Fibrosarcoma tumor cells (2×10^8) to be elutriated were resuspended in 20 ml media with 5% FCS. The elutriator was operated at a constant 1510 rpm while varying the flow rate of media through the rotor from about 5 to 29 ml/min. 12 50-ml fractions were obtained and analyzed.

The tumor cells to be separated by the STAPUT method were loaded (6×10^7 in 20 ml of 0.5% BSA) through the bottom of the 884 ml chamber followed by a 2-4% bovine serum albumin (BSA) gradient. The cells were allowed to sediment for 2.66 h in a cold room at 4 °C. After discarding the volume of the cone, 33 fractions were collected of 20 ml each. All fractions obtained from either elutriation or STAPUT were analyzed for cells per fraction, cell volume and ^{3}HTdR incorporation.

Figure 1 presents the results obtained in separating FSa tumor cells by centrifugal elutriation. The top panel shows the percent cell recovery as a function of sedimentation velocity (SV) or fraction number. The majority of the cells are removed in fraction 4 which relates to a SV of about 11 mm/h. Subsequent fractions have between 10 and 4% recovery of tumor cells. The open circles in this panel show where pulse labeled (^{3}HTdR) tumor cells are removed from the rotor. It is apparent that these labeled cells are larger and sediment at a more rapid rate than the smaller cells which predominate in fractions 3-5. The bottom panel shows the modal volume of cells obtained from the various elutriator fractions. The cells varied in a linear fashion from about 700 to 2000 μm^3 with the DNA synthesizing cells approximating 1800 μm^3.

Figure 2 shows similar results using the STAPUT method at unit gravity. Because the cells separated by the STAPUT method were collected in 33 fractions as opposed to 10 fractions in the elutriation experiments, the percent cell recovery per fraction was significantly lower in the STAPUT experiments. The peak in the ^{3}HTdR labeled cells was noted in fractions representing cells with cell volumes larger than average. This effect was not as apparent using the STAPUT as it was in cells separated by elutriation. The bottom panel of figure 2 shows the relationship of modal cell volume to sedimentation velocity. The cell volumes varied from 585 to 2800 μm^3 in the fractions collected. The line was linear with a correlation coefficient equal to 0.998.

Both centrifugal elutriation and the STAPUT methods are extremely effective in separating tumor cells on a basis of size. Cells experiencing uniform conditions of exponential growth increase steadily in size with age during the division cycle[12]. Since the SV of a cell is proportional to two-thirds power of its volume, velocity sedimentation methods can be used to separate and isolate subpopulations of cells having similar sizes and DNA contents from asynchronous populations. The enrichment of tumor cells on a basis of size allows the cell cycle phase effects to be studied in relation to many disciplines such as tumor biology, experimental chemotherapy and experimental radiation therapy[5-7].

Both separation methods can be accomplished under sterile conditions. Elutriation, although faster, requires an expensive and sophisticated centrifuge; while the STAPUT method can be accomplished cheaper but requires additional time for separation.

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Ciliary structures in the branchial unicellular glands of the grass shrimp, *Palaemonetes pugio*[1]

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Summary. A unicellular exocrine gland possessing an epicuticular ductule occurs in grass shrimp gills. This gland displays ultrastructural changes in relation to the molt cycle. These changes include an increase in the quantity of secretory granules during late premolt, and the development of ciliary axonemes in relation to ductule formation.

Both unicellular[2,3] and multicellular[4] exocrine glands of Crustacea resemble the class III dermal glands of insects[5] in having a cuticular ductule. The process of ductule formation has thus far been elucidated in the case of a variety of insect dermal glands[6-11] and a tricellular dermal gland in a crustacean[4], but not in the case of unicellular dermal glands of Crustacea. To bridge this gap in the literature, we examined the ultrastructure of unicellular glands in the branchial epithelium of the grass shrimp, *Palaemonetes pugio*.

Materials and methods. Gills from grass shrimp at various stages of the molt cycle[12,13] were fixed and processed for electron microscopy[4,14]. Sections were stained with lead citrate and uranyl acetate and viewed with a Philips 201 electron microscope.

Results. Unicellular exocrine glands with a short ductule sparsely populate the subcuticular regions of the pleurobranchiate gill axes on the sides facing the cephalothorax (figure 1). The gland cell (30-45 µm) is surrounded by epithelial cells except for a narrow (about 3 µm) neck which contacts the branchial cuticle (figures 1 and 2). A septate desmosome (about 1 µm) joins the basal portion of the cytoplasmic neck to the adjacent epithelium. The cytoplasm contains a relatively dense nucleus, numerous mitochondria and cisternae of rough endoplasmic reticulum, as well as occasional golgi and multivesicular bodies. Additionally, the cytoplasm contains secretory granules (0.2-1.8 µm diameter) with extremely dense or moderately lucent contents (figure 1). The secretory granules are most abundant during late premolt and nearly absent during

