death[1-6], then it becomes clear that in cases of acute liver failure early prophylaxis of cerebral edema is necessary, and intensive therapy should be initiated as soon as the first changes in consciousness appear.

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Effect of pretreatment with prednisolone on the phagocytic activity of mouse peritoneal macrophages in vitro

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Summary. The phagocytic activity on in vitro cultured mouse peritoneal macrophages derived from animals treated with 6-alpha-methyl-prednisolone was examined. The statistical evaluation of results showed an increase of phagocytic activity of macrophages derived from treated animals in comparison with controls.

Corticosteroids possess different effects on phagocytic activity. These substances, in fact, inhibit the adhesion of foreign particles and of microorganism on mononuclear phagocyte surface[2] and reduce the avidity of surface receptors for immunoglobulins and complement which are involved in the early stages of phagocytosis[3,4]. These effects can be related with the changes in the cell surface properties induced by prednisolone in epithelial-like cells cultured in vitro[5].

The phagocytic process which follows the recognition and adhesion of foreign particles or bacteria on the cell surface is energy-dependent. The main source of this energy is represented by glycolytic pathway, while the Krebs cycle activity is less important[6].

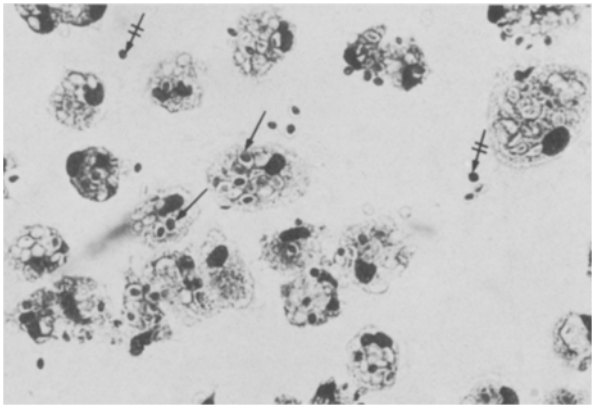
High concentrations of cortisone depress respiration and stimulate glycolysis of in vitro cultured cells, while lower concentrations of this drug stimulate the activity of the respiratory chain[7]. In this paper we have studied the phagocytic activity on in vitro cultured mouse peritoneal macrophages derived from animals treated with 6-α-methyl-prednisolone, in order to obtain further information on the influence of corticosteroids on the phagocytic process.

Materials and methods. Animals. In all the experiments, male albino mice (Swiss strain) 8 weeks old and weighing 38–40 g were used. Prednisolone was administered by i.m. injection at the dose of 2.5 mg/kg/day for 7 days. A batch of animals was treated with plain saline solution as control. This treatment was discontinued for 2 days and then 2 ml of a starch suspension were injected i.p.

Reagents. 6-α-methyl-prednisolone emisuccynate (Hoechst) was dissolved in bidistilled water at the concentration of 0.20 mg/ml. This solution was sterilized by filtration through Millipore GS (diameter 0.22 µm) filters. Sterile potato starch (Merck) was suspended in saline solution (1.5 g/100 ml). Zymosan (ICN Pharmaceuticals) was suspended in Parker's 199 medium (Difco) at the final concentration of 1.5×10^7 particles/ml. This concentration was 10 times higher than the number of the cells in culture.

Cell cultures. 1 day after starch i.p. administration the animals were killed by cervical dislocation. Macrophages were obtained from mouse peritoneum and cultured according to the technique described elsewhere[8] in Leighton tubes containing a cover slip at 37 °C. The nutrient medium consisted of Parker's 199 medium (Difco) supplemented with 15% of fetal calf serum (Microbiological Associates) and 10% of lactalbumin hydrolysate (NBC, enzymatic). The cell suspension was counted and diluted, if necessary, with nutrient medium to a concentration of 1.5×10^6 cells/ml and distributed in the Leighton tubes. 24 h after cell establishment, the nutrient medium was renewed to remove the nonadherent cells. This procedure provided a more uniform cell monolayer and enhanced the macrophages survival during the culture[9].

Experiments. 8-day cell cultures were used for the experiments. The nutrient medium was eliminated from the Leighton tubes and the cell cultures were washed 3 times with medium 199 to remove traces of serum. In a 1st group of experiments, the nutrient medium was replaced by 2 ml of nonopsonized zymosan suspension, while in a 2nd group of experiments, the same amount of opsonized zymosan particles was added to cell cultures. Opsonization of zymosan was carried out by adding to zymosan suspension a 5% dilution of homologous serum in saline solution, both heat-inactivated or not. This mixture was incubated for 30 min



Mouse peritoneal macrophages phagocytizing zymosan particles. ←: intracellular zymosan particles. ⊣: extracellular zymosan particles.

