Microinjector Needle for Determination of per os-LD_{50} of Insect Viruses

Advancement in the study of virus diseases of insects, as an important branch of insect pathology, continuously necessitates the development of new techniques. A frequent test run in the study of insect viruses is the determination of their median lethal dose by the dosage-mortality method. This test has been run in the following three ways: (i) injection of the test suspension into the body cavity of the insect (*1*); (ii) feeding the insects with food contaminated with a known amount of virus (*2*); and (iii) allowing insects, previously starved, to drink a droplet of known volume of virus suspension (*3*).

Although the first method gives very exact results, it does not permit drawing practical conclusions concerning the virulence under natural conditions, for the path of infection in nature is *per os* in almost every case. The second and third methods allow the determination of the *per os* virulence of the test suspensions; however, there are insects and insect stages on which these two methods cannot be applied, owing to the animals' particular feeding habits. Moreover, starving insects (method three) introduces an unnatural factor of unknown value into the experiment.

In studying a granulosis virus of the tortricid *Zeiraphera griseana* (Hübner), the larch bud moth (*4*), I was faced with the problem of testing the *per os*-LD_{50} of the virus with fourth instar larvae of this insect (6 to 9 mm long). These larvae web the newly sprouting larch needles together to form a shelter within which they feed. Neither the feeding of needles contaminated with a given amount of virus nor the feeding of droplets of virus suspensions to larvae starved up to 48 hours was successful with this insect. Therefore, I devised a method, in 1954, that enabled me to introduce, through the mouth of the insect, the appropriate amount of virus directly into the larval gut without leaving any traumatic lesions.

The type of syringe used for this purpose is a slightly modified Dutky-Fest microinjector (*5*). The most important part is the needle (Fig. 1), which is prepared in the following way: a normal hypodermic injection needle (gage 20) is cut off 6 mm from its base, *A*, and a capillary glass tube 100 mm long, *B*, is sealed to the needle stump with melted sealing wax, *C*. The tip of the glass tube is then drawn in the flame of a bunsen burner to a very fine capillary with an outer diameter of approximately 150 μ. This thin capillary, which is very flexible, is broken with a forceps at a distance of about 20 mm from its base. The tip of the fine capillary is then heated very carefully at the bottom of a small flame. The melting of the glass occurs almost instantaneously, and some skill is required to obtain the correct shape of the needle tip, avoiding complete occlusion of the capillary (Fig. 2).

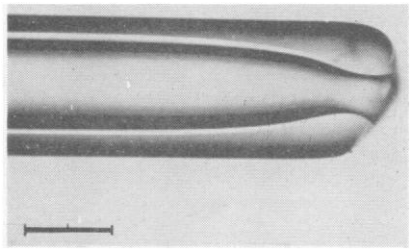

Fig. 2. Tip of the capillary glass needle after melting on a small flame. Outer diameter, 160 μ.

The microinjector is fixed on a stand, and the tip of the needle reaches into the field of the microscope. The larva to be injected is set on a 6- by 6-cm sterilized slip of paper, and this paper with larva on it is then placed in a petri dish, where the insect is anesthetized for 30 seconds under ether, in this case. The paper with the anesthetized larva is lifted out of the petri dish by means of forceps and brought under the microscope so that the larva lies with its mouth parts near the needle tip. The larva is delicately held behind the head capsule with a fine forceps and its head is lifted until the mouth parts touch the needle tip. After application of a slight pressure in the direction of the needle, the mouth opens and the head capsule is slid onto the capillary, which penetrates about 1 mm.

The hypodermic needle and the glass capillary must be sterilized previous to the sealing; the preparation of the capillary needle tip must be performed under sterile conditions. Large series of this capillary needle should be prepared in advance and stored sterile if large numbers of insects are to be tested.

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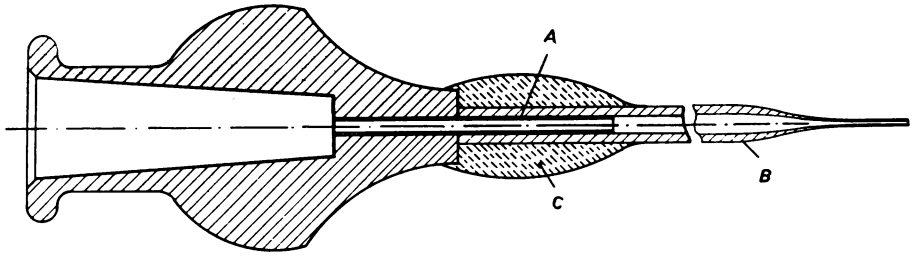

Fig. 1. Microinjection needle. *A*, stump of normal hypodermic injection needle (gage 20); *B*, capillary glass tube; *C*, sealing wax.

References

1. G. H. Bergold, *Biol. Zentr.* 61, 158 (1941); *Can. J. Zool.* 29, 17 (1951); N. Plus, *Bull. Biol. France et Belg.* 88, 248 (1954).
2. Y. Tanada, *Proc. Hawaiian Entomol. Soc.* 15, 235 (1953).
3. F. T. Bird and M. M. Whalen, *Can. Entomologist* 85, 433 (1953).
4. M. E. Martignoni, *Mitt. Schweiz. Entomol. Ges.* 27, 147 (1954).
5. S. R. Dutky and W. C. Fest, Microinjector. U.S. Pat. 2,270,804. *Offic. Gaz.*, U.S. Pat. Office 534, 673 (1942).

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High-Vacuum Filament Furnace for Gas Analysis of Metals

There are several methods currently employed to determine the gaseous content of metals, each having certain advantages as well as inherent disadvantages. The main disadvantage of all of these methods is their limited field of application.

Probably the most versatile and widely used method at present is that of vacuum fusion (*1*). This method requires complex apparatus and multiple, time-consuming operations for furnishing quantitative data on the oxygen, hydrogen, and nitrogen content of a metallic specimen. Although satisfactory results can be obtained for most metals, the method allows no further qualitative determinations and is not well suited to such operations as rate studies and others.

The so-called hot-extraction method (*2*) is a simpler technique that requires neither the complex apparatus nor the time-consuming operations, but it is strictly limited to the determination of hydrogen.

Another recently developed method that involves the measurement of equilibrium pressures (*3*) has advantages in apparatus and operational simplicity equivalent to those of the hot-extraction method, and it appears to be capable of slightly greater accuracy. However, this method has at present been applied only to hydrogen determinations in titanium.

Notwithstanding the variations in techniques, these methods have in common a dependency on direct pressure measurements for all analytic determinations.

The ideal instrument for the measurement of such gaseous components is the mass spectrometer. It is possible to trans-