7. L. Kh. Garkavi, E. B. Kvakina, A. K. Mulatova, and A. I. Shikhlyarova, Vopr. Onkol., No. 6, 48 (1980).
8. L. Kh. Garkavi, E. B. Kvakina, and E. A. Sheiko, Probl. Éndokrinol., No. 1, 45 (1987).
9. Yu. I. Ukhov and A. F. Astrakhantsev, Arkh. Anat., No. 3, 66 (1983).
10. "The evolution of the stress concept," Sci. Am., 62, No. 6, 642 (1973).

AGE CHANGES IN FIBRONECTIN LEVELS OF THE DRAINAGE SYSTEM OF THE EYE

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Fibronectin is an extracellular adhesive glycoprotein which can bind selectively with type II collagen, the main constituent of the interstitial tissue of the trabecular system of the human eye [8], it can increase resistance to the outflow of aqueous humor [4, 5], and can thus raise the intraocular pressure. Fibronectin is found in the drainage system of healthy adults [4]. It is not clear, however, whether the content of this protein changes with age. This is an important concept in connection with the sharp increase observed in the fibronectin content during progression of primary open-angle glaucoma [2].

In the investigation described below the content of fibronectin in the trabecular apparatus of the human eye was studied in individuals aged between 49 and 76 years.

EXPERIMENTAL METHOD

The investigation was conducted on eight eyes from four donors aged from 49 to 76 years, and free from eye diseases. The eyes were enucleated 5-7 h after death and fixed in 4% formaldehyde in phosphate buffer, pH 7.2-7.4. The perilimbic regions of the sclera, containing the drainage zone, the cornea, sclera, and ciliary muscle fibers were isolated. Fibronectin was detected in serial transverse paraffin sections (5-6 μ) of the trabecular zone by the indirect immunoperoxidase method [3]. The sections were incubated consecutively with rabbit antibodies to human fibronectin after which the complex was revealed with a conjugate of anti-immunoglobulin antibodies and peroxidase. Peroxidase activity was found with the aid of a substrate mixture containing H_2O_2 and diaminobenzidine. The reaction was recorded on a "Vickers M-86" scanning integrating microdensitometer (20 × objective, No. 3 probe, wavelength 550 nm, scanning time 5 sec). Rabbit antibodies to type I herpes simplex virus served as the control.

EXPERIMENTAL RESULTS

The study of sections of enucleated normotensive eyes revealed fibronectin in the form of amorphous, diffuse yellowish brown deposits. Staining extended not only along the drainage pathways (trabecular network, canals of Schlemm, collectors, venous vessels of the sclera), but also to surrounding structures. The latter include the sclera, the intermuscular spaces of the ciliary muscle, and the cornea. The intensity of staining was greater, on visual examination, in the endothelial lining of the canal of Schlemm, the collectors, and veins. Staining of the inner wall of the canal of Schlemm was brightest, that of the trabecular ap-

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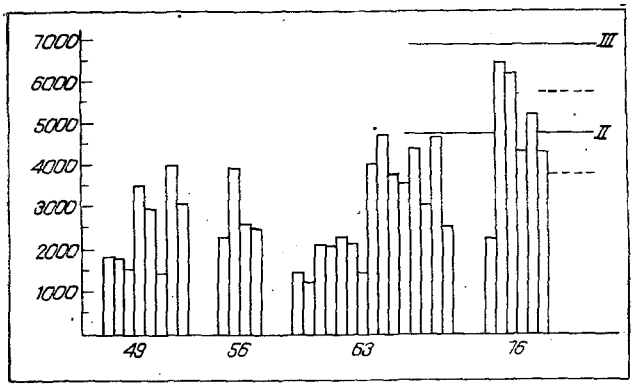

Fig. 1. Fibronectin in sections through trabecular zone of eye from donors of different ages. Continuous lines on right denote mean fibronectin level in trabecular zone in primary open-angle glaucoma in stages II and III; broken line indicates ±2 errors of the mean for stage II. Abscissa, age (in years); ordinate, fibronectin content (in relative units). Each column represents one section.

paratus less bright, and of the cornea and sclera weakest of all. With age the intensity of staining increased. Thus brightness of staining in transverse sections of the drainage system of a 49-year donor was much weaker than that of the same zone of the 75-year-old donor. In the control series, staining of the sections was not observed.

