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Studies on the Juxtaglomerular Apparatus

IV. Freeze-fracturing of Membrane Surfaces

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Summary. The juxtaglomerular apparatus of the rat was studied after freeze-fracturing with special respect to intercellular junctions. It was found that juxtaglomerular granulated cells of the vas afferens are interconnected by gap junctions to adjacent cells (granulated cells, possibly also smooth muscle cells). Gap junctions have also been found on the surface of lacis cells and mesangial cells. It is therefore concluded that these cells of the juxtaglomerular apparatus and the glomerulus—granulated cells (possibly also smooth muscle cells) of the vas afferens, lacis cells and mesangium cells—form a functional system reacting in a coordinated manner to physiological stimuli.

Key words: Juxtaglomerular apparatus (Rat) — Renin — Gap junctions — Freeze-fracturing, electron microscopy.

Zusammenfassung. Mit Hilfe der Gefrierbruchtechnik wurde der juxtaglomeruläre Apparat von Rattennieren untersucht. Dabei zeigte sich, daß die granulierten Zellen des juxtaglomerulären Apparates im Bereich des Vas afferens über gap junctions mit benachbarten Zellen (granulierte Zellen, vielleicht auch glatte Muskelzellen) verbunden sind. Gap junctions fanden sich auch auf Lacis- und Mesangiumzellen. Daher wird angenommen, daß diese Zellen des juxtaglomerulären Apparates und des Glomerulums — granulierte Zellen (vielleicht auch glatte Muskelzellen) der Arteriola afferens, Laciszellen und Mesangiumzellen — ein funktionelles System bilden, das auch auf nur lokal wirksame physiologische Reize einheitlich reagieren kann.

Introduction

The main function of the juxtaglomerular apparatus (JGA) is the synthesis and excretion of renin. Three possible parameters involved in the regulation of this function are currently being discussed: 1. the blood pressure in the afferent arteriole (Goormaghtigh, 1944; Blaine *et al.*, 1970; Blaine and Davis, 1971), 2. the tonus of the sympathetic nervous system (Gordon *et al.*, 1967; Assaykeen *et al.*, 1970; Tagawa and Vander, 1970; Passo *et al.*, 1971), 3. the sodium load at the macula densa (Thurau, 1964, 1966).

Irrespective of which of these theories is correct, all of the parameters are effective in the JGA at certain distances from the secretory cells, therefore necessitating the conduction of signals to these cells, especially if, during hypertrophy of the renin producing apparatus under extreme conditions, granulation even of the lacis and mesangial cells occurs (Barajas and Latta, 1967).

Currently gap junctions are often mentioned as an intercellular membrane differentiation, functioning not only as a site of mechanical adhesion, but also

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for metabolical and electrical coupling between adjacent cells (Dewey and Barr, 1964; Dreifuss *et al.*, 1966; Steere and Sommer, 1972; Friend and Gilula, 1972; McNutt and Weinstein, 1973).

With freeze-fracturing, a method especially suitable for investigating cell junctions, Pricam *et al.* (1974) discovered gap junctions on mesangial cells and discussed the coupling of these via lacis cells with JGA cells. In order to obtain basic morphological data concerning this assumption we investigated the entire JGA and especially the granulated Goormaghtigh-cells by freeze-fracturing.

Material and Methods

Male Wistar-rats of about 150 g, nourished on a standard diet and tap water ad libitum, were perfused through the abdominal aorta according to the modified technique of Forssmann *et al.* (1967), with 90 mM phosphate—buffered 2% glutaraldehyde after brief rinsing with procaine-containing electrolyte solution. Small pieces of kidney cortex were soaked in 30% glycerol-0.9% NaCl solution for about 1 h. The mounted specimens were frozen by immersion into liquid nitrogen after previous gas extraction by evacuation (Umrath, 1974). The specimens were then fractured at $-100°$ C in a Leybold-Heraeus freeze-fracturing unit EPA 100 (Leybold-Heraeus, Cologne), etched for 10–30 sec at a pressure lower than 10^{-6} Torr, shadowed with carbon-platinum (Glitsch, 1969) and replicated with carbon. The replicas were examined with Zeiss electron microscopes EM 9 and EM 10. 25 replicas of 3 animals were examined until one JGA showing the essential elements (macula densa, vas afferens, lacis cells) was found. The micrographs of freeze-fracture replicas are mounted with the shadows running from bottom to top; the shadows are white (direction of shadowing indicated by arrows).

