A HISTOCHEMICAL DETERMINATION OF THE DENATURATION OF THE PROTEINS OF THE NERVE CELL INDUCED BY ALCOHOL

V. V. Portugalov, I. B. Krasnov, and T. V. Ball'

Laboratory of Histochemistry (Head, Professor V. V. Portugalov), Institute of the Brain (Director, Active Member AMN SSSR S. A. Sarkisov) AMN SSSR, Moscow
(Presented by Active Member AMN SSSR I. N. Filimonov)
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Despite the wide use in bio- and cytochemistry of denaturing substances, in particular alcohol [1, 2, 4, 5, 6], their action on cell proteins has not been sufficiently studied. Previously it has been shown that protein denaturation can be studied in sections of unfixed tissue by a histochemical method [3].

In the present work we have made a histochemical study on sections of fresh unfixed tissue of the denaturation induced by alcohol in the proteins of the nerve cells of feline dorsal root ganglia. The extent of the denaturation in the neurones was determined from the increased amount of protein SH-groups, as revealed by 5-bromacetyl-3-nitrobenzoic acid (BNB) [3].

EXPERIMENTAL METHOD

Sections 15 μ thick of fresh dorsal root ganglia cut in a cryostat were incubated for 40 min at room temperature in a 1 mg/ml solution of BNB in 0.05 M medinal buffer at pH 7.4. To denature the tissue proteins, to the incubation medium we added ethanol to a final concentration of 20, 30, 40, 50, 60, and 80%.

At the end of incubation the sections were fixed in 10% neutral formal, washed free in alcohol from an excess of the reagent, and the reactions of reduction of the nitrogroup of BNB to an aminogroup, diazotization of the aminogroup, and azocoupling with N-acid as described by Maddy [8] were carried out; they were then placed in glycerine-gelatine and photographed on iso-ortho-chromatic plates with a ZhZ filter. Measurements of the negative were made with a MF-4 microphotometer. The optical density was measured in the usual logarithmic units.

EXPERIMENTAL RESULTS

A study of the sections of the dorsal root ganglia taken from the cervical region showed a correlation between the intensity of reaction of the cytoplasm and the size of the nerve cells. The intensity of the stain of the small neurones was 50-100% greater than that of the large ones. The medium-sized nerve cells were stained to an intermediate degree. In the large neurones, the cytoplasm and nucleus as a rule showed no difference with respect to their content of SH-groups.

When ethanol was added to the incubation medium, as its concentration was increased there was a steady increase in the amount of sulfhydril groups revealed histochemically in the cell structure. This effect was observed in the cytoplasm, nucleus, and nucleolus (Fig. 1, b, c, d). However, the increase in the amount of SH-groups in the structures of cells of various sizes was not uniform (Fig. 2).

In the small neurones, it was found that the concentration of sulfhydril groups was higher in the nucleus and nucleolus than in the cytoplasm. The SH-group content revealed was greatest when the incubation medium contained 40-50% ethanol. Then, in the small neurones, the nuclear and nucleolar SH-group content was $2\frac{1}{2}$-3 times greater than the cytoplasmic, and at the same time the relative increase of these groups in cytoplasm was the same for neurones of all sizes. Increase of the ethanol concentration to 60-80% reduced the amount of SH-groups present (Fig. 1, e, f; see Fig. 2).

The results we have obtained indicate that the action of ethanol on cell proteins is directly proportional to its concentration. In its action on proteins in concentrations of 20 to 50%, most of the reactions occurring are characterized by changes in the configuration of the cell proteins, as we inferred from the increased amount of sulfhydril groups.

