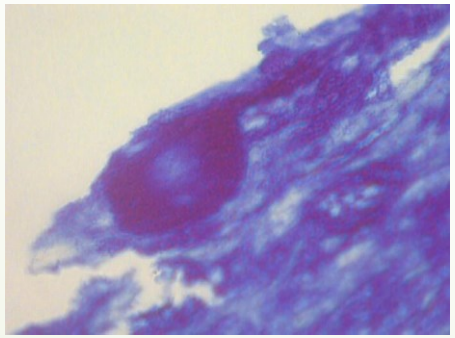
KHARKIV NATIONAL MEDICAL UNIVERSITY

RESULTS OF MORPHOMETRIC ANALYSIS OF HISTOCHEMICAL STAINING WITH BROMOPHENOL BLUE OF THE BRAIN WHITE MATTER IN MODELING ALZHEIMER'S DISEASE



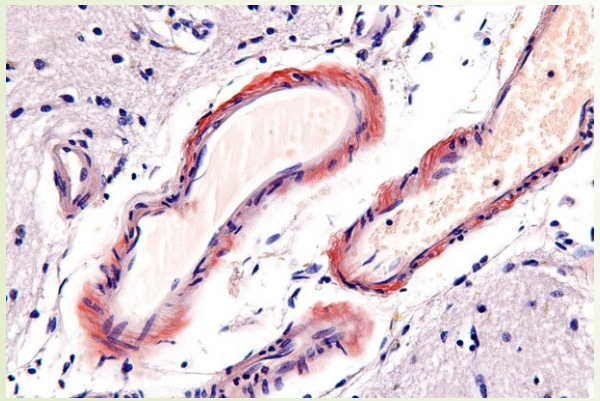
RHEA SINGH,
YEVGENIYA LUKYANOVA,
GALINA GUBINA-VAKULIK,
OLENA PAVLOVA

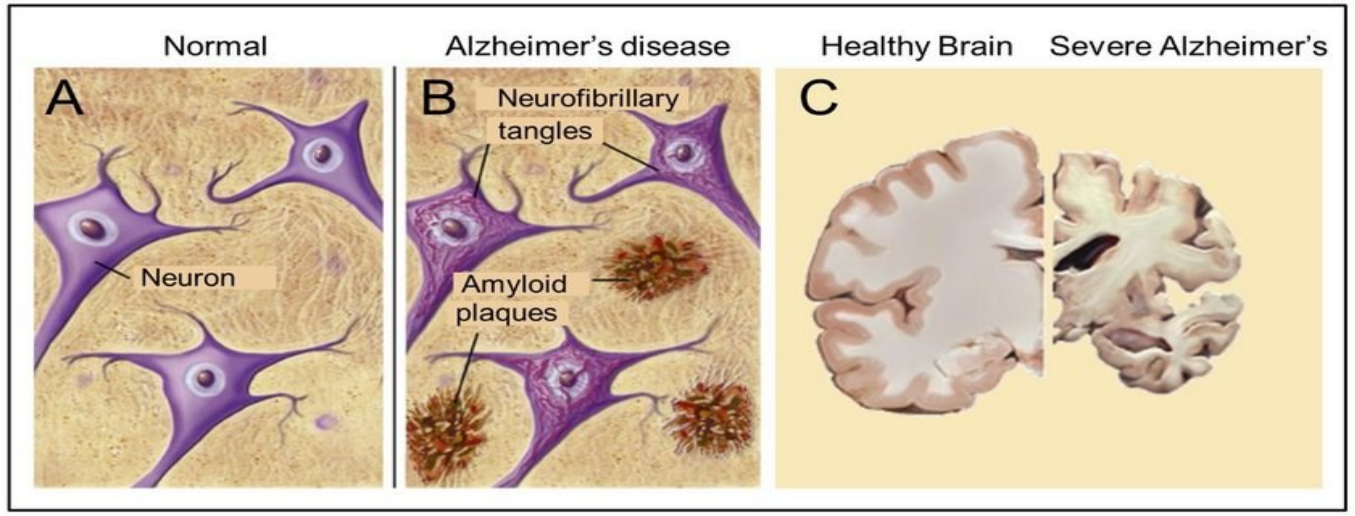
May 19, 2022

INTRODUCTION

- Alzheimer's disease (AD) is a progressive, neurodegenerative disease characterized by function loss and neuronal demise in numerous regions of the brain leading to cognitive dysfunction.
- About 50 million people suffer from dementia around the world.
- A key benefactor of the vascular dysfunction in AD is cerebral amyloid angiopathy (CAA), observed in up to 90% of AD patients due to deposition of amyloid around cerebral vessels.
- Amyloid is identified using a Congo red histochemical test or an amyloid immunohistochemical reaction.

Cerebral amyloid angiopathy



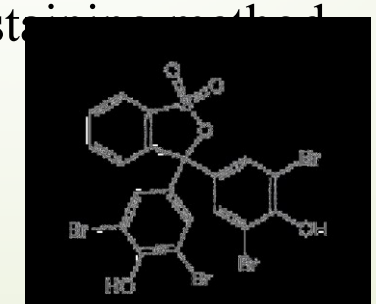


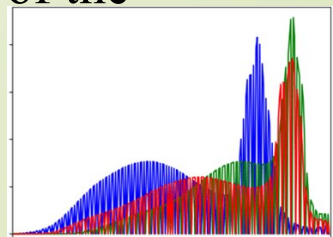
INTRODUCTION

CONTINUED

- As we know, the first step in the process of amyloid formation in the brain white matter is protein dystrophy.
- This leads to synthesis of fragments of protein molecules, as a result of which, an inert substance-amyloid is formed.
- Therefore, we decided to study the steps of amyloid formation process, using bromophenol blue (BPB) staining and with calculation of index Mikel Calvo [Davydenko IS, 2017].
- Mikel Calvo (1975) method was modified by Davydenko I. S. (2003-2016).
- The basic aim of this method is **to study the ratio of carboxyl and amino groups in proteins, their oxidative modification and dystrophy development.**

Mikel Calvo method (1975) + Modification of the st[illegible] + optical density of protein in the blue, red, green (RGB) parts of the spectrum

