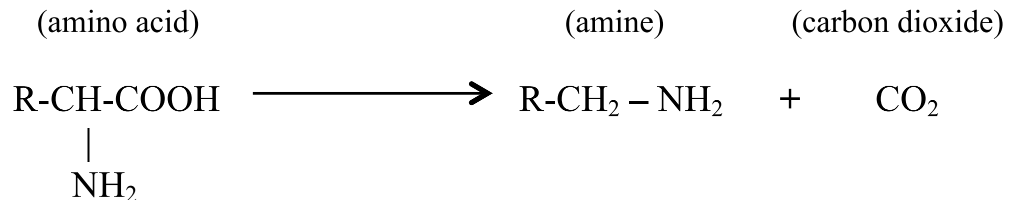
# Exercise 15-B
# PHYSIOLOGICAL CHARACTERISTICS OF BACTERIA CONTINUED: AMINO ACID DECARBOXYLATION, CITRATE UTILIZATION, COAGULASE & CAMP TESTS

## Decarboxylation of Amino Acids and Amine Production

The **decarboxylation** of an amino acid is the enzymatic splitting off of the carboxyl group ($COOH^-$) to yield an **amine** and carbon dioxide ($CO_2$). The reaction may be expressed as follows:




Bacterial decarboxylation can be demonstrated by showing either the disappearance of the amino acid (usually a fairly complex procedure) or the formation of amines and $CO_2$. **Amines** are nitrogen-containing compounds that are alkaline, volatile and foul smelling. The enzyme **lysine decarboxylase** catalyzes reactions resulting in **cadaverine** formation, while **ornithine decarboxylase** catalyzes the formation of **putrescine** (cadaverine and putrescine are amines). Since decarboxylation reactions result in the accumulation of alkaline amines, decarboxylation can also be demonstrated by measuring the rise in pH. This may be determined by using either pH indicators in the media, or by using paper strips to test the media at the end of the reaction. In either case, the test media must be covered with an airtight seal, e.g., vaspar, since volatile amines will not stay in solution.

In this laboratory we will be using an amino acid decarboxylation medium containing glucose as a carbon source and the pH indicator **Bromocresol purple** (BCP). Organisms that can ferment glucose will produce acids, and these will change the color of the pH indicator. Bromocresol purple is purple when the medium is neutral (start color) or alkaline (indicating amine formation) and yellow when the medium is acidic (indicating fermentation of the carbohydrate). Two tubes are used in this test, one containing just glucose (the control tube) and one containing glucose plus the amino acid being tested (lysine or ornithine). Both tubes are sealed with **vaspar** following inoculation. This will prevent the entry of oxygen, insure fermentation, and keep any volatile amines formed within the solution.

**Procedure:**

1. Obtain one tube of the amino acid medium available (lysine and/or ornithine) and one tube of amino acid control. Label these as you pick them up, because they look very similar.

2. Inoculate both tubes with a visible amount of your unknown culture (a blob of cell material).

3. Cover the medium in both tubes with a layer (about 1/4 to 1/2 in. deep) of molten vaspar, or place your tubes in a class basket so vaspar can be added later. All decarboxylation tubes will be incubated at $37^o$ C for 2 to 4 days.

4. Observe the tubes for gas formation and color changes. Be sure to compare your amino acid tube with your control tube, and to record your data. Then determine the results as indicated below. Compare your results with those obtained by other members of the class.

**Data and Results (Amino Acid Decarboxylation Test)**

If the medium in your **control tube is purple** (i.e., no color change has occurred) either the organisms in question are not able to ferment the glucose present, or the medium was not properly inoculated; in either case, the **test is invalid**. If the medium in your **control tube is yellowish**, the organisms in question can ferment the glucose present and the **test is valid**. Yellow color in the control medium validates the amino acid decarboxylation test, because it insures that the culture being tested can ferment glucose (glucose fermentation is positive).

When the test is determined to be valid, then the color in the amino acid medium accurately indicates amine production. If the medium in any **amino acid tube is purple**, this indicates that **amine production has occurred**, and the medium has become **alkaline** (amino acid decarboxylation is positive). If the medium in any **amino acid tube is yellowish** (acidic), amine production has not occurred and the pH is only influenced by glucose fermentation (**amino acid decarboxylation is negative**).

If the medium within your **amino acid control tube is purple** (**test is invalid**) any purple color in your amino acid tube may be due to the production of an amine or may be due to no reaction at all, and you cannot determine which is the case. Under these circumstances, the amino acid decarboxylation **test should be repeated** with a new set of tubes and fresh culture material.

If the medium within your control and/or amino acid tube appears **colorless** (the pH indicator has faded), it is possible to test the pH of these media using pH paper. This will require that the vaspar seal be broken and a strip of pH paper be wetted with the culture medium. Remember that amines are volatile and smell vile. The presence of gas ($CO_2$) under the vaspar seal (lysine or ornithine medium) can indicate amine production, but since gas may also be produced during glucose fermentation, gas alone is not a confirming test.

