EFFECT OF γ-RADIATION ON THE ABSORPTION SPECTRUM OF VISUAL PURPLE

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The study of the responses of the organism as a whole to the action of radiations has revealed an interesting fact—the formation of reflex reactions. The attention of workers investigating this phenomenon has been concentrated on receptors which, when acted upon by radiation, could initiate these reflex reactions.

In this respect great interest is attached to the investigation of the visual receptor, irradiation of which in man gives rise to the sensation of a flash of light (radiophosphene) and to electrical reactions of the retina typical of photic stimulation. Analysis of the mechanism of these phenomena has led to the hypothesis that radiation, like light, is absorbed by the light-sensitive pigment of the retina—visual purple, and that this is followed by the development of excitation of the neural elements of the retina. If this hypothesis is correct, radiation must cause decolorization of visual purple.

Lipetz [3], who used the method of subjective evaluation of the color of the retina, found that roentgen rays, in a dose of 10^7 R, cause decolorization of the retina, accompanied by denaturation of the proteins. Peskin [4] carried out a series of experiments in which he irradiated various objects: 1) intact frogs and isolated retinas (dose 3000 R), 2) segments of rods and solutions of rhodopsin (doses 10,000 and 300,000 R), and 3) rhodopsin, dried on gelatin film and powdered, dried visual purple (dose 5,000,000 R). In all the experiments save those in which dry rhodopsin on gelatin film was irradiated, negative results were obtained: the visual purple was not decolorized. Peskin concluded that radiation has little if any action on the molecule of visual purple in solution. However, by applying the calculations made by Lipetz when determining the ratio between the threshold values of energy of light and ionizing radiation giving rise to electrical reactions of the retina and the energy causing decolorization of visual purple ($1:12\cdot10^6$ and $1:200\cdot10^6$ for light, $1:100\cdot10^6$ for roentgen rays), it may be concluded that Peskin used smaller doses of radiation. This was the reason for the absence of visible changes in the rhodopsin in his experiments.

The object of the present investigation was to study, by means of an objective spectrophotometric method, the changes taking place in visual purple after irradiation of the retina in doses much larger than those used by Peskin in his experiments.

EXPERIMENTAL METHOD

Visual purple possesses the power of absorbing light of different wavelengths, and its spectral absorption curve, obtained by plotting the optical density of visual purple against wavelength, is symmetrical in shape with a maximum of absorption in the region of 500-510 mμ.

The changes in the quantity and quality of visual purple after irradiation were judged by comparing the absorption spectra of solutions of rhodopsin obtained from irradiated and unirradiated retinas. The visual purple was extracted from the frog's retina by the method described elsewhere [1, 2]. The source of γ-rays was a type OKFO-1 apparatus charged with Co^{60}. The dose rate was 96.6 R/sec, the total dose 200,000 R, and the duration of irradiation 32 min 50 sec. The optical density of the visual purple solution was measured at 8 points of the spectrum by means of a type SF-4 spectrophotometer at a low intensity of illumination.

The experimental technique was as follows. After 20 frogs had become preliminarily adapted to white light, they were placed in darkness for 18 h to allow regeneration of their visual purple. At the end of the period of dark

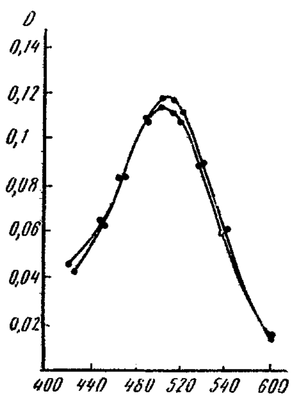

Fig. 1. Absorption spectra of visual purple extracted from retinas of right and left eyes of frogs. Here and in Fig. 2, along the axis of ordinates—value of optical density of solution of visual purple (D), along axis of abscissas—wavelength (in mμ).

