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Notes on Insect Injection, Anesthetization, and Bleeding.

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NOTES ON INSECT INJECTION, ANESTHETIZATION, AND BLEEDING[1]

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In recent years there has been a burgeoning interest in insect cytogenetics, sometimes involving in vivo cultures of haematocytes for chromosomal analysis. Mitotic poisons, such as colchicine (Tyrkus, 1971), are commonly injected to produce metaphase plates. Likewise, injection of toxins is now common-place in applied insect research. However, surprisingly little general information on injection is available in the literature.

The dictates of morphology determine the gross procedure to be used. The kind of needle and syringe, the amount of fluid to be administered, and the necessity of optical aids are a function of the size of the insect recipient. Once these decisions are made, other considerations must still be weighed, including comparative exoskeletal toughness and the insect's stage of development, which are important in determining possible areas for needle penetration.

The needle length should be adequate for injection but not so great as to make it likely that the point will be thrust through the entire organism. The needle should be of the smallest possible diameter that will permit injection without clogging or filtering particulate components of the fluid. For example, a standard colchicine solution (colcemid-lyophilized reconstituted with bacteriostatic water) can be injected with a needle as small as 25 ga. Syringe capacity need not exceed 1 cc, though 0.1 cc calibration is necessary, and 0.01 cc markings are convenient. Microsyringes may be employed for more precise measurement of small amounts of fluid, as with injection into minute insects or into specific organs in partly dissected animals. The amount of fluid can be fixed precisely in accordance with the body size of the recipient, or it can be qualitatively estimated. If the latter, a general rule for adult insects is to inject sufficient fluid so as to begin distending the abdomen. Caution is advised against over-injecting, which will produce symptoms such as bleeding at the coxae and (in male Orthoptera) extension of the genitalia.

In general, the current trend toward reusing needles should be avoided, except in the case of fast-acting toxins; this prevents possible cross-infection. Optical aids are convenient and sometimes necessary for careful injection. Head-band visor-magnifiers with three-power lenses are useful with almost all sizes of insects. A dissecting compound microscope is only needed for injecting minute insects or organs. In either case, the animal may be placed on its back with its dorsum affixed to a layer of paraffin within a petri dish.

Several things are considered when choosing the point and manner of injection. The toughness of the exoskeleton necessitates needle penetration at the sutures, except in the case of the very soft-bodied. The needle should be orientated as parallel as possible to the longitudinal axis of the body to minimize internal damage. A common point for injecting directly into the haemocoel is at the ventrocaudal abdomen, as illustrated in Figure 1. When attempting in vivo haematocyte cultures for chromosomal analysis of holometabolous insects it may be convenient to inject larvae rather than adults because of the formers' greater size, more abundant haemolymph, and relatively more flexible exoskeleton. However, inasmuch as larvae tend to be sac-like they should be injected at a point that hinders profuse bleeding upon needle removal. Such a location in many larvae of Coleoptera is the unsclerotized dorsal portion of the thorax, as illustrated in Figure 2. This area has muscle layers that prevent excessive leakage.

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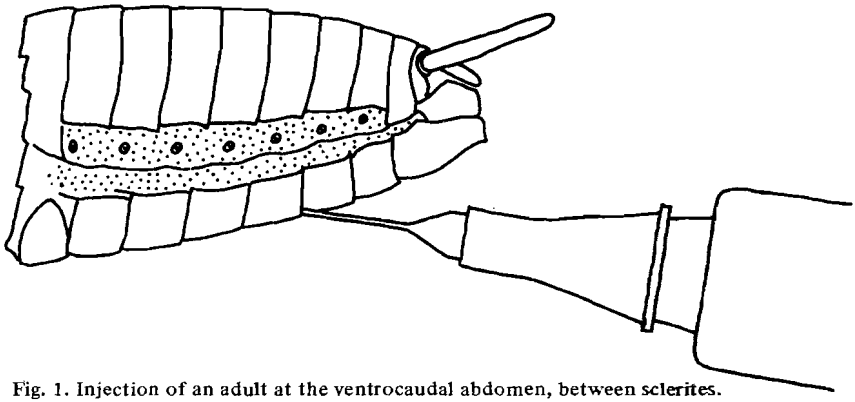

Fig. 1. Injection of an adult at the ventrocaudal abdomen, between sclerites.

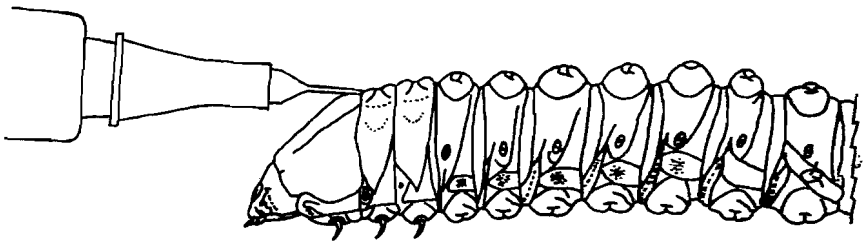

Fig. 2. Injection of a larva on the unsclerotized dorsal portion of the thorax.

It is often necessary to anesthetize large animals for easier handling and small ones to prevent damage. Chloroform is used successfully by some researchers (Lue et al., 1973), but our research with certain acridids reveals that damage (permanently extended hind legs) may result from the use of this chemical. Anesthetization with CO_2 is also common (Tyrkus, 1971), as is simple cooling of the insects in a refrigerator prior to injection or bleeding. If injection must be accomplished in the field immediately after capture, anesthetization may be done in vials using a CO_2-type cork remover sold for opening wine bottles.

The extraction of haemolymph from in vivo cultures is accomplished through several methods. Syringes can be used for bleeding large animals; the severing of a leg (preferably the hind member) at the coxa can be used for most adult insects; while a scalpel incision at the point of injection of most larvae, with squeezing, will produce sufficient haemolymph for slides or micro-tubes.

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