INVESTIGATION OF SOME HYDROLASES IN SENILE PLAQUES

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Histochemical methods revealed activity of cholinesterases, carboxylesterases, acid phosphatase, and adenosine triphosphatase in senile plaques.

The formation of senile plaques, which are frequently found in the cerebral cortex of old people, and most constantly in senile dementia and Alzheimer's disease, is associated by most investigators with various structural elements of the central nervous system.

The formation of senile plaques is interpreted by some workers as necrobiosis [2, 3, 20], by others as the precipitation of certain substances [4, 8, 18], and by a third group as the result of deposition of metabolic products [1, 6, 13, 14]. Some investigators associate the formation of senile plaques with amyloidosis [5, 7, 9, 10, 17, 19]. The conflicting opinions expressed regarding the nature of senile plaques make it essential to seek fresh ways of investigating these structures. Recently, for instance, studies of the enzyme histochemistry of senile plaques have been published [11, 12, 15, 16].

This paper presents data concerning the activity of cholinesterases, nonspecific esterases, phosphomonoesterases, and adenosine triphosphatase in senile plaques.

EXPERIMENTAL METHOD

Pieces of brain (from areas 10, 46, 6, 1, 22, 40, 17, hippocampus, cerebellum, hypothalamus) of psychiatric patients dying at the ages of between 62 and 90 years, were taken 3 h after death and were fixed for 24 h in the cold in calcium-formalin. Sections were cut on a freezing microtome and washed in ice-cold water, after which, depending on the method used, they were either placed immediately in incubation solutions or preliminarily fixed to slides.

Cholinesterases were investigated by Gomori's method with acetylthiocholine iodide and butyrylthiocholine iodide, and also by the Karnovsky–Rootes method with actylthiocholine iodide. Control sections were treated in the same incubation media, but with the addition of eserine in a final concentration of 10^{-5} M.

Nonspecific esterases were studied by Gomori's modification of the Nachlas–Seligman method. To differentiate between the esterases, control sections were preliminarily treated in 10^{-5} M or 10^{-4} M solutions of eserine and in 10^{-4} M sodium p-chloromercuribenzoate solution.

Acid phosphomonoesterase activity was studied by Gomori's lead method and alkaline phosphomonoesterase activity by Gomori's calcium-cobalt method. Adenosine triphosphatase was determined by Gomori's lead method. Control sections were placed in incubation medium with the addition of sodium p-chloromercuribenzoate in a final concentration of $2.5 \cdot 10^{-3}$ M.

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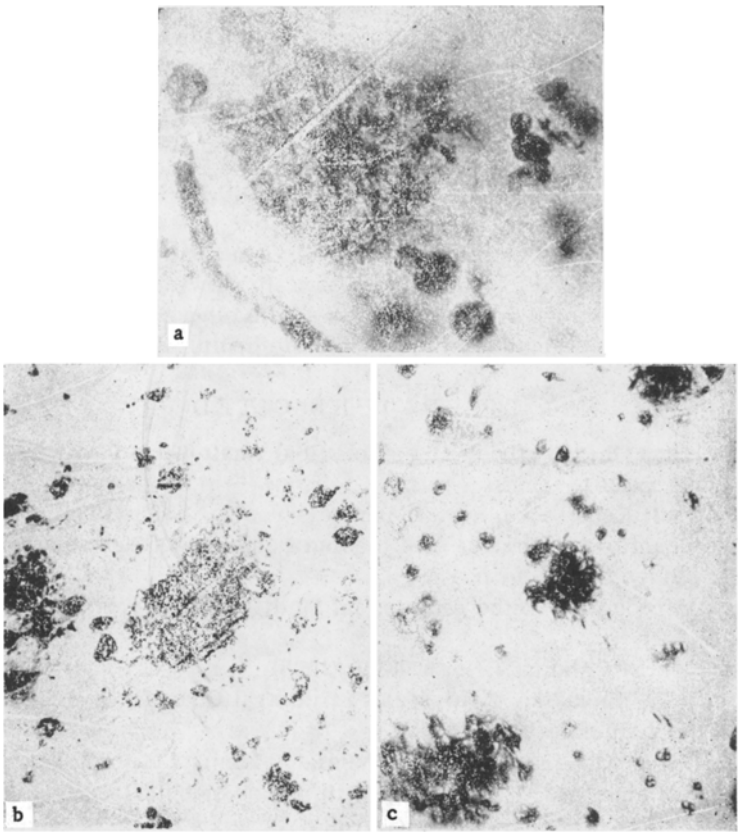

Fig. 1. Senile plaque: a) Karnovsky–Rootes method with acetylthiocholine iodide, 400×; b) Gomori's method for acid phosphatase, 200 ×; c) Gomori's method for adenosine triphosphatase, 200 ×.

EXPERIMENTAL RESULTS

Acetylcholinesterase activity was high in the senile plaques. In some plaques, a more deeply stained central part could be distinguished, surrounded by a pale zone, outside which was a halo consisting of fibrils stained slightly less intensively than the central part of the plaque (Fig. 1a). Other senile plaques appeared as collections of short fibrils. In control sections either the senile plaques could not be detected or they were only just visible. Nonspecific cholinesterase activity was low in the senile plaques, and these structures appeared as pale brown, circular formations.

In the reaction for nonspecific esterases, the senile plaques appeared as dark blue round spots, in which darker inclusions resembling degenerated microglial cells were sometimes seen. In sections preliminarily treated with 10^{-5} eserine solution the plaques were smaller, and they were less deeply stained. In sections treated with 10^{-4} eserine solution, the plaques were very much smaller. The inclusions described above were almost indistinguishable in them. After preliminary treatment of the sections in sodium p-chloromercuribenzoate solution the general intensity of staining of the sections was reduced. However, the senile plaques were clearly distinguished, and the dark blue inclusions resembling microglial cells were clearly outlined. The use of inhibitors thus shows that carboxylesterase activity is present in senile plaques.

In the test for acid phosphatase the senile plaques were revealed as circular collections of small and larger dark brown granules, among which microglial cells could be seen (Fig. 1b). Alkaline phosphatase showed up the capillary network of the cortex clearly. Only in 1 case were gray, circular structures found in the molecular layer, mainly perivascular in distribution. In all other cases the senile plaques were not revealed by the reaction for alkaline phosphates.

Two types of senile plaques could be distinguished by the character of their adenosine triphosphatase activity. One type appeared as pale brown, circular, structureless spots. In the others, a dark brown, almost black center (the nucleus) and a brown fibrous halo could be distinguished (Fig. 1c). No senile plaques could be seen in control sections.

When stained with Congo red, the plaques appeared much smaller than in the enzyme-histochemical reactions.

Hence, the results obtained by enzyme-histochemical study of senile plaques do not suggest that they are the result of a primary necrotic process. The hypothesis regarding the primary amyloid nature of senile plaques likewise is not supported by the results of staining with Congo red or of the enzyme-histochemical tests. The high activity of several enzymes in senile plaques may indicate that these structures are a manifestation of the disturbed metabolism of the senile brain.

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