Acta virol. **31**: 424—433, 1987

EXPERIMENTAL INFLUENZA INFECTION: INFLUENCE OF STRESS

L. K. CHETVERIKOVA, B. A. FROLOV, T. A. KRAMSKAYA, R. YA. POLYAK

Research In stitute of Experimental Medicine, U.S.S.R. Academy of Medical Sciences, 197 022 Leningrad, U.S.S.R.

Received July 10, 1986

Summary. — The effect of immobilizatoin stress on the course of various forms of influenza infection has been investigated. Influenza was produced in 10—14-week-old inbred mice by intranasal infection with pathogenic influenza virus strain A/PR/8/34 (H1N1) at different doses. Immobilization for 6 hr resulted in the appearance of virus-inhibiting activity in the serum of mice. This activity suppressed the reproduction of test-virus in tissue culture, it was resistant to acid pH 2.0 treatment and to heating at 56 °C. However, the high level of virus-inhibiting activity failed to protect the animals from subsequent development of lethal influenza infection. Immobilization stress caused a transient depression of virus induced interferon (IFN) production, as revealed by the use of virus inducer at early intervals after stress. Contemporarily, the stress could aggravate the course of virus infection promoting its transition from non-lethal form into a lethal one and virus penetration into brain.

Key words: influenza infection; α-interferon; stress

Introduction

It has been recently shown that various forms of stress can adversely affect natural mechanisms of antiviral resistance. It has been demonstrated, in particular, that stress affects interferon production (Rasmussen, 1969; Sukhikh *et al.*, 1984; Frolov *et al.*, 1986), inhibits the activity of natural killers (Toge *et al.*, 1981; Lukomska *et al.*, 1983; Sukhikh, Meerson, 1983) and also reduces bactericidal and cytotoxic ability of mononuclear phagocytes (Lockard *et al.*, 1973; Pavlidis, Chirogos, 1980; Starling, Norback, 1981). Since the mentioned mechanisms of nonspecific resistance block the development of viral infectious process at early stages, it may be assumed that stress will be associated with marked alterations of the susceptibility of organism to viral infections. However, the available data are controversial (Rasmussen *et al.*, 1957; Johnson *et al.*, 1963; Marsh *et al.*, 1963; Jensen, Rasmussen, 1963; Friedman *et al.*, 1969; Solomon, 1969; Ebert *et al.*, 1979; Frolov *et al.*, 1986). This discordance can be explained, on one hand, by the

depth and character of physiologic changes elicited by different stressors, and on the other hand, by reproduction of a particular pathogen resulting in a specific infectious process.

Our aim has been to investigate the influence of immobilization stress on the development of different forms of influenza infection.

Materials and Methods

Experimental influenza infection was produced in 10—14-week-old mice [male mice of CBA or (CBA × C57 Black)F_1 strains obtained from the Nursery of the U.S.S.R. Academy of Medical Sciences "Rappolovo"] by intranasal (i.n.) administration in various doses (per 0.05 ml) of pathogenic influenza virus strain A/PR/8/34 (H1N1). Infective activity of the virus was assessed by conventional titration of the virus-containing material in 11 — 12-day-old developing chick embryos.

Detection of influenza virus in the brain of infected animals was made using 3 mice for each determination. Groups of 3 chick embryos (CE) were infected with 10% brain tissue homogenate (in Hank's solution) prepared from each animal separately with initial material or diluted 1 : 2. If the virus was not found in the allantoic fluid of infected embryos, an additional "blind" passage was carried out in further CE.

Interferon (IFN) in the blood of experimental animals was measured by titration of its antiviral activity using a micromodification of conventional technique (Pavlushina, Orlova, 1981) in a continuous cell line of mouse L_{929} fibroblast against 100 cytopathic doses of mouse encephalomyocarditis (EMC) virus *. IFN-containing mouse serum prepared 6 hr after intraperitoneal injection of Newcastle disease virus was used as reference IFN. For inactivation of the inducer virus, the serum was kept for 5—7 days in the cold at pH 2.0.

