

RAD7

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*CO-DIRECTED INFLUENCE OF MITAGENES
AND MODULATED ULTRASOUND ON CELLS
OF VARIOUS ORIGIN*



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Materials and Methods



To study the *in-vitro* effects of low therapeutic intensity ultrasound (US) in animal cells, a 3-day transplanted MDBK cell culture was employed. For *in vivo* exposure, outbred adult male mice of 10–12 weeks of age, weighing 24–26 gr, were applied. Irradiation of mice was carried out in a thermostatic cuvette (25°C); the testicles of US-treated mice were at the beginning of the far zone of the emitter. The passed into tissue US-intensity was 90 % of the nominal intensity. On the third day after irradiation, the cellular (stage 7 of the cell cycle) elements of the seminiferous tubules of the testes of experimental animals were counted.

Cell culture

Cultivation of the treated with stimulants and control cells was carried out at a temperature of 37°C in the Eagle's medium with 10% of bovine serum, with antibiotics (100 µg/ml) lincomycin or kanamycin, with 5% of glutamine and sodium bicarbonate until neutral *pH*.

The initial planting concentration of cells was 8.0×10^4 – 1.0×10^5 per 1 ml.

- Plant mitagens Concanavalin A (Cohn A) and phytohemagglutinin (PHA) were tested in concentrations from 1×10^{-4} to 10 mg per 1 ml of suspension.
- The multiplicity of mitagen adding into the suspension of transplantable cell culture was 1:5. The MDBK culture cells was grown by the traditional method in 50 ml mattresses. A monolayer in the control was formed for 2–3 days.

