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Cytoplasmic sequestration phenomena in smooth muscle cells of kidney resistance vessels and epithelioid cells of the juxtaglomerular apparatus*

R. Metz, R. Nobiling, M. Harlacher, L. Rosivall[1], and R. Taugner

I. Physiologisches Institut der Universität Heidelberg, Im Neuenheimer Feld 326, D-6900 Heidelberg, Federal Republic of Germany
[1] Department of Pathophysiology, Semmelweis University, Budapest, Hungary

Summary. The occurrence of vacuoles in cells of contractile tissues and especially in media cells of resistance vessels has been known for quite some time. Recently, it has been widely accepted that these vacuoles, characteristically lined by a double membrane, result from herniation of one vascular smooth muscle cell into the other as a result of vasoconstriction. In our electronmicroscopic investigations we found double membrane-bounded vacuoles not only in kidney resistance vessels of rats and mice under conditions of vasoconstriction, but also in control animals and animals with maximal renal vasodilation. Part of our observations are compatible with the assumption that such vacuoles arise from a damage of club-shaped, musculo-muscular contacts due to shape changes of media cells during maximal vasoconstriction or vasodilation. However, serial thin sectioning revealed that some of the cytoplasmic vacuoles have no connections with neighbouring cells. This finding and various parallels to the generation of autophagic vacuoles indicate that the so-called herniations may also represent demarcations of large cytoplasmic areas within an individual cell. Irrespective of the origin of these vacuoles, their contents show different stages of deterioration. At later stages, the vacuoles appear to be adjacent, with only one membrane, to the extracellular space, into which they are believed to discharge finally. Cytoplasmic vacuolization has not only been observed in smooth muscle cells, but also in juxtaglomerular epithelioid cells of the afferent arteriole. Here the vacuoles – besides other organelles – also contain secretory granules; it is therefore proposed that autophagic phenomena with final extrusion of cytoplasmic material may be involved in the programmed down-regulation of the granular renin store following inhibition of renin synthesis and secretion.

Key words: Cytoplasmic vacuoles – Preglomerular arterioles – Juxtaglomerular apparatus – Vascular smooth muscle cells – Epithelioid cells

Introduction

During the last two decades there have been several reports on large, double membrane-bounded vacuoles in smooth muscle cells of arteries from different organs (Goldby and Beilin 1972; Joris and Majno 1977). The incidence of these vacuoles was reported to be increased drastically in vessels contracted by infusion of angiotensin II and noradrenaline, as well as in various models of experimental chronic hypertension (for reference see Lee et al. 1984).

In early studies, the vacuoles were considered to be related to necrotic cell processes (Esterly and Glagov 1963; Gardner and Matthews 1969; Salgado 1970). Later, most of the authors assumed that they result from the herniation of one smooth muscle cell into another as a result of vasoconstriction (Joris and Majno 1977 and 1981; Lee et al. 1984).

Since we had repeatedly found similar vacuoles in media cells of kidney resistance vessels without any sign of cell death or herniation into neighbouring cells, this study was designed to determine the origin of these vacuoles using serial thin sections. Smooth muscle and epithelioid cells were examined

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Offprint requests to: R. Nobiling

