Table 2. Mortality of B10 female mice, nonsensitized or preimmunized with LAF-17 or L1210 lymphomas, challenged with syngeneic S-1033 tumor and treated with graded doses of BCNU

| Group | Challenge No. of S-1033 cells (day 0, i.p.) | Treatment BCNU mg/kg (day+5, s.c.) | Mortality data Nonsensitized MST[c] | D/T[d] | $P_1$[e] | Sensitized with LAF-17[a] MST | D/T | $P_1$ | $P_2$[f] | Sensitized with L1210[b] MST | D/T | $P_1$ | $P_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1-3 | $10^3$ | - | 19.0 | 8/8 | - | 22.0 | 6/8 | - | B | 20.0 | 7/8 | - | C |
| 4-6 | $10^6$ | - | 14.0 | 8/8 | - | 14.0 | 8/8 | - | C | 14.0 | 8/8 | - | C |
| 7-9 | $10^6$ | 10.8 | 17.5 | 8/8 | A | 18.0 | 8/8 | A | C | 20.0 | 8/8 | A | C |
| 10-12 | $10^6$ | 18.0 | 23.0 | 8/8 | A | 20.0 | 8/8 | A | C | 25.0 | 8/8 | A | C |
| 13-15 | $10^6$ | 30.0 | 26.5 | 8/8 | A | >60.0 | 4/8 | A | B | 26.0 | 6/8 | A | C |
| 16-18 | $10^6$ | 50.0 | >60.0 | 4/8 | A | >60.0 | 1/8 | A | B | 26.5 | 6/8 | A | C |

[a]Viable LAF-17 cells given i.p., uding the same treatment schedule described in footnote a) of table 1. [b]Viable L1210 cells given i.p. as for LAF-17. [c]MST, medium survival times. [d]D/T, dead mice over total animals tested. [e]$P_1$, probability values calculated according to the Mann-Whitney 'U'-test, comparing control mice (nonsensitized or immunized) not treated with BCNU, with hosts subjected to BCNU chemotherapy; A, $p<0.01$; B, $p<0.05$; C, $p>0.05$ (not significant). [f] $P_2$, probability values calculated as for $P_1$, comparing nonsensitized mice with animals preimmunized with lymphoma cells, subjected to the same challenge and treatment.

mals presensitized with LAF-17 lymphoma. The data obtained in male mice showed that enhancement might also have occurred in animals presensitized with allogeneic RIL and challenged with $10^3$ lymphoma cells (groups 19-21), or inoculated with $10^7$ neoplastic cells and treated with 10.8 mg/kg of BCNU (groups 25-27). However, the influence of presensitization on the host's survival was marginal and the differences in MST's did not reach statistically significant levels. When high doses of BCNU were used, (i.e. 30 and 50 mg/kg) no influence of presensitization was found in either male or female recipients.

Similar studies were carried out in female B10 mice presensitized with LAF-17 or L1210 lymphoma cells and challenged with syngeneic S-1033 cells. The results illustrated in table 2 show that no enhancement produced by presensitization could have been detected. On the contrary, marginal graft resistance seems to have been produced by presensitization with allogeneic RIL. This was detectable in mice challenged with $10^3$ cells (groups 1 and 2) or with $10^6$ cells and treated with 30 or 50 mg/kg of BCNU (groups 13-18). In conclusion, the results of the studies described in the present report evidenced that presensitization with allogeneic lymphomas can produce enhancement of syngeneic tumors sharing, presumably, the same TATA. On the other hand, protective effects can also be obtained, although they appear to be less pronounced and detectable mainly following appropriate chemotherapy. No data is available at present to explain the mechanism underlying the observed enhancement of lymphoma growth. It can be hypothesized that this effect may be the results of activation of suppressor cells[9-11] induced in mice by presensitization with allogeneic RIL. In any case, the present findings should make us aware of the potential risk of indiscriminate use of sensitization with allogeneic tumor cells for clinical tumor immunotherapy[12,13].