Results

At low magnification all parts of the JGA in Fig. 1 are readily visible, the fracture plane being oblique to the vascular pole of the glomerulus. The macula densa cells with characteristic high nuclear-cytoplasmic ratio show no basal infoldings, scattered mitochondria throughout, and a Golgi apparatus at the basal pole of the cell. At the apex of the macula cells short microvilli are observed, the adjacent cell membranes are connected by tight junctions (Fig. 1, insert bottom left, Fig. 2). In addition to granulated cells, smooth muscle cells and lacis cells, the Bowman-capsule cells are easily identified (Fig. 1); the latter also showing ridges of tight junctions (Fig. 1, insert top right).

At higher magnification the surface of the granulated cells seems to be rather smooth, showing numerous groups of membrane associated particles (arrows Fig. 3). These particles sometimes form plaques (Fig. 4) or beaded ridges (Fig. 5). In fractures exposing the B face the corresponding impressions described for gap junctions are also seen (Fig. 5).

Lacis cells at the vascular pole are recognized by their fusiform to polygonal shape and their topography adjacent to podocytes and mesangial cells on one

Fig. 1. Low power magnification of a vascular pole of rat kidney glomerulus showing macula densa (left), the wall of the afferent arteriole, and part of glomerulus (right). × 5500. *N* nuclei of macula densa cells, *BI* basal invagination of macula densa cell, *GC* granulated cell, *SM* smooth muscle cell, *BC* Bowman's cell capsule, *LC* lacis cell, *PC* podocyte, *VL* vascular lumen. Insert at the top right: tight junctions of Bowman cell. × 34000. Insert at the bottom left: tight junction of macula densa cell. × 37000

