PATHO-MORPHOLOGICAL AND HISTOCHEMICAL CHANGES IN THE ORGANS OF TURTLES ON BOARD THE "ZOND-5" PROBE

by N. A. Gaidamakin, G. P. Parfenov, V. G. Petrukhin,
V. V. Antipov, P. P. Saksonov, and A. V. Smirnova

Paper presented at the 18th IAF Conference, La Plata, Argentina, 1969

Translated from Russian by Morris D. Friedman

Edited by George R. Zug and James A. Peters

SMITHSONIAN HERPETOLOGICAL INFORMATION SERVICES

1970

SHIS NO. 24

Additional copies available from:

Division of Reptiles and Amphibians
United States National Museum of Natural History
Washington, D. C. 20560

Some reptiles, particularly turtles, are convenient for specific biological investigations in space for a number of reasons. The organization level in these animals is not much lower than in mammals, moreover, no complex special systems are needed, and they may be fixed rigidly on board the spacecraft. Taking account of seasonal differences, the subject is accessible for research at any time of the year. For these general considerations, tests with turtles were included in the space biology research program.

Steppe turtles (Testudo horsfieldi Gray) were on the "Zond-5" which flew around the moon together with other biological subjects. In all 8 adult turtles 6-7 years old, and 340-400 g in weight, were examined. Two animals (the test group) were on the probe (Fig. 1), two (the control group) were transported to the cosmodrome and back, and four turtles (intact) were in a vivarium.

The animals were delivered to the laboratory two months before the beginning of the experiment. During this time, the turtles were weighed repeatedly, the peripheral blood was investigated and an EKG was recorded at three standard terminals. Moreover, the alimentary activity of the animals was observed carefully. Their daily ration was meat (2 g), cabbage (10 g), carrots (10 g), bread (10 g).

The experimental animals were placed in individual narrow cages on the probe, in which they were practically unable to move. Contained in these same cages were the control animals. The experimental and control turtles received no food or water throughout the whole experiment, the intact turtles were in free cages in a customary environment.

The turtles were put on board the "Zond-5" on 2 September 1968. From that time on they ceased to receive food. As is known, lift-off was on 15 September. After the circumlunar flight and a return to earth, the probe splashed down on 21 September in the Indian Ocean. The subject reached Bombay 3 October, and was returned to Moscow on 7 October. Patho-morphological investigations on the turtles were carried out on 11 October.

As is seen from the cited sequence of carrying out the experiment, the test animals were subjected to 39 days of starvation, flight factors lasting 7 days, the effect of a tropical climate and conditions associated with a stay in the ocean after splashdown, and with transportation via ship and aircraft. According to results of dosimetry conducted on the probe, the total radiation dose received by the test animals did not exceed 3.5 rad.

Excluded from the control group was the influence on the animals of not only space flight factors, but also the tropical climate and additional transportation conditions.

The biological effect of the complex of space flight factors and other conditions originating during the experiment was estimated by using some hematological tests, electrocardiography, a number of patho-morphological and histochemical methods of investigation. The electrocardiography was carried out prior to the beginning of the experiment, after circumlunar flight, and return of the animals to the laboratory.

During dissection of the animals, combined tissue blocks from pieces of intestine, spleen, testes and seminal vesicle, liver, kidney, and heart were formed by an original method developed specially for subsequent histological and histochemical investigations.

The principle behind the method of combined tissue blocks is that an organ taken from the test animal is glued to filter paper together with the same organ removed from a control and intact animal. The tissue blocks thus prepared are frozen in dry ice and combined sections are prepared in a cryostat for histochemical investigations. Analogous blocks, not frozen, are placed in a fixing mixture, carried out by dehydrating and compressing media to obtain sections treated by histological methods. Preparation of the sections on a microtome of combined blocks, enclosed in paraffin and stained by appropriate methods, was carried out in the customary order. Therefore, under one cover glass we had an identical thickness, prepared under identical conditions, of combined sections of organs of the different animals. This permitted a reliable comparison between the structural changes in the organs of the test, control and intact animals. The sections were colored by hematoxylin-eosin, ribonucleic acid (RNA) was revealed by methyl green pyronine according to Brash, glycogen according to Shabadash, lipides by scarlet, iron according to Perls. Also assayed were the activities of succinate-dehydrogenase (SDG) according to Nachlas et al., monoaminooxidase (MAO) according to Glenner, alkaline phosphatase (AP) according to Gomor (E. Pierce, 1962), and of alpha-glycerophosphate dehydrogenase according to D. Quadlino et al. (1960) in a modification by R. P. Nartisissov (1968).

