AN APPARATUS AND TECHNIQUE FOR THE CONTINUOUS ANESTHETIZATION OF HAEMATOPHAGOUS INSECTS WITH DRY ICE

I. BARRY TARSHIS [1]
Biological Warfare Laboratories, Fort Detrick, Frederick, Maryland

Carbon dioxide has been used for a long time as an anesthetic in entomological research. In their studies on the genetics of *Drosophila,* l'Heritier and Teissier (1937) routinely employed carbon dioxide for anesthetizing these flies. Williams (1946) and Willis and Roth (1949) have described techniques for anesthetizing small insects with carbon dioxide. Maramorosch (1953) used carbon dioxide in handling leafhoppers in his transmission studies of plant viruses by these insects, and, in 1956, described a technique for anesthetizing insects with carbon dioxide so that he could inject them with viruses and other substances. Caldwell (1956) employed dry ice for anesthetizing house flies for toxicological studies. In studying the effects of different anesthetics on the behavior of honey bees, Ribbauds (1950) concluded that carbon dioxide and nitrogen stop respiration; whereas ether and chloroform affect the nervous system.

Though the above mentioned works all utilized carbon dioxide in one form or another for the anesthetization of different insects, none of the insect chambers used for these studies made use of transmitted light, which the author found invaluable in the work of separating live unfed arthropods from blood-fed arthropods. Therefore, a new carbon dioxide anesthetizing chamber, or "arthropod stunner," was designed. The equipment of Willis and Roth is somewhat similar in design to the arthropod stunner in that the anesthetizing chamber can be fitted into the grooves of the stage of a dissecting microscope, but their apparatus does not have a transparent bottom and several other features are somewhat different.

The arthropod stunner is basically a topless, rectangular stainless steel box with a partial glass bottom (Fig. 1A). A tube, rectangular in cross-section, ¾ inch wide by 1 inch high, through which the carbon dioxide is piped, forms the four sides of the box. The floor of the box is 6¼ inches long by 5⅛ inches wide. The tube is set in 5/16-inch from the edge of the floor so that a flange extends out from it; this flange slips into the grooves of a dissecting microscope normally used to hold the glass stage. The bottom of the inside of the anesthetizing chamber is of glass. This glass piece is secured tightly against a soft rubber gasket with two metal strips screwed to the underside of the metal portion of the bottom (Fig. 1B). In the side wall of the tube toward the chamber, just above the glass bottom, are 28 holes, each ⅛-inch in diameter. These holes are screened on the inside of the tube with 80-mesh stainless steel screen cloth. There is a ⅛-inch needle valve welded into one of the long outside walls of the tube. Rubber tubing connects this inlet with the rubber tubing leading from the carbon dioxide supply.

The carbon dioxide used in these studies was obtained by placing crushed dry ice in a reagent bottle and piping off the gas that formed as the dry ice evaporated at room temperature, through a second reagent bottle half filled with water, and then into the anesthetizing chamber (Fig. 2). The water bottle is used only so that the worker can be sure (by the bubbles produced in the water) that the carbon dioxide supply is flowing freely from the first bottle. A 1,000 cc. reagent bottle half filled with crushed dry ice will produce enough carbon dioxide to last for approximately five hours.

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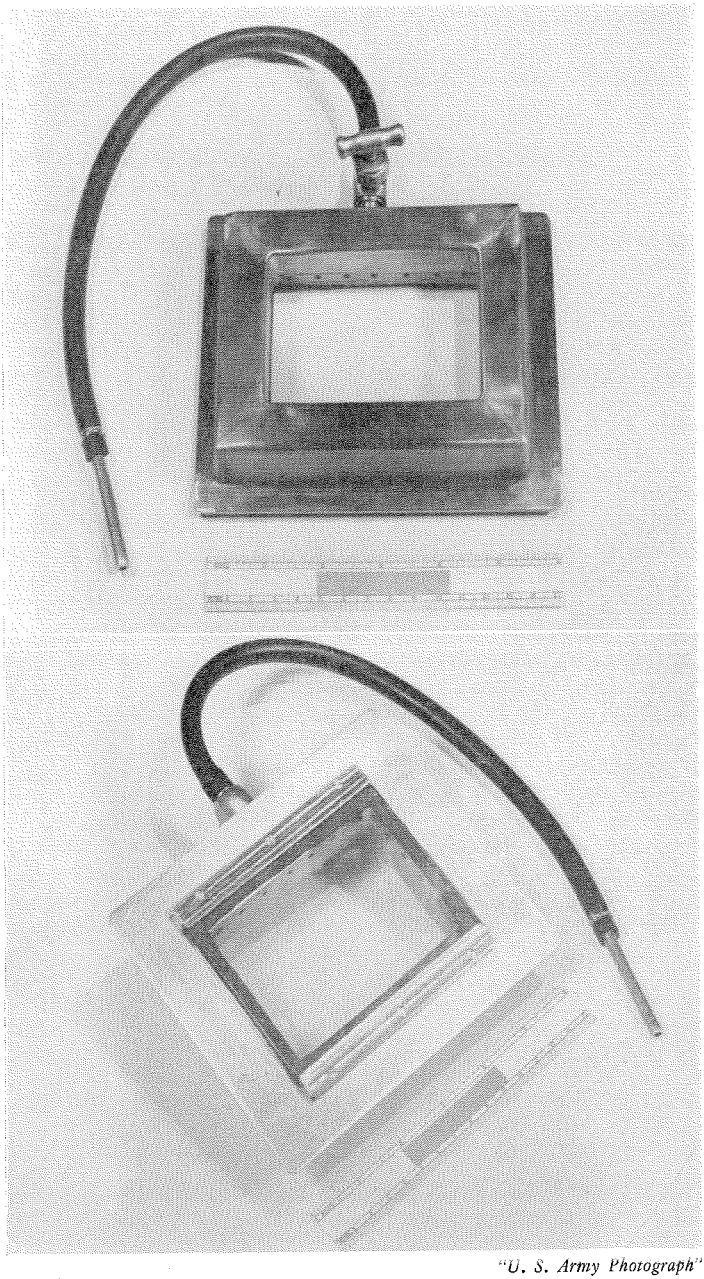