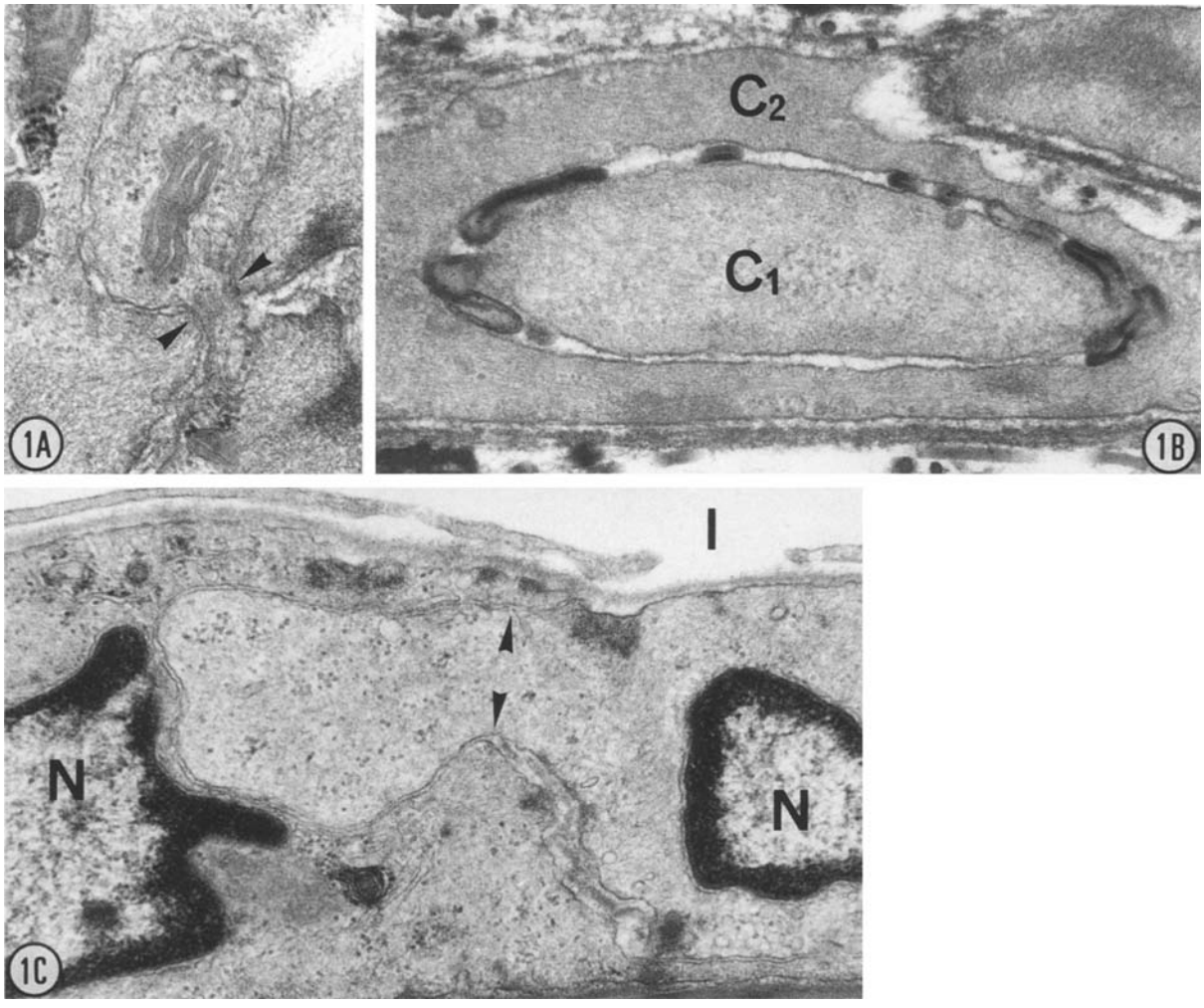

Fig. 1. Musculo-muscular contacts between media cells of afferent arterioles and interlobular arteries. **A** Longitudinal section through club-shaped contact with distinct narrowing (*arrowheads*) at the site of its invagination into the neighbouring cell (afferent arteriole from mouse kidney, 7 days after adrenalectomy). ×43500. **B** Transverse section through musculo-muscular contact with the large process of one cell (C_1) invaginating into the cytoplasm of an adjacent smooth muscle cell (C_2). Note the basal lamina material accompanying the protruding process (interlobular artery from control mouse kidney, perfused with 4% tannic acid). ×51500. **C** Longitudinal section through club-shaped contact with some narrowing at the site of its invagination into the neighbouring cell (*arrowheads*). The cytoplasmic content of the process is less electron dense than that of the parent cell (rat kidney, angiotensin II infusion for 30 min). *N*: nucleus; *I*: interstitium. ×35000

from afferent arterioles of rat and mouse kidney under different conditions of vasoconstriction and vasodilation. Control animals and animals with stimulation of the renin-angiotensin-system were used.

