

Carbon assimilation pathways

Part one: Brief summary of the four pathways for assimilation of C1 compounds

The elucidation of the Serine Cycle up to 1973

Part two: The solution of the complete Serine / Ethylmalonyl-CoA cycle

Gordon Research Conference: Magdalen College, Oxford, 2006

Molecular Basis of Microbial One-Carbon Metabolism

The Biochemistry of Methylootrophs: a historical perspective

Chris Anthony, University of Southampton, UK



Dedicated to the memory of J. Rod Quayle
(1926 – 2006)

- | | |
|-------------|--|
| 1946 – 1951 | PhD in physical-organic chemistry
[University of Wales; with ED Hughes]

PhD on aphid pigments [Cambridge with
Alexander Todd] |
| 1953 --1954 | Calvin's lab at Berkeley |
| 1955 --1963 | Krebs' MRC Unit at Oxford |
| 1963 – 1983 | University of Sheffield |
| 1983 – 1992 | Vice-Chancellor, University of Bath |

Many of my slides are from this lecture
dedicated to Rod Quayle

Carbon assimilation pathways of methylotrophs

Ribulose biphosphate [RuBP] pathway [Key contribution from JRQ]

Plants, autotrophic bacteria and a few methylotrophs

Pathways first proposed by Quayle and mainly elucidated by him and his colleagues:

Ribulose monophosphate [RuMP] pathway

Type I methanotrophs and obligate methanol or methylamine utilisers

Dihydroxyacetone [DHA] pathway

Methylotrophic yeasts

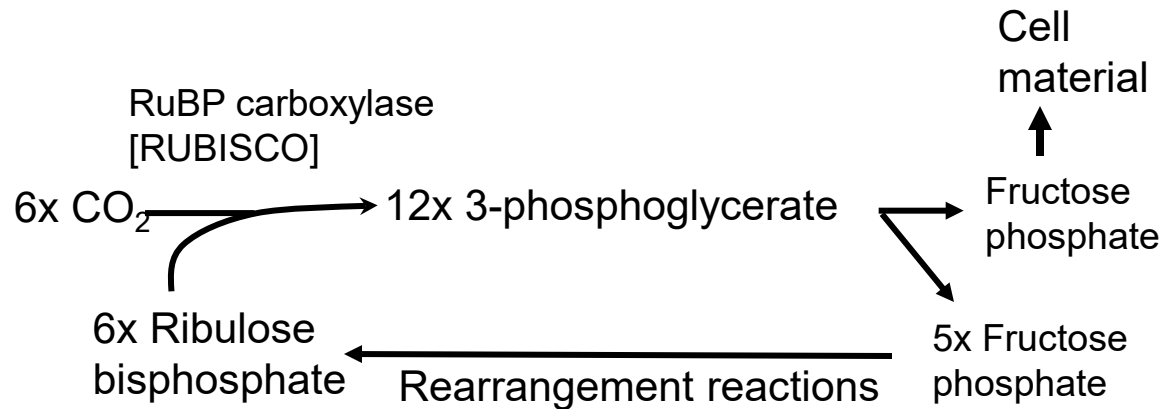
Serine pathway

Type II methanotrophs and facultative methanol or methylamine utilisers

I will summarise the first three and spend more time on details of serine pathway

Calvin-Benson cycle for CO₂ fixation in plants [1950 – 1960]

The key demonstration of the specific RuBP carboxylase activity in extracts was published by Quayle in JACS in 1954



RADIATION LABORATORY AND
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

RECEIVED MAY 24, 1954

J. R. QUAYLE
R. C. FULLER
A. A. BENSON
M. CALVIN

This pathway was soon shown to be the path of carbon dioxide fixation in aerobic autotrophic bacteria and it was commonly assumed that methylotrophs growing on methane or methanol would assimilate their carbon by this pathway after their oxidation to CO₂

JRQ showed that this is the route for formate assimilation by *Pseudomonas oxalaticus* [1959].

He later showed that the facultative autotroph *Paracoccus denitrificans* assimilates methanol by this pathway.

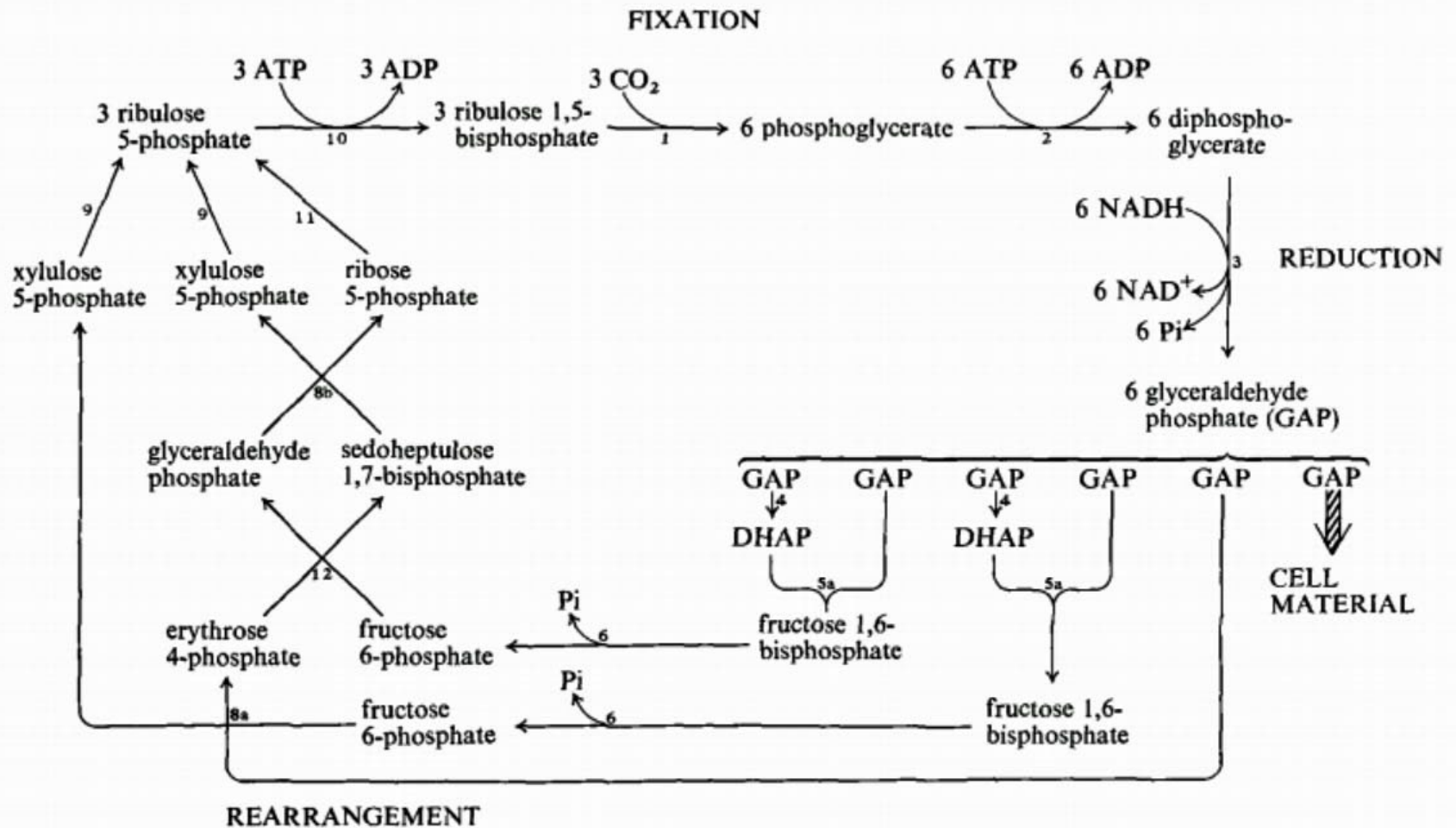


Fig. 5. The ribulose biphosphate cycle of CO_2 assimilation (transaldolase variant). The enzyme numbers refer to those in the text: (1) ribulose biphosphate carboxylase; (2) phosphoglycerate kinase; (3) glyceraldehyde phosphate dehydrogenase; (4) triose phosphate isomerase; (5a, b) aldolase; (6) fructose biphosphatase; (8) transketolase; (9) pentose phosphate epimerase; (10) phosphoribulokinase; (11) pentose phosphate isomerase; (12) transaldolase.

