ECOLOGY

Effect of Environmental Thermal Factor on Synthesis of Specific Proteins in Albino Mice

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Changes in protein synthesis in the hypothalamus, pituitary, adrenals, liver, myocardium, and femoral muscle during exposure to thermal factor were studied. Protein synthesis was activated in all studied tissues during the initial period of exposure to environmental temperatures of 35 and 40°C. At higher temperature (45°C) the synthesis was notably suppressed in all tissues except the pituitary, where it remained high (higher than in the control). Analysis of protein composition showed a series of polypeptides, identical to stress proteins, synthesized *de novo*.

Key Words: *thermal factor; protein synthesis; stress; stress proteins and adaptation*

Study of metabolic processes in the organism during exposure to extreme environmental factors is essential for practical solution of a complex of ecological and physiological problems. Adaptation to extreme environmental conditions is realized through common nonspecific reactions, which depend on the energy resource mobilization, plastic metabolism, and defense mechanisms of the organism. The mechanism of common adaptation syndrome or stress underlies total nonspecific adaptation [1,3]. Specific reactions of the organism, maintaining homeostasis, unfold in the presence of total adaptation syndrome. The hypothalamic-pituitary-adrenal system plays an important role in the realization of total adaptation; the degree of activation of this system reflects the intensity of stress reaction [3]. On the other hand, adrenocortical hormones induce mobilization of plastic reserve of the organism, which manifests in creation of a pool of free amino acids and intensification amino acid transamination. Both these processes should be regarded as purposeful preparation to adaptive protein synthesis [1,3]. Recently specific proteins and peptides were detected in prokaryotic and eukaryotic cells; the synthesis of these peptides is induced by exposure to extreme environmental factors. These proteins were called stress or thermal shock proteins (SP), as they were first detected during overheating of the organism [4,7]. Enhanced synthesis of these proteins protects cells from stress-induced uncontrollable catabolism of cell proteins. In addition, SP are involved in the regulation of protein synthesis in cells and stimulate immune response in infectious diseases caused by bacteria, protozoa, fungi, and nematodes [8]. Hence, activation of SP synthesis in response to extreme exposure is a mechanism protecting vital components from damage caused by stress or infection.

We investigated specific changes in protein synthesis during exposure to stress factors of different intensity (thermal stress).

MATERIALS AND METHODS

Experiments were carried out on random-bred albino mice (20-25 g). Control and experimental animals were intraperitoneally injected with 0.2 MBq [U-^{14}C] pro-

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tein hydrolysate (Chemapol) in normal saline. Experimental animals were kept in special cages at 35, 40 and 45°C, controls at neutral temperatures. The animals were decapitated at certain terms and fragments of the hypothalamus, pituitary, adrenals, liver, myocardium, and thigh muscles were plunged in 7% trichloroacetic acid. After 24 h the tissues were dried on filter paper and solubilized in scintillation vials in 1 M hydroxyhyamine 10 X in methanol (Serva) for 24 h at 45°C. Radioactivity of solubilized tissue was measured on a Mark-3 counter (Tracor Analytic) in toluene scintillator. Protein content was measured by the method of Lowry [6].

Corticotropin and corticosterone were measured in mice exposed to 35, 40, and 45°C and at room temperature in the morning. The animals were decapitated 15, 30 min, 1, 2, and 3 h after the start of exposure and the blood was collected. Plasma concentrations of corticotropin and corticosterone were measured by radioimmunoassay using commercial kits.

Protein synthesis at normal temperature, overheating (45°C), and in the control (37°C) was studied *in vitro* and on a model of thermal shock (43°C) *in vitro*. The choice of this latter temperature is explained as follows: similar experimental conditions were used in many studies [7]; in our experiments with exposure to 45°C core (rectal) temperature reached the above-mentioned temperature and was 41-42°C one hour after the start of exposure. For evaluation of molecular weight of newly synthesized proteins, the labeling was carried out as mentioned above or in tubes with medium 199 containing 2 MBq/ml [U-^{14}C) protein hydrolysate saturated with carbogen. In this latter case fragments of tissues (hypothalamus, pituitary, and adrenals) were incubated 1 h at 43°C. After incubation the samples were dried on filter paper and homogenized in 0.01 M Tris-HCl buffer (pH 6.8). Sodium dodecyl sulfate (Sigma) (final concentration 2%) and 2-mercaptoethanol (Serva) (Final concentration 5%) were added to each sample and the samples were heated to