Methods

The experiments were performed on male NMRI mice (b.w. 20–35 g) and male Wistar rats (b.w. 150–300 g). 12 mice were adrenalectomized and two of them sacrificed 1, 3 and 4 days, 6 of them 7 days after the operation. 4 mice served as controls. Before fixation, two Wistar rats were infused via the jugular vein with angiotensin II for 30 and 60 mins respectively and two others with noradrenaline for 60 min. Angiotensin II amide (Hypertensin, Ciba Geigy, Basel, Switzerland) and noradrenaline (Norepinephrine bitartrate, Sigma, St. Louis, MO, USA) in normal saline were given at rates of 1–5 and 2.5–10 μg/kg/min, respectively. As controls, two rats were infused with corresponding volume of saline. To monitor the hypertensive response, arterial pressure was recordered from the carotid artery, using a strain gauge transducer. Finally two isolated perfused rat kidneys were examined (cf. Schwertschlag et al. 1978). Before fixation, these kidneys were maximally vasodilated by the addition of Nimodipine (10^{-5} mol/l for 30 min), acetylcholine and hydralazine (10^{-5} mol/l for 8 min) to the perfusion medium.

All manipulations, including the perfusion fixation were performed under Nembutal anesthesia (50 mg/kg i.p.). Most of the animals were fixed by retrograde perfusion via the abdominal aorta as follows: first a rinsing solution was used, containing 9.0 g NaCl, 25.0 g polyvinylpyrrolidone (PVP, mo-

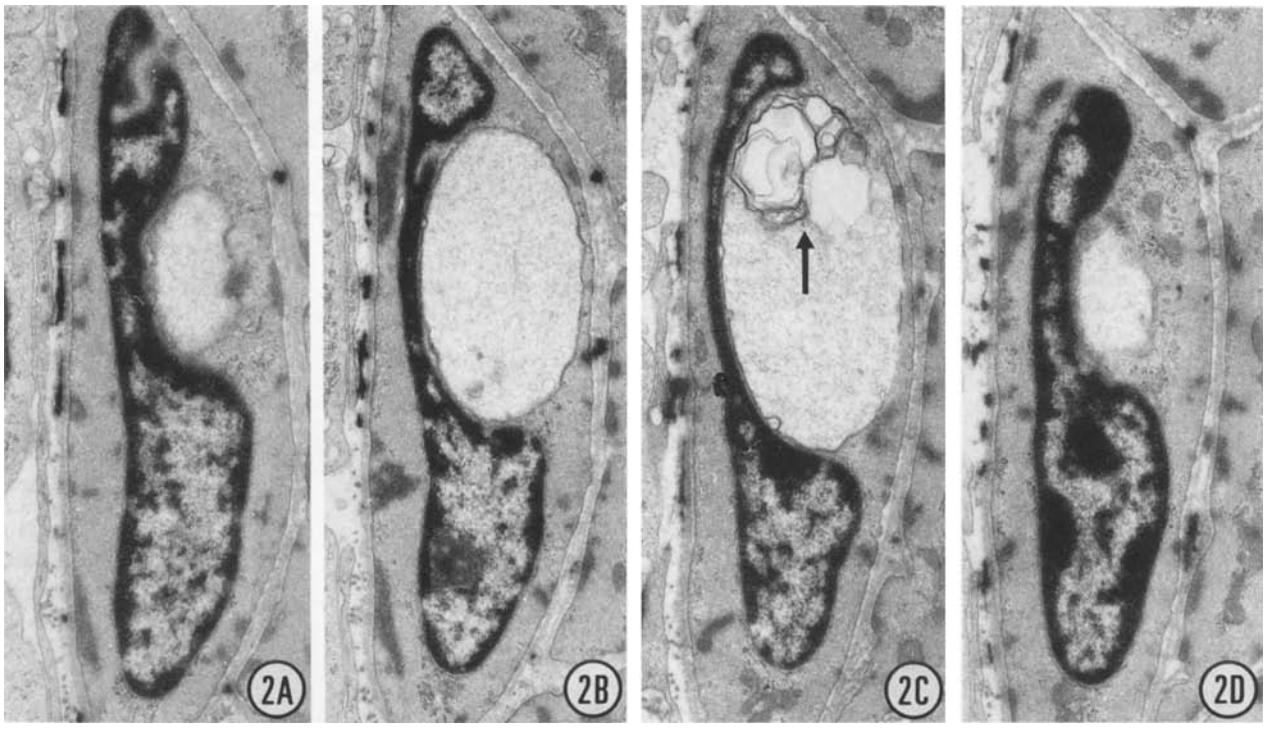

Fig. 2. Thin section series showing double membrane-bounded vacuole in the perikaryon of a smooth muscle cell in the wall of an interlobular artery from mouse kidney. The distances between the sections depicted are 1 µm (**A** and **B**), 2 µm (**B** and **C**) and 1 µm (**C** and **D**), respectively. Connections with neighbouring cells could not be detected in all other sections of the series examined. Note the electron lucent, widely homogeneous content of the vacuole pointing to an advanced stage of digestion. *Arrow*: myelin figures. × 16500

