thelium cells by chimiotactic attraction[10], the latter present a syncytial aspect, probably caused by lytic activity of the former[11]. Since the rims of the ovarian cortex in birds are much less advanced in development than the central parts, it may be possible that such phenomena still occur in our experimental conditions. Where the central part of the surface of the quail's ovarian cortex comes in close contact with the corresponding central part of the chicken's ovarian cortex, no invasion of quail cells into the latter (or vice-versa) can be seen (figure 2).

B. Part of the cortex of some transplanted ovaries from 16-day-old quail embryos penetrates into the underlying CAM mesenchyme. The ingrowing quail cell mass may invade even the deeper layers of the CAM mesenchyme, at a considerable distance (300 µm) from its point of origin. Several cortical buds may develop from a common stalk (figure 3). In the central part of the buds, usually a lumen appears. The lumen is lined by a narrow layer of quail oocytes and quail epithelial cells, separated from the CAM mesenchyme (composed of chicken somatic cells) by the PAS-positive basement membrane. Between the chicken somatic cells of the CAM mesenchyme surrounding the buds, some quail cells may be seen. These cells are accompanying ovarian stroma or medullary cells. The buds formed by the ingrowing quail ovarian cortical cells have some resemblance to the acini found in the gonads of gastropods[12].

Developing transplants are usually progressively enclosed in a paraovarian CAM cavity. After a prolonged sojourn (for instance after 2 successive transplantations on CAM), the quail germinal epithelium and oocytes are able to separate themselves completely from the medullary tissue, by invading the epithelial lining of this paraovarian CAM cavity (figure 4). As contrasted with the opinion of other authors[13,14], our results seem to indicate that an isolated germinal epithelium (completely free from adhering ovarian cells) is no longer able to give rise to/or to induce the formation of medullary tissue. Our observations are in agreement with the conclusions of Erickson[15] that the factors that control the differentiation of the female germ cells reside in the cortex ovarii.

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Association of asymmetric unit membrane plaque formation in the urinary bladder of adult humans with therapeutic radiation[1]

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Summary. Asymmetric unit membrane (AUM) is a component of the luminal membrane of urinary bladder in many species. In normal human adults it is inconspicuous, but it becomes prominent following incidental exposure to therapeutic irradiation.

One component of the luminal membrane of normal bladder urothelium is 'asymmetric unit membrane' (AUM) plaques. These plaques are present in many species[2]. They are found in humans during childhood, but are relatively inconspicuous in adults[3]. The current report describes an increase in AUM-plaques in areas of normal appearing urothelium of adults who have been irradiated for transitional cell carcinoma elsewhere in the urinary bladder.

Materials and methods. Biopsies of histologically normal appearing urothelium were obtained during surgery from 2 categories of patients: a) 7 adult patients without a history of bladder irradiation; 4 who had benign lesions, i.e. urethral obstruction or benign prostatic hyperplasia, and 3 who had transitional cell carcinomas elsewhere in the bladder; and b) 7 adult patients who had histories of irradiation for transitional cell carcinomas. The 7 irradiated patients had received 5000 R to the bladder. One was irradiated a month and a half, four 3 months, one 4 months and one 4 years prior to the biopsy. We also examined histologically normal bladder urothelium obtained at the autopsies of 3 human neonates who were free of genitourinary disease and were never irradiated. The specimens were cut into 1–2 mm³ tissue blocks and fixed in cold 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, for 1 h. They were post-fixed in 0.1 M cacodylate buffer with 1% osmium tetroxide, dehydrated through graded ethanol solutions and embedded in Epon 812. 50–70 nm thin sections were cut for electron microscopy on an LKB 8801 A ultramicrotome. They were stained with uranyl acetate and lead citrate and photographed with a Philips EM 300 electron microscope. In addition, glutaraldehyde-fixed 1–2 mm³ tissue blocks from 2 irradiated bladders were soaked in 20% glycerol in 0.1 M Millonig's phosphate buffer and then freeze-fractured at –100 °C in a Balzers model BAF 301 M freeze-etch machine, by the method of Moor and Mühlethaler[4].

