EFFECT OF ARTIFICIAL GRAVITY IN SPACE FLIGHT ON PROTEIN AND RNA CONTENT IN ANTERIOR HORN MOTONEURONS OF THE RAT SPINAL CORD

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A previous investigation revealed metabolic disturbances in the structures of the spinal reflex arc (anterior horn motoneurons of the spinal cord and nerve cells of the spinal ganglia) of rats after orbital space flights lasting 19.5 and 22.5 days [4]. It was decided to analyze the results obtained in experiments on rats exposed on the "Kosmos-936" biosatellite. Besides factors of space flight, the rats were also exposed to the effect of artificial gravity, created by spinning on a centrifuge (radius 34 cm) throughout the period of space flight, with a constant acceleration of 1 g (in the spine-sternum direction). It was considered that the results of this analysis could help to decide whether the metabolic changes observed were the result of exposure to weightlessness or to other flight factors.

EXPERIMENTAL METHOD

Material for investigation was taken from rats exposed to weightlessness (ten animals) and to artificial gravity on a centrifuge (nine animals) during a flight lasting 18.5 days, 4.5-9.5 h and 25 days after returning to earth. Samples of spinal cord tissue at the level of the lumbar enlargement, 2-3 mm thick, were fixed in Carnoy's fluid and embedded in paraffin wax. Anterior horn motoneurons were isolated from sections 40 μ thick with the aid of a de Fonbrune's micromanipulator under the control of an MBI-3 microscope. RNA was determined in separate isolated neurons by Edström's micromethod [5], modified by Maksimovskii [2]. The RNA content in a single cell was calculated by the method of Slagel and Edström [8]. The dry mass of cytoplasm and nuclei of the motoneurons was determined by interferometry in sections 7 μ thick, on a "Leitz" dual-beam interference microscope. The volume of the cell bodies and nuclei was determined by the equation for an ellipsoid of rotation. The numerical results were subjected to statistical analysis [3]. Ten rats kept in the animal house served as the control.

EXPERIMENTAL RESULTS

The RNA content in the motoneurons of the anterior horns of the spinal cord of rats under conditions of weightlessness and artificial gravity during flight, determined 4.5-9.5 h after landing, remained at the control level (Table 1). The volume of the motoneuron bodies and nuclei was significantly reduced in rats exposed both to weightlessness and to artificial gravity, by 37 and 35% and by 22 and 13%, respectively (Table 2). The RNA concentration in the cells, calculated per unit volume and per unit dry weight, was higher than the control in rats exposed to weightlessness by 43 and 60%, respectively, and in rats exposed to artificial gravity by 21 and 43%, respectively (Table 1). The protein content in the cytoplasm of the motoneurons from rats exposed to weightlessness was lower than in rats of the control groups by 42%, and in rats exposed to artificial gravity by 30%; the protein content in the nuclei was lower by 31 and 24%, respectively (Table 3).

The volume of the bodies of the motoneurons in rats exposed to artificial gravity during flight, measured 25 days after the flight, was at the control level, whereas the volume of the nuclei of the motoneurons in rats exposed to weightlessness was 12% greater than in the control animals (Table 2). The RNA content in the motoneurons of rats exposed to weightless-

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TABLE 1. Content and Concentration of RNA in Anterior Horn Motoneurons of Spinal Cord after Space Flight Lasting 18.5 Days (M ± m)

Group of animals	Time after flight	Number of neurons	RNA content, pg	RNA concentration	
				per unit volume of cell, pg/μ^3	per unit dry weight of cells†
Control	—	109	480±16	0,014±0,0007	0,06±0,003
Rats under conditions of weightlessness	$4^1/_2$—$9^1/_2$ h	84	436±17	0,02±0,0012*	0,096±0,0069*
	25 days	154	587±16*	0,018±0,001*	0,074±0,0056
Rats exposed to artificial gravity	$4^1/_2$—$9^1/_2$ h	79	458±18	0,017±0,00096*	0,086±0,0061*
	25 days	102	516±17	0,016±0,0014	0,076±0,0064

*Here and in Tables 2 and 3, difference compared with control is significant.
†Units not given in original Russian — Consultants Bureau.

