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# NEUROPATHOLOGY OF ALZHEIMER DISEASE. CONNECTIONS WITH CEREBRAL SENESCENCE

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***Senile dementia results from the progress of age. Man loses** his sensibility along with the free use of **the faculties of understanding,** before arriving at an extreme state of **decrepitude. Senile dementia is established slowly. It commences with feebleness of memory, particularly of recent impressions.** The sensations are feeble; the attention, at first fatiguing, at length becomes impossible; the will is uncertain and without impulsion; the movements are slow and impracticable....*
***A man in a state of dementia** is deprived of advantages he formerly enjoyed; **he was a rich man who has become poor.** The idiot, on the contrary, has always been in a state of want and misery.*

**Jean-Étienne Dominique Esquirol** (1772 - 1840),
French psychiatrist, a favorite student of Philippe Pinel,
*Des Maladies Mentales* (1830)

**ABSTRACT.**
***Introduction.*** Alzheimer disease (AD) becomes "disease of the century" by its prevalence, morbidity, prediction and economic impact. Paper aims of present and our following studies on AD were represented by the achievement of a global and unitarian bio-medical research of this inflammatory-degenerative pathology, the description of AD alterations in brain structures and by the correlation with the same changes from cerebral senescence.
***Materials and methods.*** Human brains from AD patients and old people, as well as aging brains from Wistar rats and guinea pigs were investigated by macro- and microscopic morphological methods.
***Results and Discussions.*** In AD patients, gross, imagistic and sectional anatomy revealed severe diffuse cortical atrophy (gyral narrowing and sulcal widening), ventricular dilatation and intense atrophy of the hippocampus and amygdala. Using microscopic anatomy, histology and cytology investigations, as AD structural hallmarks, we observed neuronal loss, amyloid plaques and neurofibrillary tangles, especially in cerebral cortex, hippocampus, amygdala and nucleus basalis of Meynert. In addition, we found neuropil threads, vascular amyloidosis, granulo-vacuolar degeneration, Hirano and Lewy bodies. All these neuropathological changes coexist with important lipopigment storages (lipofuscin and ceroid), landmarks of brain aging. The same modifications are presented in old human and animal brains, but much more reduced as number and intensity. Authors discuss the epistemological evolution of these pathological structural concepts and their pathophysiological significance.
***Conclusions.*** This study is the first Romanian research, where AD brains were investigated from anatomo-histologico-tissual level to cellular-subcellular and extracellular pathology. Also, the authors achieved a comparative and correlative research between AD and old brains from humans to animals.

**Key words:** Alzheimer disease, cerebral senescence, neuropathology, morphological correlations, selective brain atrophy, amyloid plaques, neurofibrillary tangles, ceroid and lipofuscin pigments

## INTRODUCTION

### *Prevalence and morbidity*

Research and medicine - prevention, therapy and recovery of Alzheimer Disease (AD), Senile Dementia of the Alzheimer Type (SDAT) or merely Alzheimer is the bio-medical challenge of the 21st century. AD, a disabling psychiatric-neurological disease that afflicts about 11% of the population over age 65, represents the most common form of dementia. Moreover, dementia is now recognized as the 4th commonest cause of human morbidity and mortality among aged people after cancer, cardiovascular disease and cerebrovascular disorder. Generally, AD affects 5% of all persons in their 60s, 20% in their 70s and an incredible 50% in their 80s. The impact of so many, mostly old, people with AD on relatives, caregivers or society in the main is incalculable, in both personal and social terms, as well as in the next future for its economic sustain. Actually, AD is one of the most costly diseases to society (Bonin-Guillaume et al., 2005). Aging of so-called baby boomers and aging of human society on the whole transform this pathology in "disease of the century". In 2006, there were 26.6 millions of persons with AD globally. For the near and distant future there are bad news. In 2050, AD is predicted to affect 1 in 85 people wordwide (Brookmeyer et al., 2007).