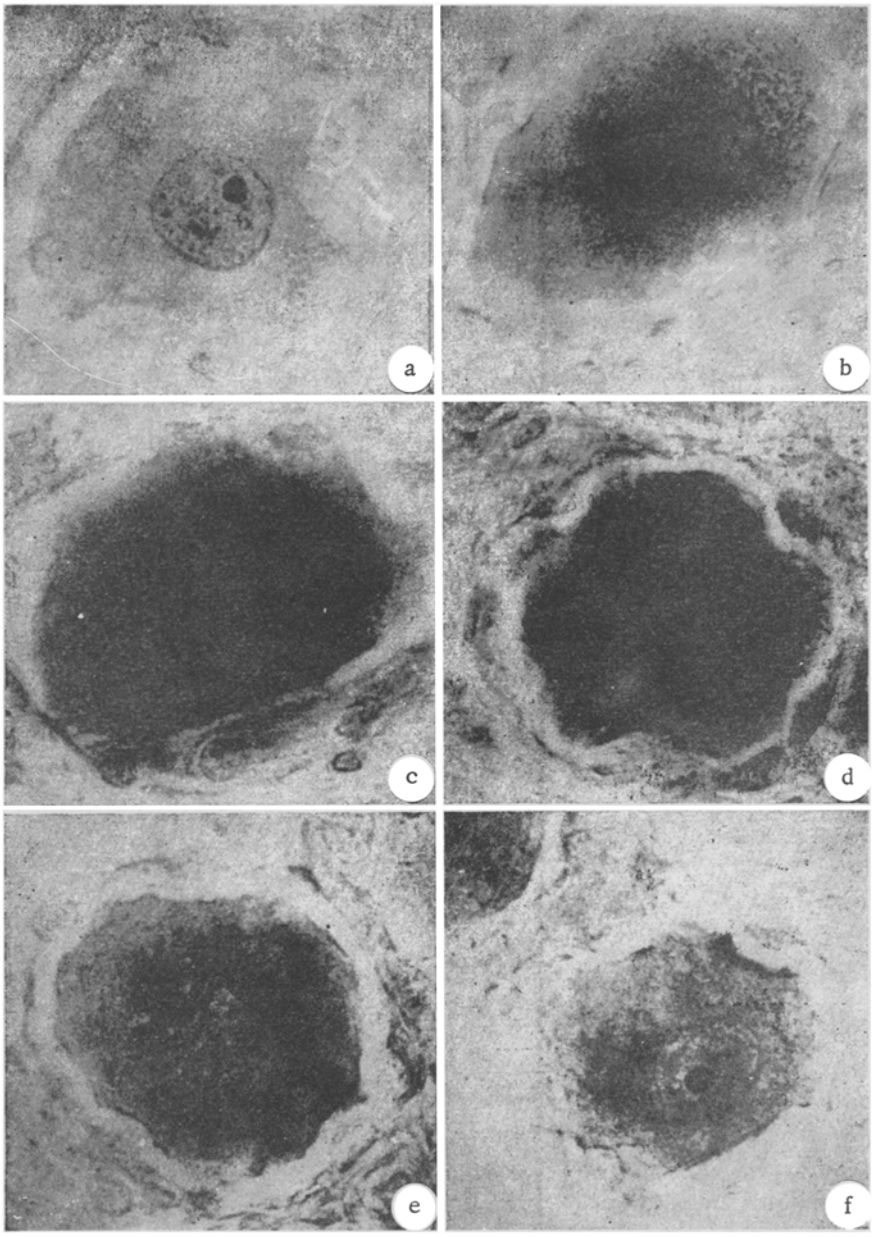

Fig. 1. Distribution of the sulfhydril groups of proteins in the neurones of the dorsal root ganglia of the cat. a) Normally; other cases after the addition of: b) 30%, c) 40%, d) 50%, e) 60%, and f) 80% ethanol. Method includes use of BNB. Objective 90×, ocular Gomal' VI.

Increase in the amount of the measured SH-groups in protein denaturation, which has been observed by many authors, has been used as a test for such denaturations [7, 10].

For concentrations of 60-80%, dehydration and coagulation of the proteins proponderate over denaturation; they may bring about changes of the special relationships within the protein molecules and hinder access of the reagent to the functional groups of the proteins. The studies of Ostrowski, Komerder, and Kwarecki [9] have shown that when tissues are treated with absolute alcohol 99.75% of the protein is preserved, and they hold that the reduction in the number of manifest SH-groups with increase of ethanol concentration from 50 to 80% is not due to the alcohol-soluble proteins being washed out from the section.

The wide range of changes of the nucleus, cytoplasm, and the nucleolus in sensory nerve ganglia of different sizes induced by ethanol suggest that the proteins of these structures differ in their organization and reactivity.

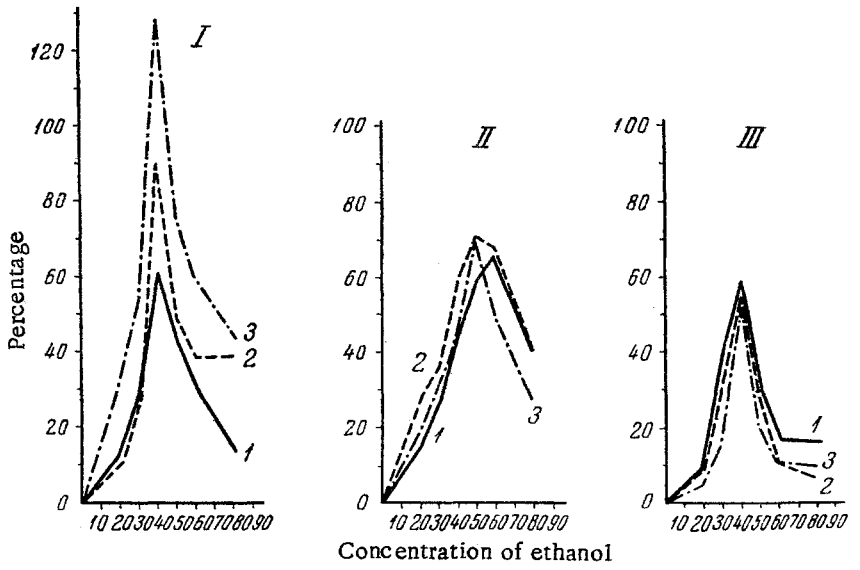

Fig. 2. Change in the amount of protein SH-groups in small (I), medium (II), and large (III) neurones of the dorsal root ganglia of the cat in response to treatment with various concentrations of ethanol.

The results indicate convincingly that different concentrations of alcohol may cause either denaturation or coagulation of the protein of the nerve cells. The significance and relative importance of each of these types of change in relation to the general histochemical picture remains unclear. Nevertheless in interpreting our results we must take into account the possibility of a change of the histochemical picture under the influence of organic solvents (particularly ethanol) present in the fixatives.

SUMMARY

The effect of various concentrations of alcohol on the proteins of the nerve cells of the dorsal root ganglia were studied in cats by a histochemical and photometric method. Denaturation of the proteins was measured in terms of the change in the number of SH-groups, the groups being detected histochemically by means of 5-bromoacetyl-3-nitrobenzoic acid.

The amount of SH-groups in the neurones decreased in sections treated with concentrations of ethanol ranging from 20 to 50%, and were also decreased by concentrations of 60 to 80%.

Denaturation of protein was induced by lower concentrations (20-50%), whereas higher concentrations (60-80%) evidently caused more complex changes.

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