7. THE SUMARRY

This work deals with optimization of cell culture conditions of RAW 264,7 cells and optimization of LPS concentrations. We have been trying to form an anti-inflammatory model.

This work is composed of acknowledgements, the introduction, the theoretical part, materials and methods, results, the discusion, summary, abbreviations, references and the content.

In the theoretical part cell cultures are described; how to work with them, how to obtain them, common problems like contamination, environmental requirements like the culture medium and the application of cell lines. Futhermore this part is focused in macrophage cell lines, their roles in the immune system and inflammatory response. There are described the Griess reaction, which is used for determination of NO production, and the growth cycle of cells.

In the materials and methods all used materials, reagents, equipments and methods are described. The methodology is focus in conditions of incubation, subculturing of cells, counting of cells, plating of cells; growth cycle (counting of cells twice a day for six days) and determination of NO production in LPS-treated RAW 264,7 cells – Griess reaction. Griess reagents (NED and sulphonilamide in phosphoric acid) combined with NO_2^- form a chromophore, which is detected by a spectrophotometer.

Cell line was incubated in an incubator in 37°C saturated by 95% of oxygen and 5% of carbon dioxide (CO_2). Every two days or if necessary were subcultured. Cells were washed by Hank's Balanced Salt Solution (BBS). As a culture medium was chosen Dulbecco's Modified Eagle's Medium (D-MEM) mixed with serum, antibiotics to protect the cell culture against contamination, and glutamine. All works with cells were realized in a biology safety cabinet. For maintaining aseptic conditions was used 70% ethanol, sterile gloves and laboratory coat.

Results consist of two chapters: results of growth cycle and results of determination of NO production in LPS-treated RAW 246,7 cells. The growth cycle is characterized by the growth curve which has sigmoidal shape. The cells were plated and twice a day counted in the hemacytometer, in the morning and in the afternoon, for six days. From a growth curve, the lag time, population doubling time, and saturation density can be determined. [3, 4, 5] The lag phase was defined from the seeding cells untill 54 h ($8,8 \times 10^5$). The cell number exponential increased from the 54 h untill 94 h ($4,1 \times 10^6$ cells). This period was expressed like a log phase. At 101 h counting showed decreasing the cell number ($3,81 \times 10^5$), the plateau phase.

The anti-inflammatory activity was tested by measuring the production of nitric oxide (NO) (Griess reaction using spectrophotometer).

It was made tree experiments. In the first experiment cells were stimulated with 0,1; 1 and 10 μM/mL of LPS. The first experiment was repeated tree times (1., 2., 3.) with the same conditions (seeding concentration: 5×10^5 cells/mL, 0,1; 1; 10 μM/mL of LPS). In the second and third experiment RAW 246,7 cells in concentration 4×10^5 cells/mL were treated with LPS (0,125; 0,25; 0,5; 2; 8 μM/mL).

In the first experiment cells treated with 0,1 μg/mL of LPS released 0,179 μM of NO, with 1 μg/mL of LPS 0,755 μM of NO and with 10 μg/mL of LPS 0,759 μM of NO.

In the second experiment cells treated with 0,125 μg/mL of LPS did not release any NO. Cells treated by 0,25 μg/mL of LPS released 0,051 μM of NO, 0,5 μg/mL of LPS released 0,14 μM of NO; by 1 μg/mL of LPS 0,2 μM of NO; by 2 μg/mL of LPS 0,253 μM of NO and by 8 μg/mL of LPS released 0,364 μM of NO.

In the third experiment cells treated with 0,125 and 0,25 μg/mL of LPS did not release any NO. Cells treated by 0,5 μg/mL of LPS released 0,066 μM of NO, 1 μg/mL of LPS released 0,090 μM of NO; by 2 μg/mL of LPS 0,115 μM of NO and by 8 μg/mL of LPS released 0,246 μM of NO.