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***Definition and evolution***

AD is a progressive degenerative brain disease of unknown etiology, characterized by diffuse atrophy throughtout the cerebral cortex, with distinctive lesions termed amyloid plaques and clumps of fibrils named neurofibrillary tangles. There is a loss of choline acetyltransferase activity in the cortex, and many of the degenerating neurons are cholinergic neurons projecting from the substantia innominata (especially nucleus basalis telencephali - nucleus basalis of Meynert) to the cortex (Anderson, 2003). Great loss of neurons in specific regions (nucleus basalis telencephali, hippocampus and cerebral cortex), plaques of abnormal proteins deposited outside neurons (amyloid plaques) and tangled protein filaments within neurons (neurofibrillary tangles) are the three distinct neuropathological changes of AD. This gradual dementing illness is getting on four stages: the 1st stage, pre-dementia, a preclinical stage called mild cognitive impairment; the 2nd stage, early dementia; the 3rd stage, moderate dementia; and the 4th stage, advanced, severe dementia, last stage. In this final stage, AD involves widespread intellectual impairment, personality changes, sometimes delirium, and dementia, the loss of reason and ability to care for oneself (Summers and Korneva, 2009). The AD patient dies from intercurrent infections such as pneumonia or complications that affect bedridden patients.

***Objectives***

This paper belongs to a succession of investigations to establish a global and unitarian description of AD, containing historico-clinical and anatomo-clinical information, macromorphological data (gross, imagistic and sectional anatomy), micromorphological researches (microscopic anatomy, histology, cytology, light, fluorescence and electron microscopy, cytochemistry and cellular biology), biochemical and genetic aspects and facts. The first objectiv of present paper is the exploration and description in the AD brains of anatomo-histologico-tissual damages and the study of cellular-subcellular and extracellular pathology. The second objectiv is the determination of patho-biological correlations between AD and aging processes, two entities which are present in humans over age 60.

## MATERIALS AND METHODS

***Human and animal brains***

We investigated and compared 14 human AD brains (patients died between 62 and 93 years) with other 14 aging brains (humans died between 60 and 95 years through non-psychoneurological diseases). For extension of neuro-pathological data on aging processes, we also studied 30 brains of old Wistar rats (26.6 months of age) and 14 brains of old guinea pigs (48 months of age).

***Clinical methods***

ICD-10 (World Health Organization, 1992), NINCDS-ADRDA Alzheimer's Criteria (McKhann et al., 1984) and DSM-IV-TR (American Psychiatric Association, 2000) clinical descriptions and diagnostic guidelines were simultaneously applied to individualize AD. In addition, MMSE (Mini-Mental State Examination), CDR (Clinical Dementia Rating) Scale and CDT (Clock Drawing Test) were used to select patients in stage 3 or stage 4 of AD.

***Macroscopic investigations. Gross, imagistic and sectional anatomy*** Gross, imagistic and sectional anatomies were used for bring out the general, regional and zonal modifications.

***Microscopic methods. Microscopic anatomy, histology and cytology*** Light microscopy (histochemical stains and silver impregnation techniques) fluorescence and transmission electron microscopy pointed out complex tissual, cellular-subcellular and extracellular damages from AD and senescence. AD and old human brains were post-mortem removed and fixed by immersion in formalin for 6-8 hrs. Afterwards brains were divided by frontal sectionalization in coronal slices. Smaller pieces from different regions were formalin-fixed for 10-12 hrs. and then processed automatically (fixation, dehydration and paraffin-embedding) for light microscopy. Parrafin blocks were later cut at 6 μm. Silver impregnation techniques (Bielschowsky, Palmgreen, von Braunmuhl and Bodian) were used for amyloid plaques, neurofibrillary tangles and neuropil threads identification; standard stains (Haematoxylin-eosin and Congo red) for vascular amyloidosis; and specific staining methods (Oil red O, Sudan black B, periodic acid-Schiff-PAS, Nile blue, long Ziehl-Neelsen's acid fast and Schmorl's ferric-ferricyanide) were selected for lipopigment distribution and histochemical characteristics. Also, 6 μm unstained sections were examined in fluorescence microscopy. For transmission electron microscopy, small pieces formalin-fixed were subsequently processed by postfixation in 2 % osmium tetraoxide, dehydration in graded acetone series, and embedding in Epon 812. Semithin sections were stained in toluidine blue O and ultrathin sections were double-stained with uranyl acetate and lead citrate. For animal brains, in order to avoid the interactions of blood constituents with the fixative and to eliminate the tissue damages caused by low aldehyde concentrations, the fixation by cardiac perfusion was started by a pre-washing with Tyrode solution containing 1 % gum acacia. This was then followed by rapid fixation with phosphate buffered 19 % glutaraldehyde, then with a slightly hypertonic buffered 4 % glutaraldehyde for 20 min. Afterwards the fixed brain were removed and sectioned in frontal slices. The subsequent processing stages were the same with the above-mentioned ones for human brains.

