each C atom (c.p.m./mg of BaCO_3)	Sum of sp. radioactivities of individual C atoms	Sp. radioactivity of barium [^{14}C]carbonate obtained from total combustion of isolated serine $\times$ no. of C atoms in molecule	Percentage of total radioactivity in molecule contributed by each C atom
CO_2H	1	94	2.42	13.77	15.09	17.6
CHNH_2	2	64	10.41			75.6
CH_2OH	3	69	0.94			6.8

precipitation with barium chloride (5%, w/v) and then plated in triplicate on filter-paper discs (2.69 cm²) as detailed by Sakami (1955). Weighed samples of barium [¹⁴C]carbonate were suspended in 7 ml of NE213 liquid scintillator containing Cab-O-Sil (4%, w/v) (both from Nuclear Enterprises Ltd., Sighthill, Edinburgh, U.K.) as described by Kemp & Quayle (1967) and radioactivity was measured with a liquid scintillation counter (Nuclear Chicago Corp., Des Plaines, Ill., U.S.A.). Quench corrections were applied by the channels-ratio method.

Serine and glycine were degraded as described by Large *et al.* (1962a).

Assay of phosphoserine phosphohydrolase (EC 3.1.3.3)

Measurement of this enzyme was used to confirm the phenotype of mutant 20S of *Pseudomonas* AM1. The enzyme was assayed in ultrasonic extracts, by measuring the P_i liberated from DL-*O*-phosphoserine, by the procedure of Heptinstall & Quayle (1970).

Chemicals

[2,3-¹⁴C₂]Succinic acid was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.) and neutralized with sodium hydroxide before use. DL-*O*-Phosphoserine was obtained from Koch-Light Laboratories Ltd. (Colnbrook, Bucks., U.K.) and neutralized with sodium hydroxide before use.

Results

Incorporation of [2,3-¹⁴C₂]succinate by wild-type *Pseudomonas* AM1 growing on methanol or succinate

A culture of wild-type *Pseudomonas* AM1, containing 32 mg dry wt. of organisms growing exponentially on methanol, was centrifuged and suspended in 5.5 ml of resuspension medium containing 10 mM-sodium phosphate buffer, pH 7.0, 10 mM-NH₄Cl and 100 mM-methanol. The suspension was incubated at 30°C with sodium [2,3-¹⁴C₂]succinate (73 μmol; 100 μCi) as described in the Materials and Methods section. The distribution of radioactivity among non-volatile compounds formed during the first 2 min after addition of labelled succinate is recorded in Table 1. Curves showing the percentage of total radioactivity incorporated into individual metabolites expressed as a function of time (Fig. 1) suggest that malate, fumarate, aspartate, citrate and glycine were early-labelled compounds. The initial positive slopes for glutamate, phosphorylated compounds and serine indicate less-rapid incorporation of radioactivity from labelled succinate into these metabolites.

The finding of a negative slope to the curve for glycine, together with its high percentage of radioactivity (9%) in the earliest sample, implies a direct

Table 3. Distribution of ¹⁴C among the non-volatile labelled compounds of ethanol extracts of methanol-grown mutant 20S of *Pseudomonas* AM1 incubated with [2,3-¹⁴C₂]succinate

A suspension of mutant 20S (32 mg dry wt. of organisms) grown on methanol (0.5%, v/v) was incubated with sodium [2,3-¹⁴C₂]succinate and treated as described in Table 1.

Time (s)	Radioactivity (c.p.m.)										Total
	Malate	Glycine	Serine	Glutamate	Aspartate	Citrate	Trehalose	Alanine	Compound H	Phosphorylated compounds	
5	4495	79	34	25	367	533	185	56	5423	1309	15412
12.5	2769	96	55	46	515	199	246	767	3577	749	11275
19	3370	231	97	108	944	228	173	1447	3120	1598	15444
30.5	2391	333	71	158	1196	563	239	1851	2705	1426	13716
64	2799	278	106	749	1153	902	987	2919	2063	3229	19578
114.5	5449	473	149	3251	2482	958	4513	7038	4878	7230	44929

pathway for its formation from succinate, not involving the phosphorylated pathway. To determine whether these results were peculiar to the methanol-grown organism, the experiment was repeated with cells grown on 50 mM-sodium succinate, the methanol in the resuspension medium being replaced by 10 mM-sodium succinate. Under these conditions radioisotope appeared predominantly in tricarboxylic acid-cycle intermediates and derived amino acids such as aspartate and glutamate, and a much smaller percentage of radioactivity appeared in glycine (<2%) than with the methanol-grown organism. Furthermore, labelled serine did not appear in succinate-grown cells until very much later than with methanol-grown cells, and even then in negligible amounts.