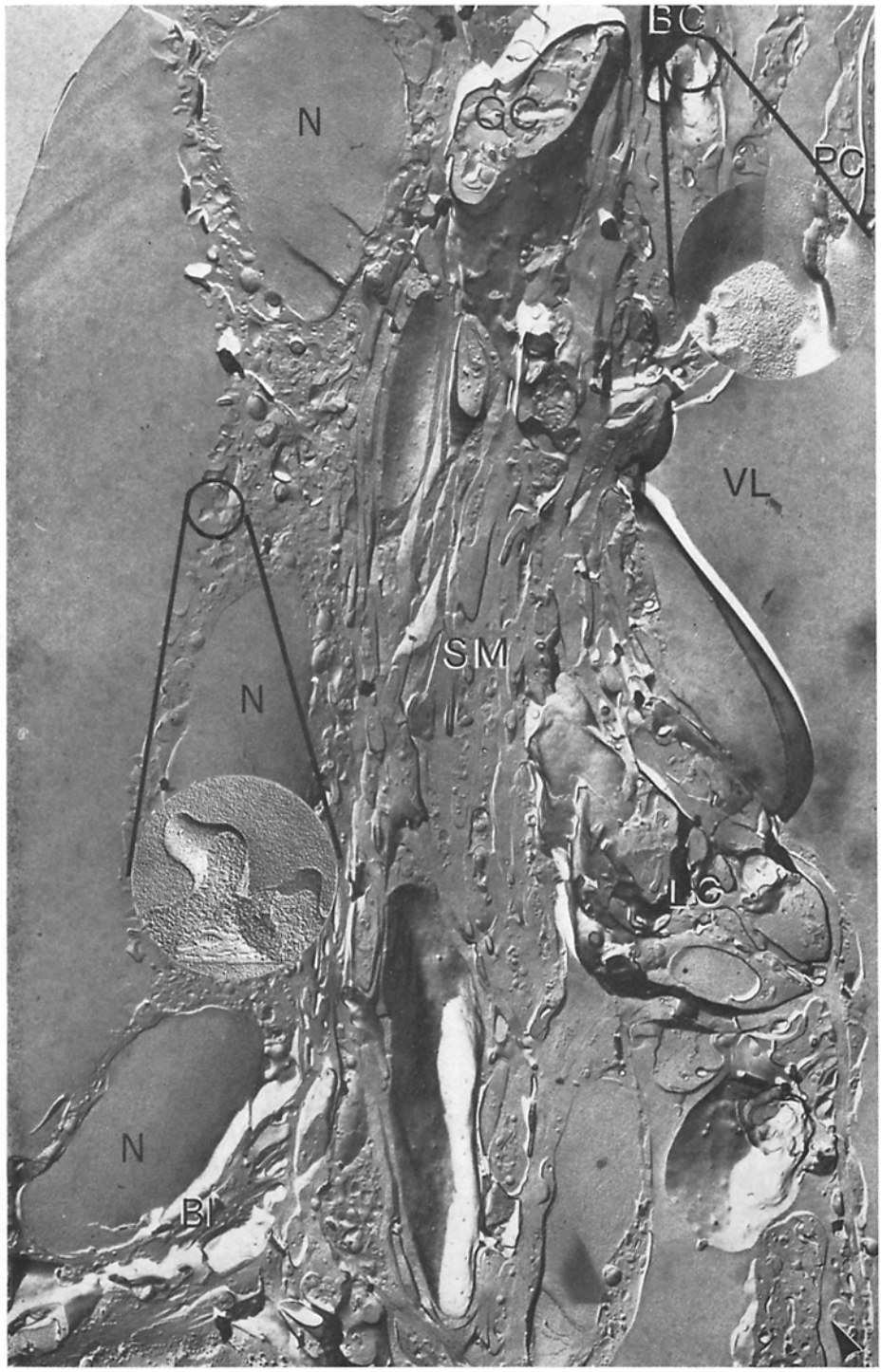


Fig. 1

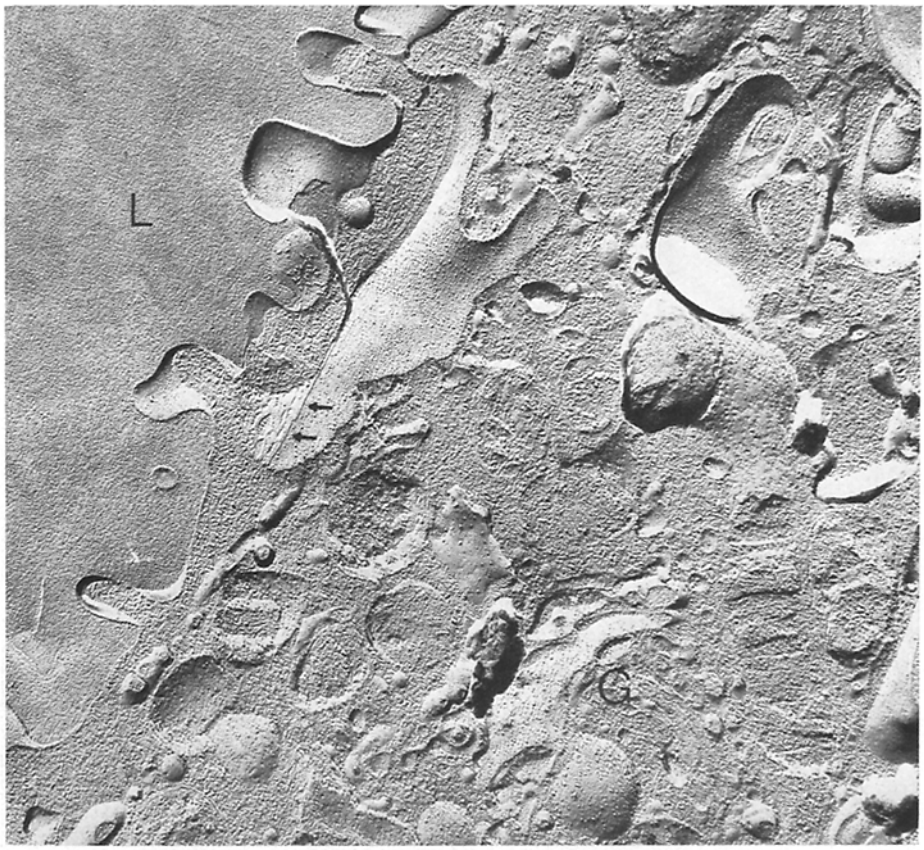


Fig. 2. Tight junction (arrows) of macula densa cell. ×40000. *L* tubular lumen, *G* part of the Golgi apparatus

side and to smooth muscle cells of the afferent arteriole on the other; between lacis cells and podocytes there is a distinct basement membrane (Fig. 6). Gap junctions at the membrane surface of lacis cells similar to those seen on JGA cells are found not only between lacis cells themselves (Fig. 7) but also where they contact smooth muscle cells of the afferent arteriole (Fig. 8). As can be seen in Fig. 9 and Fig. 10 the surface membrane of mesangial cells also shows gap junctions in our preparation. The variability of these gap junctions and both their A and B faces are clearly visible at higher magnification (Fig. 10).

