# IMMUNOCHEMICAL IDENTIFICATION AND CHARACTERISTICS OF A SPECIFIC ANTIGEN OF THE HUMAN RETINA

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An organ-specific antigen among the water-soluble antigens of the human retina was identified with the aid of monospecific antisera. It does not stain by the PAS-reaction and it has the electrophoretic mobility of $\alpha_1$ globulins in agar gel or of fast $\alpha_2$ globulins – transferrin in polyacrylamide gel. The organ-specific antigen was detected by the indirect immunofluorescence method in the region of the inner segments of the cones. By the use of the agar diffusion test the organ-specific retinal antigen was detected in extracts of the eye after the 21st week of intrauterine development of the human fetus.

Organ-specific antibodies are unique markers of cells of a certain type and stage of differentiation. The protein S-100 [8, 11], two glycoproteins with the mobility of $\alpha_2$ globulins [6, 10], and not less than two alcohol-insoluble thermostable BE-antigens [9] can serve as markers for human nerve tissue in this way.

It has been shown in Vyazov's laboratory [1, 2] that the retina of hens contains one organ-specific antigen with the mobility of $\alpha_2$-globulins and three antigens with narrow organ-specificity (retina – brain), which appear in the late stages (11-18 days) of development of the chick embryo.

This paper describes the results of an immunochemical analysis of a specific antigen of the human retina.

## EXPERIMENTAL METHOD

Material for the investigation was obtained at autopsy on persons killed accidentally, including some with head injuries, aged from 14 to 50 years. The retina was taken from 34 eyes; in 6 cases the eye was dissected into its component parts: lens, cornea, and so on. At the same time extracts were prepared from the visual cortex and subcortex, the gray and white matter of the cortex, and other organs and tissues of the human body. Tris-glycine buffer (tris 0.6 g/liter, glycine 2.88 g/liter), pH 8.3, containing 0.1% Triton X-100, 0.04% sodium azide, and 0.005% Tween 80, was used for extraction; the samples were ground with powdered glass, frozen (–20°C), and thawed, then centrifuged for 30 min at 12,000 rpm. Rabbits were immunized with extracts of the retina or of the total preparation of the eye mixed with Freund's adjuvant. The immunization cycle lasted 40 days. Most experiments were carried out with antiserum No. 705 obtained by immunizing an animal with retinal extract.

The immune sera were exhausted with mixed donors' blood serum, dry plasma, and with extracts of the liver, kidneys, gastric mucosa, thymus, and cerebral cortex, and for the immunohistochemical investigations additionally with acetone powders of liver and brain. The technique of immunoelectrophoresis and of the identification tests – double immunodiffusion in agar, analytical disc-electrophoresis, and disc-immunoelectrophoresis in acrylamide gel – was that usually employed [3-5].

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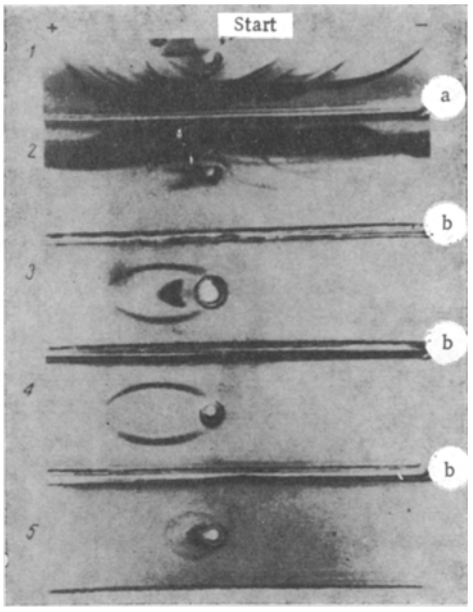


Fig. 1. Immunoelectrophoretic analysis of specific antigen of the human retina. Stained with: a) methyl green – pyronine; b) thymidine-$H^3$; and c) azure-eosin; 900×. 1, 2) Donor's blood serum; 3) extract of total preparation of eye; 4) extract of retina; 5) extract of cerebral cortex (frontal lobe); a) rabbit antiserum against donors' blood serum proteins; b) monospecific antiserum No. 705.

The protein concentration in the solutions was determined by Lowry's method [7]. Material for the immunohistochemical tests was fixed in Carnoy's fluid and embedded in parrafin wax. Sections 7 $\mu$ in thickness were treated by the indirect fluorescent antibody method and the control of immunological specificity of the reaction in the sections was carried out in accordance with Engel'gardt's recommendations [3].

## EXPERIMENTAL RESULTS

Exhaustion of the immune serum against human retina with mixed donors' blood serum, dry plasma, and extracts of various organs, monitored by the agar diffusion test, yielded a monospecific antiserum revealing only one antigen in retinal extracts. This was detected immunoelectrophoretically in the zone of the $\alpha_1$ globulins (Fig. 1). It did not stain by the PAS-reaction. On electrophoresis in acrylamide gel this antigen was located in the zone of the fast $\alpha_2$ globulins and transferrin.

