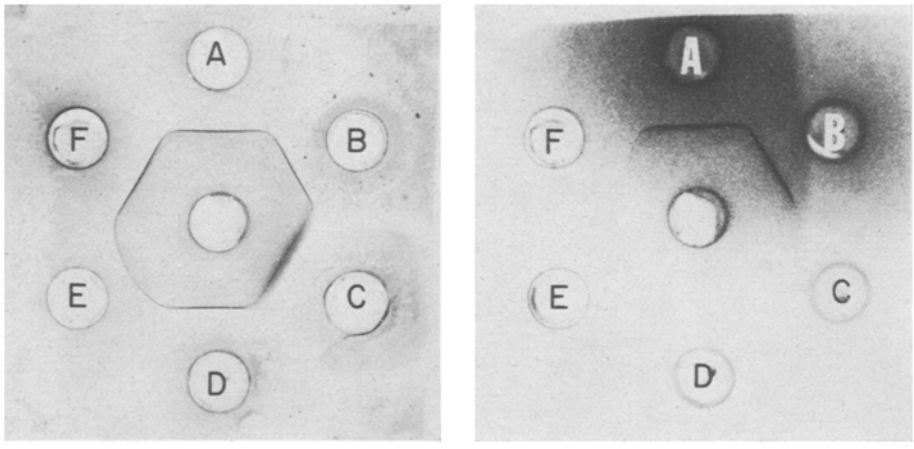

Double immunodiffusion patterns in agar (left) and radioautograph (right) obtained by incubating Hodgkin's diseae splenic tumor tissue (wells A and B) and normal appearing spleen tissue distinct from tumor nodules from the same patient (wells E and D). Wells (F) and (C) contained unlabelled human ferritin from spleen and liver respectively.

from tumor nodules did not produce demonstrable radioactive labelling of ferritin. In one such incubation, however, a trace amount of labelled ferritin was detected. This could be due to either a low level of ferritin synthesis by normal spleen/tissue or synthesis of ferritin by small foci of tumor cells which had infiltrated the normal splenic tissue area used for incubation. In support of the latter explanation, microscopic examination of sections of normal appearing spleen tissue distinct from tumor nodules did reveal varying degrees of tumor infiltration, although some areas were tumor free.

These results demonstrate increased ferritin synthesis by Hodgkin's disease splenic tumor tissue, and suggest that this is the cause of the elevated tumor and serum ferritin concentration found in patients with Hodgkin's disease.

Summary. Increased ferritin synthesis by Hodgkin's disease splenic tumor tissue was demonstrated by incorporation of ^{14}C-leucine and radioautography. This suggests that elevated tumor and serum ferritin concentrations found in patients with Hodgkin's disease is derived from tumor tissue per se.

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Patterns of Membrane Organization in Toad Bladder Epithelium: a Freeze-Fracture Study[1]

The urinary bladders of amphibians have been widely used as models of the collecting tubules of the mammalian kidney in respect to the transport of sodium, water and the hydrogen ion[2–4]. In its upper part, the collecting tubule is composed of two cell-types, the 'dark' and the 'clear' cells. In a recent freeze-fracture study[5], characteristic plasma membrane differentiations in each of these cell-types were defined: the membrane of the dark cell contains rod-shaped particles, whereas that of the clear cell exhibits square arrays of small particles. Using the freeze-fracture technique in a similar study of the toad bladder, we present evidence that the plasma membrane of one cell-type of this epithelium, the mitochondria-rich cell, also contains rod-shaped particles, while another cell-type, the granular cell, displays numerous large particles luminally in the B-fracture face of its plasma membrane. These membrane features differ sharply from the general membrane pattern seen in most other cells, as revealed by this technique[6].

Material and methods. Toads (*Bufo marinus*) were obtained from Mogul-Ed Co., Oshkosh, Wisconsin, USA. After doubly pithing the toads, we excized the urinary bladders and mounted them in glass chambers. The serosal side of the epithelium was exposed to a Ringer's solution of standard composition[7] and the mucosal side to the same solution diluted 10 times. After exposure to these solutions for various periods, the tissues were quickly removed from the chambers and fixed with a 2% glutaraldehyde solution containing 0.1 *M* phosphate buffer. For freeze-fracture studies, small pieces of epithelium cut from the original sheet were soaked in a phosphate-buffered 30% glycerol solution, then freeze-fractured in a Balzers BAF 301 unit[8]. For conventional thin-sectioning, pieces of glutaraldehyde-fixed epithelium were postfixed in phosphate-buffered osmium tetroxide, dehydrated, and embedded in Epon. Freeze-fracture replicas and thin sections, deposited on coated copper grids, were examined in a Philips EM 300 electron microscope.