1 This work was supported by Progetto Finalizzato Virus, CNR (Rome, Italy), Contract No. 770025284/1152505.
2 Acknowledgments. I wish to thank Dr J. Mayo and Mr C.R. Reeder of the Mammalian Genetics and Animal Production Section, Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda (Maryland, 20014, USA) for providing the mice used in this study. I also thank Dr H.B. Wood of the Drug Synthesis and Chemistry Branch, DCT, NCI, NIH, for furnishing the BCNU used.
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## SEM surface morphology of the contractile cells in the rat seminiferous tubules

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*Summary.* The SEM observation of the basal surface of the contractile cells in the boundary tissue of the seminiferous tubule of the rat has revealed that the contractile cells are extremely flat, vary in shape from rectangular to hexagonal, and are arranged close to each other, in the fashion of a tiled floor, around the seminiferous epithelium.

Recently, the ultrastructural organization of the boundary tissue surrounding the seminiferous tubules has been the subject of considerable interest for many investigators, in relation to such functions as mechanical supporting of the tubules and the passage governing of metabolic materials between tubule and interstitial matrix. Especially since the first description by Clermont[2] in the rat testis, the presence of a contractile cell which is characteristic of a smooth muscle cell and contains bundles of fine filaments in the boundary tissue, has been confirmed in nearly all specimens studied[3-13], and it is now generally accepted that the contractile cell may play an important role in the discharge of spermatozoa from the seminiferous epithelium and in their transport to the rat testis. However, to our knowlege

no attempts to employ SEM have been made to reconstruct the structure of the boundary tissue of the seminiferous tubules. The observation of Connell[14], who studied canine testis with SEM, was solely restricted to the interstitium of the testis.

In the present investigation the basal surface of the contractile cells in the rat testis was disclosed by removing connective tissue elements of the seminiferous tubule with HCl and collagenase, according to the original method of Evan et al.[15], and viewed with a SEM to analyse the fine surface structure of these cells.

*Materials and methods.* The testes of the adult Wistar-rats were fixed in vivo by vascular perfusion with buffered formaldehyde-glutaraldehyde (pH 7.2), cut into small blocks, and fixed for additional 3 h, and then individual seminiferous tubules were carefully teased or separated from one another with forceps or needles under a binocular stereoscope and processed according to the procedure of Evan et al.[15]. The seminiferous tubules were rinsed several times in 0.1 M phosphate buffer (pH 7.2), and placed in 8 N HCl for 60 min at 60 °C. After HCl digestion, the tissue was rinsed in 0.1 M phosphate buffer again, and placed in 0.1 M phosphate buffered collagenase (pH 6.8) at a concentration of 1 mg/1 $cm^3$ of buffer for 3 h at 37 °C. Following several rinsings with 0.1 M phosphate buffer, the tissue was postfixed in osmium tetroxide for 2 h, dehydrated with a series of acetone, dried by the critical point method using liquid $CO_2$, coated with gold and scanned by a Hitachi FSH type SEM. Some of the previously scanned samples were passed through propylene oxide and embedded in epon 812 for TEM. Normal fine structure of the seminiferous tubules were also studied by routine TEM. The tissue fixed by perfusing with buffered formaldehyde-glutaraldehyde was postfixed in osmium tetroxide without prior treatment with HCl and collagenase, dehydrated with a series of acetone and embedded in epon 812.

*Results and discussion.* As previously reported[2-4,7], the boundary tissue of the seminiferous tubules of the rat consisted of 4 main layers: namely, 1. the inner non-cellular layer comprising varying amounts of collagen fibrils sandwiched between 2 basement membranes, 2. the inner cellular layer of contractile cells characterized by the bundles of fine filaments, 3. the outer non-cellular layer contained scattering collagen fibrils and associated with a thin basement membrane, and 4. the outer cellular layer of very flat cells without fine filaments, probably fibroblastic and referred to as the endothelium of peritubular lymphatic sinusoids by some authors[6,14].

The contractile cells appeared extremely elongated in section and contained bundles of the fine filaments, as well as numerous pinocytotic vesicles situated beneath the cell membrane, dense plaques associated with bundles of fine filaments being observed occasionally (not illustrated). The contractile cells closely resembled the smooth muscle cells in an ultrastructural respect. The adjacent contractile cells usually met or overlapped each other in a fashion of typical tight junction, but, there were occasionally intercellular spaces of various dimensions, including a gap between the processes of the cells. Penetration of tracer materials, such as lanthanium and horseradish peroxydase, to the seminiferous epithelium through such gaps between the contractile cells has been recently shown in TEM[6,11].

