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The effect of spinal electrostimulation on the testicular structure in rabbit

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SUMMARY

Background: *The main objective of this study was to create an experimental model of idiopathic scoliosis (i.s.), and to assess the effect of Lateral Electrical Surface Stimulation (LESS) on the organism both intra vitam and post mortem. The experiment made it possible to determine the extent to which LESS affects overall development of the organism, apart from its positive clinical effect in correcting i.s. in children and youth. An attempt is also made to explain the basis of systemic complications accompanying this method.*
Material and methods: *Studies were carried out on 10 white New Zealand male rabbits aged 3.5 months. They were divided into two groups, 5 animals in each group. The LESS group was stimulated using an SCOL-2 apparatus, 9 hours a day. The second group served as controls. After three months, the animals were sacrificed. Detailed macroscopic and microscopic examinations were performed on the rabbits' testicles. Scraps were collected immediately after the animal's death, from the free brim of the testis. The ultrastructure was examined with a TESLA BS-500 electron microscope.*
Results: *In the LESS group, histopathological examination of the testicles revealed considerable necrosis of the seminiferous epithelium, frequently coupled with peritubular fibrosis, atrophy of seminal tubules, and proliferation of Leydig cells. Ultrastructural examination revealed a multi-layered basal lamina, collagen appearing in the proper membrane of the seminiferous epithelium and blood vessels, lysis of supporting and sex cells of the tubular epithelium, mitochondrial damage, and the formation of myelin-like bodies in the round spermatids and the middle segment of the elongated spermatid tails. Lysis of the cytoplasm of Leydig cells was observed in the testes.*
Conclusion: *Traditional electrostimulation induced regressive changes in the testes, in the form of necrosis of the seminiferous epithelium, atrophy of seminal tubules, and destruction of Leydig cells.*

BACKGROUND

The problem of systemic complications accompanying the Lateral Electrical Surface Stimulation (LESS) method, which is used in the clinical treatment of idiopathic scoliosis (i.s.) in children and adolescents, is often mentioned in the literature [1–6]. LESS is usually applied in the form of 9-hour night-time cycles, with treatment lasting from ca. one to several years [5–10]. Emotional and psychical discomfort are quite frequent complications, and sometimes body weight gains are disturbed

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under the influence of systematic therapy [1,3–7, 9,11].

Objectives of the study

The main objective of the study was to create an experimental model of i.s., and to assess the effect of LESS on the organism, both intra vitam and post mortem. To achieve this, a three-month experiment with rabbits was designed, in order to determine the direct effects of LESS on animal behavior and body weight in the course of treatment. Changes in adrenal gland and testicle structure were also observed. The experiment made it possible to determine the extent to which LESS affects the overall development of the organism apart from its positive clinical effect in correcting i.s. in children and youth. An attempt was also made to explain the basis of systemic complications accompanying this method.

MATERIAL AND METHODS

The research was conducted on 10 white New Zealand male rabbits aged 3.5 months, of body weight 2000 to 2200 g. The animals were purchased from the Breeding and Reproductive Rabbit Farm of the Ministry of Agriculture and Food, Department of Animal Production, Central Animal Breeding Station DI/421-2/92. They were divided into two groups, 5 animals in each group. The LESS group was stimulated using a SCOL-2 apparatus, 9 hours a day. The other group served as controls. The rabbits in this group were not stimulated, but otherwise all experimental parameters were the same.

LESS was performed with the SCOL-2, a Polish stimulator produced by the 'ELMECH' Electromechanical Medical and Laboratory Equipment Enterprise (Warsaw, ul. Burakowa 9, Poland) [11–14]. The SCOL-2 apparatus was placed to the right of the spine, at the level of Th3-Th8, 2–3 cm below the pectoral vertebrae. The electrodes were attached to the skin with a Codafix-net produced by Tricomed Ltd. Medical Articles (Łódz, ul. Piotrkowska 270, Poland), in a special leather box designed by Kowalski [11,12]. The SCOL-2 parameters – the condition of the wires and electrodes – were checked daily. Periodically, 2 to 3 times a week, hair was removed from the places where the electrodes were attached to the rabbit's skin. Once a month the batteries were changed. The LESS parameters were the same as those used in clinical i.s. treatment:

- Triangular interrupted impulse, lasting 0.1–0.2 ms, with impulse series of 3.5–4.5 sec.
- Frequency regulated within the range 20–50 Hz, with breaks of 4–12 sec.
- Amplitude adjustable between 5 and 75 mA.
- Power supply from a 9V battery, type 6F22.
- Maximum power uptake during stimulation of 15 mA at f=50 Hz, and 8 mA at f=20 Hz; < 3 mA during breaks.
- Precise adjustment of intensity amplitude by means of a two-stage switch, multiplying x 1 and x 3.

