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Cytochemical localization of acid phosphatase activity (cerium-based method) in the proximal convoluted tubule of the kidney of the gerbil *Meriones crassus* with reference to the GERL complex and lysosomal-vacuolar apparatus

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ABSTRACT

Ultrastructural localizations of acid phosphatase activity were established in the proximal convoluted tubule cells of the gerbil *Meriones crassus* using β-glycerophosphate as a substrate and cerium chloride as a capture agent. The reaction product of the cerium phosphate is finely precipitated in different sites of the Golgi associated Endoplasmic Reticulum involved in the formation of Lysosomes (GERL) complex including its cisternal and tubular elements, and the lysosomal-vacuolar apparatus. Prominent connection between GERL elements and vesiculated lysosomes was found, indicating the possible origin of the primary lysosome. However, the GERL elements are numerous and extensive through the matrices of the cells with different patterns and textures located, as usual, in the vicinity of the nuclei and the Golgi apparatus. The study confirms the presence of GERL elements similar to that seen in related studies. The Golgi apparatus and the apical surface of the cells showed no reaction. Neither the basal infoldings nor the membrane-bound bodies of the thick basal lamina showed any activity, and this may cast doubt upon the lysosomal origin of these bodies. The enzymatic activity also indicates heavy deposits of acid phosphatase-positive lysosomes which are distributed as regular, spherical to irregular structures. Some appear dumbbell-shaped, others contain inclusions, while still others show remarkable fusion activity. The fine cerium depositions illustrate the complexity in the GERL elements which when tilted at few degrees from the flat, show their anastomosing pattern on the one hand, and their parallel tubular elements on the other. It also shows the clear detection of the peripheral space between the limiting membrane of the lysosome and its stained contents, as well as the connection of some lysosomes to each other with a thread-like structure.

INTRODUCTION

The histological and ultrastructural features of the kidney cells of the desert gerbil *Meriones crassus* are very complex. The presence of developing membrane-bound bodies that characteristically lie in a thick basal lamina of the proximal convoluted tubule, as well as in other nephronic segments, are some of the many recognized and detailed structures of the cells (Al-Ajmi 1989; Safer *et al.* 1990; Safer 1992).

Investigators have attempted to correlate anatomical findings and the distribution

of some cytochemical phosphatases i.e. ATPase, ALKase with the behaviour of *Meriones crassus* with respect to water economy and adaptation to extreme conditions (Safer & Hussain 1989, Safer *et al.* under publication). Although these attempts have provided valuable information, the correlations are still far from clear. These two enzyme-markers were applied to study the apical and the basolateral surfaces of the cells.

The present study aims to expand our knowledge of the fine structure of the proximal convoluted tubule cells, with the view to providing an insight into the cytochemistry of these cells, with particular reference to the lysosomal-vacuolar apparatus, the Golgi associated Endoplasmic Reticulum involved in the formation of Lysosomes (Novikoff 1964) and other cytoplasmic organelles. In addition, this study aims to confirm the GERl concept in this animal as demonstrated in other mammals using acid phosphatase which is the most reliable marker for these two systems (Holt & Hicks 1961; Novikoff 1964; Maunsbach 1966; Novikoff & Novikoff 1977; Safer 1978; Robinson & Karnovskey 1983a) and which is also found on trans-Golgi saccules and some Golgi vesicles (Kinara & Ichihara 1985; Ning *et al.* 1992).

Ultrastructural localization of acid phosphatase activity in the proximal convoluted tubule cells of the kidney of the rat and several other species have been widely studied (Novikoff 1963; Ericsson 1964; Ericsson & Trump 1964; Maunsbach 1966; Maunsbach 1969; Larsson & Maunsbach 1975; Safer 1978), but this localization is still unrecognized in the kidney of the desert-adapted gerbil. In this investigation attention is focused on the GERL and lysosomal systems as typical acid phosphatase positive sites. This may explain the transport of this enzyme and the complexity and patterns of the GERL, and the establishment of the primary lysosomes. The distribution, texture, and pattern of acid phosphatase staining are related to the structure and the function of the two systems.

MATERIALS AND METHODS

Five male adult gerbils *Meriones crassus* weighing 100–120 g were used for this study. Under deep anaesthesia with 25% urethane (0.6 ml/100 gm of body weight), the animal was perfused via the ventricle with ice-cold 1% glutaraldehyde in 0.1 M cacodylate buffer containing 5% sucrose, pH 7.2 for 5–10 minutes. The kidneys were separated and decapsulated, the cortex was cut into small cubical blocks (1–2 mm), and immersed in the same fixative for 1 hr. at 0–4°C. Thirty μm sections were cut on a freezing microtome (Leica 1320), and processed in acid phosphatase medium consisting of 0.1 M acetate buffer pH 5, 1 mM sodium β-glycerophosphate, 2 mM cerium chloride, 5% sucrose (Robinson & Karnovesky 1983b). To this medium 1.5 μl Triton X-100 was added to facilitate membrane permeability (Robinson 1985). For control, some of the sections were incubated in a substrate-free medium.

After completion of incubation for 1 hr at 37°C, the sections were washed twice in acetate buffer for 15 min, followed by 0.1 M cacodylate buffer at 0–4°C overnight. The tissues were postfixed in 1% OsO_4 for 1 hr at room temperature, and gradually dehydrated in ethanol. They were then suspended in fresh Araldite M for 24 hrs. followed by embedding in Araldite M where each section was flattened in the cover of the polyethylene capsules and left for 48 hrs at 60°C. One micrometer thick sections and silver-gold ultrathin sections were cut on LKB ultra microtome with a diamond knife. The latter were mounted on copper grids and some were counterstained with

lead citrate. The sections were examined in a Jeol-1200 EXII electron microscope operated at 60 kV. Photographs were printed on Kodak RC papers.

RESULTS

The basal part of the proximal convoluted tubule cell possesses a thick basal lamina containing various membrane-bound bodies (Plate 1) that appear to be pinched off from the basal infoldings with their extended tips (Plate 2). Acid phosphatase activity was detected in two main reactive sites; the GERL complex and the lysosomal-vacuolar apparatus.

