The Ames Test

Background

Humans and other animals are surrounded by a variety of chemical substances, both naturally occurring as well as synthetic, that have the potential to act as **mutagens**.
Some of these substances are in the food we eat, others in the air we breathe, and still others can be absorbed through the skin or via other contact. Mutagens act in a variety of ways but they all have the ability to alter the DNA base sequence (e.g. point mutations, frameshift mutations, etc.) within the genome. Cancer researchers and clinical oncologists would likely agree that most (though not all) mutagens have the potential to act as **carcinogens** and can play a role in the induction many cancers.

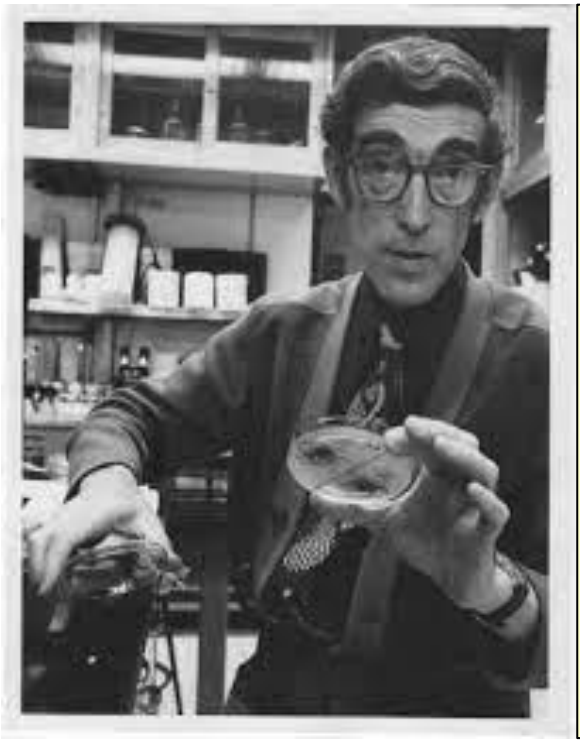

Figure 1: Bruce Ames

The Ames test was developed in the **1970s** by **Bruce Ames**, Professor of Biochemistry at UC-Berkeley, as a fast and sensitive assay of the ability of a chemical compound or mixture to induce mutations in DNA. Because the assay does not use a live animal model, it is inexpensive, easy, and fast. Bruce Ames published his work in a series of papers, including "Identifying Environmental Chemicals Causing Mutations and Cancer" in the journal Science (volume 204, 1979).

The Ames test utilizes specific strains of the bacteria *Salmonella typhimurium* as tools to detect mutations. These strains of *S. typhimurium* used are known as **auxotrophs**. A bacterial strain is defined as an auxotroph if it is unable to produce a required nutrient (the test organism in this experiment cannot synthesize the amino acid histidine) and thus will not grow unless the nutrient is supplied in growth media.
Auxotrophs are usually produced as a result of a mutation(s) that occurs in a **prototroph** (a bacteria that is able to synthesize the particular nutrient). The

mutant *S. typhimurium* strains are histidine auxotrophs (denoted his- in order to distinguish it from the original his+ prototrophic strain they were derived from) The Ames test determines the ability of a tested substance to cause a reversal, also called a **back-mutation** of these auxotrophs to the original prototrophic state. During the test auxotrophs are grown on **glucose-minimal salts agar plates** that contain all required nutrients but only trace amounts of histidine (as well biotin). The auxotrophs are able to growth for several generations until the histidine in the media is exhausted, at which time they will stop growing unless they have sustained a back-mutation that has restored their ability to synthesize histidine (these his+ cells can be described as **revertants**).

Suspected mutagenic substances are tested for their ability to stimulate backmutations by placement onto paper disks which are set on the surface of the minimal agar plates previously inoculated with the auxotroph. The test substance diffuses into the surrounding media and, if mutagenic, will induce back-mutations which will then allow growth into visible colonies. The more revertants observed near the test substance, relative to experimental controls, the more mutagenic the substance is likely to be. The trace amount of histidine included in the minimal media plates allows hisauxotrophs to divide several times in the presence of the potential mutagen.