Ribulose Bisophosphate pathway in plants, autotrophs and some methylotrophs



Figure 1. Some members of the photosynthesis research group (about 1953). They are in front of Old Radiation Laboratory at UCB. This building was later demolished to make space for a new Department of Chemistry building, Hildebrand Hall. *Left to right:* Clinton Fuller, Robert Norris, Hans Kornberg, Alice Holtham, Melvin Calvin, J. Rodney Quayle, Malcolm Thain, James Bassham and Jaques Mayudon. Andrew Benson agrees that he probably took this picture, accounting for his absence from the picture.

The ribulose monophosphate pathways

Quayle, Johnson, Strom, Ferenci, Kemp, [1965 – 1974]

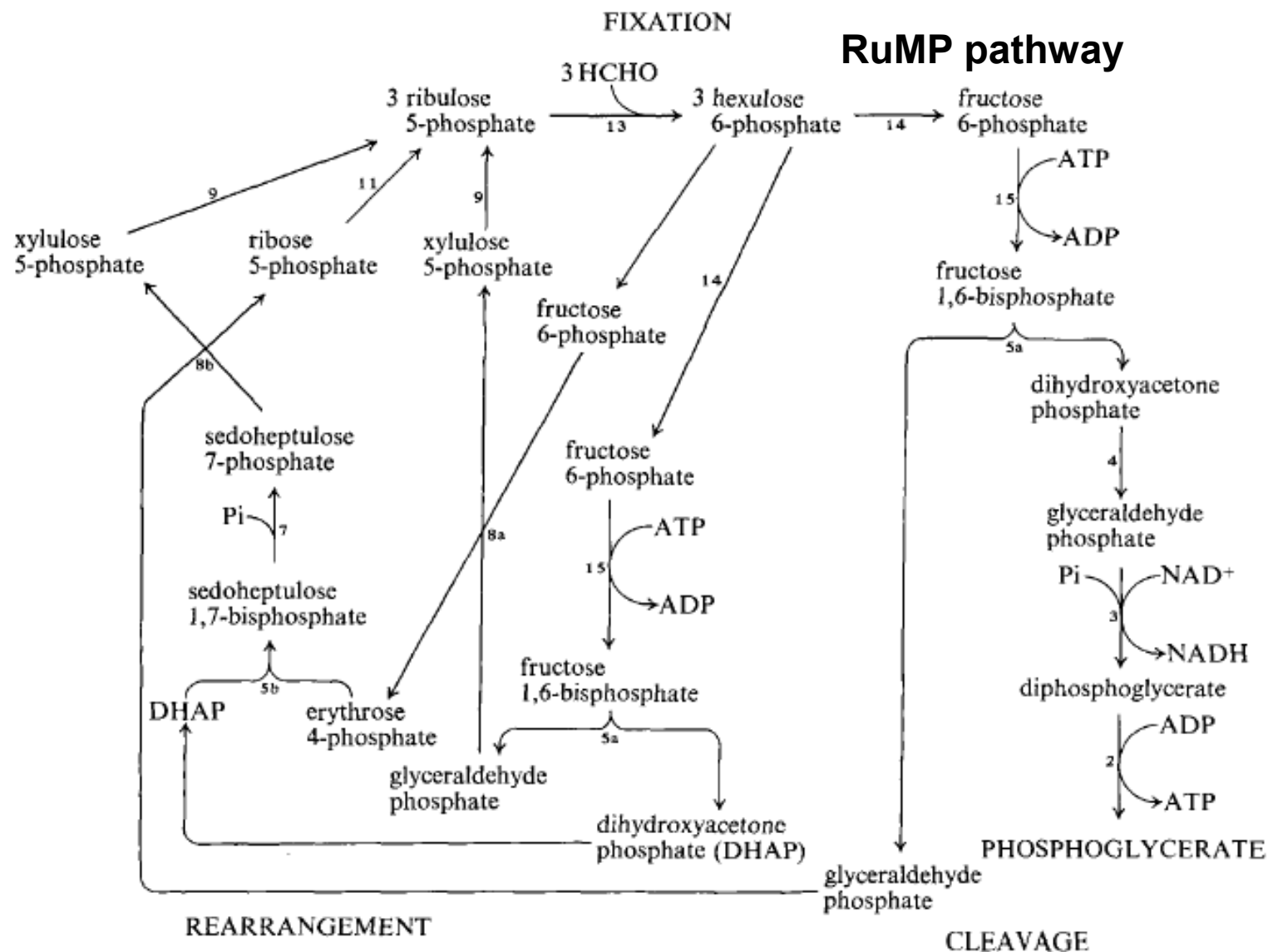
Occur in Type I methanotrophs and in the obligate methanol or methylamine utilisers. There are 4 variants; three of these have been demonstrated in different bacteria.

Methods: Short term labelling experiments; analysis of position of label in metabolites, purification and characterisation of enzymes; measurement of all enzymes of the pathway.

Similar to Ribulose biphosphate (Calvin) cycle except for 'first reaction'

Condensation of formaldehyde with RuBP to give a novel hexulose phosphate; this is then isomerised to fructose 6 phosphate. The novel synthase and isomerase were isolated and characterised.

Subsequent reactions of the pathway are similar to the rearrangement reactions of the Calvin cycle.



RuMP pathway

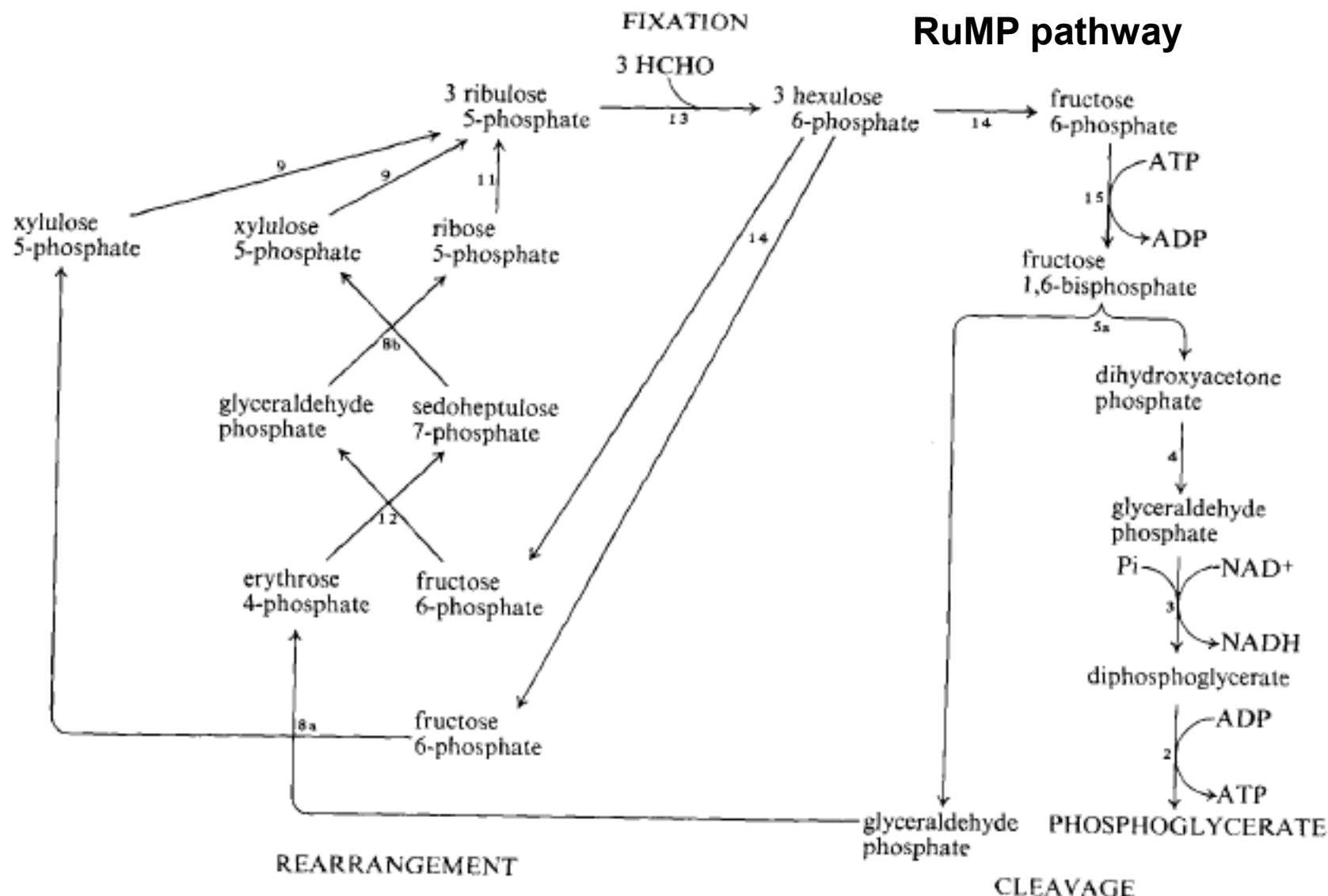


Fig. 8. The ribulose monophosphate (RuMP) cycle of formaldehyde assimilation (Fructose bisphosphate aldolase/transaldolase variant). This variant does not occur frequently in methylotrophs. The enzyme numbers refer to those listed in the text: (2) phosphoglycerate kinase; (3) glyceraldehyde phosphate dehydrogenase; (4) triose phosphate isomerase; (5) aldolase; (8) transketolase; (9) pentose phosphate epimerase; (11) pentose phosphate isomerase; (12) transaldolase; (13) hexulose phosphate synthase; (14) hexulose phosphate isomerase; (15) phosphofructokinase.