Most of the lysosomes located in the apical cytoplasm of the cell and the ones found deeper were strongly stained. Some of the secondary lysosomes showed engulfing of apical vacuoles. The brush border, mitochondria, basal infoldings, the basal lamina and its membrane-bound bodies showed no reaction (Plate 3).

The reaction product of acid phosphatase was observed in the cisternae and the connecting tubules of the GERL complex that occur near to the nucleus. The latter was devoid of reaction products (Plate 4).

The most interesting findings in this study were the presence of numerous profiles of GERL complexes that show typical heavy deposits of acid phosphatase. Such GERL complexes, with well elaborated cisternal and tubular elements found in the cytoplasmic matrix (Plates 5–7), were of varying complexity.

In many instances, the acid phosphatase reaction is variably shown in the GERL complex which extends near to what appear to be primary lysosomes. The latter seem to be pinched off from the ends of the tubular elements of GERL (Plates 4–6). Sometimes, stained secondary lysosomes are located adjacent to the GERL complex (Plates 4, 5, 7). The GERL also shows varying degrees of complexity (Plates 6–8). It is however, distinct from the cis-region of the Golgi complex (Plate 6), and its location is usually in the vicinity of the nucleus (Plates 4, 5, 7).

The regular and the intense staining of extensive profiles of the cisternal and the tubular elements of the GERL complexes may demonstrate their maturity stages and textures (Plates 8, 9). They appear as a perforated network (Plate 8, inset a) or as compacted stacks (Plate 8, inset b). Most of them are associated with the lysosomal bodies (Plate 8). Another GERL is extended along the axis of the cell where it shows both the cisternal and tubular parts (Plate 9).

Smooth-surfaced endoplasmic reticulum (SER) is moderately abundant, while rough-surfaced endoplasmic reticulum (RER), is frequently observed (Plates 5, 8).

Acid phosphatase deposits are clearly localized in the lysosomal-vacuolar apparatus, with lysosomes appearing as spherical to irregular shapes with different sizes and densities (Plate 10). Some of them are dumbbell-like structures situated near the GERL (Plate 11). Others are large, rounded or tubular and contain inclusions (Plate 12, inset). Many large lysosomes show multiple fusion (Plate 13). The fine reaction product of cerium phosphate indicates the visible membrane of such lysosomes (Plate 14).

The acid phosphatase positive contents of the lysosomes are also variable (Plates 15, 16), and some of the round lysosomes showed extensions from one or both poles (Plates 8, 16). Other lysosomes are irregular in shape, and also exhibit their extensions. Some groups of lysosomes show continuity with each other (Plate 17).

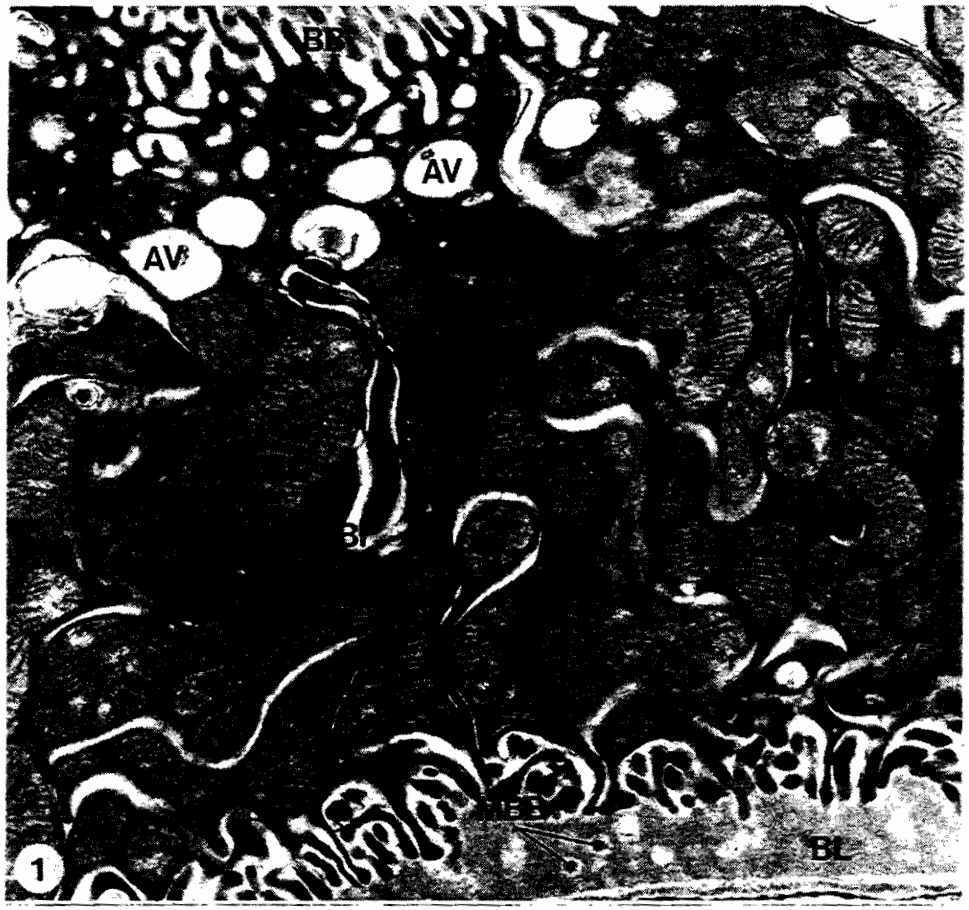


Plate 1. Survey picture of a proximal convoluted tubule cell of an adult gerbil kidney showing the general features of the cell. Pb stain. ×20,000. AV, apical vacuoles; BB, brush border; BI, basal infoldings; BL, basal lamina; M, mitochondria; MBB, membrane-bound bodies; Many mitochondria, basal infoldings are clearly visible. Note the basal lamina is relatively thick loaded with membrane-bounded bodies.

