Immunomodulating Effect of Salmozan Injected at Different Times of the Day

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Immunomodulating effect of salmozan injected at different times of the day was assessed by changes in 5-nucleotidase activity of mouse peritoneal macrophages. The effect of the immunomodulator correlates with the level of 5-nucleotidase activity in control animals. When this value was high in the control, it was decreased in experimental rats, and vice versa. The effect of the immunomodulator on the enzyme activity and on immune and neuroendocrine parameters is governed by the "mirror symmetry" principle.

Key Words: *immunomodulating effect; mirror symmetry principle; 5-nucleotidase; salmozan*

Recent publications report a relationship between immunomodulating effect and time of immunomodulator administration [2,3]. We studied immunomodulating effect of salmozan administered at different times of the day. Salmozan, a polysaccharide of *Salmonella typhi* O-somatic antigen, exhibits high immunomodulating activity. The drug efficacy was assessed by changes in the activity of 5-nucleotidase (5-N) of mouse peritoneal macrophages (PM) [6].

MATERIALS AND METHODS

Experiments were carried out in winter on male BALB/c mice weighing 16-18 g from *Stolbovaya* Breeding Center (Russian Academy of Medical Sciences). Salmozan was injected subcutaneously in a single dose of 100 μg/mouse at different times of the day. In group 1 the drug was injected at 12:00, in groups 2-4 at 18:00, 24:00, and 6:00, respectively. Time course of 5-N activity was followed up in each group: the enzyme activity was measured 6, 12, 18, and 24 h after the drug administration [7]. The activity of 5-N was expressed in arbitrary units (multiscan data×100 per 10^6 cells).

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RESULTS

Changes in 5-N activity were phasic (wave-like) in all groups (Fig. 1, *a*). The profiles of curves reflecting changes in enzymatic activity of PM varied in different groups. In group 1, the maximum decrease in 5-N activity was observed only 18 h after injection, in group 2, 12 h postinjection, and in group 3, 6 h after administration of the immunomodulator.

Comparison of the time course of 5-N activity in experimental animals and controls (Fig. 1, *b*) shows that this metabolic parameter in experimental animals is a mirror reflection of the enzyme activity in the controls.

Analysis of our results showed that changes in 5-N activity in experimental group for a certain period correlate with the activity of this enzyme in control animals during the same period. When 5-N activity was high in the controls, it was low in experimental group. When the activity of 5-N was low in the control, it was high in experimental animals.

These results (mirror experimental *vs.* control curves) permit us to hypothesize that this relationship is the main factor, determining the direction and expression of the studied parameter in experimental group. If so, comparison of different experimental groups will show that the differences between them