Externally, and in behavior, the animals of all three groups displayed no differences at the time of examination.

The alimentary activity in both the test and control animals flying to the cosmodrome and in the intact animals in the vivarium was identically high. Weight loss of the animals that flew in the probe was about 10%. Weight loss of the control animals was only 5%. No substantial differences in the peripheral blood of the examined animals was detected.

An analysis of the electrocardiograms recorded at different times before and after the flight did not disclose any noticeable differences in the cardiac activity of either the test or control animals. According to our data, the frequency of cardiac contraction in the animals in the active

state in the vivarium fluctuated between 14 - 48 contractions per minute. A definite arrhythmia evidently of vagus origin is characteristic for turtles. In our observations the R-R interval fluctuated between 20-49 sec. The repetition of cardiac contractions by 10-12 beats per minute occurred under a greater stimulus (injection by a needle in the gastrocnemius). Return of the pulse frequency to the original level occurred relatively quickly, in about 1-2 minutes.

On the 21st day after termination of the flight an EKG was recorded and some results of this investigation are presented in Table 1 and Fig. 2.

TABLE 1. CHARACTERISTICS OF CARDIAC ACTIVITY OF TURTLES WHO FLEW IN "ZOND-5"

No.	Animal Number	Heart Contraction Frequency per Minute	R-R Interval in sec. Drawing Rate 15 mm/sec
1	22 Test	28	30-49
2	37 Test	28	32-34
3	49 Control flying to cosmodrome	32	29-31
4	47 Control in vivarium	30	22-23

As is seen from the Table and the EKG, no substantial differences between the test and control were detected in a number of the indices studied, (cardiac contraction frequency, R-R interval). As regards the change in the individual EKG peaks, it is impossible to make any definite conclusions because of insufficient data.

In a macroscopic investigation of the internal organs it was clear that the thickness of the intestine diminished over the whole extent of the test and control turtles. The liver surface in the test animals was intensely brown; in the intact turtles, it was dark cerise, and was intermediate between these colors in the control animals.

Significant differences were observed in a comparative microscopic investigation of the organs of the experimental and the intact turtles. The diameter of the intestine and the thickness of the muscle layer diminished in the test animals, the villi were shortened in places (Fig. 3). The mitotic activity of the epithelial crypts was suppressed. Certain epithelial cells had a pycnotic nucleus (Fig. 4). The cells of the stroma of the villi in the cytoplasm and some epithelial cells contained clumps of brown pigment not discernable in the intact animals. The content of the PAS - positive substances was diminished (Fig. 5). The number of beaker-like cells diminished, particularly in the depth of the crypt, the MAO and alpha-glycerophosphate dehydrogenase (GFD) activity decreased.

The spleen follicles were diminished and none contained mitotic figures. Some lymphocytes had pycnotic nuclei, which were not noted in the intact animals.

The seminiferous tubules in the testes were diminished in diameter (Figs. 6, 7), and brown pigment accumulated in the interstitial tissue. It was manifest only in individual cells in the intact animals. This pigment was also detected in many remaining germ cells, had a dark orange tint in uncolored preparations, gave a negative reaction on iron, and was colored moderately by sudan. Conglomerates of the pigment possessed birefringence. The number of germ cells and the concentration of RNA therein diminished considerably (Figs. 6, 7). The SDG activity increased somewhat, but the MAO and alpha-GFD activity decreased.

The seminal vesicles of the test turtles were destroyed (Fig. 8), their lumen was filled with spermatozoids (Fig. 9) in the intact animals. The RNA concentration and ferment activity in the seminal vesicles was changed as compared with the intact control animals exactly as in the testes.

