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A Syringe Carrier and Egg Clamp for Intravenous Inoculation of Chick Embryo¹

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The great range of potentialities of the chick embryo for the study and utilization of the relationships between viruses and susceptible cells has been exploited, in part, by various methods of inoculation: allantoic, amniotic, chorio-allantoic, intracerebral, intravenous, and yolk-sac. Inoculation by the intravenous route was first used by Polk, Buddingh, and Goodpasture in 1938 (1), and the current improved technic was described by Eichhorn in 1940 (2). It has been stated, however, that widespread application for the intravenous method has not been found (3, 4).

A study, undertaken by one of us (R.G.), required a collection of serial blood samples taken at 1-2-hr intervals, from the same, 10-16-day-old chick embryo. To overcome the prohibitively high casualty rate resulting from "free-hand" injection and bleedings, an apparatus was designed and built which consists of a rigid movable syringe carrier and an adjustable egg-clamp (Fig. 1).

The syringe carrier consists of two rigid rods. One, 30 cm × 2.5 cm and one 40 cm × 2.5 cm; the larger, a vertical one (1) fixed to the work table, and the smaller, a horizontal one (2) attached to the upright rod with a bar-clamp (3) allowing for coarse vertical and horizontal movement. Attached to one end of the vertical rod is a revolving stage (4 and 5) consisting of 2 circular plates (10 cm diam.), a fixed one (4) carrying a movable disc (5), actuated by a small spur gear (6) meshed into the geared edge of the moving plate. The center of the stage revolves about the long axis of the horizontal rod. A machine slide

with bedded strip (7) is fixed to the revolving stage along the diameter. At 90° to the slide, which is fixed to the revolving stage, and bisecting it, is a second machine slide (8) and bedded strip. This slide and bedded strip is actuated up and down along the first slide and bedded strip by a worm-drive screw (9). The second (the outer machine slide), also actuated by a worm-drive screw (10) has a carriage (11), for holding various sizes of hypodermic syringes.

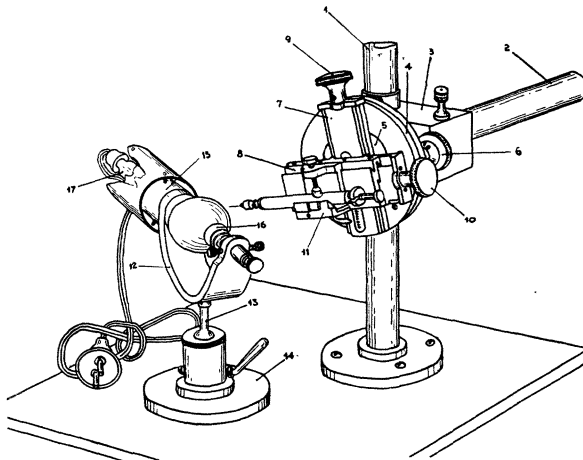


FIG. 1.

The carrier thus allows for the forward and backward motion of the entire syringe, by actuating the outer slide (10); the raising and lowering of the syringe by moving the inner slide (9); and adjustment of the angle of attack of the needle attached to the syringe by rotation (6) of the circular stage. Fine movement along the cylindrical axis of (2) can be achieved by incorporation of a rack and pinion in clamp (3).

The device permits the accurate and precise placement of the hypodermic needle at the proper angle, and entry into, and removal from the blood vessel, without erratic motion.

The other unit, the egg-clamp, consists of a U-shaped cradle, (12), mounted from below to a ball-and-joint swivel, (13), attached to a heavy but movable base (14). The egg is held at the air chamber and opposite ends. The air-chamber end fits into a fixed tube (2.0 cm diam.) (15), while the opposite holding point is an adjustable, spring-loaded rod with a small (0.75 cm diam.) soft rubber cup (16). The air-sac-holding tube extends beyond the U-cradle and contains a 25-w lamp for transillumination (17) of the embryo.

Selected eggs are transilluminated, the position of one of the large fixed veins lying in the chorio-allantoic membrane is marked on the shell, and the direction of the blood flow (which is toward the point where the fixed veins join the free allantoic vein) is indicated. A triangular segment of eggshell over the selected vein, of about 1-1.5 cm on each side, is re-

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moved in the usual manner using a dental engine with a drill or an abrasive disc. Care is taken to avoid damaging the eggshell membrane. A drop of sterile liquid paraffin placed on the exposed area makes the membrane transparent. The egg is placed in the holder with the air-sac toward the light source (15) and with the selected vein on top. The long axis of the blood vessel is set approximately parallel to the work-table and in line with the syringe. The syringe-carrier is adjusted so that the needle forms an angle with the blood vessel of about 15° , and rests on the surface of the eggshell membrane over the blood vessel. The needle is then driven forward, and into the vein, by manipulating the screw (10).