Discussion

The distribution and size of gap junctions in the smooth muscle of various tissues are quite different: in contrast to hollow organs, where they are found more frequently (Dewey and Barr, 1964), in the muscular coat of blood vessels they are restricted to certain vessels or parts thereof (gizzard: Bennett and Cobb, 1969; Cobb and Bennett, 1969; portal vein: Holman *et al.*, 1968). It was suggested that

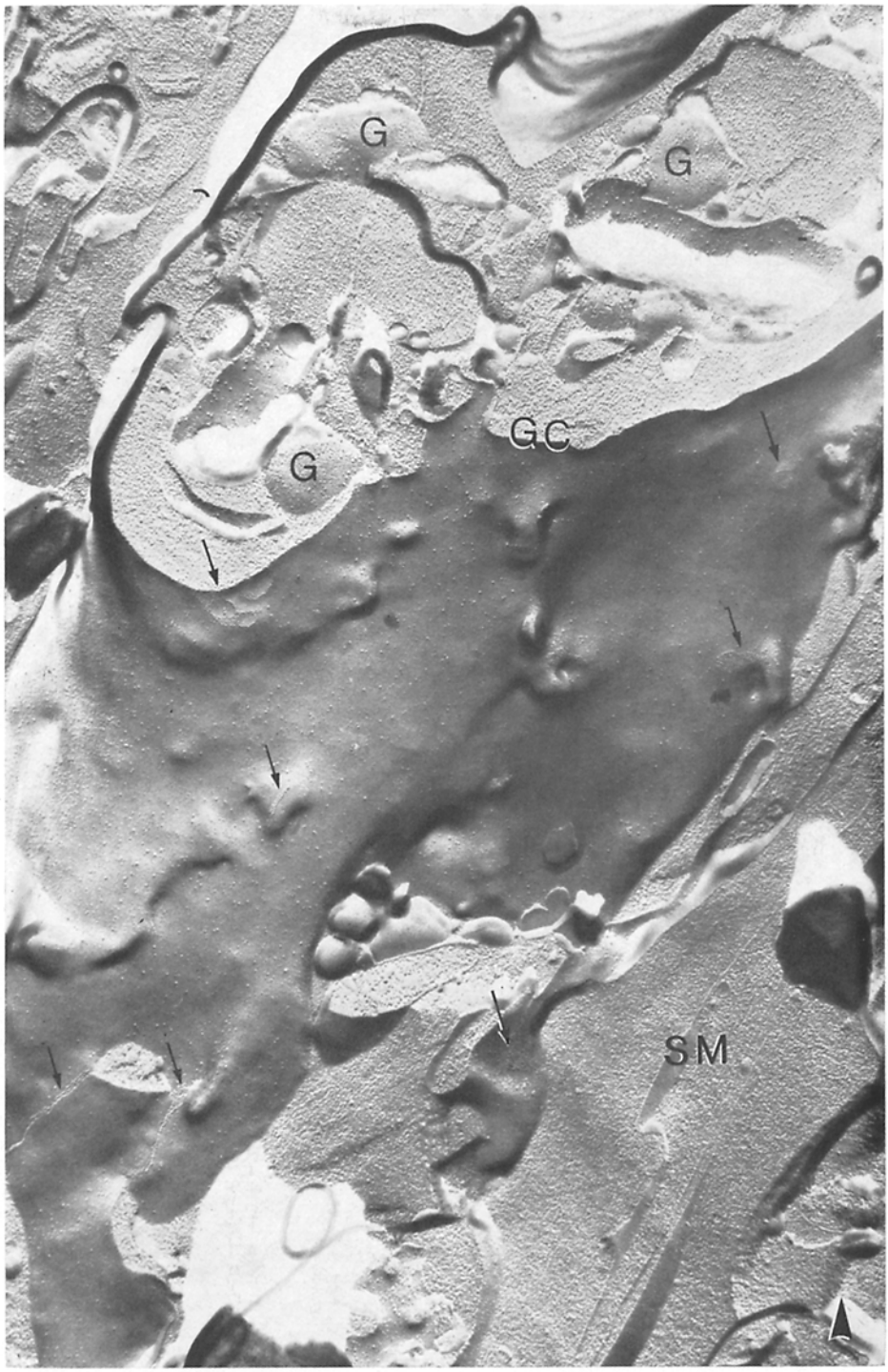
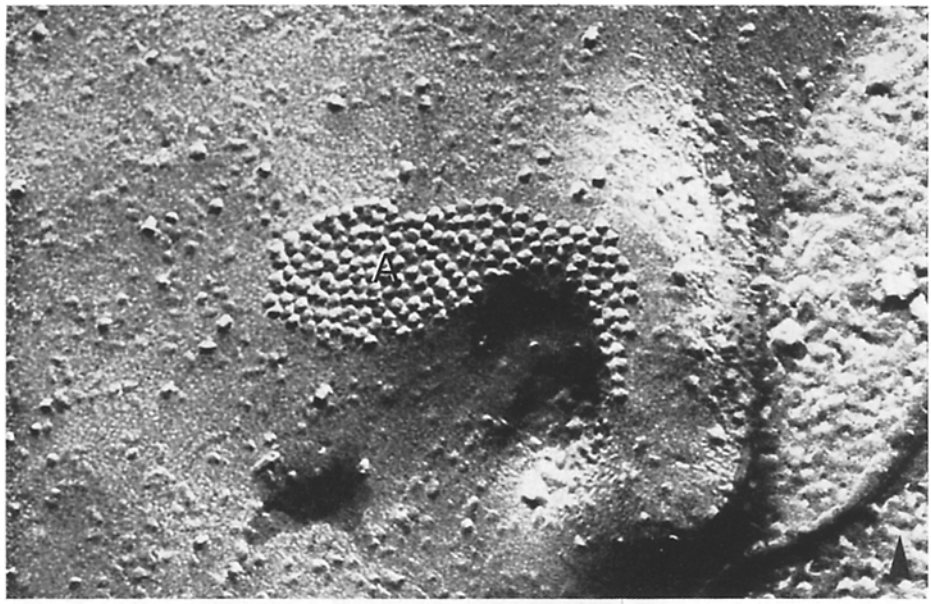