A diminution in the size of the hepatocytes and their nuclei was observed in the liver, the cell cytoplasm became basophile (Fig. 10). The quantity of brownish, big-clump pigment was increased at places where the endothelial-reticular cells accumulated, and dustlike granules of trivalent iron increased in the lumen of the bile capillaries. Ribonucleic acid was contained in the nucleoli of liver cells and in a small quantity in the perinuclear zone. The RNA concentration was negligible, but higher than in the intact animals. The glycogen content (Figs. 11, 12) in not only the hepatocytes but also in the peripheral blood cells was also elevated, principally in the leucocytes and in the free reticular cells in the lumens of the vessels. Fat vanished from the liver cells, while it appeared in a significant amount (Fig. 13) in the intact animals. In the liver of the intact turtles there was almost no outcropping of formazane corresponding to the SDG activity, they appeared in some hepatocytes in the test animals, in many Kupfer cells, the vessel walls, and the bile ducts; the AP activity was also elevated. On the other hand, the MAO activity in the hepatocytes was clearly reduced (Fig. 14), and suppressed altogether in the peripheral blood elements.

The volume of cell nuclei of the epithelium of the twisted ducts was diminished in the kidneys, some nuclei became pycnotic, the cell outlines unclear, grains of brown pigment sometimes appeared in the cell cytoplasm, which gave no reaction with iron and was not colored by sudan, and dustlike granules of an iron-containing pigment appeared. Moreover, RNA appeared more weakly in the nucleoli in the epithelium cells of many twisted ducts. The SDG activity, and to a lesser degree, the AP activity, was elevated, while the MAO and alpha GFD activity was lowered.

No changes were noted in the hearts of the test animals.

The same changes were outwardly noted in the control turtles which reached the lift-off point of the probe and were subjected only to the effect of hunger, as in the test animals. The degree of the changes in some organs was less evident. There were less cells with hyperchromic nuclei in the epithelial crypts of the intestine in the control turtles, single mitoses were evident in the crypts of one of the turtles. The mitotic activity of the cells was noted also in the spleen follicles, which while they were diminished as compared with the spleen follicles of the intact animals, they were still somewhat coarser than in the test animals, i.e., the size of the ducts and the number of germ cells were diminished, and an increase in lipofuscin was observed in the interstitial tissue. In the seminal vesicles of the control animals, in contrast to the test turtles, spermatozoids were present (Fig. 15). Moreover the SDG, AP, alpha GFD activity in the testes and walls of the seminal vesicles changed slightly. Less definite changes in the fermentative activity, particularly of MAO, were detected in the liver also.

Therefore, the diameter of the intestine, the thickness of the muscle layer, and the length of the villi of the mucosa diminished in the test and control turtles. Cells with pycnotypic nuclei and lipofucsin inclusions appeared in the epithelial crypt, the number of beaker-like cells diminished. All this indicates the development of atrophy of the intestine connected with starvation. Its functional activity was reduced, as shown by the suppression of the mitotic activity of the epithelial crypts, and the reduction of the RNA concentration in the epithelial cells of the mucosa.

Starvation and dehydration of the organism caused changes of atrophic character in other organs also: disappearance of lipids from the liver; diminution in the magnitude and volume of liver and kidney cells; accumulation of lipofucsin in these organs, particularly in the interstitial tissue of the testes; diminution in the diameter of the sperm channels and the number of germ cells of the spermatogenic epithelium; disappearance of spermatozoids from the seminal vesicles.

In our opinion, a certain increase in the RNA concentration and the glycogen content in the liver cells is associated with the decrease in the cell volume and with their dehydration.

The rise in iron content in the liver and kidneys is possibly associated with hemolysis of the erythrocytes, as well as with the diminution in its demand for hematopoesis purposes, which are suppressed during starvation.

Atrophy is caused by changes in the fermentative activity of the tissues: in the intestine walls, in the testes, liver and kidneys the SDG activity increases, as to a lesser degree does the AP activity, while the MAO and alpha-GFD activity is reduced.

However, all the listed changes in the turtle organs cannot possibly be explained by just the effect of starvation. The fact is that changes were less definite in the control turtles which were starved at the same time as the test animals. Thus, pycnotypic cells were rarely encountered in the epithelial crypt of the intestines in the control animals; single figures of mitosis could be encountered. Spermatozoids were detected in the seminal vesicles in almost the same quantity as in the intact turtles. The diminution in follicle volume was less definite. Mitotic cell fission was manifested in some follicles. The activity of the ferments in the tissues also changed less noticeably.

