To study the possible role of central cholinergic and adrenergic mechanisms in the action of the increased gravitational field on the hypophyseo-adrenal system the effectiveness of preliminary injection of the central muscarinic cholinolytic drug methyldiazine (10 mg/kg) or the adrenergic blocking drug chlorpromazine (10 mg/kg) was studied. Neither substance had any significant effect on the manifestation of the adrenocortical response to a single spinning (Fig. 2). Consequently, stimulation of the function of the hypothalamo-hypophyseo-adrenal system during a single exposure to radial acceleration evidently is not dependent on integrity of the central cholinergic or adrenergic mechanisms.

LITERATURE CITED

1. M. L. Belen'kii, Elements of Quantitative Evaluation of a Pharmacological Effect [in Russian], Leningrad (1963).
2. V. V. Parin, Selected Works, Vol. 2, Space Biology and Medicine. Cybernetics [in Russian], Moscow (1974).
3. V. E. Ryzhenkov, "The action of central neurotropic drugs on the pituitary–adrenocortical system in its various states," Author's Abstract of Doctoral Dissertation, Leningrad (1968).
4. N. A. Yudaev and S. A. Afinogenova, Probl. Éndokrinol., No. 2, 12 (1962).
5. N. A. Yudaev and Yu. A. Pankov, Probl. Éndokrinol., No. 2, 35 (1958).
6. K. Lissak and E. Endröczi, Neuroendocrine Regulation of Adaptive Activity [in Russian], Budapest (1967).
7. H. Selye, Essays on the Adaptation Syndrome [Russian translation], Moscow (1960).
8. G. Telegdy, E. Endröczi, and K. Lissak, Acta Physiol. Acad. Sci. Hung., 18, 211 (1960).

EFFECT OF CYTOSTATIC AGENTS ON DEVELOPMENT OF THE FEBRILE REACTION TO INJECTION OF BACTERIAL PYROGEN

A. V. Sorokin, O. M. Efremov, O. A. Él'kina, E. G. Rybakina, and I. K. Ukhanova

UDC 615.277.3.015.4:612.57+612.57.014.46:615.277.3

The development of the febrile reaction to injection of bacterial lipopolysaccharide (pyrogenal) in rabbits after preliminary treatment with actinomycin D and cortisone was studied. This treatment did not change the reactivity of the temperature regulating centers of the rabbits to endogenous pyrogen. After intravenous injection of the bacterial pyrogen the febrile reaction was considerably shortened, and after intracisternal injection of the pyrogen the reaction was sharply inhibited. These results indicate an important role of polymorphonuclear leukocytes and of endogenous pyrogen formation by these cells in the mechanism of fever in response to the action of bacterial pyrogen.

KEY WORDS: *Fever; pyrogens; inflammation; cytostatic agents.*

In the modern view the development of fever in various pathological conditions is due to endogenous pyrogens formed by the blood cells, mainly by polymorphonuclear leukocytes and monocytes. Endogenous pyrogens are considered to be natural and adequate stimuli for the temperature regulating centers, and the role of bacterial pyrogens is simply to stimulate the synthesis of endogenous pyrogens by the leukocytes [2, 4, 6, 10]. At the same time, it is known that a high fever can be observed in patients with agranulocytosis or severe granu-

Department of General Pathology, Institute of Experimental Medicine, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Veselkin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 11, pp. 1314-1317, November, 1976. Original article submitted April 2, 1976.

