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Effect of water exposed to a magnetic field on the morphological picture induced by hypobaric hypoxia in rat renal glomeruli

Dissertation for the degree of Doctor of Medical Sciences

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1. Introduction

a) magnetic field

When James Clerk Maxwell, in 1873, published in a work entitled: “Treatise on Electricity and Magnetism” his theories concerning the magnetic field, rejecting the views then prevailing, rooted in mesmerism and medieval distortions, experimental studies became the basis for evaluation and inference in the field of biomagnetism (1, 15, 31, 35, 48, 63).

Research conducted in recent years on the effects of magnetic fields on living organisms has provided a great deal of new information. This has occurred to a large extent because the U.S. Food and Drug Administration confirmed the need for basic research to explain the mechanisms underlying the therapeutic effects observed in medicine and obtained through the action of magnetic fields (33, 63, 64, 65, 70, 72, 74).

In parallel with progress in research on the use of magnetic fields in medicine, research in this area has also developed in industry. It mainly concerns water-transport technologies, technologies for liquid fuels, and the production of all kinds of electrical devices generating magnetic fields (including electrical equipment used in households) (3, 52, 64).

To a greater or lesser extent, living organisms, during the evolution of life on Earth, adapted without sensory involvement to perceive the effects of the geomagnetic field (2, 73). Over the last century (since the discovery of electric current), this adaptation has also incorporated interaction with magnetic fields as a phenomenon accompanying electric currents.

The results of studies conducted in recent years (4, 10, 12, 14, 15, 19, 24, 31, 34, 35, 45, 63, 64, 65, 71, 72, 73, 74) allow the basic mechanisms, or hypothetical assumptions, concerning the effect of magnetic fields on living organisms to be classified as follows:

- Effects on weakly compensated magnetic spins (rotational motions about their own axes) of paramagnetic elements that are components of coenzymes and prosthetic groups of enzymes.
- Displacement of moving electric charges that generate biocurrents in the body as a Hall effect (resulting from the Lorentz force on electric-current carriers in a material), as a consequence of the Lorentz force (the force with which a magnetic field acts on a moving charged particle).
- Changes in the state of crystals in extracellular plasma membranes, as well as intracellular membranous structures whose layers have liquid-crystalline properties.
- Induction, in electrolytic cellular structures, of potential differences associated with moving ions.
- Phase shifts in the biological rhythms of cells.
- Disturbance of adhesion processes of multinucleated neutrophilic granulocytes to vascular endothelial walls.
- Changes in the physicochemical properties of water (viscosity and surface tension).
- Modification of depolarization processes in cell membranes that use their intrinsic automaticity (in nerve cells).

Clinical data based on detailed and thorough laboratory studies, well documented in the scientific literature (18, 63, 64, 65) concerning the effects of magnetic fields are manifested as:

- Increased oxidation-reduction processes in mitochondria (24, 65).
- Stimulation of protein synthesis accompanied by acceleration of cell division, leading to intensification of tissue regeneration processes (7, 11).
- Stimulation of angiogenesis (62).
- Anti-inflammatory, analgesic, and anti-edematous effects (16, 63).
- Changes in the rheological properties of blood.
- Effects on hormonal and enzymatic activity, sometimes resulting from the need to widen calcium channels in cell membranes (25, 69, 75).

Attempts to explain the observed effects of magnetic fields encounter many difficulties associated with the methodology of the experiments performed and with the lack of highly sensitive measuring devices. The observed result most probably reflects the combined activation of many mechanisms at the molecular level. Nevertheless, the use of magnetic fields in magnetotherapy for numerous disorders is beneficial and only slightly less effective than pharmacological treatment (9, 63, 72).

b) water

Water is the only inorganic liquid occurring in nature. It is the only compound occurring in all three states of matter as a solid, liquid, and gas (vapor). In living organisms, water accounts for the largest quantitative and volumetric percentage of organic matter (70-

90%). Despite numerous studies of its place among liquids, its physical and biochemical properties are still not fully understood (13, 71).

Water is a commonly used solvent for a wide variety of chemical and biochemical reactions. It is a structural component of cells and tissues. By forming close associations with proteins, nucleotides, carbohydrates, and lipids, it participates in the formation of polymers and biological macromolecules. Water contained in protein crystals constitutes 40-50% by weight and determines the liquid-crystalline structure of various organelles (61, 65).

Membranes surrounding tissues, organs, and individual cells, as well as intracellular structures such as cell nuclei, mitochondria, Golgi apparatus structures, lysosomes, endoplasmic reticula, and membranes forming the intracellular cytomembrane system and having liquid-crystalline properties, are the subcellular structures most susceptible and sensitive to all exogenous stimuli. The actual effects of such action result from the presence in biological membranes of various types of enzymatic and receptor proteins that regulate all metabolic processes (61, 67).

Water is essential for the normal course of most reactions occurring in living organisms, serving not only as the medium but also as a reaction substrate. It has been demonstrated that under a magnetic field, water changes its physical properties. Water from a normal water-supply system, exposed to an external magnetic field, shows accelerated crystallization and an increased concentration of dissolved gases (mainly O2). Sedimentation and coagulation processes are accelerated; pH also changes, viscosity decreases, and electrical conductivity changes as well. The use of such treated water, e.g. for irrigating plants, increases their growth by 20-40%, and also accelerates the proliferation of microorganisms in cultures (31, 54, 63, 71, 74).

c) morphological structure of the renal glomerulus

The structural correlate of the kidney's most important function, namely the production of final urine, is the renal corpuscle (corpusculum renale), historically called the Malpighian corpuscle (23, 66). During embryonic development it arises from a spherical epithelial vesicle into which a tuft of capillaries invaginates, transforming the vesicle into a cup that forms the primordium of the two-layered Bowman's capsule.

Within the renal corpuscle, the following are distinguished:

- Bowman's capsule (capsula glomeruli) with a vascular pole (polus vascularis) and a urinary pole (polus tubularis),
- the vascular glomerulus (glomerulus)
- mesangial cells and extracellular mesangial matrix (mesangium)

The vascular glomerulus fills the renal corpuscle fairly tightly, leaving a narrow space called the urinary space between the two layers of Bowman's capsule, i.e. the outer layer (paries externa parietalis), which forms the external covering of the renal corpuscle and is a simple squamous epithelium, and the inner layer (pars interna), which as the visceral layer (paries visceralis) is formed by podocytes. Their secondary processes adhere to the wall of the capillaries of the vascular glomerulus. The vascular pole is the common site for the afferent arteriole entering Bowman's capsule (arteriola afferens) and the efferent arteriole leaving the renal corpuscle (arteriola efferens).

The urinary pole is the beginning of the pathway through which the glomerular filtrate (primary urine) passes from the urinary space into the system of tubules and collecting ducts of the urinary tract.

For normal exchange processes in the renal microcirculation, the filtration barrier of the vascular glomerulus plays an important role; it consists of three structural elements:

- The endothelium lining the glomerular capillaries, consisting of a single layer of fenestrated squamous epithelium resting on the glomerular basement membrane (GBM) - with a specific ultrastructural organization. Endothelial cell thickness is 20-30 nm except at sites containing cell nuclei. The extensive cytoplasm lining the vessel lumen is not continuous; it contains numerous pores 50-100 nm in diameter. It is additionally coated with a layer of negatively charged glycosaminoglycans, which, together with the small pore diameter, constitutes the first obstacle that molecules penetrating the filtration barrier must overcome (47).
- The basement membrane (GBM) forms a uniformly continuous structure in the wall of the renal glomerular capillaries and inhibits the passage of high-molecular-weight plasma particles, which accumulate on the surface of the membrane or within it. The basement membrane is formed by fusion of two separate basement membranes produced by vascular endothelial cells and epithelial cells (podocytes) (39). It is considerably thicker than other basement membranes (320-370 nm). In the electron microscope it appears as three layers: the endothelial inner light lamina (lamina lucida interna), the middle dark lamina (lamina densa), and the epithelial outer light lamina (lamina lucida externa). The light laminae are visible

as electron-lucent layers, while the dark lamina is electron-dense.

• Podocytes, the cells of the visceral layer of Bowman's capsule, play an important role in filtration and also provide supportive functions. A visible adaptation to this function is the formation of foot processes. These are numerous, regularly arranged cytoplasmic projections adhering to the outer light lamina of the GBM and wrapping around the glomerular vessels. Between individual podocyte foot processes, gaps 30-60 nm wide are formed. At the site where adjacent podocyte processes adhere to the GBM, the gaps are bridged by a delicate membrane - the slit diaphragm. In mature renal glomeruli it shows a porous structure, which affects renal filtration processes. This membrane divides the podocyte cell membrane into two regions (domains), of which the smaller adjoins the basement membrane and forms the lower surface of the foot processes, while the other, much larger region faces the urinary space. These separated regions simultaneously provide differentiation of the biochemical and biophysical properties of plasma filtration (23, 26).

Within the vascular pole are structures responsible for sensing sodium concentration in urine and for regulating blood pressure. These structures form the juxtaglomerular apparatus consisting of:

• myoid cells producing renin, located mainly in the wall of the afferent vessel,
• **extraglomerular mesangial cells located within the triangle**

whose base is formed by the macula densa cells and whose sides are the walls of the afferent and efferent vessels,

• columnar macula densa cells located in the specialized region of the distal tubule adjacent to the vascular pole of its own renal corpuscle.

The function of the juxtaglomerular apparatus is still not fully understood; through the macula densa cells it is probably responsible for monitoring sodium and chloride ion concentrations. Renin secreted by myoid cells catalyzes the conversion of inactive angiotensinogen to angiotensin I, which in lung tissue, after further conversion, is transformed into angiotensin II, stimulating the secretion of aldosterone in the zona glomerulosa of the adrenal cortex. Aldosterone affects regulation of the sodium-potassium pump in the cell membrane and regulates the content of these ions and water in the glomerular filtrate, thereby participating in normalization of plasma volume and blood pressure.