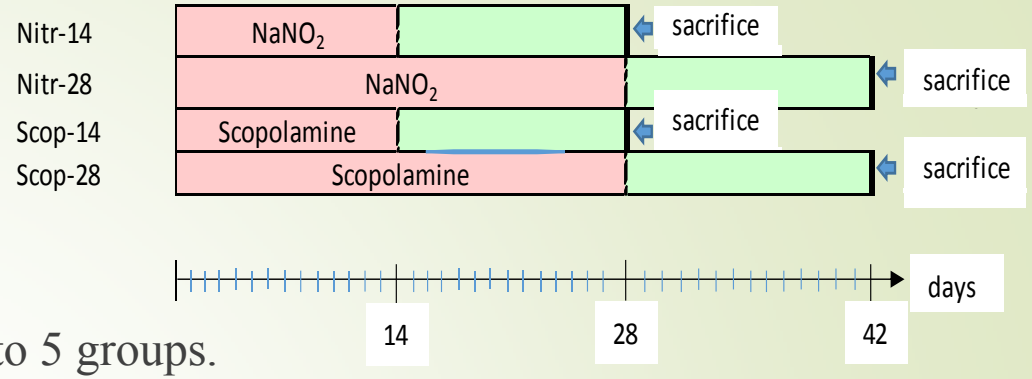




AIM OF THE STUDY

- To analyze the morphometric results of the brain white matter after histochemical staining with bromophenol blue in rats with experimental Alzheimer's disease caused by different ways.

MATERIALS & METHODS



- 48 male WAG rats weighing 180-250 g were divided into 5 groups.
- Rats from group Nitr-14 (2 weeks, n=8), Nitr-28 (4 weeks, n=8) and Scop-14 (2 weeks, n=8), Scop-28 (4 weeks, n=8) were injected with aqueous solution of sodium nitrite (NaNO2) and scopolamine butylbromide at a dose of 50 mg/kg and 1 of mg/kg body mass intraperitoneally for 2 and 4 weeks respectively which resulted in the development of AD.
- Control group (n=16) received 0.9% sodium chloride solution at the same period of time.
- The animals were sacrificed on the 14th day after all injections.
- The brain slices were stained with Congo red and Bromophenol blue 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 using software GIMP . **The R/B ratio was determined to evaluate the level of oxidative modification of neuropil proteins.**
- The optical density (D) of the neuropil of cerebral hemispheres in the blue, red, green parts of the spectrum were determined.