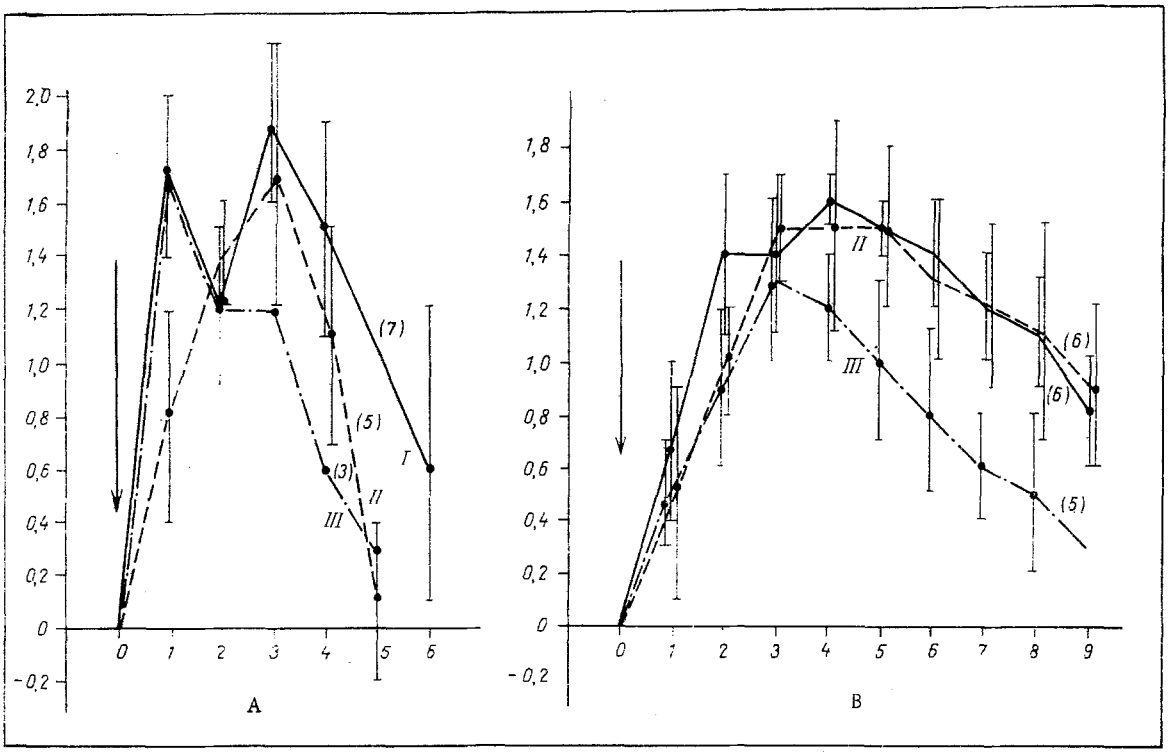

Fig. 1. Changes in body temperature of rabbits treated with actinomycin D or hydrocortisone after injection of pyrogenal. A) Intravenous injection in a dose of 5 MPD/kg body weight; B) intracisternal injection in a dose of 0.3 MPD per rabbit. Abscissa, time (in h); ordinate, increase in body temperature (in °C). Arrow marks time of injection. Vertical lines represent confidence limits. Number of animals shown in parentheses. I) Control; II) treatment with actinomycin D; III) treatment with hydrocortisone.

locytopenia and with depression of bone marrow function at certain stages of radiation sickness should infectious complications develop. The question of the possibility of other mechanisms of the pyrogenic action of bacterial pyrogens besides their stimulation of leukocytic pyrogen formation therefore requires study. It was decided to examine the development of fever in response to bacterial pyrogen when the functional activity of the leukocytes was depressed experimentally and inflammation inhibited. Data in the literature on this question are contradictory, possibly because highly toxic chloroethylamines were used to produce severe granulocytopenia [7, 8].

In this investigation the effect of some less toxic cytostatic drugs, cortisone and actinomycin D, on the febrile reaction to pyrogens was studied. Actinomycin D effectively inhibits the initial stages of endogenous pyrogen formation by blood cells in experiments *in vitro* [9].

EXPERIMENTAL METHOD

Experiments were carried out on 91 chinchilla rabbits weighing 2.5-3.5 kg of both sexes. Actinomycin D (Reanal, Hungary) was injected intravenously in single doses of 100 or 500 µg/kg or in a dose of 100 µg/kg daily for 4-5 days; hydrocortisone (Richter, Hungary) was injected intramuscularly in doses of 5 and 10 µg/kg twice or three times on alternate days. The blood leukocytes were counted in a Goryaev's chamber and the leukocyte formula determined in films stained by Romanovsky's method. Pyrogenal was injected intravenously in a dose of 5 minimal pyrogenic doses (MPD) per kilogram body weight and into the cisterna magna in a dose of 0.3 MPD. To do this, the tissues above the cisterna magna were punctured by means of a needle with a stilette at the edges of the external occipital protuberance under local anesthesia with 1% procaine (0.2-0.3 ml). Escape of cerebrospinal fluid from the needle after removal of the stilette indicated that the cisterna had been penetrated. The drugs were injected from a tuberculin syringe in a volume of 0.2 ml. In control experiments the intracisternal injection of 0.2 ml pyrogen-free 0.85% NaCl solution caused no ele-