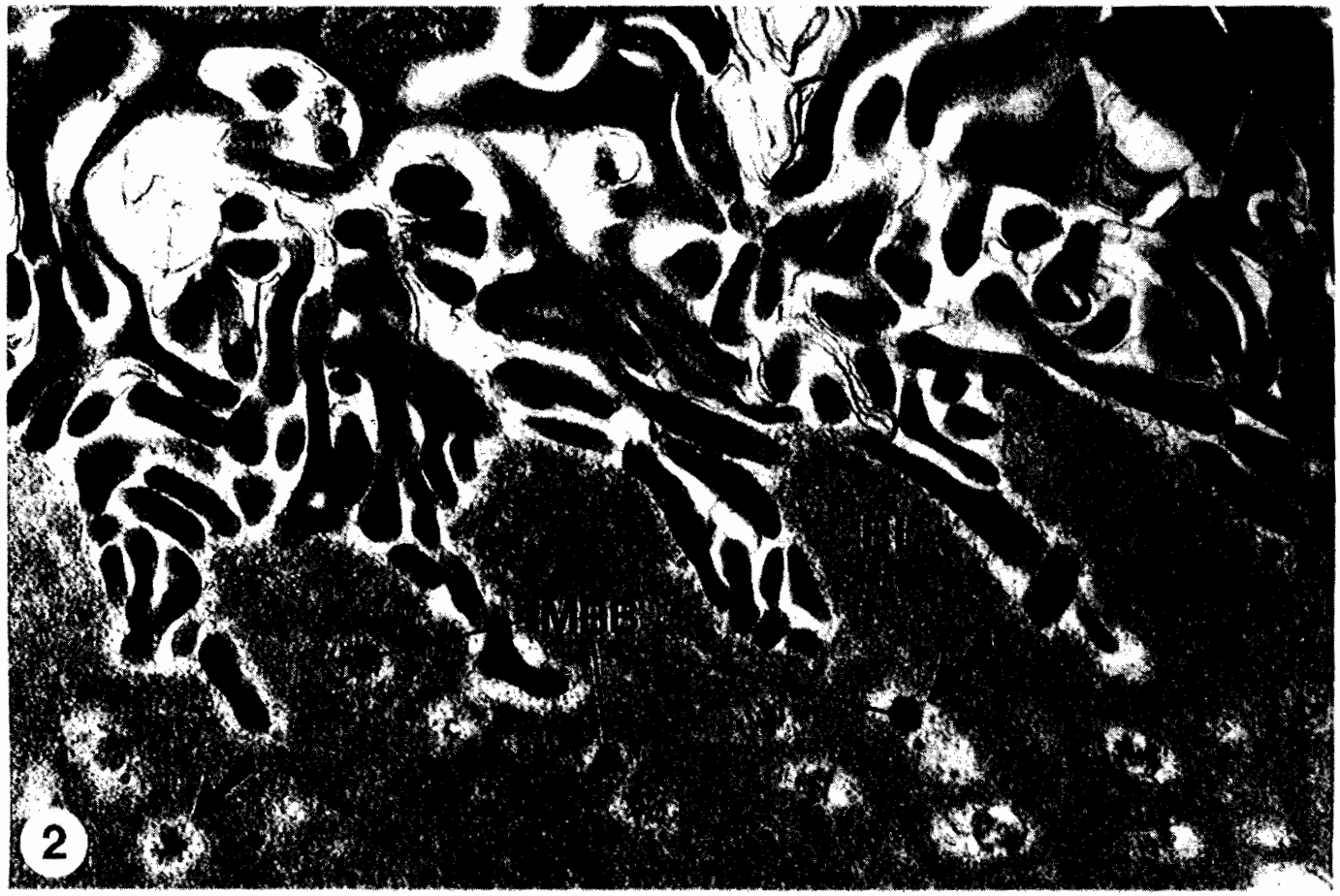


Plate 2. Enlarged area of the basal part of the proximal convoluted tubule cell. The basal infoldings with their tips are extended into the matrix of the basal lamina ending with prominent dense membrane-bound bodies. Some of which are surrounded by clear halo (arrows). Pb stain. ×37,800. BL, basal lamina; MBB, membrane-Bound bodies.

Plate 3. Thirty μm section of the proximal convoluted tubule cell of the gerbil kidney incubated for the demonstration of acid phosphatase medium containing β-glycerophosphate as a substrate and cerium chloride as a capture agent. The reaction product is intense in the lysosomal-vacuolar apparatus with different opacities where some lysosomes are seen in contact or already fused with the apical vacuoles (arrows). Note the other cellular components; brush border, mitochondria, basal infoldings, basal lamina and membrane-bound bodies have not reacted. Pb stain. ×25,000. BB, brush border; BI, basal infoldings; Bl, basal lamina; Ly, lysosomes; M, mitochondria; MBB, membrane-bound bodies.

