ary phenomenon related to an unknown biochemical defect. All these results, though referring to just solitary cases, should be taken into consideration because samples for investigation from human subjects are not easily available. There are virtually no data on the number of collagen hydroxypyridine cross-links in human costal cartilage in health and disease. Analysis of hydroxypyridine cross-links in costal cartilage samples may help understand the etiopathogenesis of the disorders observed in connective-tissue diseases complicated by thoracic deformations. According to published data, thoracic deformations are not a characteristic sign of the majority of EDS types, but analysis of a sample of patients hospitalized in the Chest Department has led us to the conclusion that EDS is often associated with complications and appears to contribute to PE/PC formation. The diverse patterns of changes in the content and spectrum of collagen cross-links in patients with EDS and MS most likely confirm the genetic heterogeneity of these nosological entities.

REFERENCES

1. D. Amiel, F. L. Harwood, M. F. Abel, and W. H. Akeson, *Collagen Relat. Res.*, **5**, 337-347 (1985).
2. D. Black, A. Duncan, and S. P. Robins, *Analyt. Biochem.*, **169**, 197-205 (1988).
3. P. H. Byers, *The Metabolic Basis of Inherited Disease*, Eds. C. R. Ceriver *et al.*, 6th Ed., New York (1989), pp. 2805-2842.
4. Z. Deyl and M. Adam, *J. Chromatogr.*, **488**, 161-197 (1989).
5. D. R. Eyre, M. A. Pas, and P. M. Gallop, *Ann. Rev. Biochem.*, **137**, 380-388 (1984).
6. D. R. Eyre, T. J. Koob, and K. P. Van Ness, *Analyt. Biochem.*, **137**, 380-388 (1984).
7. D. R. Eyre, *Meth. Enzymol.*, **144**, 115-139 (1987).
8. D. R. Eyre, J. R. Dickson, and K. Van Ness, *Biochem. J.*, **252**, 495-500 (1988).
9. D. Fujimoto, *Biochem. Biophys. Res. Commun.*, **76**, 1124-1129 (1977).
10. I. T. James, D. Perrett, and P. W. Thompson, *J. Chromatogr. Biomed. Appl.*, **525**, 43-59 (1990).
11. J. A. Last, L. C. Armstrong, and K. M. Reiser, *Int. J. Biochem.*, **22**, 559-564 (1990).
12. S. Ricard-Blum and G. Ville, *Cell.Molec.Biol.*, **34**, 581-590 (1988).
13. E. E. Safronova, N. V. Borisova, S. V. Mezentseva, and K. D. Krasnopol'skaya, *Biomed. Sci.*, **2**, 162-168 (1991).

EXPERIMENTAL BIOLOGY

Effect of T-activin on Macrophage 5-Nucleotidase Activity and Blood Cortisol Level as a Function of the Time of Day

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The long-standing investigation of immunomodulators of various chemical structure and origin has revealed that the reaction of the organism to an immunomodulatory influence involves complex relationships between phagocytosing cells and the neuroendocrine component of the regulation of immunity, in particular, between ecto-5-nucleotidase (5-n) activity in peritoneal exudate macrophages (PEM) and cortisol level [3,5].

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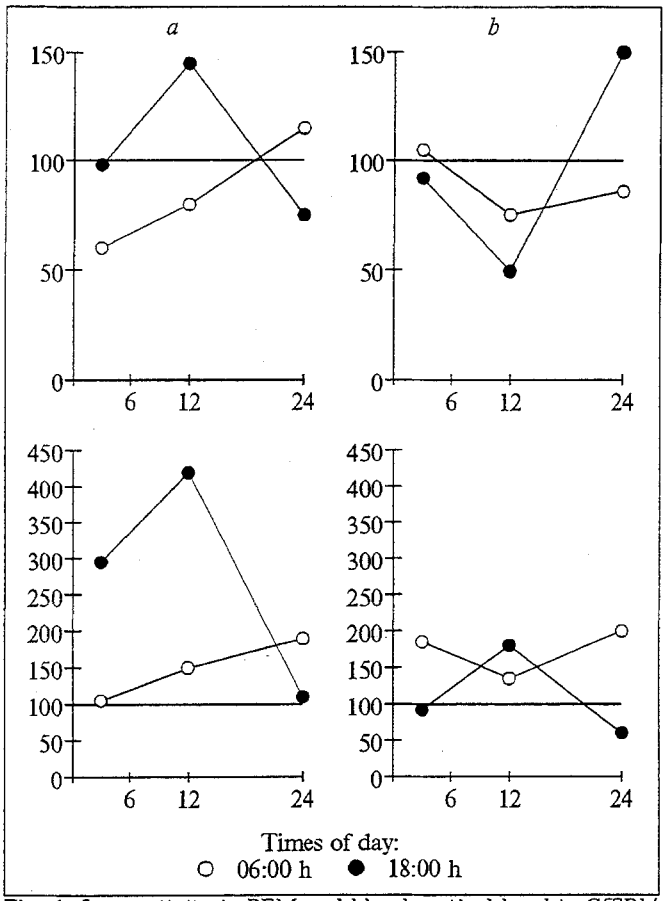

Fig. 1. 5-n activity in PEM and blood cortisol level in C57Bl/6 (*a*) and CBA (*b*) mice after T-activin injection in a dose of 1 μg at different times of the day. Abscissa: time of observation, hours; ordinate: 5-n activity, % of control, and cortisol level (K), % of control.

