A possible lethal synthesis of monofluoromalate

PETERS and his colleagues showed that monofluoroacetate *in vivo* can be converted to fluorocitrate[1,2] which blocks the tricarboxylic acid cycle and causes citrate to accumulate. DAGLEY AND WALKER[3] found citrate accumulation when a vibrio[4] utilized certain carbon sources for growth with monofluoroacetate present, but when other substrates were used, relatively high concentrations of pyruvate accumulated instead. It was suggested that pyruvate oxidation might be blocked by fluoropyruvate formed by the cells from the fluoroacetate added; but the difficulties of accepting a synthesis of the type $C_1 + C_2 \longrightarrow C_3$ were also discussed. Dr. H. L. KORNBERG has since drawn our attention to the possibility that the inhibitor which is "lethally synthesized" by the cells might be fluoromalate; and a study of the effect of synthetic (±) sodium monofluoromalate on growth of this vibrio[4] was made possible by a generous gift from Dr. P. W. KENT.

Overnight cultures of the vibrio grown in the presence of fluoromalate accumulated pyruvate which was identified as its 2:4-dinitrophenylhydrazone by paper chromatography[5,6] and absorption spectroscopy[7]. The effect of fluoromalate on a growing culture is shown in Fig. 1. Two large boiling tubes (8 in. × 1½ in.) each contained 100 ml of a medium containing 2 g/l KH_2PO_4, 1 g/l $(NH_4)_2SO_4$, 0.2 g/l $MgSO_4 \cdot 7H_2O$ and 0.01 *M* DL-malic acid. The pH was adjusted to 7 by addition of NaOH. A stream of sterile air was blown through and each was inoculated at 30° with cells from 25 ml of a culture grown in a similar medium. Samples were withdrawn at intervals; growth was measured using a Spekker photoelectric absorptiometer[3] and pyruvate concentrations by the method of FRIEDEMANN AND HAUGEN[7]. It is clear that the addition of sodium (±) monofluoromalate resulted in a blockage of pyruvate utilization by the cells. Oxidation of 8 μmoles of each of various tricarboxylic acid cycle components by non-proliferating suspensions was inhibited in the presence of 2.3 μmoles fluoromalate (reaction vol., 3 ml) in the order: pyruvate > malate >

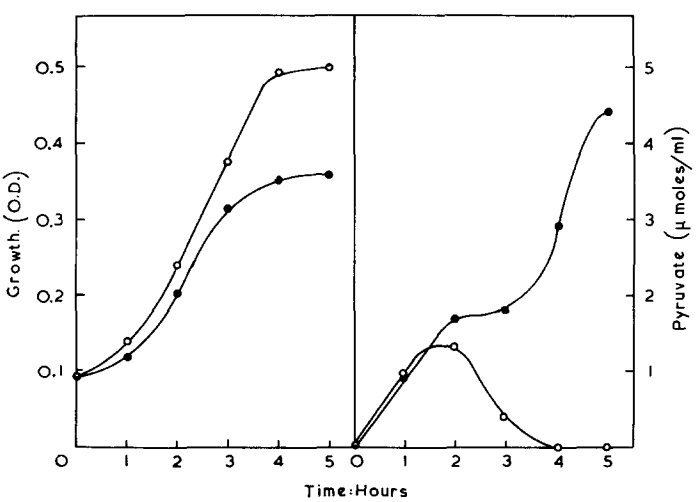

Fig. 1. Effect of 10^{-4} *M*-sodium (±) monofluoromalate on growth (left) and pyruvate accumulation (right). Open circles, no drug present; blacked-in circles, growth with addition of fluoromalate.

fumarate > succinate. Pyruvate oxidation was largely abolished, and analysis of flask contents at the end of the experiment showed accumulations of pyruvate from other substrates that were proportional to the inhibition of O_2 uptake. The addition of fluoromalate to cultures at a concentration of 10^{-4} *M* during a growth period of 18 h caused pyruvate to accumulate from various sources of carbon. The concentrations of pyruvate produced were about the same as those reported[3] for growth in the presence of 10^{-4} *M*-fluoropyruvate, namely 1–2 μmoles pyruvate/ml for cells utilizing C_4 acids and parahydroxybenzoate, with negligible amounts accumulating from acetate. Citrate accumulation did not result from any of the carbon sources by the action of either drug, but citrate accumulation after addition of fluoroacetate to cultures was confirmed[3].

The action of malate synthetase is similar to that of the condensing enzyme which forms citrate, because the methyl group of acetyl CoA condenses with a –CO–COOH group in both cases. The presence of malate synthetase in this vibrio has been demonstrated[8] and accordingly the synthesis of fluoromalate from fluoroacetate is feasible. The present work shows that fluoromalate, if formed, could be expected to block pyruvate metabolism and there is no reason to postulate another route by which fluoropyruvate could be synthesized instead. No conversion of synthetic fluoromalate to fluoropyruvate could be demonstrated for an active malate decarboxylase preparation from *Lactobacillus arabinosus*[9].

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Effect of neoplasia and fasting on phospholipid turnover rate in rat liver

Previous studies have shown that the phospholipid content of the Novikoff hepatoma was markedly lower than that of the normal rat liver[1,2]. It was also demonstrated that this change was specific to the neoplastic liver because there was no such alteration present in the rapidly growing regenerating liver. Studies on the physiological behavior of liver phospholipids showed that even after 6-days fasting 50 % of the phospholipids were present in the average liver cell. On the other hand, the average hepatoma cell