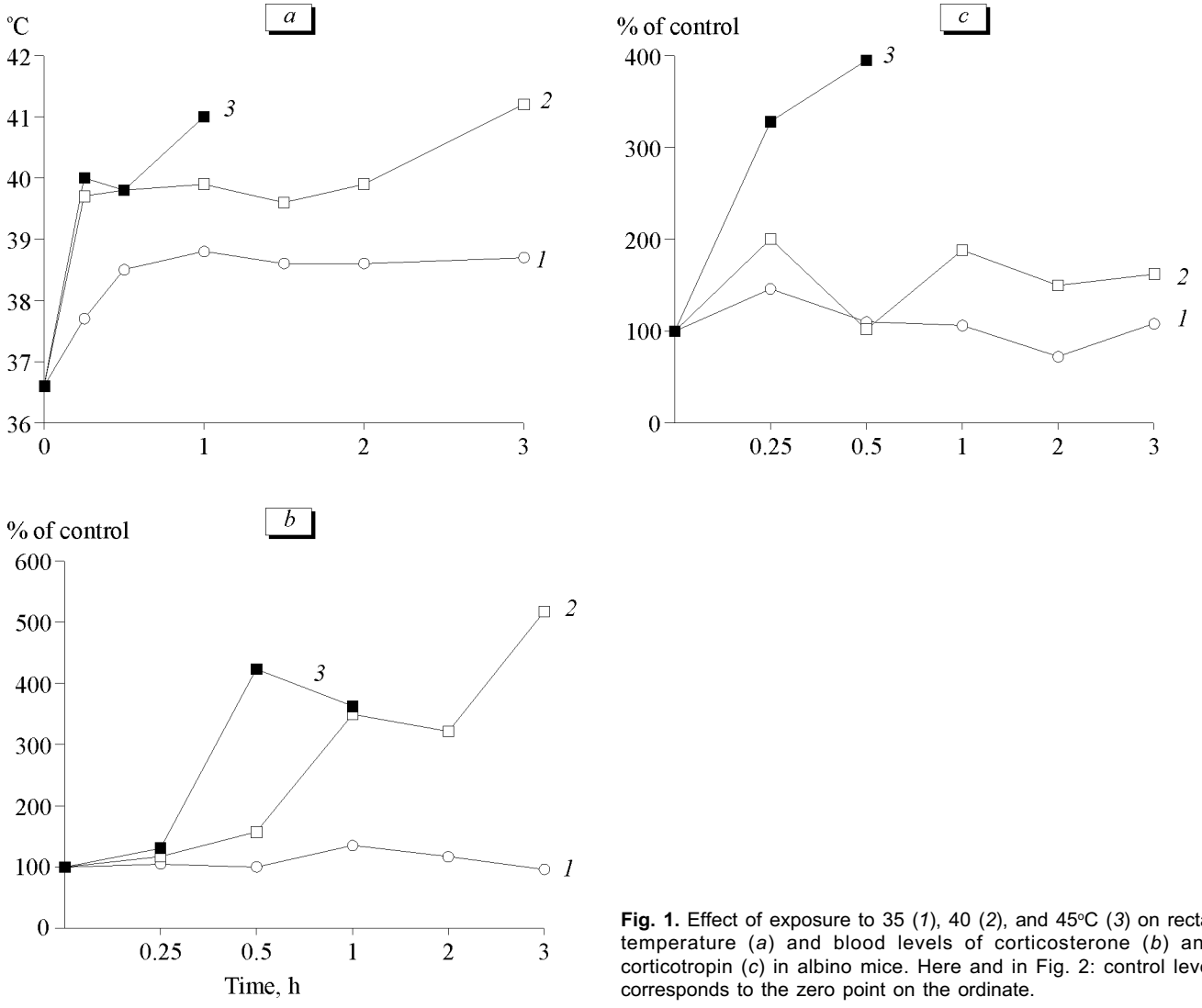

Fig. 1. Effect of exposure to 35 (*1*), 40 (*2*), and 45°C (*3*) on rectal temperature (*a*) and blood levels of corticosterone (*b*) and corticotropin (*c*) in albino mice. Here and in Fig. 2: control level corresponds to the zero point on the ordinate.