The dihydroxyacetone [DHA] cycle of formaldehyde assimilation in yeasts

Nobuo Kato, O'Connor (Mary Lidstrom), Sahm, Babel, van Dijken, Quayle
[1977-1981]

This is similar to the RuBP and RuMP cycles

Two specific enzymes are required for formaldehyde fixation:

DHA synthase and triokinase

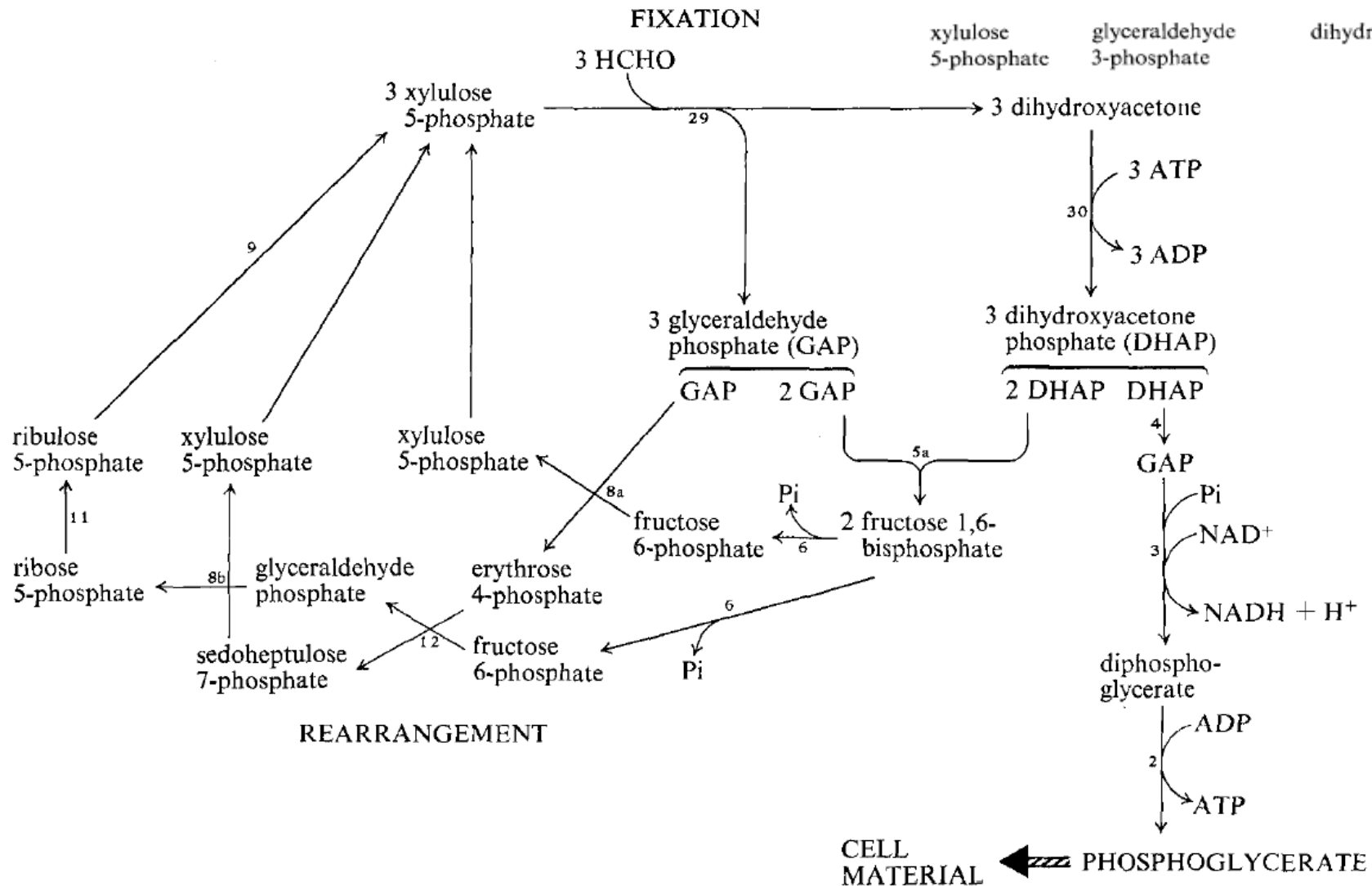
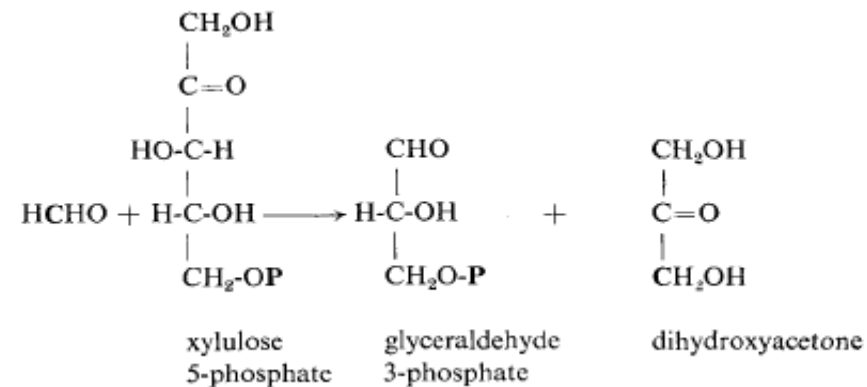
These were purified and characterised

Short term labelling pattern from ^{14}C methanol was consistent with the cycle proposed by Quayle and distribution of labelled carbon in the proposed intermediates was consistent with the cycle

Mutants lacking the key enzymes were unable to grow on methanol

DHA cycle in yeast

Fixation: xylulose phosphate + HCHO →
glyceraldehyde phosphate + dihydroxyacetone

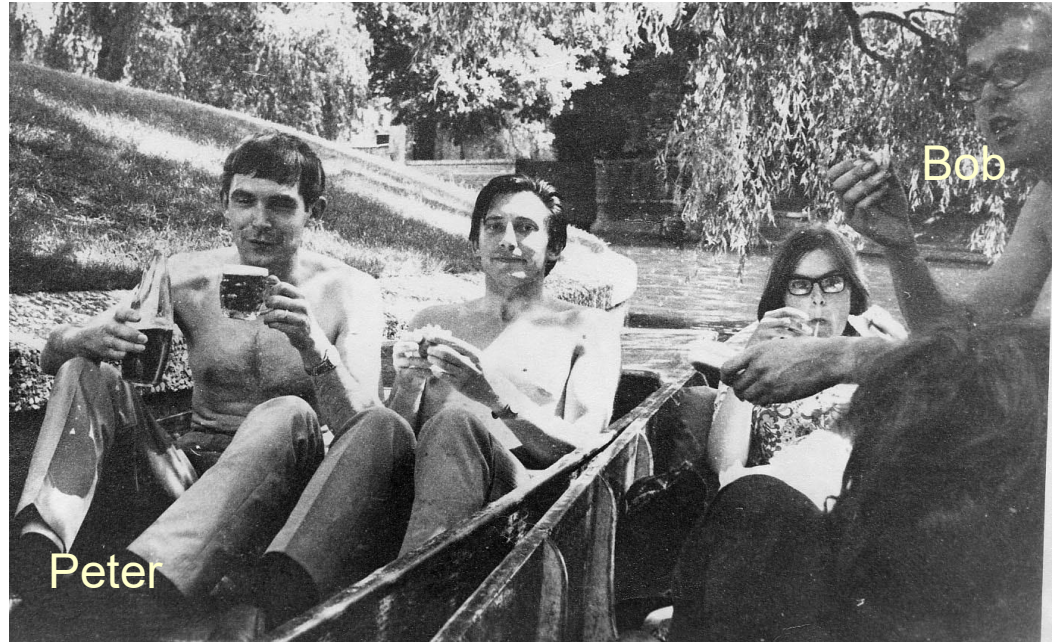


The discovery of the Serine cycle in *Methylobacterium extorquens* AM1 (*Pseudomonas* AM1)

