If this were the case, then one may consider that the single administration of δ-ALA, *i.e.* the stress, caused the initial activation of δ-ALA dehydratase, which then showed cyclic fluctuation due to some unknown stimulation after δ-ALA concentration had decreased to normal values.

The enzymes between porphobilinogen and protoporphyrin probably normally have a much higher turnover than δ-ALA dehydratase. As another possibility there may be sequential activation of all of the enzymes functioning up to protoporphyrin. Studies are in progress to elucidate the possible role of δ-ALA dehydratase and other enzymes including those in the tricarboxylic acid cycle in the metabolic regulation of porphyrin synthesis.

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Early products of [^{14}C]acetate incorporation in resting cells of *Rhodospirillum rubrum*

The enzymic conversion of (+)-citramalate to acetate and pyruvate has been characterized in extracts of *Clostridium tetanomophum*[1–3] and *Pseudomonas ovalis* Chester[4] which, in contrast to the reaction in liver mitochondria[5], is freely reversible. LOSADA *et al.*[6] have suggested the condensation of acetyl-CoA and pyruvate to form citramalate in *Chromatium* extracts probably represents an intermediate step in the synthesis of glutamate during the photometabolism of acetate. The relative importance of the reactions which condense acetate with oxaloacetate or pyruvate has not been assessed in photosynthetic bacteria.

Rhodospirillum rubrum was grown anaerobically on glutamate and malate as described by KOHLMILLER AND GEST[7]. For [^{14}C]acetate-incorporation studies log-phase cells were harvested by centrifugation, washed with 0.05 *M* KH_2PO_4 buffer (pH 7.2) and equilibrated 30 min under N_2 in an illuminated Warburg bath at 30°.

Sonicated cells were prepared for enzyme studies by removing the chromatophores and cell debris from the supernatant by centrifugation at 144000 × *g*. [^{14}C]Acetate incorporation into the organic acids of resting cells or soluble supernatant was analyzed by extracting the cells with boiling 80% ethanol, removing the protein by centrifugation and placing the extract through Dowex-50 (H^+) and Dowex-1 (formate) columns. The organic acids were eluted from the Dowex-1 resin with 8 *N* formic acid[8], concentrated *in vacuo* and applied to Whatman No. 1 for chromatography in butanol–formic acid–water (4:1:5, v/v/v). The radioactive acids were located by exposure of the chromatograms to X-ray film and counted to ± 2% efficiency with a gas-flow counter[9]. The counts recorded for the radioactive acids represented 85–90% recovery. The acids were identified by co-chromatography with authentic compounds in acidic and basic solvents.

The incorporation of [2-^{14}C]acetate into the individual organic acids of resting cells is seen in Table I. It should be made quite clear that the organic acids were the prime recipients of the [^{14}C]acetate in early time-incorporation studies for at this

TABLE I

[2-^{14}C]ACETATE INCORPORATION INTO THE ORGANIC ACIDS OF RESTING CELLS OF *R. rubrum*

70.0 mg (dry wt.) cells were exposed to 10.0 μC of [2-^{14}C]sodium acetate for 5.0 sec under N_2 in an illuminated Warburg at 30°.

Compound	*Radioactivity of [^{14}C]acid (counts/min)*
Glutamate	91
Citrate	173
Malate	167
Glycolate	244
Citramalate	824
Succinate	1179
Unknown	271
Fumarate	200

time the radioactivity in the ether-soluble components and amino acids was always low. The two major radioactive acids after a 5-sec exposure of the cells to [^{14}C]acetate are succinate and citramalate while the other members of the tricarboxylic acid cycle such as citrate, malate and fumarate are low in radioactivity. A direct comparison of the acetate incorporation in resting cells to the enzymic incorporation (Table II) indicates the above pattern prevails. It is apparent that depending on the addition of the C-2 acceptor (pyruvate or oxaloacetate) that citramalate or citrate is formed thus reflecting the presence of pyruvate transacetase or the conventional condensing enzyme. The appearance of radioactivity in citramalate is dependent on the addition of CoA, ATP, and pyruvate which would be true if the activation of acetate to acetyl-CoA were required prior to the condensing reaction. The appearance of [^{14}C]acetate in glycolate is in agreement with HARLEY AND BEEVERS' observation[10] in corn-root tips and is yet unexplained. The radioactivity in succinate both in resting cells and sonicates seems to parallel the activity in citramalate but may not be different from the appearance of [^{14}C]acetate into succinate in other bacteria[11]. As yet there is no indication of the occurrence of a "Thunberg-Condensation" of two acetyl-CoA units.

These results highlight the routes of acetate into resting cells of *R. rubrum* and shows that this route is directed toward citramalate and citrate. In agreement with

TABLE II

THE EFFECT OF CO-FACTORS ON THE ENZYMIC INCORPORATION OF [2-^{14}C]ACETATE INTO CITRAMALATE AND CITRATE IN EXTRACTS OF *R. rubrum*

The reaction mixture contained in μmoles: CoA, 0.1; ATP, 10; $MgCl_2$, 5; GSH, 10; potassium phosphate buffer (pH 7.2), 100; potassium [2-^{14}C]acetate, 5 and 10.0 μC; potassium pyruvate or oxaloacetate, 20; and 1.0 ml (2 mg protein) of *R. rubrum* supernatant. Reaction mixture incubated at 35° for 30 min.

Compound	*Radioactivity of [^{14}C]acids (counts/min)*					
	Complete	*Minus CoA*	*—ATP*	*—ATP —CoA*	*—Pyruvate —CoA —ATP*	*Plus oxaloacetate*
Citrate	2239	1174	142	530	117	38 384
Malate	1031	1110	329	376	416	1607
Glycolate	998	692	567	787	363	1137
Citramalate	17 924	27 163	6953	794	707	19 008
Succinate	2266	1677	1228	5126	997	6377
Unknown	829	776	416	788	408	666
Fumarate	923	640	570	742	440	1398

ELSDEN AND ORMEROD[12], the radioactivity in the tricarboxylic acid intermediates from [^{14}C]acetate may reflect the presence of the citric acid cycle enzymes. In longer-time experiments of [^{14}C]acetate incorporation into cells radioactive acids corresponding in R_F's to itaconate and mesaconate have been observed and it seems possible the reactions[2] involving the fermentation of glutamate may be operative in this organism grown on glutamate. Thus the early appearance of [^{14}C]citramalate from acetate could be explained by a back-equilibration of acetate with these fermentation reactions. The importance of the tricarboxylic acid cycle in glutamate and acetate metabolism is now under investigation.

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