CULTURE OF PENICILLIN

USE ASEPTIC TECHNIQUE THROUGHOUT THIS PROCEDURE

Step 1

Prepare a penicillium culture by exposing a slice of bread or citrus peel to the air at 70 deg. F until a bluish-green mold develops.

Cut two slices of whole wheat bread into ½ inch cubes and place in a 750ml Erlenmeyer flask with a cotton (non-absorbent) plug. It is important that the bread does not contain any mold inhibitors such as “mycoban”. Sterilize the flask and contents in a pressure cooker for at least 15 minutes at 15 pounds. An alternate method is to place in an oven at 315 deg F for one hour.

Using a sterile transfer loop (flamed) transfer the spores from the bread or peel into the flask containing the bread cubes.

Allow the cubes to incubate in the dark at 70 deg F for 5 days. After incubation, store in the refrigerator for not longer than two weeks.

Step 2

Prepare one liter of the following media:

- Lactose Monohydrate 44.0 gm
- Corn Starch 25.0 gm
- Sodium Nitrate 3.0 gm
- Magnesium Sulfate 0.25 gm
- Potassium Phosphate Mono 0.50 gm
- Glucose Monohydrate 2.75 gm
- Zinc Sulfate 0.044 gm
- Manganese Sulfate 0.044 gm

Dissolve in order in 500ml of cold tap water and add sufficient cold tap water to make one liter.

Adjust pH to 5.0-5.5 using HCL. Fill a series of milk bottles with a quantity of this media. Use only enough media so that when the bottle is placed on its side the media will not touch the cotton plug.

Sterilize the bottles and media in a pressure cooker or stove as previously outlined. When cool, inoculate with spores from the bread cubes. Use approximately the equivalent of one tablespoon.

Allow bottles to incubate on their sides at 70 deg F for 7 days. It is important that the bottles are not disturbed during this time. At the end of 7 days if your culture is capable of producing penicillin it will be dispersed in the liquid portion of the media.

Filter fermentation media, plug with cotton and refrigerate immediately. Use as soon as possible.

Step 3

To extract the penicillin the following procedure may be attempted. **Do the following technique as rapidly as possible.**

Adjust the cold fermentation filtrate to pH 2.2 using .01/N HCL. Mix cold filtrate with cold ethyl acetate in a separatory funnel and shake well for 30 seconds.

Drain the ethyl acetate into a beaker which has been placed in an ice bath and repeat the process until all filtrate is depleted.

Add 1% potassium acetate and mix. Permit ethyl acetate (flammable) to evaporate. This can be induced by a constant flow of air over the top of the beaker.

The remaining crystals are a mixture of potassium penicillin and potassium acetate.

WARNING: DO THE EXTRACTION AS RAPIDLY AS POSSIBLE!