[7, 8, 12]. The postradiation change in the cyclic AMP level in the lymphocytes, which was increased by isoproterenol, is therefore difficult to account for unequivocally by the use of the same approaches as used to study changes in the steady-state concentration of the cyclic nucleotide.

The findings described above suggest that the cyclic AMP system or, at least, the system regulating the cyclic AMP concentration in the lymphocytes, is damaged by ionizing radiation.

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RHODOPSIN RESYNTHESIS IN RATS WITH HEREDITARY RETINAL DYSTROPHY

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Rhodopsin resynthesis was studied in vivo in the retina and optic cup of two strains of rats with hereditary dystrophy: Campbell albino rats and Hunter rats with pigmented eyes. Wistar and MSU rats, respectively, were used as the controls. The rate of reduction of rhodopsin after its decolorization in the retina in the affected animals was shown to be much slower than in healthy animals and to decrease as the disease developed. In the period of marked morphological changes, only 50% of the decolorized pigment was reduced during 2 h of dark adaptation (the time for complete regeneration of rhodopsin in healthy rats). In Campbell and Hunter rats the breakdown and resynthesis of rhodopsin take place not only in the retina, but also in the layer of fragments of outer segments of the photoreceptors, located between cells of the pigmented epithelium and the retina.

KEY WORDS: rhodopsin resynthesis; retina; hereditary retinal dystrophy.

One of the manifestations of hereditary retinal dystrophy – a serious disease leading to blindness – is a change in the quantity of visual pigment (rhodopsin) in the retina [3, 5]. If animals with retinitis pigmentosa are kept in darkness, development of the disease is considerably retarded [3, 4], evidence of serious disturbances in the systems responsible for resynthesis of rhodopsin after its decomposition under the influence of light. This is confirmed by changes observed in the activity of enzymes concerned in rhodopsin resynthesis [5]. A characteristic feature of retinitis pigmentosa in rats is the formation of a layer of "fragments" of the

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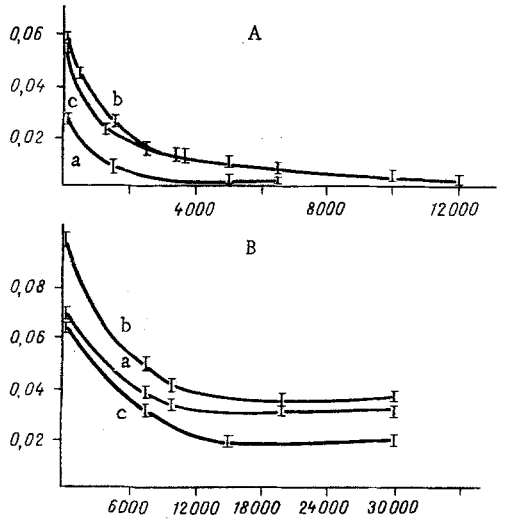

Fig. 1

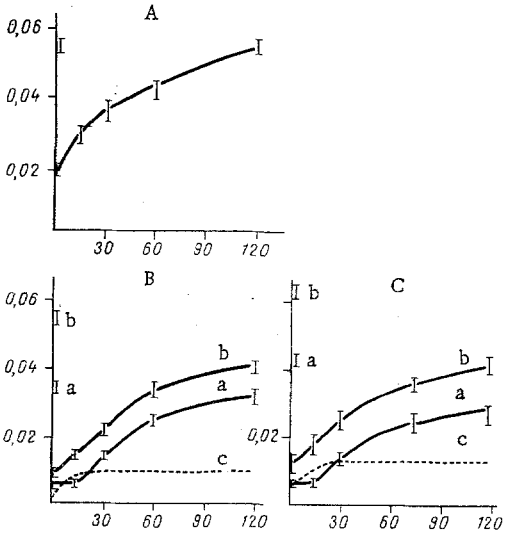

Fig. 2

Fig. 1. Decolorization curves of rhodopsin in eyes of rats (normal and with hereditary retinal dystrophy). A) Decolorization of rhodopsin in Campbell and Wistar albino rats: a) in retina of Campbell rats, b) in optic cup of Campbell rats, c) in retina of control rats; B) decolorization of rhodopsin in rats with pigmented eyes: a) in retina of Hunter rats, b) in optic cup of Hunter rats, c) in retina of control rats. Abscissa, exposure (product of intensity of illumination, in lux, and time of decolorization, in min); ordinate, quantity of rhodopsin in one retina (optic cup).