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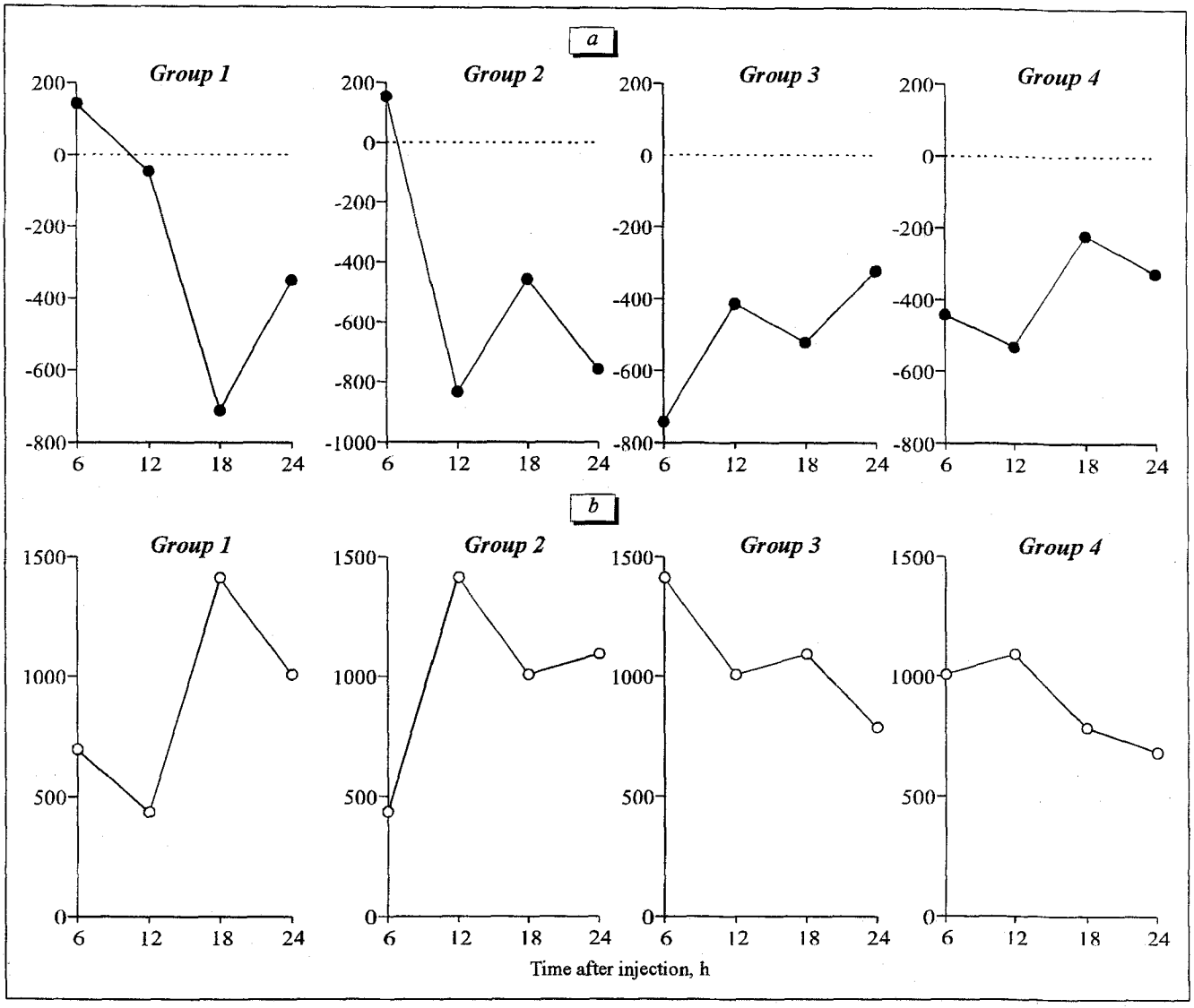

Fig. 1. Time course of changes in the activity of 5-nucleotidase of peritoneal macrophages in experimental (*a*) and control (*b*) BALB/c mice after injection of salmozan at different times of the day. Here and on Fig. 2: ordinate: *a*) activity, arb. units; *b*) difference between 5-nucleotidase activity in experimental and control animals.

for each period are determined by the difference in the level of the studied parameter in the reference groups. Six reference groups have been distinguished. Reference group I was compared with experimental groups 1 and 2, reference group II with experimental groups 1 and 3, reference group III with experimental groups 1 and 4, reference group IV with experimental groups 2 and 3, reference group V with experimental groups 2 and 4, and reference group VI with experimental groups 3 and 4 (Fig. 2). Figure 3 shows that the curve representing changes in experimental Δ is the mirror reflection of control Δ curve. The maximum difference in the enzyme activity in the controls (reference groups I and III) is paralleled by the maximum difference in experimental groups. Minimal differences in the controls are paralleled by minimal differences in experimental groups (reference group VI).

Thus, the effect of immunomodulator on the activity of 5-N depends on and is determined by the activity of this enzyme in the control at the moment of investigation. When the value is high in the control groups, in the experimental groups this parameter decreases, when the enzyme activity is low in the control — it increases in the experiment.

The effect of the immunomodulator on the activity of 5-N is determined by the principle of mirror symmetry. The gist of this principle is as follows: the direction and intensity of immunomodulating action on the studied parameter is inversely related to the level of this parameter in control animals at the moment of investigation.

