INVESTIGATION OF THE ACTIVITY OF SOME HYDROLYTIC ENZYMES IN ALZHEIMER'S DISEASE

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In Alzheimer's disease, with typical changes in the neurofibrils, increased acetylcholinesterase, acid phosphatase, and adenosinetriphosphatase activity was demonstrated by histochemical methods.

Changes in the neurofibrils characteristic of Alzheimer's disease are frequently found in cortical neurons of old persons. However, they are discovered most constantly in Alzheimer's disease itself and in senile dementia. Different views are expressed on the nature of this unique metamorphosis of nerve cells: thickening of the neurofibrils [6], proliferation of newly formed neurofibrils [11], deposition of foreign material of thread-like structure unconnected with neurofibrils [7], distinctive swelling of the neurofibrils followed by deposition of argyrophilic substances in them [3], the effects of disturbances of metabolism in the nerve cells [4, 14], and the appearance of cerebral amyloidosis [1, 5, 9, 10, 19].

To study the nature of changes in the neurofibrils in Alzheimer's disease the methods of enzyme histochemistry must be used. In this way the activity of acid phosphatase [16] and of succinate and NAD.H_2 dehydrogenases can be demonstrated [12].

This paper describes the results of a study of the activity of choline esterases, nonspecific esterases, nonspecific phosphomonoesterases, and adenosinetriphosphatase (ATPase) in neurofibrils showing the changes of Alzheimer's degeneration.

EXPERIMENTAL METHOD

Pieces of brain tissue (areas 10, 46, 6, 1, 40, 44, and 17, hippocampus with the parahippocampal gyrus) of ten patients with senile dementia who had died between the ages of 64 and 90 years were taken 3 h after death, during the cold period of the year, and fixed for 24 h in calcium-formol. Sections cut on a freezing microtome were washed in ice-cold water, from which, depending on the method to be used, they were either placed immediately in the incubation solutions or first stuck to slides.

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

Nonspecific esterases were investigated by the Nachlas–Seligman method in Gomori's modification.

Acid phosphatase was determined by Gomori's lead method and alkaline phosphatase by means of Gomori's calcium-cobalt method.

ATPase was determined by Gomori's lead method. Control sections were placed in the incubation medium together with sodium p-chloromercuribenzoate (final concentration 2.5×10^{-3} M), a specific ATPase inhibitor [8]. In addition, when choosing an inhibitor, consideration was played to the statement [15] that mitochondrial ATPase is inhibited when p-chloromercuribenzoate is added to the incubation medium. The

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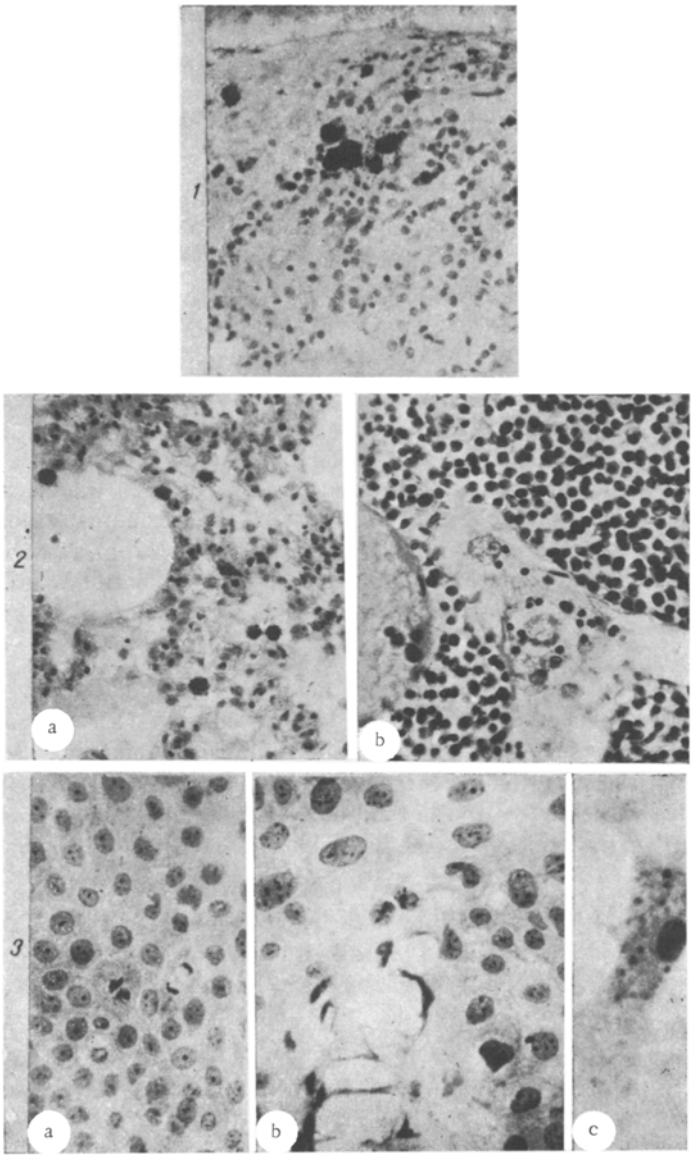

Fig. 1. Changes of Alzheimer's degeneration in neurofibrils in the cerebral cortex. Upper row – reaction for acid phosphatase; middle row – reaction for APTase; bottom row – Karnovsky – Rootes reaction with acetylthiocholine iodide. 400×.

reversibility of the inhibition was tested by subsequent treatment of some control sections in incubation solutions containing 2,3-dimercaptopropanol (BAL) in a final concentration of 4.9×10^{-3} M.

