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ASSESSMENT OF EFFECT OF WATER EXPOSED TO MAGNETIC FIELD ON FUNCTION AND STRUCTURE OF LIVER CELL

Thesis for M.D. degree

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CONTENTS

1. INTRODUCTION

Many studies are currently being conducted to explain the effects of variable magnetic fields on humans. Many of these are ultrastructural and experimental studies.

The dynamics of this research stem from the development of physical medicine, which today can be regarded as a new discovery in contemporary clinical practice. This results from the possibility of using physical methods in various classical medical disciplines, including orthopedics and traumatology, sports medicine, surgery and rheumatology.

Many major research centers and various foundations are interested in and involved in this field, including the Biomagnetic Research Foundation at Evanston University (USA), the Agency Hydrographic Center in Washington, and finally the American Food and Drug Administration (USA) and NASA (National Aeronautics and Space Administration) (5, 8, 47, 57, 77, 79).

Complex factors of molecular interdependence, sometimes difficult to resolve using currently available research instrumentation, stimulate scientists to continuously seek solutions in various fields of biophysics, biology, biochemistry, pathomorphology and related sciences (21, 23, 30, 77, 79, 81).

This direction of work by researchers in different scientific specialties is necessary and should be maintained, continued and expanded in order to clarify doubts concerning the nature of magnetic-field action (3, 4, 5, 6, 8).

Recent years have been characterized by a dynamic development of research into the effects of magnetic fields on the surrounding living structures.

This applies to both plants and animals (2, 3, 30, 37, 46, 55, 58, 71, 73, 76).

This period is particularly characterized by the development of experimental and clinical studies aimed at identifying possibilities for using magnetic fields for the benefit and health of humans (20, 41, 44, 45, 50, 74).

However, observations concerning problems arising from the mere presence of the geomagnetic field (GMF), which is a universal, imperceptible factor coordinating all biophysical and biochemical reactions of the body, should not be overlooked (27, 78).

Data from recent literature allow certain basic mechanisms (1, 3, 4, 10, 30-36, 72), or hypothetical assumptions concerning the effects of magnetic fields on living organisms, to be classified as:
- effects on uncompensated magnetic spins (rotational motions about their own axes) of paramagnetic elements that are components of coenzymes and prosthetic groups of enzymes (6),
- displacement of moving electrical charges that form biological currents in the body, as the Hall effect resulting from the Lorentz force (the force with which a magnetic field acts on a moving charged particle),
- changes in the state of extracellular crystals and plasma membranes, as well as intracellular membrane structures whose layers have liquid-crystalline properties (37-40, 49, 53, 54, 60),
- induction, in cellular electrolyte structures, of potential differences associated with moving ions (7, 9, 51, 52),
- changes in the physicochemical properties of water (viscosity and surface tension), the most common solvent and at the same time the most ideal semiconductor, with exceptional properties of

proton and electron recombination under the influence of an electromagnetic field (5, 27),
- modification of cell-membrane depolarization processes that use their inherent automaticity (nerve cells) (7, 28, 40).

Clinical data (7, 12, 16, 24, 72, 73, 74, 77), based on detailed and thorough laboratory studies well documented in the scientific literature, indicate that the effects of a magnetic field are manifested as:
- increased oxidation-reduction processes in mitochondria (1, 2, 36),
- stimulation of protein synthesis and acceleration of cell division, leading to intensification of tissue-regeneration processes (18, 19, 20, 25, 26, 63, 70),
- stimulation of angiogenesis (80),
- anti-inflammatory and anti-edematous effects (41, 56),
- analgesic effects involving the endogenous opioid system (56, 67),
- changes in the rheological properties of blood (16),
- effects on hormonal and enzymatic activity, sometimes associated with the opening of calcium channels in cell membranes (9, 10, 46, 68).

Magnetic fields are also used in technology and incorporated into objects of everyday use. For years, the effects of magnetic fields on hydraulic systems have been known; they reduce deposits in transmission-pipe systems. In technologies involving liquid energy carriers (gasoline), they promote more economical combustion.

Assuming that the influence of magnetic fields is undeniable

in various areas of our lives, it seems particularly important to determine to what extent water whose physicochemical properties are changed by a magnetic field affects living organisms.

Water, constituting 70% of body mass, is structurally bound in various ways and is responsible for all biochemical processes occurring within the cells, tissues and organs of the entire body.

It is particularly important to determine to what extent water, when used for a prolonged period as the sole source of fluids and thus effectively replacing the body's entire water pool, affects the living organism through its physicochemical properties altered by the magnetic field.

It is known from the literature (27, 69, 77, 78) that water exposed to a magnetic field produces specific biological effects, such as accelerated plant growth and more favorable development of unicellular organisms (75). These data support the hypothesis that the observed and reported effects of magnetic fields in humans may depend in a particular way on their influence on the water ubiquitous in the body. However, studies on laboratory animals also show that shielding them from the geomagnetic field (GMF) leads to far-reaching destructive structural changes (79).

In the available literature (70, 72) devoted to the effects of this field on humans, there are no data confirming or refuting whether magnetized water is beneficial or harmful to human health.

No answer to such an important question has yet been obtained. This is due to many factors, including the difficulty of creating a randomly selected (randomized) group of people drinking water exposed to a magnetic field in whom studies could be performed to clarify this issue.

2. ASSUMPTIONS AND OBJECTIVE OF THESIS

Until today we do not have an unambiguous answer about the mechanism of influence of magnetic fields producing positive effects in treatment of some chronic diseases. However, it has been ascertained that magnetic fields accelerate the healing of injuries, including burns. Magnetic fields also influence vascular function causing a vasodilatation effect and facilitate osteosynthesis processes, especially in case of retarded union of a fractured bone or false joints.

It cannot be excluded that the reason for such effects on a molecular level is, besides Lorentz force, Hall effect and the effect of the field with diamagnetic molecules and paramagnetic atoms - the effect which water exposed to a magnetic field has on tissues.

The explanation of the effect of water previously exposed to a magnetic field on a living organism is also important as many water systems supplying water to houses employ magnetisers - the systems of permanently fixed magnets generating magnetic fields influencing the water flowing through their area.

Both of the above factors affected the attempt to answer the question about the systemic effect which water exposed to a magnetic field has on the condition of tissues and organs.

A liver is the most active organ as far as metabolism is concerned. Due to this fact the principal objective of my work was to answer the following question:

- How does water previously exposed to a magnetic field influence processes taking place in a liver cell?

In order to reach the principal objective I have set the following partial objectives:

- to ascertain the morphological and ultrastructural changes in hepatocytes of rats supplied with water exposed to a magnetic field,
- to analyse selected biochemical parameters reflecting the liver function of those animals.

3. MATERIAL AND METHODOLOGY

A - Material

Tests on animals were conducted after receiving a consent from the Commission of Ethics for Tests on Animals of the Silesian Medical Academy in Katowice.

Spraque Dawley rats were provided by the Central Animal Farm of Silesian Medical Academy in Katowice Ligota run by A. Stojko, Professor Doctor Habilitated.

The rats used for experiments were sexually mature males whose weight amounted to 250g (±20). The animals were kept in standard laboratory conditions. The room temperature amounted to 20-22°C, the light was changed according to a 12-hour cycle (7am-7pm – a light phase, 7pm-7am – a dark phase). The rats were fed with standardised Murigran feed.

A control group: 15 rats received any amount of drinking water from a normal water supply system.

A test group: 15 rats received also any amount of water, which, however, was previously exposed to a magnetic field (according to the description presented in Section "Equipment").

The group was divided into groups consisting of 5 animals watered with water which had flown through a dispersed stream magnetic field changing the time of relaxation of magnetic water as well as its viscosity and surface tension.

Group I – 6 days' watering

Group II – 10 days' watering

Group III – 14 days' watering

B - APPARATUS

Tap water from a normal water-supply system, at a temperature below 30 C and a flow rate not less than 0.5 m/s, was magnetized in a specially designed device called RAM (pipe magnetic apparatus), owned by Feniks. The device is protected by patent no. 155856 (Fig. 1).

The above device for magnetizing liquids contains a magnetic stack in a tubular element, consisting of permanent magnets made of sintered anisotropic barium or strontium ferrite with an energy density of not less than 26 kJ/m3 and a magnetic-field strength of not less than 150 kA/m, together with non-magnetized barium or strontium ferrite discs having remanent magnetization equal to half the saturation magnetization, amounting respectively to not less than 420 mT and 210 mT.

