



RESEARCH ARTICLE

A Scanning Electron Microscopic studies on the podocytes of the kidneys of rat (*Rattus norvegicus*) and blind mole rat (*Spalax leucodon*)

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Özet

Nur İH, Yoldaş A, Alan A, Akçay A. Kör fare (*Spalax leucodon*) ve rat (*Rattus norvegicus*) böbreklerinde podositler üzerinde elektron mikroskopik bir çalışma.

Abstract

Nur İH, Yoldaş A, Alan A, Akçay A. A Scanning Electron Microscopic studies on the podocytes of the kidneys of rat (*Rattus norvegicus*) and blind mole rat (*Spalax leucodon*).

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Amaç: Kör fareler yer altında kendi kazdıkları tünellerde oksijen oranı oldukça düşük ortamlarda yaşayabilmektedirler. Çoğu kemiricilerden farklı olarak, kör fareler bu koşullarda metabolik faaliyetlerini yüksek oranda yerine getirebilmektedirler. Mevcut çalışmada kör fare ile ratların glomerulusların, fonksiyonlarını ve normal yapılarını sürdürebilmeleri için önemli olan podositlerinin incelemesi amaçlandı.

Gereç ve Yöntem: Çalışmada altı adet erkek kör fare ve altı adet erkek rat kullanıldı. Rat ve kör farelerin podositleri morfolojik özellikleri Scanning Electron Mikroskopu (SEM) kullanılarak incelendi.

Bulgular: Kör farelerin böbrek uzunluğu, genişliği ve kalınlığı 15.6±0.25, 9.60±0.24, 7.80±0.37 cm iken, bu ölçüler ratda sırası ile 16.00±0.89, 9.40±0.74, 6.20±1.35 cm olarak belirlendi. Bowman kapsulu çapının ve Bowman aralığının ratlarda, kör farelerden daha büyük olduğu tespit edildi. Filtrasyon aralığı kör farelerde 0.049±0.003 µm iken, ratlarda 0.073±0.004 µm olarak ölçüldü. Podosit pedicel'lerinin şekillerinin birbirine benzer ve iğne şeklinde oldukları, uzunluklarının 0.88 ile 1.02 µm arasında değiştikleri ve her iki türde de hücrelerin kenar uzunlukları boyunca birbirine kenetlendikleri tespit edildi.

Öneri: Kör farelerin podositlerinin morfolojik olarak ratlarla aynı olduğu tespit edildi. Her iki türde glomerulusun çapları arasında istatistiksel olarak bir farklılık olmadığı belirlendi (P>0.05). Bunun yanında böbreklerin uzunluk, kalınlık ve genişlikleri arasında da istatistiksel bir farklılık olmadığı tespit edildi.

Anahtar kelimeler: Rat, spalax, SEM, böbrek, podosit

Aim: Blind mole rats can live in tunnels which are goosed by them underground, in environments that have quite low-oxygen ratio. Contrary most rodents, they can carry out high levels of metabolic rate under these conditions. The present study aimed out to compare the blind mole rats and rats's renal podocyte, an important cell for maintaining the normal structure and function of the glomerulus.

Materials and Methods: Blind mole rat (Male) (n=6) and rats (Male) (n=6) were used in this study. The morphologic features of the renal podocytes of blind mole rat and rat kidneys were examined by using scanning electron microscopic (SEM).

Results: While the length, width and thickness of blind mole rat kidney were 15.6±0.25, 9.60±0.24 and 7.80±0.37 cm, in rats were 16.00±0.89, 9.40±0.74, 6.20±1.35 cm, respectively. When compared Bowman's capsule diameters and Bowman slits of blind mole rat, the rat was measured to be larger. The mean length of Pedicel spacing (Filtration slits) of blind mole rat and rats were found to be 0.049±0.003 µm and 0.073±0.004 µm, respectively. The podocyte pedicels were uniform needle-like shapes, and its length was ranging from 0.88 to 1.02 µm. They were in contact with each other intertwined processes in both species investigated.

Conclusion: The podocyte of blind mole rat is morphologically similar to rat's. In both species, between to diameter of the glomerulus were not statistically significant differences (P>0.05). Furthermore, there were not statistically significant differences in the length, the width and the thickness of both species' kidneys (P>0.05).