An important component of the renal glomerulus is the mesangium, which consists of mesangial cells and extracellular mesangial matrix. Mesangial cells have an irregular shape and numerous cytoplasmic processes characterized by a large amount of actin and tropomyosin fibers. The matrix is an acellular substance produced by mesangial cells. In the electron microscope it is visible as an element contributing to the structure of the GBM - the outer light lamina of the vascular glomerulus. It provides support for the capillary loops and, through the myosin-angiotensin system, simultaneously regulates blood flow through the renal glomerular loops and also performs phagocytic functions.

d) hypoxia

Hypobaric hypoxia is defined as a state of relative oxygen deficiency (hypoxia) in tissues and cells resulting from reduced partial pressure of oxygen (PO2).

A reduction in the amount of oxygen leads to accumulation of toxic metabolic products, damage to the integrity of cell membranes and the cellular cytomembrane system, and changes in the electrolyte composition of intracellular fluid.

Such a situation provides a stimulus for activation of mechanisms aimed, on the one hand, at normalizing metabolic processes and, on the other hand, may lead to chronically progressive pathological changes in multiple organs (heart, lungs, kidneys). The main role in these processes is played by vascular endothelial cells, which form an environmental barrier, and by the basement membranes on which these endothelial cells are located (7, 17, 20, 36, 37, 38, 46).

Ultrastructural, molecular, and immunological studies conducted in recent years have provided insight into the mechanisms of changes occurring on the surface and within the cytoplasm of endothelial cells. In these processes, receptors play a considerable role; their stimulation causes changes in the function of cell groups or individual cells (7, 8, 22, 30, 32, 58, 68, 76, 77).

When induced by hypoxia, these changes lead to:

- increased HIF-1 activity (hypoxia-inducible factor), causing activation of genes leading to expression of erythropoietin, endothelin-1, platelet-derived growth factor, and interleukin (IL-6) (5, 7, 11, 27, 28, 55),
- flattening and stretching of podocyte plasma membranes, which may

disrupt glomerular permeability and normal filtration processes (6, 53),

- stimulation of renin secretion (24, 37),
- proliferation of mesangial cells and stimulation of synthesis of the extracellular mesangial matrix (57, 60),
- cellular hypertrophy leading to chronic kidney disease (tubulointerstitial fibrosis) (57, 59),
- increased hypothalamic production of vasopressin and oxytocin,
- angiogenesis through induction of pericyte and endothelial-cell proliferation (62),
- production of reactive oxygen species (8, 40, 41, 49),
- increased production of adrenomedullin (44).

Hypoxia may result from various pathological conditions, mainly involving constriction of pulmonary microcirculation vessels and hypertrophy of vascular endothelium, which may equally affect other organs (20, 26, 38, 78). It may also be a physiological state occurring in a very large population of people living at high altitudes, e.g. in the Andes and Himalayas (4,000-5,000 m above sea level) (21, 42, 43, 51).

2. Assumptions and aim of the study

Basic research analyzing the effects of drinking water exposed to a magnetic field is justified in an era of the widespread use of various types of equipment that generate magnetic fields. Among the long list of such devices, examples include magnetic water conditioners used to improve the quality of drinking water (3, 4, 24, 52, 54).

It is currently known that water exposed to a magnetic field changes its physicochemical properties, manifested by reduced viscosity dependent on the duration of magnetic-field exposure, changes in electrical conductivity, changes in pH, and increased concentrations of dissolved gases, mainly oxygen. It is also known that the biological effects of water exposed to a magnetic field result in accelerated metabolism in plant and animal organisms (72).

These circumstances, in the biological and medical sciences, must raise the question of whether consumption of such treated water causes changes in organismal homeostasis and whether it may cause adverse consequences.

No answer to this question has yet been obtained. This is due to many reasons, including the difficulty of creating a randomly selected group of people drinking water exposed to a magnetic field in whom studies clarifying this issue could be performed.

Earlier studies conducted in our Laboratory showed that animals given water exposed to a magnetic field demonstrated beneficial normalization of subcellular structures within the cytoplasm of hepatocytes. A clearly positive effect on compartmentalization of hepatocyte cytoplasm was found, which may indicate a direct effect of magnetically treated water on the structure of the cytoskeletal membranes of liver cells (24).

Similarly beneficial effects were observed in studies of pulmonary-tissue microcirculation in animals subjected to experimental hypoxia while simultaneously being given water exposed to a magnetic field to drink (38).

The present study focuses on assessment of ultrastructural changes in the microcirculation of renal glomeruli in animals that, under experimental hypoxic conditions, were given water from an ordinary water-supply system or water exposed to a magnetic field.

The motivation for the present exploratory study is the possibility of answering the following questions:

- **what changes does experimental chronic hypoxia cause in the renal corpuscle?**
- **how does water exposed to a magnetic field, compared with water from an ordinary water-supply system, affect structural changes in the renal corpuscle caused by chronic experimental hypoxia?**

To address this problem, I used:

- **morphological examinations at the light-microscopy level**
- **morphological and ultrastructural examinations evaluated by electron microscopy**

This study was carried out as statutory research funded under Agreement No. NN-5/150-00.

The study was approved by the Local Ethics Committee for Animal Experiments in Katowice, No. 35/00 dated 27 February 2001.

3. Material and methods

a) material

Thirty female Sprague-Dawley rats were used in the study, 3 months old and weighing 250 g (±20) (this strain was also used in two previous experiments (24, 38).

The rats were divided into the following groups:

Ia
5 rats kept under normoxic conditions and given ordinary water. (control group)

Ib
Five rats kept under normoxic conditions were given water with modified parameters of its physical properties (control group).

IIa
10 rats kept in the hypoxia chamber and given ordinary water.

IIb
Ten rats kept in a hypoxic chamber were given water with modified parameters of its physical properties.

Low-pressure chamber parameters:

- temperature 20-23°C
- humidity 65.7%
- oxygen content 10% by volume
- pressure 380 mm Hg (corresponding to an altitude of 4,000-5,000 m above sea level)
- volume 3 m^3

The rats remained in the hypoxia chamber for 30 days. They were removed daily for 1 hour to replace bedding and replenish food and drinking water.

Magnetically treated water was obtained using a specially designed device called RAM (Tubular Magnetic Apparatus), manufactured by Feniks. (Details are described in the Apparatus section)

b) apparatus

Running water from a standard water-supply system, at a temperature below 30°C and a flow rate of at least 0.5 m/s, was exposed to a magnetic field in a specially designed device called RAM (Tubular Magnetic Apparatus), owned by Feniks. The device is protected by patent No. 155856 (Fig. 1).

The device contains a magnetic stack within a tubular element, consisting of permanent magnets made of sintered anisotropic barium or strontium ferrite with an energy density of at least 26 kJ/m³ and a magnetic-field strength of at least 150 kA/m, as well as unmagnetized barium- or strontium-ferrite discs having a remanent magnetization equal to half the saturation magnetization, amounting to 420 mT and 210 mT, respectively.

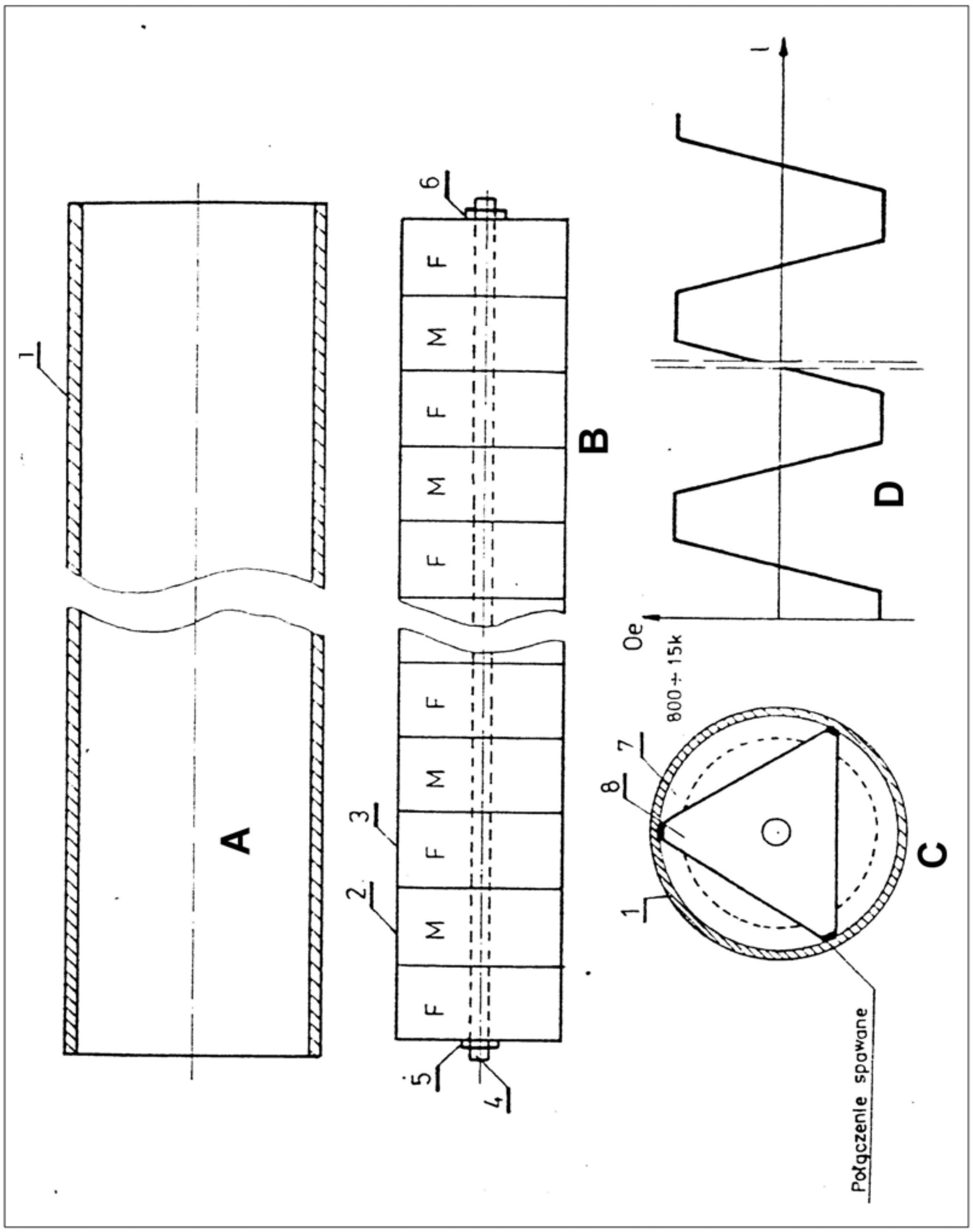
The apparatus used in the study contains a magnetic stack that produces a magnetic-flux dispersion effect, as a result of which the magnetic relaxation time of water flowing through RAM (the time required for a water molecule to rotate about its own axis) is prolonged, thereby changing the viscosity, surface-tension, and electrical-conductivity parameters of the outflowing water.

