is predominantly activated by the calcium supplied via the voltage-dependent and partially via the receptor-controlled channels.

REFERENCES

1. T. P. Storozhevykh, D. V. Zagulova, V. G. Pinelis, Kh. M. Markov, *et al., Byull. Eksp. Biol. Med.*, **116**, № 10, 374-376 (1993).
2. M. F. Shuba, N. I. Gokina, A. V. Gurovskaya, et al., in: *The Mechanisms of Excitation and Contraction of Smooth Muscle in Brain Vessels* [in Russian], Kiev (1991), p. 167.
3. S. F. Flaim, P. H. Ratz, S. C. Swigart, and M. M. Gleason, *J. Pharmacol. Exp. Ther.*, **234**, 63-71 (1985).
4. T. Godfraind, *Acta Pharmacol. Toxicol.*, **58**, Suppl. 11, 5-10 (1986).
5. K. Ijima, A. Nasjletti, and M. S. Goligorsky, *J. Physiol.*, **260**, № 5, Pt. 1, C982-C992 (1991).
6. K. Okada, S. Ishikawa, and T. Saito, *J. Cardiovasc. Pharmacol.*, **17**, Suppl. 7, 124-126 (1991).
7. C. van Renterghem, P. Vigne, J. Bargamin, *et al., J. Cardiovasc. Pharmacol.*, **13**, Suppl. 5, 186-187 (1989).
8. A. Saito, R. Shiba, S. Kimura, *et al., Europ. J. Pharmacol.*, **162**, 353-358 (1989).
9. M. S. Simonson and M. J. Dunn, *Hypertension*, **15**, № 2, Suppl. 1, 5-12 (1990).
10. M. S. Simonson and M. J. Dunn, *Exp. Cell Res.*, **192**, 148-156 (1991).
11. M. Yanagisawa, H. Kurihara, S. Kimura, *et al., Nature (London)*, **332**, 411-415 (1988).

The Possibility of Using Glutathione as a Protector during Exposure to Hypoxia

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The disturbance of energy metabolism in the cell during hypoxia is primarily associated with restricted activity of the respiratory chain at the level of the 1st enzyme complex [2-5,8,9].

Recovery of the electron-transporting function of the NAD-dependent site of the respiratory chain may appreciably reduce the damage caused by hypoxia and raise the organism's resistance to oxygen deficiency. One of the possible methods of thus restoring the work of the respiratory chain is the use of artificial electron acceptors, which can directly transfer electrons from NADH to the 3rd enzyme complex, bypassing the blocked site [4,5,9,13].

Possible protective, antihypoxic properties of glutathione, a natural metabolite of the organism and an active acceptor of reduction equivalents, were studied in the present work.

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MATERIALS AND METHODS

The study was carried out on females and offspring of albino nonpedigree rats kept on a standard diet in the vivarium. Resistance to hypoxia was assessed in the offspring of rats administered an aqueous solution of reduced glutathione in a dose of 15 mg/kg intragastrically on days 16-19 of gestation. The time of survival at an "altitude" of 11,000 m (reserve time, RT), determined after Purshottam [16], served as the test of individual resistance to acute hypoxia.

The effect of glutathione on the hypoxic myocardium was studied on the model of the perfused heart. Animals with a low resistance to hypoxia