Keywords: Rat, spalax, SEM, kidney, podocyte



Introduction

There are 29 families, 426 genera and 1814 species of rodents as known. The blind mole rat (*Spalax leucodon*) is a superspecies and shows a wide spread in Anatolia, Balkans, Russian steppes and Middle East and North Africa (Nevo 1991). The family Spalacidae is separated sub-species the Myospalacinae, the Rhizomyinae, the Spalacinae, and the Tachyoryctinae (Noris 2004). Spalacinae's there are 13 species in 1 genus, Spalax (Musser et al 2005). Kivanç (1988) reports that there are two species (*Spalax leucodon* and *Spalax ehrenbergi*) known as blind mole rat in Turkey. The genus of *Spalax leucodon* spends the vast majority of their lives in their underground burrows and tunnel systems. *Spalax leucodon* extensively lives between the height of 300 meters in Israel and 2600 meters in Anatolia. Its eyes are atrophied and the orbits are covered by skin. Their tail and auricles are not developed. The mean weight of blind mole rats is about 100-570 grams (Eroğlu 2006).

Scanning Electron Microscopy (SEM) has been used in order to 3-dimensional ultrastructural images organs including the kidney (Arakawa 1970) and tissues in morphological studies since the middle of the 19th century. SEM showed that a glomerulus consists of irregular vascular network which are covered by podocytes as finger processes. According to Vodenicharov et al (2007) were determined internal structures within the glomerular ball, capillary endothelial cells, glomerular basement membranes, podocytes. The endothelial cells lie within the lumen of capillary lumen. Capillary lumen is characteristic of plenty fenestrations. The glomerular basement membranes encircled both capillaries and mesangial matrix (Shirato et al 1991). It is well known, glomerular visceral epithelial cells are named Podocytes, which are key cells involved in the process of kidney filtration. Between podocyte extensions of one side and the other side have narrow filtration space through which circulating blood can be filtered (Kriz et al 1994). The mutual relation of neighboring podocytes with their processes provides a more important problem to be settled. The pedicels, which are extends at an angle of random irregularly the appearance a mosaic-like (Iwanaga 2002). The description by Simon and Chatelana (1969) that "foot process is not adjacent to another from the same podocyte, but the arrangement seems rather to be a staggered alternation between foot processes from two, or possibly more, podocytes" may represent a rather prevailing view of today. This view, clearly illustrated diagrammatically by Yamada (1955) as early as 1955, has, however, not been based on any concrete evidence such as a reconstruction of serial sections covering two neighboring podocytes. From its lateral sides the primary process issued thinner branches of a second order. The corners at the branching were often rounded by web-like attenuations (Fujita et al 1970). On the other hand, Trump and Benditt (1962) think that the foot processes may be formed by incisions in one and the same cell, and this view was supported by the SEM study by Buss and Kronert (1969).

It was known that spalax has some specific metabolic (Arieli et al 1977) and respiratory (Widmer et al 1997, İlgün et al 2014) features. For example, it was indicated that under laboratory conditions, *Spalax leucodon* was managed to

survive for 14 hours at the level of 3% oxygen. On the other hand, rat died after 2-4 hours at the same oxygen level. Because of this specific feature, spalax was exhorted as the animal model in hypoxic experiments (Shams et al 2004). Furthermore, blind mole rat can live until 21 years, and spontaneous tumors have never been observed in spalacids (Gorbunovaa et al 2012). As the present two investigated species (blind mole rat mole rat and rat) live in different at terrestrial media; it is suspected that there must be differences in the structure of the podocyte to adapt the respective habitats. Rats, laboratory animal, have served as an important animal model for research in various medical and some urologic fields. Therefore, the anatomically differences and similarities between the rat and rat are important.

The study is also the first comparative analysis of the electron microscopically structure of podocyte in blind mole rat and rat.

