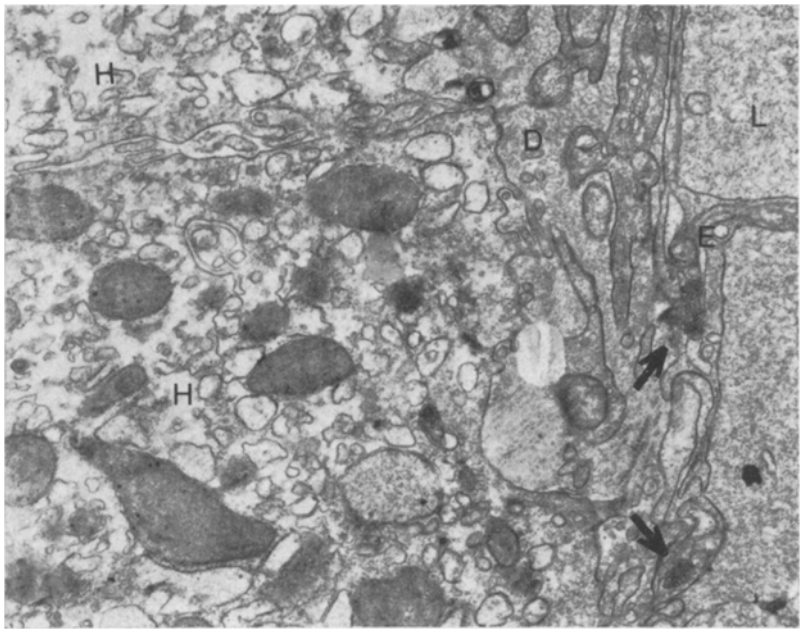

Fig. 2. Tubuloreticular structure (arrows) within dilated cisternae of endoplasmic reticulum of the endothelial lining (cappilarization) of the hepatocyte sinusoidal surface. H-hepatocyte; E-endothelial cell; D, space of disse; L, lumen.

suggest that they may reflect a cellular response to a broad range of stimuli[9–11]. The relationship between tubuloreticular structures and immunoglobulin synthesis or secretion, as demonstrated by POTHIER et al.[12], raises the hypothesis of a specificity to that response. The presence of tubuloreticular structures in the hepatic endothelium in a malignant melanoma metastatic liver may reflect a host cell response to the cellular proliferative state. A further possibility on their association with local host-tumor immunological reaction presents considerable interest as to the presence of these structures in this situation[13].

Summary. The presence of tubuloreticular structures within hepatic endothelial cells in a case of malignant melanoma liver metastasis is described. This finding may reflect a host cell response to the neoplastic proliferation in the liver tissue, possibly a host-tumor immunological reaction.

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Demonstration of Gap Junctions by Lanthanum in the Vitamin A Acid-Treated Skin Tumor, Keratoacanthoma

The topical application of vitamin A acid to the dry keratotic skin tumor, keratoacanthoma in rabbits results in a mucous metaplasia and accelerated regression in the tumors[1, 2].

In normal rabbit epidermis or in the untreated keratoacanthoma, the predominant cell junction is the desmosome. Other junctional complexes, particularly gap junctions are very sparse and are only occasionally observed. Tight junctions are never seen. In a recent paper, we have reported in the vitamin A acid-treated keratoacanthoma the numerous appearances of a cell junction having a similar substructure and dimensions that has been described for the gap junction[3–5]. These junctions form early in mucous metaplasia[6].

This study was undertaken to confirm the findings obtained with thin-section electron microscopy using the tracer material, lanthanum nitrate.

Materials and methods. 15 albino male rabbits (average weight, 2 kg) had the inner surface of their right ear

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auricles painted twice weekly with 1% 7, 12-dimethylbenzanthracene (DMBA) in equal parts of lanolin and mineral oil. After 7 weeks (14 applications), the application of carinogen to the ears was stopped with a yield of 7 to 8 tumors/ear. The tumors on 10 animals then received daily application of 3% vitamin A acid (kindly supplied by Hoffmann-La Roche, Inc., Nutley, New Jersey) in equal parts of lanolin and mineral oil. Each application of vitamin A acid (average, 0.10 g) was applied to a specific tumor by means of a wooden stick spatula. The vitamin A acid applications were continued daily for 5 days. 5 of the DMBA-treated animals received no vitamin A acid.

Five additional rabbits served as controls and had the inner surface of their right ear auricles painted only with vitamin A acid in the same amount and schedule as was applied on the ears with tumors. One separate rabbit was painted only with lanolin and mineral oil.