Summarized data from biometric analysis of the rate of zymosan ingestion in mouse peritoneal macrophages.

Zymosan particles per cell	Control Percent of cells			Prednisolone Percent of cells		
	A	B	C	A	B	C
0	18.16	48.31	31.64	6.38	28.98	29.96
1	15.72	26.68	21.52	10.64	30.71	31.13
2	26.41	17.13	21.10	17.55	19.74	24.90
3	18.24	6.18	12.24	23.40	8.28	10.12
4	10.06	0.84	6.33	16.49	5.73	2.33
5	4.50	0.56	4.22	8.51	8.95	1.17
6	2.52	0.28	1.69	10.11	0.95	0.39
7	1.26	-	0.84	3.19	0.32	-
8	1.26	-	0.42	0.53	0.32	-
9	1.26	-	-	2.66	-	-
10	-	-	-	0.53	-	-
12	0.63	-	-	-	-	-
Average	2.38	1.14	1.24	3.51	1.37	1.29
Variance	4.14	0.58	3.98	3.55	1.86	1.36
Standard error	0.16	0.04	0.13	0.14	0.08	0.07
Index of phagocytosis	1	1	1	1.47	1.20	1.04
Komolgorov-Smirnov test	-	-	-	0.001	0.001	NS

A, zymosan opsonized with homologous serum; B, zymosan opsonized with homologous inactivated serum; C, zymosan non opsonized.

at 37 °C. After 1 h of contact of cell cultures with zymosan suspension, the cover slips were extracted from the tubes, fixed in methanol acetic acid mixture and stained with hematoxylin-eosin by routine procedure.

Quantitation of phagocytosis. Phagocytosis was evaluated by direct count of intracellular particles by oil 100× immersion microscopy. For each experiment, 50 microscopic fields were examined at random. The intracellular particles of each cell were counted and these data were submitted to statistical evaluation. For each experiment, the average of phagocytized zymosan particles per cell, the variance and the standard error were determined by using the routine procedure. The index of phagocytosis was calculated as the ratio between the average of the experimental cell culture and the average of control cell culture. The Student's t-test would not be reliable because the behaviour of intracellular particles did not follow normal distribution. It was necessary to use a global non-parametric test for a comparison between the experimental data and the control ones, i.e. the Komolgorov and Smirnov test at usual significance levels (5%, 1%, 1‰)[10].

Results. The results of our experiments can be summarized as follows: a) In the control cell cultures, the phagocytic rate was positively affected by the opsonization of zymosan particles with homologous serum, while a lower level of phagocytosis was found when zymosan particles were opsonized by homologous inactivated serum, or when they were not opsonized (table). b) The macrophage cultures derived from animals treated in vivo with prednisolone always showed an increased phagocytic activity in comparison with control cell cultures when zymosan particles were previously opsonized both by homologous serum or homologous inactivated serum (0.001, significant). No significant increase in phagocytic rate was found, on the contrary, with nonopsonized zymosan particles (table). It was observed that cell concentration in peritoneal exudate was lower when the animals were previously treated with prednisolone (1×10^6 cells/ml in prednisolone-treated animals and 2×10^6 cells/ml in control animals respectively).

Discussion. The findings reported in this paper show that peritoneal inflammatory response to a foreign substance was highly affected by in vivo administration of methyl-prednisolone. In fact, this drug markedly reduced the number of macrophages recovered within the peritoneal cavity after an inflammatory stimulation. This reduced migration of mononuclear cells from the blood to peritoneum could be due to a direct effect on the vascular permeability or to a decrease of monocytes motility or, finally, to a reduction in the number of circulating mononuclear cells. The last hypothesis is very probable and it is supported by much experimental data from other laboratories. For example, Van Furth[11] reported a severe and prolonged monocytopenia amounting to more than 14 days after the administration of 15 mg of hydrocortisone acetate in mice. This phenomenon was related to a decrease in the number of promonocytes in the bone marrow. As promonocytes derive from monoblast, it could be supposed that corticosteroids affect monocytes production by depressing monoblast maturation. So it can be assumed that also methyl-prednisolone was able to reduce the number of circulating monocytes by inhibiting the monoblasts division in the bone marrow.

Our findings suggest that in vivo administered corticosteroids increased the in vitro phagocytic activity of macrophages derived from peritoneal cavity. We are unable, at present, to propose a mechanism of action for this stimulation of phagocytosis. Further investigations are required to elucidate this finding.

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