Materials and Methods

Spalax leucodon (Blind mole rat) (n=6) and rats (n=6) were used in the study. Blind mole rats were collected from region of Kayseri in Turkey. The trapped blind mole rat was taken to the Department of Anatomy at the Faculty of Veterinary Medicine, Erciyes University in cages under ideal conditions, dimensions of 1x0.7x1 cm. A cage has the capacity for 6 animals. Rat, healthy, average 12 months old, obtained from the animal house of Sutcu Imam University at Kahramanmaraş, were used for the present study. The rats were maintained under temperature (20±1oC) relative humidity (50–80%) and illumination (12 h light, 12 h dark) controlled conditions room. The animals were provided standard rat feed (procured by Feeding Company, Tavas Ltd., Turkey) and ad libitum water. All animals were accomplished according to Animal Care and Use Committee Guidelines (Approval no. 02.05.2012/16). Legal permission to conduct the animal experiments was obtained from the local responsible authorities Adana Veterinary Control and Research Institute Experimentation Ethics Committee. At 20°C room temperature, the animals were anaesthetized by intraperitoneal injection of combination of 10 mg/kg xylazine (Rompun® enj, Bayer Turk Chemistry Industry, Istanbul) and 100 mg/kg ketamine HCl (Ketalar® enj., Eczacıbaşı, Istanbul). Under anesthesia, they were killed by exsanguination from abdominal aorta before they regained consciousness. Following lateral abdominectomy, the kidneys were removed from the abdominal cavity. Immediately after removal, completing electron microscopic sample preparation procedures photographed under a scanning electron microscope (Leica, model Leo S440, Leica Cambridge, Cambridge, UK).

The dimensions of the kidneys were obtained with the use of a metric tape and a vernier caliper. The following dimensions of the left and right kidneys were taken:

Width: Measured from the medial to lateral border.

Length: Measured from the upper to lower pole.

Thickness: Measured from the visceral to the parietal surface.

The software SPSS for windows version 14.01 (Serial: 9869264) was used in statistical analysis of data. Spalax





Table 1. Comparative morphometric values of the kidney in spalax and rat.

	Spalax		Rat (WR)		Student T test
	n	Mean±SEM	n	Mean±SEM	
Kidney length (cm)	12	15.6±0.25	12	16.0±0.89	P>0.05
Kidney width (cm)	12	9.60±0.24	12	9.40±0.74	P>0.05
Kidney thickness (cm)	12	7.80±0.37	12	6.20±1.35	P>0.05

Table 2. Comparative morphometric values of the kidney in spalax and rat.

	Spalax (µm)		Rat (µm)		Student T test
	n	Mean±SEM	n	Mean ± SEM	
Glomerulus diameter	24	72.39±1.340	24	72.62±0.459	P>0.05
Bowman's capsule diameter	24	79.02±1.128	24	83.71±1.344	P<0.05
Bowman's slits	24	3.985±0.154	24	6.014±0.457	P<0.001
Pedicle spacing (Filtration slits)	24	0.049±0.003	24	0.073±0.004	P<0.001
Pedicle lengths	24	1.025±0.059	24	0.882±0.067	P>0.05
Pedicle thickness	24	0.127±0.005	24	0.172±0.012	P<0.001

and rat were compared for kidneys morphological data, using student T test. P<0.05 level was accepted statistically significant.

Results

Blind mole rat kidneys were seen to be more blackish dark red in color than rat during dissection. Each kidney had a cranial and a caudal surface, a medial and a lateral border and an upper and a lower pole. Adipose tissue surrounded hilus and sides of kidneys. The lateral borders were convex in shape and medial borders were concave and indented at hilus. The major renal vessels had their entry exit at the hilus. The length, width and thickness of the kidneys were shown as in Table 1. As shown in Table 2, glomerula diameter, Bowman's capsule diameter, pedicle spacing (filtration slits), pedicle lengths, Bowman's slits were seen to be difference between blind mole rat and rat.