100°C for 5 min for inactivation of enzymes. A kit of labeled standard proteins for electrophoresis in a system with sodium dodecyl sulfate (Amersham) was used as molecular weight markers. Molecular weights of newly synthesized proteins were evaluated as described previously [5]. In order to detect protein bands with labeled polypeptides, the gels were plunged in 20% 2,5-diphenyloxasole in acetic acid and dried [2]. Fluorography was carried out on a PM-B radiographic film (Tasma). The gels were exposed for 1 month at 4°C and films were developed according to manufacturer's instruction.

Heating was evaluated by measuring rectal temperature with TPEM-1 medical electrothermometer.

RESULTS

After 1-h exposure at 35°C rectal temperature increased by 2.4°C, at 40°C by 3.3°C, and at 45°C by 4.4°C (Fig. 1, *a*); in the latter group 80% animals died after 3-h exposure.

Protein synthesis increased after 30-min exposure to 35 and 40°, after which it returned to normal and decreased after 3 h (Fig. 2, *a*, *b*). On the other hand, in animals exposed to 45°C protein synthesis was activated in the pituitary and suppressed in the hypothalamus. In the adrenals protein synthesis increased during the initial period of exposure to 35°C, while during exposure to 40 and 45° it decreased after 30 min and 1 h of exposure, respectively (Fig. 2, *c*). In the liver protein synthesis was activated during the initial period (1 h) of exposure to 35°C and suppressed during exposure to 45°C (Fig. 2, *d*). In the myocardium protein synthesis was also activated during the initial period (up to 1 h) of slight and medium thermal exposure, and then was suppressed; at extreme exposure it decreased (Fig. 2, *e*).

In the femoral muscle protein biosynthesis increased only after 3 h of exposure to 40°C and was suppressed during exposure to 45°C (Fig. 2, *f*).

Hence, a characteristic sign of exposure to 35 and 40°C was a significant increase or a trend to increase in protein synthesis in all studied tissues. After 1-h overheating (45°C) protein synthesis was suppressed in all tissues except the pituitary, in which an opposite effect was observed.

The contribution of common nonspecific mechanism of adaptation to the realization of specific reactions of homeostasis maintenance can be evaluated by the degree of activation of the hypothalamic-pituitary-adrenal system. In winter the concentration of corticotropin in the blood of mice was 712±72 ng/liter, in spring 283±54 ng/liter; for corticosterone the respective values were 32±5 and 9±2 nmol/liter.

At 35°C the concentration of corticotropin peaked after 15 min, that of corticosterone after 1 h; later blood concentrations of both hormones did not differ from the control. At 40°C corticotropin level increased in 15 min, then decreased to the normal by the 30th min of exposure, and again increased after 1 h of exposure. The concentration of corticosterone under these conditions increased starting from the 30th min and by 3 h reached 419% of the control. A potent release of both hormones into the blood was observed during strong thermal exposure (Fig. 1, *b*, *c*).

Therefore, a slight stress exposure induces a short-term activation of the hypothalamic-pituitary-adrenal system, while a stronger stimulus induces more intense activation of the system, which does not return to the initial level.

Hence, activation of protein synthesis during exposure to a slight environmental factor correlates with a slight release of corticotropin and corticosterone into the blood; the parameters normalize after 3 h of exposure. Increase in protein synthesis is a form of adaptation to a slight environmental factor and is not related to activation of the hypothalamic-pituitary-adrenal system. Exposure to a strong stress factor (45°C) stimulates a strong release of corticotropin and corticosterone into the blood, which mobilizes the mechanism of common nonspecific adaptation [3,4]. The function of the hypothalamic-pituitary-adrenal system in this case consists in the creation of a fund of free amino acids and induction of the synthesis of amino acid metabolism enzymes [4]. Hence, two oppositely directed mechanisms co-exist during exposure to high temperature: one realizes specific reactions of maintaining the thermal homeostasis and inducing adaptive protein and nucleic acid synthesis and the other is the mechanism of nonspecific adaptation, aimed at creation of the fund of free amino acids at the expense of protein catabolism.

Therefore, the contribution of nonspecific adaptive reactions to an extreme factor increases with an increase in the intensity of this factor in comparison with the specific thermoregulation reactions, which manifests by suppression of protein synthesis.

We can expect that changes in protein and RNA synthesis observed in thermal exposures of different intensity influence the protein pool synthesized in the cell.