The magnetic relaxation times for ordinary water, water after passage through RAM, and demineralized water are respectively: 1100 µs, 1300 µs, and 1500 µs (results obtained at the Institute of Nuclear Physics in Kraków). The viscosity times for distilled water and water after passage through RAM are respectively 262.69 s and 243.84 s (measurements performed in the Rybnik Power Plant Laboratory using a capillary viscometer of the TAMSON TV-2000 AKV type).

It should be emphasized that the use in this experiment of the tubular magnetic apparatus (RAM) to magnetically treat the drinking water given to the animals should be regarded as unique. In the available literature I found no description of studies similar or comparable to those conducted here.

Diagram of the RAM device

A - section of steel pipe   B - magnetic stack consisting of ferrite discs and permanent magnets.   C - steel fitting centering the magnetic stack and housing   D - magnetic-field profile



c) morphological examinations

Both control rats and rats in the study groups were given, for general anesthesia, a 2.5% thiopental solution intraperitoneally at a dose of 40 mg/kg body weight. Kidney cortical specimens 1 mm wide were collected and placed in cold fixative (3% glutaraldehyde buffered with cacodylate buffer, pH 7.2), in which they remained for 2 hours at 4°C. They were then rinsed twice (12 hours each) in cacodylate buffer. Tissue specimens were post-fixed for two hours at 4°C in a 1% solution of osmium tetroxide (OsO_4) buffered with cacodylate buffer, pH 7.2.

The tissues were dehydrated through an ascending alcohol series ending with propylene oxide, after which the tissue samples were embedded in a mixture of epoxy resins - Epon 812 - which was polymerized in gelatin capsules in an incubator for 36 hours (12 hours each at 36°C, 42°C, and 60°C).

Epon blocks were cut with a Reichert ultramicrotome (OmU-3) into 0.5-1 µm semithin sections, mounted on glass slides, and stained with aqueous toluidine blue. These preparations were used for light-microscope observation, to localize and determine sites for ultrathin sectioning, and to obtain histomorphological documentation.

Ultrathin sections 0.05 µm thick were mounted on copper grids (300 mesh) and contrasted with uranyl acetate and lead citrate solutions (23, 56, 66). The ultrastructure of tissue material from selected areas of the renal cortex was analyzed and photographic documentation was obtained using a JEM 100CX electron microscope manufactured by JEOL.

4. Results

Photographic documentation

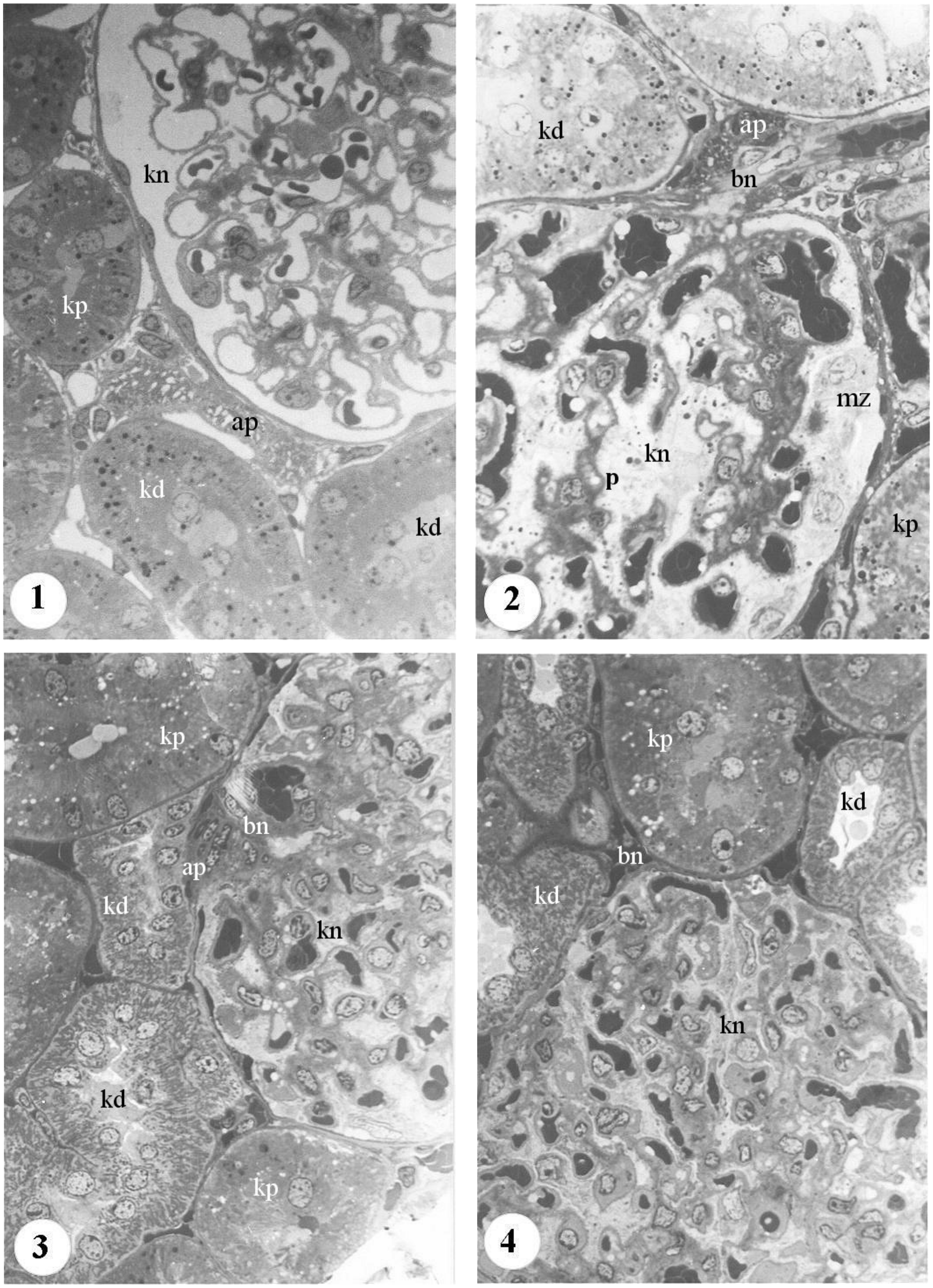


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Plate 1 presents photomicrographs of semithin sections of kidneys from control animals. Tissue samples were embedded in epoxy resin (light-microscope evaluation).

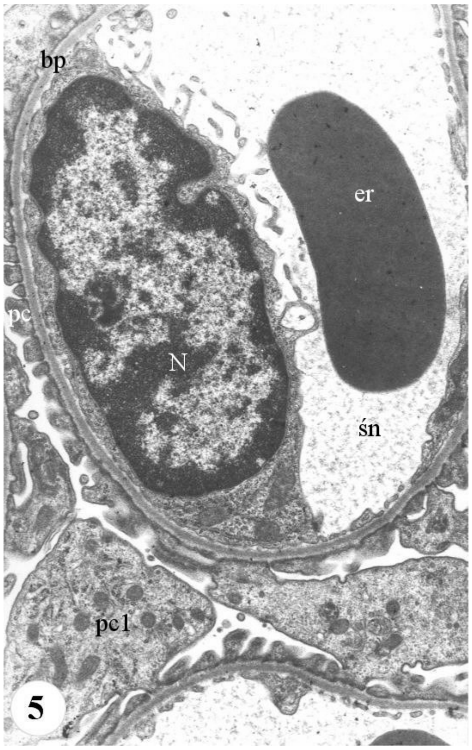
• Figures 1 and 2 show kidney specimens from animals given water from the ordinary water-supply system. • Figures 3 and 4 show kidney specimens from animals given water previously exposed to a magnetic field. Magnification: oil immersion 100x10.

Fig. 1. Fragment of renal tissue from the cortical region with a renal glomerulus (kn), showing clearly visible capillaries of the glomerular tuft together with podocytes and mesangium. Nearby are cross-sections of proximal tubules (kp) and distal tubules (kd). A fragment of the juxtaglomerular apparatus (ap) is visible.

Fig. 2. Large fragment of a renal corpuscle (kn) with the vascular pole (bn), a fragment of the juxtaglomerular apparatus (ap), and partial cross-sections of distal (kd) and proximal (kp) tubules. Not all capillaries in the renal corpuscle (kn) are patent. Both podocytes (p) and mesangial cells with extracellular matrix (mz) are visible.

Fig. 3. Semithin section of the renal cortex with a fragment of a renal glomerulus (kn) and surrounding proximal (kp) and distal (kd) tubules. To the left of the glomerulus, a fragment of the juxtaglomerular apparatus (ap) is visible near the vascular pole (bn) and the adjacent distal tubule (kd).