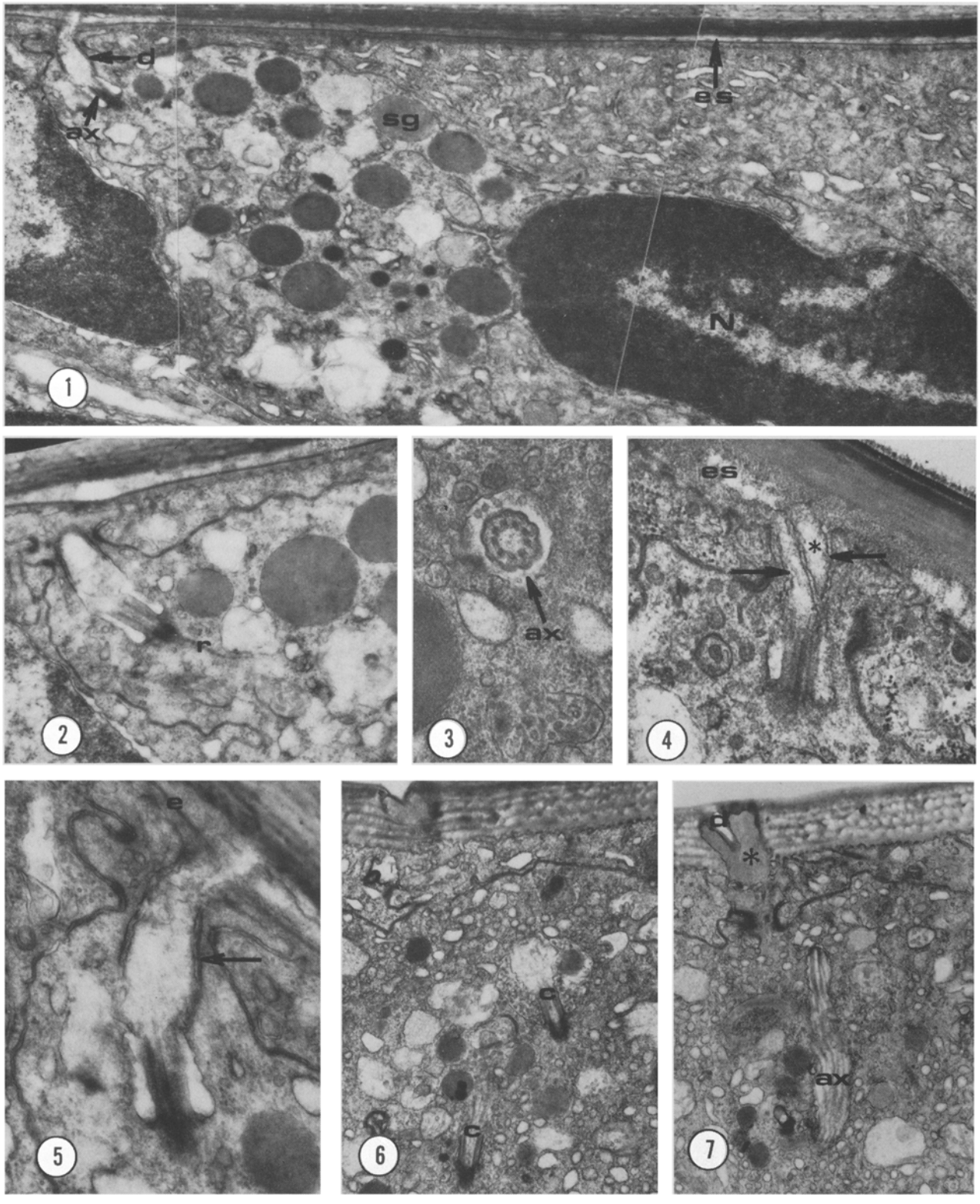

Fig. 1. Overview of branchial unicellular gland during early premolt showing the characteristically electron dense nucleus (N) and the abundance of morphologically variable secretory granules (sg). The exocrine ductule (d) is being formed around ciliary axonemes (ax) which extend into the exuvial space (es). × 10,000. Fig. 2. Higher magnification of ciliary structure showing the rootlets (r) of the ciliary basal body. × 15,000. Fig. 3. Cross section of basal region of the axoneme (ax) which reveals the 9 + 0 arrangement of microtubule doublets. × 30,000. Fig. 4. Growth of 2 ciliary axonomes (arrows) through an invagination of the gland apex and into the exuvial space (es) during apolysis. Notice the apparent reduction of microtubules (*). × 30,000. Fig. 5. The cuticular ductule (arrow) formed around the ciliary axonemes appears continuous with the new branchial epicuticle (e). × 30,000. Fig. 6. Section through gland shortly after ecdysis showing displacement of the 2 ciliary structures (c) away from the gland neck. × 15,000. Fig. 7. An adjacent section showing persistent ciliary axoneme (ax) derived from the basal body at the bottom of figure 6. Note the cytoplasmic extension (*) which envelops the most distal regions of the exocrine ductule (d). × 15,000.

early postmolt, indicating that maximum secretory activity occurs during ecdysis.