Fig. 2. Regeneration of rhodopsin in albino rats with retinal degeneration. A) In retina of control rats; B) in Campbell rats aged 20 days; C) in Campbell rats aged 1 month. a) In retina, b) in optic cup, c) in layer of "fragments" of outer segments. Here and in Fig. 3: abscissa, time of dark adaptation (in min); ordinate, quantity of rhodopsin in one retina (optic cup).

outer segments, located between the photoreceptor cells and cells of the pigmented epithelium [2], as early as in the second week of life. The appearance of this layer is evidently connected with a disturbance of phagocytosis. The properties of extracellular rhodopsin contained in the fragments of the outer segments and, in particular, its functional normality (ability to undergo decolorization and regeneration), remain unexplained.

Previously the writers showed in Hunter rats [6] that changes in the electroretinogram correlate with the extracellular rhodopsin content, as judged from the difference between the rhodopsin content in the optic cup and in the retina [1].

The object of the present investigation was to study the following problems: a) To what extent is the rhodopsin of the fragments of the outer segments capable of decolorization and regeneration?; b) what are the speed and character of rhodopsin resynthesis in the retina of affected animals in the early stages of the disease?; c) do the processes of rhodopsin resynthesis differ in albino rats and in rats with pigmented eyes?

EXPERIMENTAL METHOD

Experiments were carried out on rats with hereditary retinal dystrophy belonging to the Campbell line (albino) and to the Hunter line (with pigmented eyes) and on control Wistar and MSU rats (with pigment).

To decolorize the rhodopsin in the animals' eyes, the rats were placed in a white sphere under an incandescent lamp with water filter (intensity of illumination usually 5000 lx, in some experiments 10,000 or 30,000 lx). The pupils of the pigmented rats were dilated beforehand with 1% atropine solution. Immediately after illumination or after necessary dark adaptation the animals were decapitated and rhodopsin was determined spectrophotometrically in each rat, in the retina of one eye and the optic cup of the other eye [1]. The rhodopsin content was expressed in optical density units at 500 nm. The initial level of rhodopsin in the eye was taken to be its content after dark adaptation for 2 h.

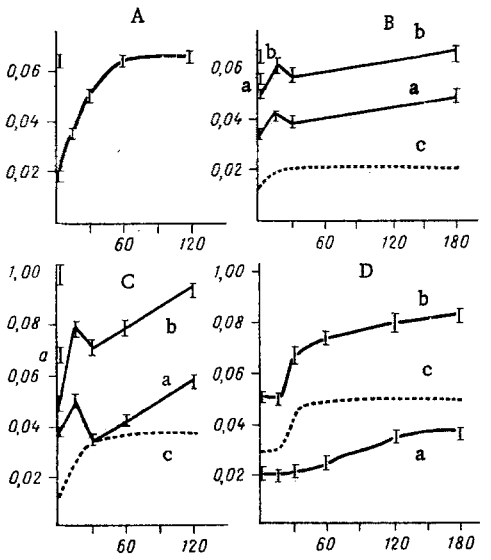

Fig. 3. Regeneration of rhodopsin in rats with pigmented eyes. A) In MSU rats (control) aged 1 month; B) Hunter rats aged 20 days; C) Hunter rats aged 1 month; D) Hunter rats aged 2 months. a) In retina, b) in optic cup, c) in layer of "fragments" of outer segments.

The data described in this paper reflect the results of 4 to 6 experiments.

EXPERIMENTAL RESULTS

To select the conditions of illumination of the animals in order to study rhodopsin resynthesis (it was intended to decolorize 60-70% of the initial content) the process of rhodopsin decolorization was investigated in vivo. It will be clear from the data in Fig. 1 that the course of the curves characterizing rhodopsin decomposition depends on the presence of the pigment in the animal's eye. In albino rats, whether healthy or with the disease, the rhodopsin decolorization curves reflected an exponential function (Fig. 1A). In animals with pigmented eyes, even in high intensities of illumination it was impossible to decolorize the rhodopsin completely; as a rule about 30-40% of the initial quantity of visual pigment remained undecolorized. This feature was probably not a result of the disease, for it was also found in healthy rats with pigmented eyes (Fig. 1B).

