5. G. N. Kryzhanovskii, Abstracts of Proceedings of the 6th All-Union Congress of Neuropathologists and Psychiatrists [in Russian], Vol. 3, Moscow (1975), pp. 546-551.
6. G. N. Kryzhanovskii and M. N. Aliev, Byull. Éksp. Biol. Med., 81, No. 4, 397 (1976).
7. G. N. Kryzhanovskii, V. N. Grafova, E. I. Danilova, et al., Byull. Éksp. Biol. Med., No. 7, 15 (1975).
8. G. N. Kryzhanovskii and S. I. Igon'kina, Byull. Éksp. Biol. Med., 81, No. 6, 651 (1976).
9. G. N. Kryzhanovskii, S. I. Igon'kina, V. N. Grafova, et al., Byull. Éksp. Biol. Med., No. 11, 16 (1974).
10. G. N. Kryzhanovskii and V. K. Lutsenko, Neirofiziologiya, 7, No. 3, 234 (1975).
11. G. N. Kryzhanovskii, M. B. Rekhtman, B. A. Konnikov, et al., Byull. Éksp. Biol. Med., 81, No. 1, 23 (1976).
12. G. N. Kryzhanovskii, M. B. Rekhtman, B. A. Konnikov, et al., Byull. Éksp. Biol. Med., 81, No. 2, 147 (1976).
13. G. N. Kryzhanovskii and V. V. Ruseev, Byull. Éksp. Biol. Med., 82, No. 10, 1115 (1976).
14. G. N. Kryzhanovsky (G. N. Kryzhanovskii) et al., Exp. Neurol., 50, 387 (1976).
15. G. N. Kryzhanovskii, F. D. Sheikhon and M. B. Rekhtman, Neirofiziologiya, No. 6, 608 (1975).
16. J. Bancaud, J. Taluirach, P. Morel, et al., Electroencephalogr. Clin. Neurophysiol., 37, 275 (1974).
17. G. Clarke and R. G. Hill, Br. J. Pharmacol., 44, 435 (1972).
18. D. R. Curtis, C. J. A. Game, G. A. R. Johnston, et al., Brain Res., 43, 242 (1972).
19. D. R. Curtis, A. W. Duggan, and G. A. R. Johnston, Exp. Brain Res., 12, 547 (1971).
20. D. R. Curtis and G. A. R. Johnston, Ergeb. Physiol., 69, 97 (1976).
21. R. A. Davidoff, Science, 175, 331 (1972).
22. R. A. Davidoff, Brain Res., 45, 638 (1972).
23. R. A. Davidoff, Brain Res., 36, 218 (1972).
24. R. A. Davidoff and M. N. Aprison, Int. J. Neuropharmacol., 8, 191 (1969).
25. H. A. Jasper and C. Ajmone-Marsan, A Stereotaxic Atlas of the Diencephalon of the Cat, Ottawa (1954).
26. W. Penfield, in: Mechanisms of Brain Activity [in Russian], Tbilisi (1975), pp. 90-94.

KINETICS OF CORNEAL FLUORESCENCE IN EXPERIMENTAL KERATITIS

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It was shown by means of a method of scanning photometry, suggested by the writers, that in rabbits with experimental keratitis the response in the zone of a corneal burn consists of three phases: an increase in fluorescein absorption in the reactive stage, loss of ability to absorb fluorescein in the degenerative stage, and a second increase in absorption in the regenerative stage. These phasic changes can be used for the diagnosis of keratitis and for an objective evaluation of its clinical course.

KEY WORDS: inflammation; trophic changes; fluorescence.

It has now been shown that the local signs of an inflammatory process and the corresponding changes in reactivity of the body are due, in particular, to a complex response of the connective tissue [1, 8]. The connective tissue is known to perform supportive and trophic functions in relation to the parenchyma [2, 9] not only under normal conditions, but also during the development of inflammation. However, the dynamics of the

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trophic function of the connective tissue in the zone of an inflammatory focus in the different stages of inflammation has not yet been studied.

In order to study this dynamics, inflammation of the cornea was produced in rabbits. The cornea is a connective tissue derivative, is free from blood vessels, and is transparent, so that trophic changes in it can be evaluated by biomicroscopy.

EXPERIMENTAL METHOD

Keratitis was induced by a measured burn of the cornea with a coil 5 mm in diameter. The temperature of the coil was 80°C and the duration of exposure 2 sec. Reactive, degenerative, and regenerative stages were distinguished in the course of the keratitis. The "fluorescein test" is widely used in clinical ophthalmology to reveal trophic disturbances in the cornea. So far, however, the results of this test have been assessed only subjectively. The method of scanning photometry [4] suggested by the writers makes it possible to record not only the extent of the lesion, but also the intensity of absorption of fluorescein by the cornea objectively over a period of time. The intensity of corneal fluorescence in the zone of injury was expressed in relative units (photometric method). Uranin (sodium fluorescein) was injected intravenously (0.3 ml/kg of the 2.5% solution) and instilled into the eyes as a 1% solution. Optical scanning investigation of the cornea was carried out in both acute (23 observations) and chronic experiments (49 observations) in the various stages of keratitis.