The results of densitometric analysis of the trabecular zone of serial sections through the same eye revealed different fibronectin levels in the trabecular tissue (Fig. 1). For instance, the fibronectin content in the trabecula of a 63-year-old donor varied from 2149 to 4700 relative units, the area of the trabecula being virtually identical (5908 and 5742 relative units respectively). In the same eye lower fibronectin levels were observed (746, 1204, and 1332 relative units), but the areas of the trabeculae of these sections were not comparable (1269, 2898, and 1269 relative units respectively). The first variant can be explained by differences in the fibronectin concentration in sections of equal area, as was confirmed by the difference in the brightness of their staining. This may indicate an uneven distribution of fibronectin in the trabecular tissue. The second variant is most probably explained by differences in the character of cutting and differences in the fragments of the ellipsoid structure in the sections.

Densitometric analysis of the eyes from different donors revealed an increase in the fibronectin content with age, especially in relation to the number of sections with an increased fibronectin content. Comparison of our previous data on the fibronectin content in the drainage system in glaucoma [2] with the observations on the fibronectin level in eyes from healthy donors (Fig. 1), described above, showed similarity of the values in some sections through the normal trabecular and the fibronectin level in stage II of open-angle glaucoma. In 49- and 56-year-old donors, this was observed in one of several sections studied. In the 63-year-old donor, sections of this kind accounted for about one-third of the total number studied, and in the 76-year-old, of six sections studied, five corresponded to stage II, and two of them, in their fibronectin content, corresponded to stage III of primary open-angle glaucoma.

The fact that fibronectin is located chiefly in the inner wall of the canal of Schlemm and the trabecular network is evidence that fibronectin, like glycosamino-glycans, may be a component of the electron-dense amorphous material which accumulates in the trabecular network and endothelial layer with age and in simple glaucoma [1, 6, 7]. The increase in the fibronectin content with age is evidence of the progressive character of dystrophic changes in the trabecular apparatus. The discovery of comparable levels of fibronectin in elderly subjects and patients with glaucoma in stage II-III raises the question of the relevance of the concept of preglaucoma as a transitional state between normal and pathological. Age can thus be regarded as a risk factor of primary open-angle glaucoma from the objective standpoint.

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LITERATURE CITED

1. V. G.Brikman and Yu. E. Batmanov, Aging and the Eye [in Russian], Moscow (1976), pp. 13-15.
2. M. V. Brodskaya, M. A. Babizhaev, and G. A. Ermolin, Vest. Oftal'mol., 104, No. 6, 10 (1988).
3. P. P. Clausen and P. Thomsen, Acta Pathol. Microbiol. Scand., 86, 383 (1978).
4. B. B. Floyd, P. H. Cleveland, and D. M. Worthen, Invest. Opthalmol., 26, 797 (1985).
5. M. M. Rodrigues, S. I. Katz, J. M. Foidart, and G. L. Spaeth, Ophthalmology, 87, 337 (1980).
6. J. W. Rohen and E. Lütjen-Drecoll, Altern und Entwicklung, ed. by H. Bredt and J. W. Rohen, Vol. 1, Stuttgart (1971), pp. 1-36.
7. J. W. Rohen and R. Witmer, Albrecht v. Graefes Arch. klin. exp Ophthalmol., 183, 251 (1972).
8. D. M. Worthen, P. H. Cleveland, J. Abare, and M. Slight, Invest. Ophthalmol., 26, 5 (1985).