Biopsies were taken daily, beginning 24 h after the initial application of vitamin A acid and continuing 48 h after the last application. The biopsies were sliced into 1-mm^3 pieces and placed in 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer (pH 6.9) containing 1% lanthanum nitrate. Each of the subsequent solutions contained 1% lanthanum up to and including 70% ethanol. After glutaraldehyde, the tissues were washed in several changes of cacodylate buffer and followed by fixation in 1% osmium tetroxide in cacodylate buffer. The tissues were dehydrated in graded strengths of ethanol and embedded in Epon 812. Sections 1 µm thick and ultrathin sections were cut on a Reichert Ultramicrotome, stained with uranyl acetate followed by lead citrate, and examined in a Siemens Elmiskop 1A electron microscope.

Results. The ultrastructural morphology of normal rabbit skin epithelium and the untreated keratoacanthoma has previously been described[7]. The Golgi apparatus and the rough-surfaced endoplasmic reticulum are sparse. Desmosomes are the evident cell junction. In sections cut from tissue blocks treated with lanthanum,

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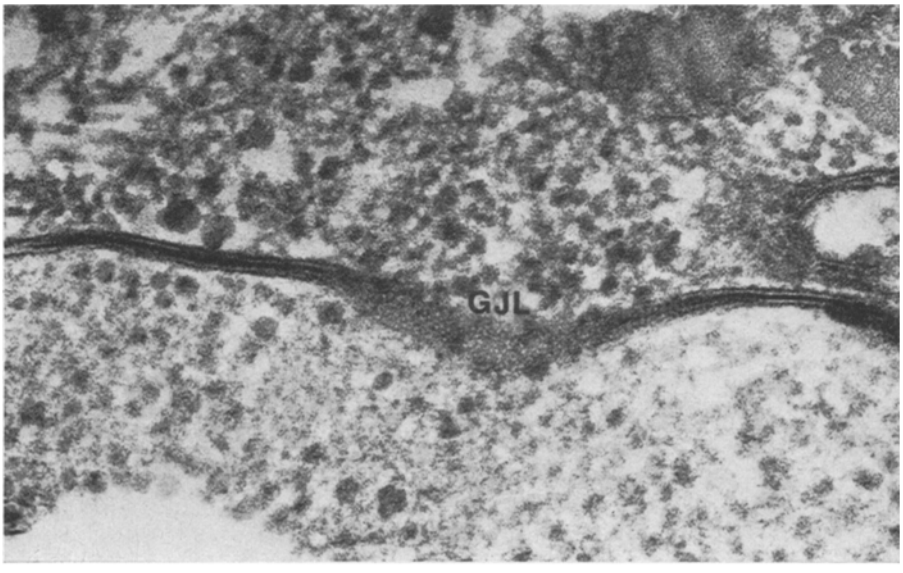

Fig. 1. The gap junction lattice (GJL) is seen in an oblique profile. ×112,750.

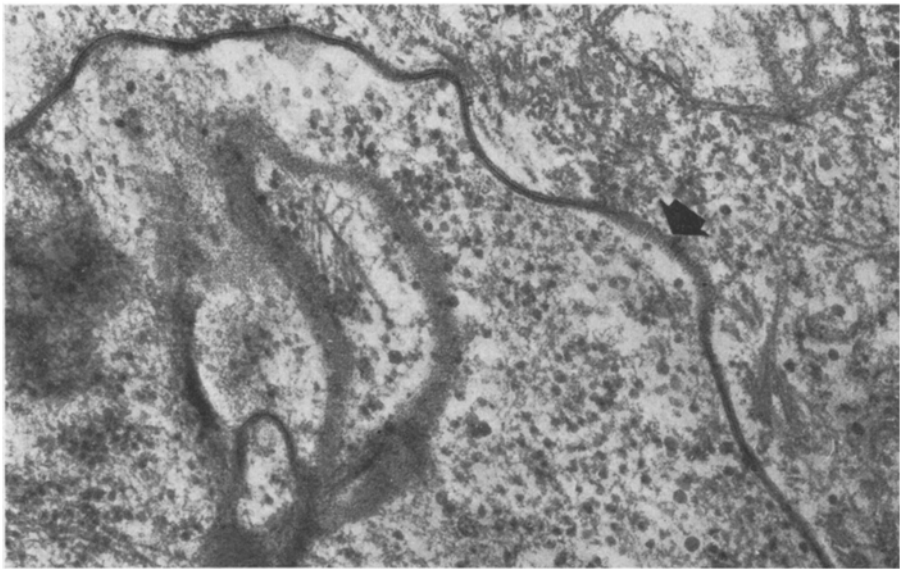

Fig. 2. The gap junction in the mucus producing tumor can assume a very long profile and measure 3 to 4 µm in length. Arrow points to gap junction lattice. ×56,000.

the intercellular spaces contain the electron dense tracer material. Occasionally, the lanthanum fills a gap junction between adjacent keratinocytes (average, 1 of 24 cells). Without lanthanum, the gap junction is difficult to find and is observed at an average of 1 of 37 cells. After 5 days of vitamin A acid treatment, the lanthanum tracer treated normal and tumor keratinocytes have gap junctions with an average of 1 of 21 cells.