It can be concluded from the results of the immunochemical tests (Fig. 2) that the specific antigen of the human retina is absent in extracts of the lens, cornea, sclera, the vascular membrane of the eye, the optic nerve, visual cortex and subcortex, gray and white matter of the frontal lobe, cerebrospinal fluid, extracts of the peripheral nerve, meninges, liver, kidneys, spleen, lung, heart muscle, mucus membrane

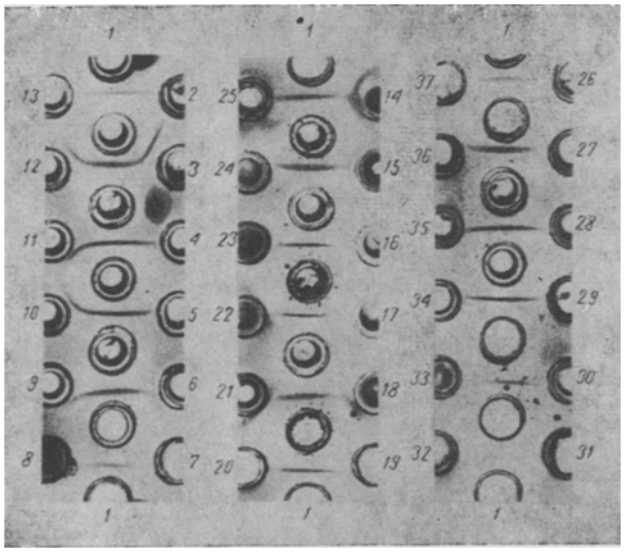


Fig. 2. Immunodiffusion analysis of the specific antigen of the human retina. Central well contains monospecific antiserum No. 705; peripheral wells contain: 1) human retina; 2) lens; 3) vitreous body; 4) vascular membrane of the eye; 5) cornea; 6) sclera; 7-12) extract of the eye from fetuses aged 9-11, 13-14, 21, 27, 28, and 29 weeks respectively; 13) donors' blood serum; 14) liver extract; 15) kidney extract; 16) lung extract; 17) spleen extract; 18) heart muscle extract; 19) extract of gastric mucosa; 20, 21) extracts of mucus membrane of small and large intestine, respectively; 22) extract of pancreas; 23) extract of fetal thymus; 24) extract of aorta; 25) of skin; 26) of frontal cortex; 27, 28) of white and gray matter of cortex, respectively; 29) of peripheral nerve; 30) of cortex of area 17-18; 31) of optic nerve; 32) of meninges; 33) of brain of a 26-week fetus; 34) of bovine brain; 35) of porcine brain; 36) of brain of domestic fowl; 37) BE-antigens of human brain.

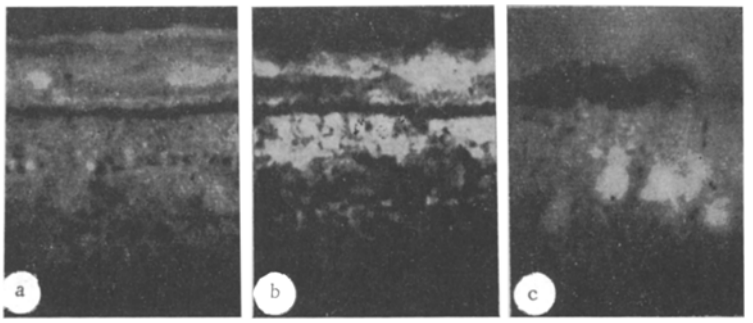

Fig. 3. Immunohistochemical detection of specific antigen of the human retina: a) section treated with antiserum exhausted by specific retinal antigen (40 ×); b, c) immunofluorescence in region of inner segments of cones.

of the stomach and large and small intestine, pancreas, adrenals, thymus, aorta, and skin, and also in alcohol-insoluble thermostable BE-antigens extracted from the human brain by Milgrum's method [9], and in extracts of bovine, ovine, and porcine brain, and the brain of the domestic fowl. It was detected in the vitreous body, simply because of the difficulty of separating this component of the eye from the retina.

After treatment of the sections of the retina by the indirect immunofluorescence method intensive fluorescence was found in the region of the inner segments of the cones. In all control sections through the retina and also sections through the liver there was an indistinct, uniform fluorescence of low intensity (Fig. 3). Neutralization of the antibodies against the specific retinal antigen abolished the immunofluorescence reaction.

Immunochemical detection of the organ-specific retinal antigen in extracts of total preparations of the eye at various periods of intrauterine development of the human fetus (9-11, 13-14, 17, 21, 27, 28, and 29 weeks) showed that this antigen is first detectable as an "inflection" of the test system of the corresponding antigen in the extract of the eye from a 21-week fetus and that its concentration in the extract rises with age of the fetus (Fig. 2).

The organ-specific antigen of the human retina identified in these experiments can thus be used as a marker of the cones.

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