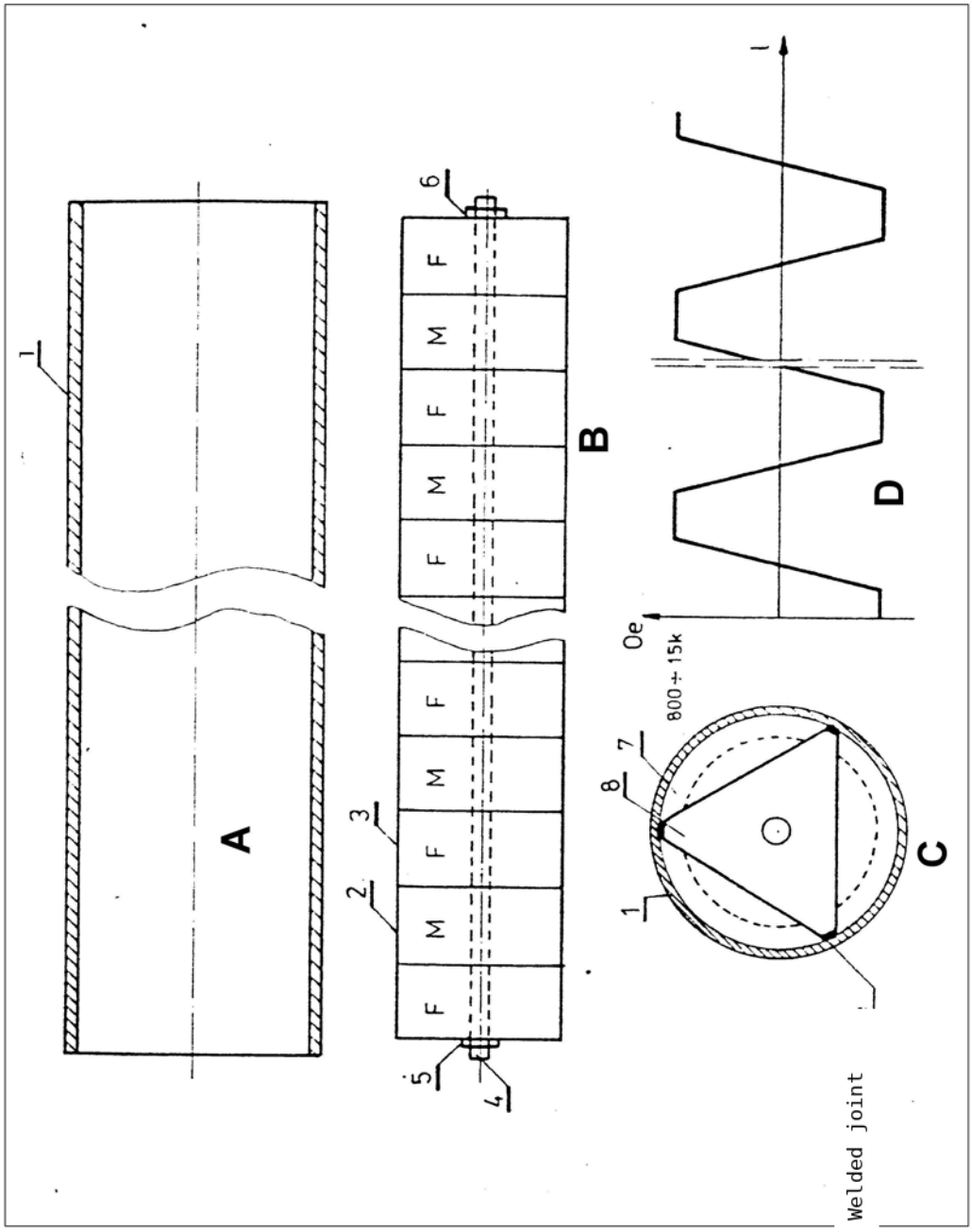
The apparatus used in the study contains a magnetic stack that produces magnetic-flux dispersion. As a result, the magnetic relaxation time in the water flowing through the RAM (the time required for a water molecule to rotate about its own axis) is prolonged, consequently changing the viscosity and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

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

It should be emphasized that the use in this experiment of a pipe magnetic apparatus (RAM) to magnetize drinking water administered to animals should be regarded as unique. I found no description of similar or comparable studies in the available literature.

Fig.

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 characteristics



C – MORPHOLOGICAL EXAMINATION

Both the rats from the control group and those from test groups were taken a segment from the left lobe of the liver (the animals were intraperitoneally given 2.5% solution of thiopental – in a dose of 40mg/kg of the body mass). The tissular specimen for morphological examination was immediately placed in a cold fixing solution (3% glutaraldehyde buffered with cacodylic buffer of pH 7.2) for 2 hours at 4°C. Afterwards, the specimen was washed two times (12 hours each time) in a cacodylic buffer. The specimens were fixed again in 1% a solution of osmium tetroxide (O_SO_4) for two hours buffered with cacodylic buffer of pH 7.2.

Routine dehydration of tissues in an increasing alcoholic series was finished by means of propylene oxide. Afterwards, the specimen were sunk in an epoxy mixture – Epon 812 and polymerised in gelatinous capsules at the thermostat temperature (12 hours at 36°, 42° and 60°C).

Epon pulleys were cut into 0.5 - 1μm semithin sections by means of the Reichert (Oum-3) ultramicrotome. The sections were put on microscopic slides and dyed blue with a water solution of toluidine. The specimens were observed in an optical microscope and used in order to obtain histomorphological documentation as well as to locate and determine the place for unltrathin cutting.

The ultrathin sections were put on copper grids (300 mesh) and contrasted by means of solutions of uranyl acetate and lead citrate (52). The observations of the ultrastructure of tissular materials and the photographic documentation were made by means of a JEOL manufactured JEM 100CX electron microscope.

The examination was conducted in the laboratory of electron microscopy of the Chair of Histology and Embryology in Zabrze of Silesian Medical Academy in Katowice.

D – BIOCHEMICAL EXAMINATION

In the homogenates of hepatic tissue taken along with the specimens for morphological examination – from both control group and test group animals, the concentration of the following enzymes in IU/g of protein was as presented below:

a) glutamate dehydrogenase	-	GLDH	-	(EC 1.4.1.2)
b) malate dehydrogenase	-	MDH	-	(EC 1.1.1.3.7.)
c) lactate dehydrogenase	-	LDH	-	(EC 1.1.1.27)
d) alanine aminotransferase	-	AIAT	-	(EC 2.6.1.2)

The tests were conducted in the Chair and Department of Biochemistry of Silesian Medical Academy in Zabrze.

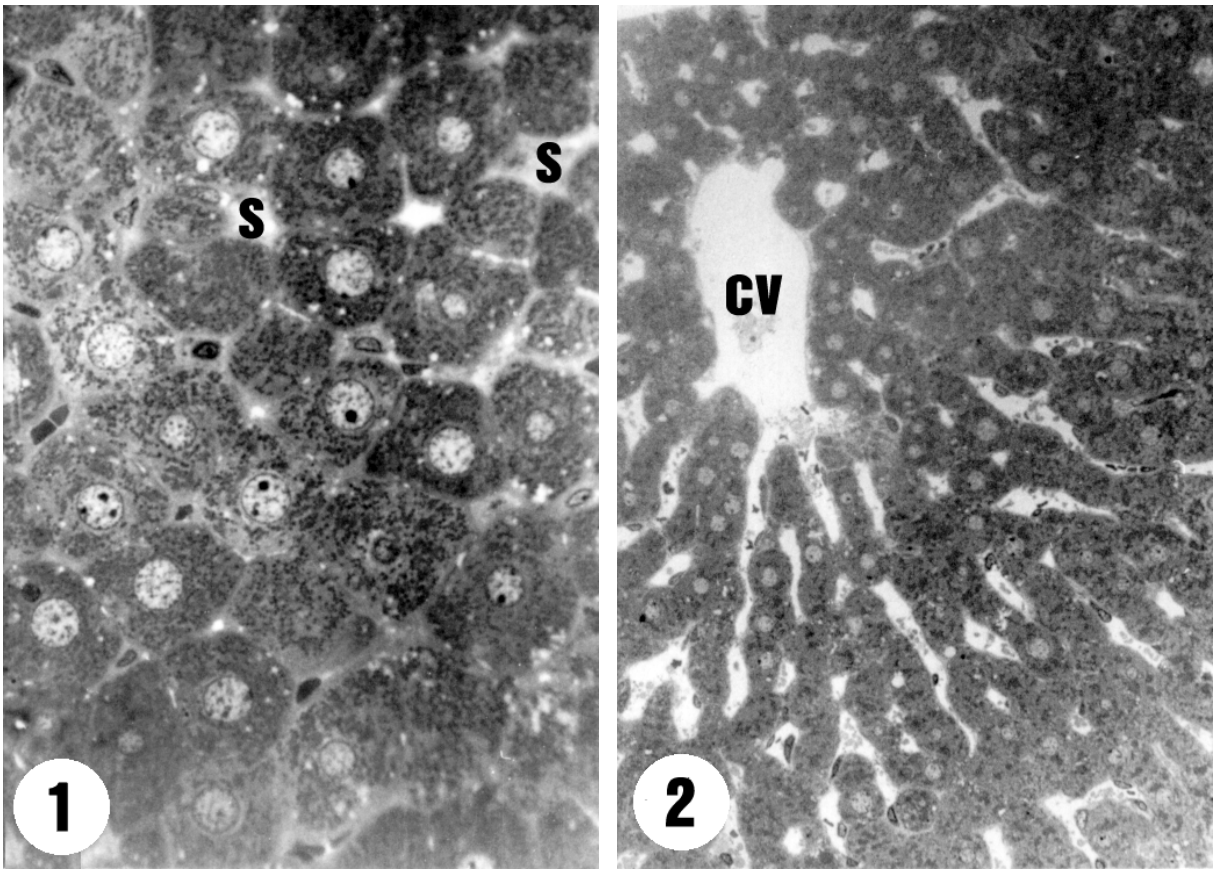
E – STATISTICAL ANALYSIS

The statistical analysis, the calculation of the average value as well as the calculation of the standard deviation and that of the statistical error of the mean value were conducted by means of STATGRAPHICS-Plus statistical spreadsheet (version 7 for DOS). Due to a small number of tests (n=5) and lack of assumptions in a normal distribution, Mann-Whitney U test was applied in order to compare the mean values. The level of $p.(a)<0.05$ was assumed as statistically significant.