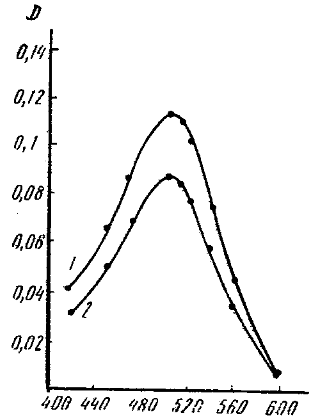

Fig. 2. Absorption spectra of visual purple, extracted from irradiated and unirradiated retinas of frogs' eyes. 1) Absorption spectrum of solution of rhodopsin from control retinas; 2) absorption spectrum of solution of rhodopsin from retinas irradiated with γ-rays emitted by Co^{60}.

adaptation the frogs were decapitated, and the retinas from the right and left eyes were extracted in red light separately into different weighing bottles. The retinas from the right eyes were then irradiated in darkness in the dose described above. The retinas from the left eyes were used as controls and were kept in darkness, and at the same temperature and humidity and otherwise in identical conditions as the irradiated retinas. Preliminary experiments showed that the absorption spectrum curves of the rhodopsin solution from the right and left eyes of the intact frogs were practically indistinguishable from each other (Fig. 1), and thereby justified the choice of solutions obtained from the unirradiated retinas of the left eyes of the frogs as controls. After irradiation of the preparations of the right eyes, both groups of retinas were treated simultaneously, at first with physiological saline, and then with dye solution. As detergent a 4% aqueous solution of saponin was used in the ratio of 4 : 1 or 5 : 1 to the weight of the thoroughly centrifuged retinas. Extraction of visual purple continued for 2 h. On the day of the experiment the absorption spectrum was determined from the values of the optical density.

EXPERIMENTAL RESULTS

The curves shown in Fig. 2 characterize the absorption of the visual purple of the irradiated and unirradiated retinas (mean data of 6 experiments). As remarked above, the height and character of the absorption spectrum are dependent on the quantity of visual purple extracted. It is clear from Fig. 2 that the absorption spectrum of the purple was modified after irradiation, and that the changes were more marked in the region of the maximum of the curve in the green, and also in the yellow-orange portion of the spectrum. The changes in the optical density of the visual purple solution were rather less marked in the blue-violet area of the spectrum.

Values of Optical Density (D) in Region of Maximum of Absorption Spectrum of Visual Purple Extracted from Irradiated and Unirradiated Retinas from Frogs' Eyes

Expt. No.	D_{max} of rhodopsin	
	Unirradiated retina	Irradiated retina
1	0.123	0.091
2	0.107	0.085
3	0.122	0.104
4	0.133	0.095
5	0.110	0.090
6	0.082	0.062
M ± m	0.113 ± 0.005	0.088 ± 0.005

The reduction in the optical density of the visual purple in certain areas of the absorption spectrum and the flattening of the curve in the region of the maximum of absorption indicate that during exposure to irradiation quantitative and qualitative changes take place in rhodopsin. The flattening of the curve in the region of the maximum of absorption (500 mμ) indicates a decrease in the concentration of visual purple in solution. These changes are characteristic of the initial stage of decolorization of rhodopsin [5].

Experimental results obtained during determination of the maximum of absorption in the 6 experiments described are given in the table. The error of the mean was calculated from the following formula:

$$\sigma = \frac{x_{max} - x_{min}}{n\sqrt{k}},$$

where σ is the error of the mean, X_{max} and X_{min} the maximal and minimal values in the control or experimental series, n the number of determinations, and k Ermolaev's coefficient. To determine the significance of the differences between the values of the optical density of the rhodopsin of the irradiated and unirradiated retinas at the maximum of absorption, Wilkinson's statistical criterion was used. By this means it was found that the assumption, that the changes obtained were fortuitous was rejected with a probability of error of less than 5%.

Hence, during irradiation of retinas with γ -rays from a Co^{60} source in a dose of $2 \cdot 10^5$ R a marked change took place in the absorption spectrum of the visual purple extracted from these retinas, a sign of the initial stage of the process of decolorization of rhodopsin. The results obtained afford further confirmation of the hypothesis of the photochemical mechanism of the action of high-energy radiation on the retina. However, the problem of the actual ways in which the effect of irradiation on visual purple is realized remains unsolved.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. ***Some or all of this periodical literature may well be available in English translation.*** **A complete list of the cover-to-cover English translations appears at the back of this issue.**