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Localization of connexin43 in rat kidney

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Localization of connexin43 in rat kidney. The localization of connexin43 (Cx 43) in rat kidney was investigated by the indirect immunofluorescence technique with polyclonal antisera raised against Cx 43. Cx 43 is a gap junction protein expressed in a variety of tissues. The typically punctuated gap junction immunofluorescence (GJI) was observed in the renal arterial and arteriolar system. In the renal artery the GJI was concentrated in the media. In the juxtamedullary nephrons, the GJI is particularly abundant in the vascular bundles. There is abundant GJI in the extraglomerular mesangium while in the afferent arteriole GJI appears decreased. Abundant GJI was observed in the inner medullary collecting ducts and pelvic epithelium. The localization of Cx 43 immunofluorescence observed in this study is only in partial agreement with the results of ultrastructural investigations on the distribution of gap junctions in the kidney. An extensive tight junctional system has been demonstrated in the collecting duct system. However, gap junctions have been reported to be absent. Further studies to resolve this discrepancy are required.

Gap junctions are composed of subunits, named connexons which join with connexons in adjacent cells to form intercellular channels for the passage of ions and small molecules. X-ray diffraction studies have visualized the connexon as a hexameric assembly of integral membrane proteins (connexins) which delineate an axial transmembrane channel. In the last few years the genes for several connexin proteins have been cloned. Amino acid primary and secondary structures have been deduced indicating that connexins have homologous extracellular and transmembranous domains with their cytoplasmic domains remaining unique. Based on sequence analysis, the connexin gene family has been divided into two groups according to their similarities in two of the four transmembrane regions [1]. In rat, group I connexins are Cx 46, 43, 40, 37 and 33 while group II include Cx 32, 31.1, 31 and 26.

We are reporting here the results of an investigation on the localization of connexin43 in the rat kidney by immunofluorescence histochemistry.

Methods

Adult Sprague-Dawley rats were fixed by transcardial perfusion with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4. Kidneys were removed and kept in the same fixative for four hours. For cryoprotection, fixation was followed by immersion of the kidney in graded sucrose concentration of 10% and 20% in PBS overnight at 4°C. Kidney were embedded in the O.C.T. compound (Miles, Naperville, Illinois, USA), and snap-freezed in liquid nitrogen. Ten micrometer-thick sections were obtained with a cryostat microtome and mounted on gelatine coated slides. Sections were washed with 0.3% Triton X-100 in 0.1 M PBS for 30 minutes. After one hour of incubation with 3% normal goat serum in 0.1 M PBS, the sections were incubated overnight with polyclonal antibody raised against synthetic peptides representing unique portions (C-terminus of connexin43; amino acids 252-271) of the rat connexin43 gap junction protein [2] (provided by Dr. E. Beyer, Washington University, St. Louis, Missouri, USA) at 1:100 dilution in PBS containing 10% normal goat serum. The primary incubation was followed by a 30 minute wash with PBS and the sections were then incubated for one hour with FITC-conjugated goat anti-rabbit gamma globulin antiserum diluted 1:40 with PBS. After a wash period in PBS, slides were coverslipped with PermaFluor mounting medium. Control sections were incubated with normal rabbit serum at 1:100 but primary antibody was omitted. The sections were observed and photographed with a Zeiss photomicroscope equipped with epifluorescent illumination using a mercury lamp and a blue interference exciter filter set at 450 to 490 nm, an FT 510 chromatic beam splitter, and an LP 520 orange barrier filter. Photographs were taken with Kodak TMZ 3200 film.

Results

Vasculature

The typically punctuated gap junctions immunofluorescence (GJI) is observed in the renal arterial and arteriolar vasculature. In the renal artery (Fig. 1A), the GJI is concentrated in the media. As the renal artery branches into smaller arteries, a relatively high concentration of GJI in the media remains (Fig. 1 B, C). In the juxtamedullary nephrons, the GJI is particularly abundant in the vascular bundles (Fig. 1D) where it can be seen down to the level of the inner medulla. There is strong GJI in the extraglomerular mesangium (Fig. 2 A, B) and GJI is also observed in the beginning of the efferent arteriole in the midcortical and superficial nephrons. On the other hand, a clear decrease in GJI is often noted in the afferent arteriole as it approaches the glomerulus.