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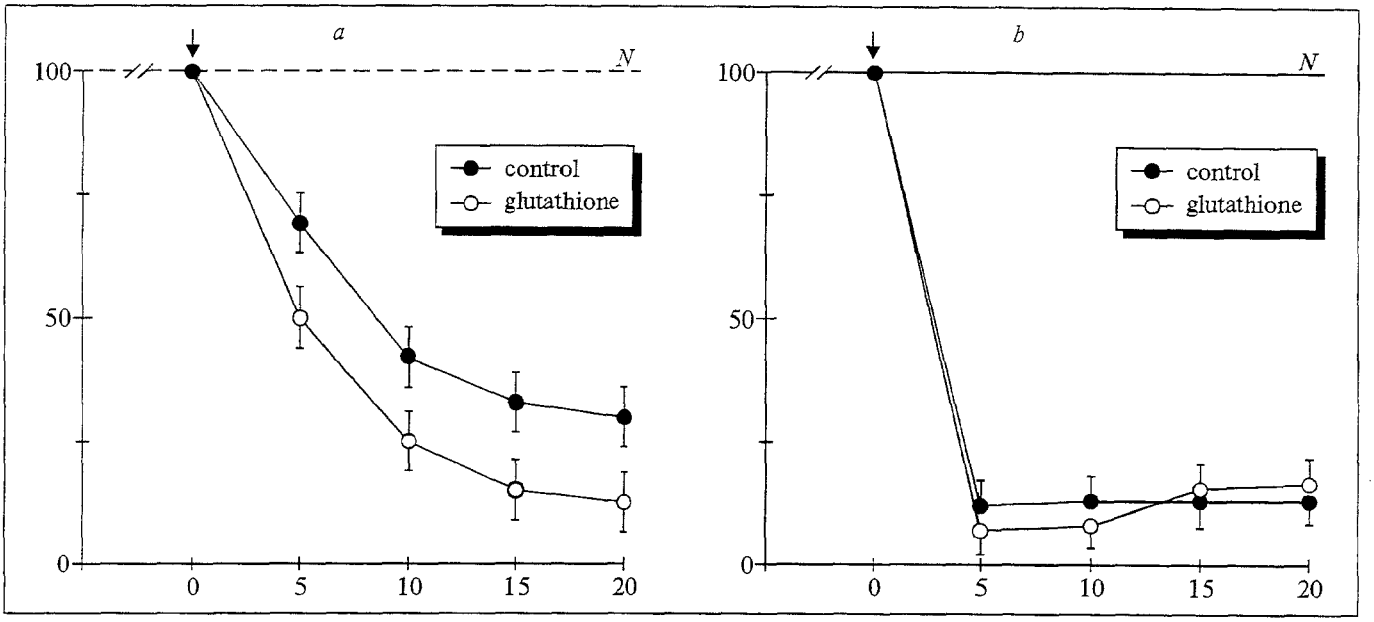

Fig. 1. Effect of glutathione on activity of hypoxic myocardium. *a*) "work" of perfused heart (*a*); *b*) rate of oxygen uptake in myocardium (VO_2);. N: normal physiological level. Arrow indicates start of hypoxic perfusion, taken as the zero point of monitoring.

are optimal objects for such testing [4,6]. In female rats, resistance to hypoxia drops sharply during gestation, bottoming out directly before the delivery (on the 20th day of pregnancy) [6]. Therefore, female rats of a 20-day gestation were used as the test objects for assessing the antihypoxic properties of glutathione. As in the previous series of experiments, glutathione in a dose of 15 mg/kg was administered intragastrically to female rats on days 16-19 of gestation. On the 20th day the animals were sacrificed; the heart was isolated and perfused after Langendorff [15]. The pefusion fluid was oxygenated with the following gas mixtures: 95% O_2 + 5% CO_2 (normoxic mixture) and 20% O_2 + 75% N_2 + 5% CO_2 (hypoxic mixture).

The heart rate and heartbeat force were recorded. The amount of "work" performed by the heart was calculated as the product of the heart rate and heartbeat force. This parameter is directly proportional to the level of energy production in the myocardium and to the ATP expenditure [4,5]. The rate of oxygen uptake was polarographically determined in the isolated heart [4].

The experimental results were statistically processed using Student's *t* test. All the numerical data are presented as $M+m$.

RESULTS

Analysis of the results of the experiments performed on the perfused heart provides evidence that glutathione has cardioprotective, antihypoxic properties. For instance, cardiac activity during hypoxia is markedly higher in the animals administered glutathione than in the control (Fig. 1). Since the "work" of the heart is an integrated parameter reflecting the degree of energy production in the myocardium [4], its increase in the presence of glutathione reflects the energy-supplying effect of the latter. However, such an effect of glutathione is not attended by an increased oxygen uptake in the hypoxic myocardium (Fig. 2), this indicating a higher efficacy of the ATP-synthesizing systems and an elevated oxidation/phosphorylation ratio.

To date, improvement of the energy state of the hypoxic myocardium has been mainly achieved by the use of drugs raising the intensity of oxygen utilization in it. For instance, the antihypoxic effects of hydroquinone and menadione cause not only a marked enhancement of cardiac activity and elevation of the ATP level, but also an increased oxygen uptake in the myocardium of animals not resistant to hypoxia [4,5,9]. The mechanisms of such an effect of quinones are associated with the fact that, being good oxidants for reduced forms of pyridine coenzymes, they are capable of bypassing the initial site of the respiratory chain blocked during hypoxia. This offers the possibility of restoring electron transport in the terminal portions of the respiratory chain, resulting in an increased oxygen uptake, and ATP production becomes possible at the terminal sites of oxidative phosphorylation.