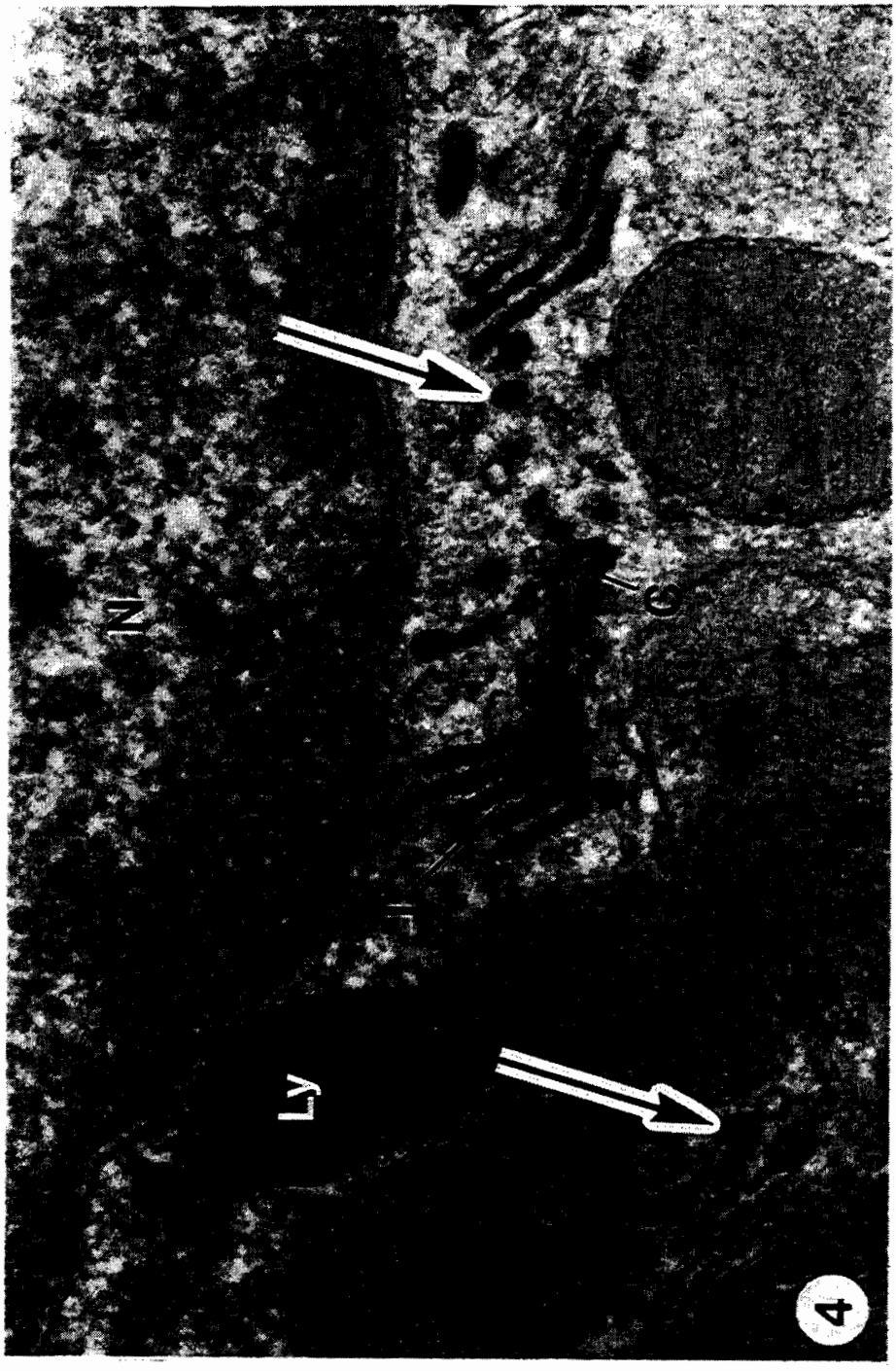


Plate 4. Part of the proximal convoluted tubule cell showing the presence of acid phosphatase reaction product in two GERL complexes with their dense cisternal and tubular elements near a nucleus. Note a large lysosome and many other small structures which appear to be primary lysosomes at the vicinity of the GERl complex (arrows). Pb stain. ×83,000. C, cisternal; Ly, lysome; N, nucleus; T, tubular elements.

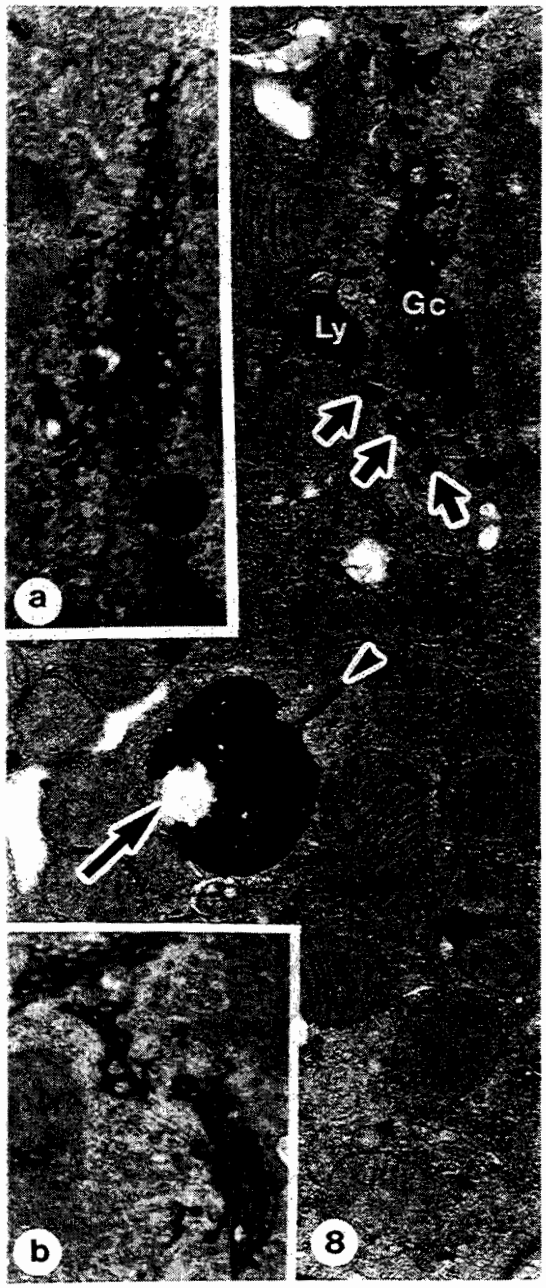


Plate 8. Electron micrograph showing a typical acid phosphatase staining of an integrated GERL complex. One lysosome is located near it, while in the center, a large lysosome seems to have some inclusions (arrow). Also, one pole is extended as a fine thread to what appears to be a GERL tubule (arrow head). Many primary lysosomes (small arrows) are seen around the main body of the GERL complex. Note the surface profile of the GERL complex showing the net-work perforated texture of its elements (inset a), while at a different angle from the flat, i.e. side view the GERL elements appeared as parallel compacted stacks (inset b). Note that the rough-surfaced endoplasmic reticulum is not stained. Pb stain. ×45,000; ×33,000; ×45,000 respectively. Gc, GERL complex; Ly, lysosome; rER, rough endoplasmic reticulum.

