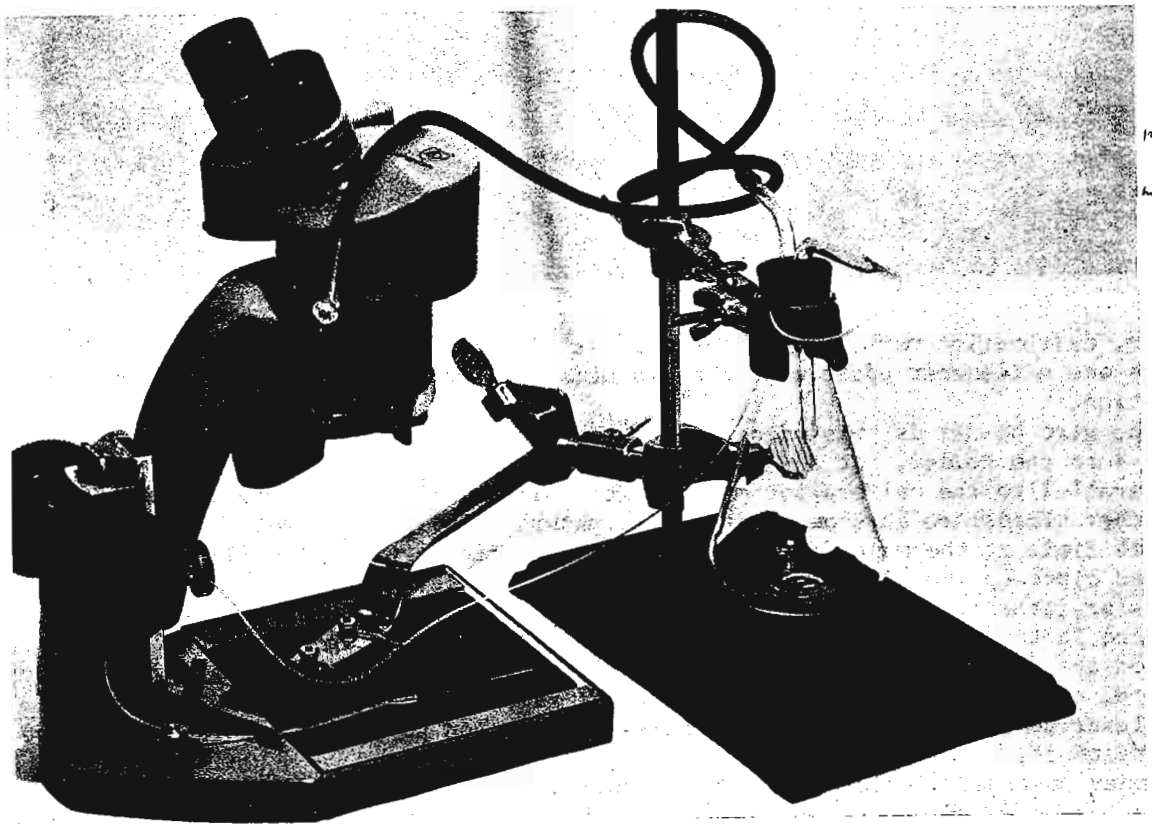
Seecof, R. L. City of Hope Medical Center.
An injection apparatus for Drosophila.

This injection apparatus is of very simple design, inexpensive and contains no wearing parts. The apparatus never requires adjustments and can therefore be operated by a technician. Many flies can be injected in a short time. Precision and accuracy are high because they are independent of micropipette size or the back pressure within the animal. The latter variables can cause inaccuracies when injections are made by methods that use a sudden blow to drive a pulse of inoculum.

