δ-Aminolevulinate dehydratase activity in liver and its relation to substrate concentration

Through the action of δ-aminolevulinate dehydratase [5-aminolevulinate hydro-lyase (adding 5-aminolevulinate and cyclizing), EC 4.2.1.24] two molecules of δ-aminolevulinate are condensed to form porphobilinogen. There are several known conditions in which the activity of this enzyme changes. An increase in δ-ALA dehydratase activity occurs in experimental porphyria[1], a metabolic derangement in which there is an overproduction of liver porphyrins. In the livers of tumor-bearing mice there is decreased δ-ALA dehydratase activity[2], which may be related to a decrease in heme synthesis[3]. The bacterium *R. spheroides* has increased δ-ALA dehydratase activity under conditions that stimulate the formation of bacteriochlorophyll[4]. KIKUCHI *et al.*[5] have discussed the δ-ALA dehydratase inhibitors that are found in certain micro-organisms. These changes in enzyme activity suggest a possible function for δ-ALA dehydratase in the cellular regulation of porphyrin synthesis, especially since the enzyme acts at a branching point in the metabolism of δ-ALA. That is, δ-ALA can be either utilized for porphyrin synthesis through the action of δ-ALA dehydratase or metabolised via the succinate – glycine cycle[6]. This report concerns the effect of varying tissue δ-ALA concentration on δ-ALA dehydratase activity and porphyrin synthesis in mouse liver.

Male Swiss mice weighing 28 ± 1 g were used. Each mouse received a subcutaneous injection of 30 μmoles of δ-ALA hydrochloride in a solution neutralized to pH 7. At several time intervals varying from 0.5 to 24 h after injection the animals were sacrificed by decapitation. The livers were perfused with physiologic saline, removed, and homogenized in 0.067 *M* phosphate buffer (pH 6.8). δ-ALA dehydratase activity was assayed, with minor changes, by the method of GRANICK AND MAUZERALL[7]. Reaction tubes contained 0.15 ml of 5% homogenate, 0.05 ml of 0.02 *M* δ-ALA and 0.30 ml of phosphate buffer. Incubation was at 37° under nitrogen. Five tubes were prepared for each homogenate. They were incubated for periods of 0, 15, 30, 45 and 60 min. The reaction was stopped and deproteinized by the addition of $HgCl_2$–trichloroacetic acid solution, then centrifuged. The color was developed by the addition of Ehrlich's reagent to an aliquot of the supernatant, and was read at 553 mμ. The results were plotted and converted to μmoles of porphobilinogen formed per h per ml of assay medium. The control values (9.2 μmoles porphobilinogen/h) were slightly higher than reported previously for rat liver[8]. Under these conditions the varying concentrations of endogenous δ-ALA did not influence the assay. δ-ALA in the homogenate was estimated colorimetrically following its condensation with acetylacetone according to the method of GIBSON, LAVER AND NEUBERGER[8]. Porphyrins in the liver homogenate were extracted with ethyl acetate–acetic acid (4:1), separated by solvent extraction, and measured fluorometrically.

The results are summarized in Fig. 1. The injected δ-ALA that was taken up by the liver was quickly metabolized. δ-ALA concentration in the liver increased from 0.02 to 0.25 μmole/g by 0.5 h after injection and returned to normal within 3 h. The δ-ALA dehydratase activity reached a maximum of 2.3 times the control activity within 1 h then decreased to near the normal range at 3 h. However, unlike the sub-

Abbreviation: δ-ALA, δ-aminolevulinate.

strate concentration, the enzyme activity continued to fluctuate. It showed a second increase at 6 h which returned nearly to normal again by 12 h. Although the data at 6 h varied over a wide range the increase seems to be significant since it was reproducible and there was no overlapping with the 3-h data. An indication of a third slight increase was seen at 24 h. Much less increase in δ-ALA dehydratase was found in starved animals.

The most notable change in liver porphyrin concentration was found to occur with protoporphyrin (Fig. 1). Coproporphyrin and uroporphyrin showed an increase in their concentration which was only 4% that of protoporphyrin. Nevertheless the changing concentrations of these porphyrins with time followed a pattern parallel to that of protoporphyrin. The liver concentrations of all of these porphyrins succeeded the concentration of δ-ALA, and reached a peak in 1.5 h. Porphobilinogen concentrations were extremely low in all cases.

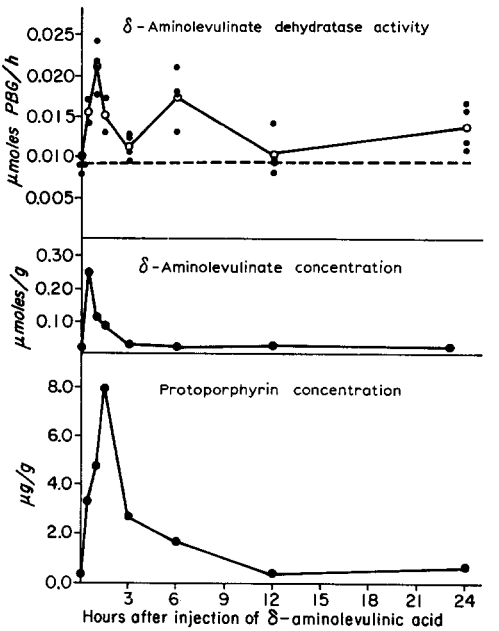

Fig. 1. The relationship between δ-aminolevulinate dehydratase activity and δ-aminolevulinate and protoporphyrin concentration in liver. Values for enzyme activity are shown for individual animals with the average being plotted. The two concentration curves represent the average obtained from two or four animals as indicated in the δ-ALA dehydratase activity curve. PBG, porphobilinogen.

Since δ-ALA dehydratase activity in liver followed so closely the concentration of δ-ALA, there is probably a direct activation of the enzyme by the substrate. Synthesis of the protein *de novo* seems less likely. This observation suggests that some of the previously reported changes in δ-ALA dehydratase activity reflected changes in available substrate. Changes in enzyme activity are found also at an earlier stage in porphyrin synthesis. Under conditions of stimulated porphyrin synthesis in certain bacteria[4] or in livers of porphyric animals[9] there is increased activity of the system that synthesizes δ-ALA from succinyl-CoA and glycine. If this increased activity is brought about by a change in succinyl-CoA concentration then the primary site for the regulation of porphyrin synthesis could lie in the tricarboxylic acid cycle.

The second and possible third increase in δ-ALA dehydratase activity in the presence of normal δ-ALA concentration were quantitatively less than the first change following injection, but nevertheless may represent a cyclic effect. LABORIT[10] has pointed out that the biological reaction to a single stress often shows a cyclic pattern.

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°.