Ames test can carry out by 2 methods:

1- Disks method

2- Tubes method (summarized in figure 3**)**

1-Disks method

Materials:

Ames agar plates
Alcohol beaker
spreader & forceps
Sterile filter disks
Transfer pipet as needed
Sterile Water
Culture of *Salmonella* (Ames strain; NR115), 1 ml
Parafilm
Vial containing mutagen : Three chemicals with known mutagenicity are available to you:

A- Ethyl methane sulfonate, EMS

B-Sodium azide (NaN3) is a white solid that is highly soluble in water. Becauseit easily kills bacteria in high enough concentration, it is used as a preservative in some chemical solutions; its degradation via electric shock is used to inflate car airbags.

C- 4-nitro-o-phenylenediame (4NOP) is an orange-red powder previously used in hair dyes. In the late 1970"s the National Cancer Institute (NCI) noticed that there was an abnormally high incidence of bladder cancer among workers in the dye industry, so they requested a bioassay of 4NOP to determine its mutagenic potential. 4NOP produced positive results in the Ames test and it is considered a potential mutagen.

Procedure

1. Place 2-3 drops of the bacterial culture on each plate and spread well with a sterile spreader. Allow the bacteria to dry for a few minutes.
2. Using sterile forceps transfer a sterile filter disk to the center of each plate and tap gently to make sure the filter is attached to the surface of the agar.
3. Using a sterile dropper, place a drop of sterile water on the filter disk which is on the Control plate.
4. Repeat the above step with your unknown sample on the plate labeled Unknown.
5. Take the plate labeled EMS to the hood area. **Wear protective gloves when handling the mutagen!** Use a sterile dropper to add one drop of EMS to the filter disk. If your gloves become contaminated with the mutagen, remove them and place them in the biohazard bag in the hood. If your gloves are not contaminated, they can be placed in the regular trash.
6. Place plates face up (do not invert) at 37°C for 48 hours. Seal the EMS plate with a strip of parafilm. Your instructor will invert your plates later today, after making sure that the chemicals have diffused sufficiently into the agar.

7. DO NOT OPEN THE EMS PLATE. Observe and record the growth and number of colonies on plates.

Note: During this exercise you will be handling strains of *S. typhimurium* and known or suspected mutagens. *S. typhimurium* is a pathogenic organism responsible for certain types of food poisoning, so be sure to follow proper microbiological technique. The mutagens we have chosen for this lab are not very harmful to humans and they are provided in low concentrations. Nonetheless, this is a good time to practice lab safety. Wear gloves when working with the bacteria or mutagens and keep these materials localized in one place on your bench. Known mutagens will only be applied to your plates in one place in the lab. Your instructor will be happy to do this application for you, if you do not wish to do so. Dispose of all gloves/plates/pipette tips in the biohazard containers (not the normal trash). Wash your hands before and after this week"s lab.