4. EXAMINATION RESULTS

A) MORPHOLOGICAL AND ULTRASTRUCTURAL EXAMINATION RESULTS

CONTROL GROUP	FOTOS 1-8
TEST GROUP I	FOTOS 9-12
TEST GROUP II	FOTOS 13-16
TEST GROUP III	FOTOS 17-20



Test group (optical microscope)

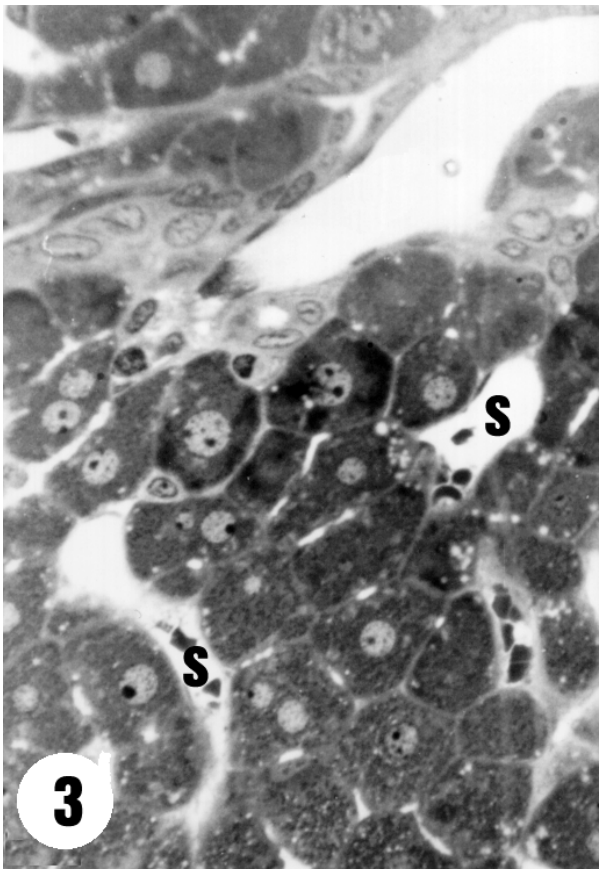
Fig 1 Microphotograph presents a fragment of a liver lobule (zone I) of a test rat. The hepatocytes have regular structure with centrally located nuclei. In some hepatocytes nucleoli are well visible. Sinusoids (S) can be seen between liver trabeculas.

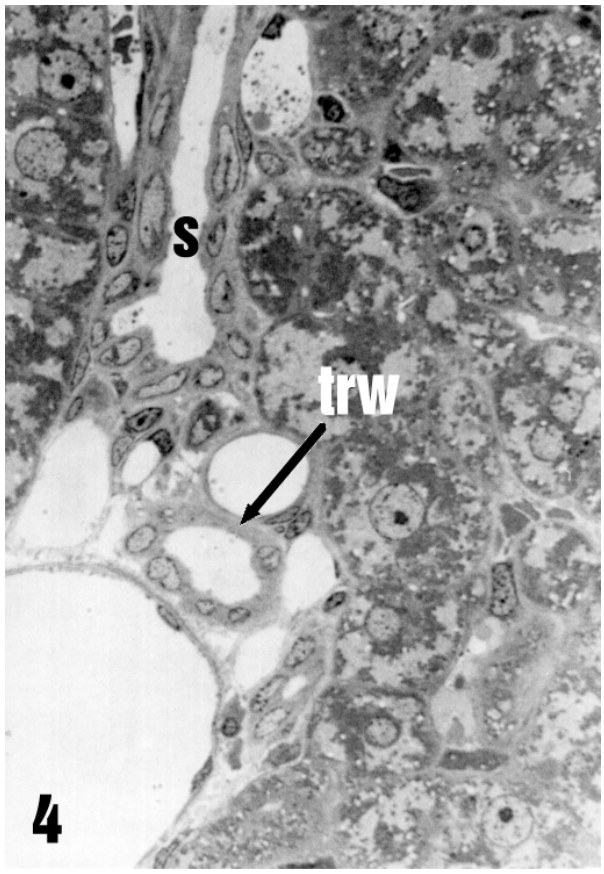
Immersion magnification – 1000x.

Fig 2 A fragment of liver tissue from the area of the central vein (CV) is convergent. The system of liver trabeculas is directed at the cenral vein, the structure of hepatocytes is regular.

Magnification – 250x

Microphotographs present the specimens of semithin sections (thickness 0.5 - 1μm) prepared by means of an ultramicrotome and dyed blue with a solution of toluidine.





Test group (optical microscope)

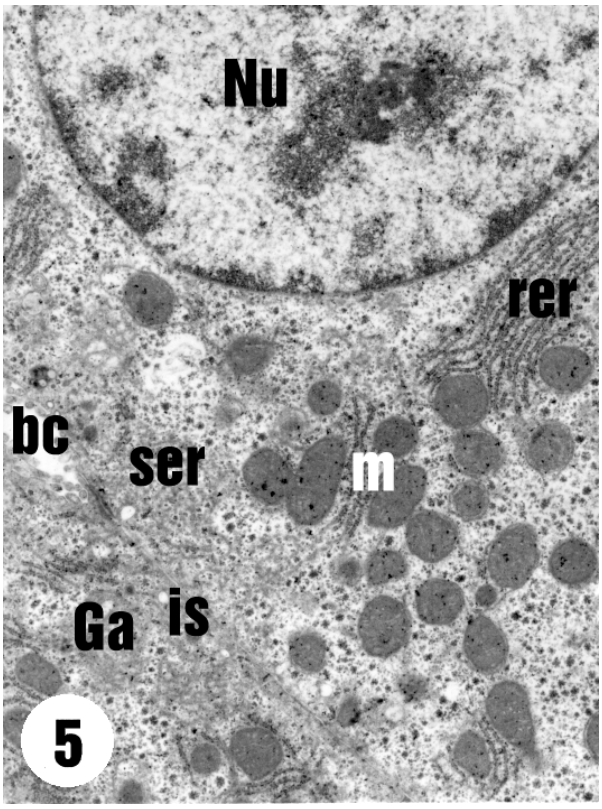
Fig 3 A fragment of liver tissue of a test rat – the area of zone I of a liver lobule. The hepatocytes have regular structure, some sinusoids (S) are slightly broadened.

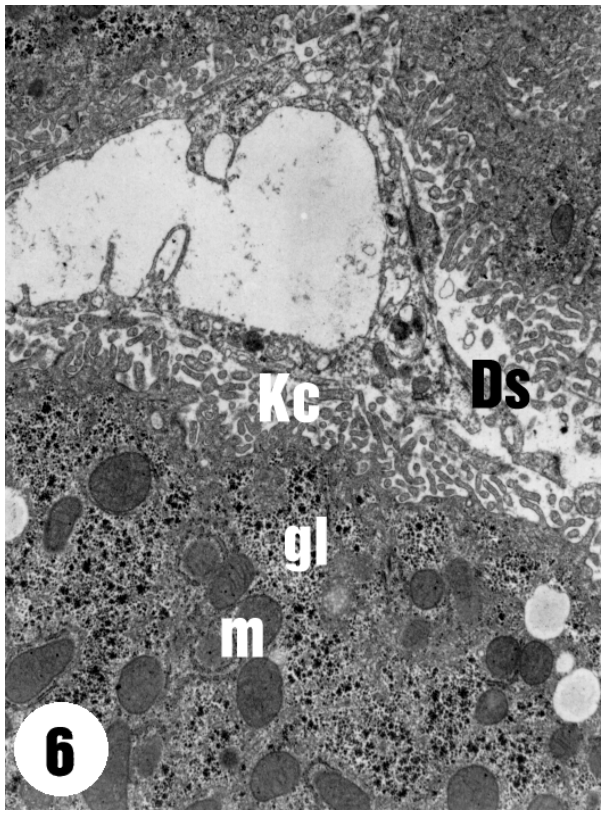
Immersion magnification – 1000x.

Fig 4 A fragment of liver tissue of a test rat – the periportal area with liver triad (trw) visible, the arrow indicates a biliary ductule. S - a sinusoid.

Immersion magnification – 1000x.

Microphotographs present the specimens of semithin sections (thickness 0.5 - 1μm) prepared by means of an ultramicrotome and dyed blue with a solution of toluidine.





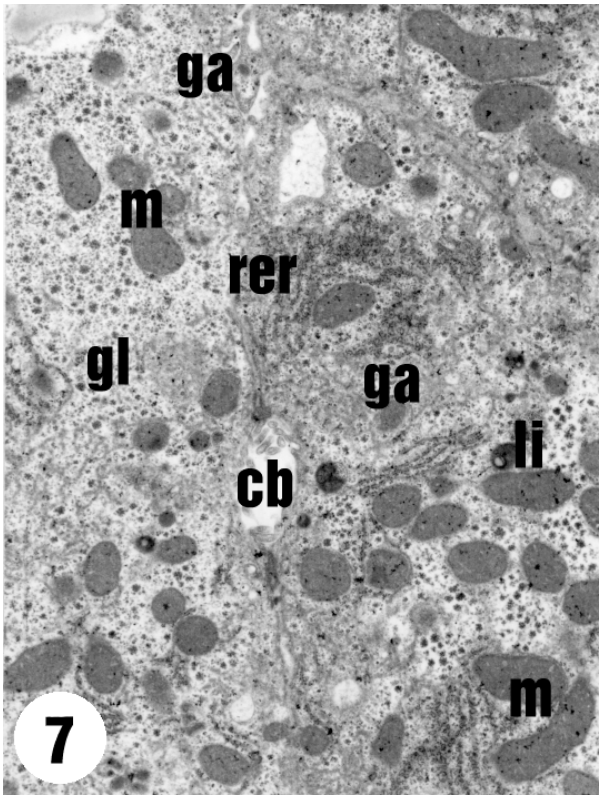
Test group (optical microscope)

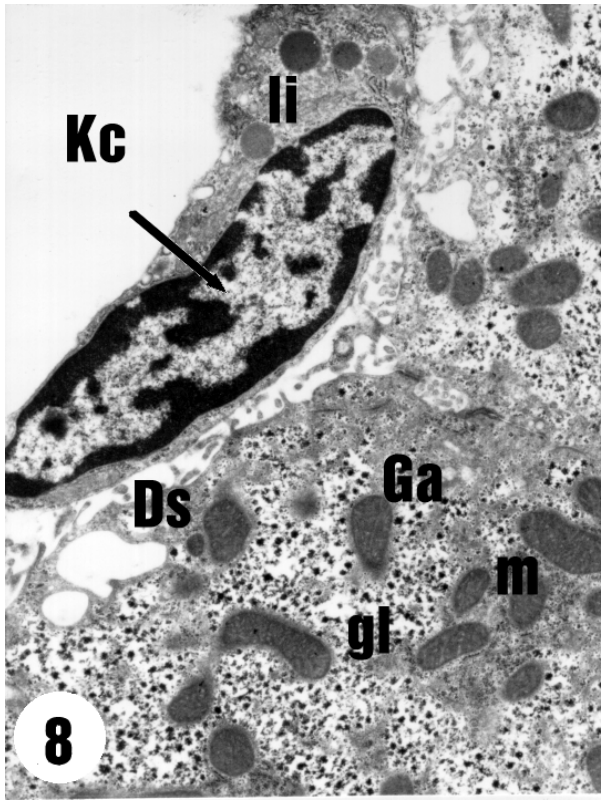
Fig 5 The fragment of a liver cell of zone I of a lobule; a visible section of nucleus (Nu) with regular membrane in karyoplasm; a visible nucleolus of granular and fibrous structure as well as peripherally located nuclear chromatin;
Oval or round mitochondria of regular structure; the basal cytoplasm contains sparse clusters of rough endoplasmatic rete (rer); sparse glycogen grains are located near the smooth endoplasmatic rete (ser).
Visible membranous structures of Golgi apparatus (Ga) are near the biliary canaliculus (bc) and intercellular space.