TABLE 2. Volume of Bodies and Nuclei of Motoneurons in Rats after Space Flight Lasting 18.5 Days (M ± m)

Group of animals	Time after flight	Number of neurons	Volume of bodies	Volume of nuclei
			μ^3	
Control	—	110	33 829±1295	2910±90
Rats under conditions of weightlessness	$4^1/_2$—$9^1/_2$ h	121	21 469±1050*	1896±82*
	25 days	69	31 065±1514	3268±129*
Rats exposed to artificial gravity	$4^1/_2$—$9^1/_2$ h	155	26 372±997*	2541±83*
	25 days	52	33 342±1386	2887±113

TABLE 3. Protein Concentration in Cytoplasm and Nuclei of Anterior Horn Motoneurons of Rat Spinal Cord after Space Flight Lasting 18.5 Days, Calculated Per Unit Volume (M ± m)

Group of animals	Time after flight	Number of neurons	Protein content, pg	
			in cytoplasm	in nucleus
Control	—	171	7884±353	477±21
Rats under conditions of weightlessness	$4^1/_2$—$9^1/_2$ h	120	4541±291*	321±21*
	25 days	80	7839±549	654±37*
Rats exposed to artificial gravity	$4^1/_2$—$9^1/_2$ h	116	5290±296*	361±18*
	25 days	90	6736±512	520±30

ness during flight was increased by 22%, whereas in animals exposed to artificial gravity it was the same as in the control. The RNA concentration calculated per unit dry weight was the same as the control in rats exposed to weightlessness, whereas if calculated per unit volume it was increased by 29% (Table 1). In rats exposed to artificial gravity neither parameter differed from the control. The protein content in the cytoplasm of the motoneurons returned to normal in animals of both experimental groups, but the protein content in the cell nuclei of rats exposed to weightlessness during flight was 37% greater than the control (Table 3).

In animals exposed to artificial gravity and to weightlessness during flight, the protein content in the motoneurons of the anterior horns of the spinal cord was reduced, as also was the volume of the cell bodies, but these changes differed in intensity in the rats of the two experimental groups. Whereas in the early period after returning to earth, the decrease in the volume of the motoneuron bodies of rats exposed to weightlessness was 37%, in animals exposed to artificial gravity it was only 22%; the volume of the nuclei was reduced by 35 and 13%, respectively. This applies equally to the other parameters studied (Tables 1-3). Comparison of the results obtained with animals of the different experimental groups suggests that the decrease in the protein content in the cytoplasm and nuclei of the spinal motoneurons was

the result of changes in the functional state of the nerve cells in weightlessness. One possible factor determining these changes in the final component of the motor system — the spinal motoneuron — was a decrease in the flow of information from the skeletal muscles and bones as a result of their deficient functional load. Changes in the cytochemical and morphological parameters of the nerve cells could be regarded as responsible for adaptation to the new conditions of existence in weightlessness and were attributable primarily to removal of the static load from the locomotor apparatus. The presence of a static load on the locomotor apparatus in the experiment with artificial gravity and maintenance of the working tone of the skeletal muscles [1] in all probability lead to intensification of the flow of impulses from the skeletal muscles and bones to the CNS, so that the exchange of information, although incomplete, is thereby stabilized.

It is an interesting fact that readaptation of animals exposed to weightlessness or to artificial gravity during flight followed a different course. Whereas 25 days after landing, metabolism of the motoneurons, so far as all the parameters studied are concerned, returned completely to normal in rats exposed to artificial gravity during flight, in animals exposed to weightlessness the RNA content in the cells, the volume of the nuclei, and their protein content were all increased, evidence of increased functional activity of the genetic apparatus of the cell. This may also be evidence of differences in the intensity of changes in the CNS neurons in animals exposed to weightlessness and to artificial gravity.

In the writers' view, the results of these investigations showing a decrease in the protein content in the cytoplasm and nuclei of spinal motoneurons of rats exposed to weightlessness may be the result of removal of the static load from the locomotor apparatus of the animals during space flight.

LITERATURE CITED

1. E. I. Il'ina-Kakueva and V. V. Portugalov, Arkh. Anat., No. 3, 22 (1979).
2. L. F. Maksimovskii, Tsitologiya, No. 4, 522 (1969).
3. N. A. Plokhinskii, Biometrics [in Russian], Moscow (1970).
4. A. V. Gorbunova, V. V. Portugalov, et al., Izv. Akad. Nauk SSSR, Ser. Biol., No. 1, 18 (1979).
5. D. Slagel and J. E. Edström, J. Cell Biol., 34, 395 (1967).