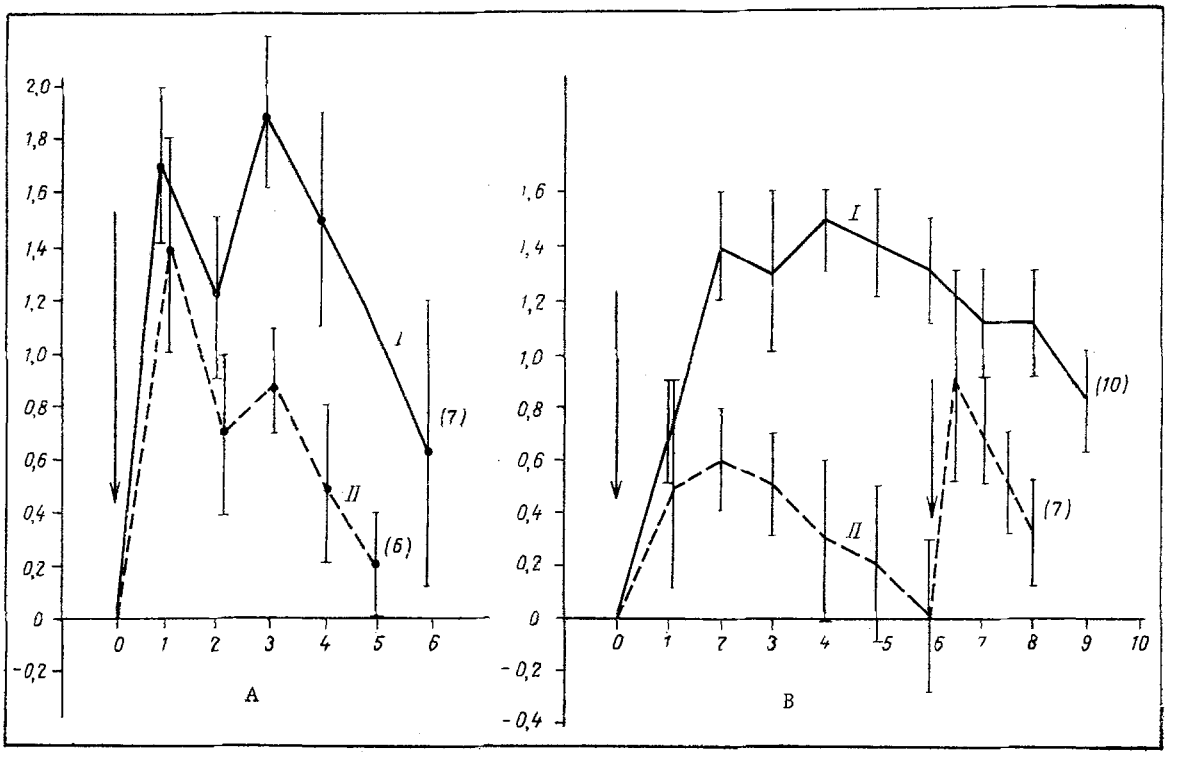

Fig. 2. Changes in body temperature of rabbits receiving combined treatment with actinomycin D and hydrocortisone, after injection of pyrogenal. Arrow indicates time of injection [in B: 1) pyrogenal; 2) leukocytic pyrogen intravenously]. I) Control; II) experiment. Remainder of legend as in Fig. 1.

vation of the body temperature. Leukocytic pyrogen was obtained by a modified method of Bennett and Beeson [3, 5].

The body temperature of the rabbits was measured in the rectum by means of a resistive electrothermometer twice or three times at intervals of 30 min to establish its initial level and at intervals of 30-60 min after injection of the drugs throughout the period of observation.

The glass ware, syringes, and needles were sterilized by dry heat at 170°C for 2 h. Solutions were made up in pyrogen-free 0.85% NaCl solution. The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

A single injection of actinomycin D in a dose of 100 µg/kg 30-120 min before intravenous and intracisternal injection of pyrogenal did not affect the character of the temperature reaction.

An increase in the dose of actinomycin D to 500 µg/kg had a marked toxic action on the animals and caused their death within 24 h. In the next group of experiments actinomycin D was accordingly injected in a dose of 100 µg/kg daily for 4-5 days. In this case, after intravenous injection of pyrogenal the first peak of the temperature reaction was somewhat reduced although the degree of the maximal rise and the duration of the response were indistinguishable from the control (Fig. 1). The character of the temperature reaction to intracisternal injection of pyrogenal was unchanged (Fig. 1).