RESULTS

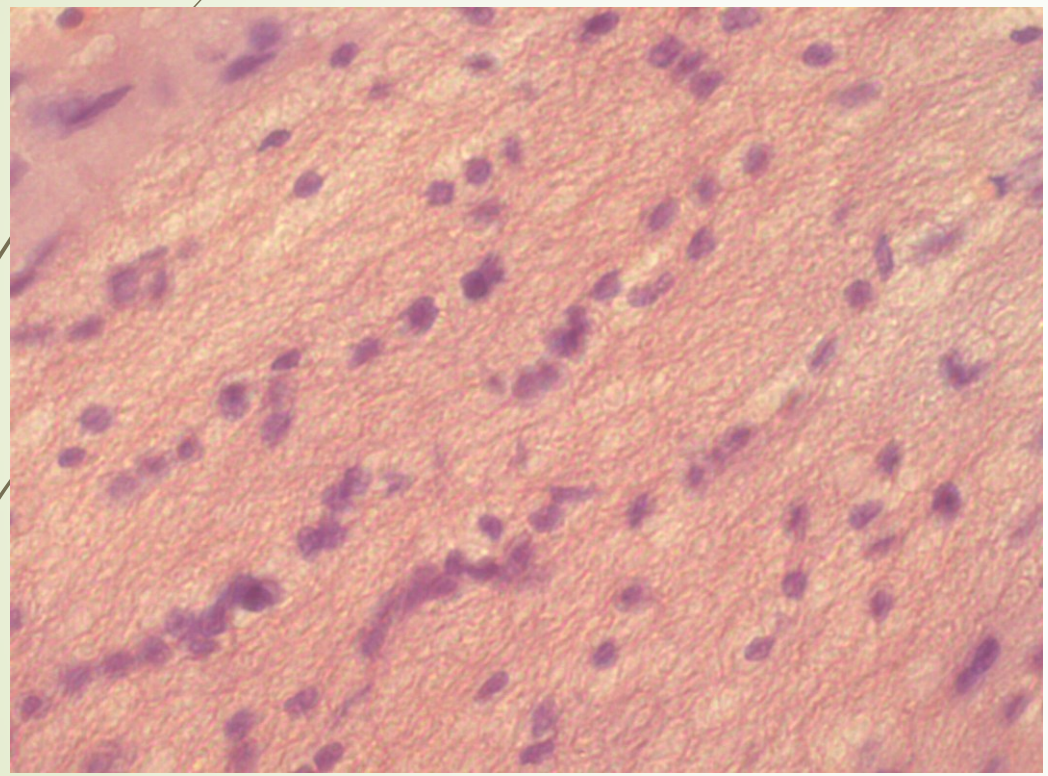


Fig 1. The neuropil-Control group with Congo Red staining. x 400

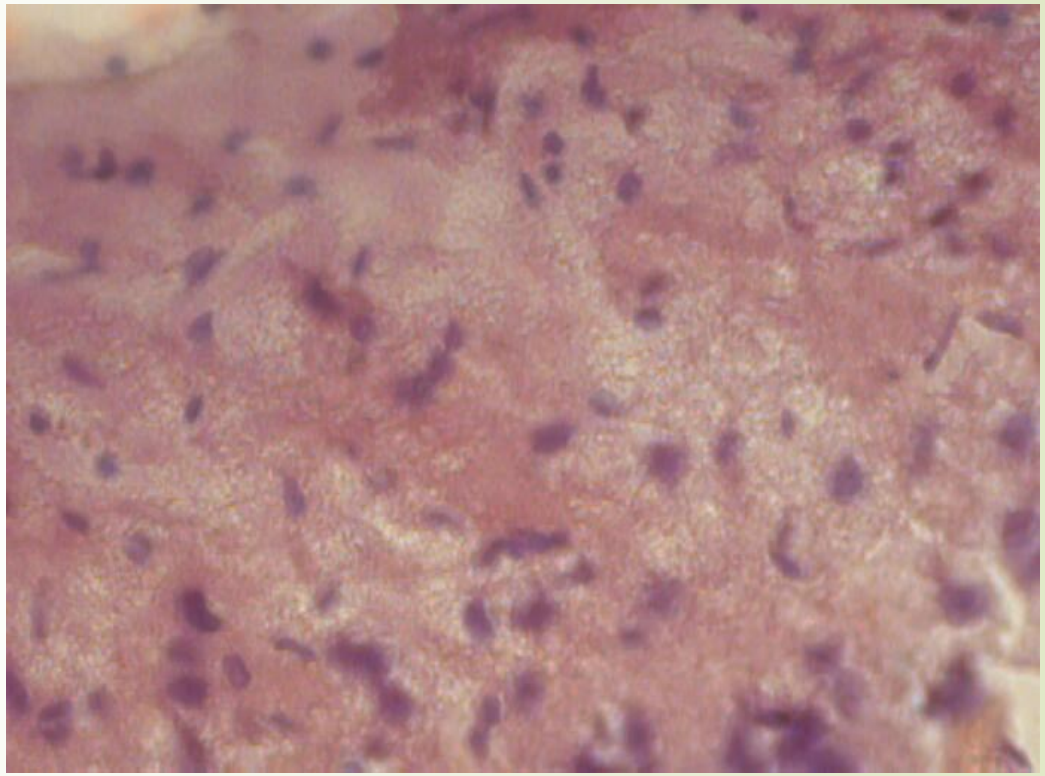


Fig 2. The foci of neuropil homogenization-Group Scop-28. Congo Red. X 400

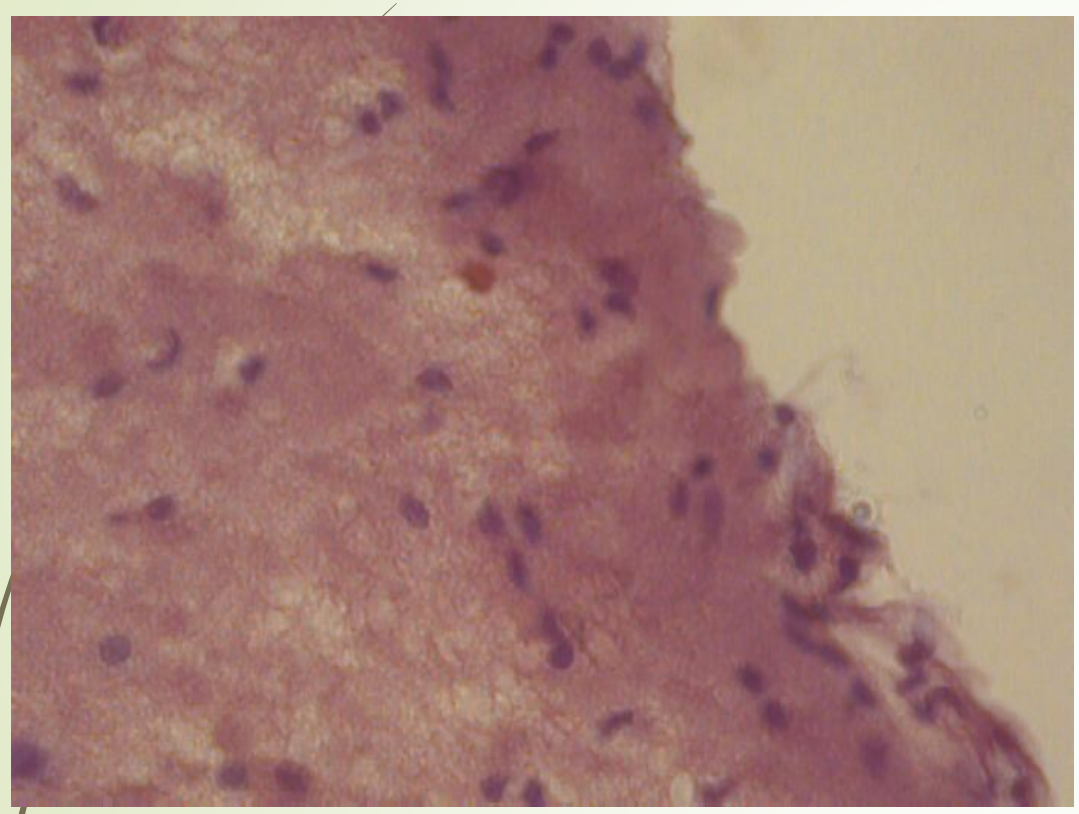


Fig 3. White matter of the surface of the large brain hemispheres. The foci of congophilic masses. Group Scop-14. Congo Red. X 400

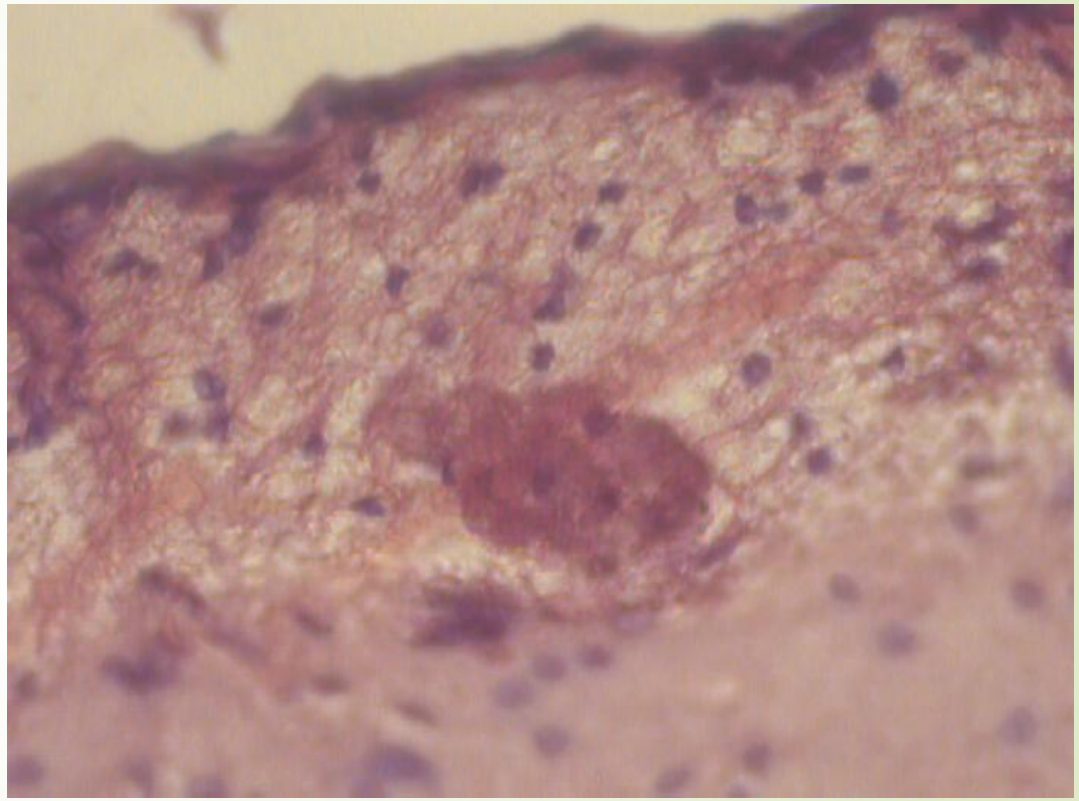


Fig 4. Accumulation of congophilic masses (amyloid plaque) and depletion of neuropil. Group Nitr-28. Congo Red. X 400