Fig. 3. Granulated cell (*GC*) in the wall of the afferent arteriole from Fig. 1. Numerous gap junctions at the cell surface (arrows) and specific granules (*G*) in the cytoplasm are seen. $\times 35000$. *SM* smooth muscle cell



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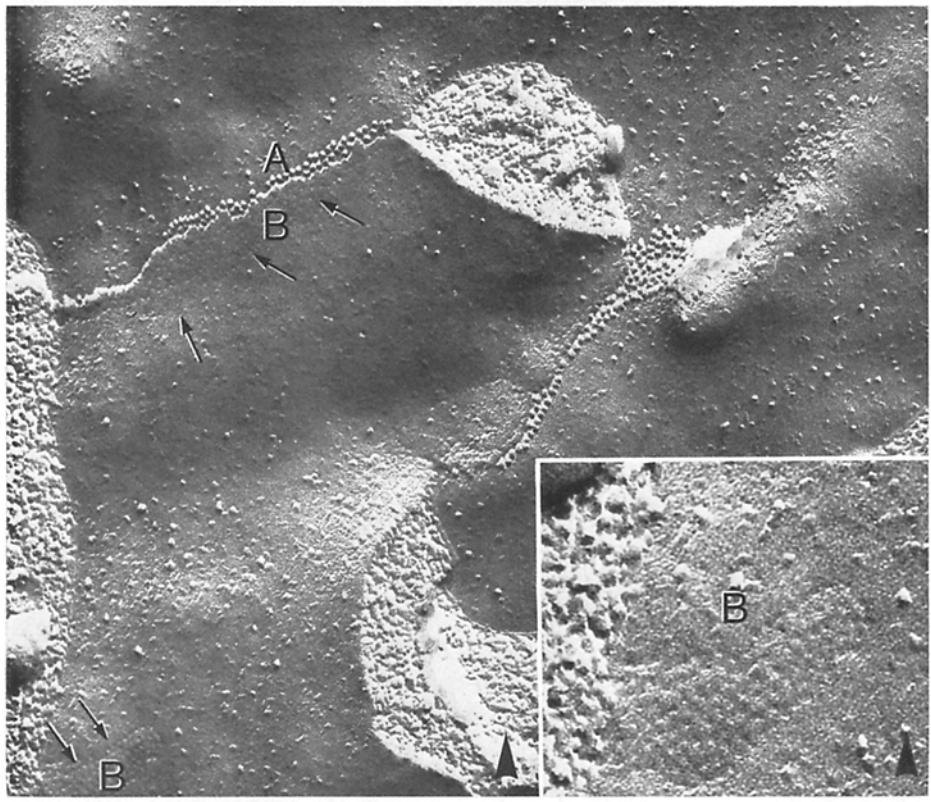