Part 1: J Rod Quayle, David Peel, Peter Large, Alan Salem
(with bits by Pat Goodwin (nee Dunstan) and Chris Anthony)



J. Rod Quayle



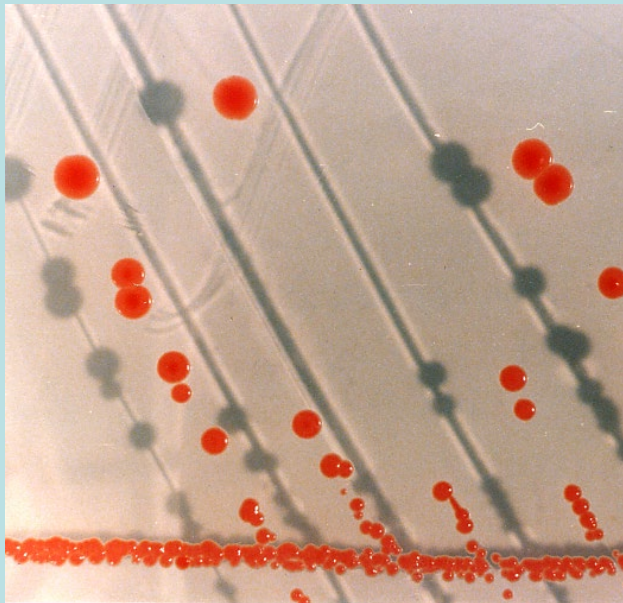
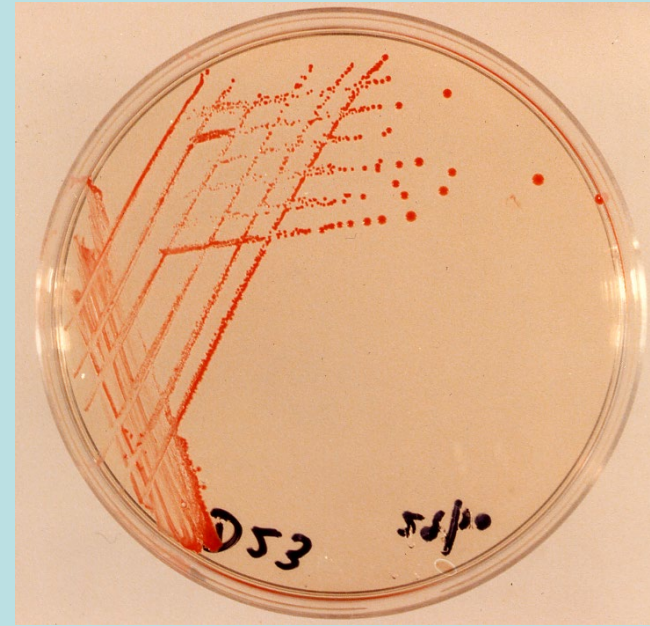
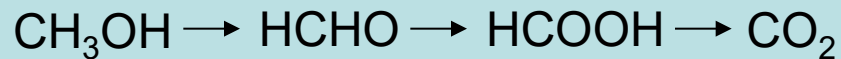
Peter

Bob

Peter Large

Methylobacterium extorquens

- *Pseudomonas* AM1 (Peel & Quayle, 1961)
- *Pseudomonas* sp. M27 (Anthony & Zatman, 1964)



The Serine Pathway; Peter Large, David Peel and Rod Quayle

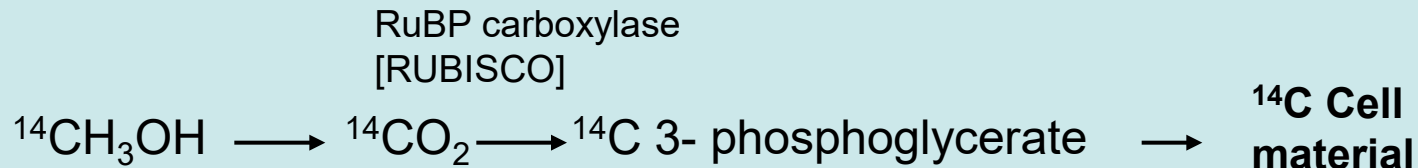
1961 - 1963

1. Large, P.J., Peel, D. and Quayle, J.R. *Biochemical Journal* **81** , 470-480 (1961).
Microbial growth on C1 compounds: Synthesis of cell constituents by methanol- and formate-grown *Pseudomonas* AM1 and methanol-grown *Hyphomicrobium vulgare*.
2. Large, P.J., Peel, D. and Quayle, J.R. *Biochemical Journal* **82**, 483-488 (1962).
Microbial growth on C1 compounds: Distribution of radioactivity in metabolites of methanol-grown *Pseudomonas* AM1 after incubation with [14C]methanol and [14C]bicarbonate.
3. Large, P.J., Peel, D. and Quayle, J.R. *Biochemical Journal* **85**, 243-250 (1962).
Microbial growth on C1 compounds: Carboxylation of phosphoenolpyruvate in methanol-grown *Pseudomonas* AM1.
4. Large, P.J. and Quayle, J.R. *Biochemical Journal* **87**, 386-396 (1963).
Microbial growth on C1 compounds: Enzyme activities in extracts of *Pseudomonas* AM1.

The Elucidation of the Serine pathway in *Pseudomonas* AM1 [now *Methylobacterium extorquens* AM1]

A pink facultative methylotroph; grows on methanol, not methane

Bacteria were grown on ^{14}C MeOH and the label in cell material recorded. If RuBP pathway is operating then passage of 'cold' $^{14}\text{CO}_2$ would decrease the label by 95%



Passage of 'cold' CO_2 through the culture during growth on $^{14}\text{CH}_3\text{OH}$ decreased label in cell material by about 50%. This shows that half the carbon enters the biosynthetic pathway as CO_2 produced from the methanol

RuBP carboxylase is absent

Short term labelling experiments showed that 3- phosphoglycerate is not an early intermediate when whole cells are incubated with $^{14}\text{CH}_3\text{OH}$ or H^{14}COOH

Short term label experiments to determine path of carbon

Incubate growing cells with $^{14}\text{CH}_3\text{OH}$ or $^{14}\text{CO}_2$ (bicarbonate)

Take samples into boiling ethanol at 2,4,8,20 secs etc

Separate all soluble components by 2-way paper chromatography

Identify labelled compounds by autoradiography (3 weeks)

Elute, count ^{14}C and confirm identity by co-chromatography with known compounds

Plot % radioactivity in each compound against time. A negative slope indicates an early intermediate.

After 1 min incubation the early intermediates were chemically analysed to determine the specific radioactivity in each carbon atom

Distribution of label in cells incubated with labelled CO₂

Negative slope = earliest intermediates
Malate [reflecting oxaloacetate, OAA]
Glycine; Later - serine

Suggests typical carboxylation of a C3 to a C4 compound [OAA / malate]

And either cleavage of C4 to glycine

Or novel carboxylation to give glycine

NB: the presence of a labelled compound at 20 seconds does not indicate an early intermediate.

Coenzyme A derivatives are cannot be seen in this sort of experiment.