The synthesis of polypeptides with molecular weights of 107, 92, 63, 54, 43, and 22 kDa was activated in the pituitary *in vivo* during development of hyperthermia (Fig 3); the synthesis of these polypeptides was also observed in many experiments on various objects of animal and plant origin [7]. However we observed a different picture *in vitro*: the synthesis of 92, 65, 46, and 23 kDa polypeptides was suppressed and there was no protein with molecular weight of 35 kDa in the pituitary; the synthesis of 130, 65, and 35

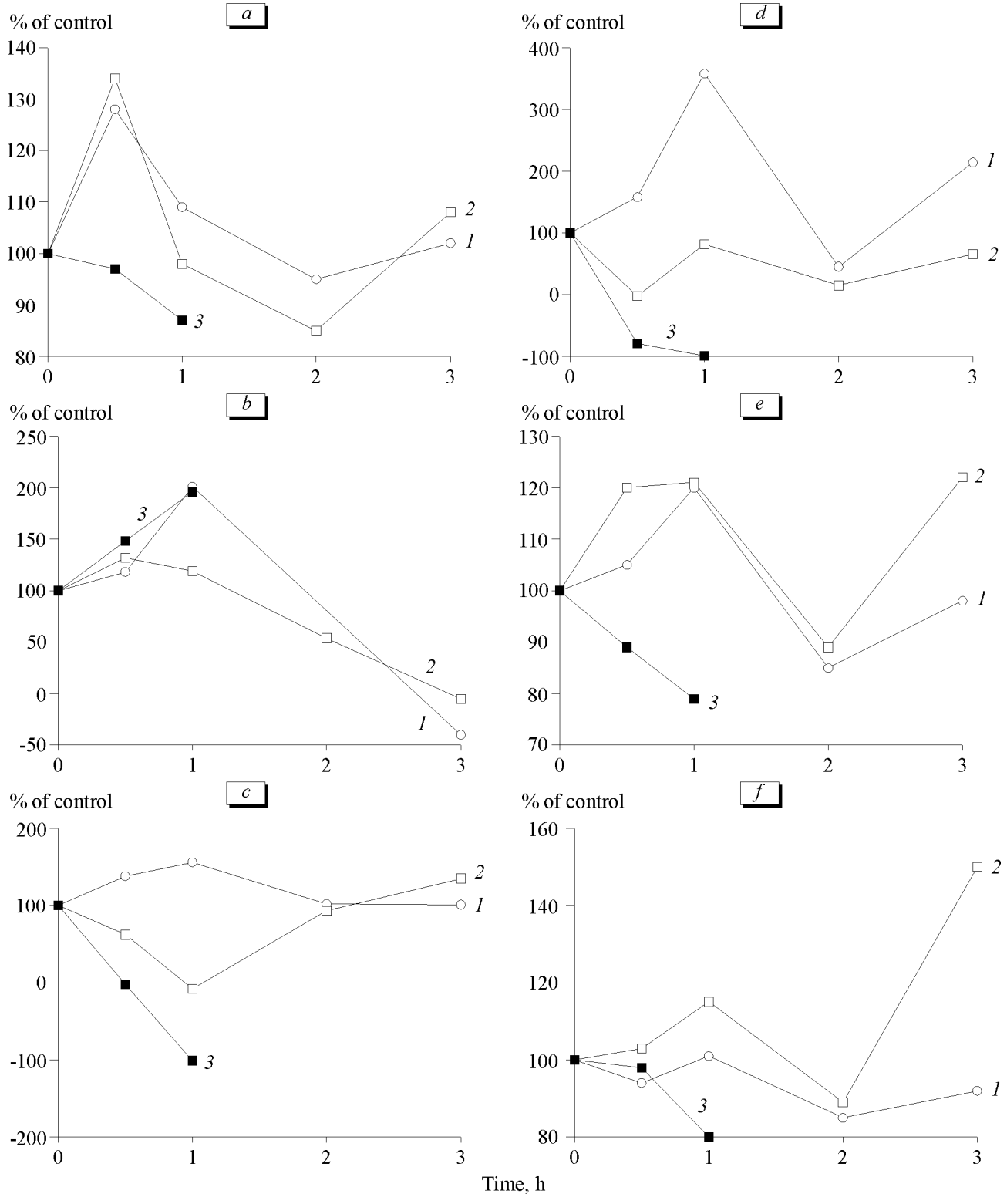

Fig. 2. Effects of exposure to 35 (*1*), 40 (*2*), and 45°C (*3*) on protein synthesis in the hypothalamus (*a*), pituitary (*b*), adrenals (*c*), liver (*d*), myocardium (*e*), and femoral muscle (*f*).

kDa polypeptides decreased in the hypothalamus; the level of protein with molecular weight of 24 kDa decreased in the adrenals, and only in the liver we observed activation of synthesis of proteins with molecular weights of 140, 82, 65, and 54 kDa, while 52, 48, and 29 kDa polypeptides were absent (Fig. 3).