Rabbit behavior was observed each day at the same time, and the animals were fed ad libitum. Once a week body temperature was measured, and once a month the rabbits were weighed. After three months, the animals were sacrificed using an intravenous injection of 6% pentobarbital solution (Vetbutal-Biowet). Detailed macroscopic and microscopic examinations were performed on the rabbits' testicles. Scraps were collected immediately after the animal's death, from the free brim of the testis. They were stained using the PAS method according to McManus, and also with HE. The ultrastructure was examined using scraps stained with osmium tetroxide, under a TESLA BS-500 electron microscope.

Statistical analysis

The results were analyzed statistically, using the Student t-test to determine the significance of differences between the means.

RESULTS

LESS group

These rabbits showed symptoms of alarm during the first month of the experiment. They were excited and hyper-active. In the second month the animals avoided contact with people, trying to stay in the distal parts of the cages. When the stimulator signal was switched on, the rabbits became very disturbed and began to tremble. These responses were often coupled with uncontrolled urination. During the last month of the experiment all the rabbits avoided the forward parts of their cages, which they entered only at meal time. They were apathetic, showing no interest in their environment, but food was readily consumed throughout the experiment.

These rabbits' growth was disturbed. Body weight decreased after the second month, although it increased again between the second and the third month. The differences between the consecutive

Table 1. Monthly increment of body weight (g ,%).

Period	LESS x±s (g)	Control x±s (g)	significance of differences
first month	110±54.8	280±44.7	4.81**
second month	-80±236.1	280±83.7	2.89*
third month	190±102.5	300±122.5	1.38
	x±s (%)	x±s (%)	
first month	5.22±2.4	13.44±3.5	5.23**
second month	-3.94±11.3	11.87±3.5	2.68*
the third month	8.79±4.0	11.56±5.3	0.84

Significance of differences: * - $p < 0.05$. ** - $p < 0.01$

Table 2. Whole body weight increment (g .%).

Period	LESS x±s (g)	Control x±s (g)	significance of differences
one month	110±54.8	280±44.7	4.81**
two months	30±263.6	560±89.4	3.81**
three months	220±294.9	860±89.5	4.15**
	x±s (%)	x±s (%)	
one month	5.22±2.4	13.44±2.0	5.23**
two months	1.16±12.8	26.88±3.8	3.84**
three months	9.98±13.7	41.46±5.3	4.27**

Significance of differences: * - $p < 0.05$. ** - $p < 0.01$

mean body weights of these rabbits were statistically significant (table 1). Body weight increment in the experimental group over the entire period was only 9.98% of the initial value (table 2).

Post mortem examination revealed atrophy of fat tissue (emaciation). Moreover, two rabbits in this group had congested livers (hyperaemia passiva hepatis), and one of these also had congested kidneys (hyperaemia passiva renum). The mean weight of the adrenal gland was 0.5026±0.0897 g.

Histopathological examination of the testicles revealed considerable necrosis of the seminiferous epithelium, frequently coupled with peritubular fibrosis, atrophy of seminal tubules, and proliferation of Leydig cells. Ultrastructural examination revealed the presence of a multi-layered basal lamina (fig.1) and the appearance of collagen in the proper membrane of the seminiferous epithelium and blood vessels (fig.2), lysis of supporting and sex cells (fig.3) of the tubular epithelium, mitochondrial damage, and formation of myelin-like bodies in the round spermatids (fig.4) and the middle segment of the elongated spermatid tails (fig.5). Lysis of the cytoplasm of Leydig cells (fig.6) was observed in the testes.

