# Effect of Experimental Desynchronosis on Immunotoxicity of Benz(a)pyrene in (CBA×C57Bl)$F_1$ Mice

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Experiments on (CBA×C57Bl)$F_1$ mice showed that constant (day and night) illumination for 2 weeks led to the development of internal desynchronosis, which significantly modulated the reaction of the immune system to benz(a)pyrene compared to those in intact animals. Presumably, the differences were associated with changed circadian rhythms of the immune parameters in experimental animals determining different ratios and, presumably, functions of immunocompetent cells during benz(a)pyrene exposure in intact controls and animals with desynchronosis.

**Key Words:** *desynchronosis; benz(a)pyrene; immune response; lymphoid organs*

The immune system is one of the priority adaptation mechanisms. Exogenous or endogenous factors always cause changes in cellular or humoral immunity components [3]. All biological processes in the immune system are characterized by rhythmic course [1,7]. Phasic discoordination of circadian rhythms of body functions (desynchronosis, DS) can modulate the degree or direction of the effects of some factors. However, the effect of biorhythm desynchronization on sensitivity/resistance of the immune system is little studied. Chemical pollution causing increased morbidity at technogenically loaded territories is now a leading trend in ecological hazards [2,3,5,6]; According to WHO criteria, benz(a)pyrene (BP) is the most hazardous and prevalent pollutant [5]. BP is an immunosuppressant modulating differentiation of T-cell precursors, leading to reduction of their reaction to IL-2 [5,8]. We studied the effects of BP on parameters of the immune system in mice with experimental DS.

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## MATERIALS AND METHODS

(CBA×C57Bl)$F_1$ were exposed to permanent 24-h (day and night) illumination for 2 weeks [1]. Control groups were kept under usual day/night light regimen. On day 15 the animals were decapitated at 10.00, 15.00, and 19.00 (5-6 animals from each group per term) in order to evaluate circadian variations in the studied parameters. Other mice of both groups (6 per group) were intraperitoneally injected with BP at 10.00 for 3 days in a total dose of 60 mg/kg in 0.2 ml olive oil (OO). Injection of the same volume of OO served as active control. These animals were decapitated at 10.00 24 h after the 3rd injection of BP and OO. Leukocyte count and differential blood count were determined. In lymphoid organs cell count, percentage, and total counts of lymphocytes and blasts, and weight organ indexes were determined. The content of lymphocyte subpopulations in the thymus and spleen was evaluated at 10.00 by flow cytometry with monoclonal FITC- and PE-labeled antibodies to CD3, CD4, CD8, and CD25 antigens (Pharmingen) on a FACSCalibur flow cytometer (Becton Dickinson). Humoral immune response to T-dependent antigen (sheep erythrocytes) was evaluated by the number of antibody-producing cells in the spleen on day 4 after intraperitoneal injection of the antigen as described previously [4].

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The results were statistically processed using Statistica 5.0 software. The significance of differences was evaluated using Mann-Whitney nonparametric test and MANOVA dispersion multidimensional analysis at 95% significance level.

## RESULTS

The mice exposed to constant illumination developed the following shifts in comparison with intact mice. The percentage of blood lymphocytes increased and the level of polymorphonuclear leukocytes at 10.00 decreased (Fig. 1, *a*, *b*). The content of lymphocytes in the thymus increased at 10.00 and 14.00 and the percentage of blasts decreased at 14.00. An increase of the spleen weight and weight index were noted at 10.00, 15.00, 19.00, total splenocyte count and lymphocyte count in the spleen increased at 10.00 and 15.00, that of blasts at 10.00, 15.00, and 19.00, while the percentage of lymphocytes decreased at 10.00 and 19.00 and the percentage of blasts increased at 10.00. The pattern of circadian variations in the studied parameters was changed (Fig. 1).

Constant illumination modified the subpopulation composition of cells in lymphoid organs (Figs. 1, 2). In the thymus the content of mature $CD3^+25^-$ T-cells, $CD3^-25^+$ prethymocytes, and $CD25^+$ cells with IL-2 receptor decreased ($p<0.05$ for all parameters), which can be regarded as suppression of differentiation and activation processes. In the spleen the content of $CD4^+$ T-helpers increased (30.5±0.8 *vs.* 22.4±1.15 in intact mice, $p<0.01$), the $CD4^+/CD8^+$ ratio (1.79±0.09 and 1.39±0.11, respectively, $p=0.02$) also increased. Presumably, these changes reflect the compensatory reactions of peripheral lymphoid organs to suppression of the central immune functions.