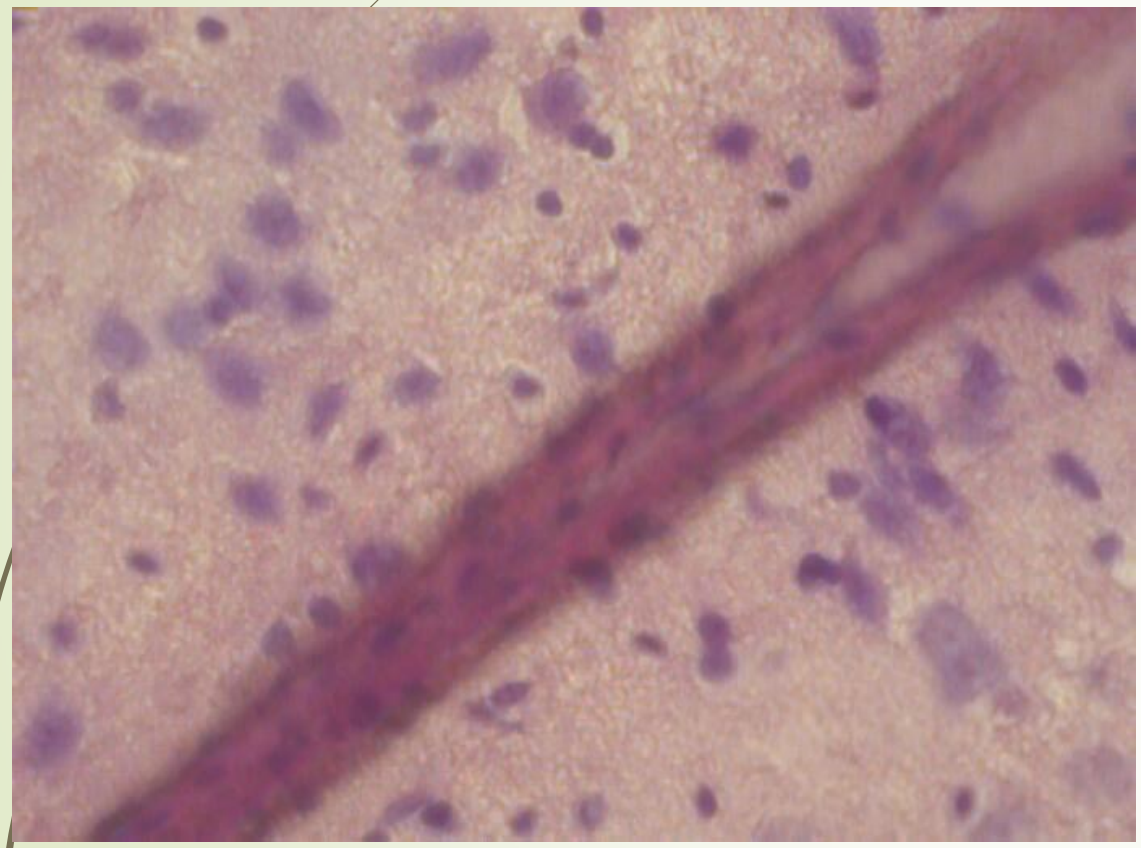


Fig 5: Formation of congophilic masses in the walls of small arteries. Group Scop-28. Congo Red. X 400