In the present study peculiarities of the effect of T-activin on 5-n activity in PEM and the endogenous cortisol level depending on the time of day have been investigated.

MATERIALS AND METHODS

Male mise of the CBA and C57Bl/6 strains were obtained from the Stolbovaya Laboratory Animals Nursery and kept under standard light, temperature, and chow regimes. The animals were allowed to acclimatize for at least two weeks prior to use. The experiments were carried out in the fall. The mice were injected subcutaneously with T-activin in doses of 10^{-6}, 10^{-3}, and 1 μg/mouse at 06:00-18:00 h. The 5-n activity in PEM [7] and the endogenous cortisol level in the serum [8] were determined 3, 12, and 24 hours postinjection.

RESULTS

Figure 1 shows the dynamics of 5-n in PEM and the blood cortisol level in CBA and C57Bl/6 mice during 24 hours after subcutaneuos injection of T-activin in a dose of 1 mg at 06:00 and at 18:00 h. As seen from the figure, T-activin administration induced changes in both indexes depending to a great extent on the time of injection. When the changes of 5-n activity in PEM were compared in the two strains of mice, the enzyme activity in CBA mice was found to be a mirror image of that in C57Bl/6 mice in both morning and evening.

A comparison of the dynamics of 5-n activity and blood cortisol level showed that in C57Bl/6 mice the changes in 5-n activity followed in general outline the cortisol level. In CBA mice injected with T-activin in the morning a positive correlation between 5-n activity and cortisol level was observed, similar to that in C57Bl/6 mice. In contrast, when T-activin was injected in the evening, the changes in blood cortisol level were a mirror image of the dynamics of 5-n activity.

Thus, in this study a correlation has been established between 5-n activity in PEM and the blood cortisol level. In C57Bl/6 mice we observed a direct correlation, whereas in CBA mice this relationship depended on the time of injection, being positive in the case of the morning administration and negative for the evening administration.

The data presented in Fig. 2 show the dose dependence of the parameters studied 12 and 24 hours after T-activin administration to CBA mice, suggesting a correlation between the macrophagal and hormonal indexes. For example, 12 hours after the morning injection of the preparation the dose-effect curve for 5-n activity followed in general features the curve for the cortisol dynamics, whereas after the evening injection the curves were mirror images of each other.

From the dose-effect curves of the parameters studied 24 hours after T-activin administration it follows that the 5-n-cortisol relationship in CBA mice depends on not only the time of injection but on the time interval after it as well. Thus, one day after injection the dose-effect curve for 5-n was the mirror image of that for the cortisol dynamics, i.e., in contrast to the 12-hour period, the dependence became negative in character.

Previously we found a correlation between 5-n of PEM and the blood cortisol level under the influence of the bacterial immunomodulators salmosan and staphilococcal enterotoxin A [5].

Just as with the T-activin administration, marked differences were established between the strains in the nature of the changes of the two indexes. However, in contrast to the present results, a negative 5-n - cortisol correlation was observed in C57Bl/6 mice. The above differences in the character of the correlation under the influence of the bacterial prepara-