Apparently such a difference should be explained by the additional effect of the space flight factors. A higher degree of reduction in the MAO activity in the organs of the test animals can indicate this in particular. As is known, monoaminooxidase is a self-oxidizing ferment, participating in the regulation of the exchange of biologically active compounds, including serotonine. The serotonine level in the blood changes under the influence of individual flight factors such as overloads, vibrations, penetrating radiation (V. V. Parin, et al., 1964-1965; V. V. Antipov, et al., 1967).

If peculiarities in ecology, the quite definite seasonal fluctuations in physiological activity which is elevated in turtles during the warm part of the year, are taken into account, then it is here impossible to exclude also the influence of residence of the animals under tropical climate conditions. Also, ship transportation conditions from the splashdown point could be important.

Therefore, the results obtained indicate that the complex of space flight factors combined with starvation caused changes of atrophic nature in the turtle organs: a diminution in the intestine walls and in the diameter of the sperm channels, in the volume of liver and kidney cells, and in the number of germ cells of the epithelium of the testes, an accumulation of lipofucsin in the organs, suppression of the mitotic activity of the epithelium of the mucous of the intestine, and the hematopoetic tissue of the spleen. Also the fermentative activity of the cells changed. Starvation and transportation to the cosmodrome resulted in less definite atrophy of the tissues.

A comparison of the changes occurring in the test and control animals showed that the fundamental structural changes in the turtles were caused by starvation, and to a lesser degree, by space flight factors.

FIGURE LEGENDS

Fig. 1. Turtles Nos. 22 and 37 on board the "Zond-5" during flight to the moon.

Fig. 2. Electrocardiogram of the turtles (Second terminal, tape rate 30 mm/sec). 1 - Turtle No. 22 (Test); 2 - Turtle No. 47 (control)

Fig. 3. Intestine. Combined tissue block. Intestine diameter and layer thickness diminished, villi shortened in test turtles (Brashe, lens enlargement.)

Fig. 4. Test turtle intestine. Cells with hyperchromic nuclei, some of which "drop out" in intestine lumen, are seen in the epithelium crypt (Hematoxyline-eosine. Gom. IV, ob. 40, 800 X magnif.)

Fig. 5. Intestine. Combined tissue block. In test turtles PAS - positive reaction deep in the crypt clearly weakened (Shabadash, lens enlargement.)

Fig. 6. Testes of turtles on board the "Zond-5". Duct diameter diminished, embryo epithelium thinned, united by cells. Considerable outcrops of brown pigment in interstitial tissue (Brashe, Gom. VI, ob. 20, 240 X)

Fig. 7. Testes of intact turtles. Compare with Fig. 6 (Brashe, Gom. VI, ob 20, 240X)

Fig. 8. Seminal vesicles of turtles on board the "Zond-5". Spermatozoids missing from lumen. (Brashe, GOM. VI, ob. 16, 115X)

Fig. 9. Seminal vesicles of intact turtles in the vivarium. Lumens filled with spermatozoids. (Brashe, Gom. VI, ob. 16, 115X)

Fig. 10. Liver. Combined tissue block. In turtles on board the "Zond-5" the cell size diminished, their cytoplasm is darker and basophilic; the amount of pigment is increased (Hematoxyline-eosine. Gom VI, ob. 40, 470X)

Fig. 11. Liver of intact turtles. Glycogen in cells in moderate quantity (Shabadash, Gom. VI, ob. 40, 470X)

Fig. 12. Liver of test animals. Glycogen content increased. Compare with Fig. 11. (Shabadash, Gom. VI, ob. 40 470X)

Fig. 13. Liver. Combined tissue block. In turtles on "Zond-5", no fat in cells. (Scarlet red. Gom. IV, ob. 40, 800X)

Fig. 14. Liver. Monoaminooxidase activity in turtles on the "Zond-5" reduced (a) and significant (b) in intact animals. (Glenner et al., Gom. VI, ob. 16, 115X)

Fig. 15. Seminal vesicles of turtles at the cosmodrome. Small quantity of spermatozoids remain in lumens. (Brashe. Gom. VI, ob. 16, 115X)

The figures are not reproduced in this translation.