GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Pathophysiological Effects of Psychoemotional Stress and Cyclophosphamide

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Effects arising in mice acutely or chronically exposed to cyclophosphamide, psychoemotional stress, or both are described. These factors produce similar physiological effects which become synergistic when they act jointly.

Key Words: *psychoemotional stress; cyclophosphamide; combined effects*

All living organisms on the Earth are constantly exposed to physical, chemical, and biological factors or agents whose effects are often impossible to differentiate. Hence, models for the investigation of the effects of individual agents and two or more factors acting jointly are necessary.

Stress manifests itself as a general (nonspecific) response of the body to various environmental stimuli [4]. For mammals, including man, the most common is psychoemotional stress (PES). While pathophysiological consequences of acute and chronic PES have been investigated in detail, little is known about the dynamics of changes occurring in the body during chronic stress, particularly when PES is combined with chemical mutagens.

Our objective was to evaluate temporal variations of the combined effects of PES and the standard chemical mutagen cyclophosphamide (CP) in mice. The results obtained in this study provide the basis for comparing such effects with genetic and biochemical changes observed in the same model [1].

MATERIALS AND METHODS

Four groups of male (CBA×C57Bl/6) F_1 mice were used. Mice of the first group were exposed to PES by being immobilized for 4 h daily over a period of 20 days [3] and sacrificed on days 1, 5, 10, 15, 20, and 25 after the start of the experiment (i.e., after the first immobilization session). Mice of the second group were immobilized and given an intramuscular injection of CP (10 mg/kg) 24 h before sacrifice on the indicated days. Group 3 mice were treated with CP but were not exposed to PES. The fourth group consisted of intact mice and served as the control.

The severity of PES was evaluated by changes in the morphology of the gastrointestinal tract and adrenals, in the weight of thymus and adrenals, and in the differential white blood cell count. Two replicate experiments were carried out. The data for the test groups were compared with the respective data for the control group. The results were statistically analyzed by Student's t test.

RESULTS

Figure 1 shows temporal variations in the weigh of thymus and adrenals. The weight of these organs varied more or less concurrently in each group, and its deviation from the control was the highest on days 10 and 15. Histological studies revealed cicatrizing ulcers in stressed and CP-treated mice on day 10 and secondary ulcers on day 15; the main histological findings on days 20 and 25 were atrophic changes in

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the gastrointestinal mucosa and sclerotic changes in the gastric tissues, respectively.

Pronounced eosinophilia (an indicator of allergic reaction) was observed in mice exposed only to one immobilization session (the eosinophil count was 2 times as that in the controls); in CP-treated mice the release of juvenile cells and reduction in the eosinophil count were observed (Fig. 2). On day 5, the eosinophil count was at the control level in both groups. On day 10, pronounced monocytosis and a slight increase in the band cell count occurred in both groups. On day 15, the band cell count in stressed mice was twice as that as in the controls. On day 20, it was the same as in the control. Differential white blood cell counts in CP-treated mice on days 15, 20, and 25 were near-normal. In general, the patterns of differential cell counts in the CP-treated and stressed mice were similar (Fig. 2, *a*, *b*).

Mice exposed to both PES and CP developed gastric ulcers on days 5-10, while cicatrizing ulcers appeared later (on days 15-20) than in the stressed and CP-treated mice (Fig. 1).

In stressed mice treated with CP (Fig. 2), eosinophilia and basophilia were observed on day 1 but then they disappeared; the band cell count was high on day 10, decreasing dramatically by the 15th day. The monocyte count was high from day 5, reaching the maximum on day 10. Five days after the end of experiments, i.e., on day 25, cell counts were near their control values; however, eosinophilia suggested that the animals had developed an allergic reaction. Only the monocyte count correlated with histological data and changes in the weight of thymus and adrenal glands, which are generally accepted physiological indicators of stress severity.

Changes occurring in the weight of thymus and adrenals and histological evidence indicate that 1) chronic exposure to CP induces a stress reaction (or another reaction with similar manifestations) by day 5, and 2) reparative processes proceed in mice during the period when they are still exposed to stress or CP and 3) during at least 5 days after exposure to stress or CP had been discontinued.