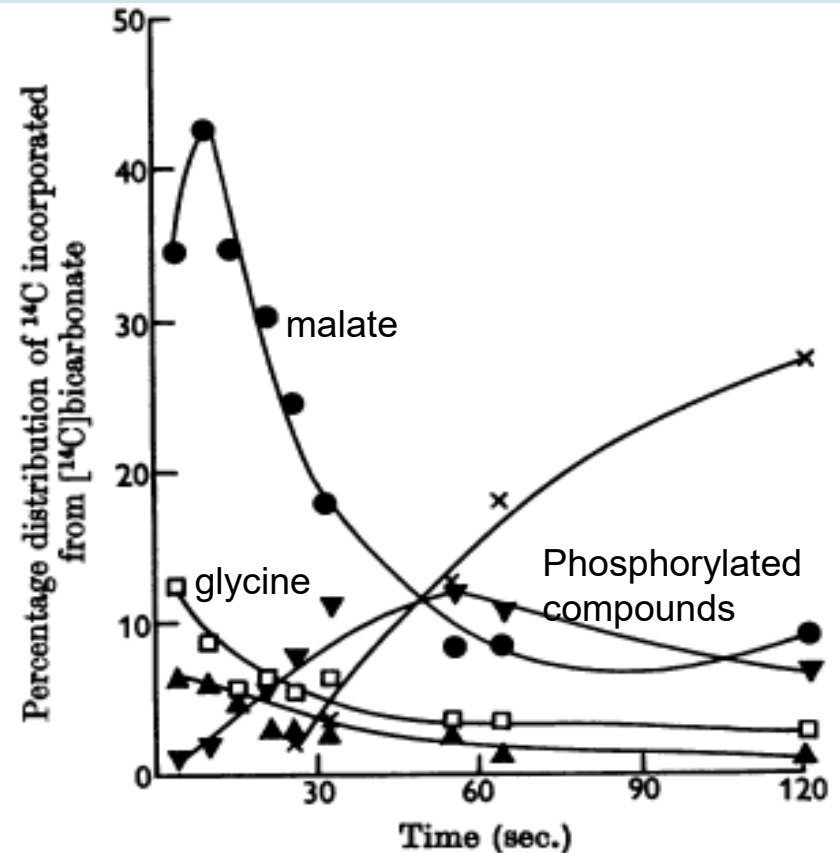


Fig. 3. Variation with time of the percentage distribution of ¹⁴C incorporated from [¹⁴C]bicarbonate into the constituents of the ethanol-soluble fraction of *Pseudomonas* AM1 growing on methanol. ●, Malate; ▲, serine; □, glycine; ▼, phosphorylated compounds; x, trehalose.

Similar results were obtained using *Hyphomicrobium vulgare*

Distribution of label in cells incubated with methanol

Negative slope = early intermediates

Serine Malate Aspartate Glycine

Suggests:

Addition of HCHO to glycine to give serine

A derivative of serine is carboxylated to OAA / malate / aspartate

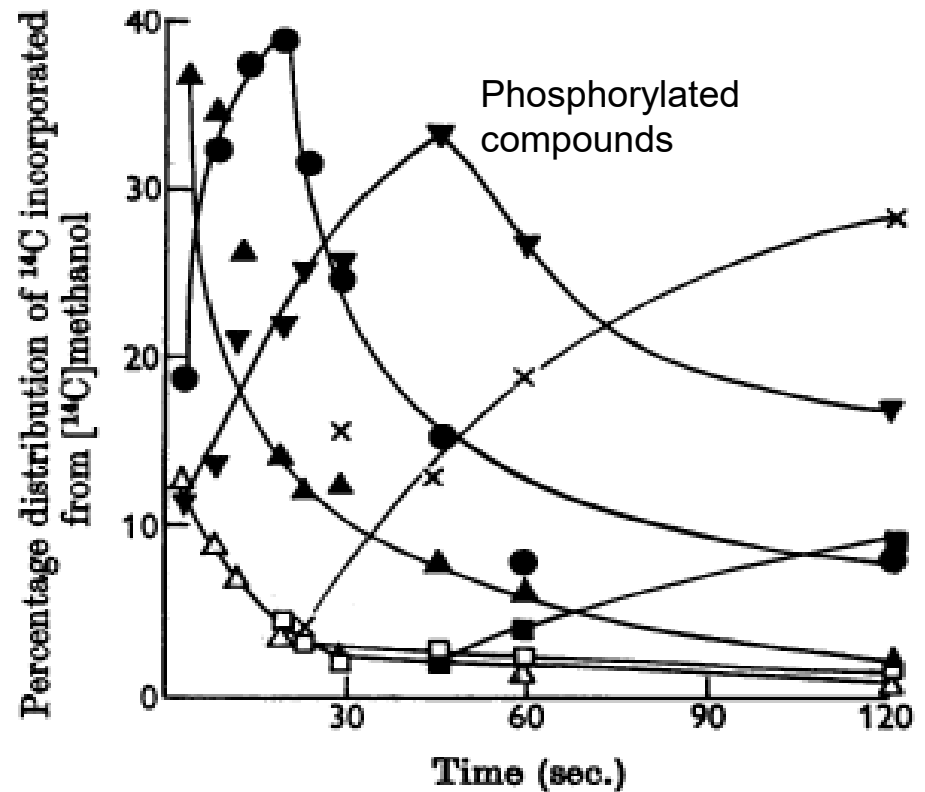


Fig. 2. Variation with time of the percentage distribution of ^{14}C incorporated from $[^{14}\text{C}]$ methanol into the constituents of the ethanol-soluble fraction of *Pseudomonas* AM1, growing on methanol. ●, Malate; Δ, aspartate; ▲, serine; □, glycine; ■, glutamate; ▼, phosphorylated compounds; ×, trehalose.

Similar results were obtained using *Hyphomicrobium vulgare*

Distribution of ^{14}C in carbon atoms of early intermediates

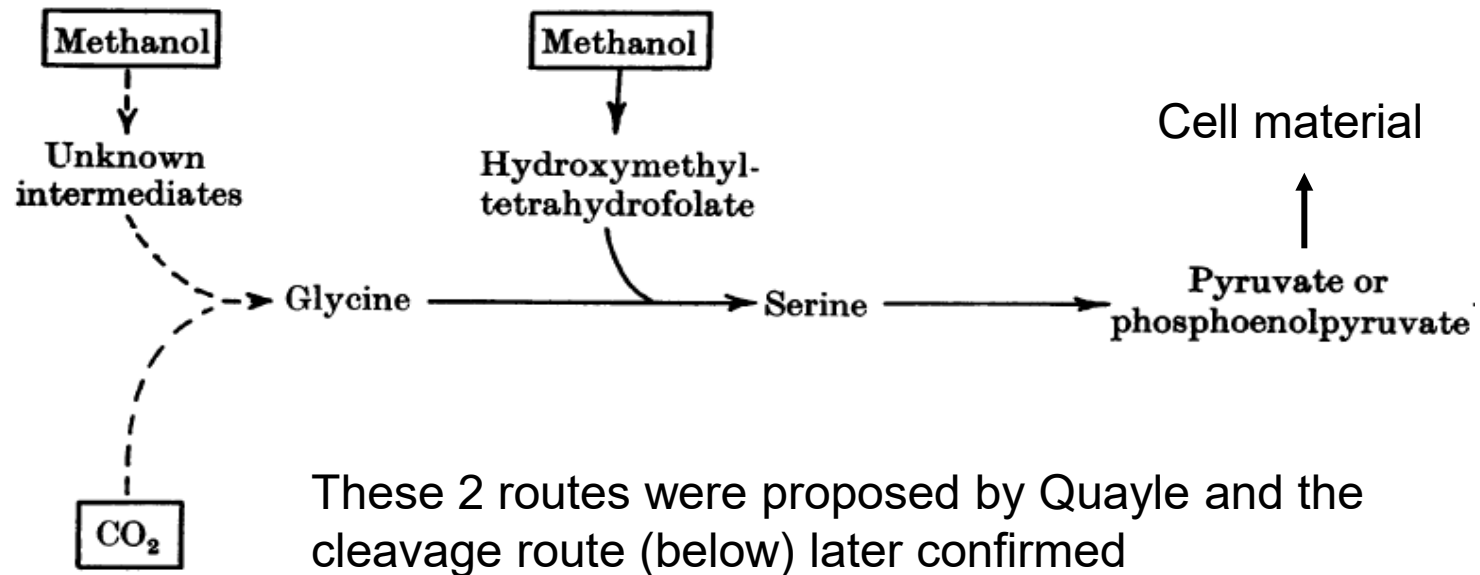
Cells were incubated for 1 minute with ^{14}C MeOH or $^{14}\text{HCO}_3$; Intermediates were purified, chemically degraded and ^{14}C in each C atom determined and expressed as % of total counts in the compound

	From methanol	From bicarbonate
Glycine		
CH_2NH_2	50	15
COOH	50	85
Serine		
CH_2OH	50	2
CHNH_2	25	15
COOH	25	83