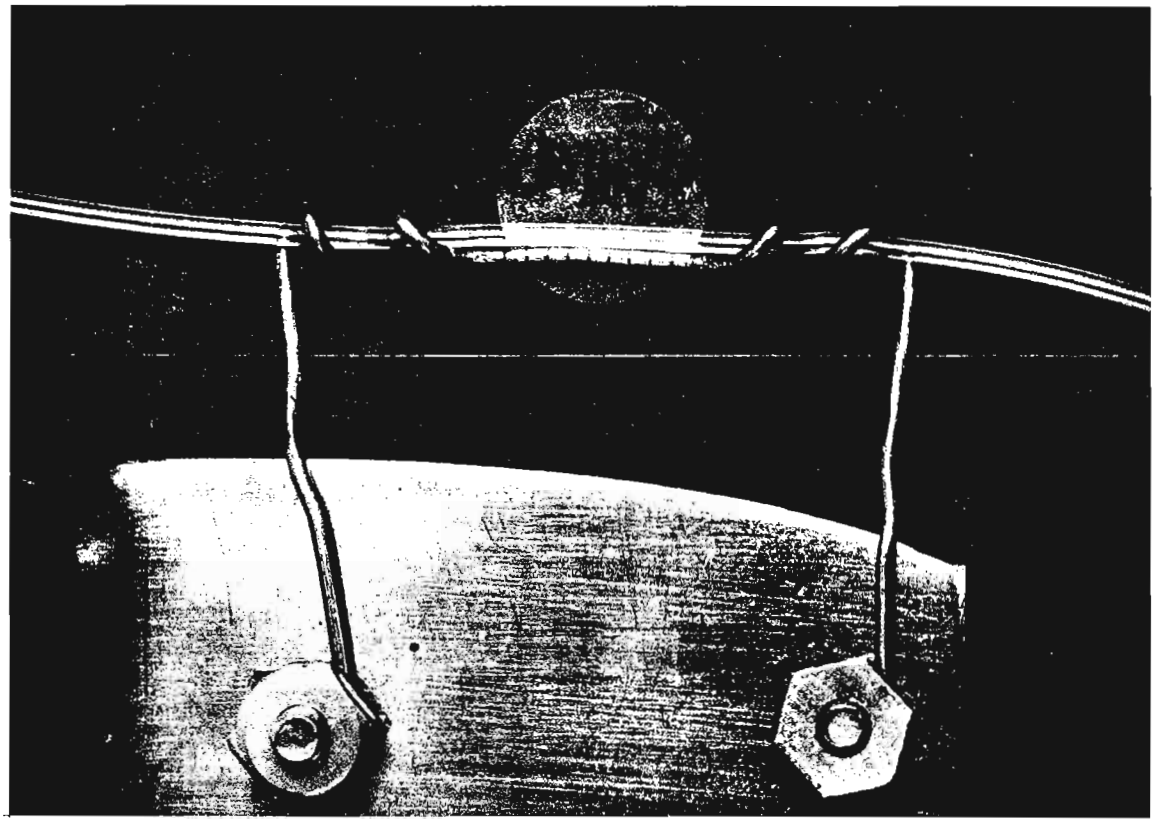
The injection apparatus is a mouth-pipetting arrangement (see illustration). The trap is a rubber-stoppered 125 ml Ehrlenmeyer flask connected to a glass mouthpiece by surgical tubing. The micropipette is attached to the trap by about three feet of polyethylene tubing (Clay Adams, PE 20, Aloe Catalogue)*. The polyethylene tubing is flared at one end to receive the tapered, fire-polished glass tubing from the trap and flared at the other end to receive the blunt end of the micropipette. Flaring is accomplished by holding the end of the tubing a few inches above a very small flame. Micropipettes, drawn from capillary tubing (OD 0.7-1.0mm), can be changed repeatedly without endangering the seal to the



* I.D. O.D.
mil .1015 .1043
mm .38 1.09

polyethylene tubing but their blunt ends must always be carefully fire-polished.

The polyethylene tubing is held in the microscope field by a wire attached to a supporting metal bar. The bar in the illustration is a bent table knife and the wire is a twisted common paper clip (Gem, no. 1, tinned steel wire 0.036 in diameter). The wire is shaped by twisting it around a piece of straight, rigid wire. After the wire is fastened



to the bar, calibration markings are cut into it with a razor blade. It is convenient to make marks one millimeter apart with the aid of a millimeter rule and twenty-power binocular magnification.

If the wire holder is twisted as shown, the polyethylene tubing need not be threaded end-first into the holder, but a loop of tubing can be folded into it. The tubing is held closely parallel to the calibration marks by the wire twists. When mounted in the field of an AO Spencer microscope as shown, seven millimeters of wire are readily visible at the side of the field at the magnification used for injection (20X). The light circle in the illustrated close-up shows the approximate size of the microscope field.

Flies are etherized and fastened in a row on a card by sticking their wings on to double-faced Scotch tape. The card can be pushed across the stage, beneath the wire holder, to position the flies in the microscope field for injection. The calibrated section of wire and the tubing against it are in nearly the same plane as the flies. No part of the wire is closer to the card than is the calibrated section so that the card can be moved freely beneath it.