Immobilization stress was produced using the procedure of Selye (1952). The limbs of animals were fastened for 6 hr keeping them on their backs. The design of experiments is shown in Fig. 1. Five groups of mice were used in each experiment. Two groups consisted of uninfected animals: intact (group 1) or stressed (group 2). Infected groups included animals without stress (group 3) and those underwent immobilization stress either one day before (group 4) or 5 days before infection (group 5). IFN in the blood serum of animals was tested on days 1, 3, and 5 after stress (group 2) or on days 2, 4 and 7 after infection (groups 3 to 5). At the same periods, concentration of virus in the lungs of infected animals was measured. Three to 5 mice were examined at each observation interval. Parallel registration of the animals' deaths in experimental groups was carried out. Each observation group consisted of 19 to 25 mice.

Results

It has been shown that immobilization of mice for 6 hr resulted in the appearance of virus-inhibiting activity in blood serum, which suppressed the reproduction of test-virus in tissue culture. Testing of the sera from each animal has shown that there was a wide individual variation in this activity (Fig. 2). In particular, on the next day after the stress this activity was detected in the sera at dilutions from 80 to 320 and on days 3 to 5 at dilutions 120—640. It is noteworthy, that the highest virus-inhibiting effect has been found on days 3 to 5 after the immobilization and was 4 to 6 times higher than analogous activity in intact mice.

To investigate the character of stress-induced inhibition activity, its resistance to treatment in cold at pH 2.0 for 3 days and to heating at 56 °C

* The virus and the cells were kindly provided by the Laboratory of Molecular Virology, the N. F. Gamaleya Institute of Epidemiology and Microbiology, U.S.S.R. Academy of Medical Sciences, Moscow.

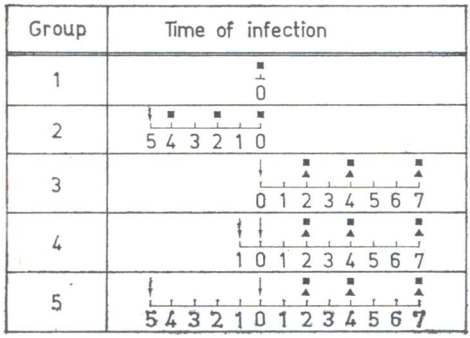

Fig. 1.
The experimental procedure
Curved arrow — stress; direct arrow — — infection; square — IFN determination; triangle — determination of virus concentration in the lungs. Abscissa: time before and after infection with influenza virus (days).

for 30 min has been tested. These treatments were without noticeable effect on virus-inhibiting activity of the sera obtained at various intervals after stress, suggesting that α-IFN might have been responsible for this effect. Doubtless, immobilization stress for 6 hr induced the appearance of a virus--inhibiting activity in the blood of stressed animals.

The data obtained were taken into account in the analysis of the influence of stress in the course of influenza infection. It seemed important to find out how IFN may influence the infectious process, and if it has been stimulated in the body of stressed mice prior to infection. The animals were, therefore, infected on days 1 to 5 after stress, when IFN was detected at titres from 80 to 1320 and from 120 to 640, respectively (Fig. 2).Three series of experiments have been carried out in each animal group with different doses of influenza virus. I.n. infection of CBA mice with lethal doses of the virus A/PR/8/34 (8.5 and 5.5 log EID_{50}/0.2 ml) led to deaths of 100% of animals

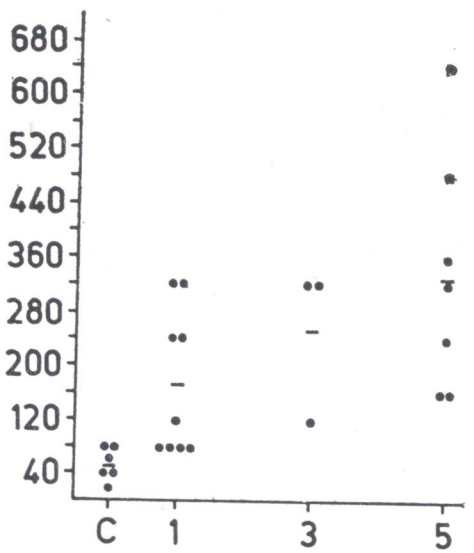

Fig. 2.
Registration of virus-inhibiting activity in the blood of CBA mice exposed to immobilization stress
C — intact mice (group 1); black dots — individual data (group 2); bars — mean values. Abscissa: time after immobilization (days); ordinate: titre of antiviral activity (dilution reciprocals).

by observation day 9. First death occurred on days 4 or 5 p.i. and the incidence of deaths reached a maximum on day 6 or 7 (Fig. 3). Immobilization stress before the infection had no effect on the survival rate. However, in the presence of a high dose of the virus (8.5 log EID_{50}) the time interval between infection and death was significantly shortened in mice infected on the day next after stress.

