Crown Gall Disease: Model of Carcinogenesis

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The use of a laboratory experiment that stimulates thought and also illustrates a concept is of benefit to a student. The experiment described in this paper can be used to stimulate students in two areas of biology. In introductory biology it can be used to investigate some aspects of abnormal growths (cancer) and in microbiology it provides the student with an opportunity to test Koch’s postulates. The abnormal growths begin to develop on carrot tissues within three to five days after inoculation with a virulent culture of the crown gall pathogen, Agrobacterium tumefaciens.

Methods and Materials

Stock cultures of A. tumefaciens are maintained on nutrient-agar slants. Cultures such as ATCC 15955 and ATCC 233308 work very effectively. They can be obtained from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md. 20852.

Fresh, firm carrots (Daucar carota) of any variety respond well to A. tumefaciens.

There are three ways of transferring the cells from the stock culture to the carrot slice. The first technique is to take a loopful of organisms and streak them over the surface of the carrot. The second technique is to suspend several scrapings of the bacterial cells in sterile distilled water and then spray the surface of the carrot with this suspension. The third technique is the development of a nutrient-broth culture of the bacterial cells; then this material may be transferred directly to the surface of the carrot. To eliminate any extraneous material that may be present in the third preparation, the broth culture may be centrifuged and the pellet of cells resuspended in sterile tap water. In our laboratories we have received uniform results using all three methods.

Sterilized disposable materials may be used in any part of this experiment. Petri dishes and metal instruments may be sterilized by dry- or wet-heat methods. Any metal instruments, such as the paring knife, forceps, and razor blades, should be individually wrapped, sterilized, and then used only once. Resterilization is necessary before reuse.

The procedure for carrot sterilization is a modification of that devised by Ark (1961).

1. Scrub the entire carrot thoroughly in a soapy water solution to remove all soil particles.
2. Soak in 85% denatured ethanol solution for two minutes.
3. Replace the ethanol solution with a 7:1 water to chlorox (sodium hypochlorite 5.25%) solution and soak the roots in this material for 55 minutes, agitating the carrots every 10 to 15 minutes.
4. Rinse with sterile water four times over a 15-minute period, agitating every 3 to 5 minutes.

For inoculation, the carrots are sliced with a sterile knife or razor blade into slices 0.5 cm thick. The carrot slices are placed on a sterile piece of filter paper in a sterile petri dish. The upper surface of each slice is then inoculated with 0.5 ml of the bacterial suspension prepared by one of the previously mentioned methods. After the carrot has been inoculated the filter paper is saturated with sterile dis-
tilled water, the petri dish cover is replaced, and the entire culture is incubated at 25 C in the dark. The culture should be checked periodically to make sure that the filter paper remains moist. As the filter paper begins to dry out it should be resaturated by aseptically (with sterile pipet) adding sterile distilled water directly onto the filter paper. Additional carrot slices should be prepared as controls. The same procedure is followed, except that they are sprayed with sterile tap water instead of the bacterial solution.

Expected Results

Three to four days after inoculation the cambial area of the carrot slice develops a raised appearance. However, on approximately the seventh day small white spherical growths begin to appear randomly over the surface of the carrot. These incipient tumors will continue to grow for approximately the next four days. By the 10th or 11th day many of the incipient tumors will begin to decrease in size, but a marked increase in tumor growth will occur on the cambial region of the carrot (fig. 1). This concentrated tumor growth will continue until the tumors have developed to approximately 5 mm in diameter (fig. 2). Tumors often appear on the rays radiating from the cambial ring. These rays will parallel the development of secondary roots (fig. 3). The controls will show no signs of tumor growth.

Discussion

Some experiments have been developed that draw an analogy between crown gall disease and animal cancers (Pelczar, 1965; Peltier, Georgi, and Lindgren, 1959). Several investigators have found that the cells produced in crown gall disease are similar morphologically and physiologically to those of cancer in animals (Braun, 1962; Braun and Wood, 1969; Braun, 1970; White and Braun, 1942).

An animal cancer has been described as a cell that has overcome the population-controlling mechanisms and multiples without restraint (Ham, 1965). The predominant tumor growth in the area of the cambium in the described carrot system suggests that the cell-division potential there may be linked with the tumor (cancer) development. The appearance of early incipient tumors suggests that many of the cells wounded by the slicing retained some totipotency but that some factors were limiting, such that only those cells near a cambial region were capable of sustained proliferation. In other words, only those carrot cells that possessed the capacity to divide were capable of producing tumors in response to infection with A. tumefaciens. Other experiments provide support for this contention, since tumors occur at the site of wounds on wounded intact plants (Pelczar 1965; Peltier, Georgi, and Lindgren, 1959).

In these cases intact tomato or sunflower plants were generally used. Limited cell-division will occur at wound sites for repair of plant tissue but extensive proliferation will occur primarily in regions where cell division is still readily inducible. The relationship between cancer and cell division is vividly illustrated by using this procedure. Comparative histologic and cytologic studies of the healthy and the neoplastic tissues are very instructive, since the cellular characteristics of the crown-gall-diseased tissue are similar to those found in the neoplastic tissues of animals. Additionally, extracts from control and infected carrot slices may be used for electrophoretic studies of the influence of this disease upon the carrot’s metabolism. Such influences are evident from changes in amounts and kinds of proteins present and from extensive changes in the dehydrogenases and phosphatases present.
new tumors are formed, new tumors should be used to once again isolate the causative agent. This agent will be *A. tumefaciens*—the same organism that was isolated from the previously infected slices.

**Summary**

This experiment has two purposes. First, it can be used to study carcinogenesis on the organismic, histologic, cellular, and physiologic levels. Secondly, it can be used as an illustration of Koch’s postulates. This experiment has several advantages. It eliminates the need for special conditions for growth and development of sunflowers or other suitable plants that normally are hosts for the pathogen and take several weeks to produce. The sterilization of carrots obtained directly from the local grocery will take a little less than an hour and a half. Turnips, rutabagas, radishes, sweet potatoes, and Irish potato tubers can also be used as experimental materials.

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**REFERENCES**


**Anadromous Fish Booklet.**