sponse. This effect can be explained on the grounds that the instrumental response itself acquired the properties of a reinforcing stage [3, 10] and led to a unique type of "sensory satiation" of the fed animals. In other words, the intermediate result achieved at the stage of the instrumental response, against the background of true satiation of the animals, was effective for suppression of the subsequent course of the response. In this case, inhibitory control of the response was thus preserved, so that the animal's behavior did not lose its adaptive character.

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ENDOGENOUS PYROGEN FORMATION BY MONONUCLEAR PHAGOCYTES

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Incubation of alveolar macrophages from rabbits and of peritoneal macrophages from peritoneal washings of albino mice did not lead to liberation of endogenous pyrogen. Peritoneal macrophages obtained after intraperitoneal injection of thioglycollate, glycogen, or heterologous blood cells into mice likewise did not secrete pyrogen on incubation without additional stimulation. Macrophages isolated after intraperitoneal injection of heterologous blood cells did not possess pyrogenic activity, probably because of the long time after phagocytosis of foreign agents. The process of pyrogen formation by macrophages in these experiments may have been triggered by phagocytosis of corpuscular particles — staphylococci or heterologous blood cells — *in vitro*.

KEY WORDS: fever; endogenous pyrogen; macrophages.

In the modern view the development of a febrile reaction in various pathological processes is due to the formation of endogenous pyrogens (EP) in the body by cells of the professional phagocyte system [2, 5, 15]. There is evidence that, besides neutrophilic granulocytes, EP may also be formed by mononuclear phagocytes: blood monocytes [6, 9, 10], alveolar [4] and peritoneal [7, 8, 13] macrophages, and the Kupffer cells of the liver [12].

The object of this investigation was to study some conditions of activation and formation of pyrogen by mononuclear phagocytes.

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TABLE 1. Effect of Stimulus on Cell Yield, Percentage Composition of Albino Mouse Peritoneal Exudate and Pyrogenic Activity of Incubation Medium

Stimulus	Cell yield per animal, $\times 10^6$	Percentage composition of exudate			Pyrogenic activity of incuba. medium (M ± m), °C
		macrophages	lymphocytes	granulocytes	
Peritoneal washings of intact mice	5	75	25	—	0,1±0,05
Thioglycollate	25	76	24	—	0,1±0,05
0.3% glycogen solution	9	72	27	1	0,2±0,05
Rabbit erythrocytes	4	46	53	1	0,0±0,05
Rabbit granulocytes	6	58	40	2	0,1±0,05

EXPERIMENTAL METHOD

A general condition of the work was strict observance of rules preventing the possibility of bacterial contamination [3]. To obtain peritoneal macrophages (PM) 300 noninbred albino mice weighing 20-30 g were used, and alveolar macrophages (AM) were isolated from 11 rabbits weighing 2.5-3 kg. PM were obtained by washing out the cells from the peritoneal cavity of intact mice with medium No. 199, and also for 96 h after intraperitoneal injection of 3-4 ml sterile thioglycollate, with 3-4 ml of 0.3% glycogen solution in 0.85% NaCl, obtaining 1 billion rabbit blood erythrocytes or 50 million granulocytes from peritoneal exudate of rabbits, 90-95% of the cells of which were granulocytes [1, 3]. The rabbit granulocytes and the hen and rabbit erythrocytes used to stimulate the macrophages *in vitro* were treated with 3% glutaraldehyde [8] to preserve their corpuscular nature.

The viability of the macrophages was determined by staining the cells with 0.25% trypan blue solution.

In the control experiments PM were suspended in a concentration of 10 million cells in 1 ml medium No. 199 with the addition of 15% heated rabbit or mouse serum and incubated without additional stimulation for 18 h. In cases of incubation of PM in mouse serum, the rabbits were used only once for determination of pyrogenic activity. Since pyrogen was liberated identically in mouse and rabbit serum, in most experiments the incubation was carried out in rabbit serum, which is convenient for repeated tests of pyrogenic activity.