Non-vasculature

Abundant gap junction immunofluorescence was observed in the inner medullary collecting ducts (Fig. 3 A, B). No GJI could be detected with certainty in other tubules, although in the proximal tubules, the strong lysosomal autofluorescence may have interfered with the detection of the GJI. The inner layers of the pelvic epithelium also shows very profuse GJI (Fig. 4 A, B). No definite gap junction immunofluorescence was seen in the superficial cell layer.

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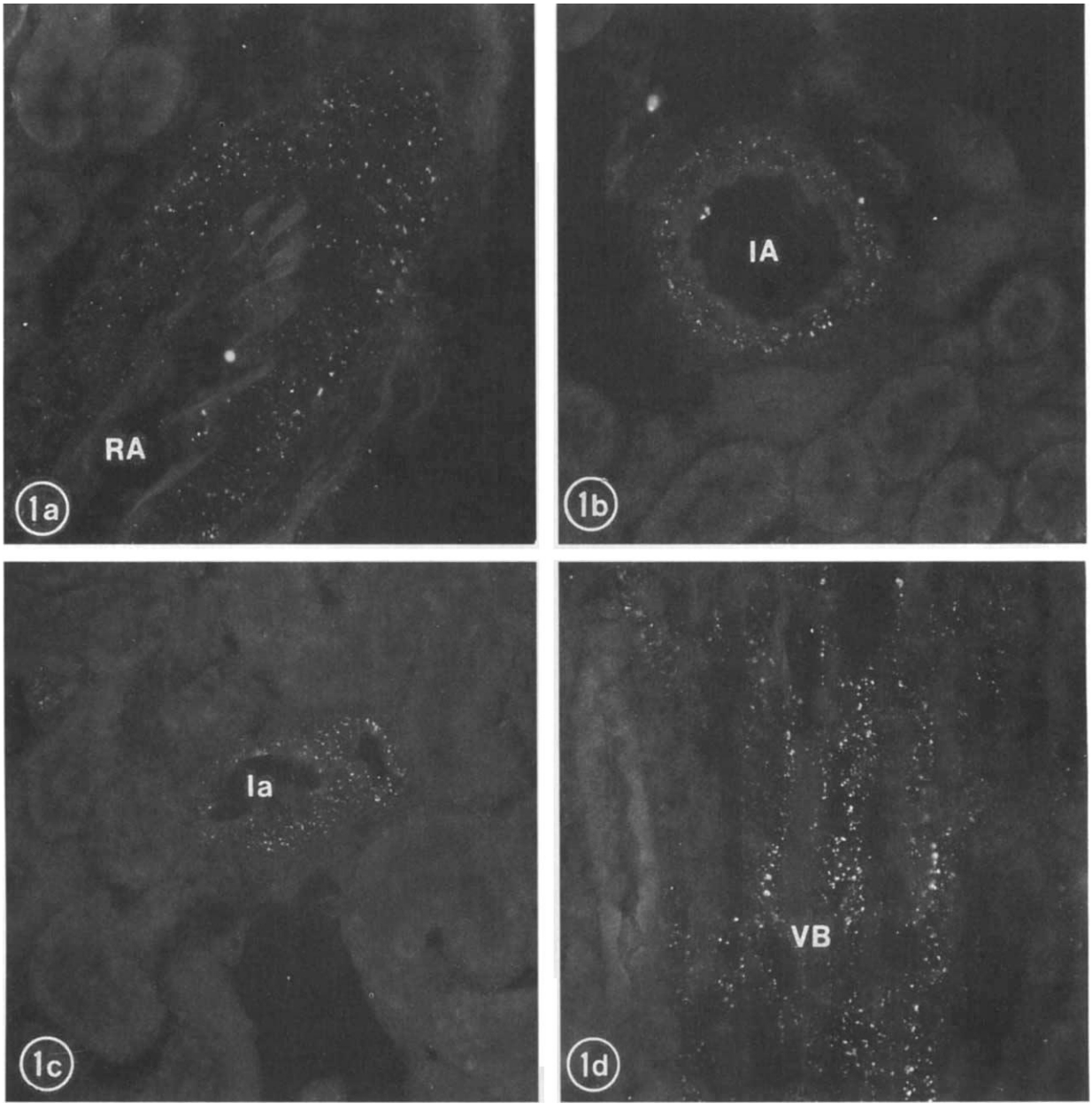