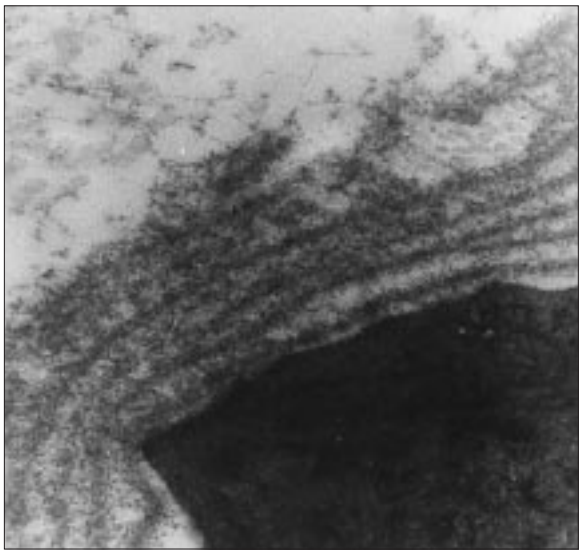

Figure 1. LESS group. Multi-layered basal lamina of the seminiferous epithelium. ME. x18000

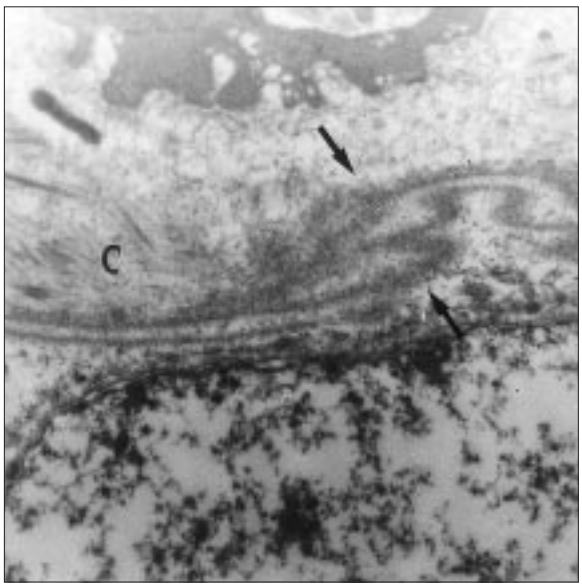

Figure 2. LESS group. Irregular and multi-layered basal lamina (between arrows) and collagen fibres (C) in the vascular wall. ME. x8000.

Control group

The behavior of the rabbits in the control group was normal throughout the three months of the experiment. These animals were interested in their environment and exhibited a lively reaction to contact with humans. They also readily consumed their food.

Individual body weight increased, and the differences between consecutive mean values were statisti-

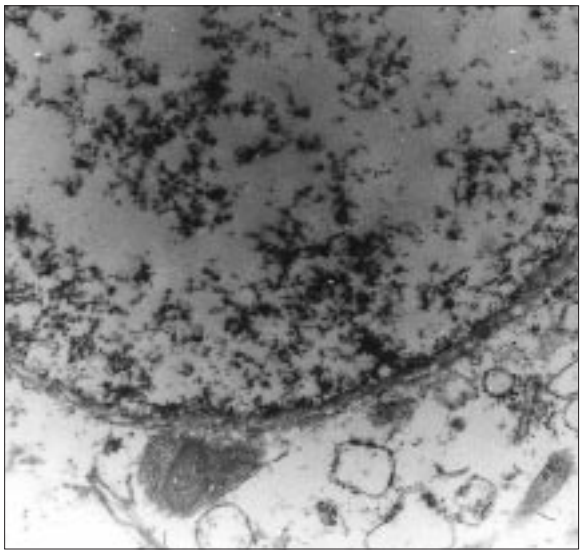

Figure 3. LESS group. Lysis of the round spermatid. ME. x8000.

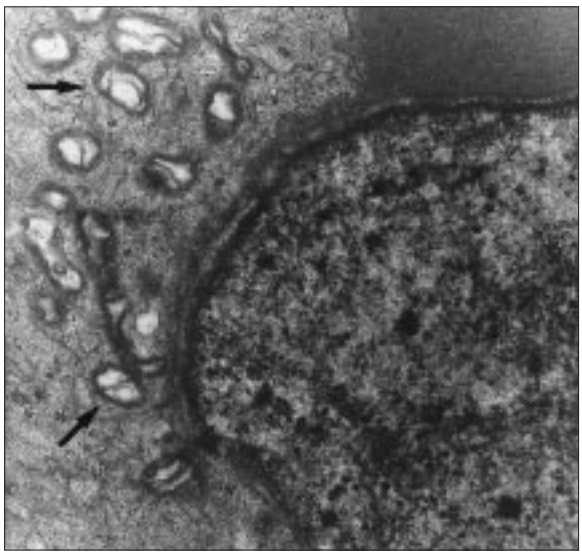

Figure 4. LESS group. Myelin-like bodies (arrows) in the round spermatid. ME. x8000.