Fig. 4. Image similar to that described in Fig. 3. In the renal corpuscle (kn), between capillary loops filled with erythrocytes, the larger nuclei correspond to mesangial cells and the smaller nuclei to podocytes; kp - proximal tubules, kd - distal tubules, bn - vascular pole.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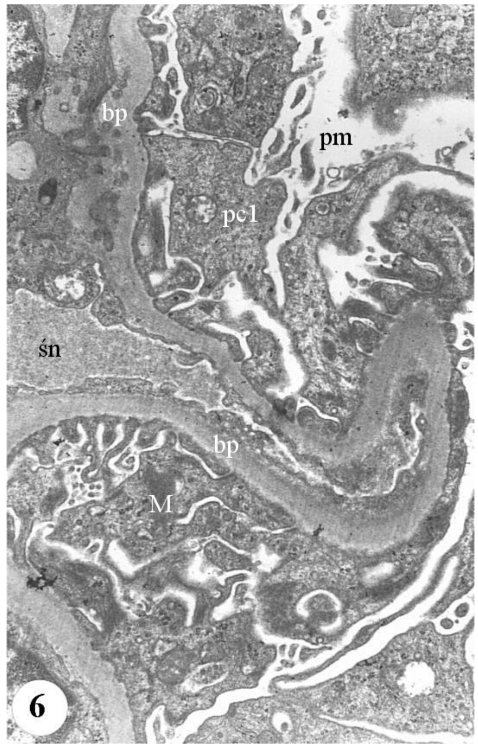
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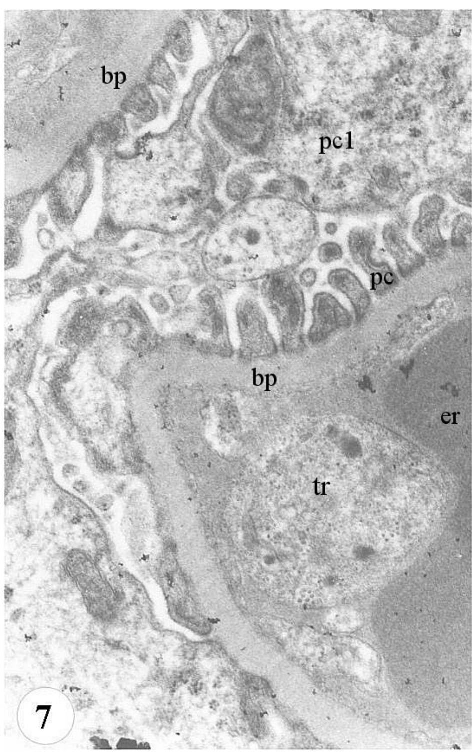
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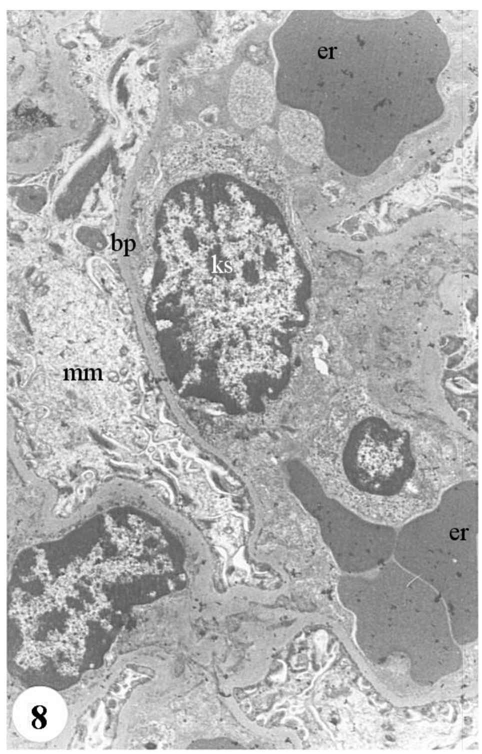
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Plate 2. Electron micrographs of ultrathin kidney sections from control animals (electron-microscope evaluation).

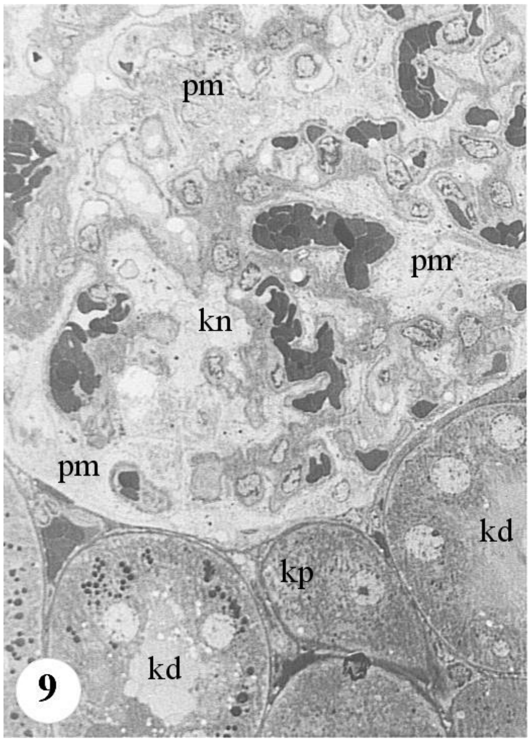
• Figures 5 and 6 - kidney specimens from animals given water from the ordinary water-supply system. • Figures 7 and 8 - kidney specimens from animals given water exposed to a magnetic field.

Fig. 5 shows a fragment of a capillary in the vascular loop of a renal glomerulus, with an endothelial cell resting on the basement membrane (bp), an oval nucleus (N), and cytoplasm lining the vessel lumen (śn), in which an erythrocyte (er) is visible. The external wall of the capillary layer is covered by cytoplasmic processes of podocytes. Fragments of podocyte cytoplasm (pc1) are visible in the lower part of the electron micrograph. Final magnification 5000x. Fig. 6 shows a fragment of an "empty" capillary with a distinct trilaminar basement membrane characteristic of renal glomeruli (bp). The stroma of the glomerular tuft consists of podocytes (pc1), with their cytoplasmic processes resting on the outer layer of the basement membrane, and extracellular mesangial matrix (M); pm - urinary space. Final magnification 12000x.

Fig. 7 shows fragments of capillaries surrounded by the basement membrane (bp), a cross-section through a platelet (tr), and erythrocytes (er). On the outer side of the basement membrane, regularly arranged cytoplasmic podocyte processes (pc) are visible. The stroma of the glomerular tuft consists of podocyte cell bodies (pc1) and extracellular mesangial matrix.

Final magnification 12000x. Fig. 8. Lower-magnification view of a fragment of a renal glomerulus. Cross-section through several capillaries of the glomerular tuft. Nuclei of vascular endothelial cells (kś) are visible in the center of the electron micrograph and in the lower left corner. Erythrocytes (er) are present in the vessel lumina. Extracellular mesangial substance (mm) is visible between the vessels.

Final magnification 4000x.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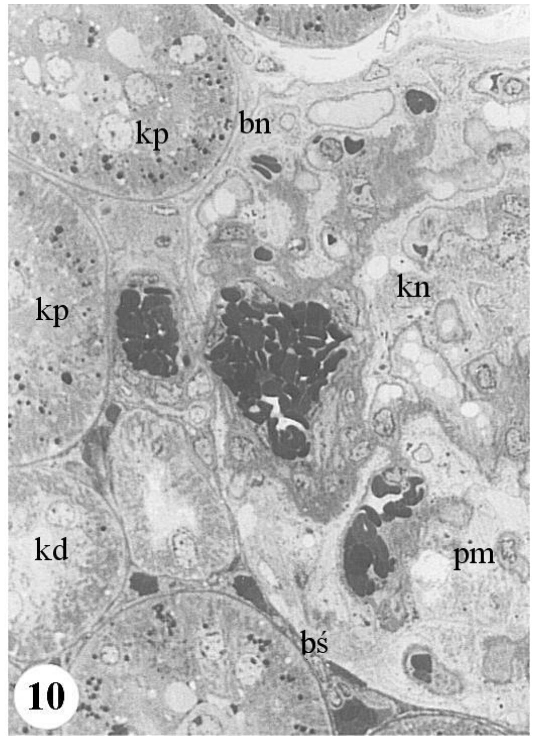
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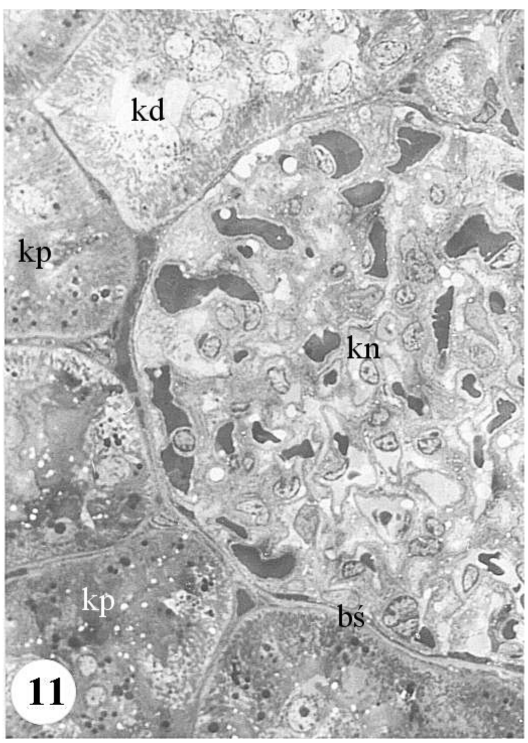
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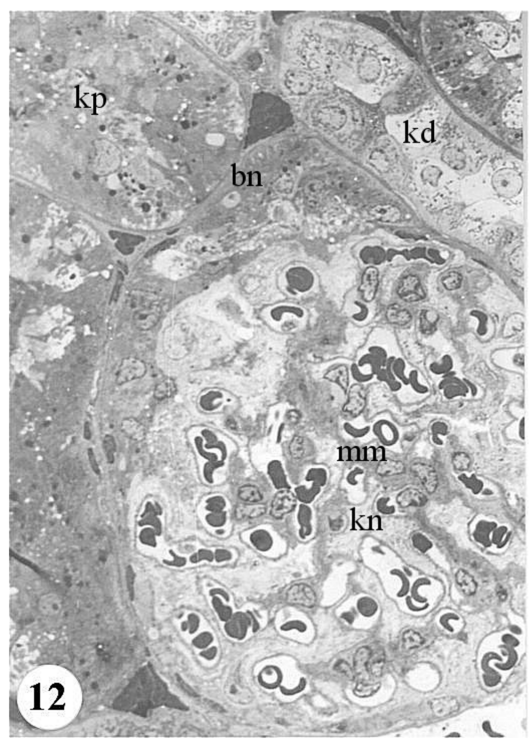
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Plate 3 presents photomicrographs of semithin kidney sections from rats exposed to hypoxic conditions and given water from the ordinary water-supply system (epoxy resin, toluidine-blue staining). Oil-immersion magnification 100x10.

Fig. 9. Large fragment of a renal glomerulus (kn) with numerous "empty" loops and wide urinary spaces (pm). Other vessels of the renal glomerulus are heavily filled with erythrocytes. Cross-sections of proximal (kp) and distal (kd) tubules are visible close to the glomerulus.