Fig. 1. *Punctuated immunofluorescence for gap junction protein Cx43 in the wall of the renal vessels.* The fluorescence is concentrated in the media. **A.** Renal artery, RA. **B.** Interlobar artery, IA. **C.** Interlobular artery, Ia. **D.** Vascular bundles, VB. ×400.

Discussion

At least five connexin genes are known to be expressed in the kidney: Cx 46, 43, 40, 32 and 26 [3–6]. Beyer et al [2] localized connexin43 by immunofluorescence in the kidney between the epithelial cells of all the tubules and in glomeruli and connective tissue. Connexin32 was also localized in the glomeruli and in the proximal tubule, also presumably between the epithelial cells. It is interesting that in spite of the extensive coupling of the extraglomerular mesangium by gap junctions, no localization to that region of any of the known connexins has been reported.

The connexin43 cDNA clone was originally isolated from rat heart encoding a predicted polypeptide of 382 amino acids [3]. Antisera against synthetic oligopeptides from unique regions of connexin43, have demonstrated immunoreactivity characteristic of gap junctions in a variety of tissues [2]. Of particular relevance to this study is the demonstration of connexin43 messenger RNA expression in cell cultures of many microvascular and macrovascular smooth muscle cells and endothelial cells [7]. No expression for connexin32 or connexin26 was found in the same cells.

The kidney is an extraordinarily heterogenous organ with a rich vasculature. The localization of connexin43 immunofluorescence observed in this study is in agreement with the results of ultrastructural investigations on the distribution of gap junctions in the juxtaglomerular apparatus. With the use of transmission and freeze fracture ultrastructural methods, gap junctions have been found in abundance in the vascular