The cut surfaces of the renal cortex revealed dispersed renal corpuscles (Figure A-1, 2). In many of them the Bowman's capsule was cut open in such a way that the glomerulus, either intact or sectioned, was exposed sitting in the bowl-like capsule. The glomerulus was represented by the loops of blood capillaries winding like the intestine. The cell bodies or nuclear portions of the podocytes that attached to the wall of the capillaries in both blind mole rat and rat glomerulus were defined as moderate thickened bodies. Some cells lay inside of the curves of the capillary convolution extending their processes to surround the sides of the curve. In the glomerulus with collapsed capillaries, the podocytes were rounded and protruded into the space of Bowman. From five to ten processes, mainly about 1 µm thick emerged in different directions from the peripheral margin of the podocyte. The foot processes or pedicels were generally issued from the sides of the secondary or tertiary processes. Some pedicels

emerged from the primary processes (Figure A-3). Only a few originated from the cell body directly. Anastomosis or bridge formation between two adjacent processes was often observed in the primary, secondary and tertiary process levels (Figure A-3, 4).

In both species, these main or primary processes were seen to flatten on swollen capillaries, but columnar on collapsed ones. The thickness varied irregularly along their course. They ran mainly in a circular direction surrounding the capillary wall. From its lateral sides the primary process issued thinner branches of a second order. The corners at the branching were often rounded as spider web like in all cases.

The end branches called the "foot processes" or "pedicels" were issued generally as side branches from the secondary processes. A few secondary processes were ramified into tertiary branches before issuing their foot-processes. On the other hand, primary processes of a small size became "fern leaves" issuing their own foot-processes. Foot-processes occasionally originated directly from the podocyte cell body. The foot-processes were fairly constant in lengths (0.88-1.02 µm) and ended in a rounded tip. Some of the secondary or tertiary processes represented a bridge between two adjacent main processes of the same cell, thus forming a circuit of the processes (Figure B-5, 6). The main and secondary processes were thickened at some places into a wide plate. The end of the primary and secondary processes was formed variably. It was observed that ends of pedicels thickened in rat (Figure C-8). The pattern of ramification of the podocyte processes was much more regular in the rat than in the blind mole rat. In the rat, the branching of the secondary and foot processes was generally at a right angle. While pedicle thickness was about 0.12 µm in blind mole rat, it was 0.17 µm in rat. There were statically significant differences in between of the both species (P<0001).