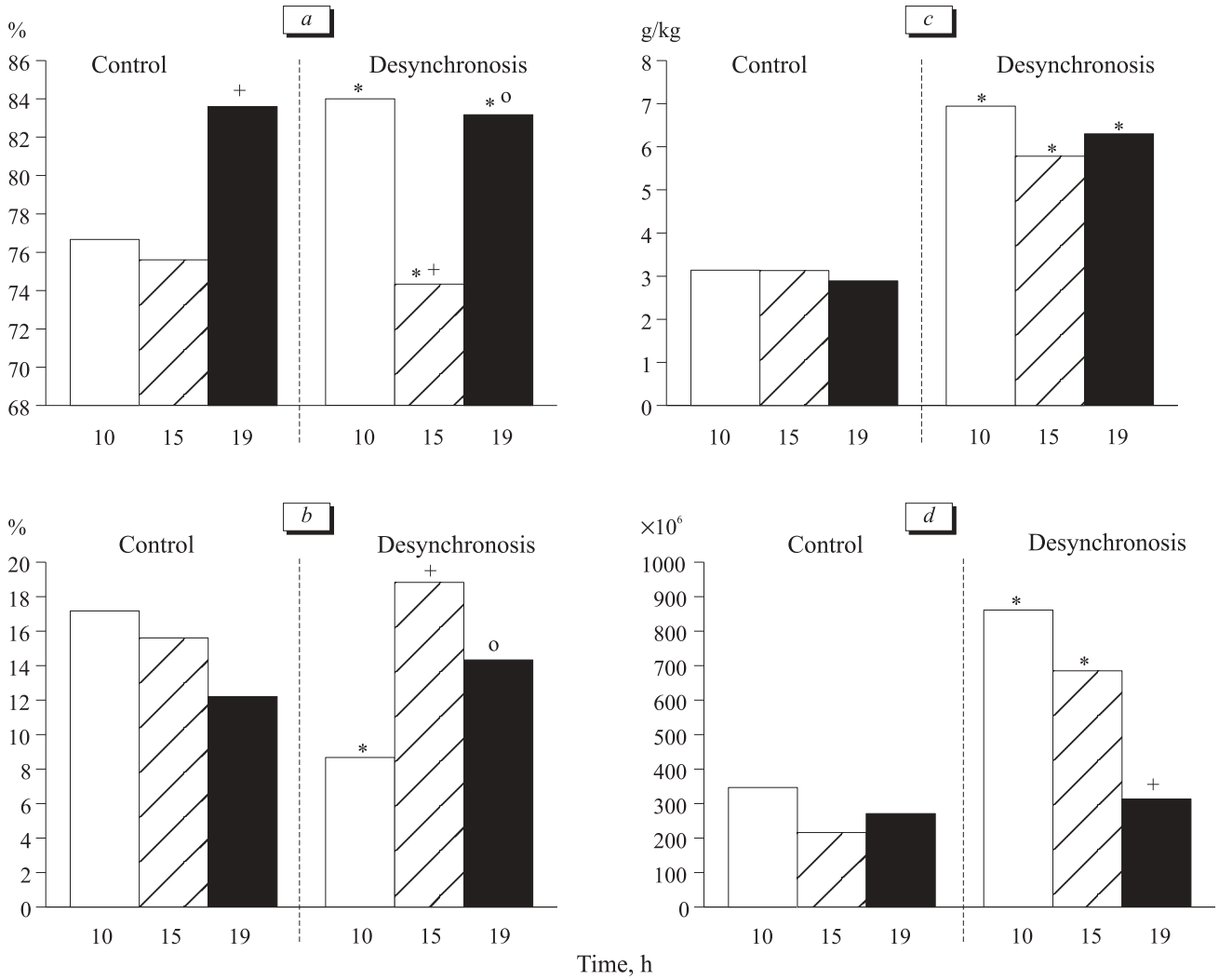


**Fig. 1.** Effect of constant illumination on circadian variations in immune system parameters in (CBA×C57Bl)$F_1$ mice. *a*) percentage of blood lymphocytes; *b*) percentage of blood polymorphonuclear cells; *c*) splenic weight index. $p<0.5$: *compared to the corresponding control, +compared to 10.00, °compared to 15.00.

