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*The pregnancy coexisting with experimentally induced  
nephrotic syndrome (NS) in rats – the histological assessment  
of kidneys in light microscopy*

The important issue in the present study is the histological assessment of pregnant rat's kidneys in which pregnancy was co-existing with experimental adriamycin-induced NS. The urinary system of females in physiological pregnancy show reversible morphological and physiological changes touching equally kidneys as well as the urinary tract (3).

The kidney longitude increases about 1 cm, and in microscopic picture glomerular enlargement is present, but it is not due to the increase in the number of cells but their enlargement. Widening of the urinary tract is accompanied by decreasing of peristaltic movement. All this picture is sometimes called gestational hydronephrosis that differs from hydronephrosis during obstructive uropathy, because it does not show signs of obstructive nephropathy. Physiological changes are due to increased perfusion of kidney and increased glomerular filtration.

Urine has lower density, and aminoacids and glucose concentration in it are higher than in the normal range (5). So during physiological pregnancy period proteinuria could appear. The influence of renal disease on pregnancy intercourse and especially on renal function is still an open issue.

#### MATERIAL AND METHODS

In the experiment were used 16 female rats with initial body weight 200–250g, aged 2.5 to 3 months. 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 the dose of 5 mg/kg of body weight intraperitoneally (to develop the NS), and female rats from control group were given 0.5 ml 0.9% NaCl, also intraperitoneally.

Once a week female urine protein concentration was analysed with stripe test. After 4 weeks from the beginning of the experiment females were paired with males. Effectiveness of copulation was assessed with presence of sperm cells in vaginal smear. On the 20<sup>th</sup> day of pregnancy pregnant females were decapitated and left kidneys were taken for histological investigation. The performed preparations were stained with hematoxyline and eosine, with PAS and Masson's methods, and semi-thin slides were stained with methylene blue and Azur II. The preparations were observed in light microscopy. Photographical documentation was performed with camera to light microscopy – Jenaval Contrast Carl Zeiss.

## RESULTS

After 4 weeks from the beginning of the experiment in urine of all animals from the experimental group protein concentration measured with stripe test was above 10 g/l. In the control group during the time of experiment it not change significantly and was from 0 to 0.3 g/l. So all rats were qualified to the further part of the experiment.

Insemination took place mainly on 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> day from paring females with males. During that time in the control group one female had stated not effective copulation. In experimental group 100% effective copulation was stated.

Histological investigation of kidneys in light microscopy showed the following results. In the control group the microscopical picture of kidneys in all individuals did not differ from descriptions of kidneys from pregnant rats, which could be found in literature (Figs. 1, 3, 5, 7). The microscopical picture of rat kidneys from the experimental group showed changes, which were similar in all individuals. The changes were focal and segmental, and they touched single glomerules and tubules (Figs. 2, 4, 6, 8).