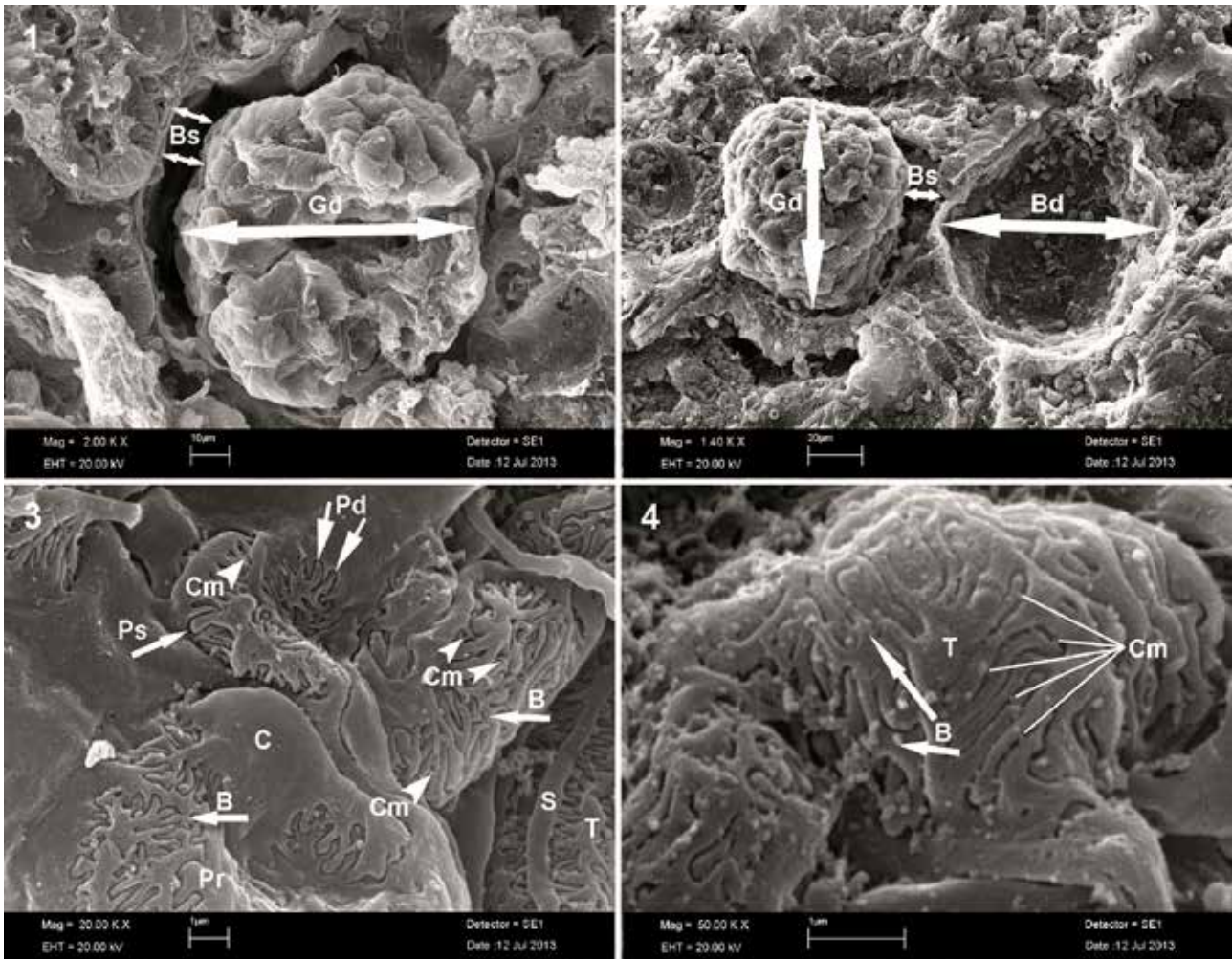


Figure A. 1: Surface view of a rat glomerulus. Glomerulus diameter (Gd) and Bowman's slits (Bs). 2: A low magnification of a glomerulus of an autopsy in spalax. The podocytes covered all the winding, well-expanded blood capillary loops. The inner and outer surface of the remaining Bowman's capsule. Bowman's diameter (Bd), Glomerulus diameter (Gd) and bowman's slits (Bs). 3: Surface view of a rat. Podocyte cell body (C) is seen in this picture micrographs at lower magnifications Show that this area processes from four different cells Primary (Pr), Secondary (S) and Tertiary (T) are interdigitated with each other. It is clear that neighboring foot processes come always from different cells and same cell. Cytoplasmic bridges (B) and between the processes of one and different cells. At the same time, However, communicans might call connection between podocyte observed (Cm). The podocyt foot processes formed indact filtration slits (Pedicel slits) (Ps). 4: Irregular ramifications as seen in the center of this picture is characteristic pattern of foot-processes in a spalax glomerulus x 20.000, Cytoplasmic bridges (B), between podocyte-shaped springs can be called communicans connections (Cm) and tertiary processes (T).

In both species, microvilli were seen also on the podocyte processes and foot processes (pedicels). Four to seven processes were emerged from the peripheral margin of the cell body. The size of these primary processes were very variable. Most of them were long and wide, distributing secondary and tertiary processes and pedicels (vide infra) to a distance (Figure C-7). Most of the primary processes issued some secondary processes from their lateral sides. Occasionally, tertiary processes were ramified from the secondary processes. The foot processes or pedicels were generally issued from the sides of the secondary or tertiary processes. Some pedicels emerged from the primary processes. Only a few were issued from the cell body directly. Anastomosis or bridge formation between two adjacent processes was often observed in the primary, secondary and tertiary process levels. The number of the connection the between of pedicels spacing in the blind mole were more than rat (Figure C-7).

The secondary processes emerged not oppositely but rather alternately. The pedicels of the blind mole rat podocyte were generally thin columnar processes, thickening irregularly at places. They often divided into two branches. Some pedicels were flattened and wide, forming into a leaf-like shape (Figure A-4).