## RESULTS

***Macroscopic changes.***

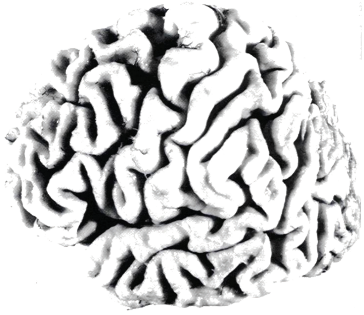
***Gross, imagistic and sectional anatomy***

AD, as commonest type of dementia and incurable, degenerative, terminal disease is a chronic progressive cortical encephalopathy. Macroscopic changes are concomitantly seen in gross, imagistic and sectional anatomy.

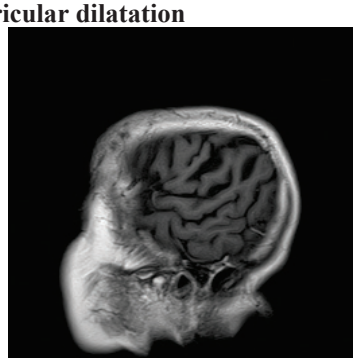
In our study all human AD brains evinced:

- severe diffuse cortical atrophy (gyral shrinking), particularly pronounced in temporal lobes and perisylvian regions;
- marked widening of the sulci, especially in the temporal, frontal and parietal lobes;
- loss (reduction) of brain weight and volume;
- ventricular dilatation (enlargement and expansion of the cerebral ventricles);
- intense atrophy of the hippocampus and amygdala;
- frequently reduction in overall size of basal ganglia.

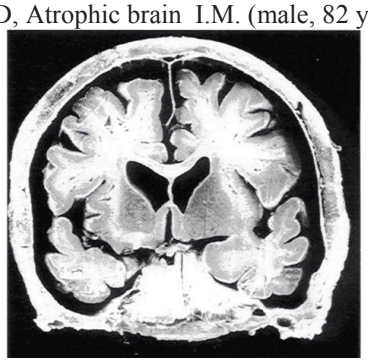
**Visualization of cortical atrophy and ventricular dilatation**



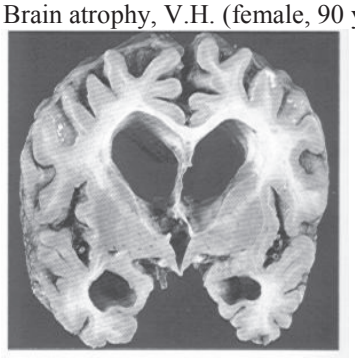
AD, Atrophic brain I.M. (male, 82 yrs.)



AD, Brain atrophy, V.H. (female, 90 yrs.)



AD, Cortical atrophy, G.N. (female, 84 yrs.)



AD, Cortical and hippocampal atrphy, A.P. (female, 77 yrs.)

In old animal brains, we found the same characteristics, but in a mild degree. We note that, about 150 years ago, in 1864, Sir Samuel Wilks (1824-1911) from Guy's Hospital London, England accuracy described brain atrophy with gyral narrowing and sulcal dilatation, in autopsy specimens. Our data confirm other previous observations and researches, performed by neuropathologists from everywhere (Esiri and Morris, 1997; Mann et al., 1994; Riga et al., 2009a; Riga et al., 2009b).

***Microscopic modifications.***

***Microscopic anatomy, histology and cytology***

We identified the microscopic changes as losses of some nervous structures and apparition of specific pathologic alterations, both within and outside of neurons. Our researches certify and complete other anterior neuropathological data (Ball, 1988; Esiri et al., 1997; Rewcastle, 1991; Riga et al., 2009a; Riga et al., 2009b).