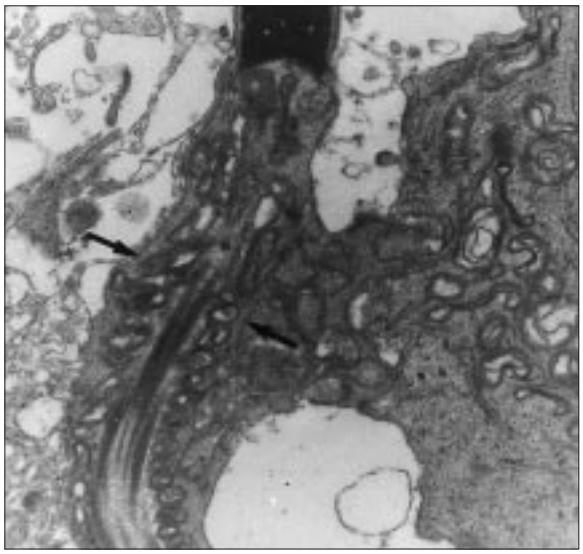

Figure 5. LESS group. Mitochondrial damage and formation of myelin-like bodies (arrow) in the middle segment of the elongated spermatid tail. ME. x8000.

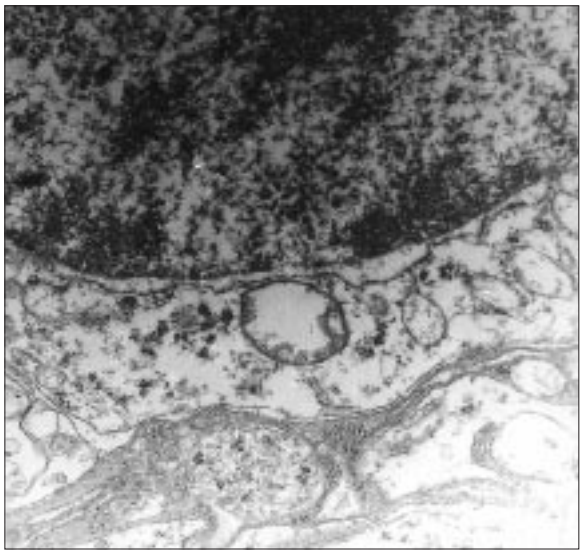

Figure 6. LESS group. Lysis of the Leydig cell. ME. x10000.

cally significant (tab. 1). The total body weight increment by the end of the three-month period reached 41.5% of the initial level (tab. 2).

Post mortem examination of the control rabbits showed no pathological changes. The mean weight of the adrenal gland was 0.2981 ± 0.087 g.

Single tubules with sperm stasis were observed during histopathological examination of the testes, as well as vacuolar degeneration and some necrotic foci in the seminiferous epithelium. Leydig cells were scattered, or formed small aggregations. Ultrastructural examination revealed a single-layer basal lamina of the tubules. Pyknosis of Leydig cells (fig.7) was observed in the testes.

DISCUSSION

Experiments carried out on animals in order to determine the impact of LESS on the behavior of a living organism usually concentrate on spinal statics [9,11,15]. Experimental papers tend to stress that the animals' age must be well selected, i.e. adapted to the age of children (so-called 'growing youth') where the progress of i.s. is characteristic [8,13,16–18]. Source publications underline the

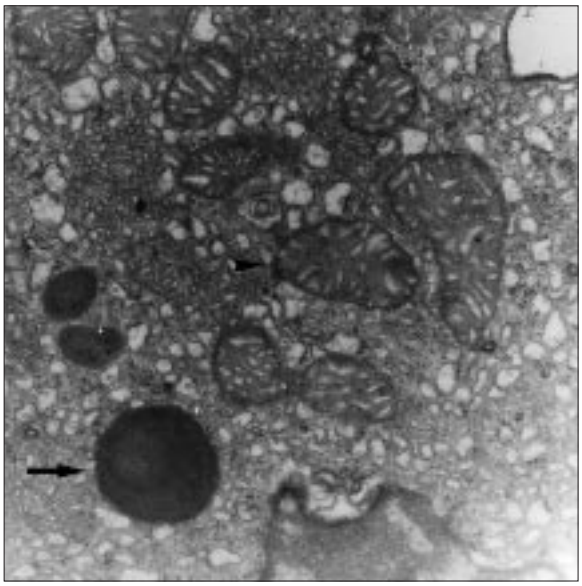

Figure 7. Control group. Pyknosis of the Leydig cell. Lipids (arrow) and mitochondrion (arrowhead). ME. x8000.