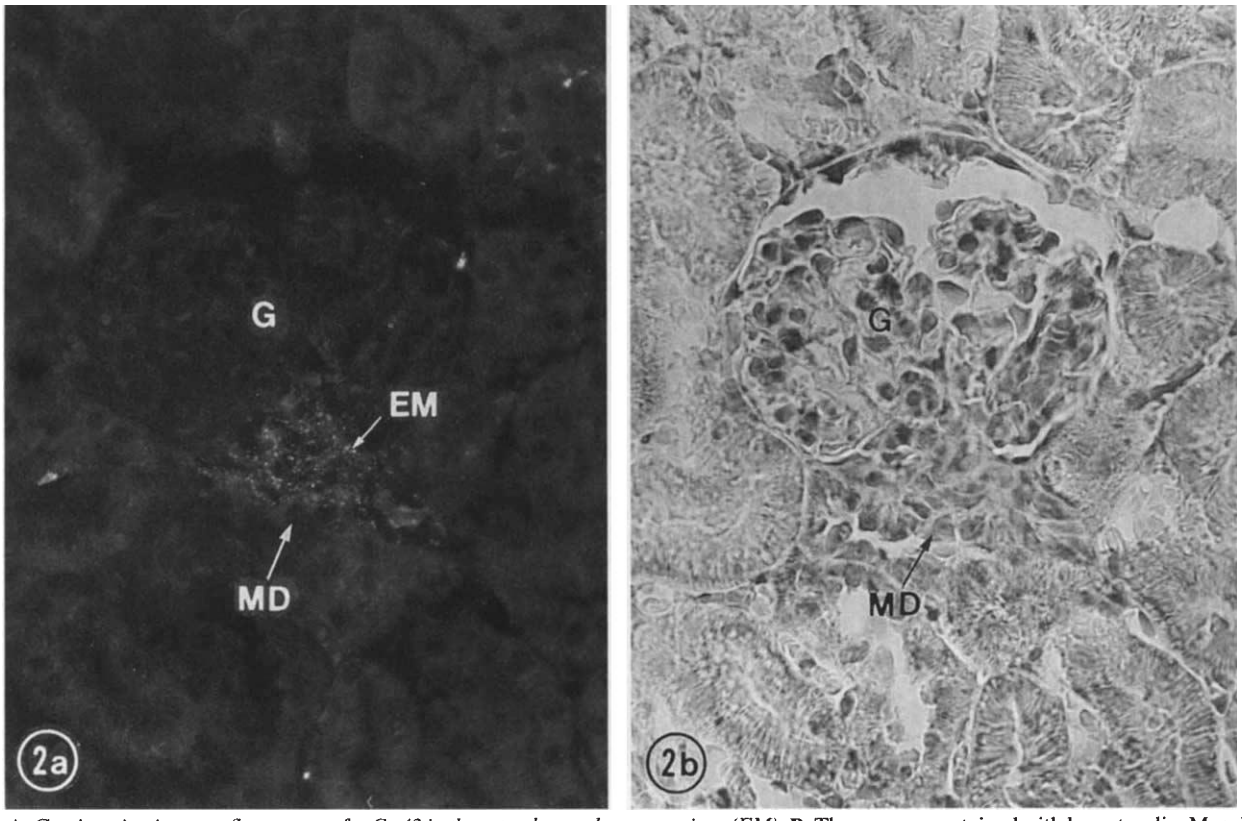

Fig. 2. A *Gap junction immunofluorescence for Cx 43 in the extraglomerular mesangium (EM).* **B.** The same area stained with hematoxylin. Macula densa, MD. Glomerulus, G. ×500.

component of the juxtaglomerular apparatus, where they are seen coupling the smooth muscle cells, JG granular cells and the extraglomerular mesangial cells [8–11]. In addition, gap junctions connect the extraglomerular mesangial cells with the intraglomerular mesangial cells [11–13]. Gap junctions have also been described between the endothelial and smooth muscle cells of the glomerular arterioles, indicating a generalized coupling between all the cellular types of the vascular component of the JGA [12, 13]. Of particular relevance to the mechanism of tubuloglomerular feedback (TGF) is the unusual abundance of gap junctions reported connecting the cells of the extraglomerular mesangium [8, 14, 15]. This region, which is in mandatory contact with the macula densa, is a required pathway for the tubular stimuli to reach the vascular effector arm of the TGF response. The abundance of gap junctions in the extraglomerular mesangium, which contains no capillaries, is consistent with the observed presence of large numbers of gap junctions on other avascular structures such as the lens or ovarian follicles, where they are thought to have an homeostatic role [1]. Our observations of a concentration of connexin43 GJI in the extraglomerular mesangium is a strong indication of its possible importance in juxtaglomerular function. The decrease in GJI at the level of the afferent arteriole may be related to its unique cellular composition since in this vessel the renin-containing juxtaglomerular myoepithelial cells are interspersed among smooth muscle cells. The abundance of GJI in the juxtamedullary efferent arteriole and vascular bundles indicates coupling of the smooth muscle cells which are known to be present in the walls of this vessels over much of their course into the medulla [14].

The gap junction immunofluorescence for connexin43 was unexpectedly high in the inner medullary collecting ducts and in the pelvic epithelium. Previous ultrastructural investigations have indicated the presence of an extensive tight junctional component between the cells of the collecting duct system. However, although gap junctions have been reported to be absent in the collecting duct [15, 16], mRNA for connexin43 has been localized in the collecting ducts by *in situ* hybridization [17]. We could not demonstrate immunofluorescence for connexin43 in the cortical renal tubules with certainty. Additional investigations using *in situ* hybridization, immunocytochemistry and electron microscopic techniques would help to clarify the presence of gap junction protein connexin43 in the renal tubules.

Given the common embryologic origin of the collecting duct and the pelvic epithelium, it is not surprising that both exhibit immunoreactivity to connexin43. It is unclear why only the inner medullary portion of the collecting ducts shows definite gap junction immunofluorescence for connexin43. It might be related to the more homogeneous cell population of this segment. It is also of interest that this portion of the nephron is closest to the pelvic epithelium which also shows strong gap junction immunofluorescence for connexin43.