Ultimate magnification 16 250x.

Fig 6 Fragments of two hepatocytes adjacent to a sinusoid; Disse spaces (Ds.) filled with numerous microvilli of sinusal surface of liver cells; the process of Browicz-Kupfer cell (Kc) separates the lumen of a sinusoid; basal cytoplasm contains oval or round mitochondria (m) and glycogen granules (gl).

Ultimate magnification 16 250x.





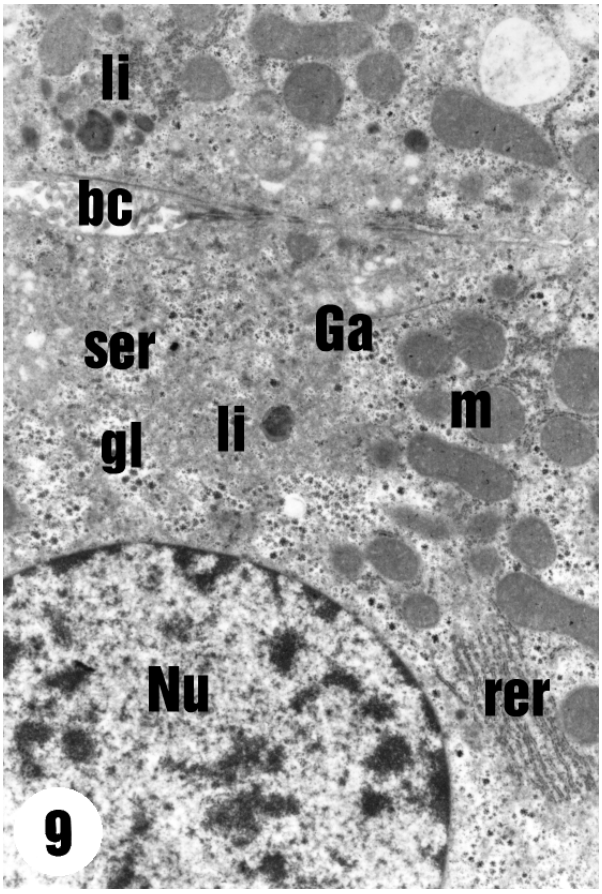
Test group (optical microscope)

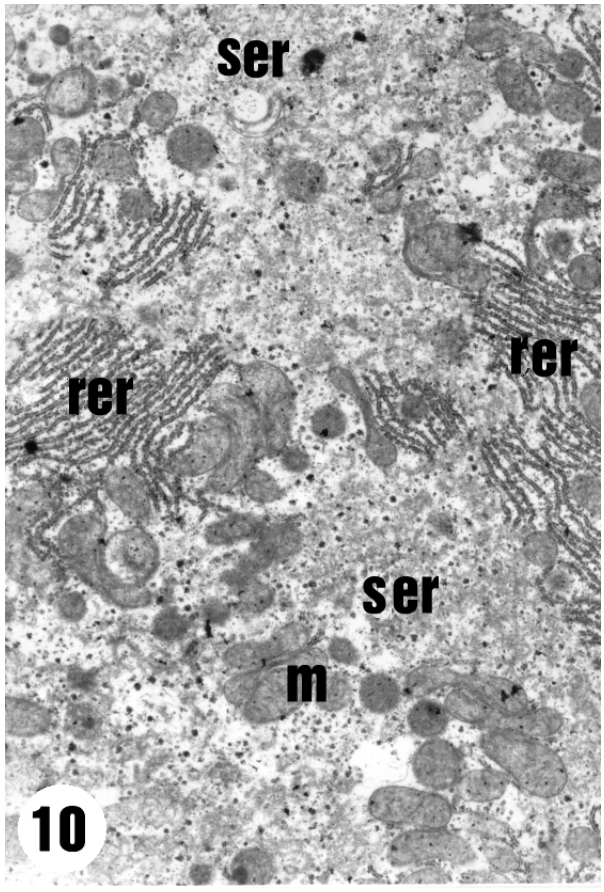
Fig 7 Fragments of two liver cells contacting each other by means of canaliculus surfaces. In the centre of the microphotograph one may see a biliary canaliculus (bc) forming a secretory pole of liver cells. Next to the biliary canaliculus, in the basal cytoplasm there are numerous lysosomes (li), mitochondria of regular structure and various sizes (m), sparse, flattened canaliculi of rough endoplasmatic rete (rer) and fragments of a Golgi apparatus (Ga) and glycogen granules (gl).

Ultimate magnification 16 250x.

Fig 8 The electronogram presents the sinusal surface of a hepatocyte with a Browicz-Kupfer cell (Kc). Microvilli penetrate the Disse space. In the cytoplasm of the Browicz-Kupfer cell are numerous lysosomes (li). In the basal cytoplasm of liver cells there are visible mitochondria of various sizes (m), fragments of a Golgi apparatus (Ga) and glycogen granules (gl).

Ultimate magnification 16 250x.





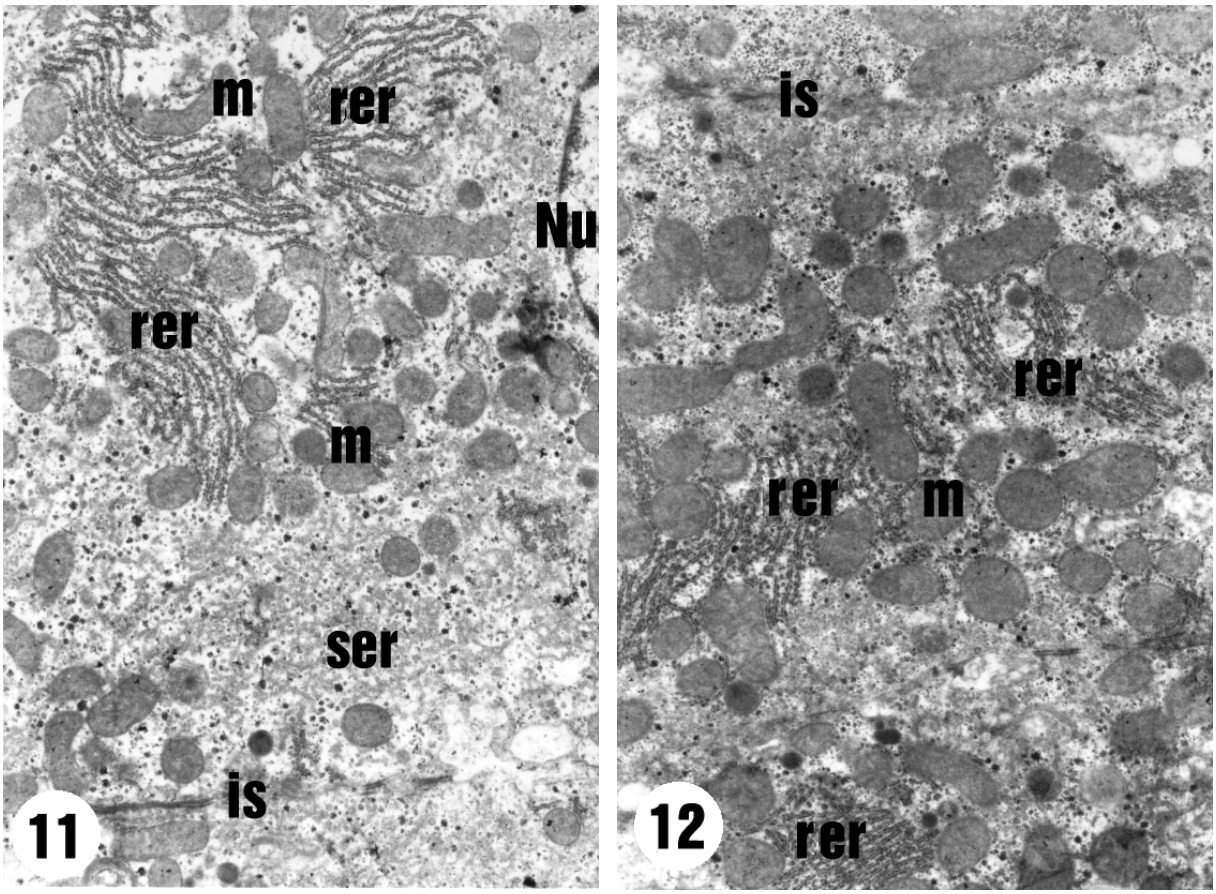
Group I after 6 days (electron microscope)

Fig 9 The electronogram presents the fragments of two liver cells contacting each other by means of canaliculus surfaces. Along the surfaces there is a biliary canalicus (bc) with microvilli. In the basal cytoplasm of liver cells, near the biliary canalicus there are fragments of a Golgi apparatus (Ga), lysosomal structures (li), mitochondria of various sizes (m), flattened sacculi of rough endoplasmatic rete (rer) and canaliculi of smooth endoplasmatic rete (ser) as well as glycogen granules (gl).