***Damage of neuronal structures***

In AD brain we noted loss of neurons, axons and synapses, especially in cerebral cortex (temporal, frontal, parietal, hippocampus) and certain subcortical regions (nucleus basalis of Meynert, corpus amygdaloideum, basal ganglia). In old brains (human and animals) we observed the same modifications, but much more reduced.

Moreover, AD and cerebral aging also bring about:

- decrease in the volume of neurosoma (neuronal shrinkage), particularly in prefrontal layer III pyramidal cells;
- simplification (decrease) of dendritic arborization by losses of dendritic trunks (processes) and ramifications and of dendritic spines;
- reductions (sometimes considerable) of cortical myelin (*Gennari's and Baillarger's striae*), as well as of subcortical myelin (*corona radiata*), and distortion (often massive) of myelin

sheaths, in transverse, oblique and longitudinal vizualization;
- pathological activation of microglia and astroglia.

***Amyloid plaques***

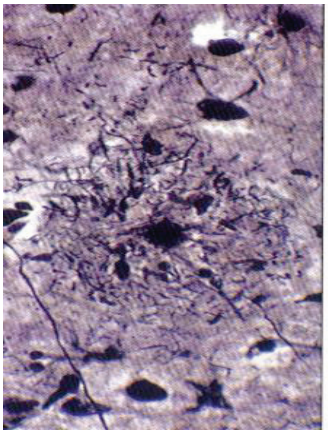
We utilized the denomination of amyloid plaques (according to their composition). These pathological structures are present in large amounts in brains with Alzheimer's disease. More than 200/mm$^3$ were identified in the frontal and temporal cortex.

Different authors use other (correct) names, as pertinent, with the same meaning :
- *senile plaques,* because they are found in small amounts in the cerebral cortex of normal elderly people;
- *argyrophil plaques,* owing to their best detection with classic silver impregnation techniques (Bielschowsky, Palmgreen, von Braunmuhl and Bodian); or
- *neuritic plaques,* due to the presence of filamentous structures, which correspond to altered nerve terminals and axons, blackened by impregnation methods.

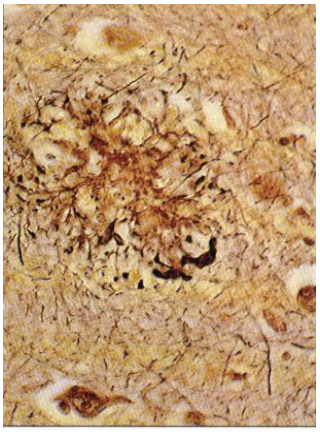
Amyloid plaques have the following morphological characteristics:
- their presence in all parts of the isocortex, but with predilection for external areas of both sensory and motor neurons,

cerebral cortex from the depth of the sulci being involved in particular;
- extracellular localization, within the neuropil of the brain gray matter is obligatory;
- practically, amyloid plaques represent pathological filaments, abnormalities of neuronal cytoskeleton; and
- great dimensional variations (diameter from 15 to 200 μm).

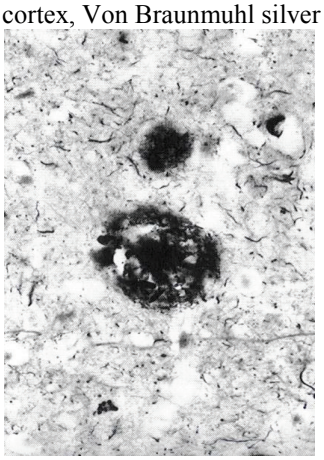
**Objectification of amyloid plaques**



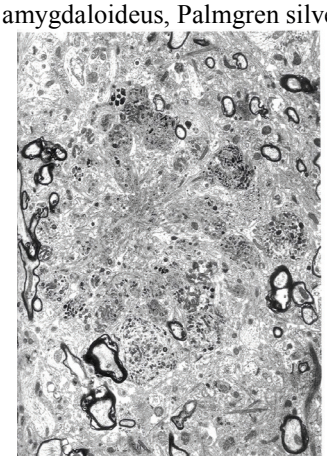
AD, Frontal cortex, Von Braunmuhl silver stain, x 420