EXPERIMENTAL RESULTS AND DISCUSSION

The experiments showed that in the reactive stage of keratitis (first to third days) the zone of the burn was clearly distinguishable as a circular opacity, the optical density of which exceeded that of the nictitating membrane (Fig. 1). This stage is known to be characterized by constriction of the pupil, edema of the eyelids, and hyperemia of the bulbo-conjunctiva. The mean intensity of fluorescence in the zone of the burn in the reactive stage was 13.0 ± 1.2 relative units, 10 times greater than the corresponding normal value (1.3 ± 0.4 units). These results indicate increased ability of the injured cornea to absorb intravenously injected fluorescein.

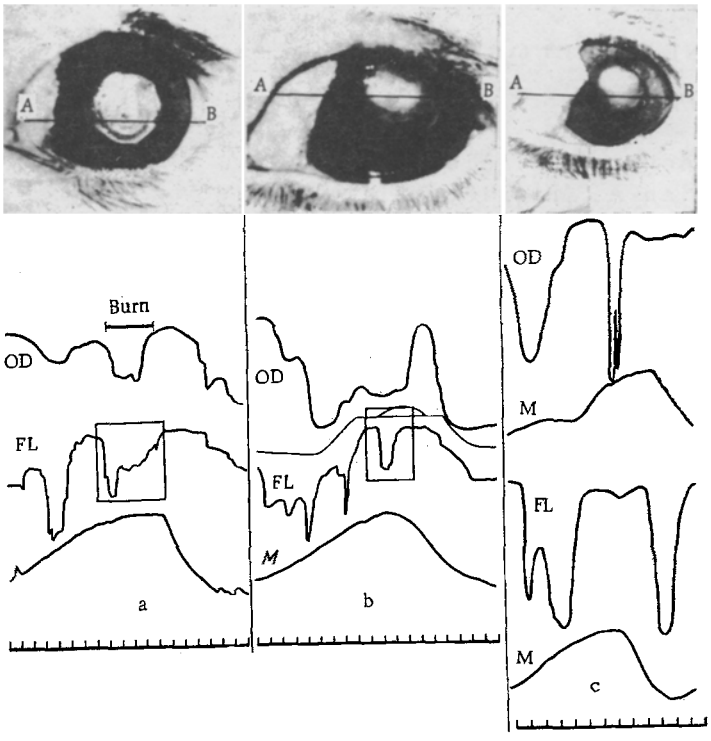

Fig. 1. Reactive (a), degenerative (b), and regenerative (the "dense scar" phase) (c) stages of keratitis (scanning photometry of anterior part of rabbit's eye). Above: photographs of eye with scanning line AB. OD) Optical density along scanning line. Upward movement of pen indicates decrease in photocurrent. FL) Intensity of fluorescence in bulbo-conjunctiva and cornea. M) Mechanogram of movements of scanning system.

As the degenerative stage develops (3rd-14th days) the degree of opacity of the burned zone began to decrease on account of the increasing edema and mucoid degeneration of the corneal stroma. These changes correspond to the period of "false optimism" when translucency of the zone of injury is due to the development of degeneration. The mean intensity of fluorescence fell from 6.0 ± 1.7 units (beginning of the degenerative phase) to 2.0 ± 1.8 units at its end. The dying tissue in the zone of ulceration gave a brick-red fluorescence instead of the ordinary green after instillation of fluorescein into the eyes. This still did not imply complete death of the corneal stroma, but it did indicate degeneration of maximal severity.

The next, regenerative stage (15th-28th days) was characterized by recovery of the ability of the corneal stroma to absorb fluorescein intensively. At the beginning of the reparative process (the "soft scar" phase), intensive fluorescence was observed in the zone of the burn after both intravenous injection of fluorescein and its instillation into the eyes. The mean intensity of fluorescence of the injured cornea was 12.0 ± 1.3 units, much greater than normal. However, later (in the "dense scar" phase) the ability of the cornea to absorb fluorescein after intravenous injection disappeared almost completely, although after instillation into the eyes the power of absorption still remained distinctly high.

These results confirm that the phasic changes in the cornea during keratitis are a characteristic example of a paranecrotic cell response [3, 5, 10]. The concept of paranecrosis now rests on a substantial basis largely because of the method of electron microscopy [7]. It has been shown at the subcellular level that under the influence of various pathogenic factors a nonspecific trophic response arises and passes through several phases: "working hypertrophy," degeneration, and "regenerative hypertrophy" [6].

The phasic changes in keratitis are to a considerable degree identical with those in paranecrosis. This phasic pattern can be used as an objective criterion of the clinical course of keratitis.

LITERATURE CITED

1. V. V. Voronin, Inflammation [in Russian], Tbilisi (1959).
2. A. A. Zavarzin, Outlines of Evolutionary Histology of the Blood and Connective Tissue [in Russian], Nos. 1-2, Moscow (1945-1946).
3. D. N. Nasonov and V. Ya. Aleksandrov, The Reaction of Living Matter to External Agents [in Russian], Moscow–Leningrad (1940).
4. I. P. Pshenichnyi, P. N. Aleksandrov, A. M. Chernukh, et al., Byull. Éksp. Biol. Med., No. 2, 121 (1975).
5. S. N. Romanov, Fiziol. Zh. SSSR, No. 1, 86 (1954).
6. D. S. Sarkisov, Regeneration and Its Clinical Importance [in Russian], Moscow (1970).
7. L. Packer, K. Utsumi, and M. Mustafa, Arch. Biochem., 117, 381 (1966).
8. H. Selye, The Mast Cells, Washington (1965).
9. A. E. Stuart, The Reticulo-Endothelial System, Longman, Edinburgh (1970).
10. V. Weimer, Exp. Cell Res., 18, 1 (1959).