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# *Nephrotic syndrome (NS) and pregnancy in rat – evaluation of kidney in electron microscopy*

The influence of pregnancy on primary kidney disease evaluation is still controversial. The pathophysiology of such complications is poorly known. In the present study we tried to make histological evaluation of kidneys of female rat in which pregnancy coexisted with existing before NS. The NS was induced by giving to rats adriamycin, antibiotic from anthracycline group with anticancer activity. The experiments performed by author (8) and other investigators (5, 14) showed that this drug developed NS in rats.

## MATERIAL AND METHODS

In the experiment there were used 16 female rats. The animals were divided into two groups: control and experimental with 8 rats in each group. At the beginning of the experiment female rats from the experimental group were given adriamycin in a dose of 5 mg/kg of body weight intraperitoneally, and female rats from control group were given 0.5 ml of 0.9% NaCl intraperitoneally as well.

Once a week female urine protein concentration was analysed with the stripe test. After 4 weeks from the beginning of the experiment female rats were paired with males. On the $20^{th}$ day of pregnancy pregnant females were decapitated and left kidneys were taken for histological investigation.

The ultrathin preparations were performed, which were stained with 8% solution of uranyl acetate in 0.5% acetic acid and plumbic cytrate according to Raynolds. Documentation was performed with electron microscop Tesla BS-500.

## RESULTS

The picture of kidneys in electron microscopy was the following. In the control group ultrastructural pictures of glomerules and convoluted tubules of kidneys rats were analogous to manuals descriptions of the norm (Fig. 1). In the experimental group changes in the electron picture of glomerules were similar in all individuals (Fig. 2).

In podocytes cytoplasm optical empty or containing non-homogeneous material vacuoles were observed. Some of them had a diameter bigger than nucleus diameter. Vacuoles were surrounded with smooth or rough membrane. Similar vacuoles were also observed in some endothelial cells. In some of podocytes perinuclear vacuoles (the feature of oedema) were observed, visible as a light "halo" around the nucleus. Diluted rough endoplasmic reticulum canals were focally visible. In podocyte cytoplasm some mitochondria were swollen. Local fusion of foot processes of podocytes were also observed. Basal membranes of glomerular vessel loops were focally thickened

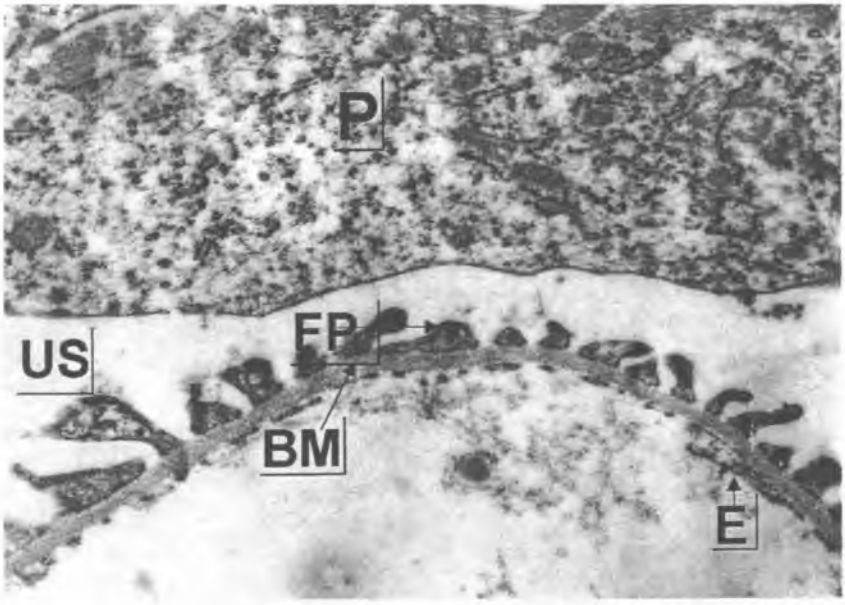


Fig. 1. Control group. Normal picture of female rat glomerulus fragment in electron microscopy. Blood-primary filtrate barrier. Magn. 14,000x; P – podocyte, FP – foot processes, N – nucleus, E – endothelial cell, V – vacuole, RBC – red blood cell, US – ureal space, BM – basement membrane, EC – epithelial cell, MC – mesangial cell

