led to complete deacetylation[2]. Paper chromatography of the hydrolysate showed one spot having the same $R_{glucose}$ as galactosamine. Furthermore, when the hexosamine obtained by deacetylation of the free sugar was treated with ninhydrin[15], the corresponding pentose, lyxose was obtained. After acid hydrolysis, UDP and UMP were the only nucleotides observed by paper chromatography in the acidic and neutral ethanol–ammonium acetate solvents. These observations indicate that the mixture of UDP-acetylglucosamine and UDP-acetylgalactosamine present in Dahlia tubers is similar to the one isolated from liver[2].

This investigation was supported in part by a research grant (No. G-3442) from the National Institutes of Health, U.S. Public Health Service, by the Rockefeller Foundation and by the Consejo Nacional de Investigaciones Científicas y Técnicas.

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Received October 19th, 1962

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PN 1177

A possible route of acetate oxidation in *Rhodopseudomonas spheroides*

ELSDEN AND ORMEROD[1] have shown that fluoroacetate strongly inhibits oxidation of acetate in *Rhodospirillum rubrum* both under dark-aerobic and light-anaerobic conditions. In this laboratory, when [^{14}C]acetate was metabolized by this organism, $^{14}CO_2$ formation was inhibited nearly completely by $1 \cdot 10^{-4}$ *M* fluoroacetate under either of these conditions[2]. Similar results were obtained with *Rhodopseudomonas spheroides* under dark-aerobic conditions. With the latter organism, however, under light-anaerobic conditions fluoroacetate inhibited $^{14}CO_2$ formation only to the extent of 40–50% (refs. 2, 5). This indicated the possibility that a pathway of acetate oxidation other than the citric acid cycle operates in *R. spheroides* under these particular conditions. To elucidate its nature, the *R. spheroides* cells were exposed

to [2-^{14}C]acetate anaerobically in the light for relatively short periods and rates of incorporation of ^{14}C into various intermediates were compared.

R. spheroides was grown anaerobically in the light in medium S of LASCELLES[4]. The cells were harvested by centrifugation, washed with 0.02 *M* phosphate buffer (pH 6.8) and suspended in 0.033 *M* phosphate buffer (pH 6.8). The suspension was placed in a specially devised vessel which was essentially a separatory funnel with one or two side arms and aerated with N_2 for 15 min. The reaction was initiated by adding [2-^{14}C]acetate to the suspension and run under illumination. Anaerobiosis and mixing were ensured by streaming N_2 continuously through the reaction mixture. At times indicated, a portion of the reaction mixture was withdrawn for analysis of ^{14}C incorporation. Fractionation, paper-chromatographic separation and ^{14}C analysis of various carboxylic acids were performed as described previously[5]. Dinitrophenylation followed by paper-chromatographic separation by the method of KOCH AND WEIDEL[6] was utilized for ^{14}C analysis of amino acids. Each intermediate was located on the paper with the aid of carrier and the following compounds were examined: glyoxylate, glycolate, pyruvate, malate, succinate, α-ketoglutarate, citrate.

