4. M. I. Shatunovskii, Ecologic Principles of Metabolism of Sea Fishes [in Russian], Moscow (1980).
5. C. A. Alexander, R. L. Hamilton, and R. J. Havel, J. Cell Biol., **69**, No. 2, 241 (1976).
6. S. Appelbaum, H. Segner, and V. Storch, Zool. Anz., **217**, No. 1/2, 54 (1986).
7. W. Byczkowska-Smyk, Zool. Pol., **17**, No. 1-2, 105 (1967).
8. A. Claude, J. Cell Biol., **47**, No. 2, 35A (1970).
9. R. L. Hamilton, D. M. Regen, M. E. Grey, and V. S. LeQuire, Lab. Invest., **16**, No. 2, 305 (1967).
10. E. Holtzman and A. B. Novikoff, Cells and Organelles, 3rd. ed., Philadelphia 1984).
11. J. F. Leatherland and R. A. Songstegard, Cytobios., **54**, 195 (1988).
12. S. Smith, J. Exp. Biol., **29**, No. 4, 650 (1952).
13. S. Walzer and M. Schönenberg, Cell Tissue Res., **196**, 75 (1979).

CONTROL OF HUMORAL TRANSPORT IN TISSUES OF THE EYE

E. G. Rapis, V. P. Tumanov, Yu. M. Levin, and N. Kh. Kurbanov

UDC 617.7-616-018-078

KEY WORDS: isolated control of humoral transport; lymphotropic agents.

Anatomical and physiological data on communication of the drainage channels of the eye with the lymphatic system and on the existence of prelymphatic formations in the eye [7-9, 12-14] have provided a basis for using the results of clinical lymphology, a new and developing trend in Soviet medicine [2, 3], in order to study regional differences in the control of humoral transport in the eye as well as differences between different structures and tissues of the eye in this respect. The first positive experimental [7, 8] and clinical results on the use of lymphotropic agents, stimulating the hydrodynamics and the lymphatic drainage channels of the eye have now been obtained. However, we could find no information in the accessible literature on the possibility of exercising isolated control of humoral transport in tissue formations of the eye. It can be tentatively suggested that a detailed analysis of this problem could reveal new aspects in ophthalmology, where the problem of the causes of selective tissue damage frequently arises, and where selective and specific action of this kind must be carried out at the tissue level rather than that of the eye itself.

The aim of this investigation was accordingly to study the possibility of exercising isolated control of humoral transport in individual eye tissues, using lymphotropic agents differing in their mechanism of action.

EXPERIMENTAL METHOD

There were three series of experiments on 65 White Giant rabbits. The agents used in the experiments of series I were dalargin [1], in a solution of 0.1% concentration and in a dose of 0.04-0.05 ml/kg body weight (30 eyes), in series II terrilytin was used in a concentration of 25,000 U in 0.1 ml physiological saline, in a dose of 0.1-0.2 ml/kg body weight, injected subconjunctivally or by electrophoresis (30 eyes), and in series III a 10% solution of mannitol was injected subconjunctivally in a dose of 0.2-0.4 ml (20 eyes). Thirty minutes after paracentesis and escape of the aqueous from the anterior chamber, an India ink—gelatin mass (India ink jelly) or Gerota's mass was injected into it or into the vitreous body (VB) in a volume equal to that of the fluid removed. The animals were decapitated 3 h later, the eyes were enucleated, and a detailed macroscopic study of the eye was undertaken with the MBS-2 microscope, and film preparations obtained from different tissues of the eye were studied under the light microscope, and the state of the optic nerve and the internal membranes of the eye also was studied. Paraffin and celloidin sections were stained with hematoxylin-eosin and by Van Gieson's method. Control experiments were carried out on 25 rabbits

Turkmenian Research Institute of Eye Diseases, Ashkhabad. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 2, pp. 211-214, February, 1991. Original article submitted August 12, 1990.