Ultimate magnification 16 250x.

Fig 10 A fragment of hepatocyte cytoplasm with numerous piles of flattened canaliculi of rough endoplasmatic rete (rer). Despite the various sizes, the structure of mitochindrias is regular.
Near the clusters of smooth endoplasmatic rete (ser) are sparse glycogen granules.

Ultimate magnification 16 250x.



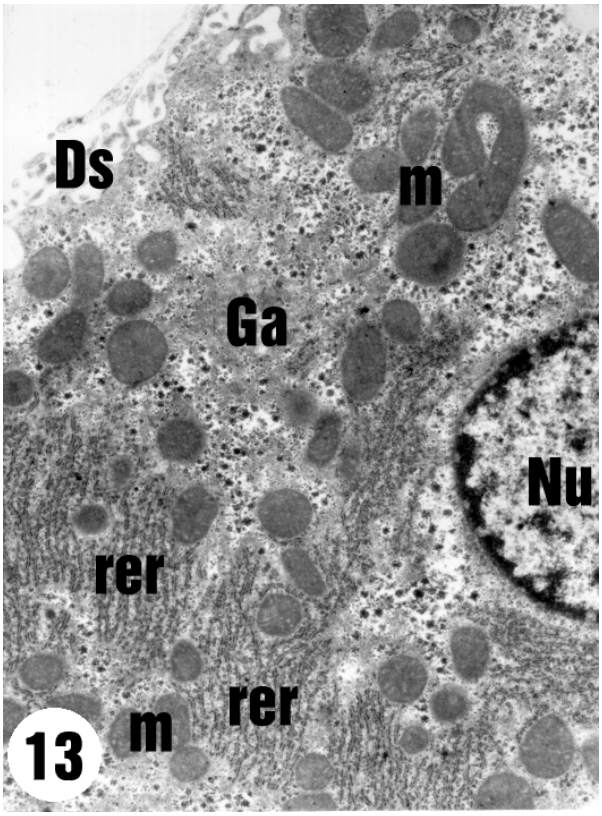
Group I after 6 days (electron microscope)

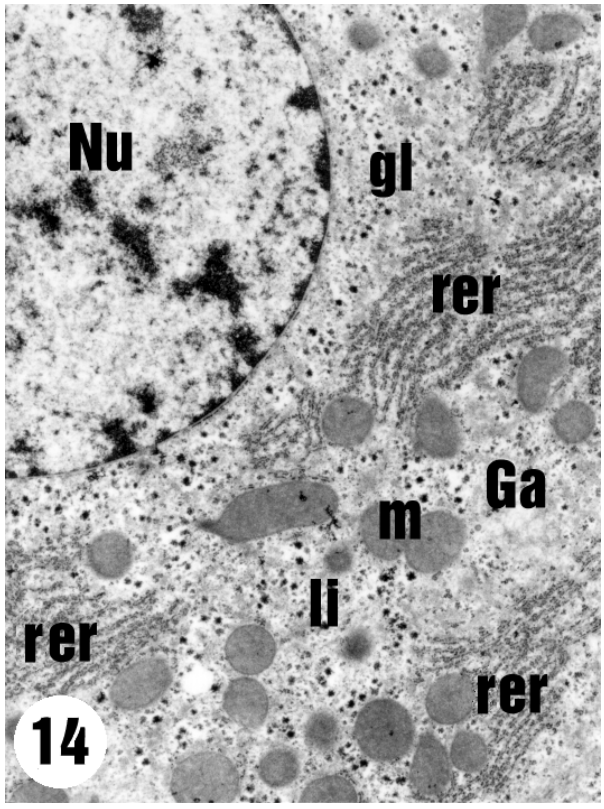
Fig 11 A fragment of liver cell cytoplasm of a test rat; in the perinuclear area (Nu) there are visible clusters of rough endoplasmatic rete (rer) and numerous mitochondria (m) of regular structure. In the area of smooth endoplasmatic rete (ser) there are visible glycogen granules. At the bottom of the microphotograph there is a visible intercellular space (is).

Ultimate magnification 16 250x.

Fig 12 A similar fragment of liver cell cytoplasm of a group I test rat with numerous mitochondria (m) and clusters of rough endoplasmatic rete (rer). In the upper part of the photogram there is a visible intercellular space (is).

Ultimate magnification 16 250x.





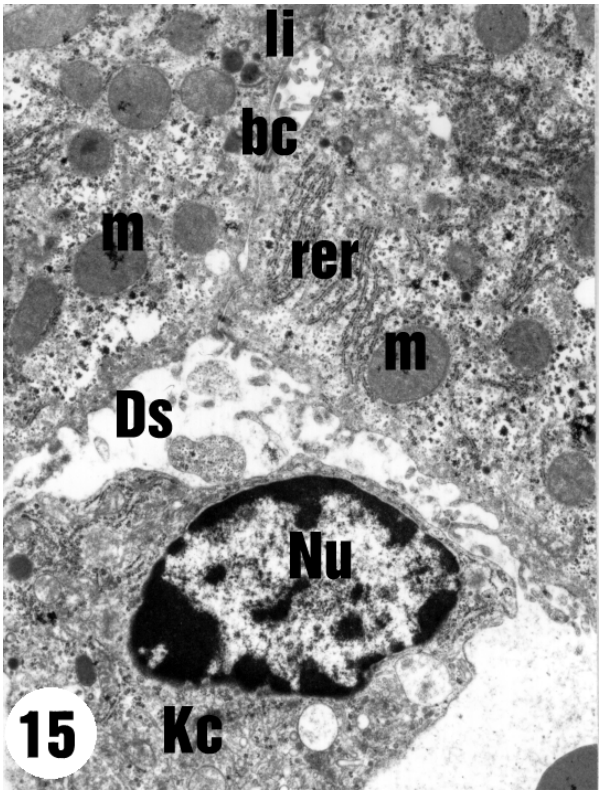
Group II after 10 days (electron microscope)

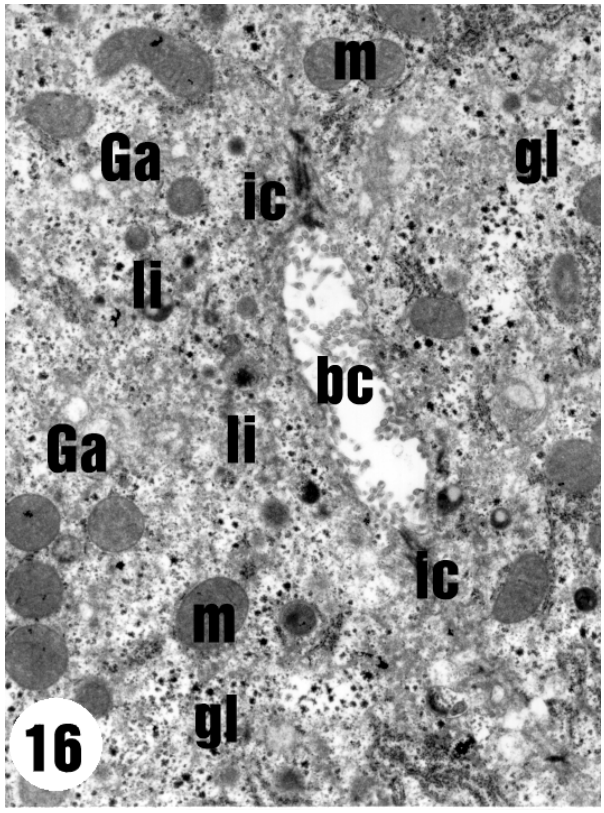
Fig 13 The hepatocyte cytoplasm with sinusal surface (Ds); a fragment of the nucleus with a distinctly border location of nuclear chromatin (Nu). Oval or round mitochondria (m) have a regular ultrastructure; numerous piles of rough endoplasmatic rete (rer); Ga – a fragment of Golgi apparatus – dictyosom.

Ultimate magnification 16 250x.

Fig 14 A fragment of hepatocyte cytoplasm from the perinuclear area (Nu); numerous clusters of rough endoplasmatic rete (rer); mitochondria of various size (m) have a regular ultrastructure. Next to the Golgi apparatus are lysosomal vesicles (li). The basal cytoplasm contains dispersed glycogen granules (gl).

Ultimate magnification 16 250x.