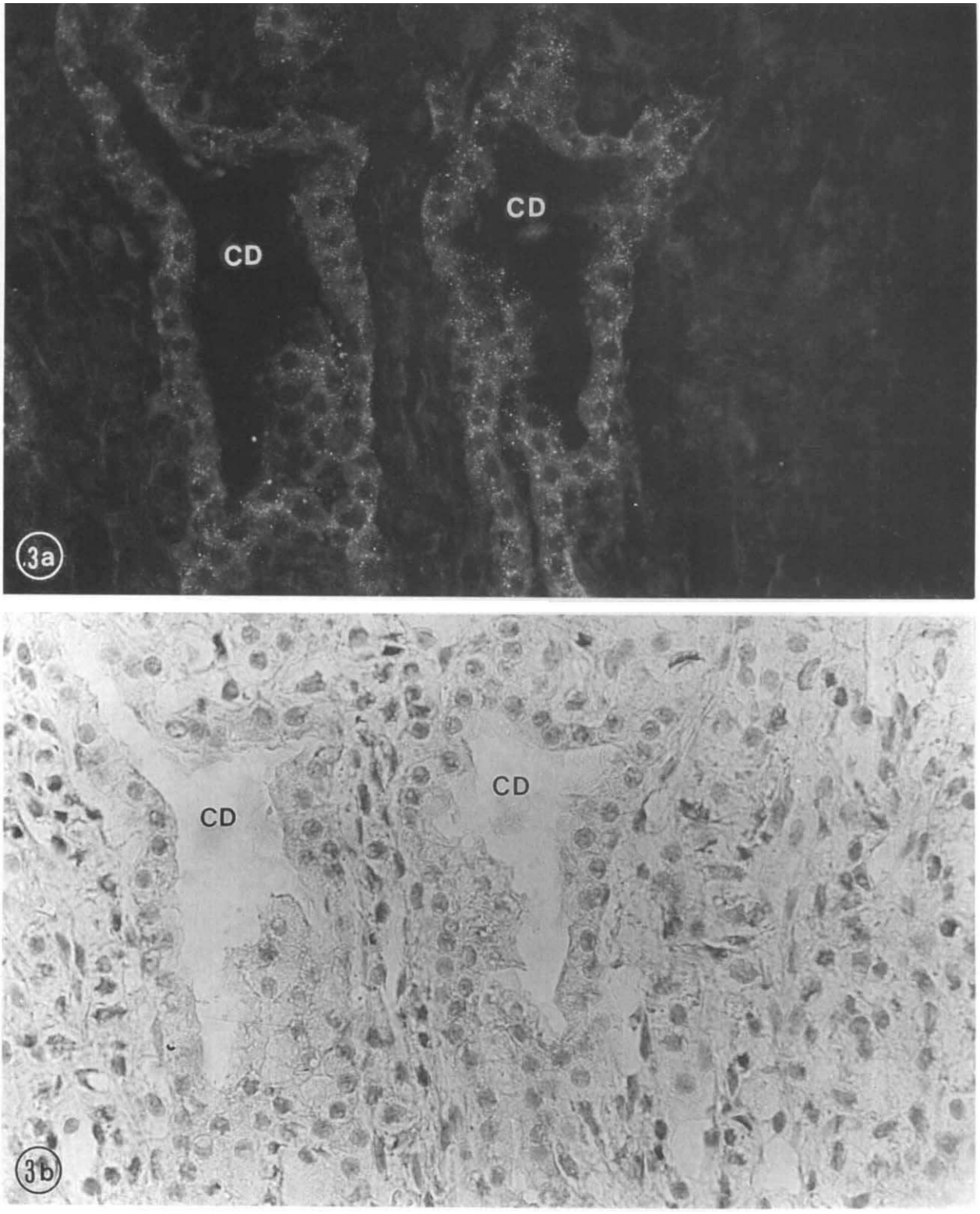

Fig. 3. A *Gap junction immunofluorescence for Cx 43 in the collecting ducts, CD.* **B.** The same section stained with hematoxylin. ×500.

