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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2 J. Pochon-Masson, J. Renaud-Mornant and P. Cals, Archs Zool. exp. gén. *116*, 123 (1975).
3 I.D. Gharagozlou-vanGinneken, Archs Biol., Liège *88*, 79 (1977).
4 D.G. Doughtie and K.R. Rao, J. Morph. *161*, 281 (1979).
5 C. Noirot and A. Quennedy, A. Rev. Ent. *19*, 61 (1974).
6 R. Barbier, J. Microsc. *20*, 18 (1974).
7 R. Barbier, J. Microsc. Biol. Cell *24*, 315 (1975).
8 S.J. Berry and E. Johnson, J. Cell Biol. *65*, 489 (1975).
9 K. Selman and F.C. Kafatos, Devl Biol. *46*, 132 (1975).
10 J. Bitsch and C. Palevody, Zoomorphologie *83*, 89 (1976).
11 L. Sreng and A. Quennedy, J. Ultrastruct. Res. *56*, 78 (1976).
12 P. Drach and C. Tchernigovtzeff, Vie Milieu *A 18*, 595 (1967).
13 P.J. Conklin and K.R. Rao, in: Pentachlorophenol: Chemistry, Pharmacology, and Environmental Toxicology, p. 181. Ed. K.R. Rao. Plenum Press, New York 1978.
14 A.L. Bell, S.N. Barnes and K.L. Anderson, Biol. Bull. *173*, 393 (1969).

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-

Days after transplantation	% Macrophages (mean)	Tumor weight (g)
14	39%	0.67
21	42%	0.70
28	51%	1.18
35	45%	1.41
42	30%	8.1

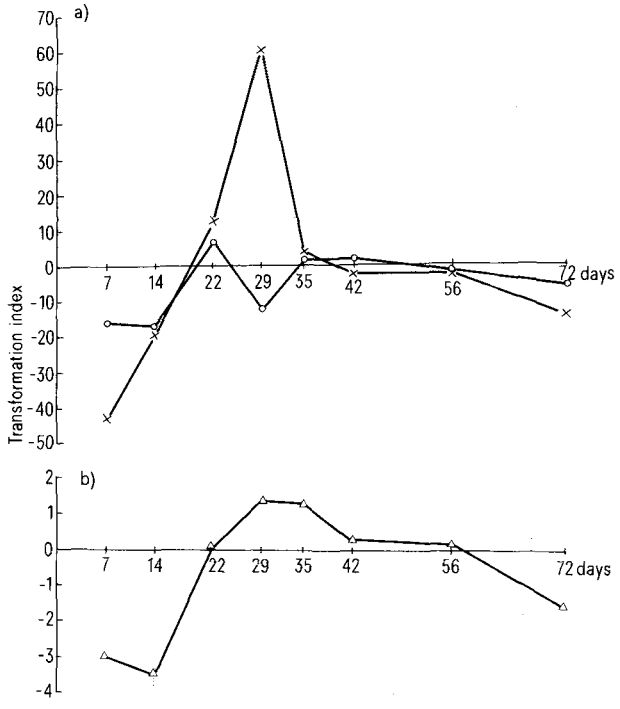

Fig. 1. Transformation index of spleen cells from tumour-bearing mice plotted against time after transplantation. The transformation index of normal spleen cells from control mice assessed simultaneously has been subtracted. *a* ×—×, Con A at 5 µg/ml; ○—○, PHA at 5 µg/ml; *b* △—△, PWM at 4×10^{-3} dilution.