Treatment of the seminiferous tubules with HCl and collagenase could almost completely remove the connective tissue elements of both outer cellular and underlying outer non-cellular layers. When viewed by SEM, the contractile cells were very thin, and flattened with various shapes from rectangular to hexagonal and arranged close to each other in the fashion of a tiled floor around the seminiferous epithelium (figure 1). This was in contrast to the results of Bustos-Obregón and Holstein[10] who studied the human seminiferous tubules with TEM and postulated that contractile cells were stellate in shape and formed a network around the seminiferous tubule. The contractile cells ranged from 21 to 40 μm in length and 16 to 27 μm in width. The basal surface of the individual contractile cells appeared generally smooth or slightly undulated, lacking any cytoplasmic protrusions such as microvilli and microprojections. Small pits could be seen scattered on the basal surface of the cells. They may be not openings of micropinocytotic vesicles, but rather artefacts due to preparation. Similar artefact was already noticed by Evan et al.[15] after prolonged exposure of the specimen to HCl. The cell boundaries between the adjacent contractile cells are clearly demarcated. They appeared linear or finely serrated and usually connected firmly with each other, but, in places, there were relatively large gaps of varying extent between the cells, especially at the corners where 3 contractile cells join (figure 2). The real nature of such gaps is not yet

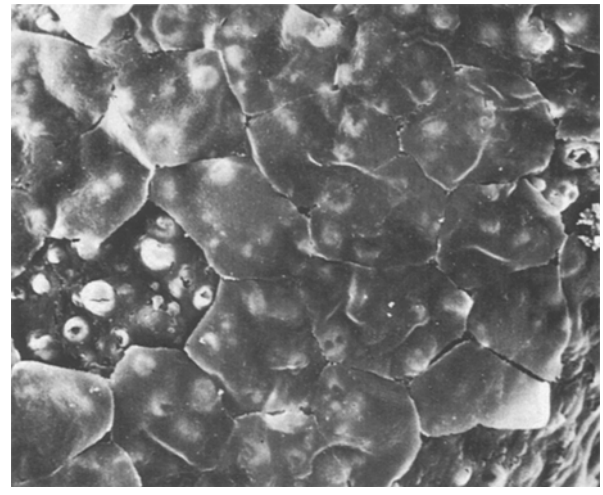

Fig. 1. Low-magnified scanning electron micrograph of HCl-collagenase-treated seminiferous tubule, to show the contractile cells. The contractile cells are polygonal in shape and arranged like a tiled floor. Patches of the cells are exfoliated to reveal the underlying seminiferous epithelium, probably due to prolonged exposure of the specimen to HCl-collagenase. × 600.

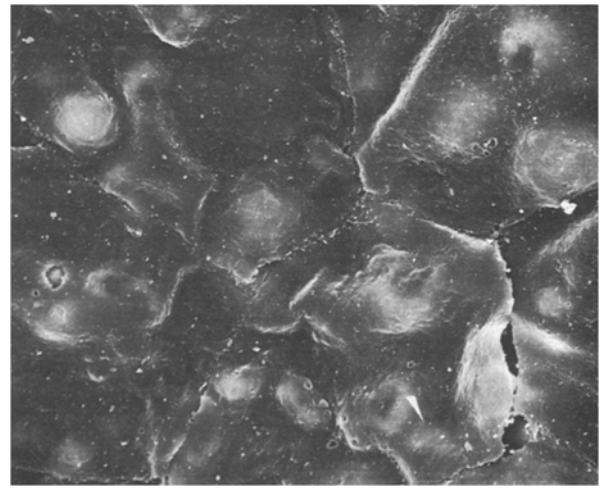

Fig. 2. High-magnified scanning electron micrograph of the contractile cells. × 2000.

known, but, this is probably an artefact due to physical cytoplasmic shrinkage involved in HCl digestion and collagenase splitting.


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## Epithelial cell processes in the development of the secondary palate in the mouse


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*Summary.* Ultrastructural studies of palatal shelves of Tuck A mice embryos aged 12.25–14.25 days show discontinuities of the epithelial basement membrane traversed by epithelial cell processes before the onset of midline degenerative changes.