lecular mass 40000), 0.25 g heparin and 5.0 g procain/l H_2O (pH adjusted to 7.35 with 1 mol/l NaOH). In animals infused with vasopressive substances procain was omitted. After a rinsing period of 1 min the animals were perfused with the fixation solutions I and II for 5 min each; solution I contained 1.5% glutaraldehyde and 1.5% formaldehyde, solution II 3% glutaraldehyde, 3% formaldehyde and 0.5% picric acid (Forssmann et al. 1977). As an exception, control mice were perfused through the abdominal aorta with 4% tannic acid and 2.5% glutaraldehyde in 0.2 mol/l cacodylate buffer (pH 6.6).

After perfusion fixation, the kidneys were removed, the renal cortex cut into small pieces and stored for 12 h in the respective fixation solution. The tissue blocks were postfixed for 60 min with 1% osmium tetroxide buffered in 0.1 mol/l cacodylate, dehydrated in graded ethanol solutions and embedded in Epon 812. Epon blocks were sectioned on an OM U 3 ultramicrotome (Reichert-Jung, Wien-Heidelberg) and examined in an EM 10A electron microscope (Carl Zeiss, Oberkochen, FRG) at an accelerating voltage of 60 kV.

Results

Club-shaped musculo-muscular contacts of a diameter of 0.5–1.5 µm were found in the media of preglomerular arterioles and interlobular arteries, often exhibiting a neck-shaped constriction at their transition to the neighbouring cell (Fig. 1 A). The cytoplasm of the processes was altogether less electron dense (Fig. 1 C) and the contained organelles showed signs of degradation. Cross-sections through the head of club-shaped protrusions might lead to their confusion with double membrane-bounded vacuoles; this, however, can be ruled out by tannin-staining the extracellular material surrounding the club-shaped contacts (Fig. 1 B).

Unlike club-shaped protrusions, double membrane-bounded intracellular structures, without any contact with neighbouring cells can be found in smooth muscle and granulated cells of the afferent arteriole (Figs. 2 and 3). The contents of these structures may vary. In smaller, probably developing, vacuoles of 0.5–1 µm the enclosed organelles may appear essentially normal (cf. Fig. 4). Larger, probably older, vacuoles contain more or less altered cytoplasmic organelles, giving the impression of advanced deterioration by autolysis (Figs. 2 and 3).

One possible mechanism involved in the development of these cytoplasmic vacuoles in smooth muscle and epithelioid cells is shown in Fig. 4 A–E. It seems as if they develop by the wrapping of cytoplasmic contents by cistern-like organelles. The increasing change of the enclosed organelles