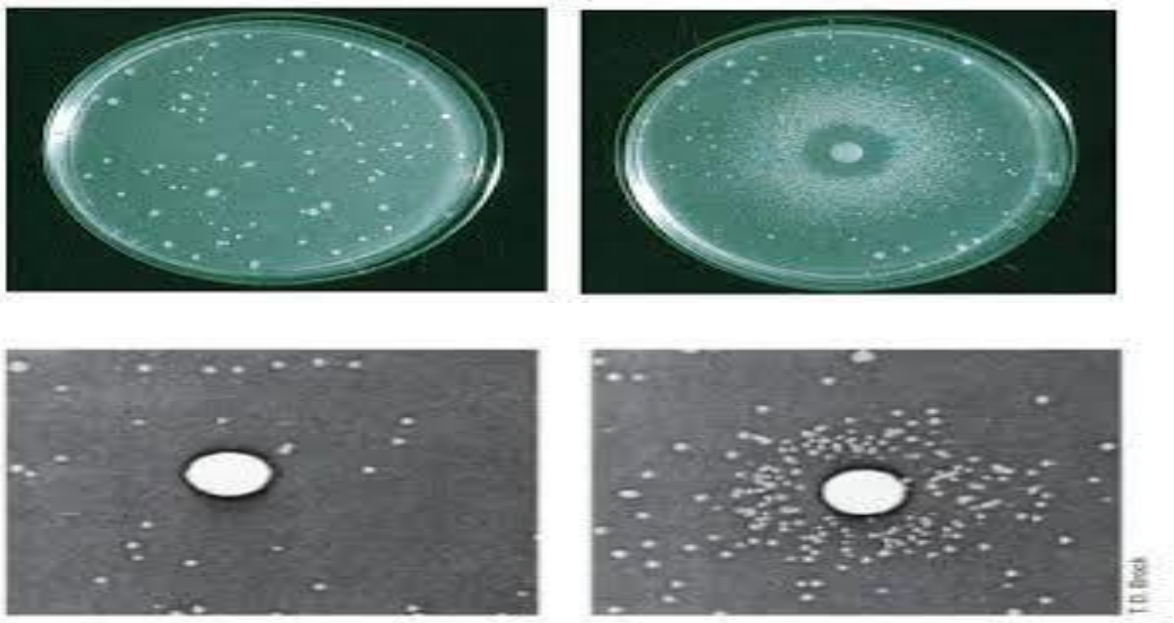

Figure 2: The dish on the left was plated with about 10^9 *his*$^-$ bacteria. In this control experiment, in the absence of the amino acid **Histidine**, such bacteria will not grow, except for a small number ($<10^2$) of white colonies derived from single bacteria that have undergone **spontaneous reversion mutations** to *his*$^+$. In the experiment on the right, the filter-paper disc in the middle of the dish contains a chemical being tested for mutagenic properties. As it diffuses outward, the chemical first kills all the bacteria (clear area around the disc), but at lower concentration is seen to **induce** reversion mutations many 10^3s of revertant (*his*$^+$) colonies. Note that concentration of the chemical drops off towards the outer edge of the plate, the frequency of revertant colonies falls to about the same as in the control.

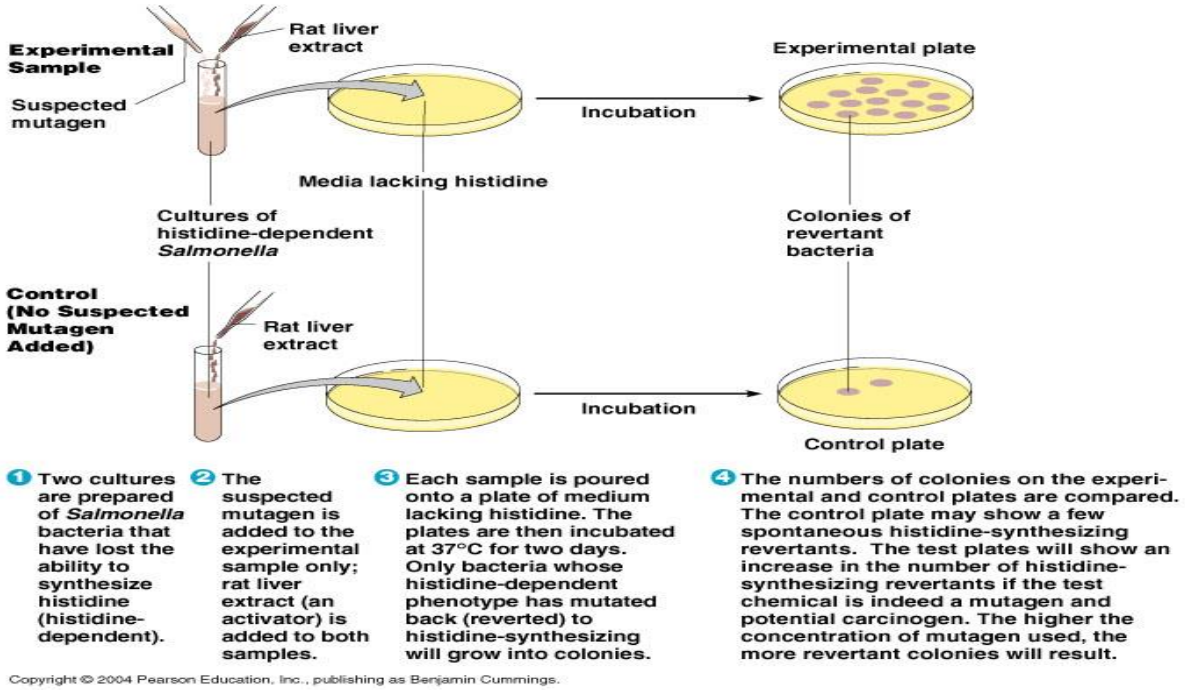

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Figure 3: Tubes method of Ames test.