AD, Corpus amygdaloideus, Palmgren silver stain, x 420



AD, Temporal cortex, Bielschowsky method, x 260



AD, Parietal cortex,

Their structure appears as microsopic argyrophilic masses (intricate feltworks), with a core of extracellular deposit of amyloid, surrounded by often ballooned processes of neurons (dendrites and fragmented axon terminals) pathologically changed and reactive astrocytes and activated microglia. Amyloid represents an extracellular protein storage, composed by the Amyloid β peptide (Aβ), a proteolytic fragment of the amyloid precursor protein (APP). In plaques, Aβ is associated with several other molecules: complement components, serine protease inhibitor α1-anti-chymotrypsin, heparin sulfate proteoglycans, and apo-lipoprotein E. The same

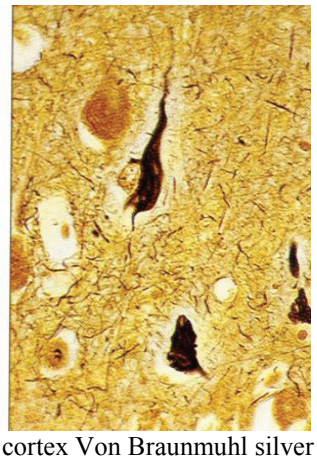
changes were observed but reduced in number and size in central nervous systems from old human and animals.

***Neurofibrillary tangles***

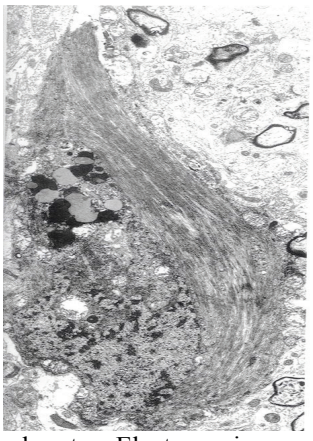
Neurofibrillary tangles have a specific structure: bands of an abnormal filamentous material (tightly packed bundles of paired helical filaments), which is formed and accumulated within the neurosoma (the perikaryon), and frequently extend into proximal portions of the dendrites and axon. In pyramidal neurons of the cerebral cortex and in hippocampus they fill the neuronal cell body and apical dendrite, often having a flame shape appearance. In amygdala, nucleus basalis of Meynert, locus ceruleus, substantia nigra and dorsal raphe neurofibrillary tangles have a more globular (globose or globiod) type. We noted an interesting relationship between neurofibrillary tangles and lipopigments (LPs) - lipofuscin and ceroid. These two subcellular structures coexist together in almost AD cases.

Neurofibrillary tangles are present also as amyloid plaques in diminished number at old people and animals.

**Demonstration of neurofibrillary tangles**



AD, Frontal cortex Von Braunmuhl silver stain, x 420

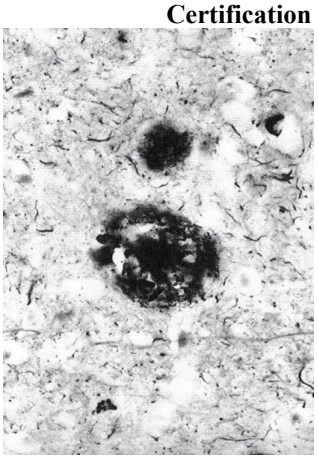


AD, Temporal cortex, Electron microscopy, x 30,000

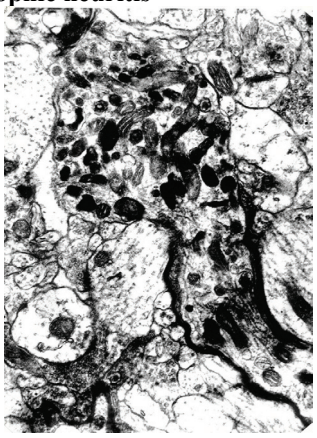
***Neuropil threads and dystrophic neurites***

Neurofibrillary pathology of AD is tripartite: neurofibrillary tangles (above described), neuropil threads and dystrophic neurites. Neuropil threads are inconspicious structures loosely scattered through the neuropil. They are constituted of small bundles of paired helical filaments contained in slender thread-like profiles, that do not cluster or accumulate in patches or columns (Braak and Braak, 1988). The threads become coated with other molecules, and develop different immunochemical reactivity, for exemple to antibodies directed against glial fibrillary acidic protein (GFAP), ubiquitin and apoliprotein E. A small fraction is also immunoreactive to the amyloid β/A4 protein. Neuropil threads are found between the plaques and tangles in the cerebral cortex, entorhinal cortex and corpus amygdaloideus.