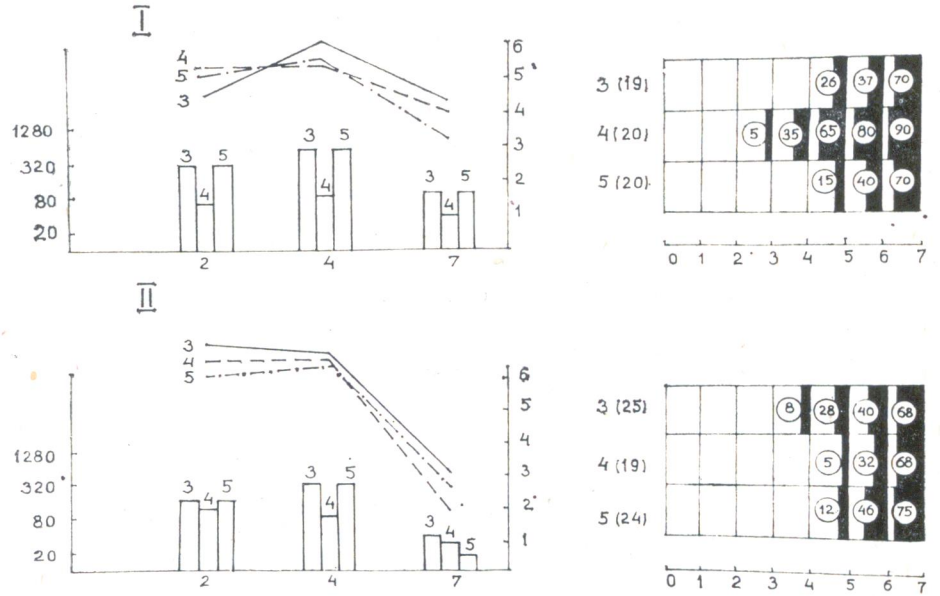

Fig. 3.
Effect of immobilization stress on the development of lethal influenza infection in mice
In the left: I — infectious dose of influenza virus A/PR/8 was 8.5 log EID_{50}/ /0.05 ml; II — 5.5 log EID_{50}; 3, 4, 5 — animal groups. In parentheses — number of animals in each group; lines — virus concentration in the lungs; columns — IFN titre; in the right — time course of deaths. Shaded section (figure in the circle) — death rate of animals (percentage). Abscissa: time p.i. (days); ordinate: in the left: IFN titre in serum (dilution reciprocals), in the right: virus concentration (log EID_{60}).

Lethal influenza infection developed in the presence of a high IFN production level in infected animals of control group 3. On day 2 postinfection, IFN activity in blood ranged from 1 : 160 (at infecting dose 5.5 log EID_{50}) to 1 : 320 (at a dose of 8.5 log EID_{50}). By day 4 the IFN titres increased up to 320—640. In the body of animals infected 24 hr after immobilization (group 4), the IFN level was significantly lower as compared to infected animals not exposed to stress. This difference was marked even on day 2 p.i. and was maintained for another 2 days thereafter. By day 7 it became less marked at an infecting dose of 5.5 log EID_{50}, but still was expressed in the

group of animals infected with a higher virus dose (8.5 log EID_{50}). However, the infection of animals on day 5 after immobilization (group 5), regardless of the dose, caused no changes of IFN production in the course of infection as compared to infected nonstressed mice.

In spite of the inhibition of virus-induced IFN production detected at early time after stress, by 2—4 days p.i. no difference in virus accumulation

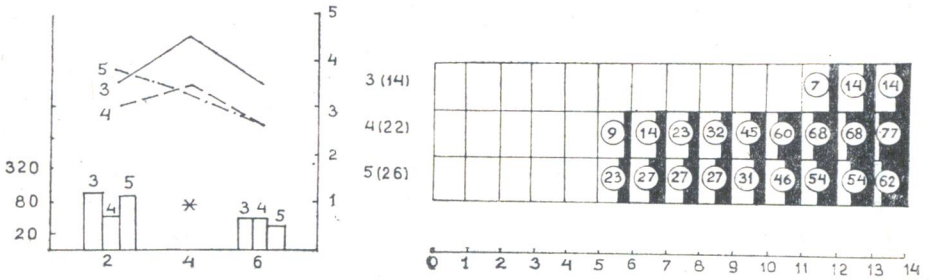

Fig. 4.
Effect of immobilization stress on the development of experimental influenza infection at a low infectious dose (3.0 log EID_{50}/0.05 ml of virus A/PR/8) For explanation see legend to Fig. 3.