Therefore, it can be concluded that changes induced by PES did not differ considerably from those induced by CP within a 25-day observation period. It should be noted that these changes were reversible, i.e., the state of strain described by Selye [4] was best defined on day 15.

Acute and chronic exposure of mice to the chemical mutagen CP (an antineoplastic drug) caused pathomorphological changes similar to those characteristic of PES. A similar situation was observed by Selye when he studied pathomorphological effects of formaldehyde solution in rats [4].

In mice exposed to both PES and CP, histological changes and those in the weight of thymus and adrenals slightly differed from those observed in mice exposed to PES and CP separately (Figs. 1 and 2).

It was logical to suppose that the time-course of the processes by which cytogenetic lesions arise and mutant cells are eliminated should correlate in all groups of mice with the parameters of the Selye triad (Figs. 1 and 2). We anticipated that the level of damage sustained by the genetic apparatus would increase from day 1 to day 15 as the exposure to PES, CP, or both was continued. The increase in thymus and adrenal weight suggested gradual normalization of all genetic parameters until the end of the experiment.

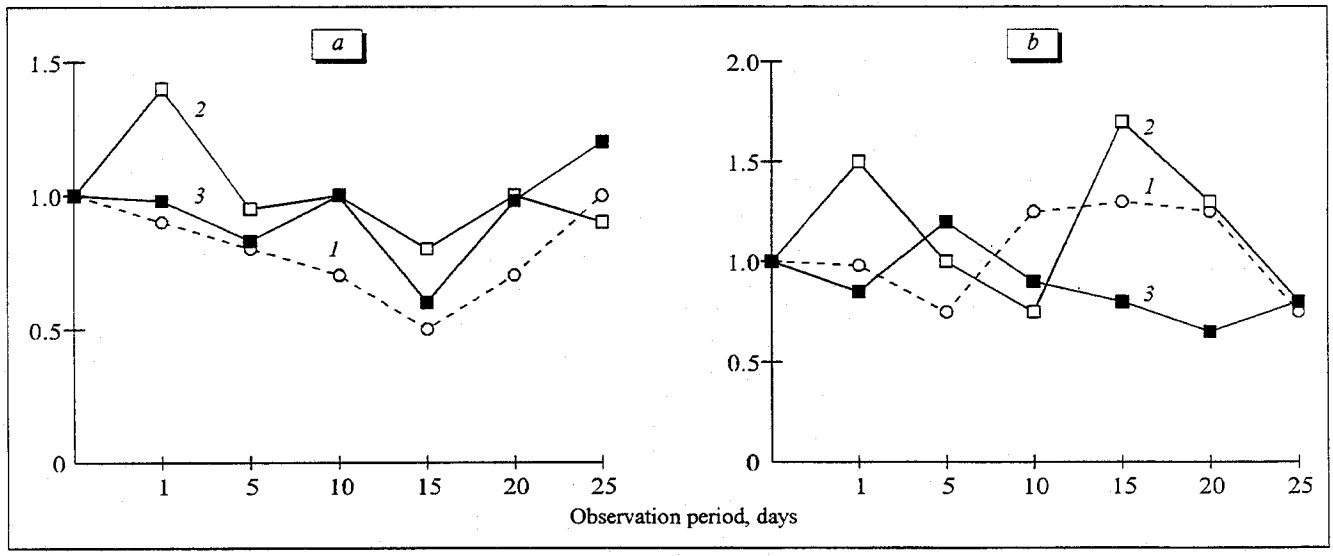

Fig. 1. Weight of thymus (*a*) and adrenals (*b*) in stressed (*1*), cyclophosphamide-treated (*2*), and stressed+cyclophosphamide-treated (*3*) mice. Ordinates: number of times the control level is exceeded.