Comparison of the rhodopsin content in the retina and the optic cup at each experimental point showed that the rhodopsin in the layer of fragments of the affected rats also undergoes decomposition under the influence of light, i.e., in this respect it is normal.

Curves of restoration of the rhodopsin content in the course of dark adaptation in albino rats, both healthy and affected, are shown in Fig. 2. It follows from these findings that rhodopsin resynthesis in the affected animals differs appreciably from normal. First, the beginning of regeneration in the retina of the affected rats is delayed by 15 min, and second, the speed of the process in these animals is considerably reduced. The decrease progressed in the course of development of the disease. For instance, in animals aged 20 days, in the course of 2 h of dark adaptation 85% of the quantity of decolorized rhodopsin in the retina was restored, compared with only 50% in the animals aged 1 month. During 10 days of life very serious disturbances thus take place in the systems responsible for the resynthesis of rhodopsin after its photo-induced decomposition, and this reflects the rapid development of the disease in the rats of this strain.

The results of analogous experiments on the animals with pigmented eyes are illustrated in Fig. 3. The rhodopsin content in the control animals reached its initial level after only 1 h of dark adaptation (Fig. 3A). In the affected rats the rate of regeneration decreased with the development of the disease. Whereas in animals aged 1 month 75-95% of the decolorized rhodopsin in the retina was restored after 2 h of dark adaptation (Fig. 3C), in animals aged 2 months the proportion was only 40-50% (Fig. 3D). The distinctive course of the rhodopsin regeneration curve in the initial phase of dark adaptation in the animals aged 20-30 days will be noted:

After an increase in absorption at 500 nm for 15 min, a decrease took place, as a result of which a "peak" appeared on the curves (Fig. 3B, C). This was a feature both of the experiments on the retina and of the experiments on the optic cup. In the period of developed pathology (animals aged 2 months) instead of an initial peak on the rhodopsin regeneration curve, delay of resynthesis was observed in the first 15 min, like that observed in the Campbell rats. The cause of this anomaly is not yet clear; what is evident is that it was the result of the disease, for no such picture was observed in the control animals with pigmented eyes.

The disturbances of regeneration of the visual pigments found in the retina in the initial phase of dark adaptation, together with the reduction in the overall rate of its resynthesis, are thus important functional features of retinitis pigmentosa. These disturbances are observed earlier in Campbell rats than in Hunter rats, in agreement with the morphological observations of Yates [6] who found that the disease develops more slowly in animals with pigmentation.

Since determination of the difference between the rhodopsin concentration in the optic cup and retina of the same animal enables its content in the layer of fragments to be ascertained, curves showing changes in the extracellular rhodopsin content in the course of dark adaptation in rats with retinitis pigmentosa were plotted (curves c in Figs. 2 and 3). In all affected animals, rhodopsin resynthesis took place in the layer of fragments of the outer segments, and from this point of view, the rhodopsin unconnected with the cell was normal. However, resynthesis in this case took place "idly," for the extracellular rhodopsin could not participate in the transformation of the photic stimulus.

It follows from the curves of regeneration of extracellular rhodopsin that this process usually begins in the layer of fragments immediately after the end of photic stimulation. The substances essential for resynthesis of rhodopsin and transported from the pigmented epithelium into the photoreceptors are evidently utilized in the layer of fragments of outer segments for the regeneration of extracellular rhodopsin, and this may explain the delay to this process in the retina.

In rats with retinitis pigmentosa (Campbell and Hunter strains) the rate of rhodopsin regeneration in the retina is thus reduced, but in the initial stage of the process there are certain abnormalities compared with normal animals. In rats with pigmented eyes these disturbances develop later.

The visual pigment found in the fragments of the outer segments, like the rhodopsin of the disks of the photoreceptors, is capable of decolorization and subsequent regeneration, i.e., it is normal with respect to these properties.

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