Plate 9. The complexity of the GERL complex shown at higher magnification. This micrograph shows an extensive GERL complex with both flat cisternae at one end and tubular elements at the other end. Note also, the elements are terminated with blebs denoting the beginning of the pinching off of the primary lysosomes (arrows). Pb stain. ×85,000. C, cisternae; T, tubular elements.

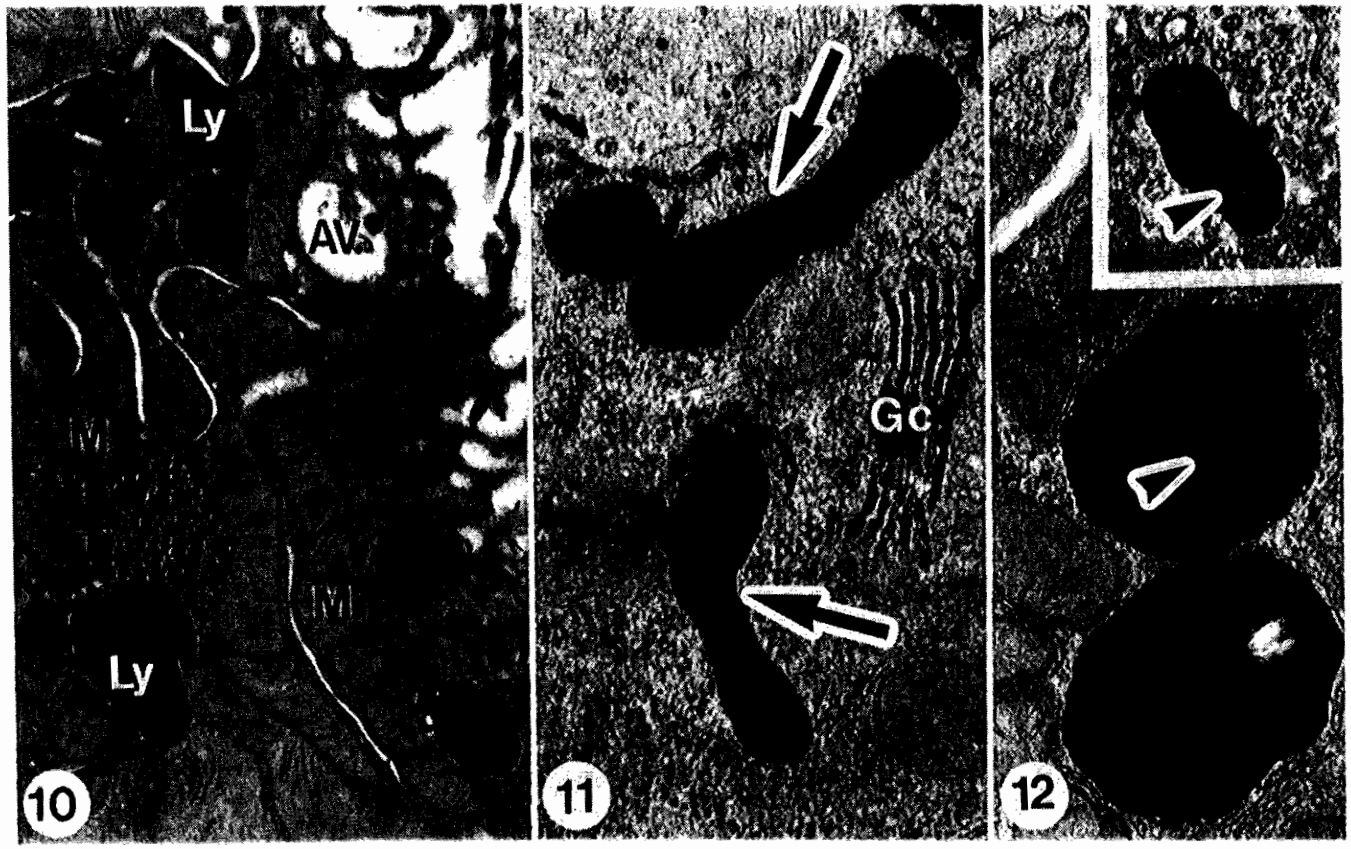


Plate 10–12. Portion of the apical part of the proximal convoluted tubule cell showing many lysosomes that are frequently stained with cerium phosphate deposits. Note the apical vacuole and mitochondria have not reacted. Fig. 10. Pb stain. ×20,000. In other areas, some lysosomes appear with elongated rod-like to dumbbell-shaped structure (arrows) located near a GERL complex. Fig. 11. Pb stain. × 40,000. Round lysosomes also show some inclusions (arrow head), and (arrow head) (inset). Fig. 12. Pb stained. ×38,000; ×24,000 respectively. Gc, GERL complex. AV, apical vacuole; Ly, lysosome; M, mitochondria.