was observed in the lungs of nonstressed mice and those which underwent stress. It is noteworthy that by day 7 p.i. the amount of the virus detected in the group of mice infected 1 day or 5 days after stress was much lower than in nonstressed mice.

Our data, therefore, indicated that immobilization stress produced 5 days prior to infection was essentially without effect on virus-induced IFN production, virus accumulation in the lungs or time course of deaths of animals. The exposure to stress 1 day prior to infection led to a dramatic inhibition of virus-induced IFN production and promoted the development of infection (see Fig. 3-I).

The next experimental series was devoted to the influence of stress in the course of non-lethal influenza infection produced in mice of strains CBA and $(CBA \times C57\ B1)F_1$ by i.n. administration of influenza virus A/PR/8/34 at a dose of 3.5 log EID_{50}/0.2 ml. In addition to evaluation of IFN content in blood and of the virus content in the lungs, the animals have also been observed for survival rate over 14 days. It was shown that in the control group (no stress) of infected animals IFN production was high: the titre of 140 on day 2 p.i. was followed by a characteristic fall by day 6 (Fig. 4, no. 3). In mice infected on the next day after the immobilization, like in the tests with lethal infection, a marked inhibition of IFN production has been observed (Fig. 4, no. 4). IFN production in this group was the same as in non stressed infected mice (Fig. 4, no. 5).

Virus concentration in the lungs of infected animals appeared to be the

Table 1. Isolation of influenza virus from the brain of mice submitted stress on day 2 after infection with influenza A/PR/8 a dose of 3.5 log EID_{50}

Mice group	Stress	Animal number	Detection of influenza virus in CE allantoic fluid															
			passage 1				passage 2											
			infecting material															
			brain homogenate				CE allantoic fluid from passage 1											
			original (a)			1 : 2 (b)	(a)						(b)					
							original			1 : 2			original			1 : 2		
3	none	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	yes	1	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
		2	−	−	−	−	−	−	−	−	−	−	n. t.			n. t.		
		3	−	−	−	−	−	−	−	+	−	−	+	+	+	n. t.		
5		1	−	−	−	−	−	−	+	−	−	+	+	+	+	+	+	+
		2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
		3	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+

Note. n. t. — non tested; the presence (+) or absence (−) of the virus in CE allantoic fluid was determined by haemagglutination.

highest on day 4 p.i. In stressed mice on days 4 and 6 p.i. the virus content was much lower than in control infected mice. This form of influenza infection was characterized in control animals by few (14% on days 12 or 13 p.i. in one series of 8) or no deaths of animals. In six series of 8 immobilizations, stress led to transition of non-lethal infection into a lethal one, the death rate of animals infected 1 or 5 days after the stress amounting from 40% to 90% by the end of the observation period.

The observed aggravation of the course of influenza infection after exposure to stress cannot be explained by changes in IFN production only. We suggest that generalization of infection may result from stress-induced impairment of the blood-brain barrier which allows the virus to penetrate into the brain. To verify this suggestion we have made attempts to isolate the virus on days 2 and 4 p.i. from the brain tissue of control and infected animals after stress. It is important to note that the virus was isolated only from those animals which underwent stress (Table 1). In primary infection of CE with the brain homogenate, the virus was isolated from 1 mouse of group 5 on day 2 p.i., whereas no virus could be detected in the brain of other stressed mice, unless virus-containing material had accumulated by repeated passage in CE. On day 4 p.i. the virus was detected neither on primary infection, nor after repeated passage in any of the samples tested. We failed to detect the virus in any of infected animals which had not undergo stress.

The isolated virus was identified in haemagglutination inhibition using standard diagnostic sera to influenza virus serotypes H1N1, H2N2 and H3N2. These data indicated that the isolated virus belonged to serotype H1N1, which was also the serotype of the strain used for infection. Our results, therefore, indicate that immobilization stress is followed by an alteration of the blood-brain barrier and thereby makes it possible for an infectious virus to penetrate the brain tissue. It cannot be ruled out that this can be of primary importance for the outcome of the infectious process and determine the lethal course of infection.