Results and discussion. The luminal membranes of superficial urothelial cells of newborn humans contain abundant AUM-plaques which are morphologically identical to those described in other species[2]. As is typical of AUM-plaques in general, the human AUM-plaques are 12 nm

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thick, with an outer leaflet that is thicker but less defined than the inner one. AUM-plaques are joined by symmetrical interplaque membrane which is approximately 10 nm in thickness. The cytoplasm of the superficial cells contains dilated fusiform vesicles, which are partially lined by AUM-plaques. The luminal membrane in human adults, in contrast to that found in infants, consists almost entirely of interplaque membrane and is devoid of AUM-plaques (figure 1). The paucity of luminal membrane AUM-plaques in adult humans and their abundance in the urinary bladders of human infants and of relatively short lived species[2], suggests that loss of plaques in human adults may be related to the aging process. Interestingly, changes in other specialized membrane components of other cell types have also been associated with aging in vivo[5,6] as well as in vitro[7]. However, other factors such as diet[8] may contribute to the loss of AUM-plaques in human adults.

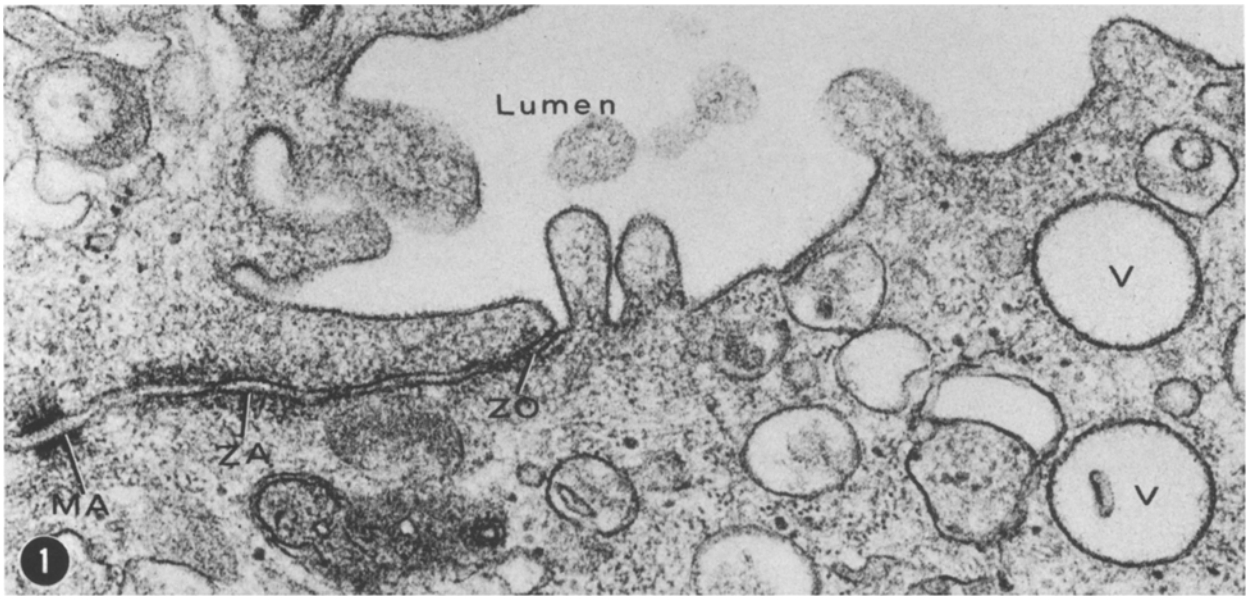

Fig. 1. Luminal surface of superficial cells in urinary bladder of a 'control' adult human. The patient was a 21-year-old female with congenital incomplete urethral stenosis and mild bladder obstruction. Bladder urothelium was essentially normal on histological examination. The luminal membrane is devoid of AUM-plaques and the cytoplasmic vesicles are lined by symmetric unit membrane. The superficial cells are joined together by a zonula occludens (ZO), a zonula adherens (ZA), and a macula adherens (MA) cell junction. ×86,000.