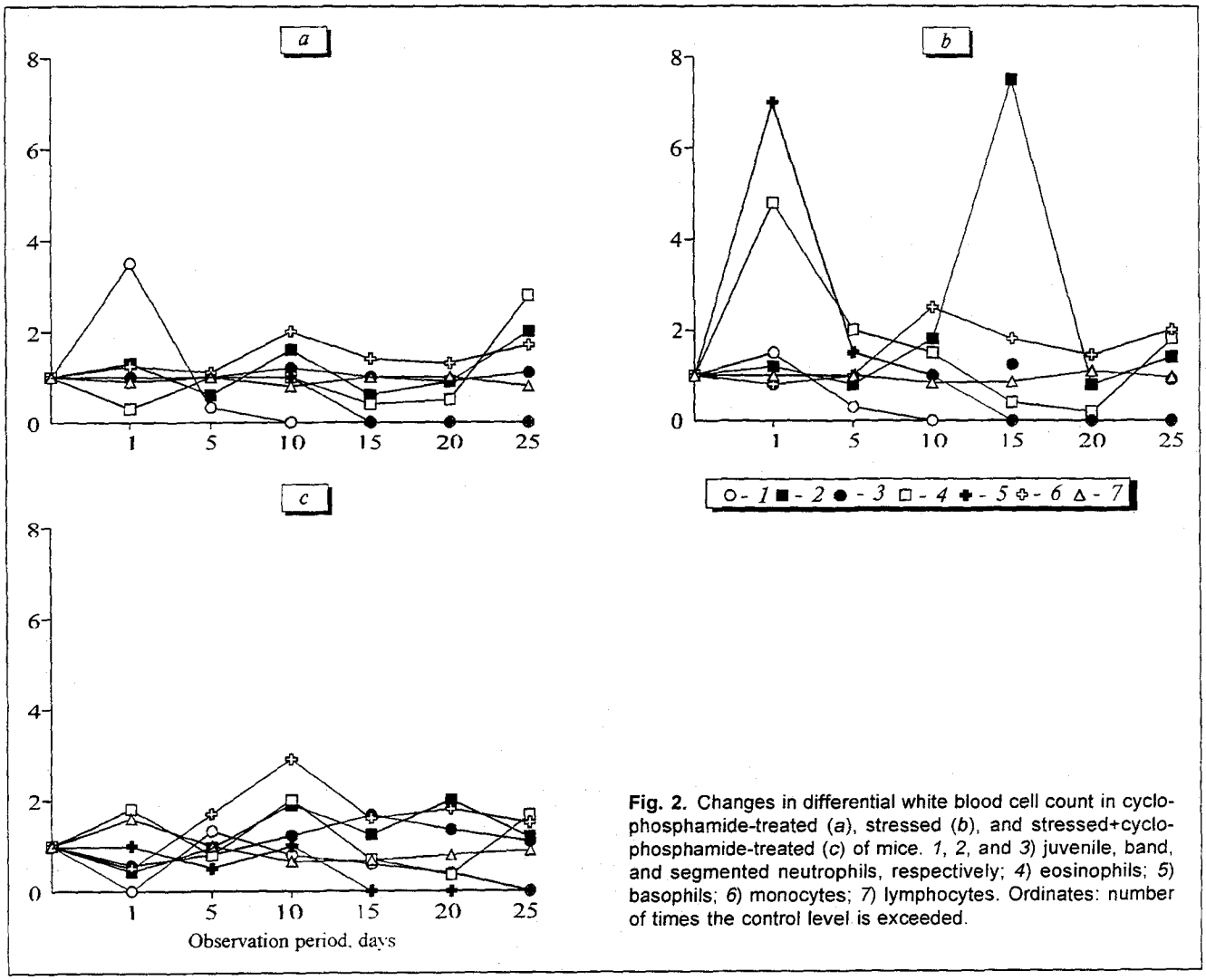

Fig. 2. Changes in differential white blood cell count in cyclophosphamide-treated (*a*), stressed (*b*), and stressed+cyclophosphamide-treated (*c*) of mice. *1*, *2*, and *3*) juvenile, band, and segmented neutrophils, respectively; *4*) eosinophils; *5*) basophils; *6*) monocytes; *7*) lymphocytes. Ordinates: number of times the control level is exceeded.

However, our previous studies showed that [1,2] changes in genetic parameters did not correlate with those in the parameters of the Selye triad. Moreover, we have found that regardless of the damaging factor the curves describing temporal variations in cytogenetic and biochemical parameters usually had more than one extremum with values for some neighboring points differing by a factor of 20.

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