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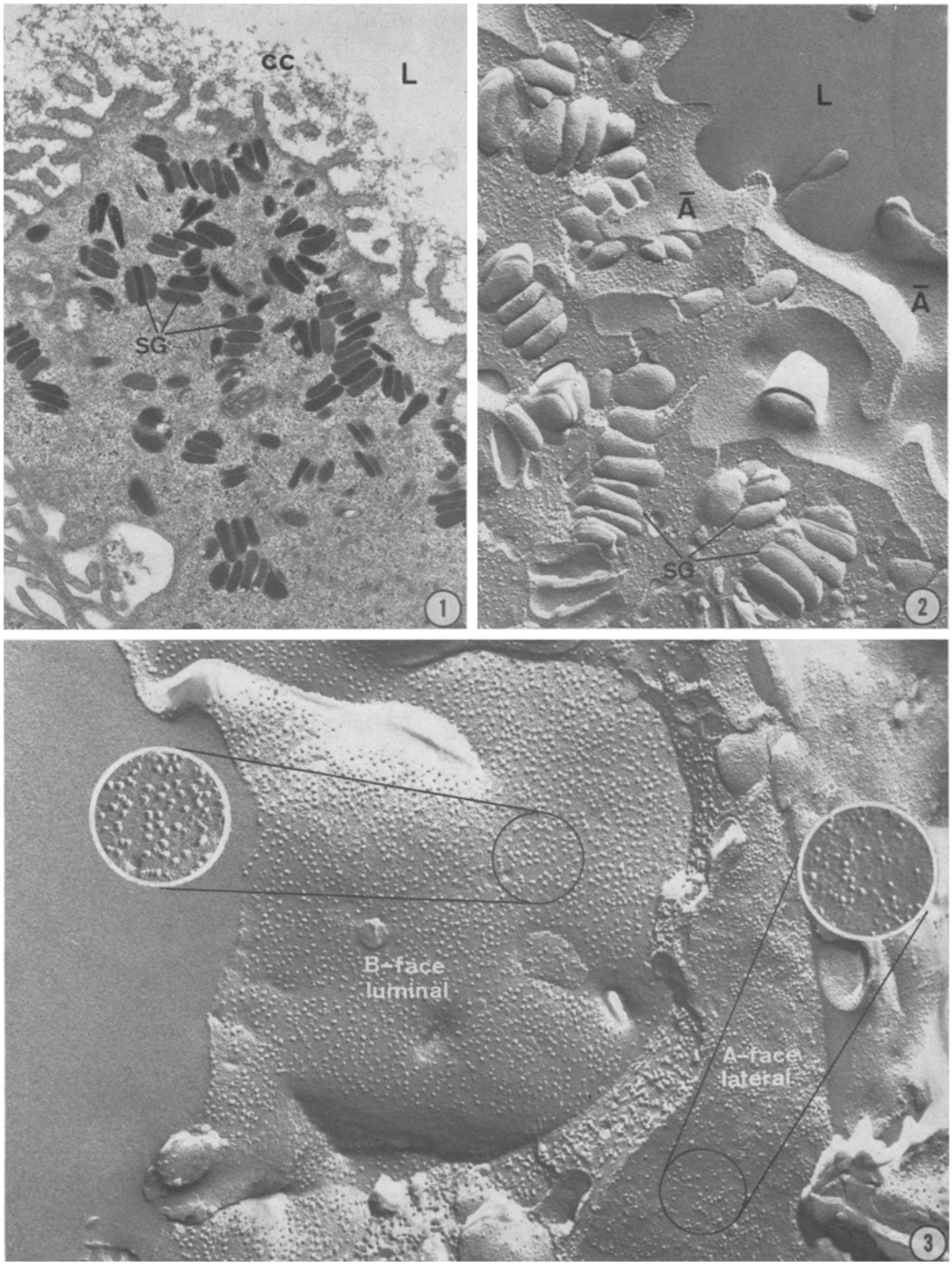

Fig. 1. Granular cell (thin section): The cytoplasm contains numerous electron-dense, flattened granules (SG), which are often stacked. The luminal surface has projections (or ridges) covered with a filamentous cell-coat (cc). × 13,000.

Fig. 2. Granular cell (freeze-fracture replica): Stacked granules (SG) are particularly evident in the cross-fractured cytoplasm. Areas of the luminal part of the plasma membrane (Ā), which represent the A-face, are poorly particulate. × 33,000.