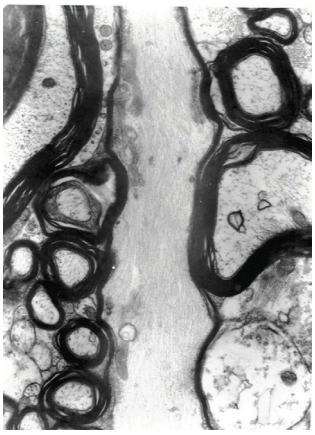
**Certification of neuropil threads and dystrophic neuritis**



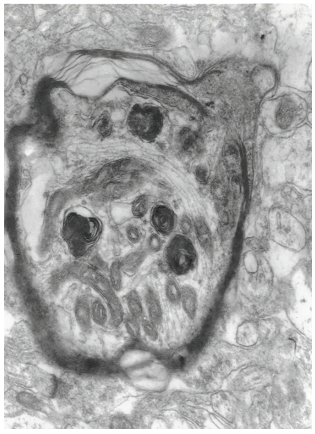
AD, Temporal cortex,
Bielshcowsky method, x 260
Mature plaque and neuropil threads



AD, Frontal cortex,
Electron microscopy, x 18,000
Neuritic dystrophy



AD, Parietal cortex,
Electron microscopy, x 20,000
Pathological neurofibrillary proliferation



AD, Temporal cortex,
Electron microscopy, x 26,000
Dystrophic neurite

Dystrophic neurites are altered axonal fragments. They are present in both neuritic plaques and throughtout the neuropil. Dystrophic neurites have modified structures: loss and dehiscence of myelin sheats, neurofibrillary proliferation in axoplasm, and/or total disorganization of neuroplasm with subcellular vaste accumulation.

***Vascular amyloidosis***

Vascular amyloidosis or congophilic (amyloid) angiopathy was described for the first time in 1938 by Willibald Scholz (1889-1971), German neuropathologist and psychiatrist. He certified amyloid depositions in the cerebral arteries and their association with pathological changes from AD (Scholz, 1938). In our AD cases, we observed the subsequent features for this angiopathy:

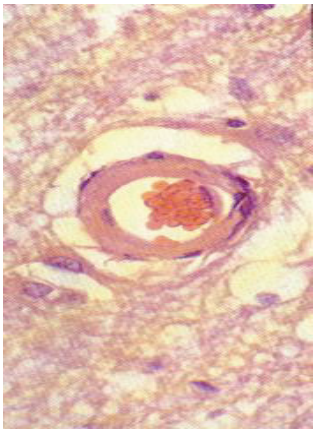
- is an almost invariable finding in AD;
- characteristically affects the smaller braches of the sulcal arteries and the penetrating arteries in the cerebral cortex;
- amyloid is deposited in the walls of the smaller arteries and arteriolae, in the following sequence:
- first tending to surround the smooth muscle cells, that comprise the muscular wall of the vessel and eventually replacing them;
- at a later stage the smooth muscle cells undergo degeneration; and
- at the final stage the entire arterial-arteriolar wall is composed of a dense meshwork of amyloid fibrils.

***Other histological changes***

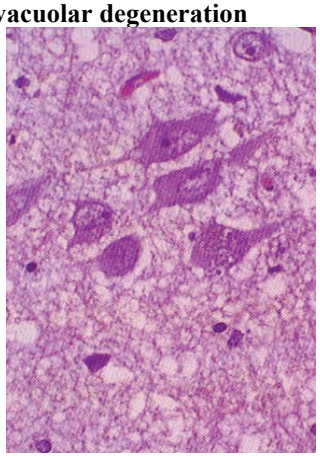
***Granulo-vacuolar degeneration***

Granulo-vacuolar degeneration of neurons in AD was identified for the first time as for back as is 1911 by Teofil Simchowicz (1879-1957), Polish neuropathologist and neurologist. He described the granulovacuolar changes in the hippocampal large pyramidal cells from brains with AD (Simchowicz, 1911). Our cases with AD confirmed there degenerative changes in pyramidal neurons from hippocampus.