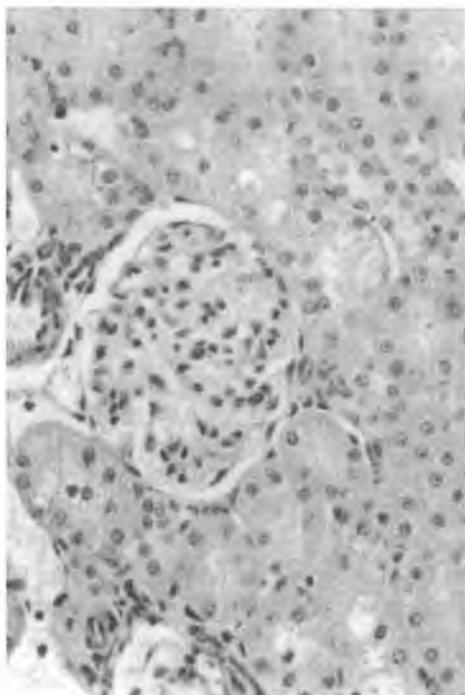


Fig. 1. Control group H+E staining.  
Magn. 320x

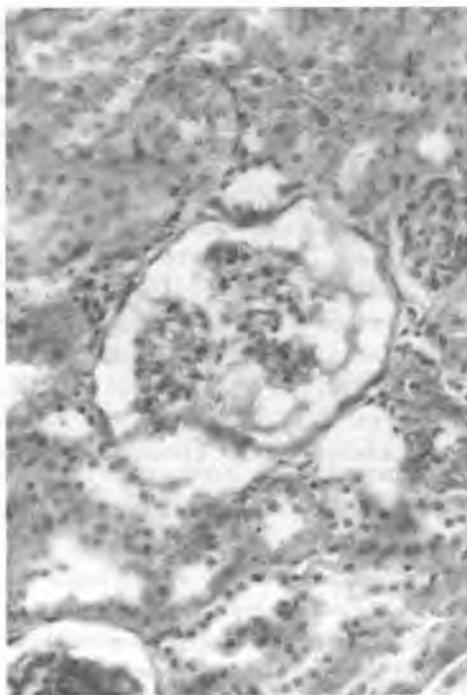


Fig. 2. Experimental group. The tubular epithelium significantly damaged. Tubular lumen dilated. Glomerules with deformed and destroyed vessel loops and significant dilatation of ureal space. Irregular shape parietal lamina of glomerular capsule. H+E staining. Magn. 320x

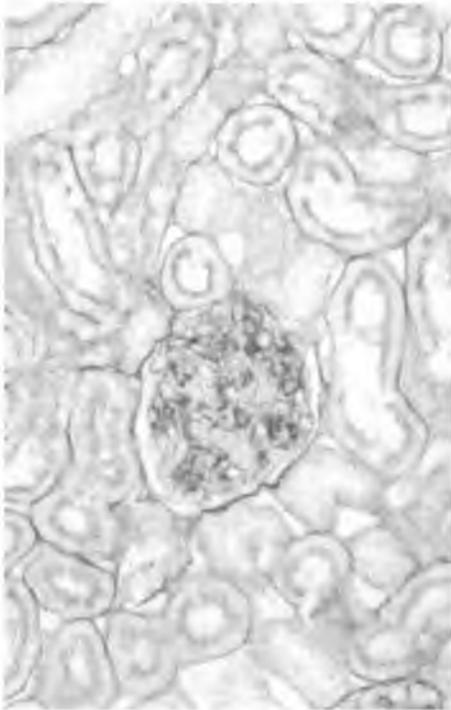


Fig. 3. Control group. PAS staining.  
Magn. 320x

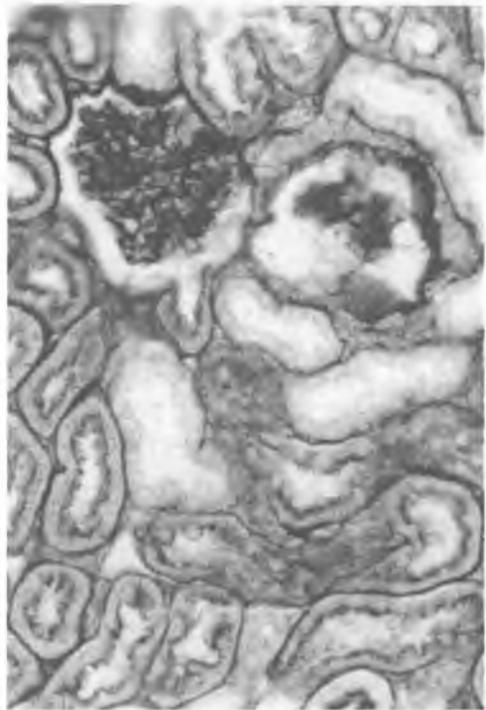


Fig. 4. Experimental group. Thickened tubular epithelium basal membrane. In glomerules: dilated, deformed and significantly damaged vessels loop. Dilatation of ureal space. Increase of amount PAS(+) positive mesangial matrix. Thickened basal membranes of glomerular capillar vessels. PAS staining. Magn. 320x

In glomerules focal thickening and deformation of an outline of glomerular capsule parietal lamina were visible in preparations stained with PAS method (Fig. 4). In some glomerules were visible thinned or completely destroyed, ruptured visceral lamina (Fig. 2). Ureal space was significantly dilated. In capsular lumen of some glomerules protein PAS(+) casts were presented, sometimes they fulfilled all ureal space (Figs. 2, 4). In glomerular vessels thickening of basal mambrane was observed, especially visible on preparations stained with PAS method (Fig. 4). Loop vessels were diluted and deformed, and in some glomerules they were destroyed partially or completely. The amount of fibres and cells of connective tissue between loops of vessels (mesangium) was increased – glomerulosclerosis (Figs. 4, 6).