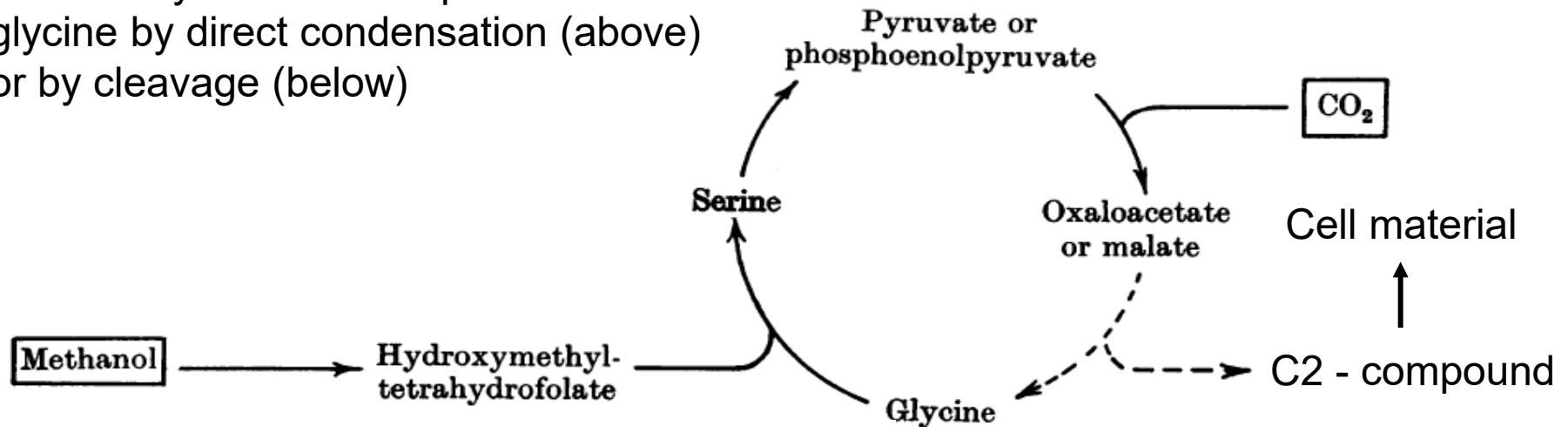
Conclusions

1. Carboxyl group of glycine comes from carbon dioxide; methylene carbon comes from methanol
2. Hydroxymethyl group in serine comes from methanol; the other 2 carbons mimic the distribution seen in glycine
3. Serine arises by hydroxymethylation of glycine

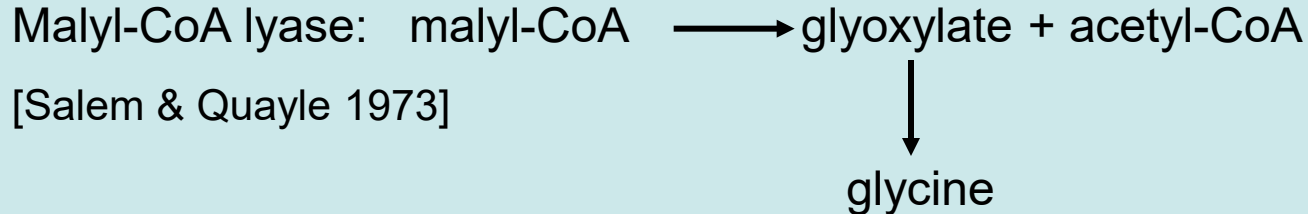
Two possible routes for conversion of methanol plus CO₂ to cell material



NOTE: key difference is production of glycine by direct condensation (above) or by cleavage (below)



The serine cycle involves a cleavage reaction



What happens to the acetyl-CoA?

In icl⁺ bacteria: isocitrate lyase is involved in oxidation of acetyl-CoA to glyoxylate; in these bacteria ICL is also involved during growth on ethanol or acetate

In icl⁻ bacteria with no isocitrate lyase [eg *Methylobacterium extorquens*]

This route is not yet fully established.

It is also involved in metabolism of C2 compounds

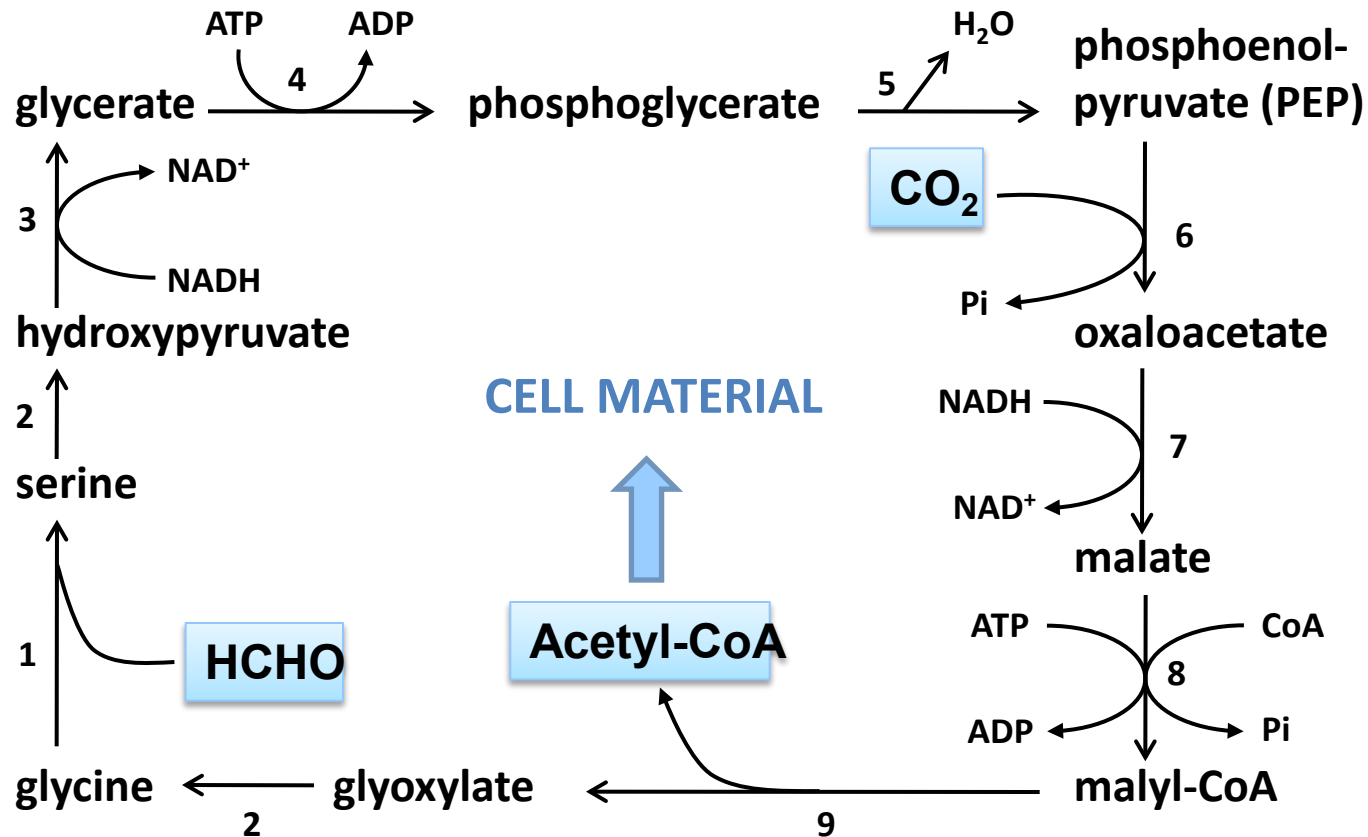


Figure 3. The serine cycle as proposed by Peel, Large, Salem and Quayle⁹⁻¹¹. The enzymes are 1, serine transhydroxymethylase; 2, serine-glyoxylate aminotransferase; 3, hydroxypyruvate reductase; 4, glycerate kinase; 5, enolase; 6, PEP carboxylase; 7, malate dehydrogenase; 8, malate thiokinase; 9, malyl-CoA lyase. After the initial proposal much further enzymological and mutant evidence was subsequently accumulated to confirm this pathway³. Note that during biosynthesis of fatty acids and poly 3-hydroxybutyrate which use acetyl-CoA as their biosynthetic starting point, this pathway is sufficient for production of acetyl-CoA from formaldehyde plus carbon dioxide.