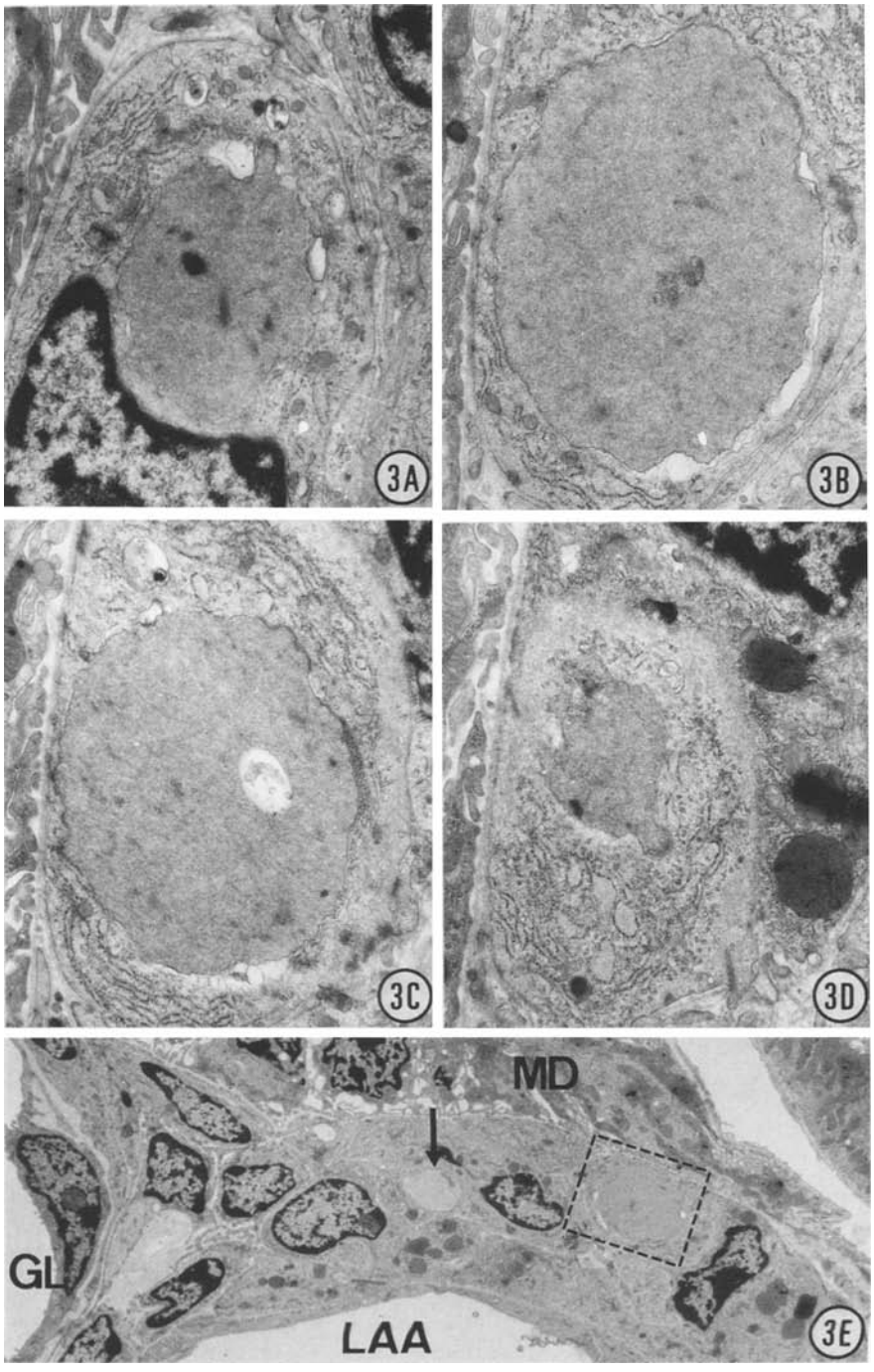

Fig. 3. Thin section series (**A–D**) and corresponding overview (**E**), showing double membrane-bounded vacuole in the cytoplasm of a granulated cell in the media of an afferent arteriole from mouse kidney. The distances between the sections depicted are 2 μm (**A** and **B**), 1 μm (**B** and **C**) and 2 μm (**C** and **D**), respectively. Connections of the vacuole with neighbouring cells could not be detected. The putative former contents of the vacuole (e.g. secretory granules and mitochondria) seem to be in the process of digestion. **B** corresponds to the rectangular area marked in **E**. *GL*: glomerulus; *MD*: macula densa; *LAA*: lumen of the afferent arteriole. The arrow points to a similar vacuole in another granulated cell with its contents in a somewhat more advanced stage of desintegration. **A–D**: ×16000; **E**: ×3400

indicates that the growing vacuoles are able to disintegrate their contents by hydrolytic enzymes, as known from autophagosomes. Figure 5 shows that in the vacuoles of epithelioid cells secretory granules are also involved. Part of the cytoplasm including secretory granules is separated by a double membrane from the rest of the cell, with the sequestered organelles apparently in the process of autolytic digestion.

Larger vacuoles with deteriorating contents are occasionally found, bordering on the interstitium with their – single – membrane. Figure 6 suggests that the largely disintegrated contents of such vacuoles will eventually be discharged into the inter-