In convoluted tubules focal lesion in tubular epithelium building was observed. The tubular building was irregular ( Figs. 2, 4, 6, 8). In most of tubules epithelial cells were flattened or completely destroyed. Intracellular barriers were blurred, the tubular light was dilated and the basal membranes were thickened (Figs. 2, 8). In other tubules epithelial cells were swollen. The cytoplasm structure was blurred, and frontiers between cells were invisible. Some of them were indented into the tubular lumen, which was significantly narrowed (Fig. 8). In cytoplasm of tubular epithelium cells, small and big vacuoles were observed, whose diameter were sometimes bigger than nuclear diameter (big or small vacuolar degeneration). Dark granules, which could be phagosomes, were also visible (Fig. 8). In tubular lumen

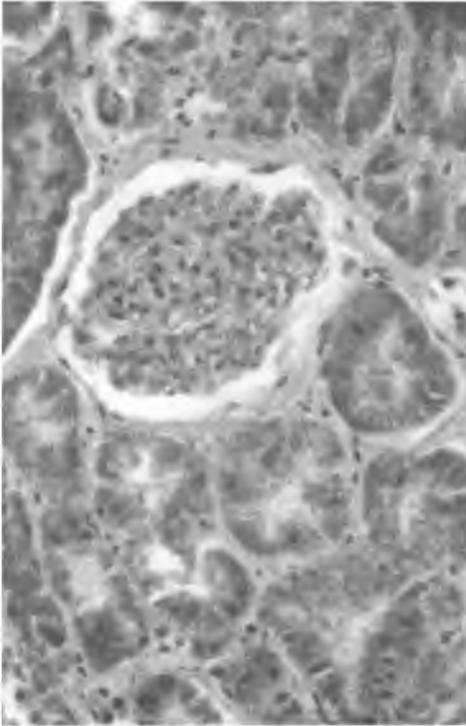


Fig. 5. Control group. Staining according to Masson. Magn. 320x

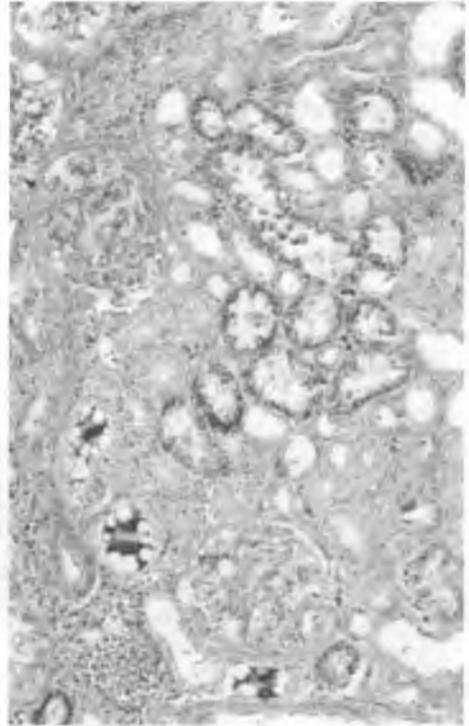


Fig. 6. Experimental group. Focally flattened epithelium in tubules, dilatation of lumen fulfilled with pathological casts. Blurred barriers between epithelial cells. Focally destroyed tubular epithelial cells. In glomerules deformed capillary vessels loops, increased amount of connective tissue. Irregular parietal lamina of glomerular capsule. Staining according to Masson. Magn. 160x

homogenous casts from PAS(+) positive material and parts and or completely exfoliated tubular epithelial cells were visible (Figs. 2, 4, 6).

In kidney's parenchyma focal hyperaemia, steatosis and partial necrosis and inflammatory infiltrations were present (Figs. 6, 8). Between tubules an increased amount of connective tissue was visible.

## DISCUSSION

There are numerous reports describing appearance of full-sign NS after adriamycin, and other antibiotics from anthracycline group. (1, 2, 6, 7, 12, 15). Some authors to develop the NS used the dose similar as in the present study – 5 mg/kg of body weight in a single dose (2, 7, 11, 14). They described full-signed NS 3, 4 weeks after adriamycin administration. Valentin (13) 3 – weeks after drug administration in a single dose 7 mg/kg of body weight, Remuzzi et al. (11) – after administration of 5 mg/kg of body weight noticed significant proteinuria already after 13 days.