"U. S. Army Photograph"

FIG. 1A.—(Above) Top view, Arthropod Stunner.
FIG. 1B.—(Below) Bottom view, Arthropod Stunner.

Commercially tanked carbon dioxide may be used with the arthropod stunner; however, the writer found dry ice more suitable since it was always available and there was never the danger of the gas supply being exhausted in the middle of an experiment. More important, the great pressure under which the carbon dioxide gas is tanked commercially makes it difficult to obtain a slow, steady release flow even with a valve system. Sometimes when tanked gas has been used the flow has been so strong as to blow the arthropods out of the anesthetizing chamber.

The glass bottom of the anesthetizing chamber enables one to use the arthropod stunner with a mirror-equipped dissecting microscope. Light transmitted from beneath illuminates the digestive tract so that it can be viewed with ease and even the slightest amount of blood within the tract of a fed insect can be detected. When light is directed on the top or side of a blood-fed insect the digestive tract is darkened to such a degree that it is most difficult to determine whether the insect has taken in blood.

The apparatus and technique have been used successfully with blood-fed fleas (*Xenopsylla cheopis*) and mosquitoes (*Aedes aegypti*). Fleas can be anesthetized for several hours and mosquitoes for two hours with carbon dioxide without lethal effects.

SUMMARY. A continous anesthetizing apparatus ("arthropod stunner") that has been especially designed to utilize transmitted light in separating live unfed from blood fed arthropods has been described. Carbon dioxide obtained from dry ice has

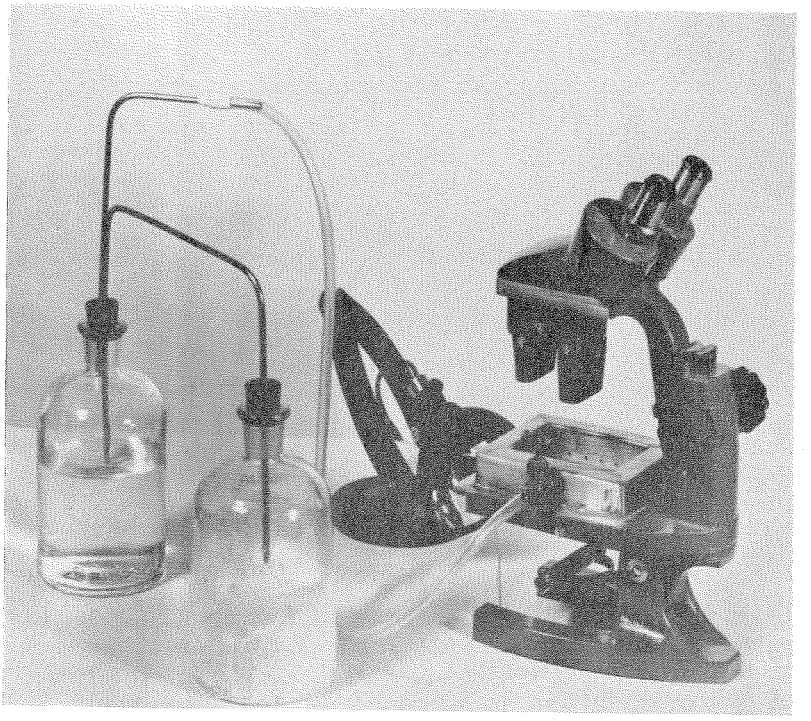

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FIG. 2—Assembled Arthropod Stunner and carbon dioxide supply.

been used as the anesthetic for fleas (*Xenopsylla cheopis*) and mosquitoes (*Aedes aegypti*).

Literature Cited

CALDWELL, A. H. JR. 1956. Dry ice as an insect anesthetic. Jour. Econ. Ent. 49:264–265.

L'HERITIER, P. and TEISSIER, G. 1937. Une anomalie physiologique hereditaire chez la Drosophile. C. R. Acad. Sci. Paris 205:1099–1101.

MARAMOROSCH, K. 1953. Use of carbon dioxide as an anesthetic in insect vector work. Plant Disease Reporter 37:352–353.

MARAMOROSCH, K. 1956. Semiautomatic equipment for injecting insects with measured amounts of liquids containing viruses or toxic substances. Phytopath. 46:188–190.

RIBBAUDS, C. R. 1950. Changes in the behavior of honey bees following their recovery from anesthesia. Jour. Exp. Biol. 27:302–310.

WILLIAMS, C. M. 1946. Continuous anesthesia for insects. Science 103:57.

WILLIS, E. R. and ROTH, L. M. 1949. A microscope stage for continuous anesthesia of insects. Science 109:230.