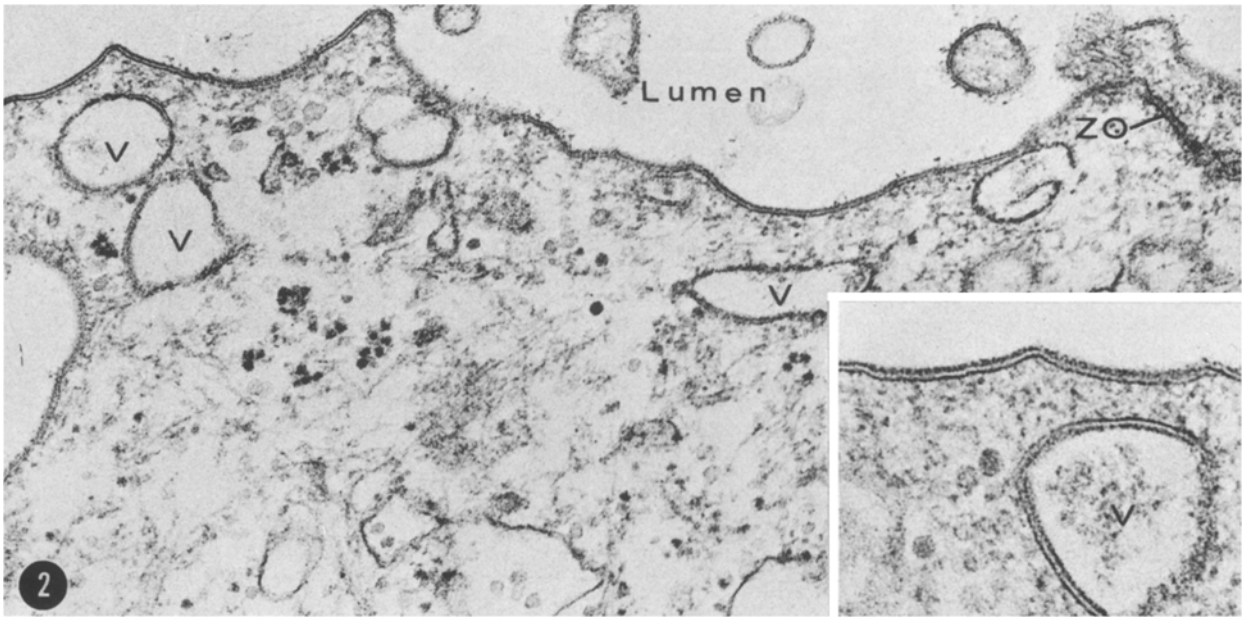

Fig. 2. Luminal urothelial cells in normal urothelium from a patient 4 years post irradiation. The patient had a transitional cell carcinoma surgically removed from a distant site in the bladder prior to irradiation. Luminal membrane is characterized by the presence of AUM-plaques, which are also components of the wall of cytoplasmic vesicles (V). The cells are joined by a zonula occludens (ZO) cell junction. ×86,000. Insert: Higher magnification of luminal and vesicular AUM-plaques. ×143,000.