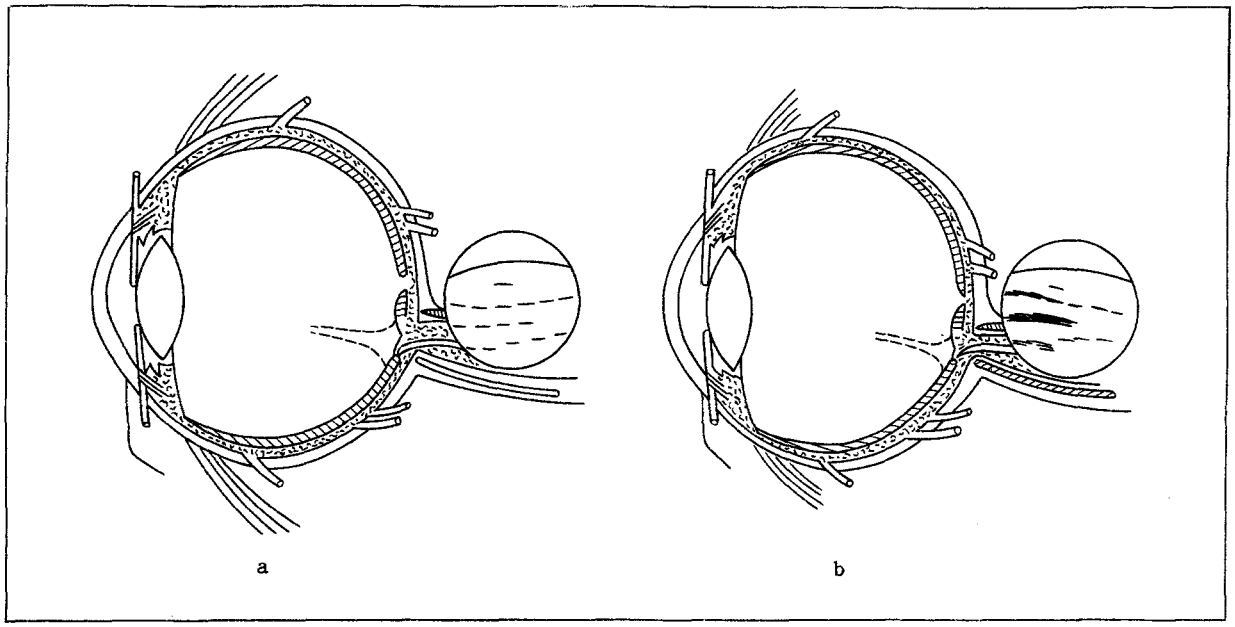


Fig. 1. India ink jelly in posterior drainage channels along optic nerve. a) Control, b) dilatation of lymphatic drainage channels under the influence of dalargin.

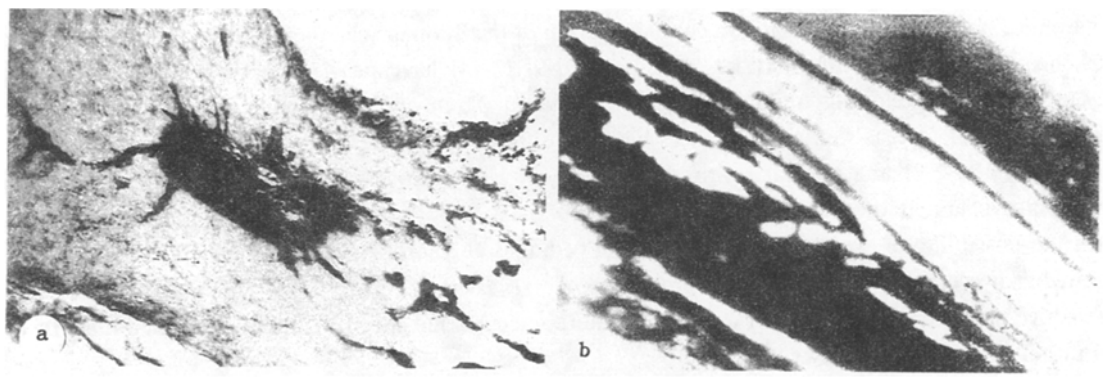
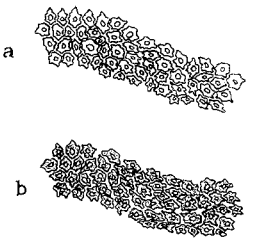


Fig. 2. Considerable dilatation of drainage channels from VB along optic nerve, compared with control (a), after subconjunctival injection of dalargin (b). 56×.

with injection of physiological saline subconjunctivally, and also without injection of physiological saline but with injection of India ink jelly or Gerota's mass into the anterior chamber and VB, without the use of therapeutic preparations.

