THE SENSITIVITY OF SKIN CHROMATOPHORES TO THE IONIZING RADIATION

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It is known from numerous published observations that the primary function of skin chromatophores of amphibia, reptiles and other animals is that of photoreception, which is expressed by the dispersal of pigment granules in their cellular outgrowths when illuminated and the concentration of pigment near the nucleus in the dark, or vice versa.

Sensitivity to light of skin chromatophores was demonstrated using animals with removed eyes [1, 4, 10, 11, 12, 15], and also in isolated preparations placed in Ringer's solution [15, 17].

In the photometric study of isolated frog's skin, Zettner [17] noted a decrease in its coefficient of light transmission after illumination. Utilizing this method, which was previously employed by Frieden, Fishbein and Hisaw [7], we attempted to study the radiosensitivity of the frog's skin photoreceptors.

Many investigators demonstrated that changes in melanophores take place as a result of exposure of frogs to ionizing radiation. G. P. Mushegyan and M. N. Abovyan [7] found narrowing of the frog's skin melanophores as a result of action of radium waves on thalamus or independently on the skin. O. S. Il'ina [4] observed that after attaching tubules with radon with radioactivity of 15-30 microcuries to the frog's skin, there is a concentration of melanophore pigment which is revealed after transillumination of the exposed skin area; whereupon, the reaction is more intense the closer radon is placed dorsally to the lumbar segments of the spinal cord.

Indications of changes in pigment cells as a result of irradiation of the frog can be found in the study of E. I. Bakina and A. I. Naumenko [1]. Ashton [9] found increase in the size of melanophores after both the total body irradiation and local irradiation of the head of frogs. A similar effect was obtained by A. V. Lebedinskii and V. V. Yakovlev [6] on pigment cells of webbed membrane of the frog's lower extremities after exposure to a dose of 4-20,000 r (the rate of administration was 500-630 r/min). In the present investigations we used isolated skin, removed from the ribs and legs of a frog (fall-winter R. ridibunda and R. temporaria).

EXPERIMENTAL METHOD

In the present investigations we used isolated skin, removed from the ribs and legs of a frog (fall-winter R. ridibunda and R. temporaria).

Prior to the experiment, the frog was kept, for one day, in the dusk at room temperature. The preparation and the subsequent changes were carried out at room temperature in red light. After a cautious and careful freeing of the remaining muscles and vessels, the pieces used for the study were washed several times in Ringer's solution (pH not less than 7.0), placed in a plexiglas frame and then into special containers, each with 10 ml of Ringer's solution. Photometric determinations were made every 30 minutes using Pul'frikh's photometer (the studies were carried out using K-2 and K-4 light filters and 633 and 541 mμ wave lengths, respectively. In the interval between measurements, the skin preparations in Ringer's solution were kept in the dark. A part of these at a definite stage of adaptation (see below) was subjected to the action of light; another part was irradiated; and some were left in the dark as controls. A daylight lamp served as the source of light. The irradiation was carried out for one hour. The test objects were illuminated with 24,000 lux. Irradiation was carried out with an x-ray machine (132 kv, 10 ma, distance of target to source 30 cm, dose rate at 44 r/min, total dose was 500 r and 1000 r; 150 kv, 10 ma, distance of target to source 19 cm, dose rate 444 r/min, total dose 5000 r) and by γ-rays from radioactive cobalt (dose rate of 6000 r/min, total dose of 540,000 r). A total of 200 preparations was studied; of these, 64 were irradiated.

EXPERIMENTAL RESULTS

From the results of the first series of experiments, we found that after immersion of the isolated frog's skin into Ringer's solution and keeping it in the dark for 2-4 hours, there occurs a concentration of pigment in the melanophores. Along with this, the transparency coefficient gradually increases and finally reaches a certain stationary level. Subsequently, there are noted certain insignificant variations in the course of a long period of time.

In the remaining two series of investigations, pieces of frog's skin were either illuminated or exposed to ionizing radiation after reaching the stationary level of the transmission coefficient. The change in light transmission coefficient of isolated frog's skin in parallel experiments in the dark, under the influence of light stimulus, as well as a result of exposure to the ionizing radiation is shown in Fig. 1, 2, and 3. In all figures, the stationary level of the light transmission coefficient is taken as 100% (columns, labeled by figure 1), and the subsequent levels are computed in relation to this level (columns 2, 3 and 4). In parallel experiments, light transmission of pieces of skin was measured at the same time intervals. Each column represents an arithmetic mean of the light transmission coefficient of a predetermined number of experiments.

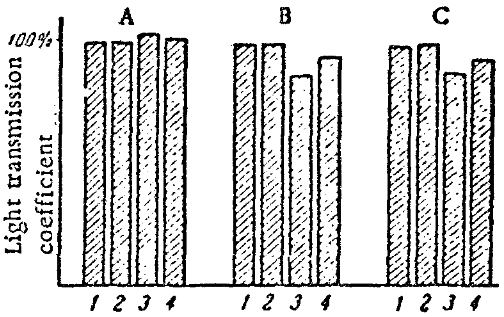

Fig. 1. Change in the transmission coefficient of the isolated frog's skin in parallel experiments (every column represents an arithmetic mean of experiments). A) Pieces of skin held in the dark for the duration of experiment (1, 2, 3, 4); B) pieces of skin illuminated (3) during a selected stage of dark adaptation (1, 2) and then left again in the dark for 1 hour (4); C) pieces of skin were x-irradiated with 1000 r (2) upon reaching the stationary level of the transmission coefficient (1) then illuminated (3), and finally were left once again in the dark for 1 hour (4).

