axonal transport of labeled protein and reduces the number of macromolecules transported into the muscle [1, 6], it can be tentatively suggested that injury to spinal α-montoneurons by the toxin delays restoration of RMP of the reinnervated muscle fibers probably as a result of disturbance of orthograde axon transport into the muscle of neuronal substances which activate the functioning of the electrogenic potassium-sodium pump of the muscle cell plasmalemma.

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CONDITIONS OF PREPARATION AND MECHANISM OF ACTION OF MACROPHAGAL PYROGEN

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Activation of mononuclear phagocytes by staphylococci *in vitro* leads to the formation of an endogenous pyrogen. The macrophagal pyrogen does not possess specific pyrogenic specificity, and on intracisternal injection sensitivity to it is enhanced by more than 100 times compared with that observed after intravenous injection. An even sharper increase in sensitivity to pyrogen was observed in animals after elevation of the body level of cyclic AMP as a result of preliminary injection of theophylline.

KEY WORDS: fever; pyrogens; macrophages.

The cell system of mononuclear phagocytes plays an important role in the mechanism of nonspecific and specific resistance [2, 5]. According to recently published data, the cells of this system play an active part in the mechanism of the febrile reaction, forming endogenous pyrogens [6-8, 11, 12]. However, some aspects of the conditions of formation and mechanism of action of macrophagal pyrogen have been insufficiently studied, and the investigation described below was accordingly undertaken for this purpose.

To obtain peritoneal macrophages (PM) 200 noninbred albino mice weighing 20-30 g were used, to isolate alveolar macrophages (AM) 11 rabbits were used, and to obtain blood mono-

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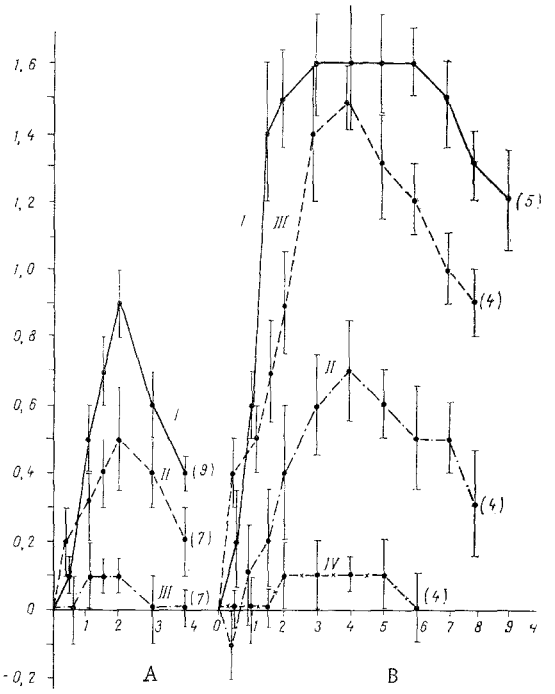

Fig. 1. Temperature reactions of rabbits to injection of pyrogen formed by mouse PM. Here and in Figs. 2 and 3; abscissa, time (in h); ordinate, change in body temperature (in °C). A) Intravenous injection of: I) dose of supernatant equivalent to 20 million cells/kg body weight, II) dose of supernatant equivalent to 5 million cells/kg body weight; III) dose of heated supernatant equivalent to 20 million cells/kg body weight; B) intracisternal injection of: I) native supernatant in a volume of 0.2 ml, II) the same, but diluted eightfold, III) supernatant diluted eightfold after preliminary injection of theophylline into rabbits, IV) native heated supernatant in a volume of 0.2 ml. Here and in Figs. 2 and 3, vertical lines represent confidence limits; number of animals given in parentheses.

cytes (FC) 7 rabbits were used. Pyrogenic activity was tested in experiments on 130 chinchilla rabbits weighing 2.5-3.0 kg.