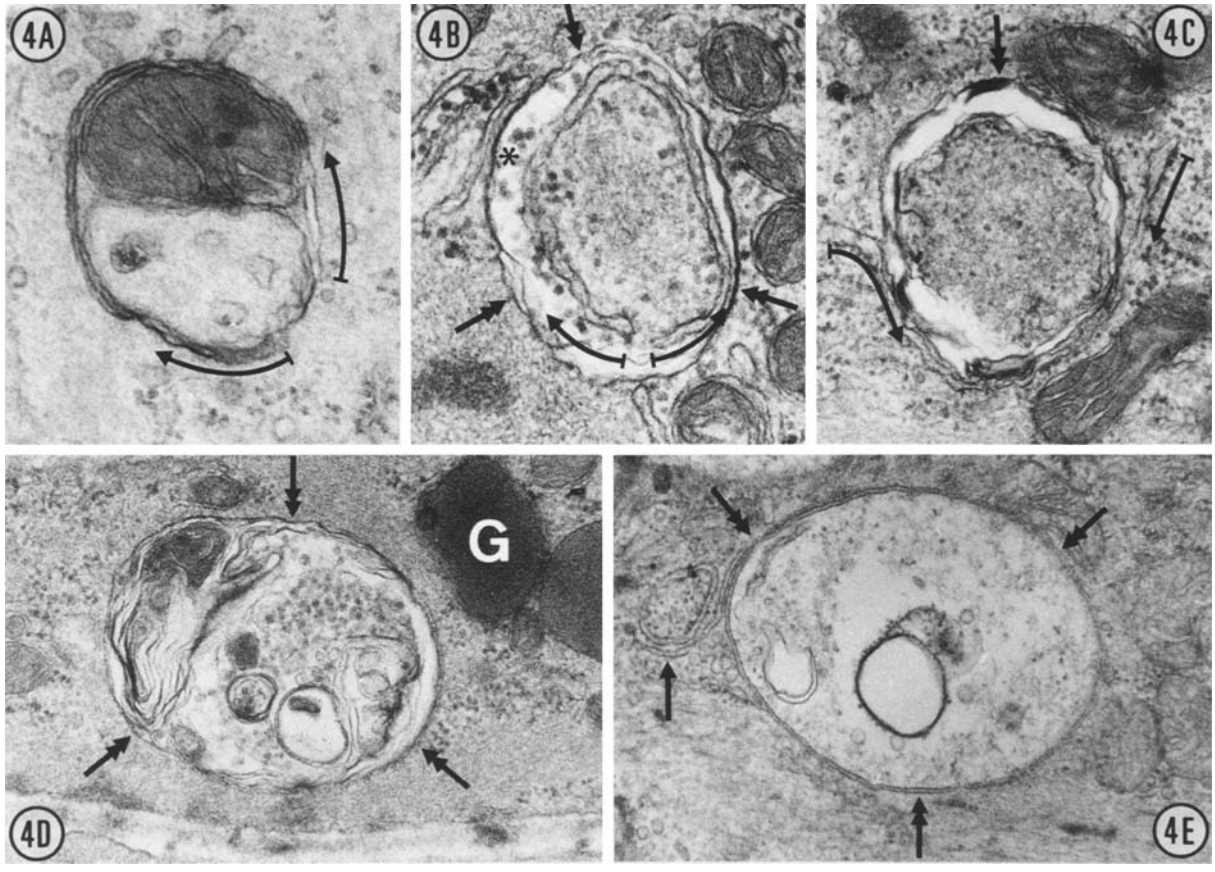

Fig. 4. Putative stages in the formation and progression of cytoplasmic vacuoles in smooth muscle and granulated cells of kidney resistance vessels. Note that in most cases cistern-like organelles lie close to and seem to wrap around the cytoplasmic area, contained in the forthcoming vacuole (*arrows*); the contents of the growing vacuoles (e.g. mitochondria and glycogen granules) show increasing signs of deterioration. **A**, **B**, **C**, and **D** are from afferent arterioles of mouse, **E** of rat kidney. *Asterisk*: glycogen granules in the electron lucent periphery of the vacuole, demonstrating that we are not dealing with a cell process surrounded by the extracellular space; *double arrows*: demarcation of the vacuoles by double membranes; *G*: secretory granule. ×72000, 73000, 51000, 41000, and 33000, respectively

stitium. In the course of such sequestration events no nuclear pyknosis or other indications of cell death such as apoptosis were observed.

Discussion

Two possible explanations for the sequestration of large cytoplasmic regions in smooth muscle cells of kidney resistance vessels can be proposed from our observations: the alteration and detachment of club-shaped musculo-muscular contacts and the development of large autophagic vacuoles.