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Fig. 4. High magnification of gap junctions at the surface of the granulated cell seen in Fig. 3. $\times$ 210000

Fig. 5. Other gap junctions seen in Fig. 3. $\times$ 100000. Insert: high magnification of the gap junctions at bottom left (B face). $\times$ 200000

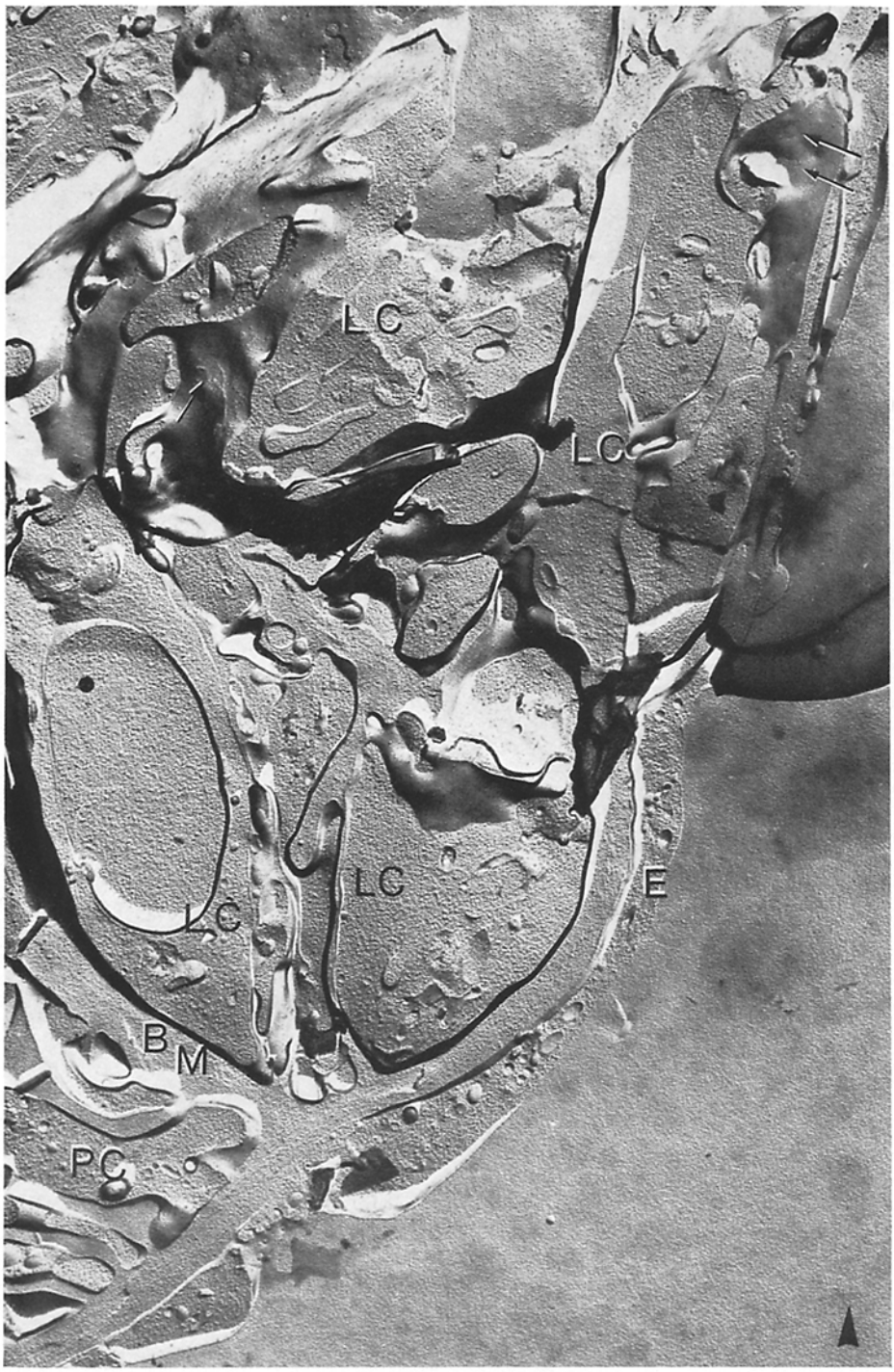

Fig. 6. Low magnification of lacis cells (*LC*) seen in Fig. 1. Arrows indicate gap junctions. $\times$ 20000. *E* endothelium, *BM* basement membrane, *PC* podocyte