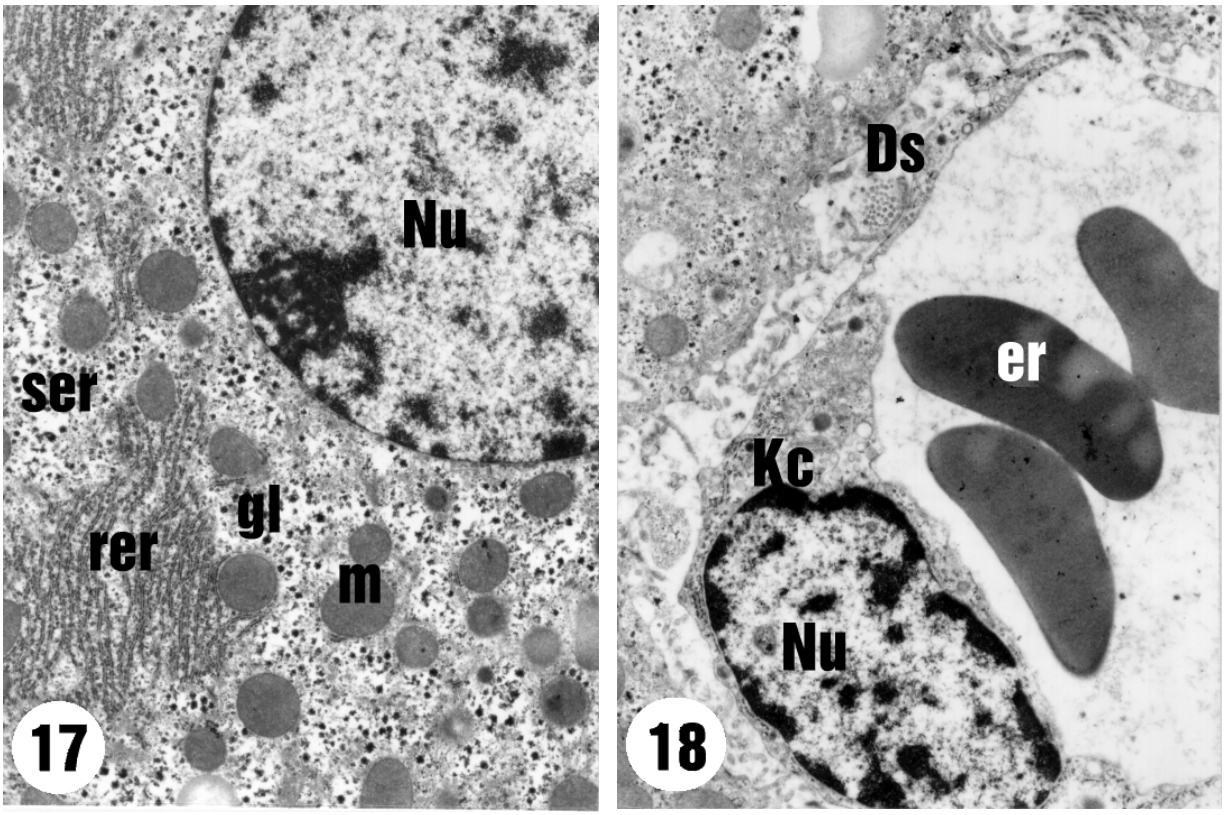
Group II after 10 days (electron microscope)

Fig 15 A fragment of the hepatocyte cytoplasm with sinusal surface (Ds). At the bottom of the electronogram there is a visible Browicz-Kupfer cell (Kc) with a nucleus with distinctly border accumulation of heterochromatin and the border location of the nucleolus in karyoplasm. In the basal cytoplasm of Browicz-Kupfer cell (Kc) there are individual primary lysosomes. The processes of hapatocytes project into the Disse space. In the cytoplasm of those cells are properly shaped mitochondria (m), clusters of rough endoplasmatic rete (rer) and primary lysosomes (li), near the biliary canaliculus (bc).

Ultimate magnification 16 250x.

Fig 16 The electronogram presents fragments of the cytoplasm of liver cells adjacent to the biliary canaliculus (bc); visible desmosomal strengthening (ic). Microvilli protrude into the lumen of the biliary canaliculus. Mitochondria (m) have a regular structure.
Next to the Golgi apparatus are primary lysosomes (li).
In the basal cytoplasm there are sparse glycogen granules (gl).

Ultimate magnification 16 250x.



Group III after 14 days (electron microscope)

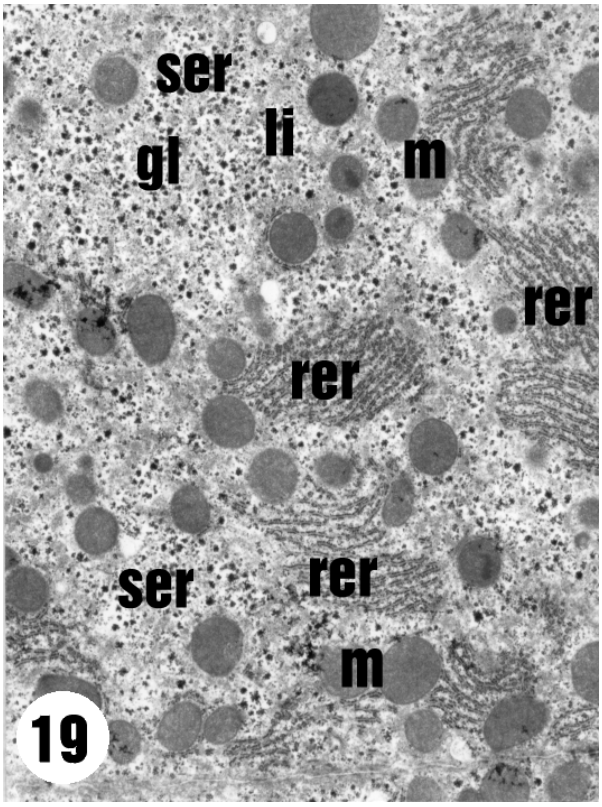
Fig 17 A fragment of hepatocyte cytoplasm from the perinuclear area of a group III test rat. The nucleus of regular contour and the border location of the nucleolus contains the clusters of rough endoplasmatic rete (rer) as well as the sacculi of smooth endoplasmatic rete (ser) accompanied by numerous glycogen granules (gl). Mitochondria (m) have a regular structure.

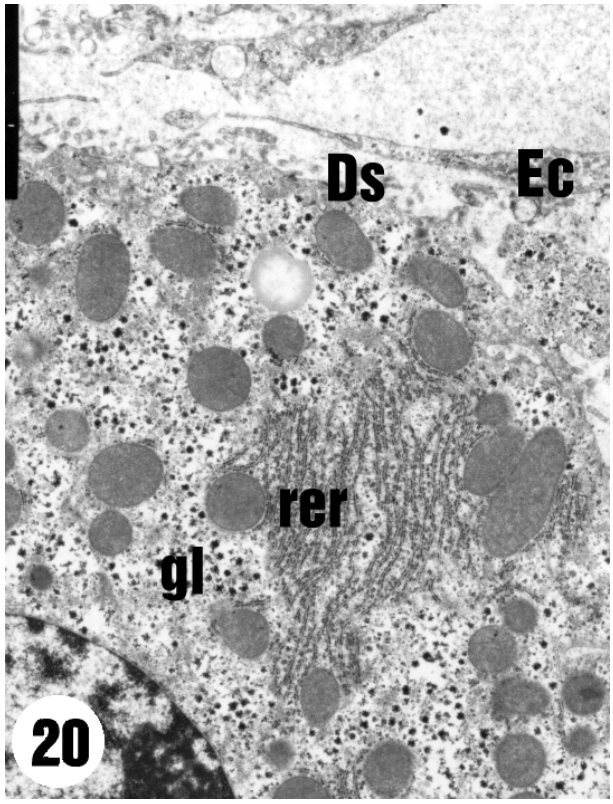
Ultimate magnification 16 250x.

Fig 18 The microphotograph presents the cross-section of a sinusoid.

In the lumen of the sinusoid there are three individual erythtrocytes (er) and a large fragment of Browicz-Kupfer cell (Kc) with a properly shaped nucleus (Nu). In the cell cytoplasm there are electron dense granules corresponding with primary lysosomes. The Disse space (Ds) with numerous microvilli and reticular fibres separates the lumen of the sinusoid with a thin trabecula of endothelium cells.

Ultimate magnification 16 250x.





Group III after 14 days (electron microscope)

Fig 19 A fragment of hepatocyte cytoplasm of a group III test rat. Attention should be paid to regularly shaped clusters of rough endoplasmatic rete (rer) and round, regularly shaped mitochondria (m).
Next to the smooth endoplasmatic rete (ser) is the cluster of glycogen grains (gl).

Ultimate magnification 16 250x.

Fig 20 The hepatocyte cytoplasm with the sinusal surface; at the top of the electrogram there is a visible lumen of a sinusoid lined with a delicate cell of endothelium (Ec) separating the cytoplasm of the liver cell with the Disse space. There are visible numerous, regularly shaped mitochondria, the clusters of rough endoplasmatic rete (rer) and regularly occurring glycogen granules (gl).

Ultimate magnification 16 250x.

B) BIOCHEMICAL DETERMINATION OF CONCENTRATION OF ENZYMES

GLUTAMATE DEHYDROGENASE

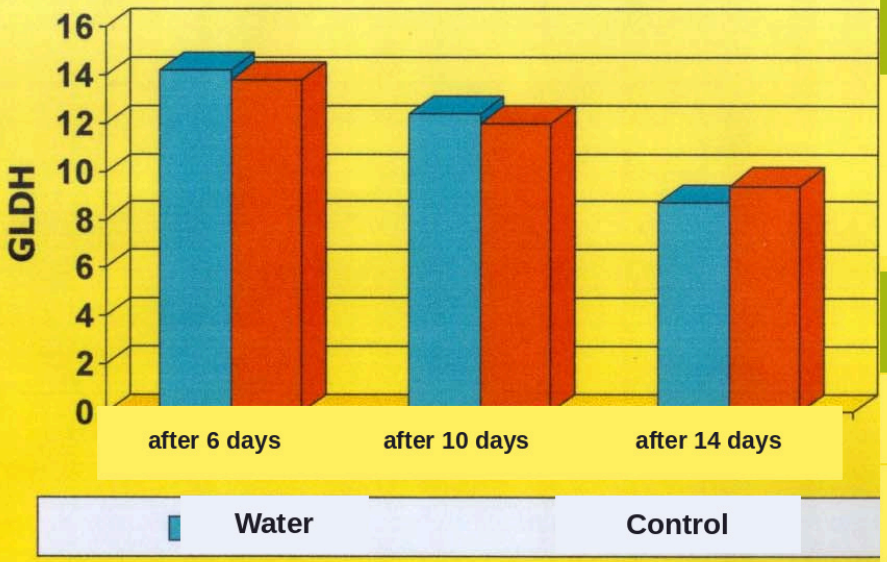
MALATE DEHYDROGENASE

LACTATE DEHYDROGENASE

ALANINE AMINOTRANSFERASE

Glutamate dehydrogenase (GLDH) level in study animal groups on days 6, 10 and 14

GLDH [EC 1.4.1.2.]	Water			Control		
	after 6 days	after 10 days	after 14 days	after 6 days	after 10 days	after 14 days
mean	14,24	12,38	8,75	13,78	11,99	9,4
SD	6,1	3,59	1,12	6,75	2,23	1,7
N	5	5	5	5	5	5
	p = 0,223			p = 0,402		