Apart from myoendothelial contacts (Rhodin 1967; Taugner et al. 1984) club-shaped musculo-muscular contacts of a diameter of 0.5–1.5 μm may be found in the media of preglomerular arterioles and interlobular arteries, often exhibiting a neck-shaped constriction at their transition to the neighbouring cell. The contents of these contacts are mostly unchanged. However, especially in animals with maximal vasoconstriction or vasodilation, that is to say, with marked shape changes of their media cells, alterations in the contents of club-shaped protrusions were observed.

One possible mechanism involved in the development of the cytoplasmic vacuoles is demonstrated in smooth muscle and epithelioid cells. It seems as if they develop by the wrapping of their cytoplasmic contents by cistern-like organelles, as is known to occur for autophagic vacuoles (Arstila and Trump 1968; Pfeiffer 1971, 1981). The increasing changes in the enclosed organelles indicates that the growing vacuoles are able to disintegrate their contents by hydrolytic enzymes, as in autophagosomes. It cannot be ruled out, however, that vacuolar structures may also develop as a re-

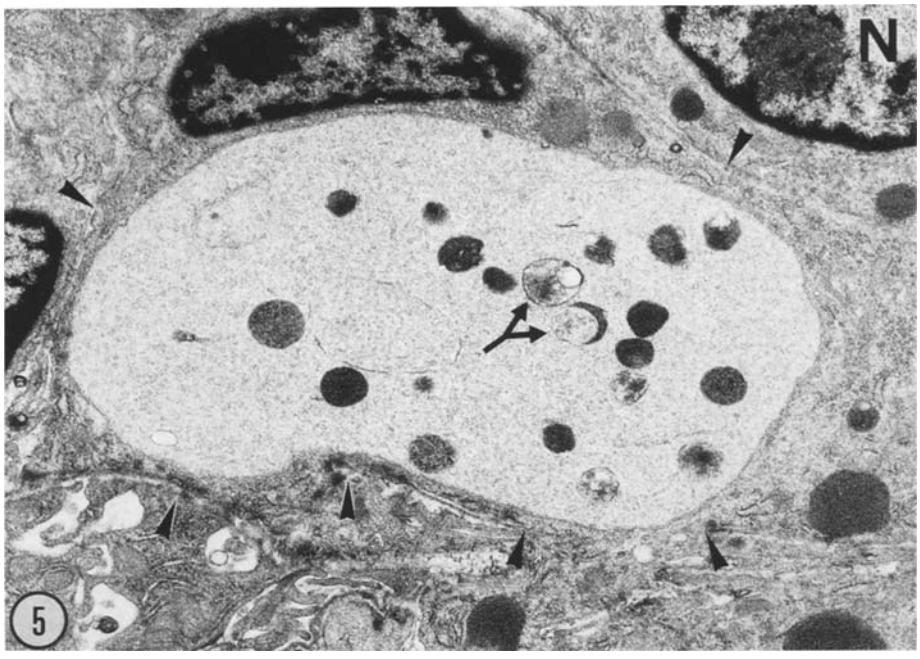

Fig. 5. Large double membrane-bounded vacuole in juxtaglomerular epithelioid cell of mouse kidney, 7 days after adrenalectomy. The electron lucent, homogeneous matrix of the vacuole contains several former cell organelles, mostly renin granules, some of them showing signs of deterioration (*split arrow*). Note that the vacuole is surrounded by a continuous small rim of unaltered cytoplasm (*arrowheads*). *N*: nucleus. ×11000

