EFFECT OF A DIRECT CURRENT ON PENETRATION OF CYSTEINE THROUGH SEMIPERMEABLE MEMBRANES

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Passage of a direct current through a cysteine solution causes more rapid breakdown of the amino acid and does not facilitate its penetration through semipermeable membranes.

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Changes in the content of protein sulfhydryl groups in the eye tissues in the presence of cataracts and the normalizing effect of cysteine have been described in the literature [5-7].

Clinical observations [2-4] have demonstrated the effectiveness of cataract treatment by administration of various drugs combined with cysteine by subcutaneous and subconjunctival injections, by instillation of cysteine into the eyes, and subsequent application of a galvanic current. Recently a method of cataract treatment by electrophoresis of cysteine through eye baths has been suggested. No experimental data serving as a basis for the use of this method could be found in the literature.

In this investigation an attempt was made to determine the effect of a direct current on a solution of free cysteine, the propulsion of cysteine by a direct current in the interpolar space, and penetration of cysteine through semipermeable membranes.

EXPERIMENTAL METHOD

Experiments were carried out with a 5% solution of free cysteine without buffer, pH 5.0-5.6, in glass vessels and in two plastic baths each of 60-ml capacity. Each bath was divided by a semipermeable membrane into three chambers of equal size. A 5% solution of cysteine in distilled water was poured into the middle chambers of the baths. The side chambers were filled with tap water or physiological saline. Bath No. 1 was the control, and the free diffusion of cysteine was measured in it. Platinum electrodes were placed into the side chambers of bath No. 2 and also into one of the glass vessels, and a direct current (4 mA) was passed for 2 h. Cysteine was detected qualitatively by the reaction between free SH groups and alkaline sodium nitroprusside. Quantitative determination of cysteine was carried out by Sullivan's method [8] (the intensity of Sullivan's reaction was measured on a photoelectric colorimeter) and by amperometric titration of free SH groups [1]. The results of determination by the two methods agreed closely.

EXPERIMENTAL RESULTS

When a 5% solution of cysteine was kept in an open or closed vessel for 24 h, the intensity of the reaction with sodium nitroprusside gradually fell, and on the 2nd day it was negative. The reaction for cysteine during and after electrophoresis remained positive; on the 5th day or later, in the solution through which the direct current had passed, a white precipitate appeared, not completely soluble in water, but the reaction still remained positive even on the 21st day.

The course of the quantitative reaction was as follows. After the cysteine solution had been kept in an open vessel for 10 min, the content of sulfhydryl groups was reduced by 35%, and after 20 min by 42%

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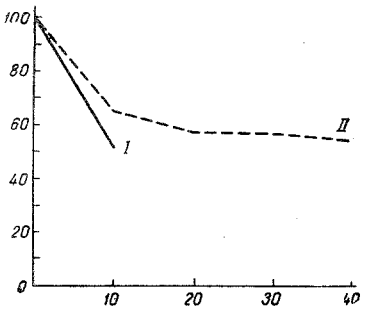

Fig. 1

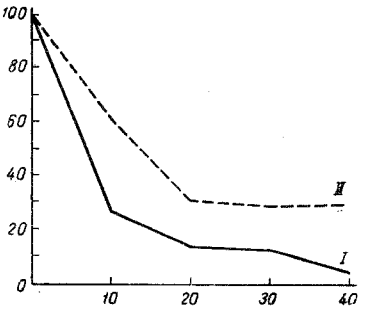

Fig. 2

Fig. 1. Content of SH groups in 5% cysteine solution as a function of time keeping in glass vessels. Abscissa, time of keeping solution (min); ordinate, content of SH groups in solution (in %). Broken line: without passage of current. Continuous line: direct current (4 mA) passed through solution for 2 h.
Fig. 2. Content of SH groups in 5% cysteine solution (in central chambers of baths) as a function of keeping time. Legend as in Fig. 1.

(the corresponding figures for cysteine kept in a closed vessel were 30 and 35%). During the action of a direct current, the content of sulfhydryl groups was reduced after 10 min by 50%, and later none could be detected (Fig. 1).

Investigations of the cysteine solution in the baths showed that 5, 10, and 20 min from the start of the experiment the intensity of staining of the solution was the same in both baths. A qualitative test for free SH groups in the solution in the side chambers of both baths was weakly positive. After electrophoresis for 30 min, the intensity of staining of the solution in the side chambers increased, after 60 min the reaction became weaker, and after 2 h it was completely negative. Possibly more profound changes took place in the molecules of the amino acid in bath No. 2, because a yellow precipitate resembling sulfur was deposited on one electrode (the cathode).

Quantitative determination of SH groups in the side chambers after 10 min showed a decrease by 20% below the initial content (in the control bath) and by 60% following the action of the direct current. The decrease in content of SH groups in the solution from the central chambers of both baths (Fig. 2) was made even greater because of diffusion into the side chambers, but the difference between the two baths nevertheless remained.

The content of SH groups in the 5% cysteine solution after irrigation of a patient's eyes by means of an eye bath (without application of a current) was 55 μmoles, but after electrophoresis through the eye bath it was only 27 μmoles.

Single experiments on rabbits with cysteine-electrophoresis through an eye bath fail to show entry of SH groups into the fluid of the anterior chamber of the eye.

Hence, if a direct current is passed through a cysteine solution, the content of SH groups falls to a greater degree than in the control. The direct current does not affect the rate of penetration of cysteine molecules through semipermeable membranes. The longer persistence of a positive qualitative test with sodium nitroprusside after the action of a direct current than in the control is evidently due not to the presence of cysteine, but to the appearance of certain unidentified conversion products (a white precipitate).

The experimental data described above do not support the view that cysteine-electrophoresis has any value in ophthalmologic practice.

LITERATURE CITED

1. B. I. Zbarskii, I. I. Ivanov, and S. R. Mardashev, Biological Chemistry [in Russian], Leningrad (1965), p. 7.
2. B. Z. Polonskii, Abstracts of Proceedings of the 4th Congress of Ophthalmologists of the Ukrainian SSR [in Russian], Odessa (1962), p. 117.
3. M. S. Sigel', Abstracts of Proceedings of the 4th Congress of Ophthalmologists of the Ukrainian SSR [in Russian], Odessa (1962), p. 116.

4. S. N. Smirnov, Abstracts of Proceedings of the 4th Congress of Ophthalmologists of the Ukrainian SSR [in Russian], Odessa (1962), p. 119.
5. K. F. Firfarova and E. M. Kedrova, Vopr. Med. Khimii, No. 3, 285 (1961).
6. K. F. Firfarova and G. A. Levdikova, Biokhimiya, No. 4, 626 (1951).
7. E. V. Cherevichnaya, Abstracts of Proceedings of the 4th Congress of Ophthalmologists of the Ukrainian SSR [in Russian], Odessa (1962), p. 130.
8. M. X. Sullivan, W. C. Hess, and H. W. Howard, J. Biol. Chem., 145, 621 (1942).