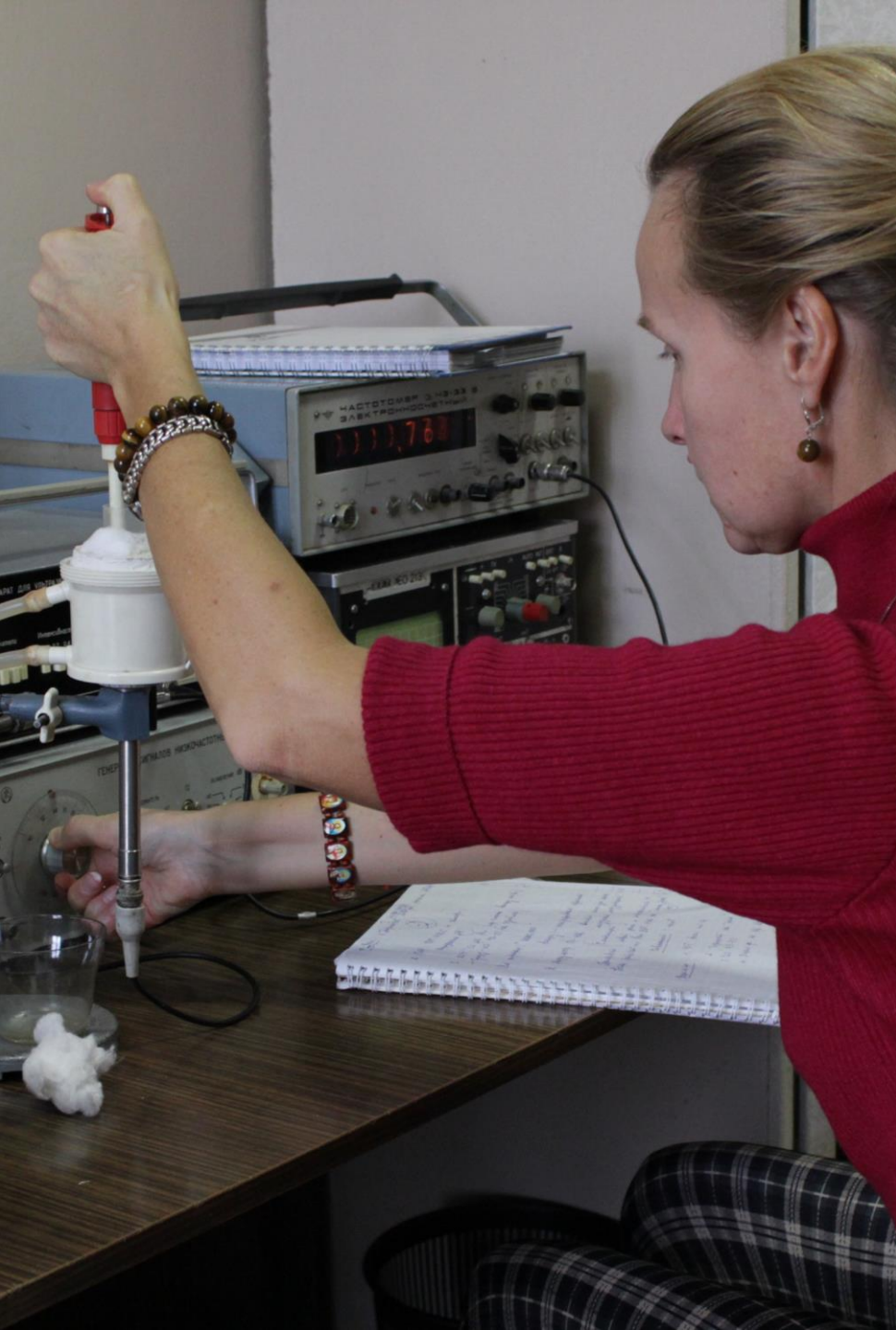
Methods of Microscopy

- US effects on cells (control and after the US exposure) were observed under a light microscope (immersion, transmitted light microscope «LOMO», optical objective– 100^x/1.25; ocular lens– 10^x/18).



Cell culture control

- For studies, the suspension of synchronized cell cultures at different stages of the cell cycle was explored. The initial number of cells with an intact membrane in the control and experience was at least 80 %. The integrate membrane of the tested cells was determined by the absence of trypan blue-staining.



Methods of sonication

- The US-exposure varied within the following limits: time from 1 to 300 sec, SATA–intensity 0.01–2.0 W/cm², generative frequency was 0.88 MHz, modulation — from 10 to 1000 Hz. Devices: UZT-1-01F; UZT-5 and UZT-1.02S.



RESULTS



1. In vitro-
experiments

US-effect

- Stimulation of the cell growth was initiated by US-intensity of 0.03–0.05 W/cm² with an exposure time of 5–30 sec.
- The increase of cell mass was 65–130% in compare with control & depended on both the duration of exposure and the stage of cell cycle. The maximum stimulating effect was registered after single “preplant” US-continuous treatment of cells (0.05 W/cm², exposure time 10 sec).
- The cell growth in control did not exceed 3.1×10^5 per 1 ml. The proliferation index increased as a result of US-stimulation from 3.8 to 9.0.

The Coh A- and PHA-effects

- Cohn A in optimal concentration of 1.0 mg/ml increased the “yield” of cells to 1.1×10^6 per 1 ml.
- Optimal PHA concentration was 0.01 mg/ml. The average cell growth was 9.6×10^5 per 1 ml. Also, in “experimental” cultures, an increase in the rate of proliferation was recorded — the monolayer was finally formed 10–12 h faster than in the control.
- Maximum increase in MDBK-harvest was obtained after "preplant" cells treatment.
- In all cases, the treatment of cells in the stage of monolayer formation with various stimulators, no positive effect was found