It is inferred from these results that glycine can arise from succinate by a route not involving the phosphorylated pathway, and that this pathway is of greater quantitative importance during growth on methanol than on succinate.

*Labelling pattern of serine formed from [2,3- 14 C₂]-succinate during growth of wild-type *Pseudomonas* AM1 on methanol*

The labelling pattern of serine formed from [2,3- 14 C₂]succinate during short-term incubation with methanol-grown cells was determined. If the serine were formed predominantly by hydroxymethylation of glycine derived from a C₄-cleavage reaction, it should be labelled predominantly in C-2. If, however, serine arose from [2,3- 14 C₂]succinate mainly by the pathway involving phosphorylated C₃ intermediates, it would be expected to be equally labelled in the C-2 and C-3 positions.

The labelling pattern of serine obtained from growing cells incubated for 90 s with sodium [2,3- 14 C₂]succinate is shown in Table 2. That 75.6% of the total radioactivity in the serine molecule occurs in C-2, whereas C-3 contains only 6.8%, is in accordance with a cleavage of the succinate carbon skeleton giving rise to [2- 14 C]glycine and hydroxymethylation

Fig. 2. Variation with time of the percentage distribution of ^{14}C incorporated from [2,3- $^{14}\text{C}_2$]succinate into (a) amino acids and (b) other constituents of the ethanol-soluble fraction of mutant 20S of *Pseudomonas* AM1 growing on methanol

○, Glutamate; ▼, alanine; ■, aspartate; ▽, glycine; ●, serine; □, malate; ▲, trehalose; ■, citrate; △, phosphorylated compounds; ×, compound H.

of the latter by a C₁ unit obtained from unlabelled methanol.

The results might, however, be explained by the formation of [2,3-¹⁴C]serine from the labelled succinate via the phosphorylated pathway, followed by an exchange reaction of C-3 of serine with a C₁ unit derived from unlabelled methanol. The availability of a mutant, 20S, deficient in phosphoserine phosphohydrolase (Harder & Quayle, 1971a), and which therefore lacks a functional phosphorylated pathway from phosphoglycerate to serine, permitted this possibility to be tested as follows.

Brief incubation of mutant 20S with [2,3-¹⁴C₂]succinate

A culture of mutant 20S of *Pseudomonas* AM1 growing exponentially on methanol was harvested and its phenotype confirmed by assay of phosphoserine phosphohydrolase: no detectable P_i was liberated when ultrasonic extracts were incubated with either DL-O-phosphoserine or 3-phosphoglycerate. The mutant was resuspended in aerated buffered medium containing methanol and the incorporation of radioactivity into non-volatile metabolites immediately after the introduction of [2,3-¹⁴C₂]succinate was determined (Table 3). The pattern of curves tracing the incorporation of radioactivity into each metabolite (Fig. 2) showed differences from the corresponding results with the wild-type organism (Fig. 1). The most striking difference was the appearance of an unknown compound H, which contained 36% of the fixed radioactivity at the earliest time of sampling and whose percentage curve fell steeply with time. Since this compound was not observed with the wild-type organism, its appearance is presumably connected with the lack of a complete phosphorylated pathway from phosphoglycerate to serine in mutant 20S. Compound H has R_F values 0.48–0.50 and 0.52–0.57 in the aq. phenol–formic acid and butanol–propionic acid solvents respectively and it is distinguishable chromatographically from fumarate, glycolate and phosphorylated compounds. Preliminary tests indicate that compound H is neither an amino acid nor a carbonyl compound, since its chromatographic properties are not altered after heating with a solution of ninhydrin and attempts to form a 2,4-dinitrophenylhydrazone have been unsuccessful. Another difference between the results obtained with mutant 20S and the wild-type organism was that glycine and serine did not contain as large a percentage of the total radioactivity in the earliest samples. Possible causes of this are suggested in the Discussion section. Nevertheless, these amino acids did become sufficiently labelled to permit determination of the distribution of radioactivity between the carbon atoms of each compound (Table 4).

The results of the degradation clearly show that both the glycine and serine produced during incuba-

Table 4. *Distribution of ¹⁴C in glycine and serine derived from mutant 20S of Pseudomonas AM1 growing on methanol in the presence of [2,3-¹⁴C₂]succinate*
Conditions identical with those described in Table 2 were used. The isolation and degradation of radioactive glycine and serine are described in the Materials and Methods section.