Interaction between ectoderm and mesoderm is a well-known embryogenic mechanism playing a crucial role in the development of many organ systems. The precise way in which mesoderm and ectoderm interact, although much studied, remains unclear.

During the development of the rodent secondary palate an epitheliomesenchymal interaction brings about appropriately timed cell death in a midline epithelial seam which is essential for mesenchymal union between apposed palatal shelves[1-3]. The basal lamina, across which any interaction must take place, is usually described as being continuous until degeneration of this epithelium is complete[4-7], although occasional discontinuities in the basal lamina of the developing mouse palate have been described on the 14th day in utero[8,9]. Epithelial cell processes (ECPs) of basal epithelial cells pass through these discontinuities into the mesenchymal cell compartment. At this stage of development extensive degenerative changes may be observed in the midline epithelial seam, including the appearance of abundant lysosomes[4,8], swelling of mitochondria and clearing of the mitochondrial matrix[5] and pyknosis[4]. The presence of ECPs antedating the degenerative processes has not been previously described.

Pregnant Tuck A females were killed by cervical dislocation at 12.25, 13.25 and 14.25 days of gestation. Fetuses were removed from the uterine horns and the palatal shelves dissected out, using the method of Goss and Avery[11], rinsed in phosphate buffer (pH 7.4), fixed in phosphate-buffered glutaraldehyde at 4 °C for 1.5 h, and postfixed in buffered osmium tetroxide for 1 h. Individual shelves were embedded in TAAB resin at 60 °C for 3 days. Ultrathin sections were cut on an LKB IV ultratome using a glass knife. Mounted sections were stained with uranyl acetate and lead citrate, and examined using an AEI EM6B electron microscope.

Although the basal lamina was generally continuous beneath the epithelium in the region of presumptive fusion at both 12.25 and 13.25 days, occasional discontinuities were seen. Processes from basal epithelial cells passing through these discontinuities into the mesenchymal compartment (figure 1) were very much more common at 13.25 than at 12.25 days. There was no evidence of lysosome

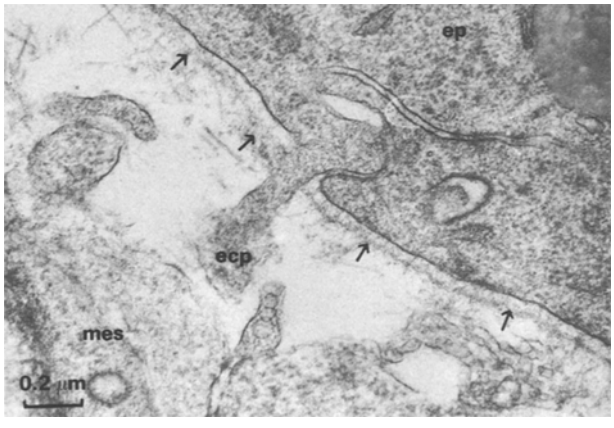


Fig. 1. Electron micrograph showing the basal lamina (arrowed) between epithelium (ep) and mesenchyme (mes) in the 13.25-day-old mouse palatal shelf. There is a discontinuity in the basal lamina which is traversed by an epithelial cell process (ecp). Bar 0.2 µm.

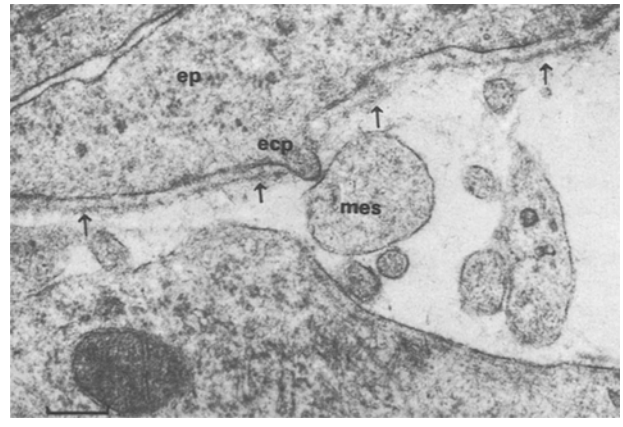


Fig. 2. Electron micrograph showing an epithelial cell process (ecp) in close contact with a subadjacent mesenchymal cell (mes). Bar 0.2 µm.