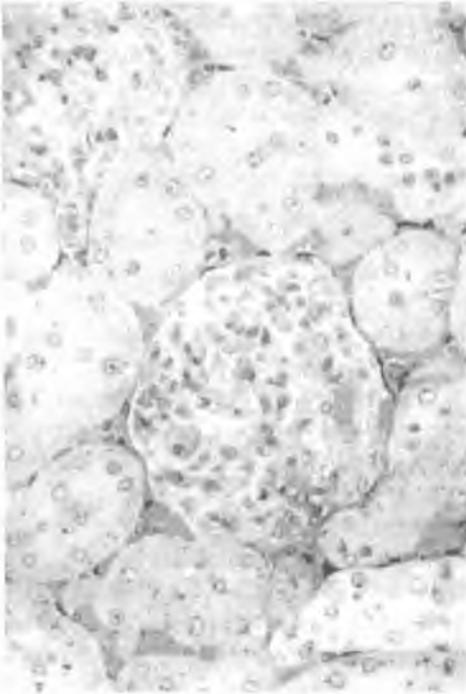


Fig. 7. Control group. Semithin slide. Methylene blue and Azur II staining. Magn. 320x

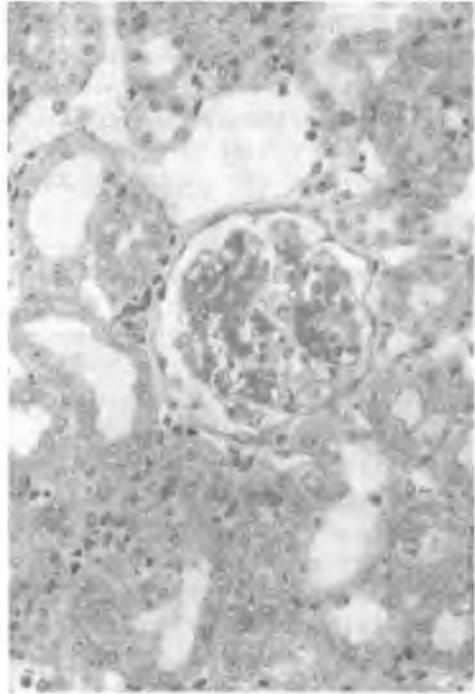


Fig. 8. Experimental group. Focally damaged tubular epithelium, blurred barriers between cells. In lumen of some tubules homogenous mass. In epithelial cells, small, empty vacuoles and irregular dark granules. Vacuolar degeneration visible also in glomerular vessels endothelium. Partly destroyed glomerular vessels loops. Dilatation of ureal space. Semithin slide. Methylene blue and Azur II staining. Magn. 320x

In preparations of female pregnant kidneys observed in own experiment in light microscopy there could be found features which were the evidence of extensive protein loss in urine. P o d j a r n y et al. (8, 9) in pregnant rats after administering adriamycin did not notice any changes in kidneys in light microscopy. P o m e r a n z (10) observed nevertheless an increased amount of mesangium. In the present study focal and segmental glomerulosclerosis was observed.

P o m e r a n z (10) noticed that during the pregnancy complicated with NS after adriamycin the relation  $TxB_2/PGE_2$  increased and stated that changes in synthesis glomerular prostanoids, especially tromboxan, could play an important role in activation of synthesis of mesangial matrix cells.

Similar observation was made by P o d j a r n y (9), who estimated the relation of glomerular tromboxan B<sub>2</sub> to prostaglandin E<sub>2</sub> in pregnant with NS as  $1.15 \pm 0.26$  and in non-pregnant females with NS after adriamycin as  $0.52 \pm 0.12$ . It was the statistically significant difference.

In relevant literature we could find discriptions of investigations about coexisting pregnancy and NS in humans. It is shown that histological changes in kidney are similar to those shown in the present experiment in rats.

Noshimoto (4) investigated pregnant women with NS. He described in kidneys focal, segmental glomerulosclerosis, also present in this study. He put forward the hypothesis that glomerular hypertrophy which develops during preeclampsia plays an important role in pathogenesis of focal, segmental sclerotic lesions in glomerules.

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#### SUMMARY

The purpose of this study was histological evaluation in light microscopy of kidneys of pregnant female rats, which before pregnancy suffered from adriamycin-induced NS. The result show numerous changes in glomerules and renal convoluted tubules, which were the evidence of increased protein loss with urine.

Ciąża współistniejąca z zespołem nerczykowym wywołanym doświadczalnie u szczurów – ocena histologiczna nerek w mikroskopie świetlnym

Celem pracy była ocena histologiczna w mikroskopie świetlnym nerek ciężarnych samic szczura, które przed ciążą cierpiały na ZN indukowany Adriamycyną. Wyniki badań wskazują na liczne ogniskowe zmiany w kłębkach i kanalikach nerkowych, świadczące o wzmożonej utracie białka z moczem.