**Visualization of vascular amyloidosis and granulo-vacuolar degeneration**



AD, Amyloidaceous parietal intraparenchimal artery.
Haematoxylin eosin stain, x 420



AD, Granulo-vascular degeneration, of hippocampal pyramidal cells, Haematoxylin eosin stain, x 280

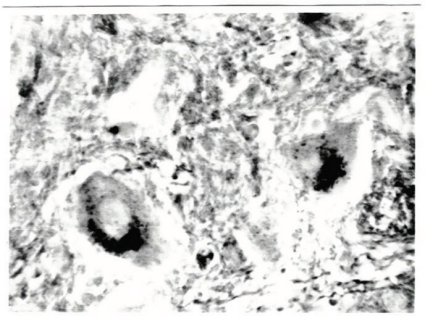
***Hirano bodies and Lewy bodies***

Presence of Hirano bodies and Lewy bodies is associated with nervous degenerative pathology. Although these structures were identified for the first time on other diseases of central nervous system, they are found in AD, too. Hirano bodies were described in 1965 and 1966 by Asao Hirano (n. 1926) in amyotrophic lateral sclerosis and in Parkinson-dementia complex of Guam (Hirano, 1965; Hirano et al., 1966; Hirano, 1994). In 1977, P. Gibson and B. E. Tomlinson communicated their occurence in the intellectually normal old people and in demented persons with AD (Gibson and Tomlinson, 1977). Lewy bodies are spherical intracytoplasmatic structures formed by abnormal aggregates of protein, which appear inside the neurons. They were identified in 1912, first in *substantia nigra* (Lewy, 1912), and represent a pathological characterstic of Parkinson disease (Forno, 1986) and of Cortical Lewy Body Dementia (CLBD), (Akashi et al., 1991; Lennox, 1992). In our investigation on AD we occasionally found Hirano bodies in hippocampus and Lewy bodies in *substantia nigra* (classical brain stem Lewy bodies) and in cerebral cortex (cortical Lewy bodies).

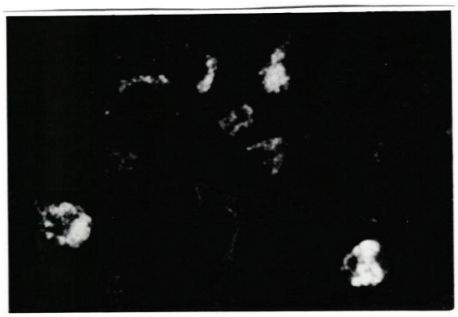
***Lipopigment storages (lipofuscin and ceroid)***

AD neuropathology, above analysed, always coexists with lipopigment deposits (lipofuscin and ceroid) They are *age, senile, wear and tear pigments* (other denominations) and represent subcellular waste (tertiary lysosomes). We found lipopigment accumulations in neurons and glia cells (especially microglia) in old human and animal brains, because lipofuscin and ceroid storages are the strongest characteristic of cellular aging. Moreover, also in AD, lipopigment storages are a tissual permanence. In the next paper we will present and discuss, the interesting coexistence between specific AD neuropathological changes and lipopigment masses (Riga et al., 2010 in press).

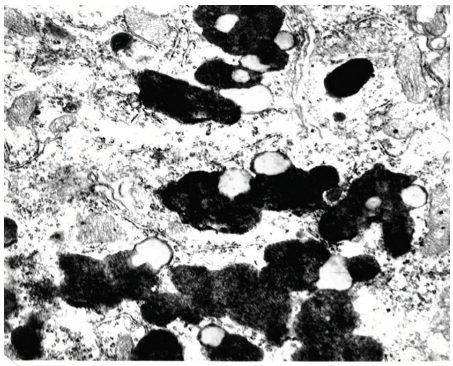
**Objectification of lipopigment accumulations**



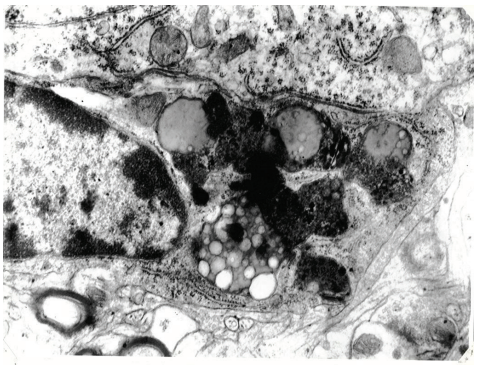
Old rat, Nucleus reticularis tegmenti pontis. Two lipopigment storages, Sudan black B, x 600