Compound	Formula	Carbon atom no.	Percentage yield of CO ₂ derived from oxidation of each C atom	Sp. radioactivity of barium [¹⁴ C]carbonate derived from each C atom (c.p.m./mg of BaCO ₃)	Sum of specific radioactivities of individual C atoms	Specific radioactivity of barium [¹⁴ C]carbonate obtained from total combustion of isolated amino acid × no. of C atoms in molecule	Percentage of total radioactivity in molecule contributed by each C atom
Glycine	CO ₂ H	1	84	0.69	15.64	14.90	4.4
	—	2	29	14.95			
Serine	CO ₂ H	1	96	0.23	4.73	5.55	94.3
	—	2	65	4.46			
	—	3	88	0.04			0.8

tion of methanol-grown mutant 20S with $[2,3-^{14}\text{C}_2]$ -succinate are labelled predominantly in C-2. Since this mutant lacks phosphoserine phosphohydrolase, glycine and serine must have been formed by a route other than the known pathway involving phosphorylated C_3 intermediates. Moreover, the labelling patterns of glycine and serine are consistent with a cleavage of the succinate carbon skeleton between C-2 and C-3 to give C_2 products, at least one of which is convertible into glycine.

Discussion

The three specific predictions, which were based at the outset on the assumption of regeneration of the glycine skeleton by cleavage of the succinate carbon skeleton into two C_2 compounds, have been verified. The possibility that the results could be explained either by reversal of the serine pathway, or by operation of the phosphorylated pathway, followed by exchange of the C-3 atom of serine with C_1 units derived from methanol, is unlikely for the following reasons.

(a) The labelling patterns of serine and malate resulting from incorporation of $[^{14}\text{C}]$ methanol and $[^{14}\text{C}]$ bicarbonate into methanol-grown *Pseudomonas* AM1 are consistent with the formation of malate by carboxylation of a C_3 fragment derived from serine, rather than the reverse (Large *et al.*, 1962a).

(b) Reversal of the serine pathway would necessitate conversion of phosphoglycerate into glycerate. It is most unlikely that reaction (5) is appreciably reversible and no evidence could be found for release of P_i from phosphoglycerate in ultrasonic extracts of *Pseudomonas* AM1.

(c) The labelling patterns of serine and glycine obtained from mutant 20S incubated with $[2,3-^{14}\text{C}_2]$ -succinate appear to eliminate a major involvement of the phosphorylated pathway in supplying the carbon skeletons of these amino acids. However, a role for the phosphorylated pathway during growth on C_1 compounds has been suggested previously by Harder & Quayle (1971a). They observed that although mutant 20S was able to grow on methanol alone, its growth rate was lower than that of the wild-type organism. The addition of serine (1 mM) increased the growth rate of the mutant, whereas that of the wild-type organism and of revertants of mutant 20S to wild type was unaffected. The function of the phosphorylated pathway during growth of wild-type *Pseudomonas* AM1 on C_1 compounds was suggested to be net replenishment of amino groups necessary for the cyclic formation of glycine and serine with serine-glyoxylate aminotransferase, when these amino acids are withdrawn for macromolecular synthesis (Harder & Quayle, 1971b). A consequence of the lesion in mutant 20S may therefore be a short-

age of amino groups normally provided by the phosphorylated pathway for net synthesis of glycine and serine, and decreased intracellular pools of these amino acids may result. Indeed, this could be reflected in the early appearance of a considerable proportion of radioactivity incorporated from $[2,3-^{14}\text{C}_2]$ succinate in compound H, especially if, for example, compound H is itself a precursor or is in equilibrium with a precursor of glyoxylate and glycine. These factors would be expected to contribute greatly to the observed differences in kinetic data on the incorporation of radioactivity into serine and glycine by mutant 20S (Fig. 2) when compared with the wild-type organism (Fig. 1).

Although the results with whole cell suggest a cleavage reaction as the source of the glycine skeleton, no such reaction has yet been demonstrated in cell-free extracts. Of the possible C_2 products, glyoxylate, glycollate and acetate have each been shown to satisfy the requirement for C_2 compounds by mutants of *Pseudomonas* AM1 unable to grow on methanol unless supplied with substrate quantities of C_2 compounds (Anthony *et al.*, 1971b; Salem & Quayle, 1971). If acetate, or a derivative, is a cleavage product, then the postulated oxidation of acetate to glycollate, which has been implicated in the growth of *Pseudomonas* AM1 on ethanol (Anthony *et al.*, 1971a), would form an essential part of the pathway of glycine regeneration during growth on C_1 compounds.

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