

## Effects of Malonic Acid on Krebs's Cycle

**Summary:** My group and I tested the effects of Malonic Acid on Krebs's Cycle functions to determine whether it serves as a competitive inhibitor to the enzyme Succinate dehydrogenase. We did this by using the dye methylene blue, which is reduced instead of FAD as the substrate Succinate is changed into Fumarate, in three different reactions to observe any interruption in respiration. Our results indicate that Malonic Acid *is* a competitive inhibitor for this particular enzyme and slows and potentially stops the cycle. Our findings are supported by the results found in another experiment where succinate dehydrogenase was found to be disabled by other external molecules, such as 3-nitropropionate (Coles *et al.*, 1978). Succinate dehydrogenase has also been confirmed to exist in many internal organs, indicating that it serves as an appropriate sample for the electron-transport system of the inner mitochondrial membrane in lab experiments (Davis & Hatefi, 1971).

**Methods & Results:** My group and I prepared three test tubes to perform three separate reactions. In each, 1mL of liver homogenate was added, which served as a source of the enzyme Succinate dehydrogenase for the experiment. We then added 1mL of 1.1% KCl solution to all three, along with 1mL of Succinic Acid solution to tube A, 1mL of Malonic Acid solution to tube B, and 1mL each of Succinic Acid and Malonic Acid solutions to tube C. Drops of methylene blue solution were added to all three test tubes until each had a distinct blue color. Finally, about 1mL of mineral oil was added to the tops of the mixtures to form a covering film which prevented oxygen from diffusing into the solution. All three test tubes were then placed into a heater block set to 38°C and we examined them at five minute intervals until a change in color occurred. After about fifteen minutes, the colors in the test tubes were as follows:

Contents	Initial Color	Final Color
Test Tube A 1mL Succinic Acid solution 1mL 1.1% KCl solution 1mL liver homogenate	Blue-Green	Brown-Red
Test Tube B 1mL Malonic Acid 1mL 1.1% KCl solution 1mL liver homogenate	Blue-Green	Blue-Green
Test Tube C 1mL Succinic Acid solution 1mL Malonic Acid solution 1mL 1.1% KCl solution 1mL liver homogenate	Blue-Green	Blue-Green

The change in test tube A indicates that the reduction of methylene blue in the place of FAD was not interrupted. However, in test tubes B and C the Malonic Acid solution effectively bound to the enzyme as a competitive inhibitor, slowing or stopping the reaction. A picture of where this interruption occurred in the Krebs's Cycle is shown below:

### References:

Coles, C.J. & Edmondson, D.E., & Singer, T.P., 1978. Inactivation of Succinate Dehydrogenase by 3-Nitropropionate, *The Journal of Biological Chemistry*, 254(12), pp. 5161-5167.  
Davis, K.A. & Hatefi, Y., 1971. Succinate dehydrogenase. I. Purification, molecular properties, and substructure, *Biochemistry*, 10(13), pp. 2509-2516.