Injection is always made in the direction of the blood flow, and withdrawal of samples preferably in the opposite direction. The needle is withdrawn slowly; but in cases following injection, only after a pause of several seconds. In our experience bleeding after removal of the needle, even from 10-12-day-old chicks rarely occurred. It was not necessary to reseal the egg in the course of these studies even though incubation was carried forward for several days after veno puncture.

In our experiments on 10- to 14-day-old chick embryos, we have injected over 2500 eggs, using the apparatus described, with a casualty rate of less than 3%, and more than 250 eggs have had blood withdrawn from 2-4 times, with less than 10% loss. Routine bleedings of 0.1-0.2 ml of blood at 1-2-hr intervals can be carried out with ease for 4-6 bleedings (up to 7 or 8 with increasing difficulty). In one series, 15 chick embryos were bled every 2 hr for 8 hr, withdrawing 0.2 ml of blood each time without any casualty.

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A Mechanical Fly-Tagging Device

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A mechanical device has been developed for tagging flies that are to be used as experimental free-flying insects. The tagging consists of gluing 1-in. lengths of brightly colored nylon thread to the thoraces of flies anesthetized with carbon dioxide. This procedure permits suitable test insects to be more readily located, recovered, and identified, and is particularly useful

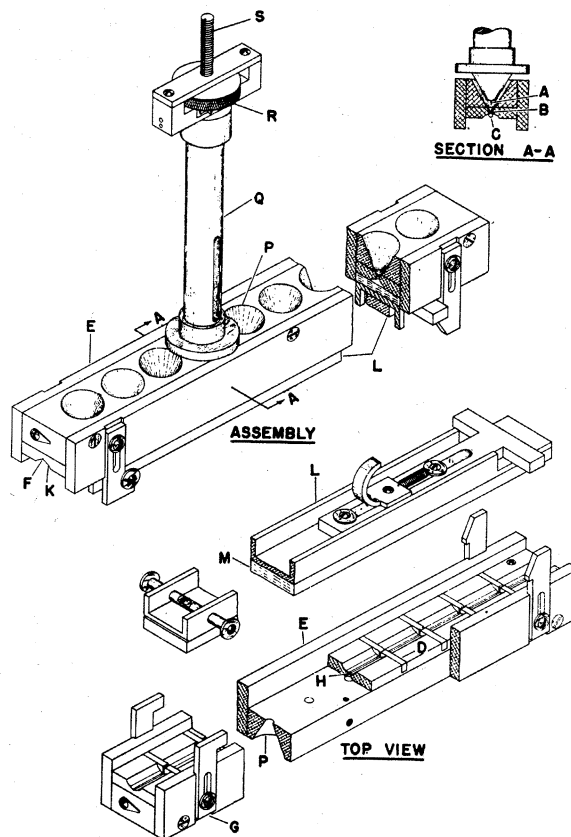


FIG. 1. Drawing of a fly-tagging device.

for aircraft-disinsectization studies. The bright colors are used to aid in locating the flies and to distinguish between different series of releases made in the same enclosure. The 1-in. length of thread extending directly back from the thorax of each tagged fly is readily grasped with forceps without agitating the fly into flight. The captured insects are then transferred to holding cages. When properly attached, the threads do not interfere with the normal activities of the flies.

Originally a manual method was used to attach threads to flies' thoraces, using high-melting-temperature paraffin. A small amount of the paraffin was put on the end of a piece of thread and this in turn was placed on the dorsum of the thorax. A warm metal rod was used to keep the paraffin melted and to hold the thread in contact with the anesthetized fly until the paraffin hardened. In about an hour the flies became accustomed to the attached threads and resumed normal activity. No fly injury has been observed, but proper precautions must be taken to prevent inactivation of the wings. Using this method, a highly skilled worker has sexed and attached threads to as many as 300 female flies an hour for short periods. The procedure is quite tiresome when large numbers of flies are to be tagged, and relatively unskilled workers have been able to produce only about 800 tagged flies per day. With the mechanical device, workers