Fig. 10. Image similar to that described above, with the vascular pole (bn) visible. Some markedly dilated vascular loops are filled with erythrocytes, while others appear as empty capillaries; wide urinary spaces (pm) are visible between them.

kd - distal tubules, kp - proximal tubules, bś - parietal layer of Bowman's capsule.

Fig. 11. Fragment of the renal cortex with a renal glomerulus (kn), whose capillary loops are unevenly filled with erythrocytes and closely adjoin the parietal layer (bś). Nearby are cross-sections of proximal (kp) and distal (kd) tubules.

Fig. 12. Image similar to that described in Fig. 11, with the vascular pole (bn), adjacent cross-sections of a distal tubule (kd) and a proximal tubule (kp). Components of the juxtaglomerular apparatus are present near the vascular pole. Capillaries of the vascular loop in the renal glomerulus (kn), filled with blood cells, are separated by extracellular mesangial matrix (mm) and more darkly stained mesangial cells that form a supporting framework for the vascular loop.

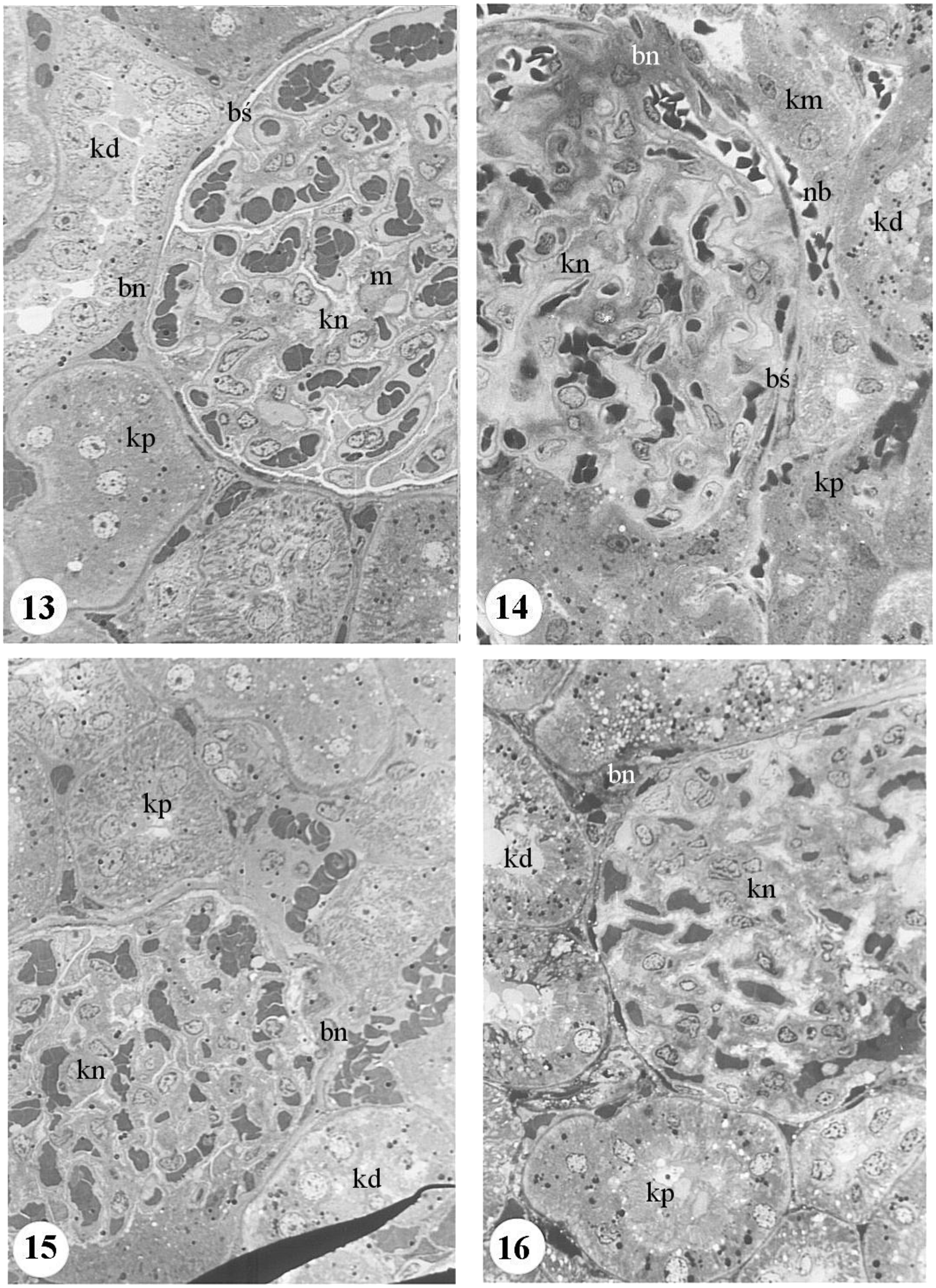
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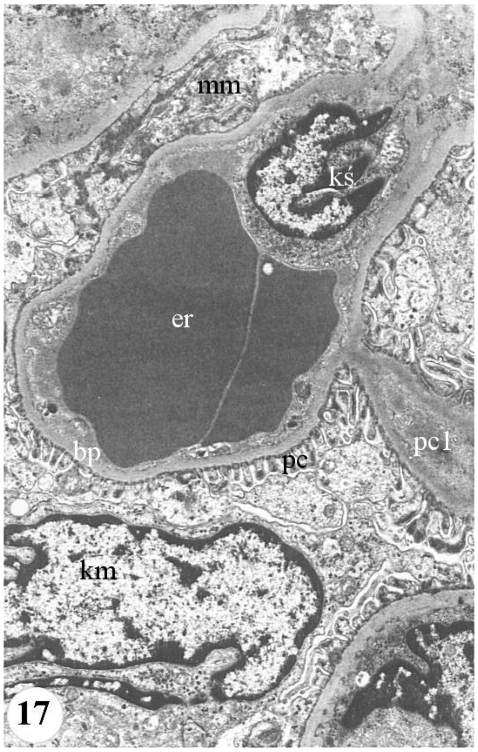
Plate 4 presents photomicrographs of semithin kidney sections from rats exposed to hypoxic conditions and given water exposed to a magnetic field (epoxy resin, toluidine-blue staining). Oil-immersion magnification 100x10.

Fig. 13. Fragment of renal cortical tissue with a renal glomerulus (kn) and a partially visible vascular pole (bn). Most capillaries of the glomerular loop are filled with erythrocytes. Between the capillaries are podocytes with smaller nuclei and mesangial cells (larger nuclei) with extracellular mesangial substance (m). Cross-sections of proximal (kp) and distal (kd) tubules are visible close to the vascular pole; bś - parietal layer of Bowman's capsule.

Fig. 14. Photomicrograph similar to Fig. 13, with a clearly marked vascular pole (bn). Nearby are distal tubules (kd) and extracellular mesangial cells (km) of the juxtaglomerular apparatus; bś - parietal layer of Bowman's capsule, kp - proximal tubule.

Figures 15 and 16 show similar fragments of renal cortical tissue, including renal glomeruli (kn), the region of the vascular poles (bn), and adjacent proximal (kp) and distal (kd) renal tubules.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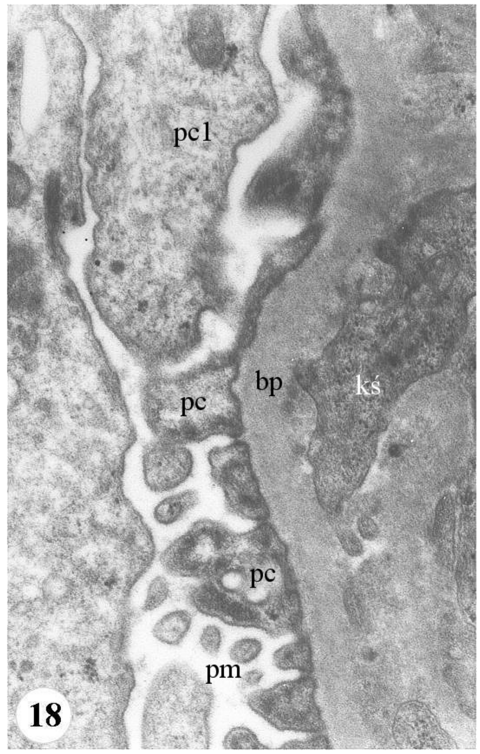
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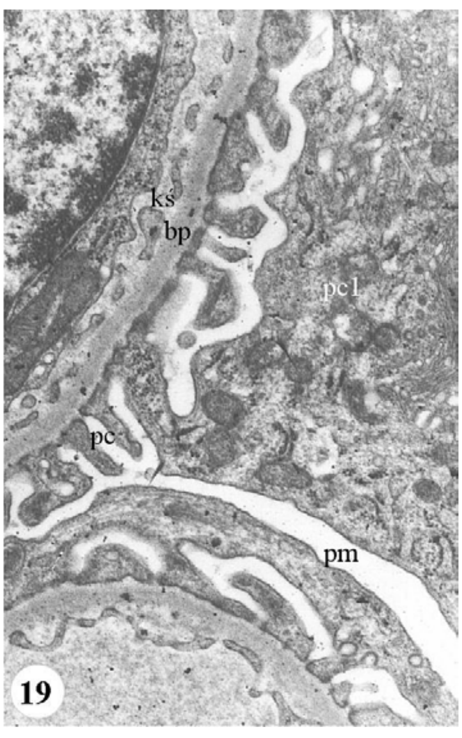
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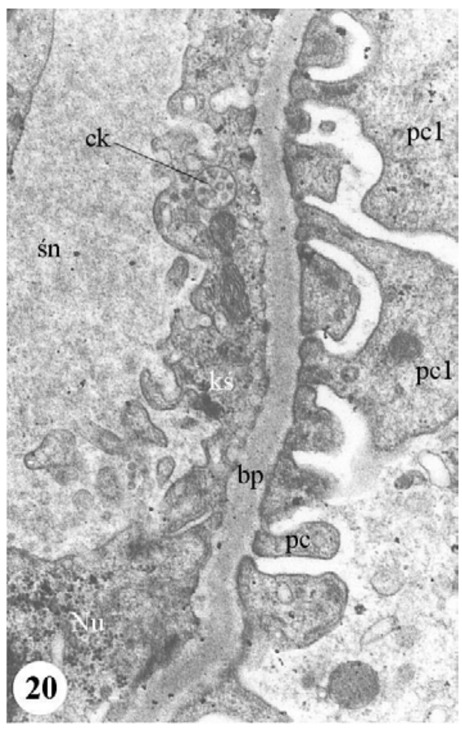
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Plate 5 presents electron micrographs of ultrathin kidney sections from rats exposed to hypoxic conditions and given water from the ordinary water-supply system (electron-microscope evaluation).