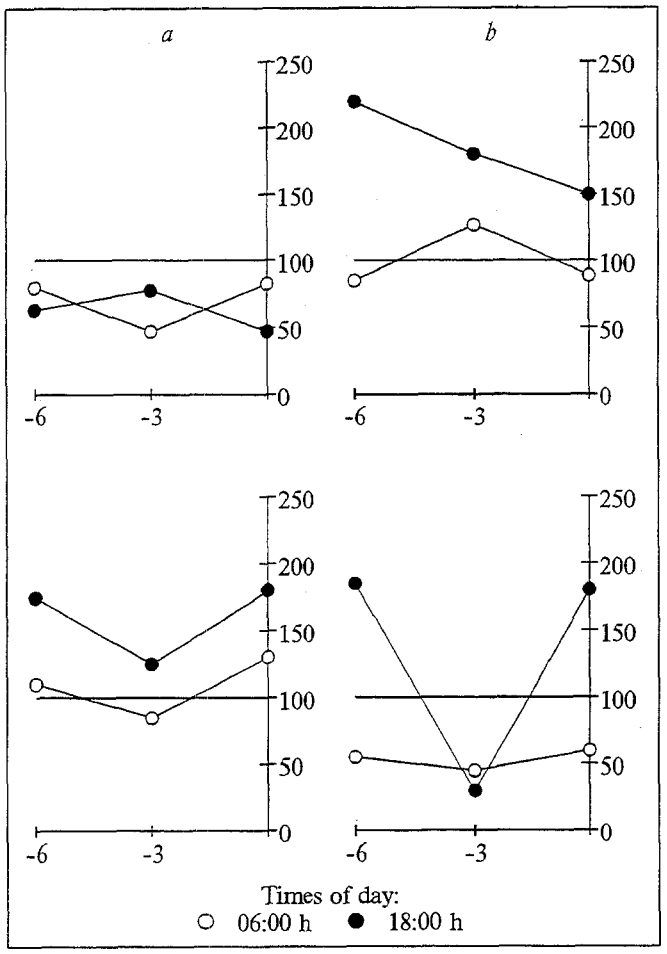

Fig. 2. 5-n activity in PEM and blood cortisol level in CBA mice after T-activin injection at different times of the day. *a*) after 12 hours, *b*) after 24 hours. Abscissa: log of dose; ordinate: 5-n activity (% from control) and cortisol level (% of control).

tion and T-activin are probably to be attributed to the different type of immunomodulator used, as well as on peculiarities of its effect on the organism. It cannot be ruled out that the time of administration also plays a role: salmosan and staphilococcal enterotoxin A were injected at 09:00 h.

The present data, taken together with previous results, suggest that the immunomodulatory influence is characterized by the appearance of a correlation between 5-n in PEM and the blood cortisol level. It should be noted that the nature of this correlation is not absolute but depends on genotypic pecularities of the organism, the time of day when the preparation is used, and the period of investigation.

Thus, T-activin has been found to have a considerable effect on 5-n activity in PEM and the blood cortisol level in a broad range of doses from 10^{-6} to 1 μg. Interstrain differences were observed in the effect of T-activin on both the macrophagal and endocrine indexes. It was shown that the effect of the immunomodulator on the parameters studied and even on the correlation relationships between them can be reversed to diametrically opposite just by varying the time of injection.

The strain-dependent distinctions in T-activin effect on phagocytosing cells and the hormone status in inbred animals provide an adequate model of genotypically determined individual variants of reactivity in the higher animals, including man. This implies that several variants of T-activin influence on the parameters studied in human beings are possible. The dependence of the effect of T-activin on the circadian biological rhythms increases the likelihood of considerable variability in its influence on the activity of 5-n in PEM and the blood cortisol level all the way to a completely opposite effect in different persons.

The activity of 5-n is known to be a factor in the resistance of the organism to infection. Correlations between 5-n in PEM and a number of immunological indexes (lymphocyte blast transformation test and others) under immunomodulatory influences have also been established [2,4,6]. The direction and expression of the immunomodulatory effect can be judged by changes in 5-n activity [7].

Moreover, there is a large body of evidence concerning the extremely important role of glucocorticoids in the mechanisms of immunoresistance [1]. Therefore, the results obtained on the effect of T-activin on 5-n activity and the blood cortislol level suggest a considerable variability in the immunomodulatory effect of T-activin, depending on genotypic pecularities of the organism, the phase of biological rhythms, and the time of investigation. Considering the broad clinical application of T-activin in a variety of pathological states, the latter circumstance should be taken into account when prescribing T-activin and when developing guidelines for its optimal usage.

REFERENCES

1. V. V. Abramov, *Immune and Nervous System Interaction* [in Russian], Novosibirsk (1988).
2. G. V. Kirillicheva, V. A. Bekhalo, M. S. Solov'eva, *et al., Immunomodulators in Infection Pathology* [in Russian], Moscow (1988), pp. 105-108.
3. G. V. Kirillicheva, V. V. Mit'kin, N. V. Bogomolova, *et al., Immune and Nervous System Interaction* [in Russian], Leningrad (1990), p. 36.
4. G. V. Kirillicheva, M. S. Solov'eva, E. V. Ermolova, *et al., I. I. Mechnikov All-Union Society of Epidemiologists, Microbiologists and Parasitologists : 17th Congress,* Alma-Ata (1989) Abstracts, p. 57.
5. G. V. Kirillicheva, V. V. Mit'kin, M. S. Solov'eva, *et al., Byull. Eksp. Biol.*, **112**, № 9, 280-282 (1991)
6. G. V. Kirillicheva, A. V. Pronin, N. P. Goncharov, *et al., First Congress of Russian Immunologists,* Novosibirsk (1992), Abstracts, p. 210.
7. M. A. Tumanyan and G. V. Kirillicheva, *Otkrytiya*, № 6, 621 (1986).
8. J. U. Eskola, V. Nanto, L. Meurling, *et al., Clin.Chem.*, **31**, 1731-1734 (1990).