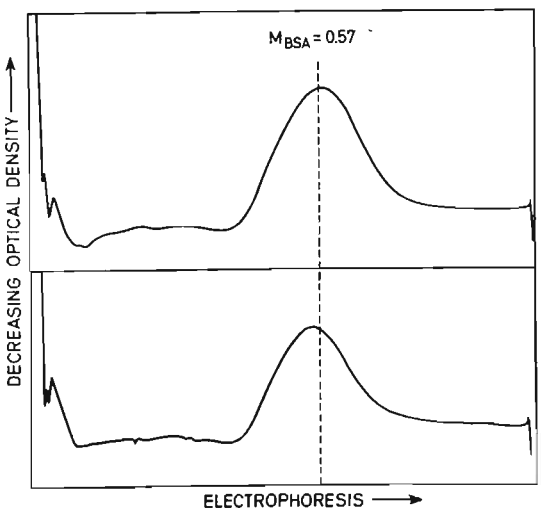
Boyd, J. B. Max-Planck-Institut für Biologie, Tübingen, Germany. Mass preparation of salivary glands from prepupae of Drosophila hydei.

To facilitate biochemical studies of the prepupal salivary glands, a method has been developed for the mass isolation of these organs. The procedure requires very little work with a microscope and permits one person to prepare about 500 glands/hour.

The developmental stage of the animals is determined to within one hour either by collecting white prepupae or by the floating method of Mitchell and Mitchell (DIS 39:135). Several hundred synchronized prepupae are placed in one side of a large petri dish which is tilted at a slight angle in a dish of ice. About 5 ml of Ringer is placed in the lower side of the dish. Each prepupae is held in the middle with blunt forceps while one tip of a pair of sharp forceps is inserted into the posterior tip of the operculum. The contents of the animal are then forced through the hole by firmly squeezing the blunt forceps, and the animal is placed in the cold Ringer.

After the desired number of animals has been so treated, 15 ml of Ringer is added and the suspension is pipetted up and down in a fire-polished 10 ml pipette that has had the tip cut off to a diameter of 6 mm. This step frees the glands from the other organs and removes all fat from glands obtained from prepupae which are eight hours or older. Pipetting is done gently, but considerable air is included to help break up the fat body. The suspension is filtered through a tea strainer into a beaker at 0° C. The cutical is washed in a stream of Ringer, resuspended in Ringer, and the pipetting and straining steps are repeated. The glands, which settle rapidly, are washed twice with 250 ml of cold Ringer.

If fewer than 100 glands are required, they can be picked out quickly under a dissecting microscope. For larger quantities, about 1000 glands are layered on a discontinuous gradient containing 8 ml 75%, 12 ml 62%, and 20 ml 20% sucrose (w/v). The sucrose is buffered at pH 7.0 with Tris. After spinning at 20,000 rpm for 7 min. and at 4° C in the Spinco SW 25,2 rotor, the glands are removed from the top of the 75% sucrose layer and washed in Ringer. The few contaminating testes are removed with forceps.



The recovery and quality of the glands depend on the developmental stage used. Yields of 50-75% are obtained from animals 8 - 14 hours after puparium formation. In white prepupae, glands are too firmly attached to the larval mouth parts to be recovered by this method. Yields from slightly older animals are low and the recovered glands have some attached fat body. At 25° C and between 16 and 16.5 hours after puparium formation glands can no longer be recovered.

This method has been used to study DNases in these glands after their separation in acrylamide gel (Boyd and Mitchell, Anal. Biochem. 13, 28, 1965). The enzyme patterns, presented in the top and bottom of the figure, were obtained from hand dissected and mass-prepared glands respectively. The gels were incubated at pH 9 in the presence of Mg^{++}. This enzyme activity, which is not detected in the larval salivary gland, is only slightly reduced by the isolation procedure. The morphology of the glands does change, however, as a result of centrifugation in sucrose. For some purposes it is, therefore, desirable to replace the sucrose with Ficoll as used by Fristrom and Mitchell (J. Cell. Biol. 27, 445, 1965).