Commonly, decarboxylation is an initial step in the degradation of amino acids to provide cells with energy and essential nutrients. However, it may also serve cells by raising the pH of the growth medium, thus countering acidic conditions. Specific enzymes that decarboxylate a number of amino acids have been described. Without exception they function only where the pH of the medium is below 7 (below neutral). The foul odors that often accompany proteolysis are due to the formation of volatile amines from the decarboxylation of amino acids.

**Citrate Utilization Test (Simmon's Citrate Agar)**

The ability of some organisms to take in and utilize citrate as a sole source of carbon is a characteristic useful in their identification. Citrate (citric acid) is one of the organic compounds catabolized via the Krebs cycle; however, citrate utilization also requires an enzyme called **citrate permease**, which allows for the transport of citrate through the cell membrane. The ability of cells to form this enzyme can be determined using the citrate utilization test.

The citrate utilization is determined by examining the inoculated and incubated slants of Simmon's Citrate Agar. This medium contains sodium citrate as the only carbon source, ammonium salts as the only nitrogen source, and the pH indicator **Bromothymol blue**; which is green in a neutral medium, and blue in alkaline. If citrate is utilized, the ammonium salts are converted to ammonia and the residual medium becomes alkaline; thus a **deep blue color** is indicative of citrate utilization and is read as a **positive test**.

**Procedure:**

1. Obtain one tube of Simmon's citrate agar and inoculate it with your unknown culture. Streak the slant surface using a zigzag or fishtail pattern (from bottom to top) **and** stab to the tube bottom.

2. Carefully label your tube and then incubate it for 24 to 48 hours at $37^{o}$ C.

3. Observe your tube, record your data, and determine and record your results. Compare your results with those obtained by other members of the class.

   **Note** – The results of the Indole, Methyl red, Voges-Proskauer, and Citrate tests are known as the IMViC reaction, derived from I (Indole), M (Methyl red), V (Voges-Proskauer), iC (Citrate). For *Escherichia coli* the IMViC reaction is ++-- and for *Enterobacter aerogenes* it is --++.

**Coagulase Test**

**Coagulase** is an enzyme that catalyzes the coagulation or clotting of blood plasma (the conversion of fibrinogen to fibrin). The ability to produce coagulase is a common characteristic of some pathogenic staphylococci, so the coagulase test can be used as a means of differentiating pathogenic from non-pathogenic staphylococci, (e.g., *Staphylococcus aureus* from *Staphylococcus epidermidis*).

**Note** – We will use the coagulase test as the definitive test for distinguishing between *Staphylococcus aureus* and *Staphylococcus epidermidis*. Differentiation indicated by the use of MSA and the ability of pathogenic *Staphylococcus* to ferment mannitol is less reliable.

**Procedure:**

1. Inoculate 0.5ml of coagulase plasma with a visible amount of growth from solid media or two loopfuls of broth culture. Incubate the tube at $37^{o}$ C for 24 hours.

2. After incubation, gently invert your tube (**do not shake**) and record your data and results. If the plasma has become **solid** (coagulated) the result is **coagulase-positive**, and if it **remains liquid**, the result is **coagulase-negative**. Compare your results with those obtained by other members of the class.

   An alternate method known as the **slide coagulase test** may be performed by making a heavy, smooth emulsion of bacteria in normal saline (0.85% NaCl), and adding 1-2 drops of rabbit plasma. The slide is rocked gently for 1-3 minutes and the formation of clumps indicates the presence of "bound coagulase" or "clumping factor". This would be recorded as a positive coagulase test.

**CAMP test**

The CAMP test is a diagnostic test commonly used in the identification of pathogenic streptococci. It is based upon an enhanced hemolysis reaction (the CAMP reaction) evident when certain partially hemolytic species of *Streptococcus* (and a few other organisms) are grown in the presence of *Staphylococcus aureus*. Pathogenic strains of *Staphylococcus aureus* produce a toxin known as **β-hemolysin** that interacts with the lytic agents of other organisms to bring about the complete lysis of human erythrocytes or red blood cells (RBCs). The β-hemolysin alone will cause complete hemolysis if blood plates containing *S. aureus* are kept cold (refrigerator temperature) for 24 hours after a period of normal incubation (24 hours at $37^{o}$ C). Prior to this hot-cold lysis procedure the β-hemolysin causes a "halo" of partial hemolysis peripheral to the zone of complete hemolysis caused by *Staphylococcus* α-hemolysin **(α-hemolysin** causes **complete lysis of RBCs** or **β-hymolysis**, while β-hemolysin causes only partial hemolysis without cold exposure or the influence of toxins from other organisms).

The CAMP test is performed by applying *Staphylococcus aureus* (or its β-hemolysin which is also commercially available) in a single straight line down the middle of a blood agar plate. Next, the cultures to be tested are applied in lines running perpendicular to and across (**but not touching**) the line of *Staphylococcus*. After a period of incubation, the enhanced hemolysis of the CAMP reaction will be evident as arrowhead shaped clear zones adjacent to and "pointing" toward the central line. Organisms that test negative for the CAMP test will not exhibit enhanced hemolysis.

**Procedure:**

1. Obtain a blood agar plate and inoculate it with *Staphylococcus aureus* in the manner described above, i.e., a single straight line down the middle of the plate.

