A SIMPLE TISSUE PRESS FOR INITIATING CELL CULTURES FROM MOSQUITO EGGS, LARVAE, PUPAE, OR ADULTS OR THEIR ORGANS

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A simple, inexpensive tissue press for dissociating cells of mosquito eggs, larvae, pupae, and adults or their organs has been devised, as a means of initiating cell cultures from these specimens. This note describes its construction and basic operation.

Among the methods used for dissociating cells for culture are (1) by using proteolytic enzymes, such as trypsin, hyaluronidase, or Pronase; (2) by using hydrolytic enzymes for certain materials; (3) by mincing tissues or organs; (4) by setting up tissue or organ explants; and (5) by using a tissue press. The latter may give successful results when explants or proteolytic enzymes fail to yield the desired cultures. Basically the press consists of two components: (a) a 1.0 cc or 5.0 cc glass or plastic syringe, and (b) a superfine mesh or sieve through which the specimen is squeezed; it is made by being punched from various grades of silk bolting cloth, and is discarded after use.

The sieve is made from standard quality Dufour Swiss silk bolting cloth, available from Tobler, Ernst, and Traber, Inc., 420 Sawmill River Road, Elmsford, New York 10523. Although the best mesh size for any particular specimen can be determined by trial and error, it was found that the following sizes were very useful: size 11, 116 meshes per inch, mesh aperture with a mode of .0057 inch or 142.5 microns; size 12, 125-mesh, aperture mode .0047 inch or 117.5 microns; size 13, 129-mesh, aperture mode .0044 inch or 110 microns; size 14, 139-mesh, aperture mode .0039 inch or 97.5 microns; and size 15, 150-mesh, aperture mode .0037 inch or 93 microns. There are altogether 20 usable sizes, and a few tests will quickly enable one to select the best ones. This bolting cloth is also available as extra heavy quality, double extra quality, and treble extra quality, referring to the thickness of the strands, as well as a material called grit-gauze which is also quite durable. "Nitex" nylon monofilament bolting cloth supplied by the same company was also used for cellular dissociation, but results were not as thorough as with the silk, although the nylon was more durable.

The sieve is punched out in the form of a disk by using a cork borer. A set of borers may consist of from 6 to 23 individual borers of gradually increasing sizes. A few trials by cutting disks of paper will quickly enable one to select the borer which will punch a disk to fit snugly at the proximal end or floor of the syringe just preceding the snout which holds the needle. A disk of approximately 4.5 mm diameter will fit at the bottom of a 1.0 cc disposable plastic tuberculin syringe or a 1.0 B-D Yale glass tuberculin syringe. A disk approximately 11.5 mm diameter will fit at the bottom of a 5.0 B-D Plastipak disposable plastic syringe, a 6.0 Roehr Monoject disposable syringe, or a 5.0 B-D Yale glass syringe; however, a 16 mm disk is best for a 5.0 B-D Yale glass syringe. Syringes of other manufacture may require different disk diameters. If a cork borer is not available, a sieve for the 1.0 cc syringe can be made by punching out a ¼-inch diameter sieve with a standard hole punch, then trimming to size with a fine pair of scissors.

To cut one or more disks, the single or multiple bolting cloth is placed upon a piece of flat, soft wood, such as balsa, or upon a rubber eraser, and pressure is applied while turning the borer alternately to the right and left. The resulting disk will have a smooth edge. Its removal from the hollow stem of the borer can be facilitated by pushing a glass rod or applicator stick through from the opposite end. If the wood is too hard, the cutting edge of the borer may be dulled, in which case the edge is restored with a borer sharpener. The extracted disk is picked up with a fine forceps. The plunger is removed from the syringe, and the disk is inserted into the distal end; the plunger is then replaced, and is then used to push the disk gradually down the syringe to the proximal end where the disk is seated with pressure. No hypodermic needle is used in this device. The syringe can be sterilized with its disk insert, or disks can be sterilized separately and inserted under sterile conditions. Disposable syringes do not autoclave very well. All subsequent operations should be carried out in a tissue culture hood, vertical-flow biological cabinet, or sterile tissue culture cubicle.