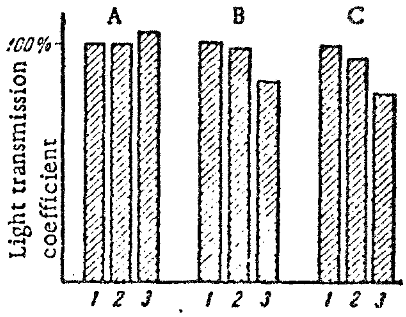

Fig. 2. Alteration in light transmission coefficient of isolated frog's skin in parallel experiments (every column is an average arithmetic mean of 8 experiments). A) Pieces of skin held in the dark for the duration of experiment (1, 2, 3); B) pieces of skin illuminated (3) during a selected stage of dark adaptation (1, 2); C) pieces of skin were irradiated with 5000 r (2) upon reaching the stationary level of light transmission coefficient (1), and then illuminated (3).

In most cases, there was observed a decrease in the light transmission coefficient in illumination experiments (see Figs. 1 and 2); i.e., there was an expansion of melanophores. We demonstrated that in 62 experiments using the light stimulus and red light filter, the light transmission coefficient was lowered on the average by 15.8%, and by 22% using the green light filter in comparison with the level prior to illumination.

Insignificant changes in the light transmission coefficient of frog's skin were observed after exposure to roentgen and Co^{60} γ-rays, with a slight tendency to decrease (Figs. 2 and 3) which was not observed during the corresponding time intervals in the parallel control experiments. However, statistical treatment of the data, carried out using the method for determining the significance of the difference of the means, showed that the changes in the light transmission coefficient after illumination are statistically not significant. However, as for the simultaneously carried out experiments with the light, it became apparent that the changes observed before and after exposure to light were statistically significant.

In experiments utilizing doses of 500, 1000 and 5000 r the irradiated pieces were illuminated after measurement of the coefficient of light transmission in order to determine whether irradiation effects the above indicated action of light. No marked changes were noted; the light brought about in this case a similar decrease as was found in the control experiments (see Figs. 1 and 2).

After remaining in the dark for one hour, both the control and the irradiated preparations, subjected to the action of light in the parallel experiments, demonstrate in certain cases a tendency to restoration of the coefficient of light transmission (see Fig. 1).

It is known from the literature that the malanophore reaction of the frog's skin is closely related with hypophyseal hormone intermedin [8] and with adrenalin. The former brings about expansion of the pigment in chromatophores, and adrenalin concentrates the pigment. The action of hypophyseal hormones on preparations of isolated frog's skin has been proven [9, 13, 14]. However, in the intact amphibian organism the endocrine glands are only the necessary link in the efferent chain. However, the central nervous system, which probably plays an important role in alteration of the melanophore reactions in amphibia in the course of irradiation, also acts as the coordinating mechanism insuring the pigment reactions of amphibia [3].

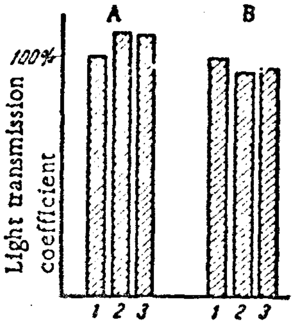

Fig. 3. Change in the transmission coefficient of the isolated frog's skin in parallel experiments (every column represents an arithmetic mean of 5 experiments). A) Pieces of skin held in the dark for the duration of the experiment; B) pieces of skin were irradiated with 540,000 r of γ-rays of radioactive cobalt (2) upon reaching the stationary level of light transmission coefficient (1), and then were again placed in the dark (3).

During the total body irradiation of frogs [6, 9] or as a result of the action of the ionizing radiation only on their heads [7, 9], as well as during local irradiation of frog's skin in the intact animal [4], there appear changes in the melanophore reaction; however, in our experiments using many-fold higher doses, there was no clear effect. All this indicates that the direct stimulation of expansion or concentration of the pigment as a result of radiation is not definitive. The melanophore effect appears as a result of irradiation either from an alteration of the nervous system in the course of irradiation, or as a result of destruction of the endocrine system, or as a result of both factors.

SUMMARY

Radiosensitivity of the skin photoceptors was studied in frogs by means of photometric method. A preparation of the isolated skin placed in Ringer solution was used as a test object. The light transmission coefficient prior to and after the irradiation was determined. As demonstrated, as distinct from light, irradiation with x- and γ-rays (CO^{60}) in large doses does not cause statistically significant changes of the light transmission coefficient of the isolated frog skin. It is suggested that ionizing radiation affects the melanophoric function of amphibia not directly, but through the nervous or endocrine system.

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