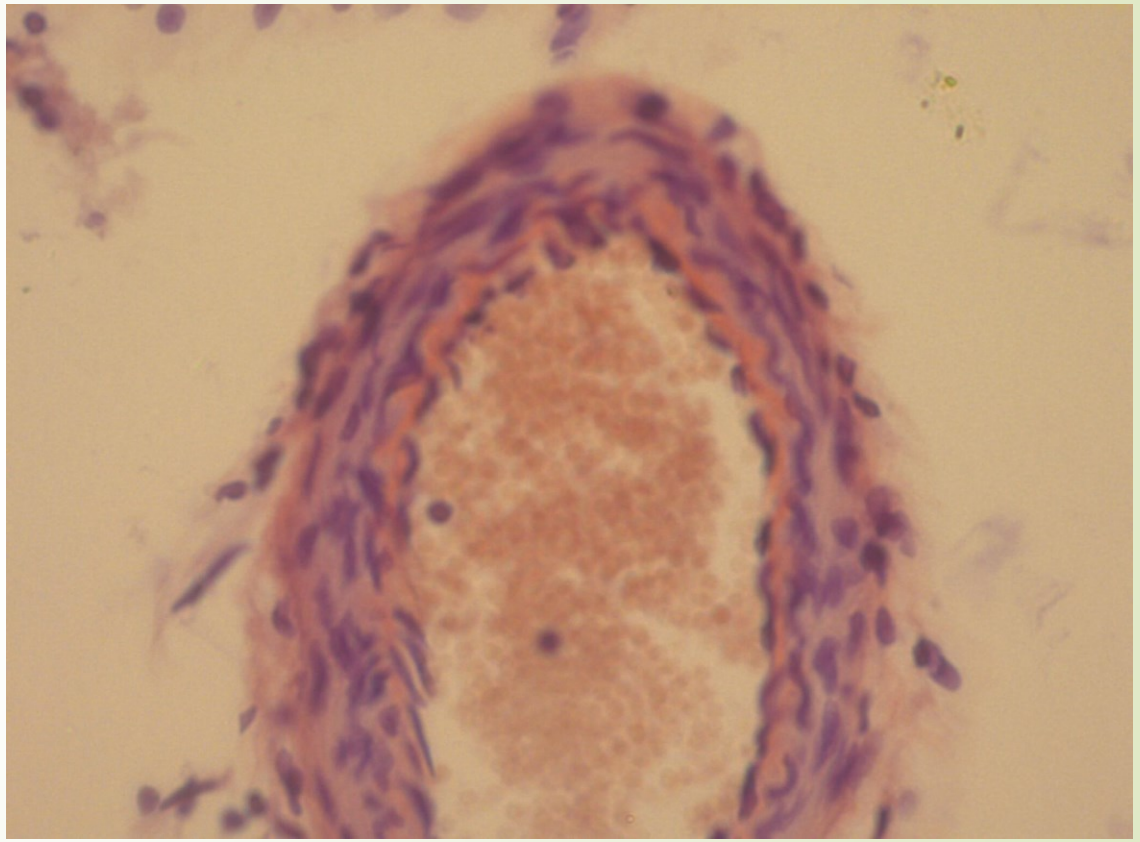
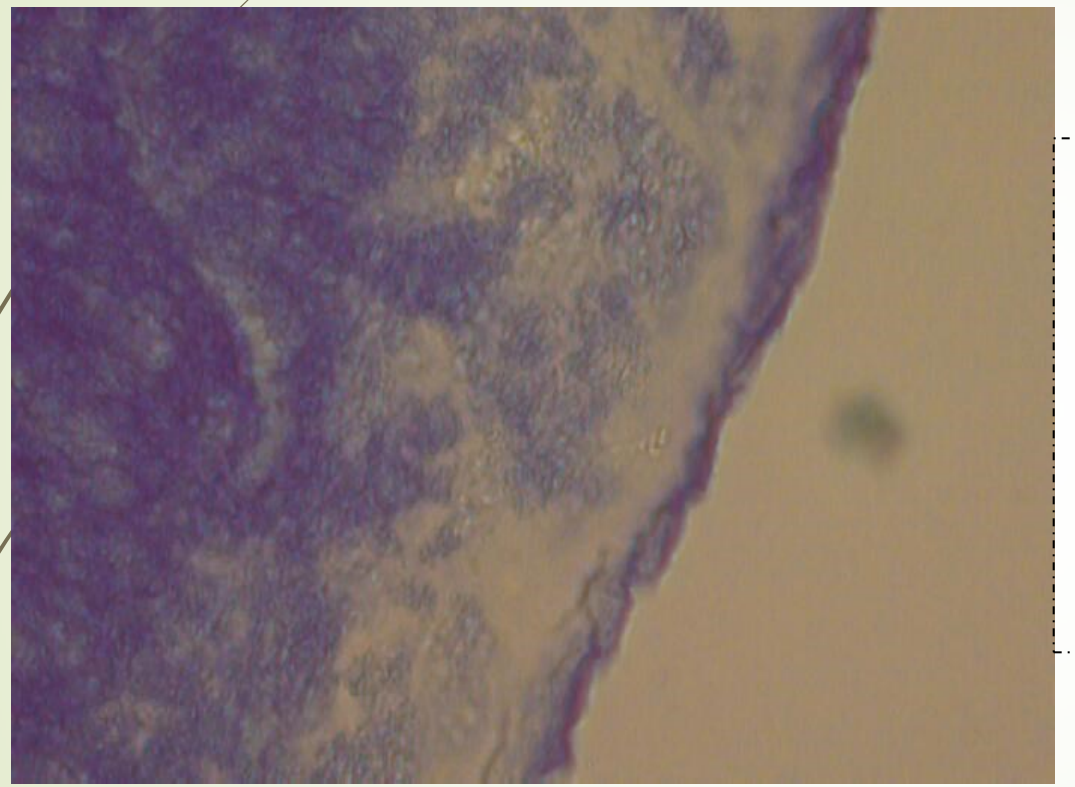
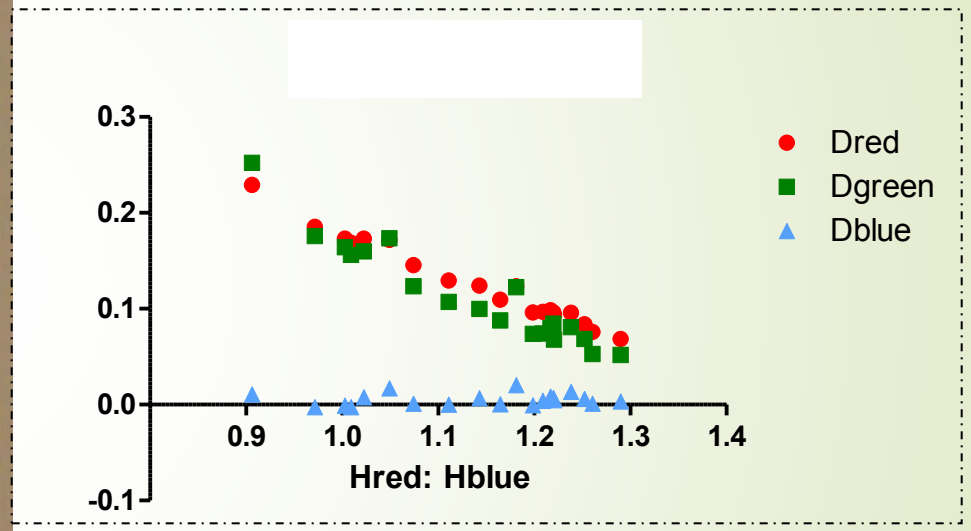


Fig 6: Subendothelial accumulation of amyloid. Group Nitr-14. Congo Red. X 400

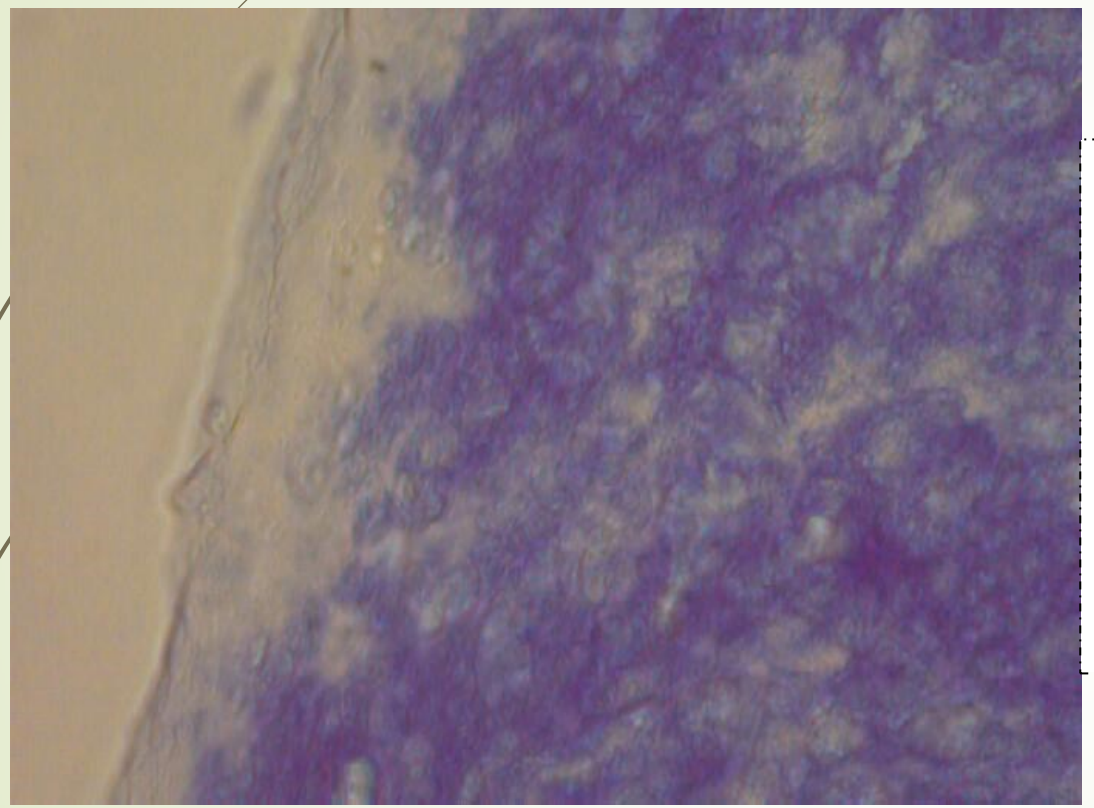
Fig. 7. The neuropil of the hemispheres' white matter. Control group