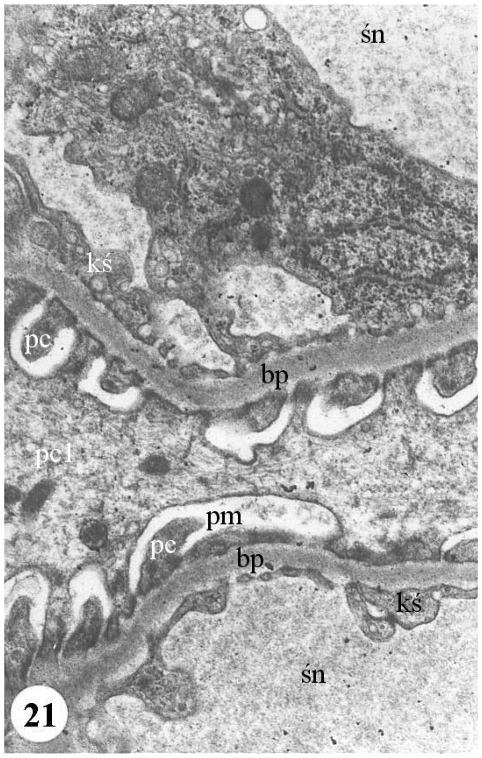
Fig. 17. Low-magnification TEM image of a fragment of a renal glomerulus with a cross-section through a capillary of the glomerular vascular loop; nucleus of a vascular endothelial cell (kś) adjacent to erythrocytes (er); pc - secondary podocyte processes adjoining the capillary basement membrane (bp); pc1 - fragment of podocyte cytoplasm in contact with the vessel basement membrane; mm - extracellular mesangium; km - nucleus of a mesangial cell.

Final magnification 3500x. Fig. 18. Fragment of a renal glomerulus (high-magnification TEM); bp - basement membrane of a capillary in the vascular loop of the glomerular tuft; pc - irregularly shaped cytoplasmic processes of podocytes (pc1); pm - urinary space; kś - fragment of the nucleus of a vascular endothelial cell.

Final magnification 14500x. Fig. 19. Fragment of a renal glomerulus; contact of a podocyte (pc1) and its processes (pc) with the basement membrane of capillary vessels of the vascular loop; kś - fenestrated capillary endothelium resting on the basement membrane (bp); pm - urinary space.

Final magnification 12000x. Fig. 20. Fragment of a renal glomerulus. On the left side of the electron micrograph is the capillary lumen (śn), lined by relatively thick endothelium (kś). A centrosome (ck) is visible in one of the cells. Endothelial cells merge with one another and do not exhibit the characteristics of fenestrated endothelium. Cytoplasmic podocyte processes (pc) vary in shape and do not show characteristic filtration slits. In some areas, epithelial cells are in direct contact with the basement membrane (pc1).

Final magnification 10000x.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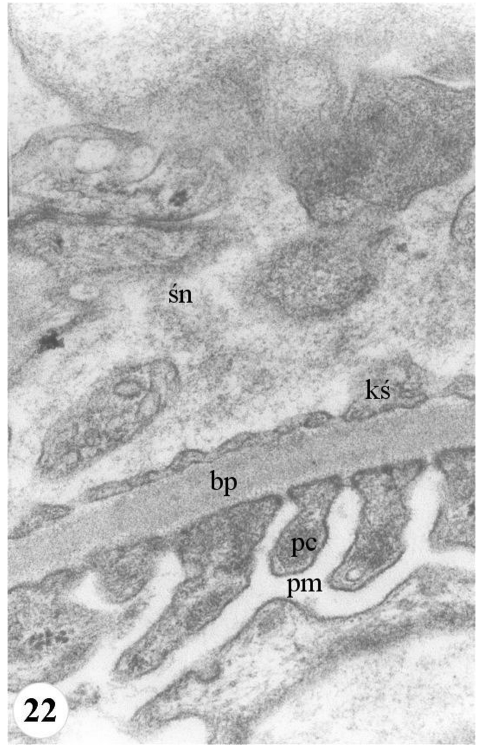
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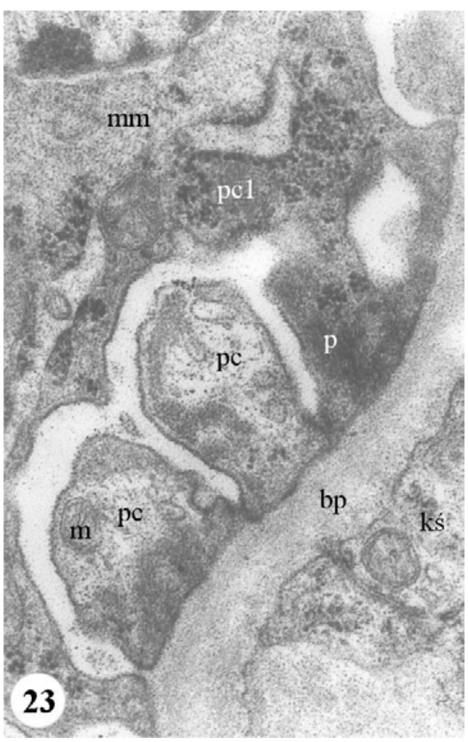
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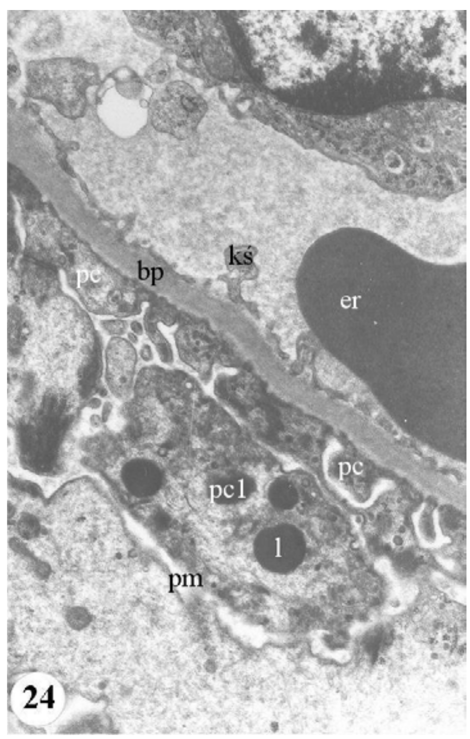
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Plate 6 presents electron micrographs of ultrathin kidney sections from rats exposed to hypoxic conditions and given water from the ordinary water-supply system (analyzed by electron microscopy).

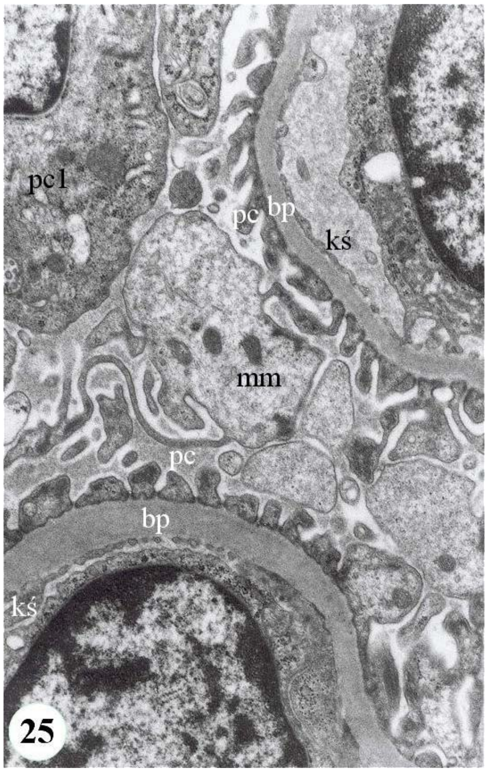
Fig. 21. Fragment of a renal glomerulus with cross-sections of two capillaries (śn). In the upper part of the electron micrograph, the vessel lumen contains a large fragment of endothelial-cell cytoplasm with typical subcellular elements, mitochondria, and rough endoplasmic reticulum. The vessel lining itself is fenestrated, slightly swollen endothelium, visible in the lower part of the electron micrograph (kś). Cells of the visceral layer of Bowman's capsule (pc1) show direct contact in places with the capillary basement membrane (bp); pm - urinary space, pc - podocyte processes.

Final magnification 10000x. Fig. 22 shows a cross-section of the lumen of a capillary in the vascular loop (śn) containing numerous blood cells. The urinary space (pm) is separated from the vessel lumen by a trilaminar basement membrane (bp). Fenestrated endothelial cells (kś) adjoin the lamina rara interna. On the urinary-space side (pm), podocyte processes (pc) also adjoin the lamina rara externa.

Final magnification 14500x. Fig. 23. At higher magnification, the electron micrograph reveals in some places fusion of podocyte foot processes (pc), absence of the filtration membrane, and irregularly shaped processes. Mitochondria (m), fragments of rough endoplasmic reticulum, and free ribosomes are observed in the cytoplasm of epithelial cells (pc1) and their processes (pc); mm - mesangial cells. Final magnification 20000x.

Fig. 24. Fragment of a renal glomerulus with a capillary in the upper-right part of the electron micrograph, containing an erythrocyte (er) and a fragment of an endothelial cell with its nucleus. The vessel lumen is lined by flat, fenestrated endothelium (kś). On the side of the relatively narrow urinary space (pm), irregularly shaped podocyte processes (pc) lie on the capillary basement membrane (bp), with fragments of epithelial-cell cytoplasm (pc1) containing numerous lysosomes (l).