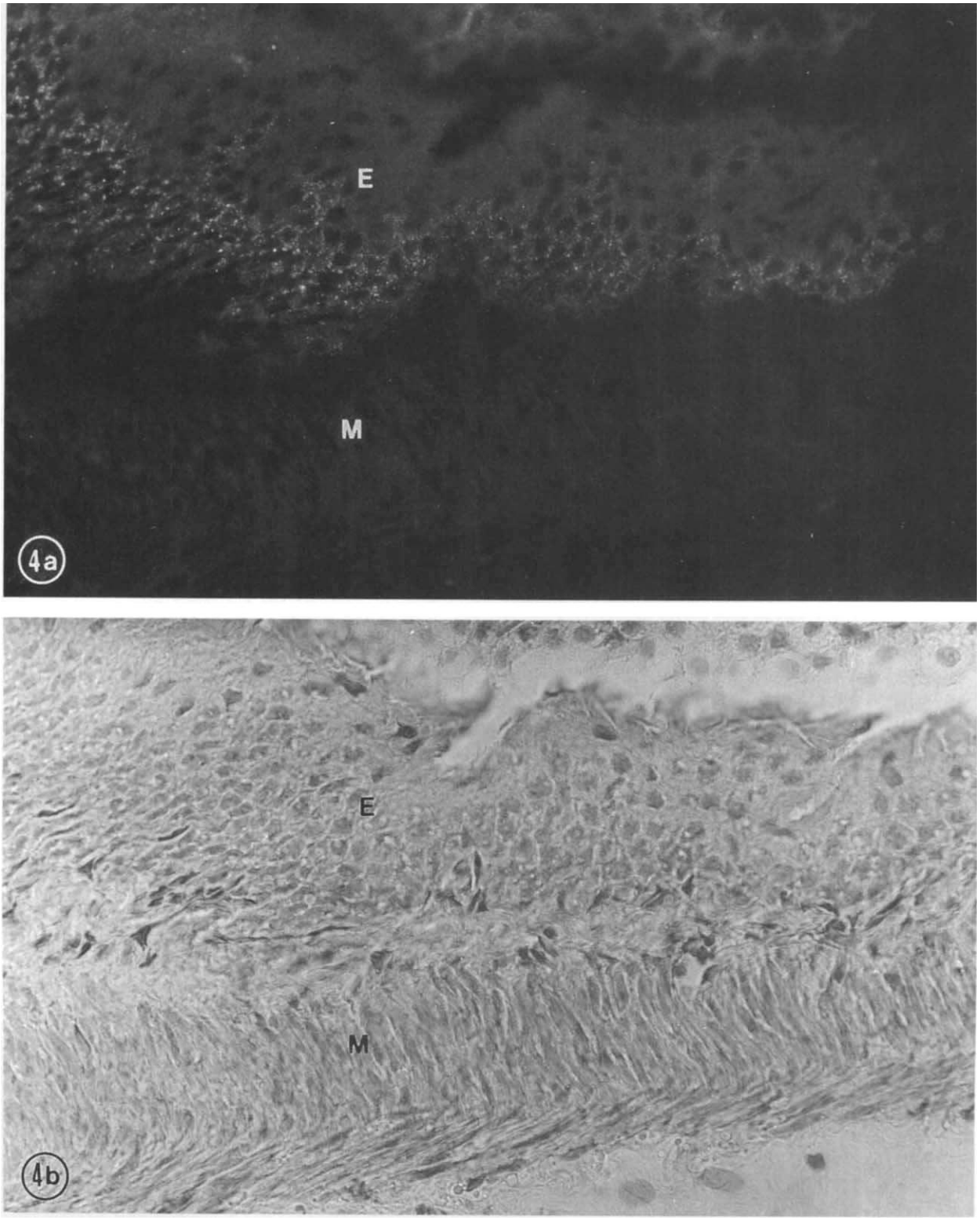

Fig. 4. A. *Gap junction immunofluorescence for Cx 43 in the pelvic epithelium.* The superficial layer of the pelvic epithelium and the muscularis layer appear devoid of fluorescence. **B.** The same area stained with hematoxylin (×500).

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