The maximum optical density of the neuropil in red and green colors (i.e. with the maximum number of corresponding proteins) was 0.2-0.3 conventional units of optical density with low Calvo index (H red:H blue) (0.6-0.9). The minimum optical density of the neuropil (i.e. with a minimum amount of the corresponding proteins) was 0.05 - 0.1 conventional units of optical density with a high Calvo index (1.1 – 1.3).

Fig. 8. The neuropil atrophy in an animal. Gr. Nitr-14



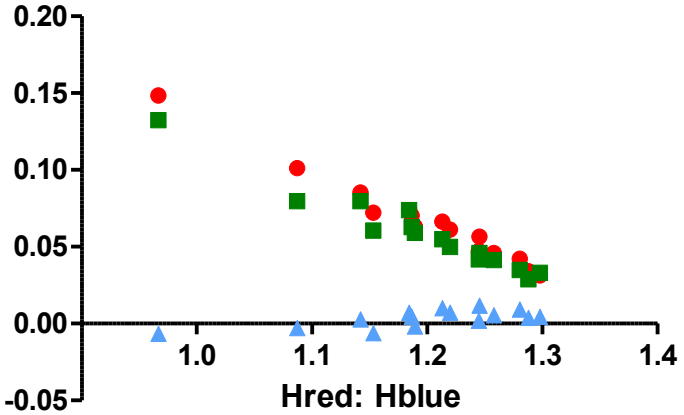
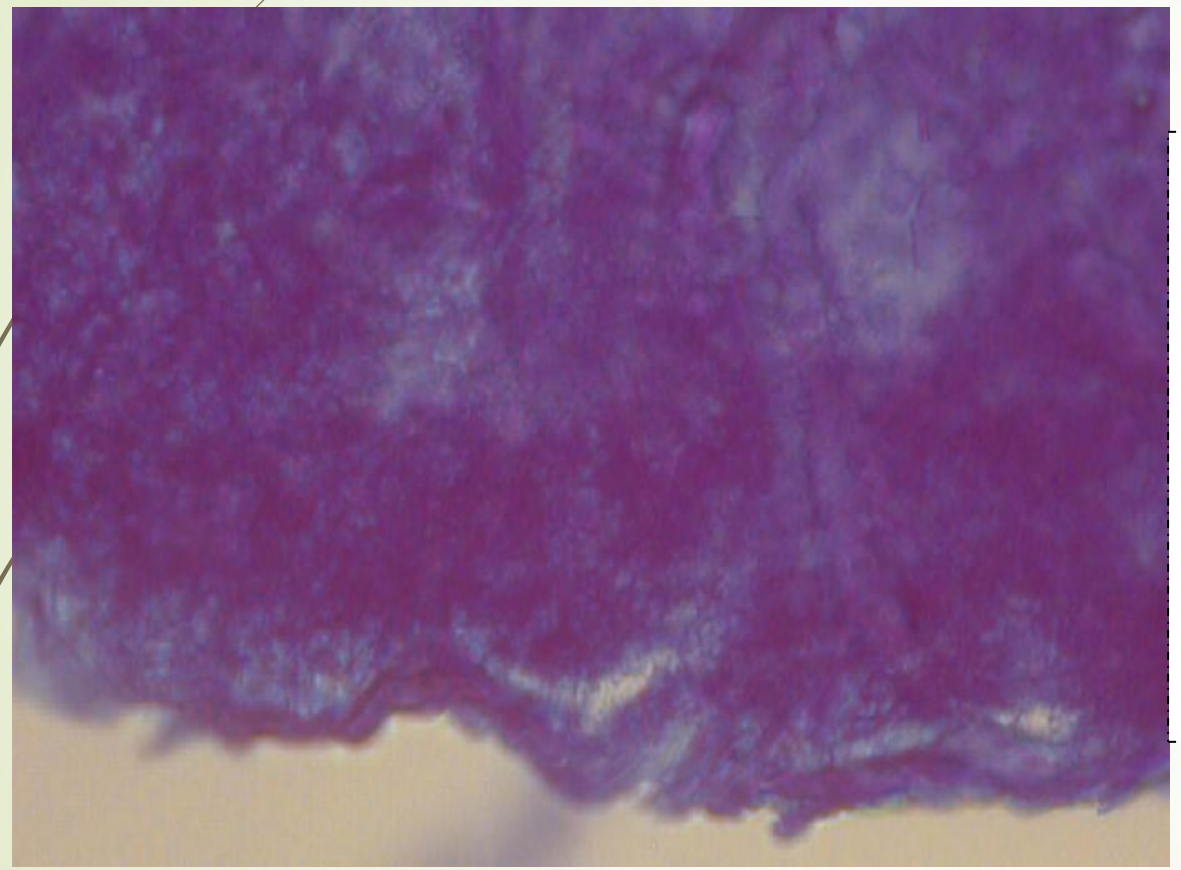
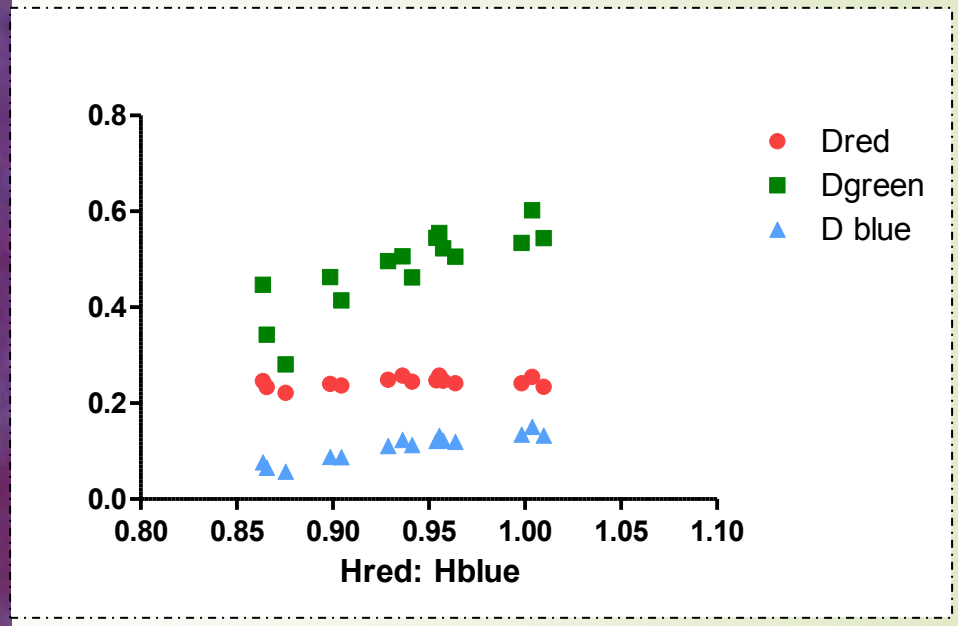