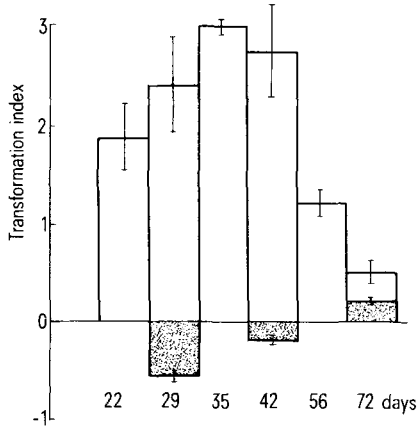

Fig. 2. Enhancing effect of adenovirus 12-induced tumour extracts on normal spleen cell transformation induced with Con A at 5 µg/ml. The effect of normal tissue extracts is shown in solid blocks. All tissue extracts at 2 µg/ml.

ing at 37 °C on a rotary shaker for 2 h and scoring as phagocytes those cells which had taken up the India ink[9]. Macrophages (phagocytes) were expressed as a percentage of the total number of cells present.

Results and discussion. Figure 1 shows a plot of the difference between the tumour-bearer and control mouse spleen cells plotted against time for each of the 3 mitogens. Early suppression was followed by a period of enhancement of transformation, particularly with Con A and PWM. The enhancement of transformation, returned to control levels by day 35 after transplant and slight suppression was then seen in the terminal stages. Early suppression of immune reactivity may allow the tumour to develop while later enhancement of spleen cell responses could be due to host-tumour interactions particularly at the site of the tumour, producing immunostimulatory substances[10]. Indeed tumour tissue extracts had an increasingly enhancing effect on normal spleen cell transformation by Con A (fig. 2) while normal tissue extracts had little or no effect. Maximum enhancement was produced by the 35-day extract, thereafter the activity declined as the tumour rapidly increased in size (table). It is interesting that the macrophage content of the tumour increased (table); this suggests an attempt by the host to overwhelm the immunogenic tumour[11,12].

It may be that the immune enhancement apparent in these studies was a result of the tumour inducing suppressor mechanisms[13,14] which would ultimately prevent the host rejecting the tumour. However, such suppressor systems may be stimulated to respond in the in vitro assays used in this study. PHA and Con A are T cell mitogens whilst PWM activates both T and B cells. Also, Con A in particular is known to activate suppressor cells in mice[15]. Alternatively, enhanced reactivity of tumour-bearer cells may be due to sequestration of active immune cells in the spleen[3,16] or the activation of existing cells, rather than a further increase in spleen cell numbers, because spleen size did not differ from that of the controls.

Tumour-bearing is not necessarily always associated with impaired lymphocyte transformation[17]. Stimulation of lymphocyte reactivity has to be interpreted with caution in view of the various methods and mitogens available for assaying immune responses by lymphocyte transformation in vitro.

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2 D.E. Schumm, D.F. Billmire and H.P. Morris, Eur. J. Cancer *12*, 689 (1976).
3 K.D. Chandradasa and R.M.R. Barnes, Eur. J. Cancer *14*, 279 (1978).
4 G.T. Strickland, A. Ahmed and K.W. Sell, Clin. exp. Immun. *22*, 167 (1975).
5 G. Janossy and M.F. Greaves, Clin. exp. Immun. *9*, 483 (1971).
6 R.C. Rees and C.W. Potter, Arch. ges. Virusforsch. *41*, 116 (1973).
7 R.C. Rees and C.W. Potter, Eur. J. Cancer *9*, 497 (1973).
8 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
9 R. Evans, Transplantation *14*, 468 (1972).
10 S.E. Salmon and A.W. Hamburger, Lancet *1*, 1289 (1978).
11 S. Eccles and P. Alexander, Nature *250*, 667 (1974).
12 I.J. Fidler and D.E. Peterson, Adv. exp. Med. Biol. *73*(B), 389 (1976).
13 C.C. Ting and D. Rodrigues, Proc. natl Acad. Sci. USA *77*, 4265 (1980).
14 K. Yamauchi, S. Fujimoto and T. Tada, J. Immun. *123*, 1653 (1979).
15 D.L. Peavy and C. Pierce, J. exp. Med. *140*, 345 (1974).
16 J.G. Kreuger, R.A. Segal and R.C. Moyer, Cancer Res. *37*, 320 (1977).
17 R.W. Gillette and C.W. Boone, Cancer Res. *35*, 3774 (1975).