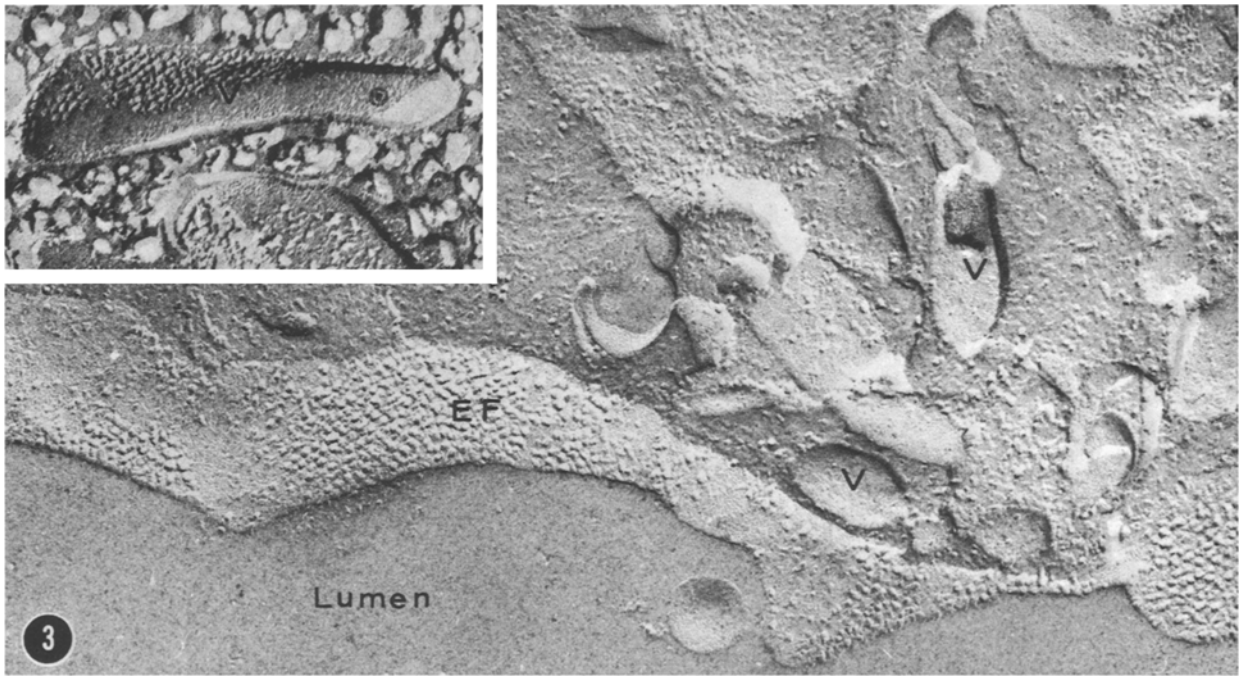

Fig. 3. Freeze fracture replica of an area of normal urothelium from a patient 4 months post irradiation. The patient had an invasive, poorly differentiated transitional cell carcinoma elsewhere in the bladder at the time of biopsy. EF face of luminal membrane is characterized by the presence of AUM-plaques, which contain arrayed particles and smooth interplaque areas. ×87,500. Insert: EF face of a fusiform vesicle (V) with AUM-plaque. 87,500.

The luminal membrane in histologically normal areas of urothelium, from adult patients who received total bladder irradiation for transitional cell carcinoma elsewhere in the bladder, contains AUM-plaques in abundance. In contrast, AUM-plaques are absent in areas of normal appearing urothelium from similar patients who have not been irradiated. In addition to the many AUM-plaques found in the luminal membrane of irradiated patients (figure 2), the cytoplasm of the superficial cells is rich in fusiform vesicles which have AUM-plaques as a component of the vesicle wall (figure 2, insert). Similar vesicles are not present in the nonirradiated urothelium. Freeze-fracturing of irradiated adult urothelium discloses polygonal plaques of various sizes packed with particles 6 nm in diameter (figure 3) on the extracellular fracture face (EF). They correspond to the AUM-plaques[9,10], while the relatively smooth areas correspond to the symmetric interplaque membrane. The protoplasmic fracture face (PF) displays the complementary polygonal plaques which instead of pits have fine granules. EF fracture of the fusiform vesicles discloses areas of packed particles (figure 3, insert) similar to those seen in the luminal surface. We have not observed the plaques in freeze-fracture replicas of nonirradiated adult urothelium.

Luminal membrane AUM-plaques are normal components of fully differentiated bladder urothelium in many species[2], including human infants[3]. The plaques are formed within the Golgi complex[11,12] and are believed to serve as membrane attachment sites for tonofilaments within luminal cells[9]. AUM-plaques are markedly reduced in number in urinary bladder urothelium in adult humans. X-irradiation influences cell differentiation as has been shown in studies on neuroblastoma cells which undergo irreversible differentiation in vitro following irradiation[15]. In vivo, X-irradiation restores in invasive urinary bladder carcinoma cells, the blood group isoantigens[16], which are a constituent of the cell membrane surface coat[17]. Furthermore, X-irradiation is known to increase the amount of Golgi complex[18], and its products[19]. This was suggested to be the key organelle in somatic regeneration in the replacement of injured membrane structures[20]. Since AUM-plaques are assembled within the Golgi complex, it is likely that AUM-plaque formation in adult human urothelium following X-irradiation is the result of Golgi complex stimulation. Our observations also suggest that loss of AUM-plaque formation by the Golgi complex may be a normal part of the aging process.

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