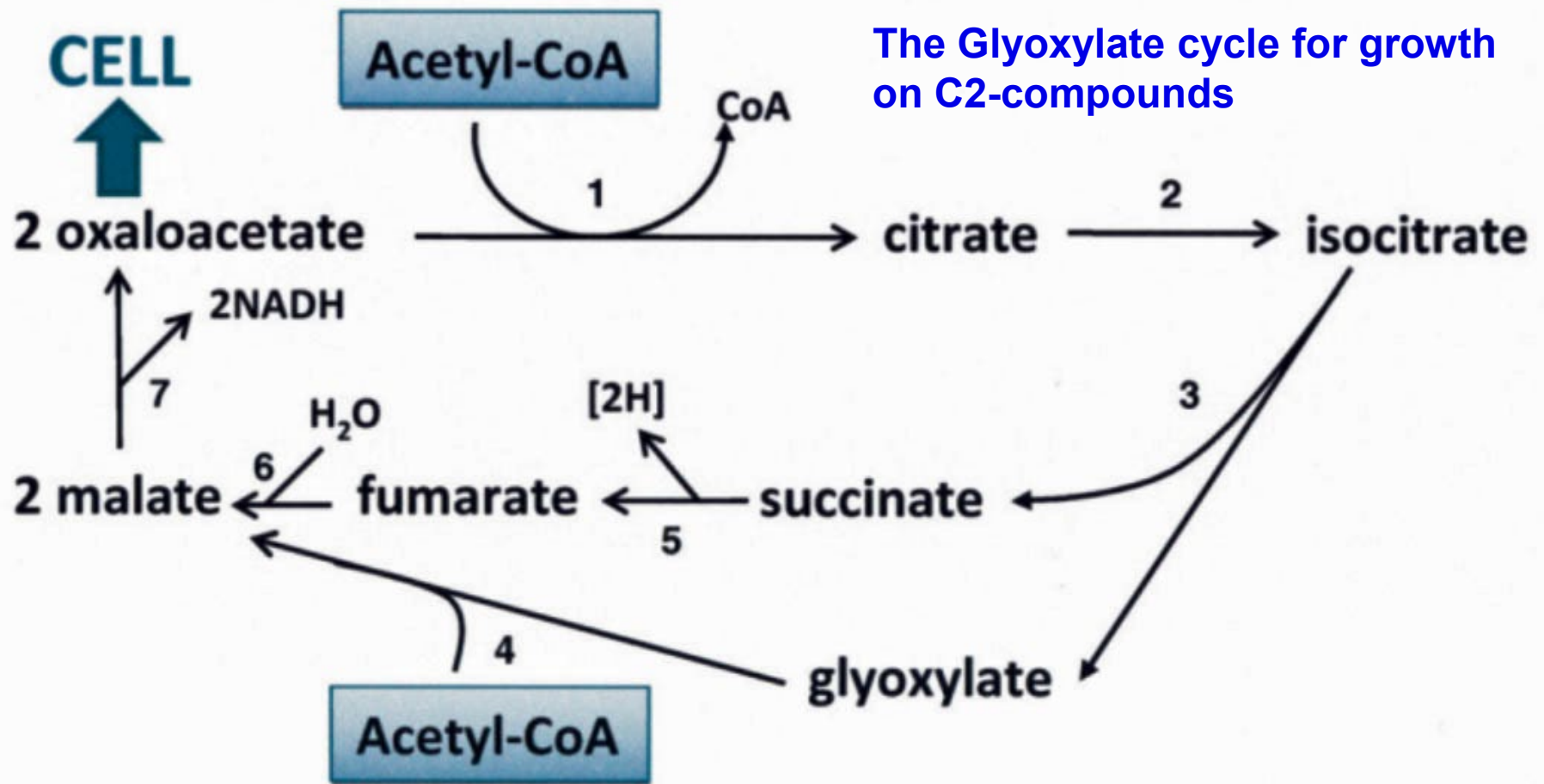
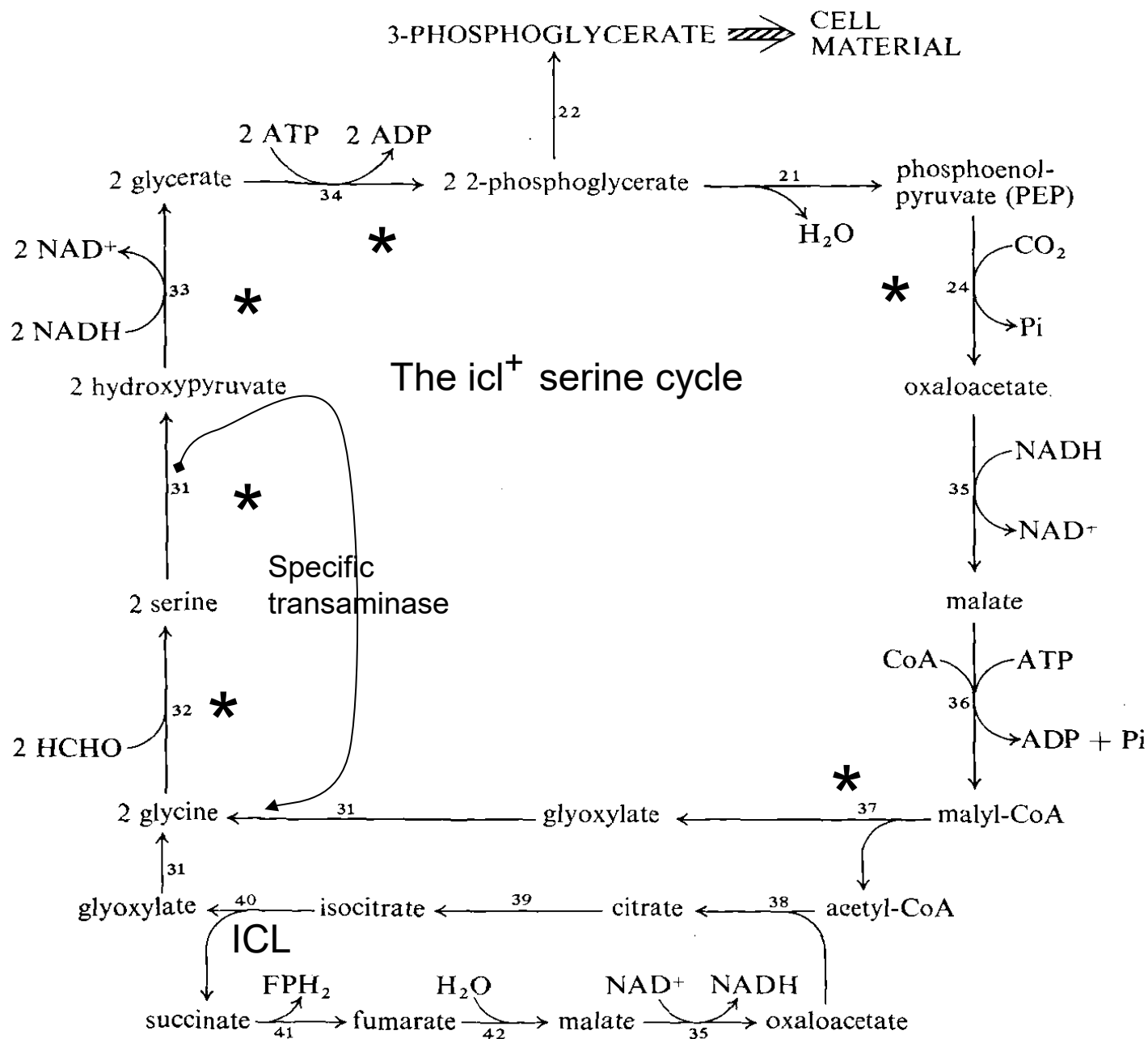


Figure 1 Kornberg's glyoxylate cycle⁴. This achieves the condensation of 2 molecules of acetyl-CoA to malate. The enzymes are: 1, citrate synthase; 2, aconitase; 3, isocitrate lyase; 4, malate synthase; 5, succinate dehydrogenase; 6, fumarase; 7, malate dehydrogenase. In effect this cycle can be summarized as $2 \text{ acetyl-CoA} \rightarrow \text{malate}$. A complete balance equation is given in Table 1 at the end of this review.



