LITERATURE CITED

1. B. V. Konyukhov and M. P. Vakhrusheva, Teratology, 2, 147 (1969).
2. A. M. Malashenko and N. I. Surkova, Genetika, No. 2, 105 (1975).
3. U. H. Ehling, Mutat. Res., 11, 35 (1971).
4. S. S. Epstein and G. Rohrborn, Nature, 230, 459 (1971).
5. Y. Masui and R. A. Pederson, Nature, 257, 705 (1975).
6. D. Mendelson, Mutat. Res., 37, 229 (1976).
7. D. Mendelson and F. H. Sobels, Mutat. Res., 26, 123 (1974).
8. S. Okada and T. Ono, Mutat. Res., 46, 145 (1977).
9. T. Ono and S. Okada, Mutat. Res., 36, 213 (1976).
10. J. P. Proust, Mutat. Res., 16, 65 (1972).
11. F. Vogel and G. Rohrborn (editors), Chemical Mutagenesis in Mammals and Man, New York (1970).
12. F. E. Würgler and P. Maier, Mutat. Res., 15, 41 (1972).

PROTEINS OF THE RETINA AND ITS PIGMENTED EPITHELIUM IN HEREDITARY DEGENERATION OF THE RETINA

I. A. Ostapenko and G. G. Chusova

UDC 617.735-007.23-008.939.6-092.9

The assortments of water-soluble and membrane proteins of the retina and its pigmented epithelium in Campbell (albino) rats with hereditary degeneration of the retina and in healthy Wistar rats were studied by electrophoresis in polyacrylamide gel. Early changes were shown to be detectable in the assortment of retinal proteins of the diseased animals; the first proteins to undergo changes were found to be neither cyclic nucleotide phosphodiesterase nor opsin. Changes in the set of proteins of the pigmented epithelium were observed much later.

KEY WORDS: retinal protein; proteins of the pigmented epithelium; hereditary degeneration of the retina.

The location of the primary lesion in the eye – whether in the retina or pigmented epithelium (PE) – in hereditary degeneration of the retina is a problem that is still unsolved [4, 10, 12].

It was accordingly decided to compare changes in the protein composition of the retina and the pigmented epithelium in relatively early stages of the disease. In the present investigation the assortments of water-soluble and membrane proteins of the retina and PE in Campbell rats with hereditary degeneration of the retina and in healthy Wistar rats at different periods of postnatal life were studied by electrophoresis.

EXPERIMENTAL METHOD

Electrophoresis of the proteins in acrylamide gel was carried out by the methods in [1, 6, 11], using the supernatant as the fraction of water-soluble proteins and the residue obtained after centrifugation of a homogenate of the retina or PE at 13,000g for 15 min as membrane proteins. Cyclic GMP phosphodiesterase (PDE) in the gels after electrophoresis was identified by the method in [7]. Opsin was identified by electrophoresis of the membrane proteins of the outer segments of the rods (OSR) of a rat, which were isolated in the same way as bovine OSR [2]. Protein was determined by Lowry's method [9]; protein in the residue was calculated as the difference between protein of the homogenate and of the supernatant.

Laboratory of the Biochemical Basis of Reception, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 2, pp. 211-213, February, 1980. Original article submitted April 9, 1979.

0007-4888/80/8902-0198 $07.50

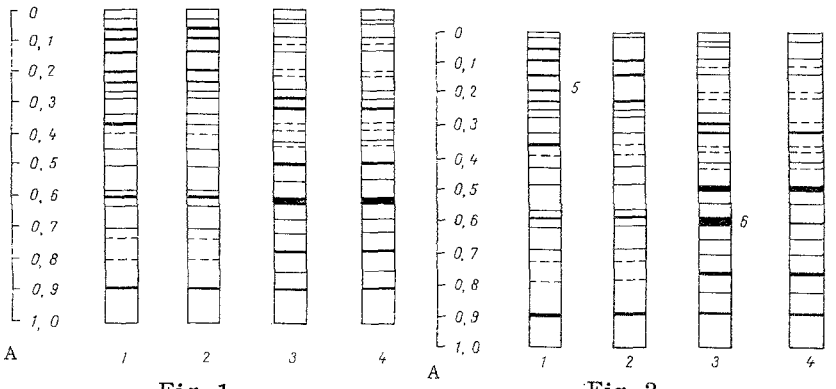

Fig. 1 Fig. 2

Fig. 1. Electrophoresis of water-soluble and membrane retinal proteins of 20-day-old Wistar and Campbell rats (scheme): A) scale of R_f values; 1) water-soluble proteins of Wistar rats; 2) water-soluble proteins of Campbell rats; 3) membrane proteins of Wistar rats; 4) membrane proteins of Campbell rats.

Fig. 2. Electrophoresis of water-soluble and membrane retinal proteins of Wistar and Campbell rats aged 3 months (scheme): 5) protein band identified as PDE; 6) protein band identified as opsin. Remainder of legend as in Fig. 1.

EXPERIMENTAL RESULTS

Comparison of the results of electrophoresis of water-soluble retinal proteins from Campbell and Wistar rats showed that in the affected 20-day-old animals the protein with relative mobility $R_f = 0.37$ was represented by a narrower band than that of healthy rats of the same age (Fig. 1); by the age of 45 days, the band corresponding to this protein of the affected rats was hardly visible, which can be attributed to a sharp decrease in the relative content of this protein. No significant changes in the set of water-soluble retinal proteins were found at the age of 20 days, although the protein band with $R_f = 0.29$ was less marked in rats with hereditary degeneration of the retina than in healthy rats (Fig. 1).

