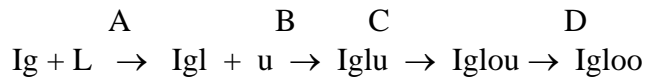


Name _____

**Pre-Laboratory Exercise
Laboratory 6**

Suppose that substance IGLOO is required for the formation of the bacterial cell wall. It is made by the following pathway:



A bacterium which is unable to make enzyme A will be unable to grow. Will it grow if I feed it Igl?

Will a bacterium which can't make enzyme C grow if I feed it Igl? _____

The A bacterium accumulates Igl. Will it be able to cross feed C? _____

The C bacterium accumulates Iglu. Will it be able to crossfeed A? _____

Laboratory 6 Biochemical Pathways

Objectives: 1) To become familiar with the growth characteristics and interactions of mutant bacteria blocked in a particular biochemical pathway. 2) To experience how bacterial mutants can be used to help to elucidate a particular pathway. 3) To become familiar with the concept of a biochemical pathway and to understand the implications of this concept.

References: Madigan et al, p.131, any biochemistry textbook, and the biocyc web site <http://biocyc.org/server.html>

Auxotrophic mutants have been used to help elucidate the pathway for the biosynthesis of many compounds. You will be provided with a set of four auxotrophic mutants which require arginine (an amino acid) for growth. These mutants are point mutants. When they were tested in pairs in a cis trans complementation test they were each found to complement the other three mutants. Thus each of these mutations affects a different cistron or polypeptide chain (remember that some enzymes are composed of multiple subunits or polypeptide chains). We will use two different techniques to help us to determine the biosynthetic pathway for arginine in *E. coli* and the rough location in that pathway of the enzyme encoded by each of these genes.

Growth on suspected intermediates in arginine synthesis.

Arginine is made from glutamate and ornithine is known to be involved early in the pathway leading to arginine and citrulline is a possible later intermediate. You will be given a plate with the four Arg mutants (H, Y, P, and C). One person at each bench should do this part of the experiment and incubate it where everyone can read the results. Using a bacteriology loop take a small amount of bacteria of each strain from the plate and resuspend them in buffer. Use the bacteria suspended in buffer to streak plates containing glucose minimal medium, glucose minimal medium plus ornithine, glucose minimal medium plus citrulline, and glucose minimal medium plus arginine (4 plates for each bacterial mutant). Observe the growth of the mutants the next day. Growth on a particular substrate is proof that the bacteria can make arginine from that substrate and so must be defective in some earlier biochemical reaction. Lack of growth on a substrate may only indicate a failure of the cells to be permeable to that substrate. (Note that these mutants are point mutants and can revert; thus the small number of colonies you will see on some plates probably represent revertants of the mutation).

Cross-feeding. (Each student should do this part)

Many biosynthetically defective cells accumulate the substance which they can no longer metabolize. If this happens then another mutant defective in an earlier biosynthetic reaction may be able to grow on the intermediate accumulated by the first cells (provided the first cells release the intermediate into the medium and that the second mutant is permeable to the intermediate). This is the basis of cross-feeding experiments.

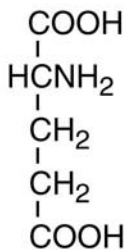
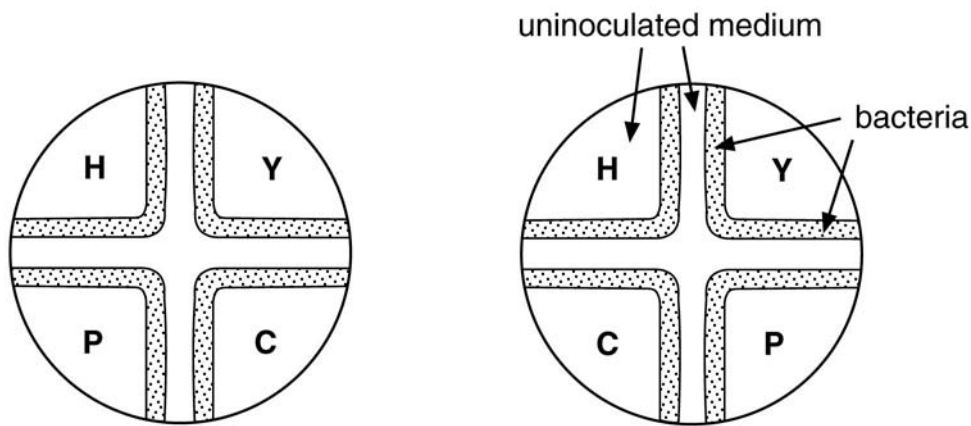
Laboratory 6
Biochemical Pathways

-2-

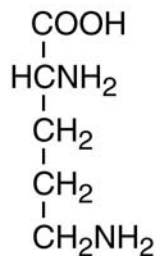
Each student should do this part of the experiment. Streak the four arginine-requiring mutants on glucose minimal medium so that each mutant is near each of the other three but be careful that the mutants do not touch each other. Use bacteria from the plates and make very heavy streaks on the surface of the agar. A possible streaking pattern is illustrated on the bottom of this page. Streak the cultures very close together.

Incubate the plates at 37°. **If you made your streaks close together record the data the next morning. If you made light streaks far apart record the data the next afternoon.** Check your platers again the next day. Note that substances released by one mutant will form a gradient and thus cross-feed those cells nearest it first.

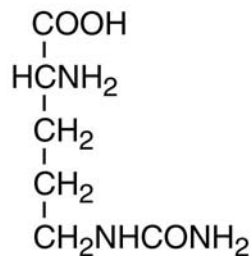
Report: Record your data in a table. Using the data from the tests you have made, attempt to order the mutants in the arg pathway. Indicate which mutants could cross-feed which other mutants. Also attempt to order the mutants with respect to ornithine and citrulline. Now look up the pathway for arginine biosynthesis in a biochemistry book (for example, Henry R. Mahler and Eugene H. Cordes. Biological Chemistry. 2nd edition, 1971. pp. 776-777 call number QP514.2 .M35 1971 or you can find the pathway at the BioCyc web site. <http://biocyc.org/server.html>



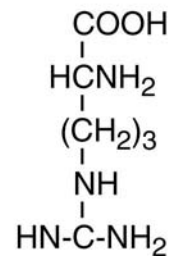
GLUTAMATE



ORNITHINE



CITRULLINE



ARGININE