Two ciliary structures, consisting of a basal body and rootlets (figure 2), are present in the cytoplasm of unicellular glands during premolt (stages D_0-D_4). During apolysis (stage D_0), the basal bodies are situated at the base of the cytoplasmic neck and produce axonemes, the basal portion of which contains a 9+0 arrangement of microtubule doublets (figure 3). These axonemes ascend through an extracellular sheath (formed from the neck apex) toward the exuvial space (figure 4). As the new branchial epicuticle is formed by the adjacent epithelial cells, the unicellular gland deposits a layer of epicuticle around the ciliary axonemes; this constitutes the building of a cuticular ductule that is continuous with the branchial epicuticle (figure 5). The cuticular ductule (1-5 µm length) possesses a terminal orifice no greater than 0.3 µm in diameter.

Subsequent to ecdysis (stage A) the ciliary basal bodies are displaced away from the gland neck, but still possess axonemic processes (figures 6, 7). The complete ductule is separated from the branchial exocuticle by a thin (0.3 µm) sheath of attenuated dermal gland cytoplasm (figure 7).

Discussion. Previous ultrastructural studies of arthropod exocrine glands revealed the occurrence of transitory ciliary structures during ductule formation in developing insect glands[6-11] and during molt-related ductule reformation in a crustacean dermal gland[4]. In these glands, the transitory ciliary axonemes seem to serve as a template for ductule deposition and degenerate shortly after ductule formation. In the unicellular glands of *Palaemonetes*, the ciliary axonemes do appear to serve as a template for ductule deposition but they persist subsequent to ductule formation and even after ecdysis. The significance of this unusual phenomenon and the overall function(s) of the unicellular glands in grass shrimp gills remain to be ascertained.

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Changes in immune reactivity during growth of an adenovirus 12-induced transplantable tumour in CBA mice[1]

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Summary. Early suppression, followed by a period of enhancement and finally, suppression, was seen when the spleen cell response to T and B cell mitogens was monitored during growth of an adenovirus 12-induced tumour in CBA black mice. The macrophage content of the tumour changed with time and these fluctuations correlated with the ability of tumour tissue extracts to enhance the normal spleen cell response to mitogen.

Changes in host immune reactivity during growth of a cancer may be important factors in tumour development[2,3]. In this study, mouse spleen cell responses to the mitogens phytohaemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM)[4,5] were monitored during the growth of transplanted adenovirus 12-induced tumours in CBA black mice. The macrophage content of the growing tumours was estimated and correlated with the modulatory effects of tumour tissue extracts on normal mouse spleen cell responses to Con A.

Materials and methods. An adenovirus 12-induced tumour of demonstrable immunogenicity[6,7] was transplanted by S.C. inoculation of 0.1 ml of viable tumour cells (approximately 0.5×10^6) into the upper flank. Groups of treated and matched control animals were killed at each time point (7, 14, 22, 29, 35, 42, 56 and 72 days after transplant) and the spleens and tumour tissue removed. Pooled spleen cell suspensions (tumour-bearer or normal) were prepared by gently pushing spleen tissue through a sterile wire mesh, washing the cells 3 times in Hanks buffered salts solution (HBSS) and resuspending in RPMI 1640 medium containing L-glutamine, penicillin 100 units/ml, streptomycin 100 µg/ml, and foetal bovine serum to give a final concentration of 10%. The cell suspension was dispensed into micro-titre plates (U-shaped wells, Sterilin Ltd), and PHA (Wellcome Reagents), Con A (Sigma) or PWM (Gibco Biocult) in sterile solutions were added at the appropriate concentrations to give a final volume of 0.2 ml. Cultures were incubated at 37 °C in a humid atmosphere of 95% air/5% CO_2 for 72 h. 0.5 µCi of ^{3}H-thymidine was added for the last 18 h. Cultures were harvested using a Titertek cell harvester and samples counted on a Packard Scintillation counter and the mean of triplicate samples determined. Transformation indices (TI) were calculated:

$$TI = \frac{\text{mean cpm cells + mitogen - mean cpm cells alone}}{\text{mean cpm cells alone}}$$

Tumour and normal tissue extracts were prepared by dialysis and lyophilisation of the supernatants of an aqueous homogenate of each tissue. Protein estimations were made using the Lowry technique[8] and sterile solutions containing a known quantity of protein added to normal mouse spleen cells treated with Con A as above. Transformation indices were determined, comparing cells with mitogen plus extract with cells plus mitogen alone.

The macrophage content of the tumours was estimated by adding India ink to a suspension of tumour cells, incubat-