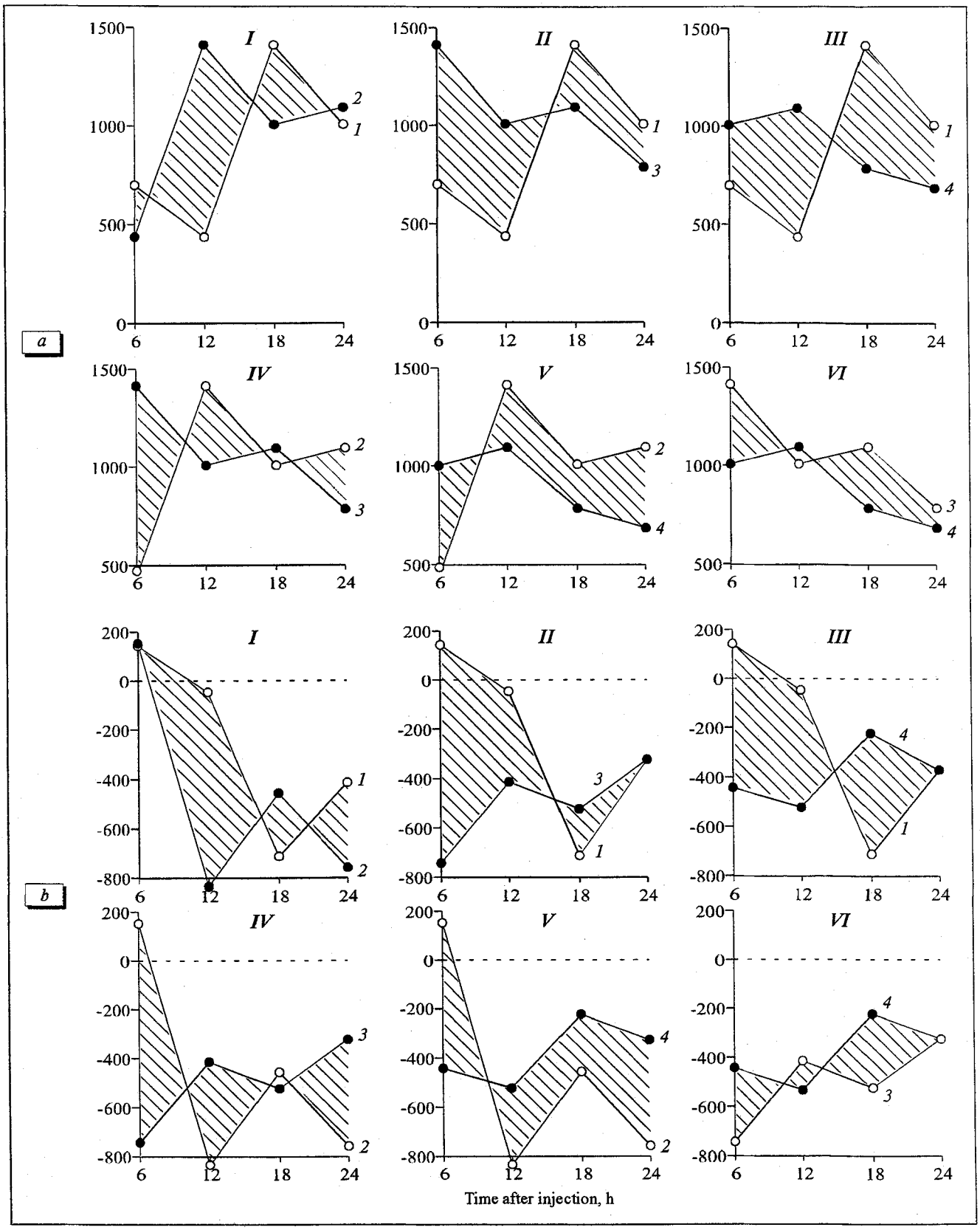

Fig. 2. Activity of 5-nucleotidase of peritoneal macrophages in control (*a*) and experimental (*b*) BALB/c mice after injection of salmozan in groups differing by the time of drug injection. *I-VI*) reference groups; *1-4*) experimental groups. Cross-hatched area: difference between the compared groups (Δ).