PM were obtained 96 h after intraperitoneal injection of 3-4 ml sterile thioglycollate into the mice. Macrophages obtained in the control experiments were suspended in medium No. 199 with the addition of 15% heated mouse or rabbit serum in a concentration of 15-20 million cells/ml and incubated for 18 h at 37°C. After incubation of PM in mouse serum, the rabbits in which pyrogenic activity was determined were used once only. Since the liberation of pyrogen took place equally in both mouse and rabbit serum, in most experiments incubation was carried out in rabbit serum, which was convenient for repeated tests of pyrogenic activity on rabbits.

AM were obtained by the method described in [13], and Ficoll–Hypaque [9] was used to isolate monocytes from rabbits' blood [9]. In control experiments AM and MC were incubated in a concentration of 20-25 million cells/ml in medium No. 199 with the addition of 15% homologous serum. Incubation was carried out for 18 h at 37°C. To obtain macrophagal pyrogen, all the suspensions of mononuclear phagocytes were incubated as in the control experi-

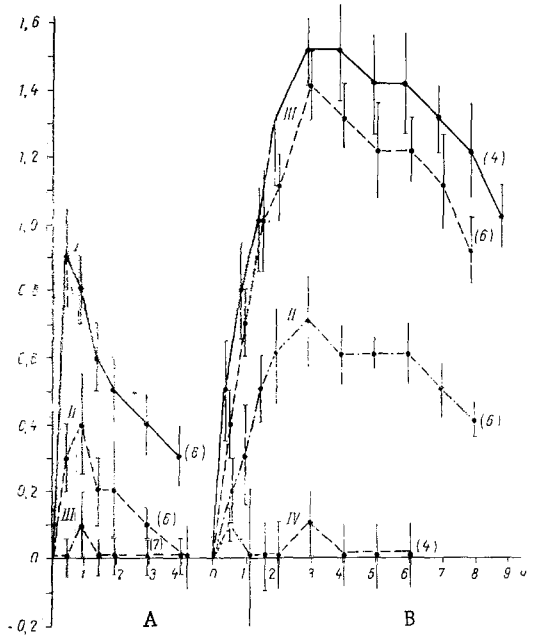

Fig. 2

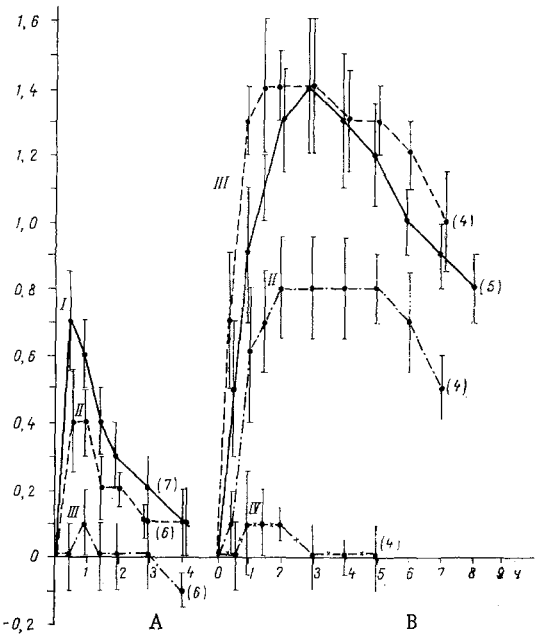

Fig. 3

Fig. 2. Temperature reactions of rabbits to injection of pyrogen formed by rabbit AM. A) Intravenous injection of: I) dose of supernatant equivalent to 25 million cells/kg body weight, II) dose of supernatant equivalent to 10 million cells/kg body weight, III) dose of heated supernatant equivalent to 10 million cells/kg body weight; B) the same as in Fig. 1B.

Fig. 3. Temperature reactions of rabbits to injection of pyrogen formed by rabbit blood MC. A) The same as Fig. 1A; B) intracisternal injection of: I) native supernatant in volume of 0.2 ml, II) the same, but diluted fourfold, III) fourfold dilution of supernatant after preliminary injection of theophylline into rabbits, IV) native heated supernatant in volume of 0.2 ml.