Preliminary administration of hydrocortisone to the rabbits in two injections each of 30 mg shortened the temperature reaction to both intravenous and intracisternal injection of pyrogenal (Fig. 1).

Counting on the possibility of mutual potentiation of the pharmacological action of the cytostatics, in the next experiments the drugs were injected together: actinomycin D in a dose of 100 µg/kg four or five times daily and hydrocortisone as three daily injections in doses of 5, 5, and 10 mg/kg. In rabbits treated in this way with actinomycin D and hydro-

cortisone the total leukocyte count in the peripheral blood fell sharply on account of granulocytes and lymphocytes (to 30-50% of the initial level).

The temperature reaction to injection of pyrogenal in this case was considerably inhibited. In the control, following intravenous injection of pyrogenal a bimodal temperature reaction lasting several hours developed, such as is typically observed after intravenous injection of bacterial lipopolysaccharides [2].

In the experimental animals, although the first peak was completely preserved, the second peak of the reaction was virtually absent and it ended more rapidly (Fig. 2). More marked inhibition of the reaction still was observed after intracisternal injection of pyrogenal. In view of the high sensitivity of this method the dose of pyrogenal was reduced to one fiftieth of that given intravenously. Injection of pyrogenal in this dose in the control caused a high and prolonged temperature reaction. In the experimental animals the reaction was sharply inhibited, for in two of the seven rabbits the rise of temperature did not exceed 0.4 °C and the body temperature of all the rabbits fell quickly to its initial level (Fig. 2). To verify the state of reactivity of the temperature regulating centers in this group of animals, after the body temperature had fallen to its original level leukocytic pyrogen was injected intravenously. A brief temperature reaction of rapid onset, typical of endogenous pyrogens, developed in all the animals (Fig. 2). This indicates that the reactivity of the temperature regulating center of these animals to pyrogenic stimuli remained intact.

In these experiments combined treatment of rabbits with hydrocortisone and actinomycin D thus led to marked inhibition of the febrile reaction to injection of bacterial pyrogen. The many-sided inhibitory effect of hydrocortisone on processes of inflammation [1] and the blocking action of actinomycin D on the formation of endogenous pyrogen by leukocytes [9] are familiar from the literature. Inhibition of the reaction was evidently connected with these properties. The results confirm the great importance of the functional state of the leukocytes and of inflammation in general for the development of fever in response to bacterial products. This emerged with great clarity from the experiments with intracisternal injection of pyrogenals, in which fever was completely suppressed in some rabbits. In the experiments with intravenous injection of pyrogenal the second peak of fever was completely abolished, thereby confirming the role of leukocytic pyrogen in the development of the febrile cycle and pointing to a sharp decrease in the ability of the leukocytes of the experimental animals to produce leukocytic pyrogens. However, in these same experiments the speed with which the body temperature rose and the height of the first peak were unchanged. Some different mechanism of action of bacterial pyrogen on the temperature regulating system in the phase of triggering of the febrile reaction besides the stimulation of endogenous pyrogen production by granulocytes by the pyrogen cannot therefore be ruled out.

LITERATURE CITED

1. P. D. Gorizontov and T. N. Protasova, The Role of ACTH and Corticosteroids in Pathology [in Russian], Moscow (1968), p. 146.
2. A. V. Sorokin, Pyrogens [in Russian], Leningrad (1965).
3. A. V. Sorokin and O. M. Efremov, in: Proceedings of the 3rd Scientific Conference of Pathophysiologists of the Northern Caucasus [in Russian], Rostov-on-Don (1969), p. 238.
4. E. Atkins and P. T. Bodel, in: Pyrogens and Fever (Ciba Foundation Symposium) (ed. by G. E. W. Wolstenholme and J. Birch), Edinburgh (1971), p. 81.
5. I. L. Bennett and P. B. Beeson, J. Exp. Med., 98, 477 (1953).
6. C. A. Dinarello, N. P. Goldin, and S. M. Wolff, J. Exp. Med., 139, 1369 (1974).
7. S. M. Gillman, Bull. Johns Hopkins Hosp., 108, 381 (1961).
8. J. C. Herion, R. J. Walker, and J. G. Palmer, J. Exp. Med., 113, 1115 (1961).
9. J. J. Nordlund, R. K. Root, and S. M. Wolff, J. Exp. Med., 131, 727 (1970).
10. B. Wood, Lancet, 2, 53 (1958).