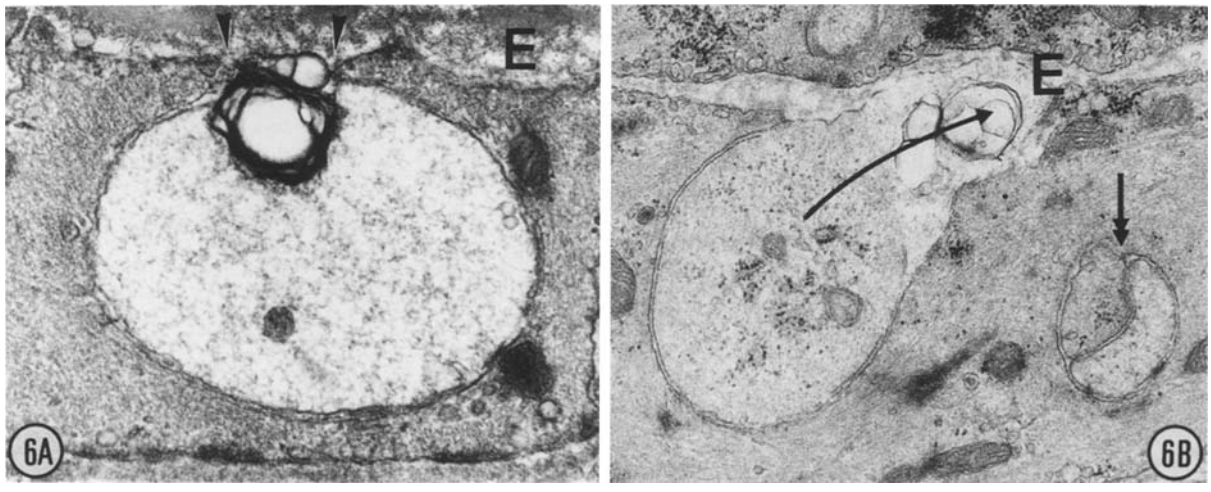

Fig. 6. Double membrane-bounded cytoplasmic vacuoles in media cells of afferent arterioles, extruding their contents into the extracellular space (*E*). Note that the internum of the vacuoles is changing its appearance on contact with the interstitial fluid. In **A**, the gap of the surrounding unaltered cytoplasm is marked by *arrowheads*; in **B**, the arrow points to the ensuing large opening of the vacuole to the interstitium. *Double arrow*: second, smaller vacuole. **A** is from mouse, **B** from rat kidney. ×46000 and 31000, respectively

sult of severance of musculo-muscular contacts; these pinched-off protrusions may be treated by the recipient cell as a pathological inclusion with the fate of a phagosome (Joris and Majno 1981).

In our opinion, in epithelioid cells, the occurrence of vacuoles which contain degenerating organelles merits special attention. The renin producing, granulated cells are formed by metaplastic transformation of smooth muscle cells in the media of the afferent arteriole (Cantin et al. 1977). The ultrastructural aspects of this transformation process upon stimulation of the renin-angiotensin-system has been described, among others, by Cain and Kraus (1971) and Cantin et al. (1977). According to these authors, swelling of the nucleus and nucleolus is followed by increase in glycogen, hypertrophy of the rough endoplasmic reticulum and the Golgi area and finally, development of the first

mature secretory granules from protogranules. Concomittantly, the number of myofilaments and attachment sites decreases, leading to intermediate cells that contain both the secretory machinary and the residuum of the contractile machinary, attachment sites and myofilaments. In fully transformed cells, myofilaments may virtually be absent, so that the resulting epithelioid cells can no longer be regarded as contractile cells (Cain and Kraus 1971; Taugner et al. 1987). There have been no reports on the participation of lysosomes in the metaplastic transformation of smooth muscle cells into epithelioid cells. This may indicate that mainly cytoplasmic enzymes are involved in the catabolic processes accompanying this drastic remodelling of media cells.

Comparatively little research has been directed to the ultrastructural alterations of the JGA upon acute and drastic inhibition of the renin-angiotensin-system. There is agreement that in the absence of mitoses, retransformation of epithelioid cells into smooth muscle cells takes place upon inhibition of the system, but, as far as we know, there have not been any reports on the associated ultrastructural changes. During retransformation of epithelioid cells into smooth muscle cells the disappearence or elimination by autodigestion, exocytosis or sequestration, of the numerous large mature secretory granules occurs. This alone shows that the process of retransformation cannot simply be the reversal of transformation and that different forms of intermediate cells have to be anticipated during retransformation as compared to those during transformation.

An example of a cell with secretory granules in a double membrane-bounded vacuole was found in the kidney of a mouse, 7 days after adrenalectomy. Generally such changes were rarely seen in control mice but were seen repeatedly several days after adrenalectomy, in animals about to recover from adrenal insufficiency (Hartman 1924). The hypothesis that the observed demarcation of part of the cytoplasm represents the first step in the retransformation of epithelioid cells into smooth muscle cells after resetting of the stimulation level is thus supported. According to this view, epithelioid cells may reduce their surplus secretory product by means of a scavenger process, that is the sequestration and degradation of parts of their cytoplasm including secretory granules following a drastic, long lasting inhibition of the renin-angiotensin-system.

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