It is not intended to review or discuss here the methods used for surface sterilization of insect eggs, or the aseptic or axenic rearing of mosquito larvae, pupae, or adults. A number of such methods have been discussed or reviewed by MacGregor (1929), Trager (1935), Trager (1959), Mitsuhashi and Maramorosch (1964), Vago (1967), Varma and Pudney (1967), Schneider (1969), Schneider (1971), Christophers (1960), Wallis and Lite (1970), and Hirumi and Maramorosch (1972). It will be assumed here that the selected specimens have been treated or reared so that they are uncontaminated.

During trials with this tissue press, the following commercially available insect tissue culture media were used to initiate cultures from the mosquitoes *Aedes aegypti, Anopheles stephensi, Culex pipiens pipiens,* and *Culex pipiens quinquefasciatus:* Grace's insect tissue culture medium, Grace's medium (modified), Schneider's *Drosophila* medium (revised), Mitsuhashi and Maramorosch leafhopper medium, and mosquito culture medium, all available from the Grand Island Biological Company, 3175 Staley Road, Grand Island, N.Y. 14072. In each case antibiotics were added to yield 100 units of penicillin and 100 micrograms of streptomycin per cc of medium. In cases where mold

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appeared after 24–48 hrs., the cultures were sometimes saved by changing the medium every day for 4 successive day, providing plating had occurred. For initiating the primary culture, 3.0 cc of medium containing antibiotics are pipetted into a sterile 2-inch Petri dish. If available, both glass and plastic Petri dishes should be used for comparison of growth and differentiation of cells upon the different surfaces. Subsequently 30 cc Falcon T-flasks may be used following the plastic Petri dishes, or glass prescription bottles following the glass Petri dishes.

As an example of how the press is used, the dissociation of uncontaminated mosquito larvae will be described. From one to six larvae can be conveniently crushed in a 1.0 cc syringe, depending on size. A sterilized syringe with bolting cloth sieve is prepared as already described. The plunger is removed and placed into a small sterile beaker for easy grasping; or, the plunger can be packaged and sterilized separately. A larva is picked up with a sterile pipette from the sterile distilled water or sterile culture medium in which it is being held, and is dropped upon sterile filter paper in a sterile Petri dish. After the water has been absorbed, the larva is lifted up with a fine needle or forceps, and deposited into the syringe as far down as possible. This procedure is repeated for as many larvae as one wishes to crush. The plunger is inserted, and the larva is gradually pushed down the barrel of the syringe until it is deposited upon the disk. The snout of the syringe is then placed into tissue culture medium in a Petri dish, and 0.2 cc of medium is drawn up. If a 5.0 cc syringe is used, 0.5 to 1.0 cc of medium is drawn up. The snout is then placed just above the surface of the medium in the Petri dish, and the plunger is pushed forward, crushing the larva while turning the plunger alternately to the right and left. The larval contents will be forced through the sieve into the medium. One can then examine for cellular dissociation immediately with an inverted microscope. If the disk has been cut properly, it will remain in place. The operation may be repeated, using other syringes. The disks, being easy to make and inexpensive, should be discarded; however, if nylon disks are used, they are more durable, and may be thoroughly washed, resterilized, and reused.

Newly-formed pupae and newly-emerged adults are so soft that they may easily be crushed in the same manner; however, it would be better to nick the pupal and adult integument with number 27 hypodermic needles (Burton, 1971), and extract the contents in large pieces prior to placing them into the syringe. The adults should have wings and legs removed, and be separated into head, thorax, and abdomen beforehand. When this procedure is used with eggs, the contents must be first extruded with needles, pooled, and dropped upon the mesh disk. If it is desired to utilize only individual organs, they should be dissected out, pooled, deposited upon the sieve, and crushed in a 1.0 cc syringe. The primary cultures should be incubated at 28 degrees C; however, they have been kept satisfactorily at 23 to 26 degrees, although growth is somewhat slowed at 23 degrees. Results should be compared with and without an atmosphere of 5 percent carbon dioxide, if a CO_2 incubator is available.

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RECOVERY OF *AEDES ALBOPICTUS* FROM USED TIRES SHIPPED TO UNITED STATES PORTS

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The importance of international traffic in the dispersal of mosquitoes and other arthropod vectors

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