Discussion

We have confirmed our previous data that stress can induce virus-inhibiting activity in the blood of experimental animals (Frolov *et al.*, 1986). It should be noted that in intact CBA mice used in this experimental series, in 2 out of 6 cases the initial serum level of virus-inhibiting activity appeared rather high (titre 80, Fig. 2), whereas in C57B1 mice it was never higher than 10 (Frolov *et al.*, 1986a). In general, although two abnormally high values cannot be consistently interpreted, they still are insignificant for the estimation of the results obtained, as the level of virus-inhibiting activity in the organism of stressed mice has always been 2 to 4 times higher than the baseline level (Fig. 2).

Preliminary experiments have shown that the treatment of sera at pH 2.0 or heating at 56 °C were without effect on the virus-inhibiting activity detected in the blood of mice that underwent stress. These data suggested the identity of stress-induced virus inhibiting activity and α-IFN, which is known to have similar properties (Soloviev, Balandin, 1981; Hayes, 1981). However, for final elucidation of the character of the described antiviral activity immunologic tests are required.

It can be speculated that stress-induced production of this factor can result from the activation of non-specific protective mechanisms. This is in a good accord with recent knowledge that the stress response promotes a systemic activation of nucleic acid and protein biosynthesis and thereby causes a general potentiation of host protective reactions (Meerson *et al.*, 1982). Moreover, it is known that increased Ca^{2+} inflow into cells is one component of the pathogenic stress-induced damage chain (Meerson *et al.*, 1984). Therefore, there are reasons to believe that this mechanism can also be of importance in stress activated IFN production. Orlova *et al.* (1985) had first demonstrated that Ca^{2+} ions neutralize in the cytoplasm the inhibitory activity of IFN repressor, which may be a necessary (though not a sufficient) condition of IFN production. Finally, it cannot be ruled out that detection of IFN in the blood of stressed animals indicated enhanced production of "physiologic" IFN which, according to Bocci (1980), is constantly present in the normal organism and can dramatically increase owing to intensive lymphocyte traffic. The latter is known to be enhanced during stress responses resulting in mobilization and redistribution of immunocompetent cells (Zimin, 1979, 1983).

Although stress itself causes the appearance of virus-inhibiting activity in the organism of mice, virus-induced IFN production appears to be suppressed in these animals as compared to that in unstressed mice (Figs. 3, 4). Several days thereafter, however, the ability of IFN synthesis becomes restored in stressed mice. The hyporeactivity phenomenon following vigorous IFN production is known, but its mechanism has yet to be elucidated (Borecký, 1986).

The established stress-induced conversion of nonlethal influenza into lethal form deserves a thorough discussion (Fig. 4). Whereas for the group of animals infected 24 hr after stress, this could occur due to the alteration of natural resistance, in particular of IFN production, this mechanism could not be of much importance for the animals infected on day 5, for IFN production in these animals was the same as in nonstressed ones. This seems to be also true of other factors of natural antiinfluenza resistance — natural killers and mononuclear phagocytes, whose functional activity had been reported to be restored 5 days after immobilization stress, or even to show a tendency to enhancement (Boranic *et al.*, 1983; Sukhikh *et al.*, 1984). Therefore, while analysing the mechanisms of death of stressed animals infected with sublethal dose of influenza virus we have considered the possibility of generalization of the virus infection, which could largely determine the severity of stress effects. A prerequisite for this was provided by the data indicating that various forms of stress were associated with impairment of physiological barriers, blood-brain barrier included (Bogdanova *et al.*, 1981; Belova, Yunson, 1983). It is known that increased permeability of the blood--brain barrier during influenza infection can result in penetration of the virus into CNS and its damage, either owing to reproduction of the virus in neurons and their destruction, or due to toxic effects of the virus (Polyakova *et al.*, 1985). This may aggravate the course of infection and thereby increase the death rate of animals.