Fig. 3. Granular cell (freeze-fracture replica): In this case, the plane of fracture reveals a large region of the luminal area of the plasma membrane. This is a B-face. Note its content of numerous large particles (also see circular inset). This organization contrasts with the membrane region visible in the lower right-hand corner – an A-face of the plasma membrane (lateral portion) of the same cell. The particles in this face are also numerous but smaller than those of the B-face (also see circular inset). L = lumen of the bladder. × 52,000. Insets: × 86,000.

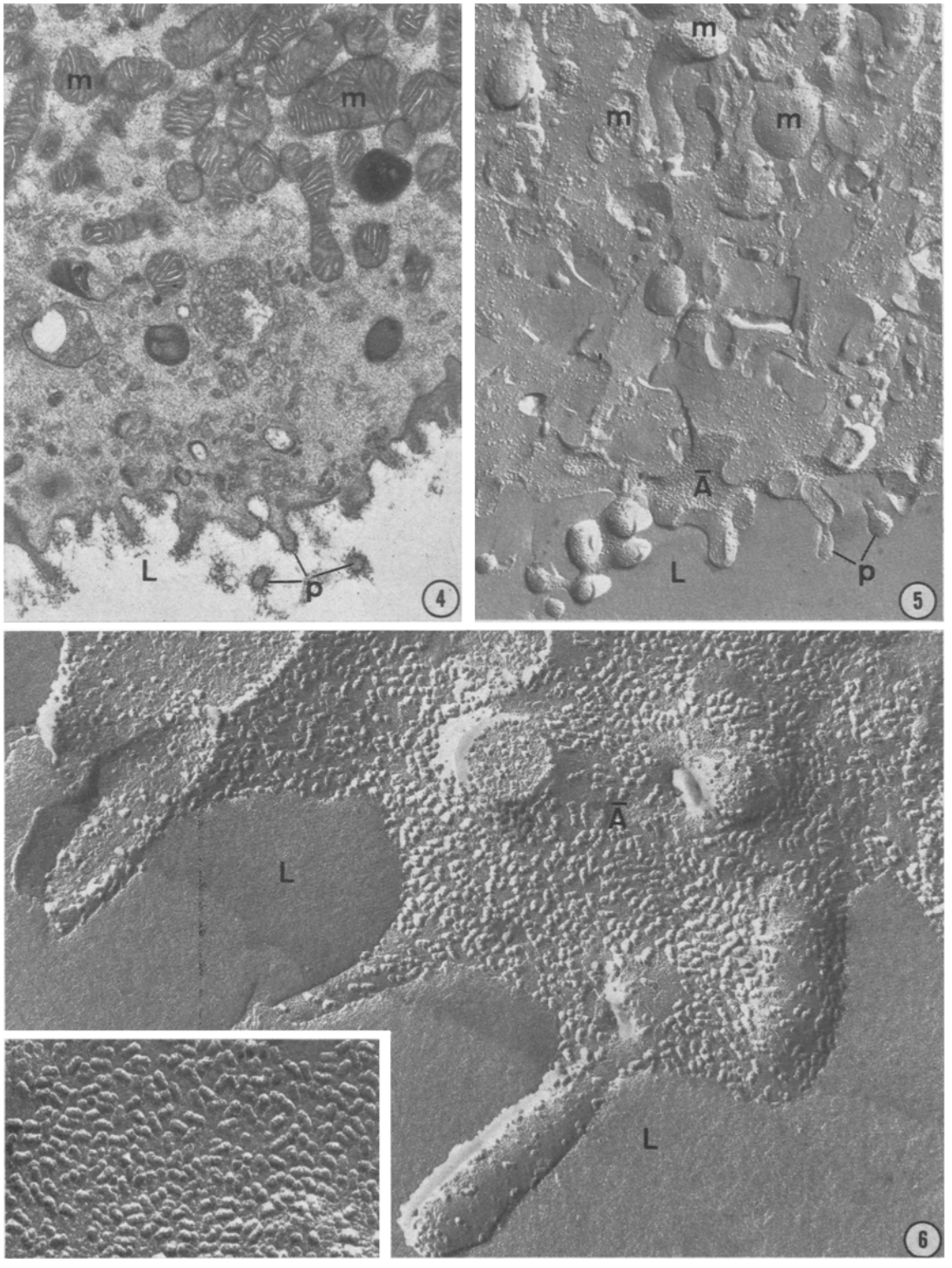

Fig. 4. Mitochondria-rich cell (thin section): The cellular cytoplasm contains a host of mitochondria (m), and, as in the granular cell (see Figure 1), the luminal surface exhibits projections (p) covered with a filamentous cell-coat. × 14,000.

