tangents of the angles of slope of these graphs are directly proportional to the volume of plasma in the liquid above the gel.

The method of determination of the fibrinolytic activity of plasmin relative to the change in height of the fibrin gel column is simple, well reproducible, and highly sensitive. Unlike methods involving the use of radioactively or fluorescently labeled fibrin, in the method described above there is no background noise. By means of the method it is possible to study the kinetics of fibrinolysis and to determine plasmin in concentrations of above 50 μg/ml for 1-2.5 h at 25°C. The sensitivity of the method at 37°C is 0.5 μg/ml. The method is also suitable for quantitative assay of plasminogen in human plasma, which may be used to verify the potential fibrinolytic activity of plasmin during thrombolytic treatment.

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FLUORESCENCE OF LENTICULAR HOMOGENATES FROM Cat^{Fr} MICE WITH HEREDITARY CATARACT

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Cataract, or opacity of the lens, is the commonest cause of blindness in the population [3]. Familiar remedies against cataract, including Vitaiodurol, Sencatalin, and Catachrome, only delay the development of opacity of the lens, and in by no means every patient: one-third of patients do not respond to treatment [6]. This state of affairs has led to a search for new remedies [11], but this is made more difficult by the fact that the pathogenesis of the disease has not been adequately studied. Cataracts of different origin probably have common stages of their pathogenesis. The development of senile and hereditary cataract has been shown to be accompanied by activation of free radical lipid oxidation (FRLO) [7, 8, 13]. Changes in morphology [1, 2] and ionic homeostasis of the lens [4] and in the electrophoretic properties of the crystallins [9] have been demonstrated for mice of the Cat^{Fr} line. Fluorescence of lipid extracts of opaque lenses have been studied in only one investigation [5].

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TABLE 1. Parameters of Fluorescence of Mouse Lenticular Homogenates (M ± m)

Parameter	Control	Strain Cat^{Fr}
Intensity of fluorescence of tryptophanyls, conventional units	1±0,06	0,16±0,03* (8)
Position of maximum of tryptophan fluorescence, nm	329,1±0,62 (5)	333,2±0,5* (5)
Half-width of protein fluorescence spectrum, nm	58,8±1,3 (5)	55,4±1,1 (5)
F_{360}/F_{293}, relative units	0,6±0,11 (10)	1,4±0,21* (8)
Ratio between intensities of protein fluorescence at 311 and 369 nm, relative units	0,78±0,07 (8)	0,98±0,03* (5)

Legend. Number of specimens tested shown in parentheses. *$p < 0.05$ compared with control. Fluorescence of tryptophan residues excited at 293 nm. F_{360} and F_{293} nm respectively.

The aim of this investigation was to study the structure of crystallins in lenticular homogenates from Cat^{Fr} mice and to estimate the quantity of FRLO products (Schiff bases) during the development of cataract by the use of fluorescence methods.

EXPERIMENTAL METHOD

A strain of mice homozygous for the autosomal-dominant Cat^{Fr} gene, provided by colleagues at the Institute of Genetics, Academy of Sciences of the USSR, was used. Noninbred albino mice of the same age were used as the control. The animals received an ordinary diet. The lenses were removed after sacrifice from mice of different ages, and their minimal age was 4 months. Opacity of the lens was observed in the 2nd week after birth of the animals and it progressed during the next 2-4 months [1-4]. Homogenates were obtained as described in [9].

After preparation of homogenates of water-soluble proteins from the cytoplasm of the fibers, fluorescence spectra were recorded with excitation at 293 and 360 nm. At the first wavelength, fluorescence of tryptophanyls was recorded, at the second, fluorescence of Schiff bases, whose concentration increased with intensification of FRLO [12].

Fluorescence of lenticular homogenates was measured in a solution of 0.15M NaCl with 0.01 M Tris-HCl, pH 7.4, on a Hitachi MPF-2A spectrofluorometer (Japan) and absorption spectra were measured on a Beckman (USA) spectrophotometer. The specimens were diluted in Tris buffer so that their optical density did not exceed 0.1 optical unit.

EXPERIMENTAL RESULTS

The data in Table 1 show that opacity of the lens in mice of the Cat^{Fr} line was accompanied by a change in fluorescence of the homogenates: a change in the quantum yield of protein fluorescence and in the shape of the spectrum. The position of the maximum of the fluorescence spectrum was shifted into the long-wave region and the spectrum was widened at the same time.

The intensity of long-wave fluorescence of Schiff bases, reduced to protein fluorescence, was much greater in mice with cataract (Table 1). This is evidence of intensification of FRLO in the cytoplasm of the fibers of the Cat^{Fr} mice. Synthesis of crystallines is probably depressed in opaque lenses, as was reported previously [9]; the result of this is a decrease in fluorescence in response to excitation of tryptophanyls or fluorophores, absorbing in the region of longer wavelength, which we observed (Table 1). A significant change in morphology of the lenses was found previously in Cat^{Fr} mice. In particular, impairment of the staining properties of the cytoplasm of the altered lenticular cells was found, further evidence of reduction of the crystallin content [4]. Several workers have mentioned a decrease in the relative proportion of intracytoplasmic protein in the lenses of animals with hereditary cataract [2, 9].

An increase in the content of products of pre-radical oxidation in the lenses of mice of this same line was reported in [5]. The content of these products was estimated on the basis of their fluorescence at 440-480 nm. However, only the lipid fraction was analyzed. In our view, because of the paucity of experimental material described in that publication and the absence of analysis of the protein fraction of the homogenates, no unequivocal conclusions can be drawn regarding the leading role of lipid peroxides in the genesis of hereditary cataract. Moreover the authors of the paper cited [5] themselves do not rule out the possibility of formation of covalent cross-linkages of proteins with lipids. An increase in the content only of lipid peroxides in the lens affected by cataract could not take place for the simple reason that integral proteins, which are in close contact with crystallins, are localized in the cytoplasmic membrane of the fibers. It is out of the question that the proteins can remain intact while lipids are oxidized.

On the other hand, there is the possibility that the increase in the intensity of non-tryptophan fluorescence which we found is connected not only with a change in the free crystallins, but may also be partly due to covalent cross-linking of lipids and proteins. Complexes of this kind cannot be separated from pure proteins by simple sedimentation [10].

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