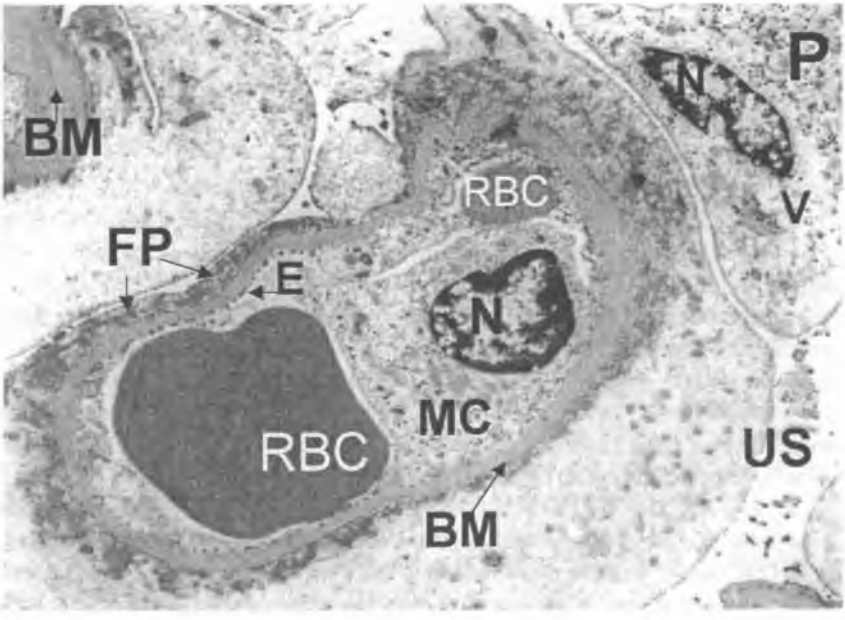


Fig. 2. Experimental group. Blood-primary filtrate barrier. In podocyte (P) cytoplasm visible vacuoles (V). Focally fusion of foot processes of podocyte (FP). Focally distinctively thickened basal membrane (BM). Magn. 6000x

In proximal tubules total indistinctness of barriers between tubular epithelial cells were observed (Fig. 3). Flattened or completely destroyed epithelial cells were visible as well. The brush broder was focally destroyed and tubular light was focally dilated. In tubular lumen the homogenous casts naked nuclei, mitochondria and single whole tubular epithelial cells were visible. Under the epithelium basal membrane was focally thickened.

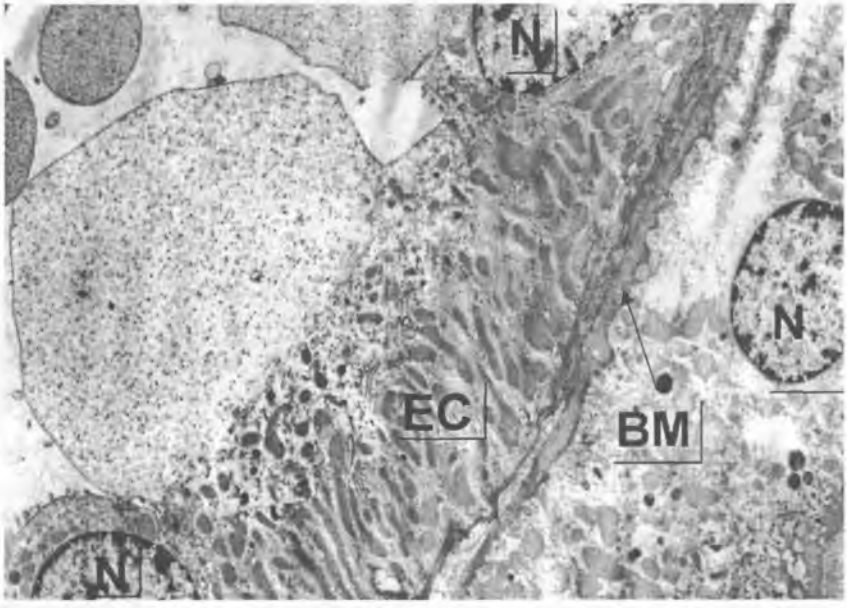


Fig. 3. Experimental group. Homogeneous substance in tubular lumen. Blurred barriers between tubular epithelial cells (EC). Thick basal membrane (BM). Magn. 3000x