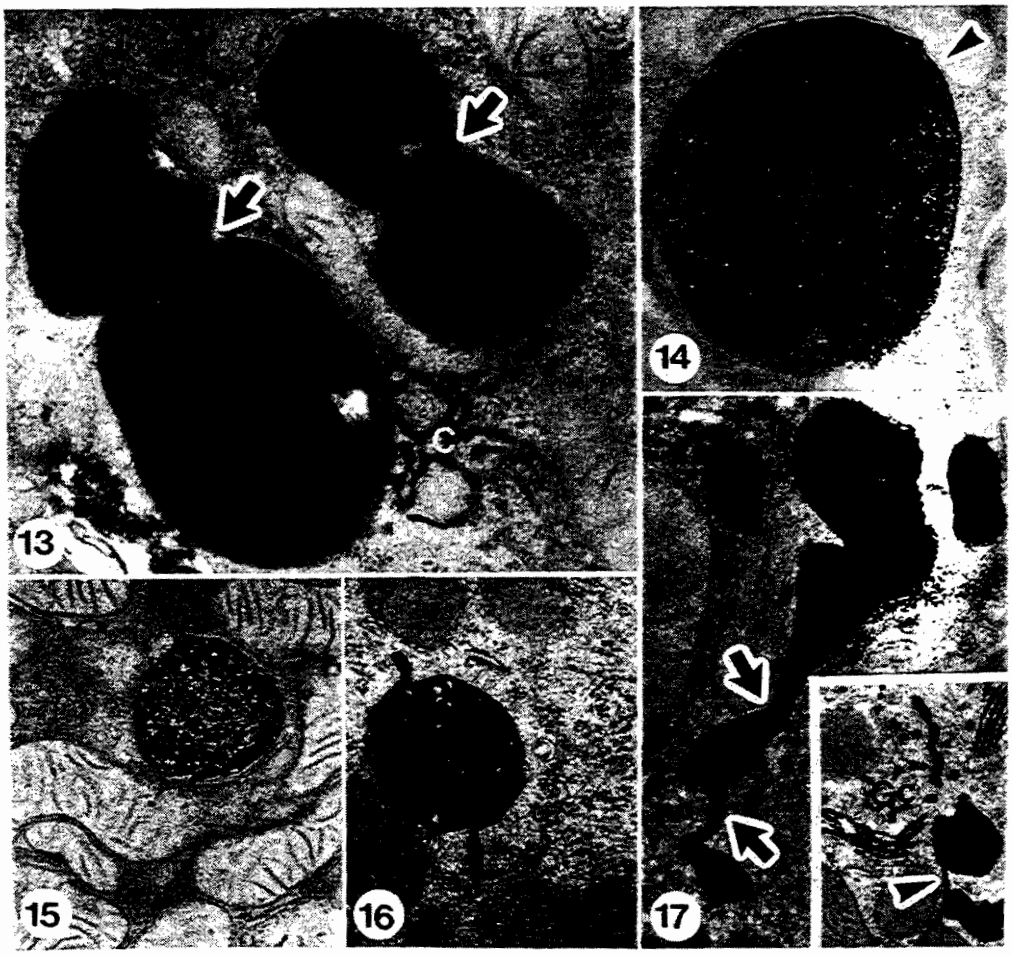


Plate 13–14. An enlarged area showing a clear multiple fusion between two pairs of lysosomes (arrows), one of which is attached to a GERL cisterna. Plate 13. Pb stain. ×50,000. Again, the fine deposits of cerium phosphate make it possible to clearly discern the space between the membrane of the lysosome and its contents (arrow head). Fig. 14. Pb stained. ×32,000. C, cisterna.

Plate 15–16. These two pictures suggest that the acid phosphatase reaction product may be variable showing the internal contents of the lysosomes in the form of coarse deposits. Fig. 15. Pb stained. ×34,000, and the one with more condensed contents with extensions of what appear to be remnants of the GERL tubules. Fig. 16. Pb stained. ×40,000.

Plate 17. A similar area to plates 15/16 indicating the complexity of the lysosomes which are irregularly expanded with continuity with each other in an irregular pattern. Some of them are connected with each other by a fine tubule (arrow). The inset shows two small lysosomes connected with a fine tubule (arrow head) nearby a GERL cisterna. Pb stained. ×42,800; 30,000 respectively. C, cisterna.