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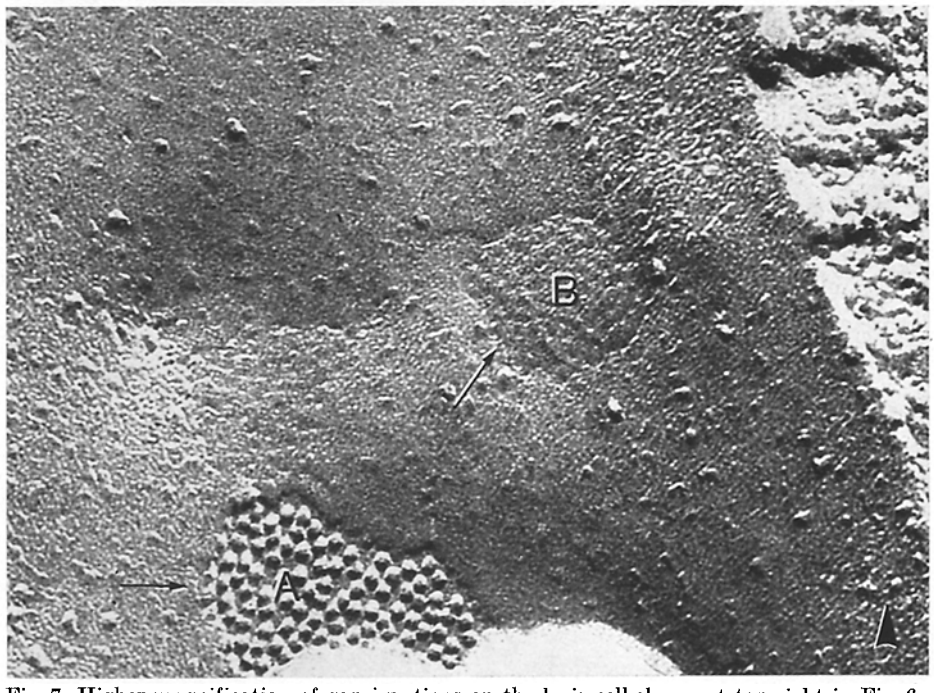

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Fig. 7. Higher magnification of gap junctions on the lacis cell shown at top right in Fig. 6. $\times 80000$

Fig. 8. Gap junction (A and B face) on the surface of another lacis cell shown at top left in Fig. 6. $\times 210000$

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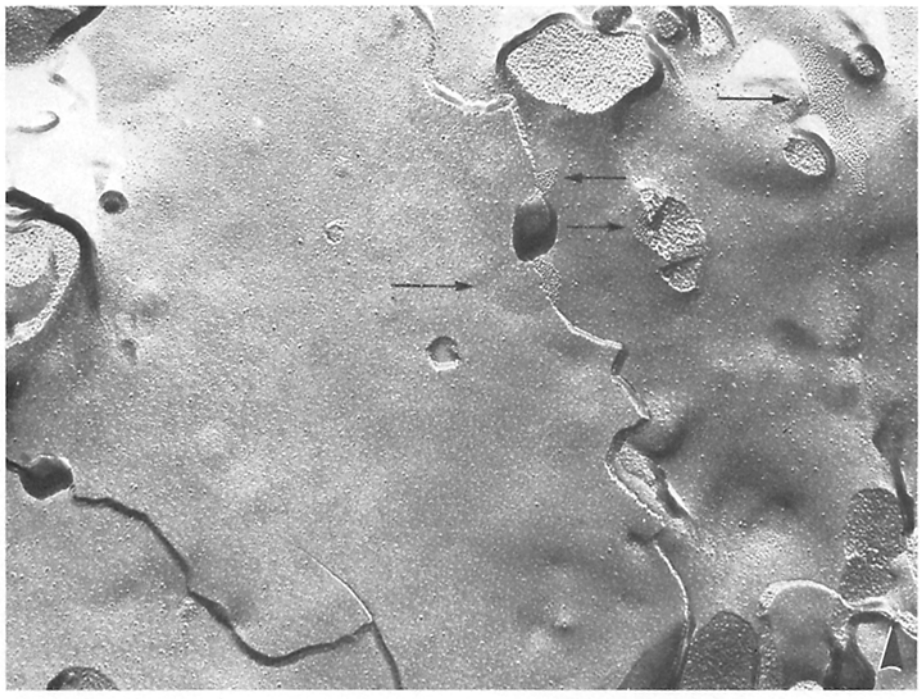