To study the conditions of pyrogen formation PM were incubated in the same medium for 18 h with the addition of heat-killed *Staphylococcus albus* cells in the proportion of 30 microorganisms per macrophage (30:1), or of rabbit erythrocytes (10:1) or granulocytes (5:1).

AM were obtained by Myrvic's method [14] with strict observance of sterile and apyrogenic conditions. The AM were stimulated by staphylococci (30:1) or hen erythrocytes (10:1) and incubated for 18 h in medium No. 199 with the addition of 10% homologous serum in a concentration of 8 million cells/ml. In control experiments AM were incubated for 18 h in the same medium, but without stimulators.

At the end of incubation the cells were removed by centrifugation and the supernatants were tested for pyrogenic activity by intravenous injection into rabbits in a dose of 1-1.5 ml/kg body weight. The body temperature of the rabbits was measured in the rectum by means of an electrothermometer twice or three times at intervals of 30 min before injection of the preparation and during the 4-5 h after its injection, at the same interval. The experimental results were subjected to statistical analysis by Student's t-test.

EXPERIMENTAL RESULTS

Cell Yield and Composition of Mouse Peritoneal Exudate and Washings from the Bronchial Tree. By washing out cells from the peritoneal cavity of intact albino mice with medium No. 199, 5-6 million cells were obtained from each animal, of which PM accounted for 75% and lymphocytes for 25%; no granulocytes were found. After intraperitoneal injection of thioglycollate into the mice the cell yield per animal was 25 million (76% PM and 24% lympho-

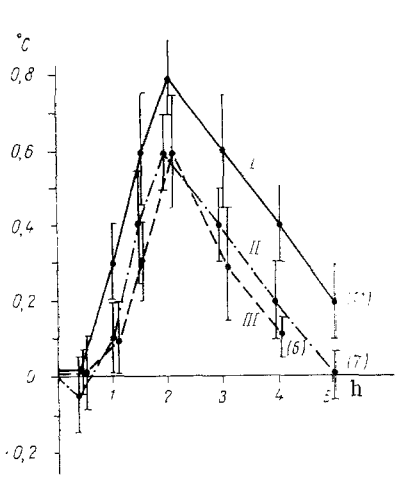

Fig. 1

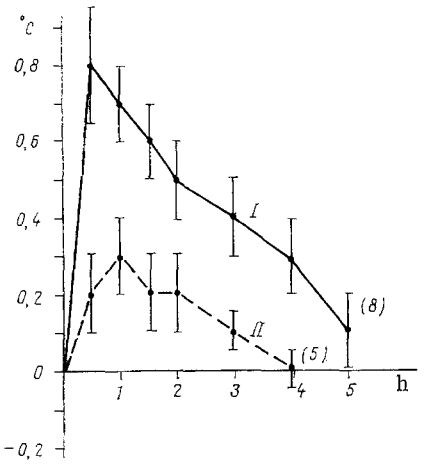

Fig. 2

Fig. 1. Pyrogen formation by PM of mice after additional stimulation with various stimuli. I) Staphylococci; II) rabbit granulocytes; III) rabbit erythrocytes. Vertical lines represent confidence limits. Number of animals indicated in parentheses. Abscissa, time (in h); ordinate, changes in body temperature (in °C).

Fig. 2. Pyrogen formation by rabbit AM following additional stimulation by various stimuli. 1) Staphylococci; II) hen erythrocytes. Remainder of legend as in Fig. 1.

cytes), and after injection of glycogen solution it was 9 million (72% PM, 26-27% lymphocytes, 1-2% granulocytes). The cell yield per animal 96 h after intraperitoneal injection of rabbit erythrocytes or granulocytes into the mice was 4-6 million, and the percentage of lymphocytes was considerably increased — to 44-45 (Table 1). Since the highest yield of macrophages was obtained after injection of thioglycollate, the subsequent experiments were carried out with the use of this stimulus.