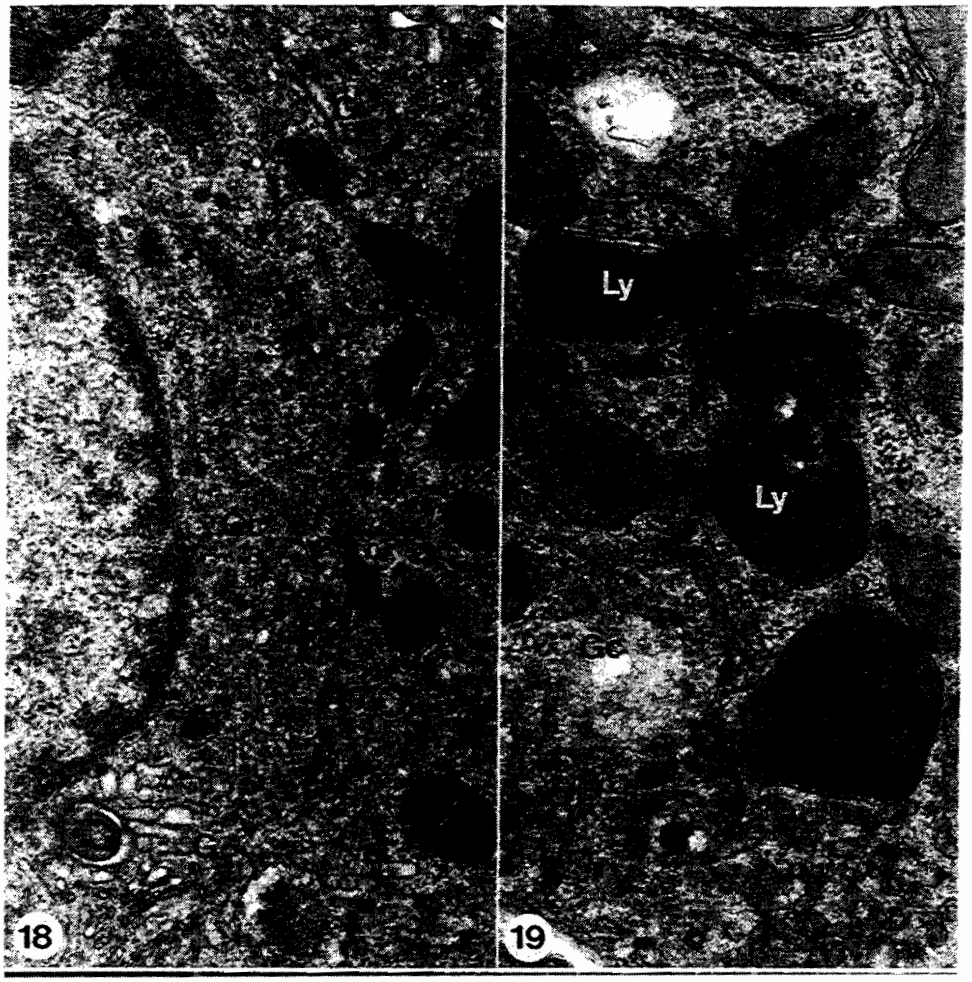
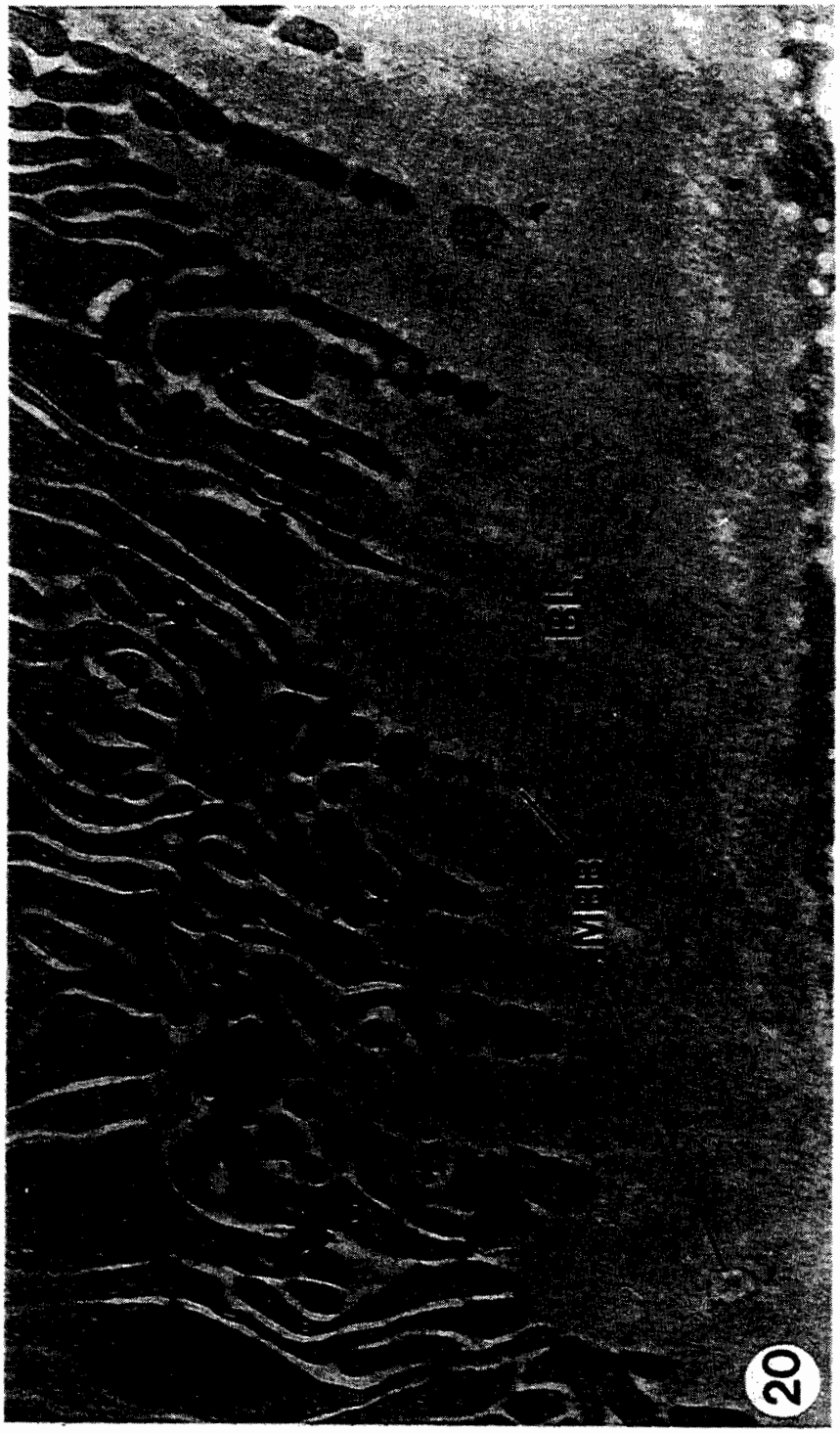


Plate 18–20. Electron micrographs showing substrate-free control preparation for acid phosphatase. Neither GERL complex with the Golgi apparatus (Pls 18, 19) nor the lysosomal vacuolar apparatus (Ly) (Pl. 19) are stained. In addition, the basal part of the cells including the membrane-bound bodies of the thick basal lamina show no activity. Pb stained. ×39,000; ×53,000; ×30,000 respectively. BL, basal lamina; G, Golgi; Gc, GERL complex; Ly, lysosome; MBB, membrane-bound bodies.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In other locations, they are directly connected together and appear close to a GERL cisterna (Plate 17, inset).

In the control preparations no cell components were stained (Plates 18–20).

DISCUSSION

Previous studies have focused on the localization of two enzyme-markers in *Meriones crassus*, namely, adenosine triphosphatase and alkaline phosphatase, in order to determine the activity of the kidney cells in their basolateral and apical borders respectively (Safer & Hussain 1989; Safer *et al.* under publication).

The main task of the present investigation is to advance knowledge regarding the cytochemistry of the proximal convoluted tubule cells in the gerbil kidney, using the ultrastructural distribution of acid phosphatase activity to demonstrate the secretory mechanisms in these cells, particularly the lysosomal systems, Golgi apparatus, the Golgi associated Endoplasmic Reticulum involved in the formation of Lysosomes (GERL complex) (Novikoff 1964; Safer 1978) and the organelles associated with it. Acid phosphatase has been widely used as a marker for these cellular components revealing their structures and functions in different tissues (Novikoff, 1964; Maunsbach 1966; Novikoff & Novikoff 1977; Borger 1973; Safer 1978; El-Mardi 1987; Ning *et al.* 1992).