Tumors treated with vitamin A acid show an increase in the Golgi complex and the rough-surfaced endoplasmic reticulum after 2 days of vitamin A acid applications. At this time period, gap junctions as visualized by lanthanum impregnation increase in their frequency of appearance (average 1 of 19 cells).

Lanthanum impregnation depicts the gap junction to be pentalaminar. The median line with its row of discontinuous electron densities is replaced by an uninterrupted electron dense line. The width of this line is 40–50 Å. In oblique section, a pattern of subunits is observed as the gap junction lattice (Figure 1). The width of the plasma membrane measures approximately 75 Å. The total thickness of the lanthanum impregnated gap junction is about 195 Å which is observed in the junction without lanthanum.

Examination after 5 days ot vitamin A acid treatment but without lanthanum depict tumor tissue with 1 of 15 cells having gap junctions between them. Examination after the 5th day of the vitamin A acid-treated tumor tissue also treated with lanthanum yield 1 of 8 cells with gap junctions between them. The intercellular contacts now can assume a very long profile and measure 3 to 4 μm in length (Figure 2).

Discussion. The present study, using the lanthanum tracer technique, positively demonstrates the appearance of the gap junction, particularly after vitamin A acid treatment. Without the use of an extracellular tracer, the gap junction is more difficult to observe and one is sometime unsure of its frequency of occurrence.

Gap junctions have been reported in human epidermis[8,9] basal cell cancer[10], wool follicle cells[11], liver[4], and cervical epithelium[12]. An increasing body of evidence has accumulated indicating the gap junction as a low-resistance pathway for cell to cell coupling. The gap junction lattice also seen in this study, has been suggested as an area to facilitate electronic coupling between cells[13]. This coupling demonstrates areas that exist for the exchange of small ions between cells. LOWENSTEIN[14], as well as other investigators, have suggested that the junction may be instrumental in exchanging substances that control cellular growth and differentiation[15,5]. The gap junction is found to increase in frequency of appearance early in mucous metaplasia. As mucus is copiously produced and secreted from the vitamin A acid-treated keratoacanthoma, gap junctions become the predominant cell junction. It is interesting to speculate that if growth and differentiation depend upon metabolic cooperation through mediators produced by other cells, then the mucus producing keratoacanthoma with its numerous gap junctions can probably act as a syncytium.

Summary. In normal rabbit epidermis or in the untreated skin tumor, keratoacanthoma the usual cell junction is the desmosome. Gap junctions are very sparse. The extracellular tracer material, lanthanum nitrate was used to confirm the definite identification and increase of gap junctions in the vitamin A acid-treated keratoacanthoma. Without the use of lanthanum, the gap may not be always apparent in conventional thin sections and can be confused with the zonula occludens.

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Actin in Tracheo-Bronchial Ciliated Epithelial Cells

In the last few years, contractile proteins, similar to actin and myosin found in smooth muscle cells and blood platelets, have been demonstrated in many non-muscular cells[1]. They have been implicated in cell activities such as motility, division, and exocytosis[1,2]. This paper describes the presence of actin in the apical portion of tracheobronchial ciliated epithelial cells.

The trachea of 7 deeply anaesthetized (i.p. Nembutal, 17 mg/100 g) male Wistar rats (200–220 g) was cannulated and, after bilateral pneumothorax, 3.5–4.5 cm^3 of 10% gelatin solution[3] were injected into the trachea at a constant pressure of 10 cm H_2O in order to prevent damage to cilia. After dissection, the trachea and lungs were dipped into liquid nitrogen for 10 min, and then brought back to a temperature of −20 °C. Longitudinal sections of the trachea and main bronchi were cut on a cryostat, and 2 consecutive sections mounted on the same slide; one was treated with a serum containing anti-actin antibodies (AAA)[4], the other with normal human serum. Both sections were then stained with fluorescein-conjugated IgG fraction of goat antiserum to human IgG[4]. Human tracheal and bronchial samples from one autopsy case without pulmonary disease were collected 1 h after death, frozen in a cryostat, sectioned and treated as above.

For electron microscopic studies, the trachea and lungs of 5 male Wistar rats (150–200 g) were fixed by perfusion[5], and those of 4 rats and 1 human lung were fixed by instillation[3]. Tissues were postfixed in OsO_4 and embedded in Epon[3].

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