In washings of cells from the bronchial tree with medium No. 199 the cell yield per rabbit was 400-450 million, consisting of 60% AM and 38-39% lymphocytes; the number of granulocytes present did not exceed 1-2%.

Incubation of Cells without Additional Stimulation. PM from peritoneal washings of intact mice, on incubation in medium No. 199 with the addition of 15% serum for 18 h, did not liberate EP. PM obtained after intraperitoneal injection of thioglycollate, glycogen solution, or rabbit erythrocytes or granulocytes into the mice likewise did not liberate pyrogen (Table 1). The proportion of viable cells was 94-95%.

On incubation of AM in medium No. 199 with the addition of 10% serum, EP formation was not found and the proportion of viable cells was 95%.

Pyrogen Formation by Macrophages during Incubation with Stimulating Agents. Incubation of PM and AM with staphylococci for 18 h led to significant ($P<0.01$) accumulation of pyrogen in the supernatant. Intravenous injection of mouse macrophagal pyrogen into rabbits led to the development of a short monophasic febrile reaction with a temperature peak 1.5-2 h after injection of the preparation (Fig. 1), whereas injection of rabbit alveolar pyrogen caused a short monophasic febrile reaction with a temperature peak 30-60 min after injection of pyrogen (Fig. 2); in both cases the duration of the temperature reactions was 4-5 h.

PM incubated with rabbit erythrocytes and granulocytes liberated pyrogen significantly ($P<0.01$; Fig. 1). Incubation of AM with hen erythrocytes led to slight accumulation of pyrogen in the supernatant (Fig. 2). Heating the supernatants to 90°C for 30 min led to complete loss of pyrogenic activity.

Incubation of AM and PM thus does not lead to accumulation of pyrogen in the incubation medium. PM obtained from inflammatory exudate after intraperitoneal injection of thioglycollate or glycogen, although activated [11], likewise do not liberate pyrogen during incubation without additional stimulation. PM isolated 96 h after intraperitoneal injection of

heterologous blood cells do not possess pyrogenic activity, probably because of the length of the period elapsing after phagocytosis of foreign cells (macrophages liberate pyrogen for 36 h [9]). Later removal of the exudate cannot be recommended because of the considerable contamination with granulocytes. The process of EP formation by macrophages can be triggered by phagocytosis of corpuscular particles: staphylococci or heterologous blood cells. EP formation by macrophages in the present experiments required additional stimulation *in vitro*. To determine the precise mechanism of EP formation by macrophages, and to elucidate the relationship between phases of activation and liberation of pyrogen, further investigations are required.

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EFFECT OF THYROID HYPOFUNCTION ON GROWTH OF THE MUSCULOSKELETAL SYSTEM AND BODY AS A WHOLE IN EARLY POSTNATAL RATS

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Depression of thyroid function by chronic administration of mercazolyl* to rats aged from 5-7 days to 4 months causes a reduction in motor activity of the animals, a decrease in the absolute and relative weights of the bones, muscles, and heart, reduced oxygen demand and cardiac activity, and also hypercholesteremia, combined with a fall in the cholesterol level in the skeletal muscle tissues. Delayed growth and development of the musculoskeletal system and the reduced oxygen consumption lead to a decrease in body weight of the experimental rats under the age of 1 month compared with that of control animals. In rats aged 1-4 months, these factors lead to an increase in the gain in weight because of disturbance of lipid metabolism, despite a decrease in weight of the muscles and bones.

KEY WORDS: thyroid gland; musculoskeletal system; autonomic systems.

Laboratory investigations have shown that in each period of postnatal development the intensity of growth, the level of energy metabolism, and activity of autonomic systems are determined mainly by the particular features of development of the skeletal muscles [1-3].

*1-methyl-2-mercaptoimidazole.

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