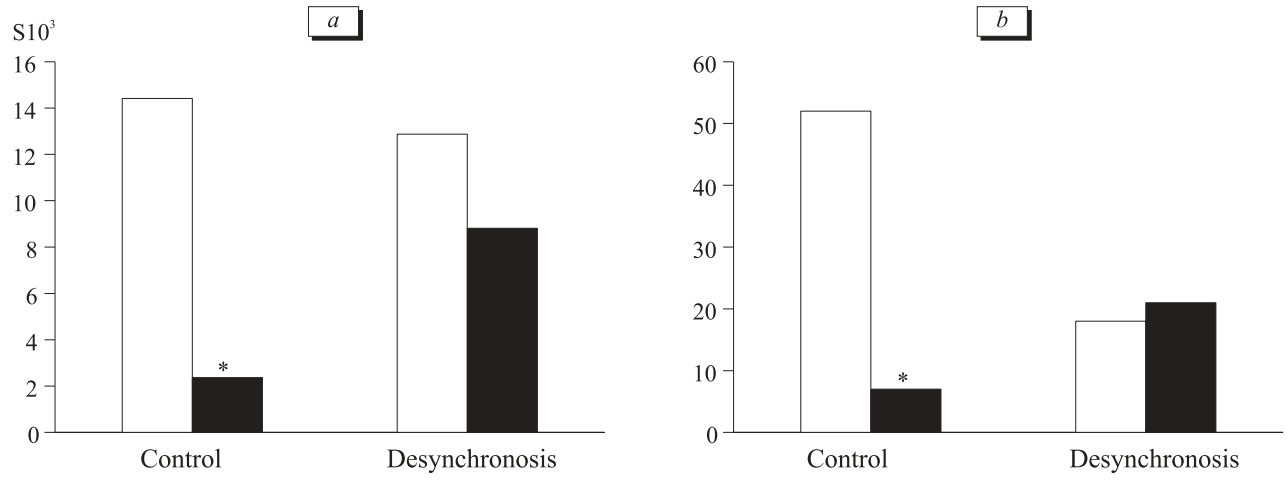


**Fig. 2.** Counts of antibody-producing cells in the spleen on day 4 after immunization of (CBA×C57Bl)$F_1$ mice (intact and exposed to permanent illumination) with sheep erythrocytes. *a*) total number; *b*) per $10^3$ splenocytes. Light bars: olive oil; dark bars: benz(a)pyrene. *$p<0.05$ compared to olive oil.

Hence, changes in the immune system parameters in experimental animals can indicate the development of internal DS and changes in the structural and functional parameters of the central and peripheral immune organs as a result of disordered day/night regimen.

The effect of BP on intact animals and animals with DS was different (Tables 1, 2). BP did not modify the percent ratio of blood cells in control mice, while in DS it decreased the level of lymphocytes and polymorphonuclear leukocytes. In the thymus (control group) BP reduced the percentage of T-helpers ($CD4^+$), increased the level of mature T-cells ($CD3^+25^-$), and decreased the level of prethymocytes ($CD3^-25^+$), which can be regarded as impairment of central T-cell differentiation with suppression of T-helper maturation. In animals with DS BP increased the level of mature T-cells ($CD3^+25^-$) more markedly than in the control and did not change the percentage of prethymocytes ($CD3^-25^+$). The increase of $CD25^+$ thymocyte level and of $CD4^+/CD8^+$ ratio can be regarded as direction of differentiation towards T-helper maturation, which can be a manifestation of the compensatory reaction.

BP had no effect on the percentage of $CD4^+$ and $CD8^+$ subpopulations in the spleen of control animals and reduced the weight of the organ; the percent of lymphocyte tended to decrease. In DS BP decreased the level of T-helpers, total counts of splenocytes, lymphocytes, and blasts. In controls BP decreased the percentage of blasts in mesenteric lymph nodes, while in DS just a trend to their decrease was observed (Tables 1, 2).