In most instances, the present findings show an extensive acid phosphatase positive GERL system and lysosomal-vacuolar apparatus in the proximal convoluted tubule cells. The reaction product was found in the cisternal and the tubular elements of GERL, which is usually located, according to the common definition close to the nucleus in a specialized region of the endoplasmic reticulum relative to the Golgi saccules (Novikoff 1964; Ehrenreich *et al.* 1973; Novikoff & Novikoff 1977). As expected, the Golgi apparatus was unstained as reported by other authors (Novikoff *et al.* 1971; Novikoff & Novikoff 1977; Safer 1978).

Further observation revealed that GERL complexes are relatively abundant. It seems appropriate to confirm that the cisternal and the tubular elements were associated with acid phosphatase vesicles, that probably pinched off to form the primary lysosomes (Safer 1978). These lysosomes may also be associated with digestible exogenic or endogenic cellular materials to form larger lysosomes (secondary lysosomes). These observations are in agreement with other studies (Novikoff 1964; Novikoff *et al.* 1971; Novikoff & Novikoff 1977; Safer 1978).

As presented, the cerium phosphate depositions clearly showed a prominent highly extensive, and complicated GERL network of compacted stacks with flattened areas that may be extended along the axis of the cell. This localization was more regular and finer than that showed by Safer (1978) in a similar area in rat kidneys. The cerium chloride method revealed the complexity of the GERL elements. When tilted from the flat, they show their anastomosing pattern and also their parallel tubular elements with many blebs coming out of them. The latter appear to be primary lysosomes.

The membrane-bound bodies lying in the thick basal laminae were negative to acid phosphatase activity. This suggests that these bodies are not of lysosomal in origin and may thus support the biogenesis concept of Safer (1992) that the membrane-bound bodies are pinched off from the basal infoldings and their extended tips.

By using cerium chloride as a capture agent it seems quite reasonable to expect widespread acid phosphatase positive lysosomes with fine depositions of cerium

phosphate and discrete membranes. The stained apical lysosomes show their contact with the apical vacuoles as described by Straus (1964), and Maunsbach (1973) while several types of dumbbell and spherical shaped-lysosomes contained inclusion-like substances. Such lysosomes play a role in autophagy and in the digestion of extracellular macromolecules (e.g. protein) (de Duve & Wattiaux 1966; Maunsbach 1969, 1976; Safer 1978; Ning *et al.* 1992). Such lysosomal activities are probably due to the activity of the acid phosphatase (Borger 1973).

The performance of the secondary lysosomes is evidently localized. Large lysosomes may commonly fuse as reported by Ericsson (1964), Maunsbach (1969), and Safer (1978). In this respect it is interesting to emphasize the fine reaction product demonstrated by the limiting membrane of the lysosome as described by Maunsbach (1969) who also suggested that the different sizes, shapes and the internal contents of lysosomes in the proximal convoluted tubule cells may be strongly correlated with functional heterogeneity within the lysosomal systems.

There are irregular lysosomes that are connected with each other even by a fine thread-like structure, and this may be related to the route of acid phosphatase transport, and secretion of other hydrolytic enzymes into the extracellular fluids.

Finally, the demonstration of high GERL and lysosomal activities may form a basis for future attempts at correlating the cytochemistry and morphology of the kidney. These structures may play a role in the adaptation of this desert animal to withstand severe conditions such as extreme water deprivation and high salt tolerance.

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الموضع الخلوي الكيميائي لنشاط الآسيد فوسفاتيز بطريقة السيريم المعتمدة في الأنيبيبة الجوارية الملتوية لكلى الجربوع ميريوناس كراسس بالإشارة الى مركب الجيرل وجهاز اللزوزومات التجويفية.

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خلاصة

المواضع الدقيقة لنشاط الآسيد فوسفاتيز ترسخت في خلايا الأنيبيبات الجوارية الملتوية للجربوع **ميريوناس كراسس** باستخدام بيتا جليسروفوسفيت كمادة خاضعة وسيريم كلورايد كعامل آسر. ناتج التفاعل للسيريم فوسفيت كان مترسبا بعناية في مختلف مواقع مركب الجيرل (ارتباط جهاز جولجي بالشبيكات الاندوبلازمية المتعلقه بتكوين اللزوزومات) شاملا عناصره الوعائية والأنبوبية وأيضا أجهزة اللزوزومات التجويفية.

وجد ارتباط واضح بين عناصر الجيرل واللزوزومات الحويصيلية والتي تدل على المنبت المحتمل للزوزومات الاولية. وأيضا كانت عناصر الجيرل عديدة ومركزة خلال النسيج الخلوي بمختلف النماذج والصفات متواجدة بجوار الأنوية ومناطق جهاز جولجي. زيادة على ذلك.. الدراسة تؤكد وجود للجيرل مشابها للدراسات المماثلة.

كان جهاز جولجي والسطح العلوي للخلايا وكذلك التعرجات السفلية أو الأجسام الخلوية الخاصة بالغشاء القاعي السميك غير مصبوغ ولا تظهر أي نشاط. وهذا ينفي فكرة الأصل اللزوزومي لتلك الأجسام.

كما أن النشاط الأنزيمي أوضح ترسبات بارزة من الآسيد فوسفاتيز في اللزوزومات والتي تكون متوزعة كدوائر منتظمة أو أشكال غير منتظمة تكون بعضها مثل الدمبل وأخرى تحتوي على أجسام ضمنية.. بينما يظهر البعض الآخر وبوضوح نشاطا التصاقيا.

ترسبات السيريم الدقيقة تؤكد تركيبة عناصر الجيرل والتي في بعض الدرجات من المستوى الافقي يظهرون نماذجهم المتلاقية من جهة وإمتداد عناصره الأنبوبية من جهة أخرى. وكما أن تلك الترسبات الدقيقة تسمح بتمييز الفراغ المحيطي بين الغشاء التحديدي للزوزوم ومحتواه الصبغي وأيضا امتداده وارتباطه ببعض اللزوزومات الأخرى بواسطة مركب يشبه الخيط.