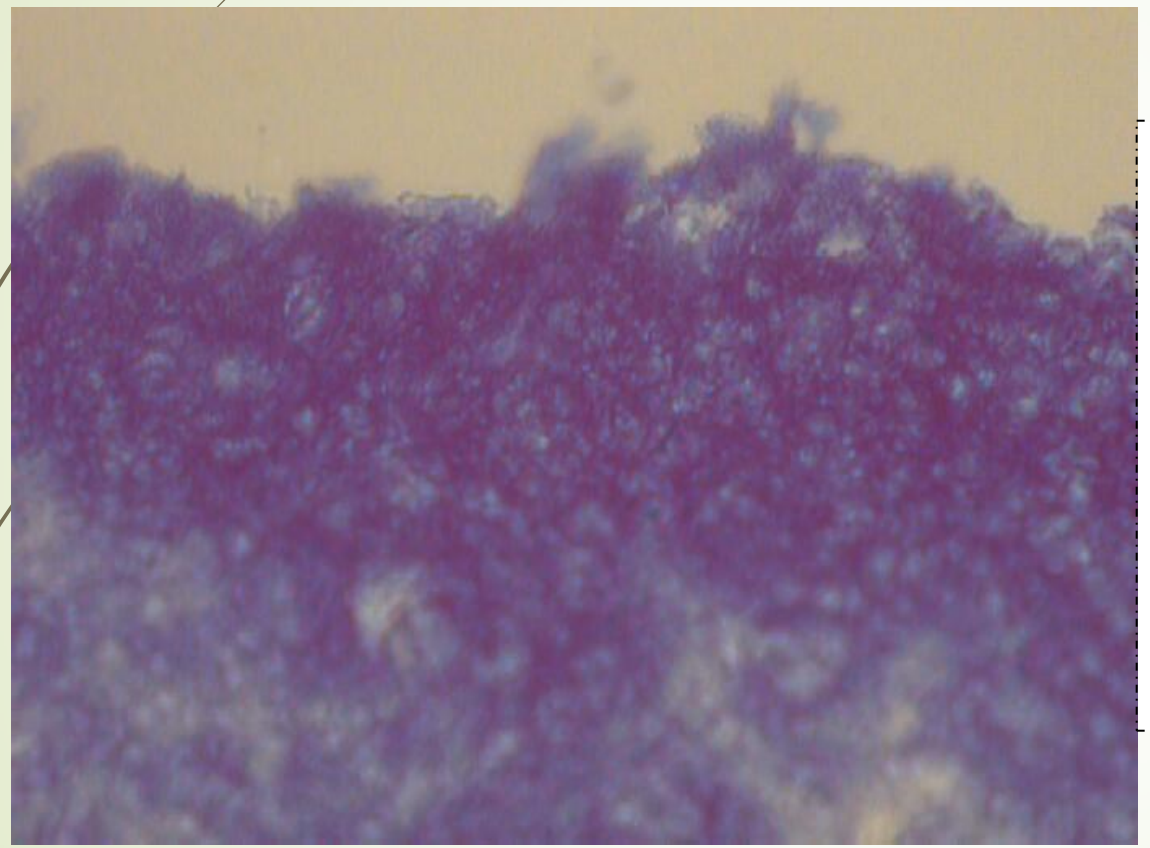
Fig 9: The neuropil dystrophy in an animal. Group Nitr-14

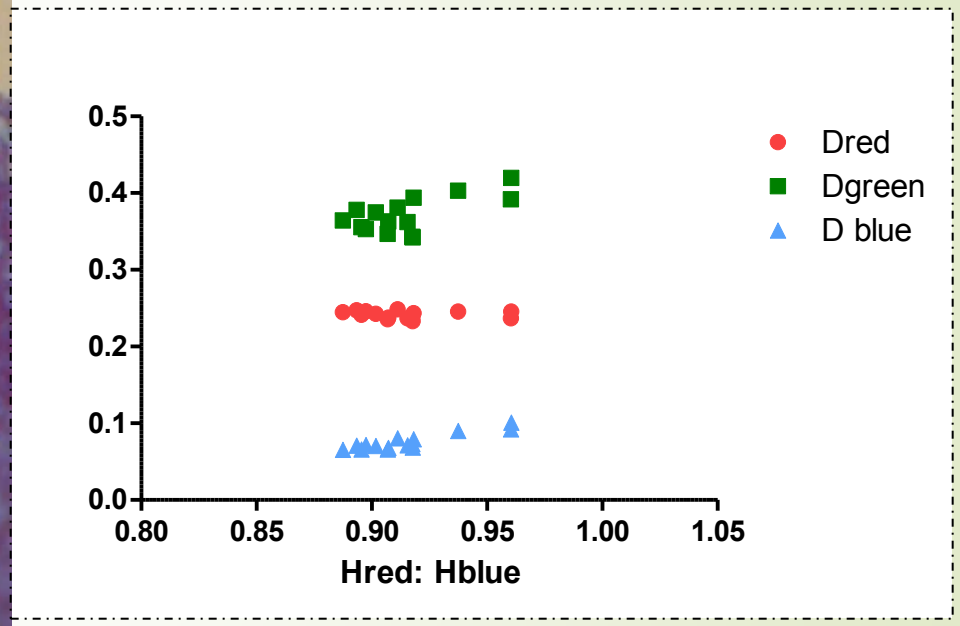




At the stage of neuropil dystrophy of amyloid formation the Calvo correlation became positive (r =+0.7), proteins with a high Calvo index were dominant, which, according to Calvo's interpretation, was due to an increase in the number of free carboxyl groups.