The results obtained suggest the following statements. First, immobilization stress causes a temporary depression of virus-induced IFN production evident after application of a viral inducer at early intervals (24 hr) after stress. Second, high level of virus-inhibiting activity detected in mice after exposure to stress failed to protect the animals from subsequent development of lethal influenza infection. Third, lethal influenza infection might develop in the presence of high IFN levels in the blood confirming the earlier suggestion of the limited role of IFN in the pathogenesis of virus infection (Mentkevich *el al.*, 1967; Ennis *et al.*, 1981). Fourth, stress can aggravate the course of virus infection and thereby promote the transition of nonlethal infection into lethal one. Impairment of the blood-brain barrier and penetration of the virus into the brain tissue can be one of the causes of this observation.

We succeeded in preventing the conversion of the nonlethal form of influenza virus infection into a lethal one due to stress by prophylactic administration of the antioxidant ionol (Frolov *et al.*, 1986*b*). One of the main mechanisms in stress is the activation of lipid peroxidation, which generates

metabolites exerting adverse effects. Ionol reverted membrane peroxidation in stress and also prevented the increased permeability of the blood-brain barrier.

References

Belova, T. I., and Yunson, Yu. (1983): Damage of the blood-brain barrier after a single immobilization stress (in Russian). *Byull. eksp. Biol. Med.* **7**, 3–4.

Bocci, V. (1980): Is interferon produced in physiologic conditions? *Med. Hypothes.* **6**, 735–745.

Bogdanova, E. D., Kagan, V. E., Kuliev, I. Ya., Meerson, F. Z., and Prilipko, L. L. (1981): Activation of lipid perixodation in the brain and appearance of antibodies to brain antigens during stress (in Russian). *Immunologiya* **2**, 65–66.

Boranic, M., Gabrilowac, J., Manew, H., Pericic, D., Poljak-Blazi, M., and Sverko, V. (1983): Phagocytic and cytotoxic activity of peritoneal macrophages of rats stressed by repeated restraint and treated with amphetamine or propranolol. *Period. Biologorun* **85**, 295–299.

Borecký, L. (1986): Current view on the perspectives of interferon therapy. *Acta virol.* **30**, 161–169.

Ebert, L. Ya., Dolgushin, I. I., Marachev, S. I., and Kovynev, A. R. (1979): Acute endogenous bacteriemia as a means to increase the resistance of the body against extraordinary stimuli under the conditions of stress response (in Russian). pp. 16–19. In: *Factors of Natural Immunity During Different Physiologic and Pathologic States*, Chelyabinsk, *No* **6**.

Ennis, F. A., Beare, A. S., Riley, D., Schild, G. H., Meager, A., Yi-Hua, O., Schwarz, G., and Rook A. H. (1981): Interferon induction and increased natural killer-cell activity on influenza infections in man. *Lancet* **8252**, 891–893.

Friedman, S. B., Glasgow, L. A., and Ader, R. (1969): Psychosocial factors modifying host resistance to experimental infections. *Ann. N. Y. Acad. Sci.* **144**, 381–393.

Frolov, B. A., Chetverikova, L. K., Kramskaya, T. A.. and Polyak, R. Ya. (1986): The effect of immobilization stress on the course of experimental influenza infection and the process of interferon production (in Russian). *Zh. Mikrobiol. Epid. Immunol.* **1986** (3), 53–56.

Hayes, T. G. (1981): Differences between human leukocyte and fibroblast interferons. *Arch. Virol.* **67**, 267–281.

Jensen, M. M., and Rasmussen, A. F. (1963): Stress and susceptibility to viral infections. II. Sound stress and susceptibility to vesicular stomatitis virus. *J. Immunol.* **90**, 21–23.

Johnsson, T., Lavender, J. F., Hultin, E., and Rasmussen, A. F. (1963): The influence of avoidance-learning on resistance to Coxsackie B virus in mice. *J. Immunol.* **91**, 569–575.

Lockard, V. G., Grogan, J. B., and Brunson, J. J. (1973): Alterations in the bactericidal ability of rabbit alveolar macrophages as a result of tumbling stress. *Amer. J. Pathol.* **70**, 57–58.

Lukomska, B., Olszewski, W. L., Engesed, A., and Kolstad, P. (1983): The effect of surgery and chemotherapy on blood NK cell activity in patients with ovarian cancer. *Cancer* **51**, 465–469.

Marsh, J. T., Lavender, J. F., Chang, S.-S., and Rasmussen, A. F. (1963): Poliomyelitis in monkeys decreased susceptibility after avoidance stress. *Science* **140**, 1414–1415.