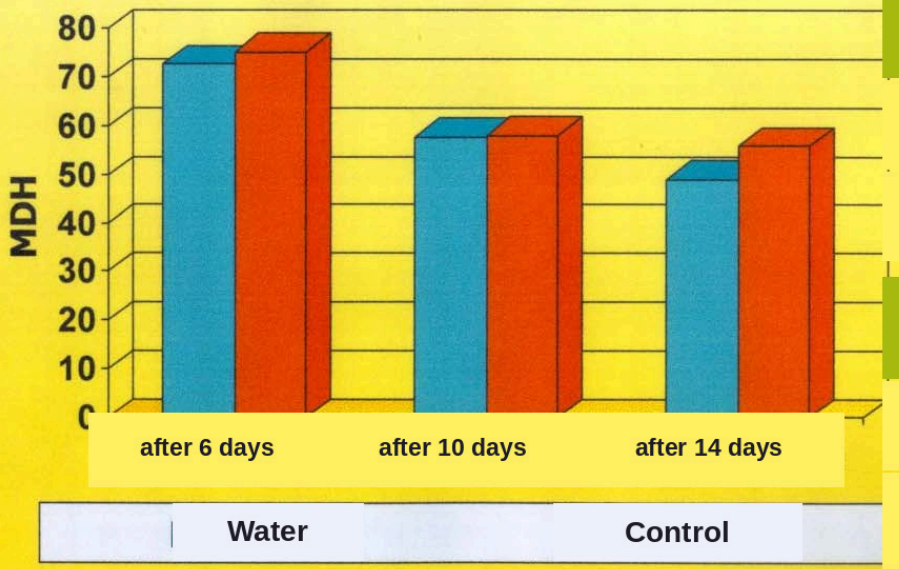


Water	after 10 days	after 14 days
after 6 days	p=0,34	p=0,17
after 10 days		p=0,17

Control	after 10 days	after 14 days
after 6 days	p=0,6	p=0,6
after 10 days		p=0,07

Malate dehydrogenase (MDH) level in study animal groups on days 6, 10 and 14

MDH [EC 1.1.3.7.]	Water			Control		
	after 6 days	after 10 days	after 14 days	after 6 days	after 10 days	after 14 days
mean	72,62	57,83	49,09	75,15	58,09	55,93
SD	17,54	12,68	4,95	14,82	6,82	7,64
N	5	5	5	5	5	5
	p = 0,055			p = 0,059		

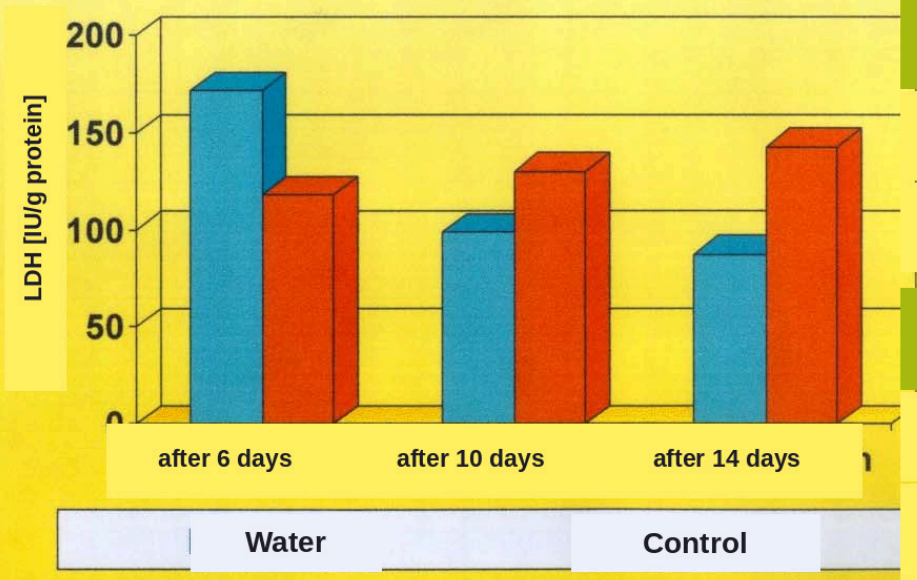


Water	after 10 days	after 14 days
after 6 days	p=0,17	p=0,02
after 10 days		p=0,17

Control	after 10 days	after 14 days
after 6 days	p=0,07	p=0,02
after 10 days		p=0,6

Lactate dehydrogenase (LDH) level in study animal groups on days 6, 10 and 14

LDH [IU/g protein]	Water			Control		
	after 6 days	after 10 days	after 14 days	after 6 days	after 10 days	after 14 days
mean	171,61	98,57	87,44	117,9	130,18	142,72
SD	87,08	48,75	17,79	71,21	46,92	75,07
N	5	5	5	5	5	5
	p = 0,22			p = 0,82		

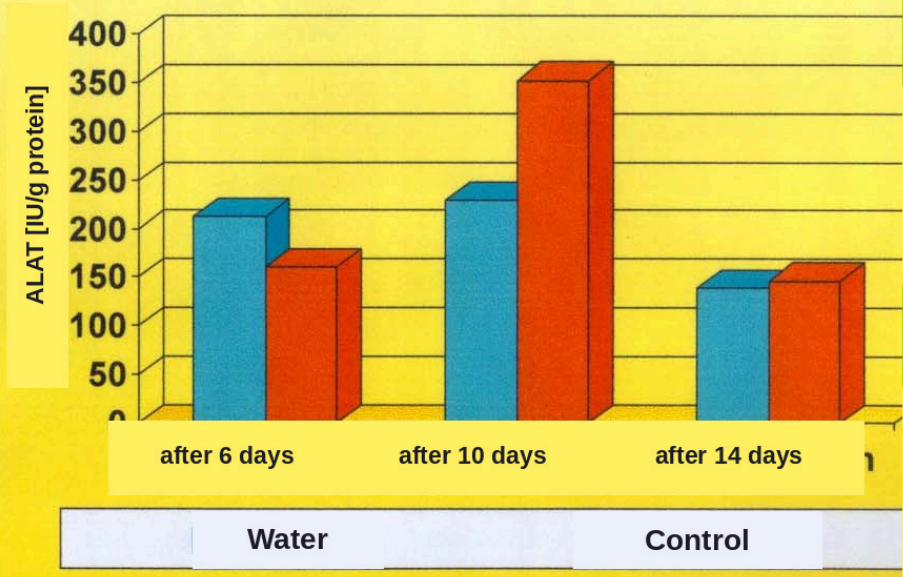


Water	after 10 days	after 14 days
after 6 days	p=0,17	p=0,12
after 10 days		p=0,92

Control	after 10 days	after 14 days
after 6 days	p=0,6	p=0,6
after 10 days		p=0,92

ALAT level in study animal groups on days 6, 10 and 14

ALAT [IU/g protein]	Water			Control		
	after 6 days	after 10 days	after 14 days	after 6 days	after 10 days	after 14 days
mean	212,7	229,18	138,62	160,58	352,29	146,54
SD	104,12	124,34	42,97	111,11	266,59	37,1
N	5	5	5	5	5	5
	p = 0,402			p = 0,223		



Water	after 10 days	after 14 days
after 6 days	p=0,91	p=0,25
after 10 days		p=0,25

Control	after 10 days	after 14 days
after 6 days	p=0,17	p=0,92
after 10 days		p=0,12

5. SUM-UP OF RESULTS

SUM-UP OF MORPHOLOGICAL RESULTS

Control group – photographs 1 – 8

All the semithin specimens of all the liver lobule areas of zones I, II and III (photographs 1 – 4) present a regular system of liver trabeculas. Individual hepatocytes do not have any deviations from the regular condition.

The electrograms (photographs 5 – 8) present various ultrastructural fragments of hepatocytes, mostly from zone I of the liver lobule. The nucleus has regular contours and mitochondria have a regular structure, despite various sizes (photographs 7 and 8). A more significant accumulation of glycogen granules (photographs 6 and 8) is to be observed in the basal cytoplasm adjacent to the vascular pole (Disse space). Numerous lysosomes are present in the cytoplasm of Browicz-Kupfer cells.

Test group I – photographs 9 – 12

All the electrograms present the hepatocytes from zone I of the liver lobule. Similarly as in case of the control group, we can observe a regular ultrastructure. The nucleus has regular contours (photograph 9) and mitochondria have a regular structure, despite various sizes and shapes (photographs 9 and 10). Numerous piles of flattened canaliculi of rough endoplasmatic rete (rer) can be observed (photographs 10 and 11).

Test group II – photographs 13 – 16

The electrograms of the hepatocytes of this group of animals present a regular structure with the rough endoplasmatic rete's (rer) tendency to grow as against the previous group (photographs 13 and 14). The nuclei of regular contours show the increase of the grain-like structure of heterochromatin (photographs 13 and 14). The mitochondria have a regular structure. Individual lysosomes adjacent to the secretory pole (biliary canaliculus) are properly located.

Test group III – photographs 17 – 20

The electrograms of the hepatocytes of the animals tested after 14 days of the experiment present a regular sub-microscopic structure. The nucleus has regular contours and a regularly shaped nuclear membrane (photograph 17). The round mitochondria have a regular structure. The rough endoplasmatic rete (rer) tends to grow in comparison to the control group (photographs 17, 19 and 20). The vascular pole of the hepatocytes separated from the lumen of the sinusoid by Disse space is regularly shaped (photographs 18 and 20).