In order to inject, water is first sucked about 30 cm into the tubing, then a micropipette is inserted and water blown down into the micropipette to fill it. A drop of mineral oil is then sucked up and, finally, the liquid to be injected is sucked into the micropipette. Air bubbles should be absent. The oil droplet prevents diffusion and is not sucked so far that it enters the plastic tubing. The plastic tubing is then drawn through the wire holder until the water meniscus within it is aligned with a calibration marking. The apparatus is arranged so that the flies, calibration marks and meniscus are visible in the microscope field (no meniscus is visible in the illustrations). The tip of the micropipette is then inserted into the ventral part of the flies' thorax, to either side of the midline, and liquid is introduced by mouth-pressure until the meniscus travels to the next calibration mark. The pipette tip need not be polished sharp; it will enter the imaginal thorax readily even though the tip is relatively blunt or poorly formed. Following the withdrawal of the pipette, bleeding will occur immediately (and the fly can be discarded) or not at all. Bleeding occurs in less than 10% of flies. The card can then be moved to align the

next fly and the injection repeated until the meniscus has reached the last visible calibration mark. Then the tubing can be drawn through the wire holder until the meniscus is repositioned at the first calibration mark and the next fly injected. Thirty flies can be fastened to the tape and injected in about 10 minutes. After injection the flies can be brushed from the tape with only minor damage to their wings. If the micropipette eventually becomes exhausted of liquid to be injected, more liquid can be sucked in without delay.

The volume of liquid delivered can be closely estimated by assuming the bore of the tubing to be uniform, sucking up a known volume of water, and measuring the length of tubing filled. An apparatus was checked by injecting dye into aliquots of buffer and found to deliver 0.164 microliters per millimeter injection (S.E. 0.014) over six millimeters. If millimeter injections were all made between the same two calibration marks the S.E. was lowered to about 0.01.

If quantitation is unimportant the apparatus can, of course, be used without the wire holder. Mortality is less than 5% if the flies are not overetherized. While the above description has been devoted to imago injection, the apparatus can be used to inject larvae or pupae or to deliver transplants if the micropipette is fashioned properly.

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Marques, E. K.,[1] Marly Napp[1], Helga Winge[2] and A. R. Cordeiro[2]. Universidade do Rio Grande do Sul[1], Universidade de Brasília[2], Brazil. A corn meal, soybean flour, wheat germ medium for *Drosophila*.

Composition:

Water	14 liters
Wheat germ	500 g
Wheat flour	250 g
Corn meal	1950 g
Soybean flour	100 g
Sugar	1550 g
Moldex (Nipagin or Tegosept M)	45 g
Salt (NaCl)	15 g
Hydrochloric acid 0,3 N.	115 cm^3

This medium is inexpensive, its components are easily stocked and less variable than the one with bananas. The wheat germ and the small amount of soybean makes it very rich and productive. It can be autoclaved at higher temperatures than the banana agar food.

These proportions of wheat germ, wheat flour and soybean flour were adopted as a result of a factorial experiment using several species of *Drosophila*.

It is advisable to mix well the weighted dry flours with the moldex, packing them in the desired amounts. These packages can be sterilized to destroy any parasites (mites, fungi, etc.). This mixture is poured in tap water in which sugar and salt were added and they are boiled about ten minutes. The hydrochloric acid solution is then added. After about ten more minutes the mixture can be poured in the vials.

Mossige, Jeanne Coyne. Norsk Hydro's Institute for Cancer Research, Oslo, Norway. Fermented yeast for egg collection.

When large numbers of eggs are to be collected over a short period of time, the addition of acetic acid and alcohol to the yeast have been reported to stimulate oviposition. These procedures have improved egg laying, but none has been found to be consistently reliable, as is the following. Mix about 1/4 teaspoon of granulated sugar with 50 g of bakers yeast along with just enough water so that the mixture can be stirred with a spoon. This is covered and left in a thermostat at 25° for an hour or more, by which time it will be a foamy, spongy mass. When stirred with a spoon the CO_2 is released and the volume decreases. This yeast can then be spread or dropped on an appropriate surface for collecting eggs. It is readily manipulated as long as it is not too moist, and it consistently stimulates the females to lay large numbers of eggs.