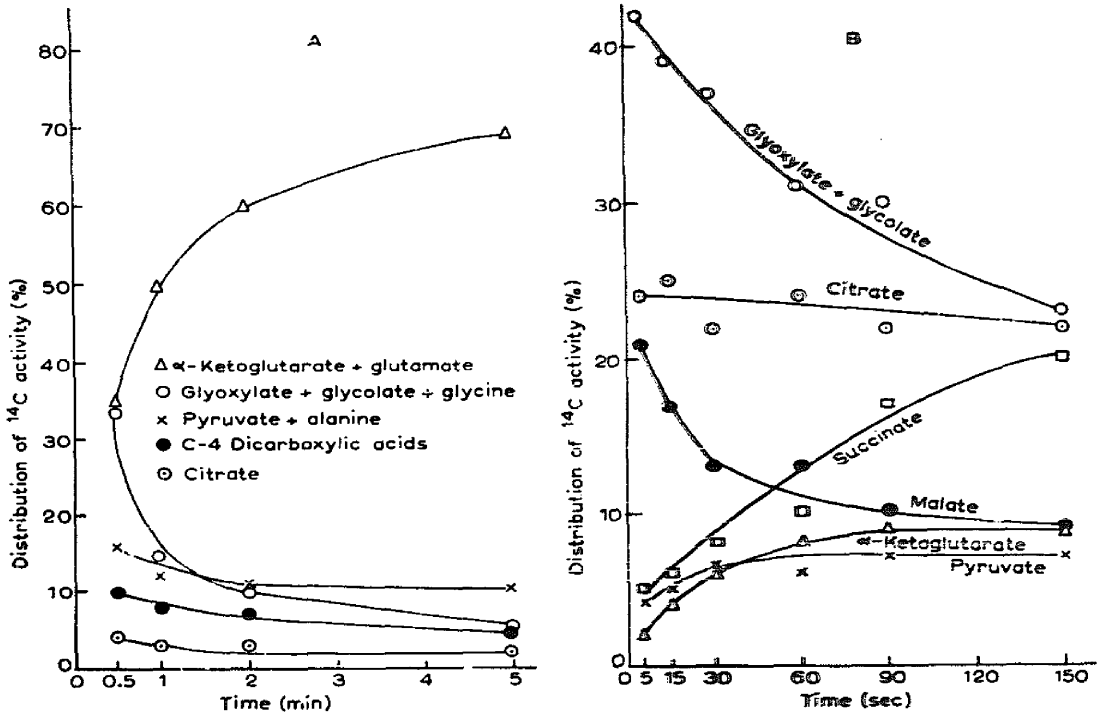

Fig. 1. Variation with time of the percentage distribution of ^{14}C from [2-^{14}C]acetate in various intermediates. A, 30 ml of the suspension of *R. spheroides* cells (360 mg dry wt.) were incubated anaerobically in the light with 1.8 ml of 0.05 *M* [2-^{14}C]acetate (0.5 mC/mmole). At times indicated, 6-ml portions of the reaction mixture were withdrawn, by opening the stopcock of the flask, into a glass tube in which 1 ml of 6 *N* H_2SO_4 had been placed. The acidic mixture was then analyzed for ^{14}C incorporation. B, the suspension was preincubated with 1.8 ml of 0.05 *M* non-labeled acetate for 4 min. Then, the reaction was initiated by adding 1.8 ml of 0.05 *M* [2-^{14}C]acetate (1.0 mC/mmole). Other conditions were similar to those for A except that the ^{14}C analysis of amino acids was omitted.

glycine, serine, alanine, aspartate and glutamate. The total ^{14}C activity incorporated increased linearly with time for at least the initial 5 min.

As shown in Fig. 1A, after incubation for 30 sec, as much as 35% of the total ^{14}C incorporated were found in C-2 compounds. However, the percentage declined sharply reaching the value of 5% after 5 min. On the contrary, ^{14}C in C-5 compounds increased steadily and after 5 min nearly 70% of the total ^{14}C was located in this fraction. Comparison of the rates of ^{14}C incorporation of the citric acid cycle intermediates (Fig. 1B) further revealed that malate was one of the earliest products of ^{14}C incorporation. Glyoxylate and glycolate also acquired ^{14}C at a very rapid rate. The presence of $5 \cdot 10^{-4}$ *M* fluoroacetate did not affect much the rapid appearance of ^{14}C in these compounds. On the other hand, incorporation of ^{14}C into α-ketoglutarate and succinate was always slow. These results strongly suggest that a mechanism of malate formation from acetate other than the citric acid cycle functions in *R. spheroides* under light-anaerobic conditions.

The mechanism for the early appearance of ^{14}C in glyoxylate and glycolate has not yet been elucidated. In agreement with KORNBERG AND LASCELLES[7], no activity of isocitrate lyase (EC 4.1.3.1) could be detected in *R. spheroides*. Thus formation of glyoxylate from isocitrate is very improbable. Malate-cleavage enzyme found in this organism[8] also does not seem responsible unless any C-4 dicarboxylic acid were formed prior to the appearance of glyoxylate. Participation of Thumberg condensation is unlikely on account of the relatively slow rate of ^{14}C incorporation into succinate. Acetate might be converted into glyoxylate by a rather direct way. If so, the route would enable the continuous generation of malate from acetate through the action of malate synthase (EC 4.1.3.2) which has been demonstrated repeatedly in *R. spheroides*[5,7]. Subsequent oxidation of malate to pyruvate, the route prevailing under light-anaerobic conditions in this organism for the oxidation of C-4 dicarboxylic acids[3], would become a site of acetate oxidation which does not pass through the stage of citrate and thus is not inhibited by fluoroacetate. In *Chromatium*[9] and in *R. rubrum*[10], a pathway is said to be working to a significant extent which transforms acetate into citramalate and possibly into glutamate. In our experiments, although citramalate appeared to acquire ^{14}C to a considerable extent under the conditions described above, the rate of incorporation was slow. Significant participation of this route in the acetate oxidation in question in *R. spheroides* is unlikely.

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Received September 7th, 1962