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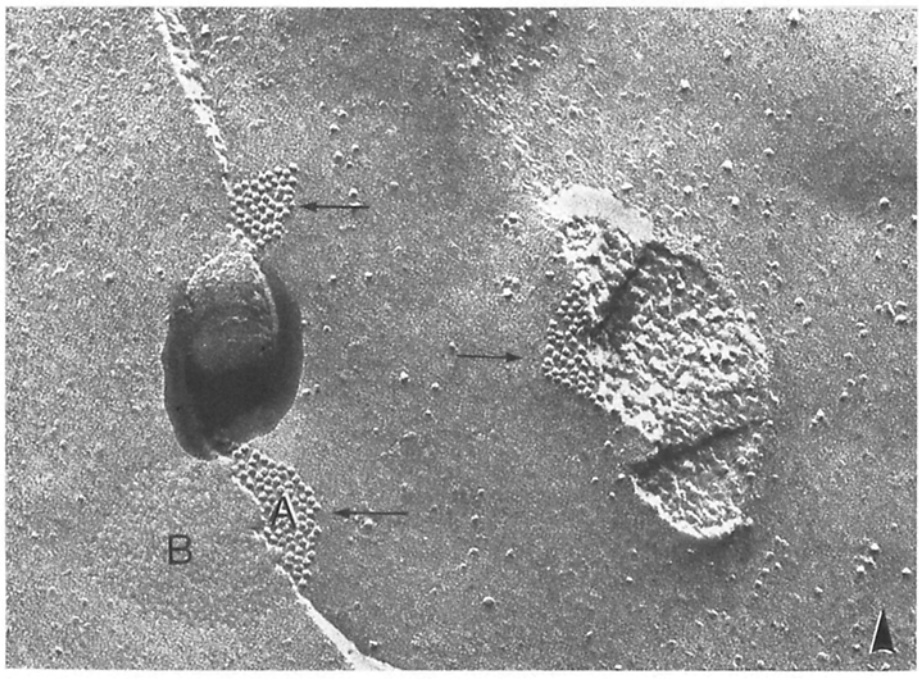

Fig. 9. Cell membranes of mesangial cells of rat glomerulus. × 60000
Fig. 10. Gap junctions between the mesangial cells shown in Fig. 9. × 120000

the presence of gap junctions in smooth muscles is correlated with the functional coupling of groups of smooth muscle cells which form units depending upon pace maker cells. Such an organisation, however, has not yet been demonstrated for the vas afferens and the JGA.

The present work shows gap junctions as a regular feature of granulated JGA cells of the vas afferens, lacis cells and mesangial cells. The plaque-like or ridge-like gap junctions described here correspond to those shown in other tissues. The existence of gap junctions in the wall of the afferent arteriole as well as between lacis and mesangial cells seems to prove Orci's presumption (see Pricam *et al.*, 1974) of a coupling of all these structures to form a functional system. A synchronized response of the JGA to local stimuli at any of these interconnected cells as discussed in the context of the three above mentioned theories is therefore possible.

In addition to the demonstration of gap junctions in the JGA, the present work also provides results concerning the pars maculata of the distal tubule: tight junctions of the macula densa do not differ from those found on the rest of the distal tubule (Friend and Gilula, 1972; Pricam *et al.*, 1974; see also Farquhar and Palade, 1963). Therefore the influence of distal tubular urine upon the JGA cells via intercellular spaces can be ruled out. It seems more probable that the influence of distal tubule sodium load on renin release is triggered by a transcellular process through macula densa cells.

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