To compare the results of the histochemical tests with the histological pictures the sections were impregnated with silver by Bielschowsky's method. Sections from analogous areas of the brain treated by all the methods specified above, from six persons dying at different ages, and in which impregnation by Bielschowsky's method revealed no changes of Alzheimer's degeneration in the neurofibrils, also were used.

EXPERIMENTAL RESULTS

In specimens impregnated by Bielschowsky's method characteristic patterns of Alzheimer's degeneration (spirals, loops, tangles) were found in the neurofibrils in neurons of cortical layers II and V and also the paramedal layer of the hippocampus in patients with senile dementia.

In the reactions for cholinesterase using acetylthiocholine iodide as the substrate, high enzymic activity was found in most neurons (Fig. 1). The reaction products, in the form of tiny granules, were diffusely scattered in the cytoplasm and axon. The nuclei remained unstained. In many cells the density of the reaction products were highest in the cytoplasmic membrane. In the large pyramidal cells these granules were darker and more numerous at the base of the cell than in its apical part. In general, judging

from the intensity of staining of the neurons, enzyme activity increased with an increase in the size of the cell. In some neurons, areas of most intensive deposition of reaction products corresponded to structures similar to neurofibrils with Alzheimer's degeneration: spirals, tangles, and loops. In control sections the cortical structures were hardly distinguishable because of their pale staining. When butyrylthiocholine iodide was used as the substrate, no connection could be found between the deposits of reaction products and Alzheimer's degeneration of the neurofibrils.

The formation of dark blue granules in the reaction for nonspecific esterases showed up the nerve cells sharply. The cell boundaries were clearly visible, and the axons, which were more palely stained than the cytoplasm, could be traced for a long distance. The nuclei were unstained. No signs of Alzheimer's degeneration of the neurofibrils were revealed by this reaction.

The nucleoli were stained most strongly by the reaction for ATPase. Reaction products were deposited unequally in neurons of different sizes. In small cells granules of sulfide were more numerous than in large cells. The large cells were hardly distinguishable, often because of their pale color. In individual cells of cortical layers III and V and in the pyramidal layer of the hippocampus, deposits of brown granules of sulfide formed loops, spirals and tangles just as was observed in cases of Alzheimer's degeneration of the neurofibrils in sections impregnated by the Bielschowsky method. All the structural elements of the cortex were hardly distinguishable in sections incubated in medium containing p-chloromercuribenzoate. If these sections were washed twice in calcium chloride solution and then treated in incubation medium with the addition of 2,3-dimercaptopropanol, the staining of the brain structures was approximately the same as in sections not treated with the inhibitor.

After the reaction for acid phosphatase the outlines of the neurons and of their pale nuclei and light brown processes were clearly visible. In most neurons deposits of sulfide were observed as tiny brown granules, the density of distribution of which was highest in the cytoplasmic membrane and lowest in the axoplasm. In the large pyramidal cells dark brown reaction products were seen in the largest quantity in the basal part. In some neurons of cortical layers III and V and in the pyramidal layer of the hippocampus the most intensive deposition of brown granules corresponded to the neurofibrils showing Alzheimer's degeneration.

Because of the high alkaline phosphatase activity the capillary network was clearly defined. The small quantity of dust-like residues of reaction products indicated low alkaline phosphatase activity in the neurons. No connection could be found between the activity of this enzyme and Alzheimer's degeneration of the neurofibrils.

The structures described above, resembling the changes of Alzheimer's degeneration of the neurofibrils, were discovered only in those cases in which they were found by impregnation by the Bielschowsky method.

In histochemical reactions resulting in the precipitation of salts of metals it is difficult to rule out the possibility of their precipitation through causes other than enzyme activity. In the case of determination of cholinesterase activity, the different results when acetylthiocholine iodide and butyrylthiocholine iodide were used as the substrates are evidence in support of the specificity of the reaction, i.e., that acetylcholinesterase activity was found in Alzheimer's degeneration of the neurofibrils. It is much more difficult to interpret the results of the histochemical reactions for acid phosphatase and ATPase. It must be remembered that the inhibition of ATPase activity by p-chloromercuribenzoate takes place on account of blocking of the sulfhydryl groups. Ability to be impregnated by metal salts may also be connected with sulfhydryl groups (by analogy with silver impregnation). However Josephy [16], who first described acid phosphatase activity in Alzheimer neurofibrils, used as his control the treatment of some sections in soda solution and others in a solution of lead nitrate with buffer, but without glycerophosphate. No staining was obtained in either batch of control sections. It cannot therefore be asserted without reservation that the precipitation of lead salts during the reaction for acid phosphatase and ATPase can be explained entirely by the affinity of the neurofibrils in Alzheimer's degeneration for salts of the heavy metals.

On electron-microscopic investigation of neurons in Alzheimer's disease [13, 17, 18] collections of various organelles are found among the tangles in the changed neurofibrils. Detection of acid phosphatase and ATPase activity thus confirms that the so-called Alzheimer changes of the neurofibrils visible in the light microscope are not simply a change in the neurofibrils, but also affect other organelles and, in particular, the lysosomes and mitochondria. This, in turn, is evidence that, as well as morphologically clearly

visible death of the cell, Alzheimer's changes in the neurofibrils at some stage are a manifestation of "organoid regeneration" [2].

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