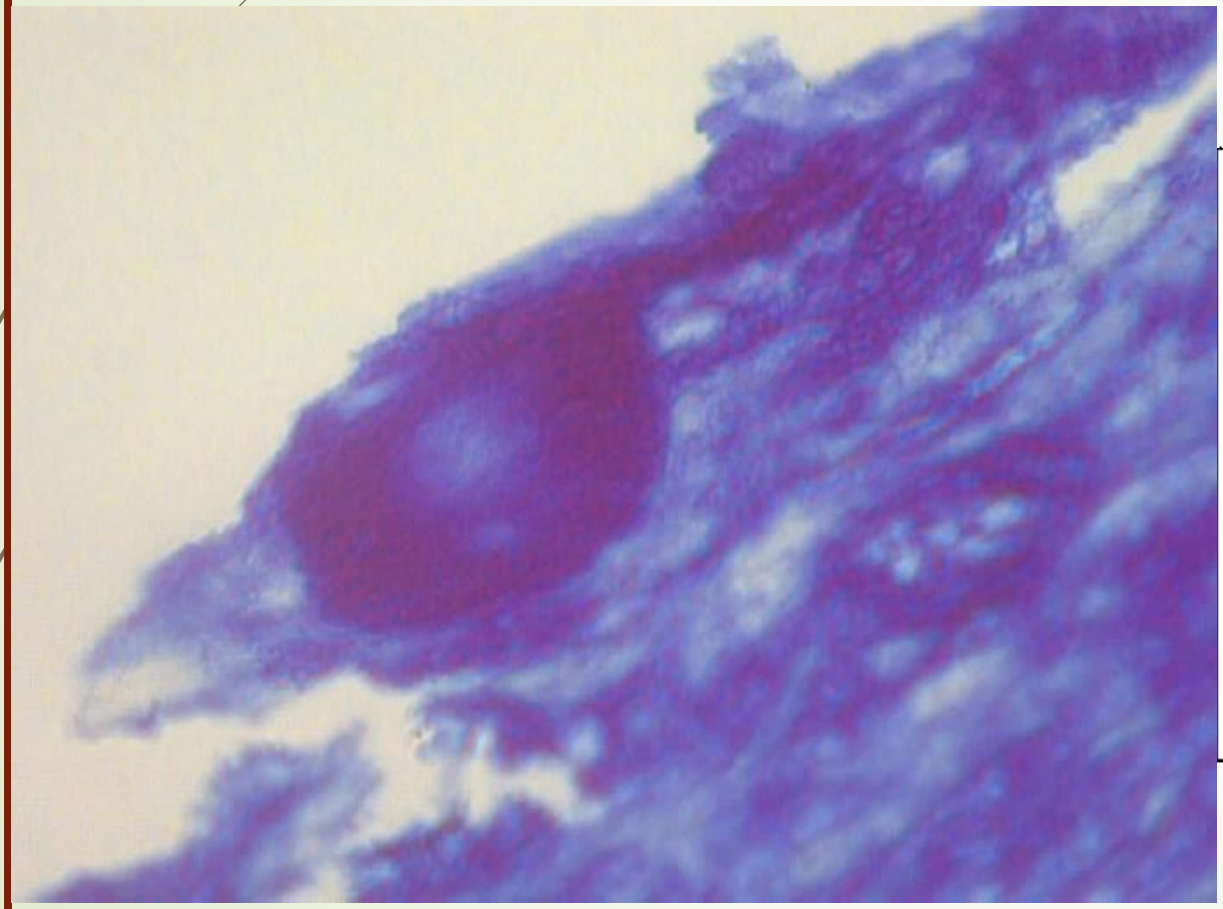
Fig 10: Amyloidosis. Group Nitr-28

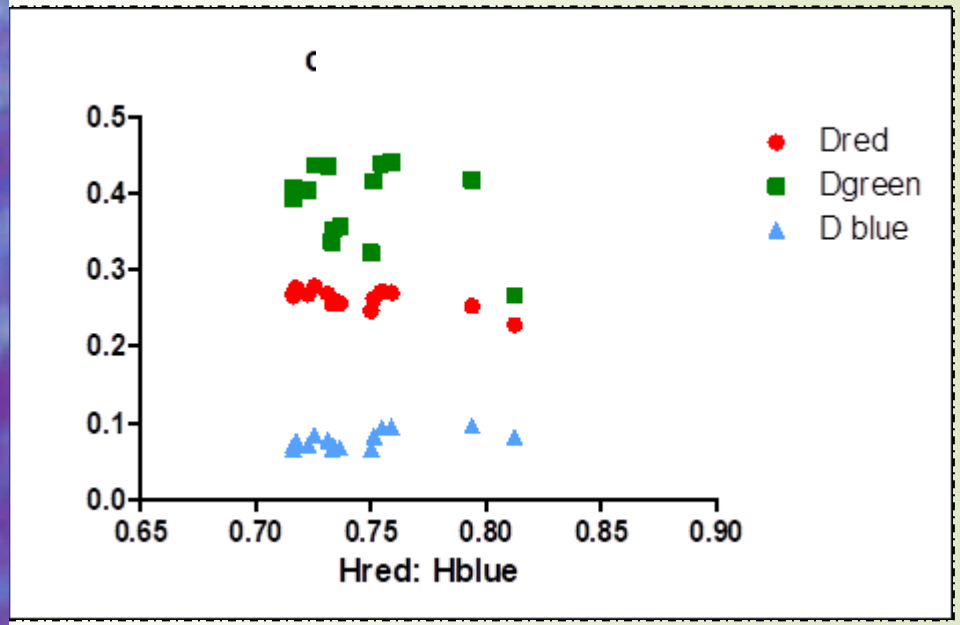




In areas of amyloid accumulation, the Calvo correlation disappeared (r= +0.2 - -0.2), the Calvo index was low (0.8-0.9), i.e. neuropil protein had no signs of degeneration and accumulation of amino groups, this substance was inert.

Fig11: The mature amyloid. Gr. Scop 28.





CONCLUSION

Additionally to the morphometric estimates proposed by Calvo, the correlation analysis using bromophenol blue staining offered determination the different histological stages of amyloid formation in the neuropil in rats with nitrite- and scopolamine-induced models of Alzheimer's disease.

ACKNOWLEDGEMENT

Immense gratitude to Prof. Gubina-Vackulick Galina for carrying out the morphological study.

thank you for your attention!