Since glutathione does not raise the oxygen uptake in the hypoxic myocardium, the mecha-

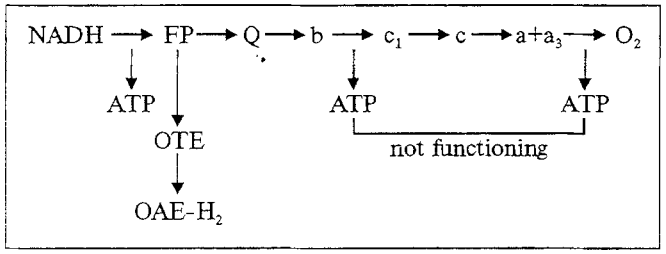

Fig. 2. Scheme of work of respiratory chain in ascarid muscles during anoxia. See text for explanation.

nism of its action is not confined to bypassing of the respiratory chain, as is true for quinones. Evidently, a direct effect of restorating respiratory chain activity may also be ruled out for glutathione, because such a process would necessarily be attended by an increased oxygen uptake.

We speculate that the following mechanism of action of glutathione takes place during hypoxia: when the respiratory chain is blocked and a large amount of reduced forms of coenzymes is accumulated at the NAD-dependent site, glutathione may act as a natural electron acceptor, reducing the hyperreduction in the initial site of the respiratory chain. At the same time, however, the electron flux is not directed toward the terminal portions of the respiratory chain (as occurs in the presence of quinones), but may be utilized in specific glutathione-mediated reactions.

Although such a mechanism of action of glutathione does not presume the recovery of the electron-transporting function of the terminal portions of the respiratory chain, its patent energy-ergic properties manifest themselves in the experiments. One may assume that the protective effect of glutathione is, in one way or another, associated with restoration of the ability of the respiratory chain to produce ATP. This may occur at the first site of oxidative phosphorylation.

Such natural phenomena are well known. For instance, in the absence of oxygen, only one, the 1st, site of oxidative phosphorylation functions in the mitochondria of ascarid muscle [1]. In this case, the electron flux from NADH via flavoprotein (FP) is perceived not by ubiquinone (Q), but by an organic acceptor of electrons (OAE); volatile fatty acids (C_3-C_6) may serve as the latter (Fig. 2). Such a protective-compensatory mechanism enables the ascarid to obtain the necessary minimum of ATP even during anoxia.

The protective, antihypoxic effect of glutathione has been confirmed by the results of our findings on the organism's individual resistance to acute hypoxic hypoxia determined in the experiments on the offspring of rats given glutathione.

Probably, the high resistance of the newborn to hypoxia which has been noted by many researchers [6,7,11,12,14] stems from the fact that energy metabolism does not depend on the NADH-oxidase oxidation pathway, which is primarily disturbed during hypoxia, and is practically entirely maintained by the oxidation of fatty acids. The state of increased resistance to hypoxia is preserved by the newborn pups over the whole period of nursing (Fig. 3). During the period of weaning (16th-30th day of life in rats), when the mother's milk starts to be gradually replaced with general hydrocarbon-rich food, a pronounced drop of individual resistance to hypoxia occurs (Fig. 3).