Final magnification 10000x.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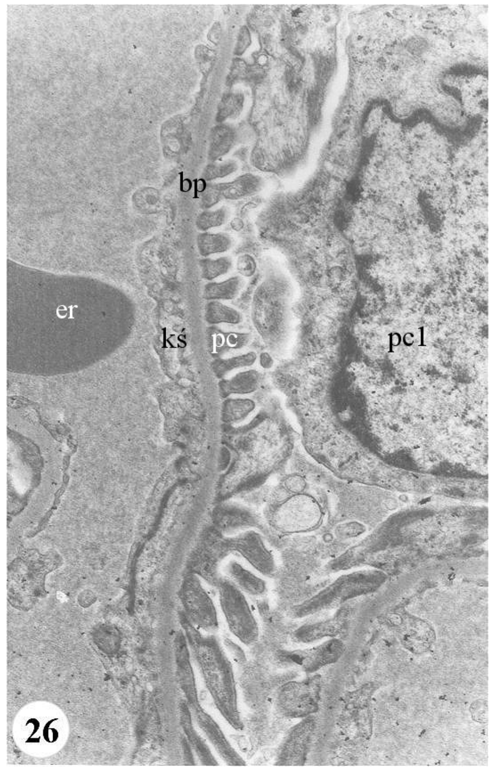
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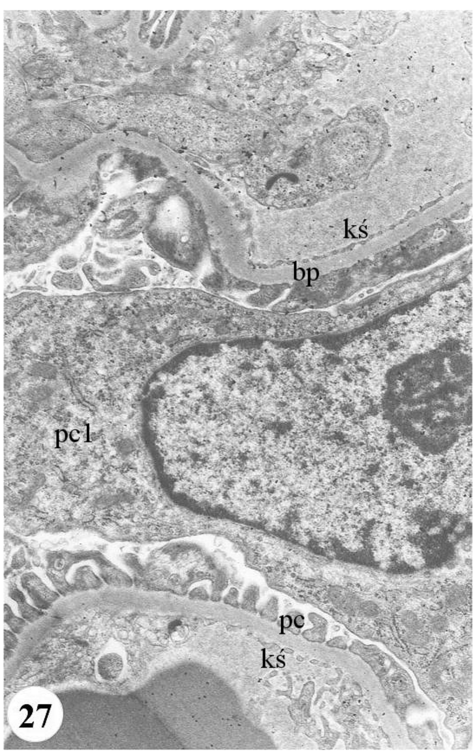
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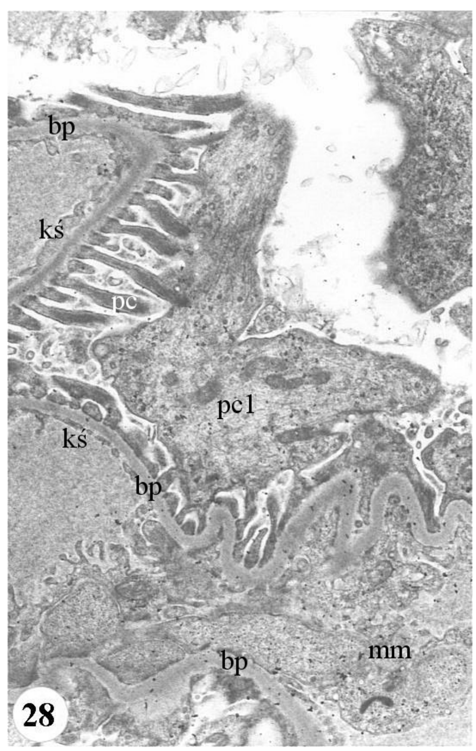
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Plate 7 presents electron micrographs of ultrathin kidney sections from rats exposed to hypoxic conditions and given water exposed to a magnetic field (electron-microscope evaluation).

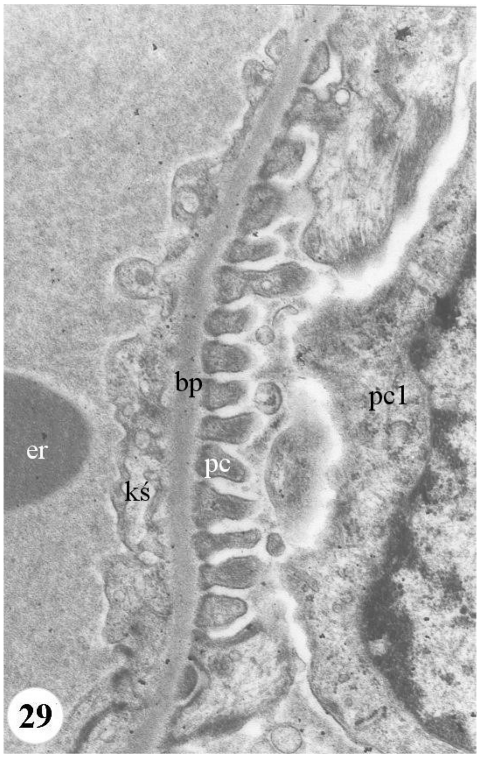
Fig. 25. Fragment of a renal glomerulus with two capillaries showing normally developed endothelium (kś) and regularly arranged podocyte processes (pc). In the intervascular stroma are extracellular mesangial matrix (mm) and a fragment of an epithelial cell (pc1) in the upper-left corner of the electron micrograph.

Final magnification 4500x. Fig. 26. Fragment of a renal glomerulus. On the left side of the electron micrograph is the lumen of a capillary with an erythrocyte (er), lined by vascular endothelium (kś). The basement membrane (bp) has a regular course; its outer surface is covered by podocyte processes (pc). An epithelial cell (pc1) is visible on the right side of the electron micrograph.

Final magnification 10000x. Fig. 27. Low-magnification fragment of a renal glomerulus. A podocyte (pc1) with its nucleus is visible in the center of the electron micrograph. Capillary lumina with normally arranged basement membrane (bp) are visible in the upper and lower parts of the image; kś - fenestrated capillary endothelium.

Final magnification 4500x. Fig. 28. Fragment of a renal glomerulus with capillaries lined by endothelium (kś); pc - cytoplasmic podocyte processes; pc1 - fragment of an epithelial cell; mm - extracellular mesangium.

Final magnification 4500x.



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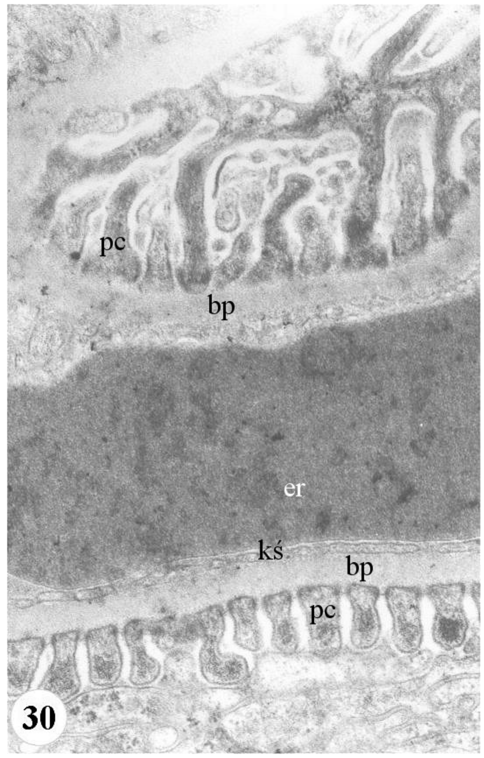

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Plate 8 presents electron micrographs of ultrathin kidney sections from rats exposed to hypoxic conditions and given water exposed to a magnetic field (electron-microscope evaluation).

Fig. 29. Image similar to Fig. 26 at slightly higher magnification, showing the normal arrangement of podocyte processes (pc) adjoining the basement membrane (bp), with visible filtration spaces and the capillary lumen on the left side of the electron micrograph; kś - vascular endothelium; pc1 - epithelial cell of the renal glomerulus; er - erythrocyte.

Final magnification 12000x.

Fig. 30. Similar arrangement of basement membranes (bp) with fenestrated endothelium (kś) and regularly shaped podocyte processes (pc), with visible filtration spaces. An erythrocyte (er) is present in the vessel lumen.

Final magnification 20000x.

5. Summary of study results

In accordance with the assumptions and aim of the study, for analysis of the findings in the light microscope I used specimens obtained from epoxy blocks, cut into semithin sections and stained with an aqueous toluidine-blue solution. Areas selected in this way in the appropriately trimmed tissue block were then cut into ultrathin sections used for evaluation of the renal glomeruli by transmission electron microscopy (TEM).

The histological structure of renal glomeruli analyzed by light microscopy in animals given ordinary water (Figs. 1, 2) and animals given water exposed to a magnetic field (Figs. 3, 4) did not deviate from the normal state. Considering that 10% patency of vessels in the glomerular tuft is regarded as the physiological norm, "empty" cross-sections of capillaries may be considered normal. The noticeable, dense filling of the renal corpuscle with vessels loaded with erythrocytes (Figs. 3, 4) may suggest better or increased blood flow in the group of animals given water exposed to a magnetic field.

Electron micrographs of renal tissue from both control groups evaluated by TEM (Figs. 5-8) do not differ and show no pathological changes. Neither the basement membranes with their characteristic trilaminar structure nor the endothelial cells of capillaries in the renal glomeruli deviate from the normal state.

In the kidneys of animals exposed to hypoxic conditions and given water from the ordinary water-supply system, specimens intended for light-microscope evaluation showed a heterogeneous structure of the renal corpuscles, presented comparatively in Figs. 9-12. Some capillary loops, markedly dilated and filled with large aggregates of erythrocytes, suggest the formation of emboli or vascular obstruction. Electron-lucent areas within the renal corpuscles may indicate enlarged urinary spaces as well as proliferation of extracellular mesangial matrix.

Analysis of semithin kidney sections from animals exposed to hypoxia and given magnetically treated water (Figs. 13-16) indicates substantial normalization of morphological structures, especially with regard to patency of the capillaries of renal glomeruli. This assessment follows from comparison of the kidney specimens presented and described in Figs. 9-10 and 13-16.

In evaluating electron micrographs of kidneys from animals exposed to hypoxia and given water from the ordinary supply system (Figs. 17-24), and animals given water exposed to a magnetic field (Figs. 25-30), particular attention was paid to the filtration barrier of the renal glomeruli, i.e. the contact, through the basement membrane, between endothelial cells of the glomerular tuft and epithelial cells (podocytes).