Discussion

The gross features of the rat and blind mole rat observed in this study showed close similarity to reports on the rat by Onyeanusi et al (2009), Nur and Yoldaş (2011) and Simon and Chatelana (1969). The shape and color was also similar for rat Onyeanusi et al (2009). However, mole rat kidneys were blackish dark red kidney than rat in during dissection. In this study, these findings confirm those of (Yamada 1955, Arakawa 1970, Fujita et al 1970, Iwanaga 2002) regarding the nature of the filtration surface of the glomerulus, and





described morphological property of podocytes and their foot processes, which are specialized cells and play an important role in glomerular filtration.

In this study shows that the finger-like pectineal extensions of pedicels are primary feature of the glomerular podocyte in both blind mole rat and rat.

According to literature dates Arakawa (1970), Shirato et al (1991), Fujita et al (1970), podocytes of both in the embryo and in the neonatal period frequently is situated flattened surfaces to the glomerular membrane. These findings demonstrate that undifferentiated podocytes be as irregular protrusions can be interpreted as podocyt (Hay and Evan 1979). In addition to the present observations, it were seen that the coverings rate of glomerular epithelial of pedicels; were quite a large proportion in rat and blind mole rat.

In this SEM study, we obtained a three-dimensional visualization of the podocytic membrane which, in fractured appearances, coincided with the diagram from Yoshinari and Fujita (1982). The primary, secondary, tertiary and even terminal processes were markedly irregular in shape, length and direction, compared with the uniform pattern of the processes in the rat and with the less uniform one of the blind mole rat. Buss and Kronert (1969) who performed the first

scanning electron microscopy of the glomerulus, using the rat, described neighboring processes as coming partly from the same cell. According to his paper, slits within cytoplasm gave rise to cytoplasmic bridges which further divided themselves and finally built up a meshwork of processes within a cell. Fujita et al (1970) using the rat and the rabbit, and the Arakawa (1970) using human and rat recognized that the interdigitation of the terminal processes occurred always between those of different cells. The present study confirmed their findings which it originated from different cells and exclusively regular.

The most important discrepancy concerns the cellular source of neighboring foot-processes. Buss and Kronert (1969) described the neighboring foot-processes as coming either from different podocytes, or from the same cell. Laying stress on the latter case, they lead to a conclusion that the slits between the end-feet represented unique incisions and pores formed within a single epithelial cell. This view is compatible with that of Trump and Benditt (1962) who, using the transmission electron microscope, described the neighboring foot processes as derived from the same cell.

The present observation on the renal podocytes of the rat and blind mole rat using the scanning electron microscope clearly indicated that the neighboring foot processes came

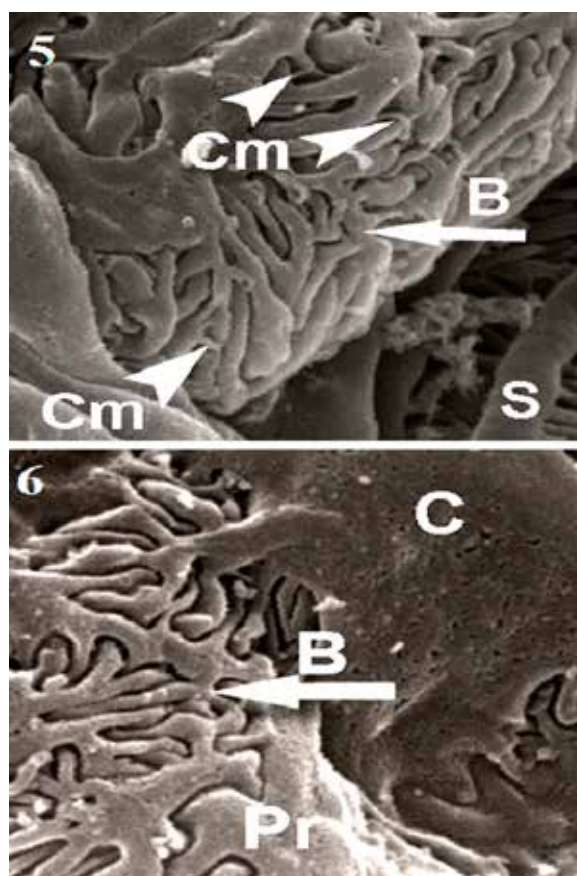


Figure B: 5: A closer view of the central part of figure 3. 6: A closer view of the central part of Figure A. The labels correspond to those in that figures. Note microvilli on the end-feet and thicker processes x 20.000.