**TABLE 1.** Effect of BP on Immune System Parameters of Intact Mice (*M*±*m*)

| Parameter | | Intact control | OO injection | BP injection |
|---|---|---|---|---|
| Thymus weight, mg | | 33.00±2.22 | 31.60±3.63 | 25.67±2.09* |
| Thymus: | blasts, % | 26.90±0.84 | 19.50±6.81 | 16.70±3.13* |
| | $CD3^-25^+$, % | 0.64±0.09 | 1.04±0.07* | 0.53±0.13$^+$ |
| | $CD3^+25^-$, % | 62.2±0.7 | 63.7±7.7 | 76.1±5.2* |
| | $CD3^+$, % | 63.90±0.64 | 66.02±3.80 | 78.45±5.40* |
| | $CD3^{+hi}$, % | 13.7±1.7 | 22.7±6.9 | 39.4±3.6* |
| | $CD4^+$, % | 93.2±0.1 | 89.9±1.3 | 86.3±1.1*$^+$ |
| | $CD8^+$, % | 87.90±1.46 | 76.12±4.06* | 65.90±2.07* |
| | $CD4^+/CD8^+$ | 1.06±0.02 | 1.19±0.06* | 1.31±0.03* |
| Spleen weight, mg | | 84.50±3.62 | 84.40±4.37 | 73.17±2.72*$^+$ |
| Splenic lymphocytes, % | | 73.50±1.47 | 64.62±3.12* | 61.80±7.13 |
| Lymph node blasts, % | | 29.9±3.17 | 14.06±3.13* | 16.23±2.35* |

**Note.** $p<0.05$ *compared to intact control; $^+$compared to OO.

**TABLE 2.** Effect of BP on Immune System Parameters in Mice with DS (*M*±*m*)

| Parameter | | DS | DS+OO | DS+BP |
|---|---|---|---|---|
| Blood: | lymphocytes, % | 84.00±1.12 | 80.17±3.20 | 73.0±3.5* |
| | polymorphonuclear leukocytes, % | 8.7±0.7 | 15.00±2.56* | 22.5±2.9* |
| Thymus: | lymphocytes, % | 72.5±2.7 | 56.1±2.5* | 66.7±3.8$^{+}$ |
| | $CD3^{-}25^{+}$, % | 0.34±0.07 | 0.35±0.06 | 0.58±0.18 |
| | $CD3^{+}25^{-}$, % | 57.8±1.3 | 56.3±3.6 | 73.3±5.1*$^{+}$ |
| | $CD3^{+}25^{+}$, % | 1.4±0.2 | 1.05±0.20 | 2.48±0.5*$^{+}$ |
| | $CD25^{+}$, % | 1.7±0.2 | 1.4±0.2 | 3.05±0.40*$^{+}$ |
| | $CD3^{+}$, % | 61.2±1.9 | 57.3±3.8 | 75.80±5.45$^{+}$* |
| | $CD3^{+hi}$, % | 13.2±0.5 | 15.60±0.34* | 58.05±8.20*$^{+}$ |
| | $CD8^{+}$, % | 86.2±0.6 | 85.0±0.6 | 48.30±7.02*$^{+}$ |
| | $CD4^{+}/CD8^{+}$ | 1.08±0.01 | 1.07±0.04 | 1.84±0.26*$^{+}$ |
| Splenocyte count, $\times 10^6$ | | 861.5±88.9 | 840.0±114.6 | 248.03±52.20*$^{+}$ |
| Spleen: | lymphocytes, $\times 10^6$ | 583.0±60.0 | 565.4±85.2 | 159.4±33.0*$^{+}$ |
| | blasts, $\times 10^6$ | 239.6±29.4 | 138.4±45.5 | 45.5±13.2*$^{+}$ |
| | $CD4^{+}$, % | 30.5±0.8 | 31.7±1.7* | 28.1±0.5$^{+}$ |
| Lymph node blasts, % | | 30.9±1.97 | 16.7±2.3* | 25.1±4.3 |

**Note.** *$p<0.05$ compared to DS; $^{+}p<0.05$ compared to DS+OO.

BP suppressed humoral immune response to sheep erythrocytes in intact mice and virtually did not influence this parameter in mice with DS, though a trend to a decrease could be seen (Fig. 2). Presumably, different effects of BP on the immune system of intact mice and animals with DS was due to differences in the circadian rhythms of their immune parameters. It seems that controls and animals with DS were exposed to BP when they had different ratios and functions of immunocompetent cells.

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