appearance of degenerative and atrophic changes in muscular and connective tissue. These are treated as secondary to the destruction of the sensory part of the spinal reflex arc and interference with blood supply due to i.s. and long-term therapy with LESS [1,3,5,7–9,11,13,14,17].

Bigard [8] observed in an experiment with monkeys that surface electrostimulation disturbed blood supply in tissues and organs, and especially in muscle capillaries. Joe [16] observed disturbances in blood supply through the spinal muscles of rats subjected to three-week stimulation. Blood supply returned to normal within an additional three-week period when no electrostimulation was applied.

Papers dealing with the effect of LESS on cell metabolism underline the possibility of atrophy or degenerative changes, sometimes even necrosis [8,11,13,14,16–19]. The analysis of the side-effects of clinical treatment based on LESS applied for 9 hours daily has revealed sleep disturbances, suggesting that there may be some systemic disturbances induced by stress [2–6,10].

Our studies showed that LESS disturbed body weight increments. This effect was confirmed by decreased individual weight in the LESS group, in contrast to the control animals, in which the weight increments were always statistically higher. In addition to this, the post mortem examination of rabbits from the LESS group showed that these animals were emaciated and had enlarged adrenal glands [11–14], both of which are probably stress symptoms. Histopathological examination showed also considerable destruction of the seminiferous epithelium and Leydig cells, probably of irreversible character. The changes observed in Leydig cells may even suggest hormonal disturbances. Several papers that have analyzed the effect of spinal electrostimulation on internal organs describe highly negative changes induced by 9-hour stimulation, particularly as regards the adrenal gland and testes [12,19].

The number of papers documenting the negative effects of stress on testicles continues to increase. Descent of the testes was observed in rats whose mothers were stressed during pregnancy. This was due to the inhibition of processus vaginalis growth by the mild antiandrogenic effect of prenatal maternal stress on the fetus [20]. Morphological and histochemical changes were found in adrenal cortex and testis (even decreases in testosterone and androstendione synthesis) after one hour of daily immobilization for seven consecutive days in rat [21]. Chronic immobilization-induced stress from puberty to sexual maturity has also been shown to reduce the amount of mature spermatids in the testis of male rats by 16%, causing a 32% reduction in the spermatozoon concentration in the cauda epididymis [22]. Inter-relationships have also observed between behavior, high cortisol concentrations accompanying chronic stress, and decreasing androgen levels in the environment of subordination. Certain behaviors can be renormalized by sex steroid support [23]. These examples illustrate the extent to which the testes are susceptible to stress, so that even slight stress can disturb testis function. They also show surprising links between behavior under stress and the levels of sex hormones. Hence, substances and treatments capable of changing testosterone or corticosterid levels in the prepubertal period in response to various stressors are risk factors in male sexual development.

In view of the results obtained here it would seem advisable to continue research on the possibility of shortening the period of daily stimulation. Daily therapy involving the application of LESS could perhaps be shortened in children and youth with slight i.s., so as to minimize general stress-related side effects. The possibility of such an approach has already been confirmed in our own clinical studies, initiated in 1987. Treatment consisted of only 2 hours of LESS, applied in the evening, at the Rehabilitation Center in Olsztyn and Ameryka [11]. As a result, the stress-related side effects disappe-

ared, while the therapeutic benefits remained unchanged. Clinical observations conducted over the last several years seem fairly promising. They are also supported by the results obtained with experimental animals, suggesting that this type of treatment should be continued [11–14,19].

CONCLUSIONS

1. Protracted stimulation disturbs rabbit behavior and body weight increments.

2. Traditional electrostimulation induces regressive changes in the testes, in the form of seminiferous epithelium necrosis, atrophy of seminal tubules, and destruction of Leydig cells.

3. More research is needed on the effects of shortening the daily duration of LESS.

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