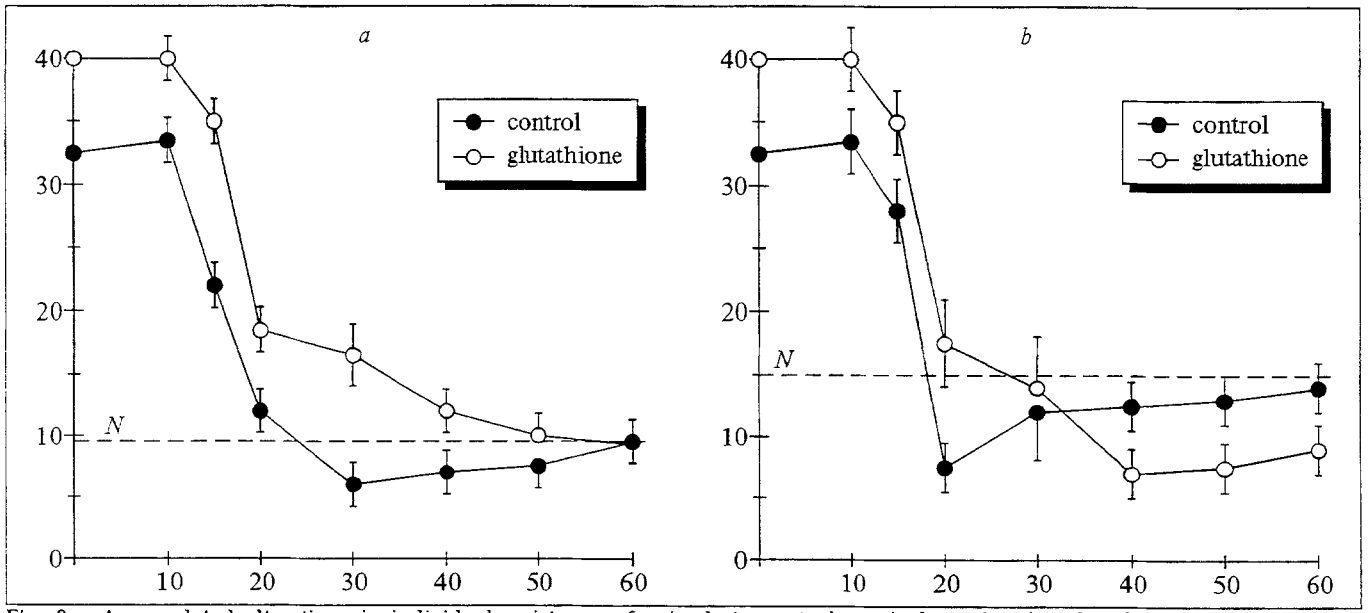

Fig. 3. Age-related alterations in individual resistance of animals to acute hypoxic hypoxia. *a*) males; *b*) females. N: average level of individual resistance to hypoxia in adult animals.

The lowest level of resistance is observed in female rat pups on the 20th day of life, and in male rat pups on the 30th day.

Following such a critical decrease of the organism's resistance to hypoxia, a gradual continuous increase occurs, until on day 60 the level typical of adult animals is attained (Fig. 3).

Intragastric administration of glutathione to female rats during gestation resulted in a higher resistance of the offspring to acute hypoxic hypoxia as compared with that in the control (Fig. 3). The newborn pups showed a sufficiently high level of resistance even during the critical period of weaning. In male pups, such an enhanced resistance to hypoxia caused by glutathione was preserved over the entire developmental period followed up, and on the 60th day of life this parameter attained the control level observed in adult animals.

Evidently, glutathione administered to the female in the last trimester (when its tolerance of hypoxia is lowest [6]) stimulates the development of adaptative-compensatory mechanisms directed toward raising the resistance to hypoxia in the organisms of both mother and fetus. These protective mechanisms continue to function actively in the postdelivery period, especially during perinatal and early postnatal development, when the risk of hypoxic damage is especially high.

To summarize the results of our findings, we may conclude that glutathione, a natural metabolite of the organism, exhibits properties of an active antihypoxic agent and/or of a protector with an energy-supplying effect. The mechanisms of its action may be associated with a decrease of the hyperreduced state of the respiratory chain and restoration of its ability to produce ATP at the first site of oxidative phosphorylation.

REFERENCES

1. E. Budding, *Proceedings of 5th International Biochemical Congress*, Moscow (1962), Vol. 3, p. 280.
2. M. N. Kondrashova, E. I. Maevskii, and T. B. Babayan, in: *Mitochondria* [in Russian], Moscow (1973), p. 112.
3. M. N. Kondrashova and E. I. Maevskii, in: *Mitochondrial Processes in the Timetable of Vital Activity* [in Russian], Pushchino (1978), p. 6.
4. A. A. Korneev, *Study of Certain Oxygen-Dependent Processes on the Isolated Contracting Heart during Hypoxia (Dissertation submitted for the degree of Candidate of Biological Science)*, Moscow (1985).
5. A. A. Korneev, O. A. Popova, S. V. Zamula, and L. D. Luk'yanova, *Byull. Eksp. Biol. Med.*, **110**, № 7, 60 (1990).
6. A. A. Korneev, G. A. Sheveleva, and N. N. Zaripova, *Akusher. Ginekol.*, № 10, 56 (1990).
7. A. A. Korneev, *Pat. Fiziol. Eksp. Ter.*, № 1, 41 (1991).
8. L. D. Luk'yanova, in: *Pharmacological Correction of Hypoxic States* [in Russian], Moscow (1989). p. 11.
9. L. D. Luk'yanova, G. N. Chernobaeva, I. G. Vlasova, *et al., Eksp. Klin. Farmakol.*, № 1, 44 (1992).
10. D. E. Metzler, *Biochemistry. The Chemical Reactions of Living Cells*, Vol. 1, Academic Press, New York-London (1977).
11. Yu. I. Savchenko and K. S. Lobyntsev, *Essays on the Physiology and Morphology of the Functional System Mother-Fetus* [in Russian], Moscow (1980).
12. G. J. Cooney, H. Taegtmeyer, and E. Newsholme, *Biochem. J.*, **200**, 701-703 (1981).
13. L. Danielson and L. Eruster, *Nature*, **194**, 155 (1962).
14. J. M. Jarmakani, M. Nakazawa, T. Nagamoto, and G. A. Longer, *Amer. J. Physiol.*, **235**, № 5, 469 (1978).
15. O. Langendorff, *Arch. Ges. Physiol.*, **61**, 291 (1895).
16. T. Purshottam and N. C. Chosh, *Aerospace Med.*, **43**, № 6, 610 (1972).