*Zorenko Yevgeniya, Singh Rhea*

**STAINING WITH BROMOPHENOL BLUE AS METHOD FOR**

**STUDYING THE NEUROPIL PROTEIN OF CEREBRAL**

**HEMISPHERES IN RATS WITH EXPERIMENTAL**

**ALZHEIMER'S TYPE DEMENTIA**

Department of General and Clinical Pathophysiology named after D.E. Alpern

Kharkiv National Medical University

Kharkiv, Ukraine

Scientific advisor: prof. Pavlova Olena, prof. Gubina-Vakulik Galina

Introduction. In Ukraine, some researchers in oncology, hematology, gynecology, experimental medicine stain the slides of different tissues with bromophenol blue (BPB) guided by the method of Mikel Calvo (1975) to study the ratio of carboxyl and amino groups in proteins. Nowadays, the Mikel Calvo method is something new, which, in fact, is a long-forgotten old one.

It is known that hypoxia, oxidative stress, endothelial dysfunction, choline deficiency leads to the formation of pathological proteins (amyloid, tau protein) and atrophy in the brain tissue. It is of interest to research how the staining of brain slides with BPB helps to determine these pathological changes in hemispheres’ neuropil in rats with experimental neurodegeneration.

Aim of study. To study the specific changes in the neuropil of the cerebral hemispheres on brain slides stained with bromophenol blue in rats with scopolamine-induced and nitrite-induced dementia. Material and methods. The experiment was performed on 74 male WAG rats with 14-day and 28-day nitrite-induced and scopolamine-induced model of dementia without and after single intravenous mesenchymal stem cells (MSC) injections (500000 cells per rat). The control group (gr.C) received 0.9% sodium chloride solution. The animals were sacrificed on the 14th day after all injections. The brain slices were stained with

BPB and studied using Zeiss Axiostar plus binocular microscope and software GIMP.

The average values of color brightness in the red (R) and blue (B) parts of the spectrum were measured on computer images of the white matter of cerebral hemispheres slides. The R/B ratio was determined to evaluate the level of oxidative modification of neuropil proteins. The optical density of the neuropil of cerebral hemispheres in the blue, red, green parts of the spectrum were determined.

Results. In gr.C the lower the R/B ratio, the higher the optical density of this protein in the neuropil in the red and green parts of the spectrum was observed. The presence of a protein that reflects blue color was close to 0. In the main groups, areas of neuropil atrophy were observed. The optical density of proteins in red and green reflected colors decreased. Higher values of the R/B ratio indicated the presence of oxidative modification of neuropil proteins. In other parts of the neuropil, a different picture was observed: the R/B ratio became lower, the optical density of the protein in green color was the higher, the greater the oxidative modification of neuropil proteins. The same dependence was noted in the reflected blue color. However, in the red color the optical density of proteins was stable with different degrees of damage. In these areas, there was an accumulation of a dense substance that was resistant to hypoxia and toxic effects, against the background of significant oxidative modification. In the groups with the maximum number of injections, especially scopolamine, there were areas of accumulation without signs of dystrophy, which corresponded to a mature amyloidplaque.

Conclusions. Histochemical staining with bromophenol blue was very indicative for studying the dynamics of the development of amyloidosis, in particular, of the brain.