Fig. 5. Mitochondria-rich cell (freeze-fracture replica): The presence of numerous mitochondria (m) gives a characteristic, bumpy cytoplasmic fracture. Small areas of the luminal portion of the plasma membrane (Ā) are revealed (A-faces) and noted to contain rod-shaped particles, illustrated at higher magnification in Figure 6. × 30,000.

Fig. 6. Mitochondria-rich cell (freeze-fracture replica): A-face of the luminal portion of the plasma membrane (Ā), indicating that the membrane contains two populations of particles: one formed of the usual globular particles, the other formed of elongated or rod-shaped particles (see inset). L = lumen of the bladder. × 94,000. Inset: × 110,000.

Results and discussion. Four basic cell types can be identified in the epithelium lining the toad bladder[9,10]: the granular cell (by far the most frequent: 85%), the mitochondria-rich cell, the goblet cell, and the basal cell. Granular cells (Figure 1), mitochondria-rich cells (Figure 4), and goblet cells span the entire width of the epithelium, from the lumen of the bladder to the basal lamina, whereas basal cells never reach the lumen[11]. In freeze-fracture, the first three cell-types manifest characteristic fracture faces easily distinguished in replicas. Also, when the fracture plane involves their plasma membranes, specific patterns can be recognized. The plasma membrane of the goblet cell has an A-face (or inner fracture face) rich in globular particles, and a less particulate B-face (or outer fracture face) – a pattern corresponding to the organization typical of cell membranes. In comparison, the plasma membranes of both the granular and the mitochondria-rich cells display a highly unusual organization at their luminal poles (Figures 2, 3, 5 and 6).

Luminally, the A-face of the granular cell membrane contains fewer particles ($800 \pm 20/\mu m^2$) than the B-face ($1500 \pm 90/\mu m^2$) (Figures 2 and 3), and the size of the particles differs as well, averaging 10 ± 0.2 nm in diameter in the A-face and 16 ± 0.4 nm in the B-face. Moreover, the number of large particles in the B-face decreases sharply at the tips of microvilli in the granular cell. In contradistinction to the luminal portion of the membrane, laterally (below the level of the tight junction) and basally, the plasma membranes display the usual freeze-fracture pattern – namely, 1700 ± 100 particles/μm^2 in the A-face, and 300 ± 30 particles/μm^2 in the B-face, with a constant diameter of 12 ± 0.6 nm in both faces.

The luminal, lateral and basal areas in the plasma membrane of the mitochondria-rich cell are also specific in freeze-fracture appearance: whereas the B-face contains few globular particles, the A-face, in addition to globular particles, shows a large population of rod-shaped particles measuring about 16×29 nm (Figures 5 and 6). Rod-shaped particles were not detected in the B-faces. While this paper was being prepared for publication, an abstract on the same subject was published by WADE et al.[12]. The observations of these authors comply with ours except for the fact that they found the rod-shaped particles *only* in the lateral and basal portions of the plasma membranes of mitochondria-rich cells. Our data on the frequency of particles in the luminal part of the granular cell membrane B-face are also consistent with the findings of CHEVALIER et al.[13], although these authors did not mention that the particles in the B-face were larger than those in the A-face.

By allowing detailed observation of the membrane morphology of granular and mitochondria-rich cells, freeze-fracturing has unveiled organizational patterns which were undetectable in former studies of amphibian bladder – either by transmission[9,10] or scanning[14–16] electron microscopy. At present, it is impossible to assign a definitive role to such patterns in the various transport phenomena occurring in toad bladder epithelium. In particular, we would emphasize that uncertainties persist concerning the sites of action of hormones such as vasopressin and aldosterone. Conflicting experimental evidence has been reported – some in favor of the granular cell, some in favor of the mitochondria-rich cell as the target of these hormones[10,17–19]. It is generally agreed, however, that the apical part of the membrane is the locus where changes in permeability to sodium and water take place, particularly in the case of vasopressin[10,16,20]. Visualized in freeze-fracture, the specific membrane organization at the luminal poles of these cell-types appears to be of importance and should be further investigated in studies designed to answer this question.

Summary. Two cell-types of toad bladder epithelium show uncommon plasma membrane organization in freeze-fractured specimens. One type, the granular cell, contains a plasma membrane in which the A-face is poorly particulate luminally while the B-face discloses multiple large particles at this site. In contrast, the lateral and basal portions of the granular-cell membrane are typical in that more particles occupy the A-face than the B-face. In the other cell-type, which is mitochondria-rich, the plasma membrane, luminally, laterally, and basally, contains rod-shaped and a few globular particles in the A-face. We suggest that these two peculiar membrane organizations be considered in the localization of both vasopressin and aldosterone action in toad bladder.

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