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).

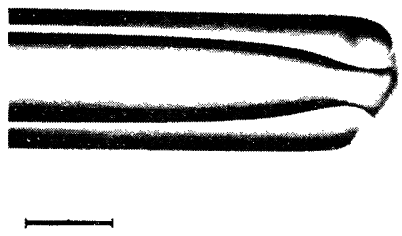

Fig. 2. Tip of the capillary glass needle after melting on a small flame. Outer diameter, 160 μ.

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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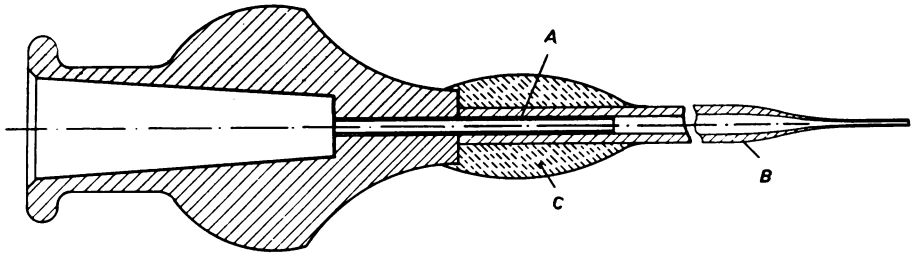

Fig. 1. Microinjection needle. *A*, stump of normal hypodermic injection needle (gage 20); *B*, capillary glass tube; *C*, sealing wax.

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30 June 1955

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-

fer to the mass spectrometer the gases collected during any of the operations previously mentioned. However, it is more desirable to study these gases directly as they are effused from a furnace assembly that is an integral part of the inlet system of the mass spectrometer (*4*).

Of the many types of apparatus that have been designed for the study of gas-metal systems, few have been suitable for adaptation to a commercially available mass spectrometer. Specialized types of mass spectrometers, such as the solid-source instruments that are not yet commercially available, usually have the furnace unit incorporated within the ion source (*5*). The filament-type furnace reported in this paper (*6*) has been designed for adaptation to the conventional mass spectrometer.

The housing of the furnace, as shown in Fig. 1, is fabricated of Pyrex. The bottom section of the housing is fitted to a brass base section through the medium of a glass-to-metal seal. The base section is bolted to a base plate; the assembly is vacuum sealed by means of a Teflon "O" ring, as shown.

The sample support rods, which also serve as electric leads, are attached to the base assembly. One of these is electrically insulated (and made vacuum tight) by means of a Teflon gasket assembly. The other is screwed directly into the base plate, through which the electric circuit is completed.

The metallic specimen to be studied is installed as a small filament between the two nickel support rods, which are connected to a low-voltage, high-current source. The use of nickel for these rods

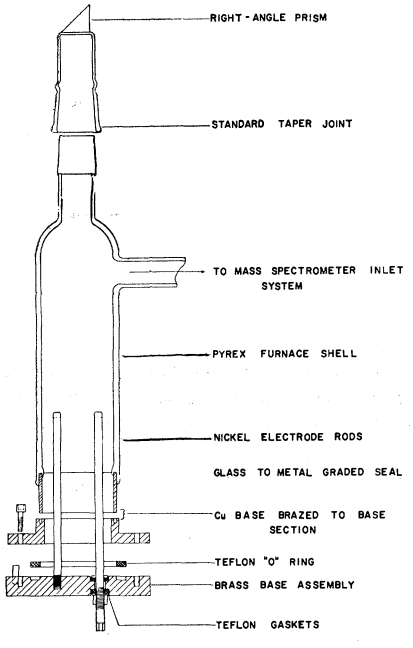

Fig. 1. Housing of high-vacuum filament furnace.

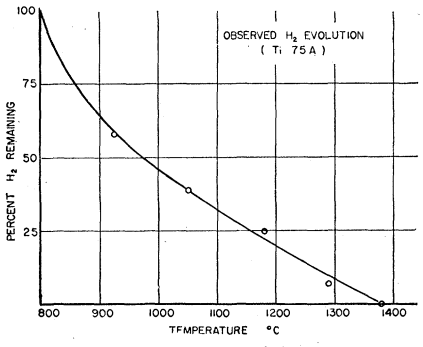

Fig. 2. Preliminary data obtained with high-vacuum filament furnace in a study of hydrogen evolution from titanium.

represents a practical compromise that was found after experimentation with several types. We sought adequate electric conductivity combined with low thermal conductivity. The effect of conduction heating and subsequent outgassing of these rods in contact with the hot filament has so far been found to be negligible. The use of resistance heating in this manner affords closer temperature control and is much simpler in operation and more economical in installation than induction heating.

In place of the sample filament, a small tantalum, tungsten or iridium coil or crucible may be installed between the rods to accommodate a massive specimen or a small heater crucible that would be conductively heated. The outgassing of this associated material can be determined and subsequent data corrected for its effect.

A right-angle, optically ground prism attached to the top of the furnace provides an optical path by which temperature measurements can be made by means of an optical pyrometer. The standard taper joint is incorporated to facilitate removal of any deposit that may obstruct the optical path. The entire furnace may be readily disassembled for cleaning or sample installation.

The furnace is connected to the inlet system of the mass spectrometer through a line that leads directly into the ionization chamber of that instrument. The entire system is evacuated by an oil-diffusion pump and a supporting mechanical pump that are an integral part of the vacuum system of the mass spectrometer.

Gaseous molecules, as they are effused from the heated sample, are passed through the line leading into the ionization chamber, where they are ionized by bombardment with electrons that are thermionically emitted from a tungsten filament. The resulting positive ions are accelerated by an electrostatic field, then deflected through a magnetic field that produces a spectrum of the ions according to their mass-to-charge ratios. By varying the strength of the electrostatic or magnetic fields, it is possible to bring into focus, at the collector plate, ions at any desired position within this mass spectrum. The signal produced by this ion current at the collector electrode is amplified and recorded.

It is possible, therefore, to scan the spectrum to identify all of the various components of the effused gases. Or, by "fixing" the electrostatic and magnetic fields of the instrument, it is possible to "sit" at any mass position. The amplitude of the recorded signal is quantitatively proportional to the partial pressure of the component being measured. By properly calibrating the inlet system, quantitative determinations can readily be made.

Various diffusion-rate determinations, investigations of outgassing characteristics, reaction kinetic studies, and so forth, as well as quantitative analytic measurements, may be made by correlating such data to imposed conditions of time and temperature.

To illustrate an application, some preliminary data obtained in a study of hydrogen evolution from titanium (commercially available Ti-75 A) are shown in Fig. 2. The observed effusion rate of hydrogen is plotted as a function of temperature with time-rate a constant (30 min between plotted points). As can be seen from the curve, the evolution of hydrogen is complete at a point just below 1400°C. Further increase in temperature resulted in no further hydrogen evolution. In the vicinity of 1400°C, hydrogen evolution is very rapid—quantitative removal is observed in less than 10 min at this temperature.

It is not intended that this method should supplant others currently in use that may be more practical or even superior for particular or routine applications. However, it is hoped that this idea may begin to answer the need for a more versatile method that is capable of furnishing some previously missing data that will aid in furthering studies of gas-metal interactions.

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6. The opinions expressed are those of the authors and do not necessarily reflect those of the Ordnance Corps.

18 April 1955