ments in the same medium and concentration, with the addition of heat-killed *Staphylococcus albus** cells (in the ratio of 30 microbial cells to one macrophage) for 18 h at 37°C. The viability of the macrophages was determined by staining the cells in 0.25% trypan blue solution. At the end of incubation the cells were removed by centrifugation, and pyrogenic activity was determined in the supernatants by injecting the preparation intravenously into rabbits in a dose of 1-1.5 ml/kg body weight. As one method of central administration macrophagal pyrogen was injected into the cisterna magna of rabbits [4]. The preparation was given in a volume of 0.2 ml. To raise their body level of cyclic AMP, rabbits were given two injections (1 h before and simultaneously with intracisternal injection of the pyrogen) of theophylline, which blocks phosphodiesterase, in a dose of 10 mg/kg body weight. Isolation of the cell suspensions and all subsequent stages of the work were carried out with strict observance of measures to prevent bacterial contamination. The rabbit's body temperature was measured in the rectum with an electrothermometer, twice or three times before injection of the preparations at intervals of 30 min, and during the period of 4-9 h after injection, at the same intervals. The experimental results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The yield of cells 96 h after intraperitoneal injection of thioglycollate into the mice was 25 million cells per animal (70% of PM and 30% of lymphocytes). Washing out the cells of the bronchial tree from one rabbit yielded 400-450 million cells (60% of AM, 38-39% of

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lymphocytes, 1-2% of granulocytes). From 100 ml rabbit blood 200-250 million leukocytes were obtained (26-27% MC, 70% lymphocytes, 3-4% granulocytes).

In the control series, after incubation of suspensions of macrophages in medium No. 199 with the addition of 15% serum for 18 h at 37°C no pyrogen was liberated. The number of viable cells after isolation and incubation was not below 90%. Stimulation of mononuclear phagocytes (PM, AM, and MC) by staphylococci led to significant ($P<0.01$) formation of pyrogen. Titration of pyrogenic activity of the supernatant showed that the optimal pyrogenic dose of mouse peritoneal pyrogen, causing a short period of monophasic fever with a temperature peak 1.5-2 h after injection, was a dose of supernatant equivalent to 20 million cells/kg body weight (Fig. 1A). The optimal dose of alveolar and monocytic rabbit pyrogen also lay within these limits, but the temperature reaction of the animals developed more rapidly after injection of the pyrogen (Figs. 2 and 3). The duration of the temperature reaction was 4-5 h. Macrophagal pyrogen, in a dose of 5-10 million cells/kg body weight, caused a slight temperature reaction in the rabbit. This dose was the threshold (see Figs. 1-3).

After intracisternal injection of macrophagal pyrogen in a dose of 0.2 ml the febrile reaction was greatly intensified compared with that to intravenous injection ($P<0.01$): The temperature began to rise after only 30 min, it reached a maximum 3-4 h after injection of the preparation, and it remained high for over 9 h (Figs. 1-3). Macrophagal pyrogen heated to 90°C for 30 min was inactive whether injected intravenously or intracisternally (Figs. 1-3). Eightfold dilution of alveolar and peritoneal pyrogen and fourfold dilution of monocytic pyrogen did not prevent the development of a significant ($P<0.05$) temperature reaction after intracisternal injection in a volume of 0.2 ml (Figs. 1-3). Preliminary injection of theophylline considerably potentiated the temperature response of the rabbit to all three types of macrophagal pyrogen.

One of the essential conditions for triggering the formation of macrophagal pyrogen is thus activation of the mononuclear cells by bacterial phagocytosis. The ability of tissue macrophages to form pyrogen was somewhat greater than that of the blood monocytes. Since mouse macrophagal pyrogen produced fever in rabbits it can be concluded that this type of pyrogen, like granulocytic pyrogen, does not possess specific pyrogenic specificity [1, 3]. On central injection of macrophagal pyrogen sensitivity to it was enhanced by more than 100 times in the present experiments compared with that after intravenous injection of the pyrogen. An even sharper increase in sensitivity to macrophagal pyrogen after intracisternal injection was observed after preliminary injection of theophylline into the rabbits. These data are evidence of the participation of cyclic nucleotides in the mechanism of fever induced by macrophagal pyrogen. High activity of macrophagal pyrogen after intracisternal injection shows that, like granulocytic pyrogen, it acts directly on temperature regulating centers [10].

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