The glomerular basement membrane (GBM) shows its trilaminar structure, particularly clearly demonstrated in Figs. 18, 20, 22, and 23.

The most evident changes concern the structure of podocyte processes adjoining the GBM. They are irregularly shaped (Fig. 18), and in places appear swollen (Fig. 23); most often they merge along their lateral basal surfaces, leaving no room for filtration diaphragms (Figs. 18, 20, 21, 23, 24). Numerous lysosomal structures are observed in the podocyte cytoplasm (Figs. 19, 24).

The capillary endothelium of the renal corpuscles also shows fairly significant structural differences. In places there is marked thickening of endothelial-cell cytoplasmic processes, without preservation of their fenestrated character (Figs. 20, 21). In other areas flattened endothelium with clearly defined fenestrations is observed (Figs. 19, 24).

In electron micrographs of renal glomeruli from animals that were given water exposed to a magnetic field and were also subjected to hypoxic conditions, normal structure is found at the endothelial-podocyte interface, especially within the epithelial elements. Podocyte processes are regularly shaped. In most electron micrographs from this group of animals (Figs. 25-30), slits with diaphragms are seen between the processes, which may indicate normalized filtration despite the hypoxic factor. This factor is manifested by slight thickening of the endothelium of vessels in the renal glomeruli (Figs. 26, 29), and in places by detachment of endothelial cells from the basement membrane (Fig. 25).

6. Discussion of results

Results of numerous studies at the interface of biophysics and physical medicine demonstrate that magnetic fields have beneficial effects on a number of conditions because of their anti-inflammatory and analgesic properties. Beneficial outcomes are also obtained when magnetic fields are used in the repair and regeneration of soft tissues and bone union, and in intensifying tissue respiration, as well as in states of disturbed homeostasis in living organisms (14, 15, 63, 69, 70).

The term "magnetic-field therapy" should be understood as a biophysical method that is becoming an alternative among non-invasive treatments, with undeniable benefit comparable to the effects of pharmacological agents. Significant achievements in this field have been made by the team of A. Sieron, author of the valuable monograph: "Application of Magnetic Fields in Medicine" (63).

Relatively few publications address the possibility of magnetic-field effects on water, which is the principal component of living organisms, simultaneously serving as an intra- and intercellular filler and as the medium for all biochemical reactions and metabolic processes (4, 13, 54, 73).

Devices now commonly used to condition drinking water in households and in industrial transmission systems make it necessary to seek the appropriate selection of an externally applied magnetic field. Uncertified devices may not only be ineffective but can cause adverse effects, which would require their removal from the market. A positive assessment of the effects of their use should support their promotion for health and economic reasons (3, 52).

The present dissertation, part of a series of studies in this field, presents one variant of analysis of magnetic-field effects on a living organism.

It also appears important to obtain an answer to the question of how water exposed to a magnetic field affects changes following previously induced experimental hypoxia in the animals studied.

The apparatus used in the present experiment, which generates an external magnetic field, makes it possible to obtain dispersion of the magnetic flux. As a result, in water flowing through RAM (Tubular Magnetic Apparatus), the magnetic relaxation time is prolonged, consequently changing water viscosity, surface tension, and electromagnetic conductivity.

This device, used to magnetically treat water flowing from the ordinary water-supply system, was also used in experiments previously conducted in our laboratory. These studies showed that water prepared in this way and given to rats to drink has a beneficial effect on the intracellular structure of hepatocytes (24). This was expressed by normalization of hepatocyte cytoplasmic compartmentalization, in which the intracellular cytomembrane system participates, proper formation of mitochondrial structure, Golgi cisternae, and the rough and smooth endoplasmic-reticulum systems.

A second experiment investigated the effect of water exposed to a magnetic field on structural changes in pulmonary microcirculation in rats kept in a low-pressure chamber under hypoxic conditions. The results, expressed by the normal formation of the cytoplasmic lining of both capillary endothelial cells and pulmonary alveoli, and by orderly subcellular structures in the stroma of interalveolar septa, likewise indicate a beneficial effect of this water when used in animals in which hypoxia induced specific structural changes (38).

Despite existing controversies concerning views on the effects of magnetic fields on living organisms, their influence on homeostasis has been confirmed, among other things, on the basis of: the effect of the magnetic field on uncompensated electron spins, its effect on liquid-crystal structures in organisms, and its effect on moving electric charges generated, for example, by the circulatory and nervous systems.

Existing differences in interpretation often result from methodological differences or from the variety of temporal, physical, or apparatus parameters used (2, 7, 14, 34, 35, 79).

In our experiments we used the RAM device with defined, precise parameters, supplying water with identical physicochemical values.

Our first experiment, concerning hepatocyte analysis, showed a beneficial effect of this water on liver cells, which was confirmed by ultrastructural studies. These results provided inspiration for further experiments.

It is known, even based only on media reports, that drinking water, apart from bacteriological purity, has many shortcomings, as evidenced by the high consumption of all kinds of heavily advertised mineral waters.

The use of water obtained with the described device as drinking water for animals exposed to hypoxic conditions was also assessed as beneficial in studies of pulmonary-tissue microcirculation.

In the present experiment, involving examination of renal microcirculation in animals exposed to similar experimental conditions, the validity of the favorable assessments of magnetically treated water obtained in previous studies was evaluated.

The premise of my study was to determine whether water exposed to a magnetic field affects structural changes in the renal corpuscle caused by hypobaric hypoxia.

Hypoxia, expressed by proliferation of vascular endothelium and basement membranes, ultimately triggers a series of enzymatic and molecular processes leading to disruption of the balance between vasoconstrictive and vasodilatory mechanisms (8, 9, 10, 11, 12, 22, 50, 55), while changes in the plasma membranes of podocytes and their processes result in disturbances of filtration and primary-urine production (39, 42, 47, 53, 60, 61).

Administering magnetically treated water as drinking water to rats exposed to hypoxia minimizes these changes, as confirmed by morphological studies evaluated, among other methods, by electron microscopy.

The experiments performed may contribute to further research in which enzymatic, molecular, and immunological processes should be examined closely to fully confirm the beneficial effect of structurally modified, magnetically treated water.

7. Conclusions

1. Chronic hypoxia in the renal corpuscle is characterized ultrastructurally by thickening of the capillary endothelium of the renal glomerulus and destruction of podocyte cytoplasmic processes, consisting of their flattening and loss of filtration diaphragms.

2. Giving rats water exposed to a magnetic field substantially minimizes the changes within podocytes that were caused by chronic hypoxia, which may indicate a beneficial effect of water prepared in this manner.

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9. Summaries

Magnetotherapy, recently used quite widely in clinics and outpatient departments, is supported by beneficial results obtained in the treatment of various disease states. The mechanism of action of the magnetic fields used in this therapy is not fully understood and requires further research. Considerable attention is devoted to magnetic water conditioners, devices generating an external magnetic field that are used to condition drinking water used in households and industry.

However, the question remains open whether consumption of such structurally modified water is beneficial to health or whether it entails adverse health effects.

The present dissertation is one of a series of experimental studies on the use of water exposed to a magnetic field and given to experimental rats as drinking water.

The present study was designed to determine what structural changes are observed in the microcirculation of renal glomeruli in rats exposed to hypoxic conditions and given magnetically treated water, compared with water from the ordinary water-supply system.

The experiment used 30 female Sprague-Dawley rats, divided into four groups: Ia and Ib - control groups of 5 animals each, kept under normoxic conditions and given water from the ordinary water-supply system and water exposed to a magnetic field, respectively; and IIa and IIb - experimental hypoxic groups given ordinary water and magnetically treated water, respectively.

After the 30-day experiment, all animals used in the study were euthanized. Tissue specimens were collected from peripheral kidney regions and routinely prepared for analysis and description by light and electron microscopy. The results indicate that experimental animals kept in the low-pressure chamber developed changes corresponding to hypoxia.

The changes were more evident in the experimental animals given water from the ordinary water-supply system, based both on analysis of histological specimens and evaluation of electron micrographs.

Structural changes in the renal glomeruli mainly concerned blood flow through the glomerular capillary network. At the ultrastructural level, the changes consisted of thickening of the glomerular capillary endothelium and destruction of cytoplasmic processes of the renal corpuscle epithelium (podocytes), indicating impaired glomerular filtration.

Giving rats kept under hypoxic conditions water exposed to a magnetic field as drinking water demonstrates its beneficial effect.

The author recognizes that the results of these experiments can only serve as a contribution to further research and investigation of the effects of magnetic fields on living organisms.

Summary

Recently magnetotherapy is quite widely employed in clinics and out-patients' departments, which is supported by adventageous results obtained in the treatment of different diseases. The mechanism of this magnetic field impact is still not well-understood and requires further research. Magnetizers, which are external magnetic field generators are especially interesting as they are employed for water conditioning.

However there is a question still under discussion: is drinking such structurally modified water advantageous for human health or there is a negative impact of such water.

Current discussion is one of the projects concering examinations of rats watered with magnetized water.

The aim of the current project is to determine whether there are any structural changes in the area of microcirculation in nephrons in rats under hypoxemia, watered with magnetized water and rats under hypoxemia, watered with tap water.

30 female rats from Spraque-Dawley tribe were used to conduct the experiment. The animals were grouped in 4 sections. Group Ia consisted of 5 control rats watered with tap water and not subjected to hypoxemia. Group Ib consisted of 5 control rats watered with magnetized water and not subjected to hypoxemia. Group IIa were 5 animals with induced hypoxemia and watered with tap water and magnetized water, respectively.

After 30 days the animals were put to death. The tissues were biopsed from the peripherial area of kidneys and prepared for light and electron microscope analysis by use of standard techniques. The results confirm that hypoxemia was induced in animals that resided in the low-presure chamber. More serious changes were found in the group of animals

(IIb) under hypoxemia watered with tap water by analysis of both histological slides and electronogrames. Structural changes in kidney tubules refer mainly to blood circulation in the capillary system of nephrons. Ultrastructural changes were also found. The epithelium of the nephron capillars was thickened and podocytes were damaged, which indicate nephron filtration disorders. Watering with magnetized water resulted in less severe changes, which points on its adventageous effect.

The author is aware that these are only preliminary results leading to further examination of the effects of magnetized water on organisms.