Meerson, F. Z., Pavlova, V. I., Frolov, B. A., and Vorontsova, E. A. (1982): Post-stress activation of nucleic acid and protein synthesis and its role in adaptation reactions of the organism (in Russian). *Patol. Fiziol. eksp. Ter.* **5**, 3–14.

Meerson, F. Z., Sikhikh, G. T., and Katkova, L. S. (1984): Adaptation of the organism to stress situations and prevention of stress-induced damages (in Russian). *Vestn. AMN SSSR* **4**, 45–51.

Mentkevich, L. M., Orlova, T. G., and Eremkina, E. I. (1967): Sensitivity of viruses to interferon and significance of this factor in pathogenesis of viral infections (in Russian). In M. V. Chumakova (Ed.): *Interferons and Interferonogens.* Transactions of the Institute of Poliomyelitis and Viral Encephalitides, pp. 173–179, Moscow.

Orlova, T. G., Bokhonko, A. I., Mamontova, T. V., and Kognovitskaya, A. I. (1985): Modulation of the induction of interferon production by calcium chloride (in Russian). *Vopr. Virusol.* **30**, 347–350.

Pavlidis, N.. and Chirogos, M. (1980): Stress-induced impairment of macrophage tumoricidal function. *Psychosom. Med.* **42**, 47–54.

Pavlushina, S. V., and Orlova, T. G. (1981): A method of rapid microtitration of interferons by

the delay of cytotoxic action of vesicular stomatitis virus (in Russian). *Vopr. Virusol.* **26**, 242—245.

Polyakova, T. G., Demidova, S. A., Bikbulatov, R. M., Ivanova, L. A., Krylov, V. F., Balnokina, S. I., Kelli, E. I., and Rudometov, Yu. L. (1985): Virologic and immunologic studies in patients with influenza and acute respiratory diseases associated with central nervous system damage (in Russian). *Vopr. Virusol.* **30**, 299—302.

Rasmussen, A. F., Marsh, J. T., and Norman, Q. B. (1957): Increased susceptibility to Herpes Simplex in mice subjected to avoidance-learning stress or restraint. *Proc. Soc. exp. Biol. Med.* **96**, 183—189.

Rasmussen, A. F. (1969): Emotions and immunity. *Ann. N. Y. Acad. Sci.* **164**, 458—464.

Selye, H. (1952): The history of the adaptation syndrome. *Acta Inc. Medical Publ.*, Montreal, Canada.

Solomon, G. F. (1969): Emotients, stress, the central nervous system and immunity. *Ann. N. Y. Acad. Sci.* **164**, 325—343.

Soloviev, V. D., and Balandin, I. G. (1981): Up to date state of knowledge about interferon. Progress and vistas (in Russian). *Immunologiya* **3**, 65—71.

Starling, J. L., and Norback, D. H. (1981): Pulmonary alveolar macrophage lysosomal enzyme activity after sublethal rotation trauma. *J. Surg. Res.* **31**, 128—135.

Sukhikh, G. T., and Meerson, F. Z. (1983): Inhibition of normal killer cell activity during emotional pain stress and elimination of this phenomenon by interferon inducer (in Russian). *Byull. eksp. Biol. Med.* **11**, 84—86.

Sukhikh, G. T., Nosik, N. N., Parshina, O. V., Vanko, L. V., Meerson, F. Z., and Ershov, F. I. (1984): Interrelations between the system of cellular cytotoxicity and interferon system during immobilization stress (in Russian). *Byull. eksper. Biol. Med.* **11**, 593—595.

Toge, T., Hirai, T., Takiyama, W., and Hattory, T. (1981): Effects of surgical stress on natural killer activity, proliferative response of spleen cells and cytostatic activity of lung macrophages in rats. *Jap. Cancer Res.* **72**, 790—794.

Zimin, Yu. I. (1979): Immunity and stress. (in Russian) In: *Scientific and Technological Achievements.* All Union Research Institute of Medical and Medico Technological Information Publishers, U. S. S. R. Academy of Sciences, **8**, 173—198.

Zimin, Yu. I. (1983): Stress: immunological aspects (in Russian). In: *Scientific and Technological Achievements.* All Union Research Institute of Medical and Medico-Technological Information Publishers, U. S. S. R. Academy of Sciences, **12**, 41—62.