EXPERIMENTAL RESULTS

The results of the control experiment with injection of India ink into the anterior chamber showed filling of the anterior drainage channels, the long venous trunks being filled first, and the shorter and flat branches and capillaries later. The whole sclera was without traces of ink. Three hours after injection of ink into VB, stippled stripes were observed along the surface of the optic nerve, greyish in color, on its dorsal and ventral aspects (Fig. 1a, b). After injection of dalargin (series I), the marked (ink) could be seen in 27 eyes, not only in the dilated drainage channels, but also in areas where it was not found in the control. For instance, in the anterior part of the eye the effect consisted of the appearance of dark round spots, resembling foci of extravasation around the limbus, and filling of vessels of a capillary network, similar in appearance to lymphatic vessels. Accompanying dark bands often appeared close to the vorticose and ciliary veins on the sclera, and the isolated filling of the anterior and posterior drainage channels was disturbed. The intrascleral channels filled with ink not only from the front, in the zone of



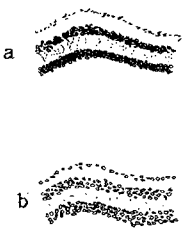


Fig. 3 Fig. 4

Fig. 3. Distribution of ink marker in PE of retina: a) control, subconjunctival injection of physiological saline. No ink present in PE of retina; b) subconjunctival injection of terrilytin. Penetration of ink into cytoplasm of PE of retina.

Fig. 4. Distribution of marker (Gerota's mass) in retina: a) penetration of marker into cytoplasm of retinal neurons after injection of mannitol; b) marker absent in retinal neurons; control — after injection of physiological saline.

drainage, but also in the posterior part of the sclera. Microscopy showed basically that the "black bands" on the sclera were due, not to diffuse spreading of the ink in the scleral tissue, but to filling of the drainage channels — collector and venous vessels. The posterior perineural, perivascular, and intraseptal drainage channels were particularly clearly dilated (five-tenfold) (Fig. 1b; Fig. 2).

The ink did not penetrate intracellularly into the eye tissues. Consequently, we observed the selective action of dalargin on the lymphatic and humoral drainage channels. The macro- and microcirculation in the efferent system was sharply increased under these circumstances. However, membrane permeability was unchanged.

The use of terrilytin (series II), by contrast with the control, gave a macroscopic picture of increased penetration of the ink perineurally, and penetration of ink in the form of spots under the retina was found if the retina was translucent. Examination of native film preparations showed that ink penetrated into cells of the pigmented epithelium (PE) of the retina, but only into the cytoplasm, for the nuclei were not stained with the ink. This was later confirmed in paraffin sections of the preparations, both unstained and stained by Van Gieson's method and with hematoxylin-eosin. Examination of celloidin sections showed that ink also penetrates into the nonpigmented epithelium (NPE) of the ciliary body and into PE of the retina, whereas ink did not penetrate into other tissues (Fig. 3).

After injection of mannitol (series III) and Gerota's mass, into VB the following data were obtained: macroscopically a blue color could be seen in vessels of the optic nerve, but the same picture also was observed in the control. On dissection of the eyeball the impression was obtained that the dye stained all contents of the eyeball diffusely, in both control and experiment. However, in unstained paraffin sections it was clear that only the retinal neurons had taken up the blue stain (Fig. 4), whereas in the control (Fig. 4b) they were unstained. The same was true also of stained preparations. A blue color was not observed in the synaptic layers and was hardly visible in the layer of nerve fibers. The blue stain likewise was absent in the choroid, unlike in the control, where the chromatophores of the choroid (or melanophores) were stained, and the sclera showed only a suspicion of being stained. In the control, blue focal staining was present with an intrachoroid, subchoroid, perivascular, and perineural distribution.