During the period of marked pathology (animals aged 2-3 months) differences in the results of electrophoresis of the proteins of the affected and healthy animals were perfectly clear (Fig. 2). Besides the deficient protein with $R_f = 0.37$, in the set of water-soluble proteins two bands with R_f values of 0.07 and 0.20 were completely absent; among the membrane proteins, besides changes in the band with $R_f = 0.29$, bands with R_f values of 0.06 and 0.27 had disappeared completely, and the band with $R_f = 0.60$ was less clearly visible.

On electrophoresis of proteins from PE of the affected and healthy rats on the 20th day of postnatal life no changes were found in the sets of water-soluble or water-insoluble proteins. Real differences among the membrane proteins of PE began to appear only on the 40th day of life (Fig. 3A): the intensity of staining of the band with $R_f = 0.12$ was much weaker in material from the affected animals than from the healthy rats. No changes were found in the soluble proteins of PE. Later (at the age of 2-3 months) during the development of hereditary degeneration of the retina in Campbell rats definite changes were found in the proteins of PE (Fig. 3B); the band of the water-soluble protein with $R_f = 0,12$ became sharply defined.

Among the various retinal proteins, in the mechanism of onset and development of hereditary degeneration of the retina special importance is ascribed to cyclic nucleotide PDE [8]. It was therefore decided to study whether any of the protein bands shown by electrophoresis of the retinal proteins of the affected rats to be changed belongs to PDE.

Staining the gels for PDE activity showed that this protein has electrophoretic mobility corresponding to $R_f = 0.20$. Since the earliest change observed was a decrease in the relative content of a water-soluble protein with $R_f = 0.37$, it is obvious that this protein is not PDE. The first changes in the relative content of PDE protein were not found until the 40th day of life. This indicates that a change in the PDE content in the animals that were studied was definitely not the first sign of hereditary degeneration of the retina.

Besides identifying PDE, opsin, the protein of visual pigments, also was identified on the gel after electrophoresis of the retinal membrane proteins. Its identification was based on the fact that the principal band

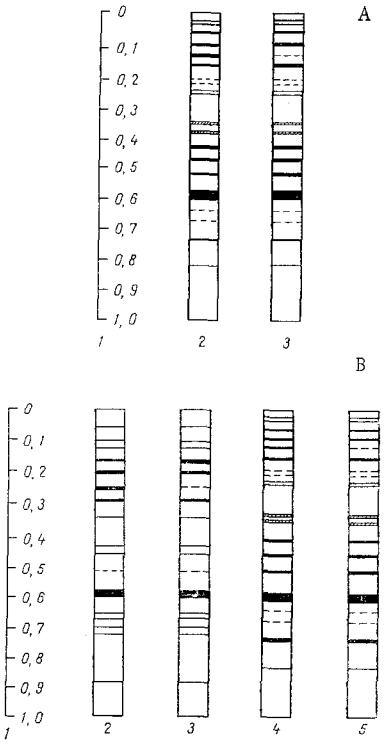

Fig. 3. Electrophoresis of water-soluble and membrane proteins of pigmented epithelium of Wistar and Campbell rats (scheme). A) Rats aged 40 days: 1) scale of R_f values; 2) membrane proteins of Wistar rats; 3) membrane proteins of Campbell rats; B) rats aged 3 months: 1) scale of R_f values; 2) water-soluble proteins of Wistar rats; 3) water-soluble proteins of Campbell rats; 4) membrane proteins of Wistar rats; 5) membrane proteins of Campbell rats.

after electrophoresis of solubilized proteins of isolated bovine OSR belongs to opsin. Comparative electrophoresis of bovine and rat OSR proteins showed that the opsin of both animals has the same mobility ($R_f = 0.6$).

In Campbell rats the protein band with $R_f = 0.6$ was appreciably reduced among the retinal membrane proteins only in animals aged 2-3 months, and in rats aged 20-45 days it was identical with that in the healthy animals, thus confirming data showing the absence of a disturbance of opsin in the early stages of the disease [3].

In hereditary degeneration of the retina changes in the set of proteins were thus observed considerably earlier in the retina than in PE. Retinal proteins showing changes in the early stages included neither PDE nor opsin.

LITERATURE CITED

1. H. Maurer, Disc Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis, De-Gruyter (1971).
2. I. A. Ostapenko, A. L. Berman, and M. P. Rychkova, Tsitologiya, 17, 947 (1975).
3. M. N. Chaitin and T. P. Williams, Exp. Eye Res., 24, 553 (1977).
4. D. Bok and M. O. Hall, J. Cell Biol., 49, 664 (1971).
5. J. Dowling and R. L. Widman, J. Cell Biol., 14, 73 (1962).
6. G. Fairbanks. T. L. Steck, and D. F. Wallach, Biochemistry (Washington), 10, 2606 (1971).
7. R. I. Hrapchak and H. Rasmussen, Biochemistry (Washington), 11, 4458 (1972).
8. R. N. Lolley and D. B. Farber, Exp. Eye Res., 22, 477 (1976).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
10. R. J. Mullen and M. M. La Vail, Science, 192, 799 (1976).
11. A. H. Reisner, Anal. Biochem., 64, 509 (1975).
12. M. Tamai, J. Takahashi, N. Tatsu, et al., Exp. Eye Res., 26, 581 (1978).