SUM-UP OF BIOCHEMICAL RESULTS

The determination of the activity of metabolism-related enzymes conducted on hepatic homogenates have not shown any significant differences. The glutamate dehydrogenase, which together with the glutamate synthetase and transaminases plays the principal role in the biosynthesis of amino acids is structurally connected with mitochondria.

The statistically significant increase of the malate dehydrogenase proves the increase of activity of Krebs Cycle, which would be consistent with the statement that a magnetic field positively affects the processes of oxygen respiration.

The lactate dehydrogenase, whose changes in concentration would imply the structural changes in hepatocytes does not statistically indicate any significant changes in the groups of animals watered with magnetised water, if compared with the control group.

The alanine aminotransferase belongs to intracellular indicating enzymes participating in the transmission of amine groups from alanine onto alpha-ketoglutaric acid with the formation of pyruvic acid and glutamate acid. The real values of concentration of this enzyme would prove the undamaged structure of hepatocytes.

The examination of the concentration of the aforementioned enzymes important for the intracellular metabolism as well as their levels in test groups not deviating from control groups well correspond with a properly maintained structure of the liver of the rats supplied with the water previously exposed to a magnetic field.

6. DISCUSSION OF RESULTS

Variable magnetic fields are being used increasingly widely in medicine. In 1998, the Food and Drug Administration approved magnetic fields for magnetostimulation of pelvic-floor muscles, thereby enabling research into treatment of urinary incontinence by a method different from those previously used (Gaalovay-Urology). The results obtained suggest that the action of an external magnetic field on the pelvic-floor muscles has a beneficial effect on the urinary bladder, reducing complaints associated with urinary incontinence.

Other studies show that variable magnetic fields have beneficial anti-inflammatory and analgesic effects. One interpretation is a vasodilatory effect and an effect associated with improved cellular utilization of oxygen (12, 13, 42, 43, 48).

Among the beneficial effects of magnetic fields, many authors also include regenerative and reparative effects on soft tissues, acceleration of bone union, and intensification of tissue-respiration processes (22, 29, 59, 62).

Many authors also emphasize the possibility that magnetic fields affect membrane transport and ion distribution (11, 14, 15, 16, 17, 64).

Until now, explanations of these effects have not considered the possibility of an effect on tissue water; attention has instead focused on mechanisms associated with the humoral regulatory system.

In recent years, variable magnetic fields have been not only one of the dynamically developing areas of physical medicine. They are also increasingly used in everyday life as a means of

treating water used in households, both for drinking and industrial purposes, in order to reduce scale precipitation in pipes and water-system equipment. Because so-called water magnetizers are now commonly used, it is not easy to answer whether the benefits resulting from reduced damage to water installations are also accompanied by benefits for the users of those installations.

An important fact is that water exposed to an external magnetic field exhibits profound ultrastructural changes, not yet fully explained, which alter its physicochemical properties. These changes are particularly related to the induction value S' of the external magnetic field acting on the water (65).

Another important fact is that changes in the physical properties of water caused by an external magnetic field persist for several dozen hours after the field is removed (72).

Among the many documented effects of magnetic fields, it is assumed that the effectors are liquid-crystalline structures constituting components of biological membranes, as well as components of membrane channels. This is often used to explain observed effects on cellular organelle systems and cells themselves. To date, however, these effects have not been linked, even hypothetically, to the influence of magnetic fields on the cellular filler that is water.

The results obtained in my study allow the conclusion that this mechanism of field action - that is, changes occurring within the ultrastructure of the water molecule - is an effector of the external magnetic field that influences changes within cells and cellular organelles,

as well as enzyme metabolism, at least within the liver.

When assessing the effect of magnetic fields used in drinking-water supply systems, every demonstrated effect is extremely important because:
- identification of negative effects should lead to elimination of water magnetizers,
- absence of negative effects, even without proof of positive effects, may justify use of such systems because they improve the patency of water-supply systems,
- demonstration of a positive effect may support promotion of water magnetizers in supply systems delivering drinking water to our homes.

In my experiment, the experimental animals were given for 14 days water that had passed through a magnetic field of variable induction, whose value substantially exceeded the induction of the Earth's field.

This was ordinary water from the water-supply system; the only difference from the water consumed by control animals was that it had been exposed to a magnetic field.

Histomorphological images of liver specimens from both control and study animals show normal hepatocyte structure, with cell nuclei clearly visible in semithin sections and intracellular structures discernible.

Electron-microscopic images of hepatocytes in the first two study groups, which received water exposed to the field for 6 and 10 days, do not differ structurally from controls. In the third group, however, which drank water exposed to a magnetic field for

14 days, features of increased "organization" of cytoplasmic compartmentation and the contained cellular organelles can be observed. Compared with controls, mitochondria show greater uniformity of shape, generally round or oval, and the tubules of rough endoplasmic reticulum are arranged regularly in stacks.

No destructive features are observed in any study group, regardless of the duration of exposure to water treated with a magnetic field. A cautious hypothesis may therefore be advanced that magnetic-field treatment of water, beneficial from the standpoint of water-supply technology, is not harmful; moreover, after longer consumption of such water, features of a beneficial effect on hepatocyte structure are observed.

Assuming that cytoplasmic compartmentation is a prerequisite for normal cellular life processes and that each compartment represents a specific environment enabling particular biochemical reactions, the structure of cytomembranes separating individual organelles plays an important role in cellular metabolic reactions.

Cell nuclei, mitochondria, lysosomes, Golgi apparatus structures, and spaces contained in the rough and smooth endoplasmic reticulum are surrounded by lipoprotein membranes.

In the biochemical studies I performed, I also demonstrated that the water exposed to a magnetic field and used in the experiment does not significantly affect markers of liver-cell damage, namely lactate dehydrogenase, glutamate dehydrogenase and alanine transaminase. This shows that use of this water under the conditions of my experiment caused no adverse effects on liver cells.

As with the ultrastructural studies, there appear to be grounds for assuming that the water used in my experiment, which

had been exposed to a magnetic field, produces beneficial effects on tissue respiration after a longer period of consumption; this could be supported by the change in lactate dehydrogenase activity observed in my study.

The results of submicroscopic liver examinations and measurements of enzyme concentrations responsible for the most important metabolic reactions in hepatocytes support the hypothesis that water exposed to a magnetic field and consumed for 14 days by laboratory animals caused no impairment of their homeostasis.

Based on the results obtained, it may be assumed that prolonged use of water treated with the magnetizer used in this study may produce certain beneficial effects on liver function and activity. To demonstrate this relationship, however, I believe a longer-duration experiment would be necessary.

7. CONCLUSIONS

1. Morphological and ultrastructural changes in hepatocytes of rats given water exposed to a magnetic field indicate a beneficial effect, particularly visible in the normal formation of mitochondria and increased rough endoplasmic reticulum.

2. The biochemical results obtained may indicate activation of the Krebs cycle in mitochondria.

3. Water exposed to a magnetic field and given to rats for drinking shows no adverse effects during the initial observation period; after 14 days of exposure, a beneficial effect on liver-cell function and structure can be observed.

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9. SUMMARY

The aim of the study was to assess the effect of water exposed to a magnetic field on processes occurring in liver cells.

Sexually mature male Sprague-Dawley rats weighing approximately 250 g, obtained from the Central Animal Facility of the Silesian Medical Academy in Katowice-Ligota, were used. The animals were maintained under standard laboratory conditions at a temperature of 20-22 C and under a 12-hour light-dark cycle.

Control rats (15 animals) received tap water, whereas experimental rats (5 animals in each group) received, for 6, 10 and 14 days, water exposed to a dispersed magnetic field, which changed the magnetic relaxation time of the water, its viscosity and surface tension.

Tap water for the experiment was magnetized in a specially designed device called RAM (pipe magnetic apparatus) manufactured by Feniks.

Ultrastructural changes in hepatocytes were assessed in control and experimental animals after administration of magnetized water. Experimental results were also evaluated on the basis of selected biochemical parameters. In liver-tissue homogenates from control and experimental animals, enzyme concentrations in IU/g protein were determined for glutamate dehydrogenase, malate dehydrogenase, lactate dehydrogenase and alanine aminotransferase, reflecting liver function.

The results of morphological and structural studies indicate a beneficial effect of magnetized water on the organization of mitochondria and rough endoplasmic reticulum, while the biochemical findings indicate activation of the citric acid cycle.

Evaluation of the influence of magnetized water on liver cell function and structure

Key words: magnetic field, water, liver cells, metabolic processes, citric acid cycle

Summary

The aim of the study was an evaluation of the influence of water exposed to magnetic field on the processes in the liver cell.

The study included male sexually grown up rats of Spraque Dawley breed, body weight 250 /-+/ from the Central Animal Quarters, Silesian Medical University in Katowice Ligota.

The animals were kept in standard laboratory conditions, at the temperature of 20-22°C/ and changeable 12-hour lighting cycle.

The rats from control group /15/ received tap water while the study rats, 5 rats in each group for 6, 10 and 14 days, water exposed to magnetic field, of disperse stream, which caused a change in relaxation time of magnetic water, its viscosity and surface tension.

Tap water for the experiment was magnetized in a special device called RAM (pipe magnetic device) by Feniks company.

The ultrastructural changes in hepatocytes in control and study animals after using magnetized water were evaluated. The results of the experiments were also evaluated on the basis of the analysis of some biochemical parameters. In homogenates of the liver tissue of control and study animals, the enzyme concentration was measured in IU/g, the measurements included: glutamate, malate, lactate, dehydrogenase and alanine aminotransferase, which are a reflection of a liver function.

The results of morphologic and structural tests show a positive influence of water exposed to magnetization on mitochondria creation and rough endoplasmatic reticulum while, in biochemical terms, on the activation of citric acid cycle.