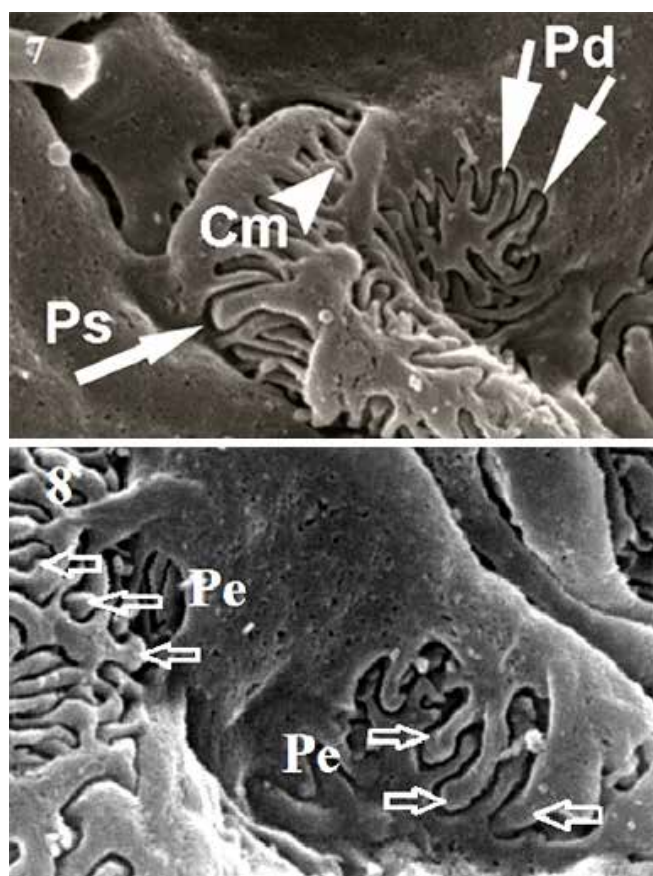


Figure C: 7: A closer view of figure 4. Pedicel slits (Ps) and), between podocyte-shaped springs can be called communicans connections (Cm) and foot processes (Pedicels) (Pd). X 20.000. 8: A closer view of Figure A. Enlargement Pedicel ends (Pe)





always from different cells and so the slits in between were the true intercellular spaces.

The second important difference of our observation from that by Buss and Kronert (1977) is on the "Cytoplasmabrücken" (cytoplasmic bridges) which they frequently found between the processes not only of the same cell but also of different cellular sources. The present observation showed many microvilli on the cell body as well as on the processes which were occasionally considerably long. Most of the "bridges" by the named authors (Simon and Chatelana 2002) are believed to correspond to microvilli which appeared, presumably by the effect of coating, as if fused at their free end with a process. A bridge by a thin cytoplasmic thread occasionally could be revealed also in our micrographs but they were always restricted within one and the same cell.

Study in different animal species on pedicels has shown that the pedicels were fine mosaic array of their pedicels (Iwanaga 2002). In this study we have observed pedicel slits of the ratio of both types where there are significant differences were detected. Also the pedicels of the spalax podocyte were generally thin columnar processes, thickening irregularly at places. They often ramified dichotomically.

Fujita et al (1970) stated that in the rabbit the pattern of ramification of the podocyte processes is irregular when compared with other species like the human and rat. According to our observations the irregularity was noticed in the size and branching mode of the primary and secondary processes, as well as in the distribution and interdigitation pattern of the end feet. The frequent anastomosis or bridge formation between two adjacent processes of primary, secondary and tertiary levels is also characteristic of the rat and blind mole rat.

Conclusions

The present study was seen that the podocyte extensions of the rat and the blind mole rat differ conspicuously in their pattern of arborization. A comparative study in different species of rodents seems to be an attractive field to which the scanning electron microscope will be effectively applied. Whereas the podocytes constriction of blind mole rat was same as rat, its value of morphometric was different from rat. We hope that the present first comparative investigations about the structure of podocyte on blind mole rat and rat will increase to concern on urinary system studies of blind mole rat.

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