Confirmation of serine cycle

The proposed pathway fits the early labelled intermediates

The distribution of labelling in the intermediates fits the pathway

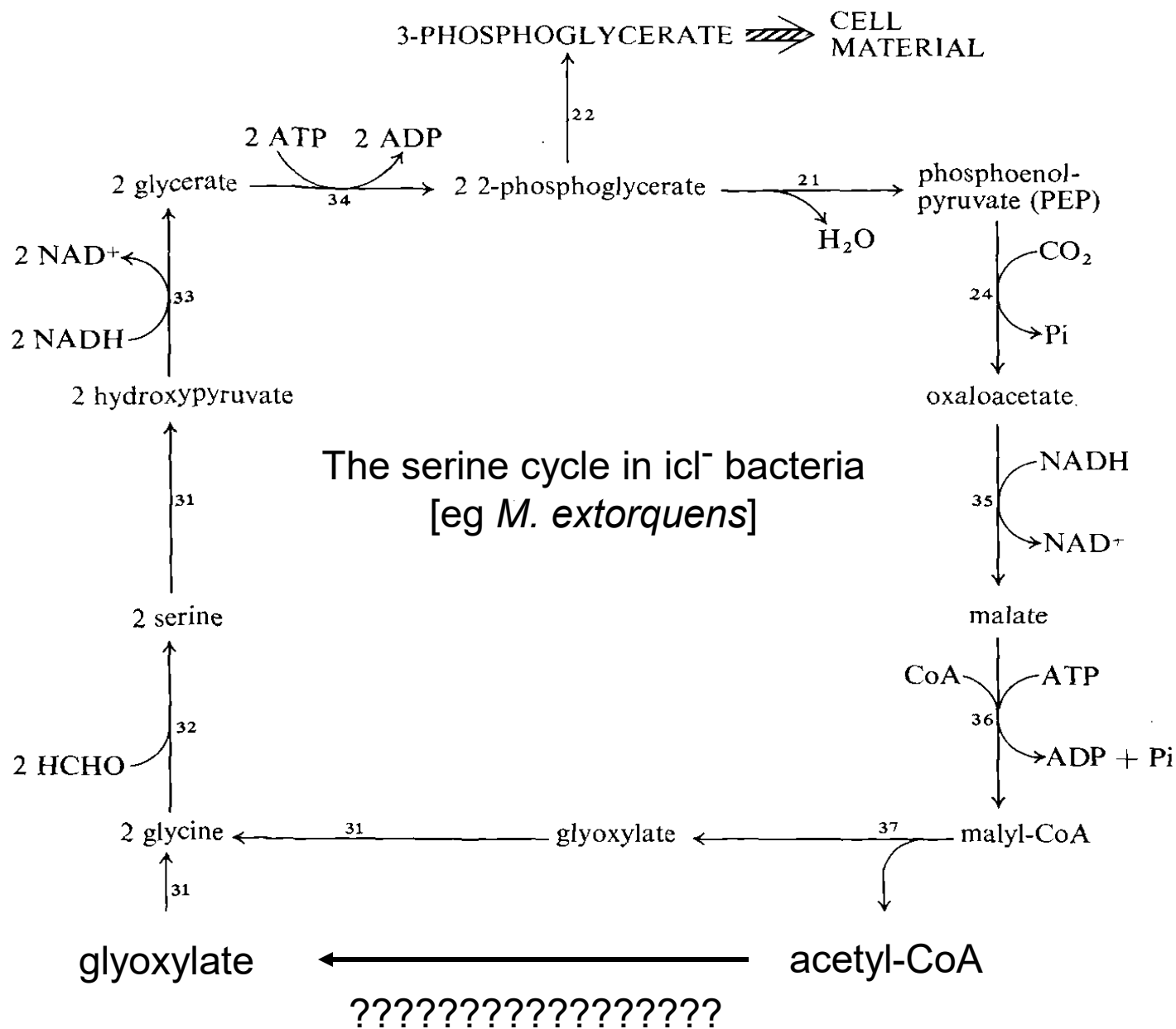
The 5 novel enzymes were purified and characterised

They were shown to be inducible on methanol

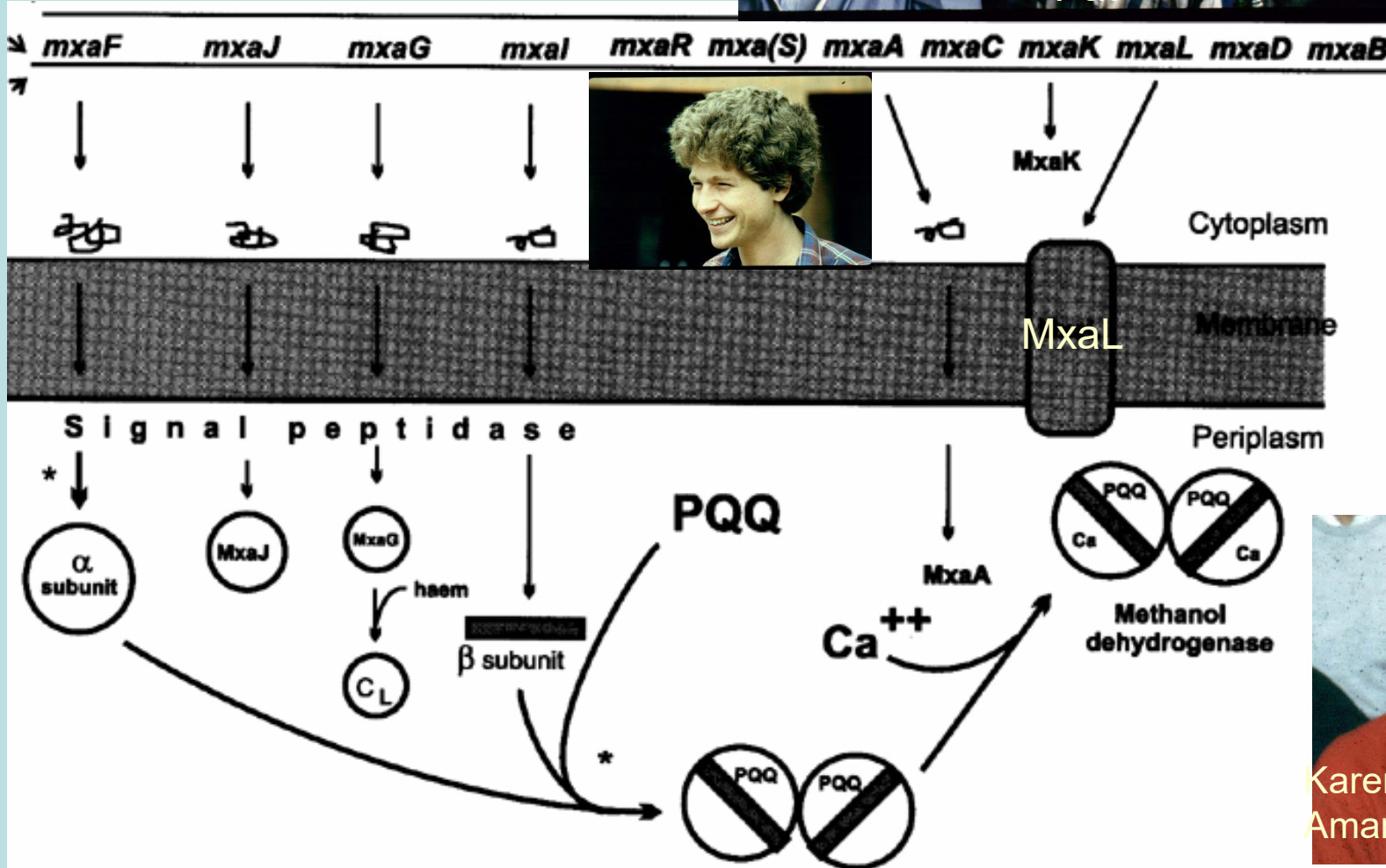
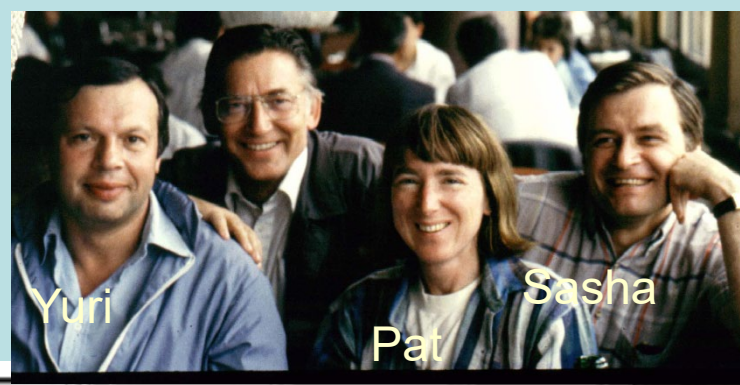
They were of sufficiently high specific activity to account for the growth rate on methanol

Mutants lacking them failed to grow on methanol; revertants had regained the enzyme

Later shown that key enzymes were coordinately regulated, implying the presence of an operon [Dunstan (Goodwin) & Anthony; Hanson & O'Connor (Lidstrom)]



Expression of the mxa operon



The genes: Nunn, Lidstrom, Amaratunga, Anderson, Anthony, Goodwin, Morris, O'Connor