## DISCUSSION

The description of NS, which had developed after a single dose of adriamycin could be found in literature (2, 8, 9, 12, 15, 16). In the present study the appearance of distinctive proteinuria 4 weeks after adriamycin administration in one dose of 5 mg/kg of body weight interperitoneally, was taken as a sure sign of NS according to literature data and own study (8). To induce NS, adriamycin was used also in a smaller dose of 3–3.5 mg/kg of body weight in one intravenous dose (9, 10, 12). Distinctive proteinuria appeared then 2 (10) to 4 weeks (9), so in approximetely the same time as in the dose of 5 mg/kg of body weight. Adriamycin was given mainly intravenously. In the present study the drug was given intraperitoneally (11). In case of rats it is an easier and less traumatic way. Results did not differ as regards time of appearance of signs and clinical-histological picture of changes comparatively to intravenous drug administration. Intraperitioneal way was also used by Papierkowski et al. and Skomra et al. (7, 11). Papierkowski et al., in order to develop signs of NS administered adriamycin in a dose of 5 mg/kg of body weight intraperitoneally two times during the experiment (on the first and 56$^{th}$ day). Extensive proteinuria they observed already 2 weeks after beginning the experiment. In the present study extensive proteinuria was observed starting from the fourth week to the end of the experiment.

Distinctive proteinuria during the pregnancy could be the result of coexisting NS or preeclampsia and could as well depend on other diseases of kidneys and urinary tract, but then it is not the sign of

glomerular insufficiency (6). Differentiation of NS and preeclampsia during pregnancy is difficult. Good solution seems to be the biopsy.

Preeclampsia is a complex of signs which is the most frequent complication of high-risk pregnancy (it is a serious danger for mother and child). Now it is known that morphological changes in kidneys in preeclampsia are distinctive and specific for that disease and let differentiate it from other diseases, which could create diagnostic problems (1, 3, 4, 13). These changes are presented in: diffuse oedema and thickening of endothelium with indention of them to vessel lumen and ischemia of loops with lack of proliferation signs; swelling and vacuolar changes in Bowman capsule epithelial cells; deposition of fibrin-like substance casts between endothelial cells and basal membrane; delicate oedema of interstitial tissue and small degenerative changes in proximal convoluted tubular cells (1, 4, 13). There were not stated any changes in basal membrane or indistinctness of podocytes foot processes building. The regression of observed changes results in renewed closing of damaged barrier and dissapearing of proteinic urine (3).

There is described a complex of morphological changes, typical of glomerular filter damage, which is the reason of increased serum protein permeability of glomerules in NS and is the direct reason of proteinuria, that is: thickening and change of basal lamina structure and blurring of building and fusion in one band foot processes of podocytes (3).

The histological picture of kidney observed in electron microscopy in the present study was similar to the picture which was described in literature in non-pregnant female with NS. It showed in kidney the features of proteinuria and morphological damage of filtration membrane (focal thickening of basal lamina, fusion of foot processes of podocytes in one band) as a reason of increased permeability of protein. The changes visible in tubular epithelium seem to be secondary to glomerular changes.

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## SUMMARY

The purpose of this study was the histological evaluation of kidneys on the ultrastructural level. In the experiment there were used pregnant female rats in which gestation coexisted with adriamycin-induced NS. Results showed numerous focal changes in kidney glomerules and tubules which were the evidence of increased protein loss and filtration barrier damage as the main cause of increased permeability for proteins.

Ciąża współistniejąca z zespołem nerczycowym (NS) wywołanym doświadczalnie u szczurów – ocena nerek w mikroskopie elektronowym

Celem pracy była ocena histologiczna nerek na poziomie ultrastrukturalnym. Do doświadczenia użyto ciężarne samice szczura, u których ciąża współistniała z ZN indukowanym adriamycyną. Wyniki badań wskazują na liczne ogniskowe zmiany w kłębkach i kanalikach nerkowych, świadczące o wzmożonej utracie białka z moczem, oraz na uszkodzenie bariery filtracyjnej kłębka jako główną przyczynę wzmożonej przepuszczalności dla białka.