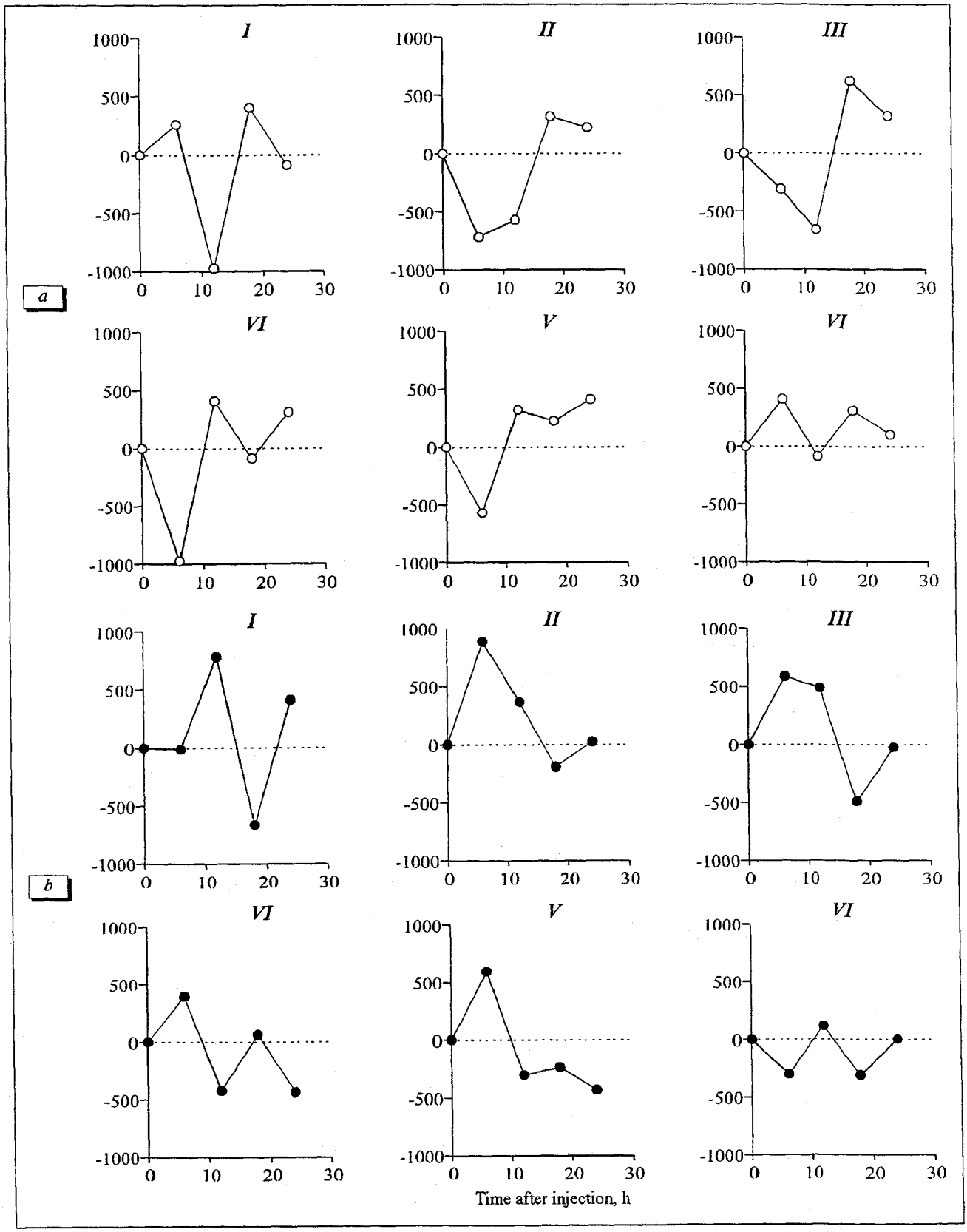

Fig. 3. Changes in the activity of 5-nucleotidase of peritoneal macrophages in control (*a*) and experimental (*b*) mice. Ordinate: activity, arb. units; *I-VI*: reference groups.

Previously we showed that during immunomodulating exposures, changes of PM 5-N activity correlate with the resistance to bacterial infections, toxic effects, level of spontaneous blasttransformation of splenic lymphocytes, blood hydrocortisone, and other parameters of immunoneuroendocrine system [4,5]. Study of the effects of immunomodulators on these parameters shows that their effects on various components of the immune and neuroendocrine systems depend on the levels of the studied parameter in control animals at the moment of investigation and are governed by the mirror symmetry principle.

Thus, the parameters of immune and neuroendocrine systems of control animals at the moment of investigation are among the main factors determining the immunomodulator effect on this or that parameter of the system. Biorhythmological fluctuations of the studied parameter in control animals should by no means be neglected when assessing the immunomodulating effect. Analysis of the activity of a parameter in control mice at the moment of investigation will show a new aspect in the interstrain differences of immunomodulating effect and will help understand the relationship between immunomodulator effect and time of the day when the exposure took place. This parameter should be taken into consideration when studying the immunomodulating effect of the drug in time and in any comparative experiments.

Our results prove that time-related modulations of biological processes (biorhythms) and reactions of the organism to any exposure, including immunomodulating, are closely related to each other, because they are components of the same process: functioning of the organism.

The mirror symmetry principle sufficiently well fits into modern concepts about "symmetry as the fundamental regularity of nature and at the same time, a principle for cognition of the properties and laws of the world of live and inorganic substances surrounding us" [1].

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