2. In vivo-
experiments

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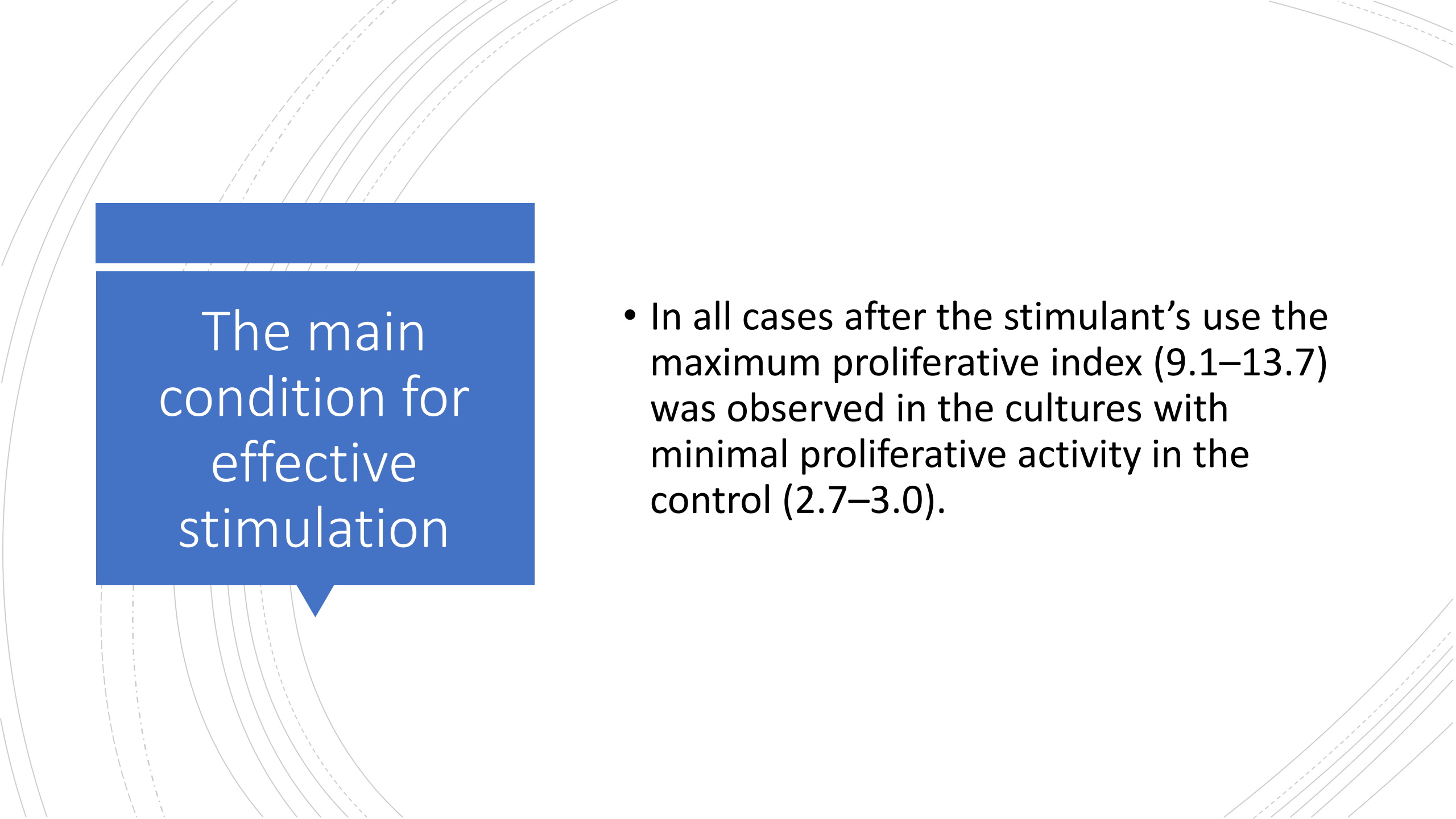
In vivo- experiments

- A similar regularity was also observed in *in-vivo*-experiments
- The irradiation of the seed epithelium cells, which were *only in the early stages* of maturation while, caused the stimulation of cell proliferation and growth



The in vivo US-effect

- A positive effect was obtained after US-irradiation of the testes with intensity of 1.0 W/cm^2 for 5 min: a significant increase ($p < 0.05$) in the number of spermatids and spermatozoa was recorded at US-modulative frequency of 250 Hz.

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The main
condition for
effective
stimulation

- In all cases after the stimulant's use the maximum proliferative index (9.1–13.7) was observed in the cultures with minimal proliferative activity in the control (2.7–3.0).

Acknowledgments



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