2. Obtain samples of the organisms to be tested and apply each one to the plate as a single line running perpendicular to and across (**but not touching**) the line of *Staphylococcus*.

3. Incubate the plate at $37^{o}$ C for 8-12 hours and then look for the presence of enhanced hemolysis. Note that if the plate is incubated for more than 12 hours, the CAMP test may be unclear due to excessive hemolysis. Record your data and results.

**Questions:**

1. Why is it necessary to run a control tube containing decarboxylase medium plus glucose but no amino acid whenever you are testing a culture's ability to decarboxylate amino acids?

2. What is vaspar? Why is it necessary to use this material whenever an amino acid decarboxylation test is being conducted?

3. What is the pH indicator present in Simmons Citrate agar? What happens to the pH of this medium when the citric acid is removed?

4. What is coagulase? What does a positive coagulase reaction look like?

5. What is the CAMP test and how does it work?

**STUDY GUIDE**
**Exercise 15B**
**AMINO ACID DECARBOXYLATION, CITRATE UTILIZATION, COAGULASE TEST AND CAMP TEST**

**Materials, Methods & Data:**

**Amino acid decarboxylation** **Date** ______________________

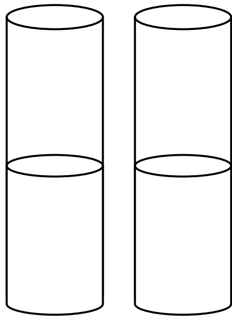
Enzyme catalyzing Lysine Decarboxylation? ____________________________________________

End products formed in Control tube ____________________________________________

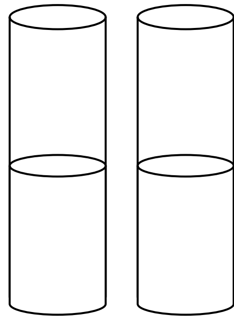
End products formed in Lysine tube ____________________________________________

pH indicator ______________________ Neutral = ☐ Acidic = ☐ Alkaline = ☐

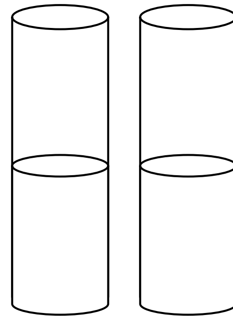
Functions of vaspar (two) ____________________________________________
____________________________________________



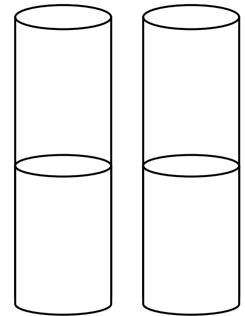
Initial color



Positive results



Negative results

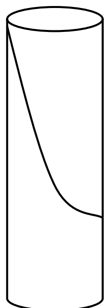


Invalid results

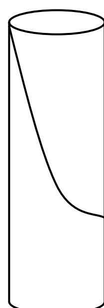
**Citrate utilization** **Date** ______________________

Enzyme being tested for ___________________ product formed ____________________________

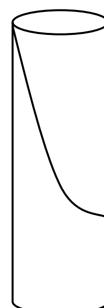
pH indicator present ___________________ Neutral = ☐ Acidic = ☐ Alkaline = ☐



Initial color



Positive result



Negative result

**Coagulase test** **Date** ___________________

Enzyme being tested for _____________________ Medium used ____________________________

Indicate results possible in the space provided below

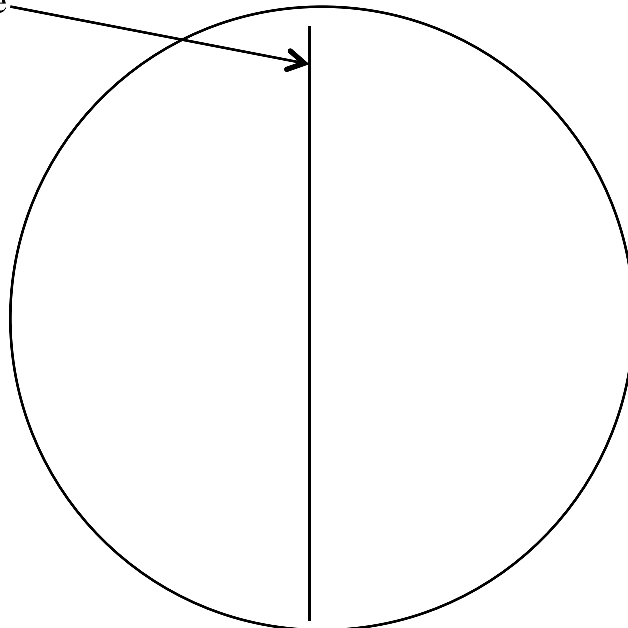
___________________ = Enzyme is present ______________________ = Enzyme is not present

**CAMP test**

Organisms most often tested for CAMP reaction? __________________________________________

*Staphylococcus* toxin causing enhanced hemolysis? ________________________________________

*Staphylococcus* culture



Show placement of cultures being subjected to the CAMP test and the data obtained.