The investigation thus showed that various substances injected into the bloodstream enable humoral transport to be selectively controlled. The action of dalargin causes dilatation of the anterior and posterior drainage channels of the eye, including those drainage channels which, under normal conditions, are not involved in the elimination of ink. However, the drainage channels, when dilated and when accompanied by activation of new channels, preserve their structural nature and do not allow diffuse penetration and spread of ink. This conclusion contradicts the views of some workers [5, 11] who consider that fluid penetrates diffusely through scleral tissue, and it is in agreement with observations showing that the rate of passage of

labeled albumin and globulin through the sclera is equal and is independent of the specific gravity of the substance [4]. It must be emphasized that the selective lymphotropic action of dalargin leads to dilatation of the humoral lymphatic channels of the eye. Terrilytin, a preparation reducing the viscosity in the intracellular components of humoral transport in general, sharply increases the permeability of membranes and cytoplasm of the cells of NPE of the ciliary body and of PE of the retina, and also dilates the humoral perineural channels. This is evidence that although terrilytin destroys glycosaminoglycans (GAG), it does so to a significant degree only in membranes of PE of the retina and NPE of the ciliary processes. This fact is interesting with the aim of selective injection of drugs into PE without involving other cells and tissues, for the treatment of retinitis pigmentosa, for example.

A new fact was discovered, namely that the osmotic agent mannitol, which creates an osmotic pressure difference between tissues (carrying liquid in and out)has an effect on permeability of retinal neurons, but almost without affecting other tissues. Thus specificity toward different conditions of osmotic pressure within the cells and in the external medium of the neurons was discovered. These experiments are in agreement with data [6, 10] showing that, by changing the osmotic pressure, it is possible to influence the tissue-blood barriers of brain tissue.

The results can accordingly provide a basis for the experimental and subsequent clinical development of a therapeutic trend in ophthalmology, consisting of controlling the humoral transport of individual tissue structures through the intervention of lymphotropic agents differing in their mechanism of action.

LITERATURE CITED

1. G. S. Varaskina, V. A. Vinogradova, and A. A. Varaskin, "Action of dalargin on development of the sea urchin," Author's Abstract of Dissertation (1986). Lodged with the All-Union Institute of Scientific and Technological Information, No. 12.54.294.
2. Yu. M. Levin and A. E. Sorokatyi, "Stimulation of lymph formation and lymphatic drainage," Patol. Fiziol., No. 4, 28 (1979).
3. Yu. M. Levin, Principles of Therapeutic Lymphology [in Russian], Moscow (1986).
4. R. S. Mirzoyan, Kh. S. Ragimov, and T. S. Gan'shina, "Cerebrovascular effects of Met- and Leu-enkephalins," Byull. Éksp. Biol. Med., **101**, No. 1, 42 (1986).
5. A. P. Nesterov, Primary Glaucoma [in Russian], Moscow (1982).
6. Yu. A. Petrovich, Selective Permeability. Tissue-Blood Barriers and Neurohumoral Regulation [in Russian], Moscow (1981), pp. 67-69.
7. E. G. Rapis, R. Kh. Khakkiev, Yu. M. Levin, et al., Problems in Lymphology [in Russian], Novosibirsk (1987), p. 57.
8. E. G. Rapis, N. Kh. Kurbanov, M. Ya. Shmerling, et al., Proceedings of a Republican Scientific and Practical Conference of Ophthalmologists [in Russian], Ashkhabad (1988), pp. 56-63.
9. V. F. Shmyreva, Vest. Oftal'mol., No. 4, 8 (1986).
10. L. S. Shtern, The Immediate Nutrient Medium of Organs and Tissues [in Russian], Moscow (1960).
11. M. W. Bradbury and D. F. Cole, J. Physiol. (London), **299**, 352 (1980).
12. J. R. Casley Smith, E. Földi-Börescok, et al., Brit. J. Exp. Path., **57**, 179 (1976).
13. M. Földi, F. Kukan, et al., Acta Anat., **53**, 333 (1963).
14. J. H. Grüntzig, Das Lymphgefässsystem des Auges, Stuttgart (1982).