These opposite effects of the thermal stimulus on hypothalamic-pituitary-adrenal structures seem to be

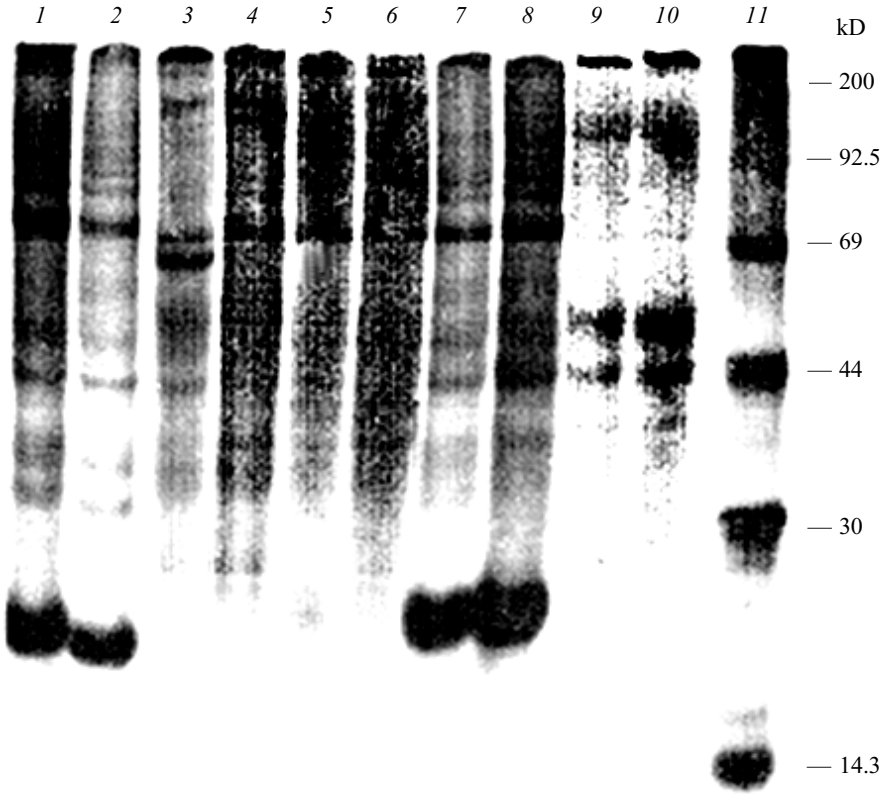

Fig. 3. Fluorograms of electrophoregrams of separation of newly synthesized proteins of different tissues of albino mice normally (*2*, *4*, *6*, *8*, and *10*) and after thermal exposure (*1*, *3*, *5*, *7*, and *9*). *1*, *2*) pituitary *in vivo*; *3*, *4*) liver *in vitro*; *5*, *6*) adrenals *in vitro*; *7*, *8*) pituitary *in vitro*; *9*, *10*) hypothalamus *in vitro*; *11*) mixture of standard proteins: myosin (200 kDa), phosphorylase b (92.5 kDa), BSA (69 kDa), carboanhydrase (30 kDa), and lysozyme (14.3 kDa).

explained by the absence of the nonspecific component of stress reaction, stimulated by nervous and humoral mechanisms and mediated through this system under *in vitro* conditions. On the other hand, the synthesis of some SP was observed in the liver.

Hence, synthesis of specific SP is a mechanism including the process of specific adaptation of cells. These proteins can function as mediators of homeostasis maintenance via various pathways: directly affect the cell structures by stabilizing them or indirectly through activation of protein synthesis or stimulation of the immune system. In addition, it was shown recently that SP, inducing an immune response in infections and cancer, protect the host from these diseases [8,9].

Therefore, homeostasis (including thermal) is maintained at the organism and cell levels. The organism level represent a complex of defense and adaptive (specific and nonspecific) reactions aimed at the maintenance of stability of internal media as the integral system and the cell level consists in adaptive cell reactions presenting as modulation of its metabolism in response to extreme factors. The leading cell reaction is activation of plastic metabolism of specific regulatory macromolecules (including proteins) increased the resistance of cells as an autonomic system of the host.

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