Old rat, Anterior horn cells of cervical spinal cord. Extensive masses of autofluorescent lipopigment, Fluorescence microscopy, x 900



AD, Frontal cortex, Layer V pyramidal cells, Intraneuronal lipopigment, Electron microscopy, x 37,000



AD, Temporal cortex, Layer V pyramidal cells, Intramicroglial lipopigment, Electron microscopy, x 30,000

## DISCUSSIONS

***Relevance of anatomo-clinical method***

Auguste Deter was the first described patient with AD. Alois Alzheimer clinically followed her from 1901 until she died in 1906. Then he performed neuropathological study on her brain and communicated the results at the 37th meeting of South-West German psychiatrists, in Tübingen, on November 3-4, 1906, followed by research publication in 1907 (Alzheimer, 1907). We must note that this case was a complete anatomo-clinical investigation,

a choice and very useful method of biomedical study in the beginning of 20th century. In addition, Alois Alzheimer had a whole biomedical training, performing and practising two connected medical specialities: psychiatry and neuropathology, which allowed him to make a correct, pertinent and complete (at that time) description and characterization of this new disease. After his death in 1915, Franz Nissl (1860-1919), close friend and collaborator, wrote about A. Alzheimer: *first and foremost a psychiatrist who strove to advance psychiatry by using a microscope* (Nissl, 1916).

***Clinical landmarks***

In this way, Alois Alzheimer (1864-1915) comprehensively described (in 1907) the first case of AD, concerning both clinical features and neuropathological modifications at a 51 year old woman (Auguste Deter) with dementia, characterizing thus the illness, which in the future will designated as AD. In 1910, Emil Kraepelin, Director of the Royal Psychiatric Clinic in Munich, where Alzheimer worked from 1903 to 1912, named the illness after his colleague, in the 8th edition of his textbook of Psychiatry (Kraepelin, 1910). Since that time the eponym Alzheimer's disease was detailed investigated, and after a century we know molecular and cellular bases of the most common cause of dementia. On the other hand, Oscar Fischer (1876-1942), Czech psychiatrist and neuropathologist, published in the same year the description of brain changes at tissual and cellular levels in 12 cases of this disease (Fischer, 1907). Besides, the end of 19th century shown important progresses in clinical specification, delimitation and classification of dementia, notions also valid nowadays:

- in 1892, Arnold Pick (1851-1924) described a form of presenile dementia (Pick's disease) due to lobar cortical atrophy (frontal and temporal lobes) and degenerating, swolling neurons (Pick cells), nerve cells with globular intracytoplasmic filamentous inclusions (Pick bodies), characterized as "amnestic aphasia";
- in 1894, Emil Kraepelin (1856-1926) the founder of contemporary scientific psychiatry, distinguished between senile dementia and arteriosclerotic dementia;
- in 1898, Otto Ludwig Binswanger (1852-1929) introduced the term and concept of "presenile" dementia, notion notably used by Kraepelin in 1899.

Then, during 1907-1911, A. Alzheimer, O. Fischer, E. Bonfiglio, G. Perusini, M. Bielschowski and T. Simchowicz described neurohistological modifications of AD, many of these researches being anatomo-clinical investigations. ***Amyloid plaques - evolution of the concept*** Amyloid (senile, neuritic or argyrophil) plaques, characteristic for AD, are present in high number in cerebral cortex. In 1892, Paul Oscar Blocq (1860-1896) and Gheorghe Marinescu (1863-1938) described for the first time senile plaques as nevroglia nodules in cerebral grey matter (Blocq and Marinescu, 1892). In 1904 and 1906, A. Alzheimer performed first report of argyrophil plaques in cases of senile dementia (Alzheimer, 1904; Alzheimer, 1906). In 1907, A. Alzheimer published Auguste Deter case: clinical (dementia between 1901 and 1906) - morphological (gross anatomy and histology) investigation, ascertaining the link between dementia and brain atrophy, argyrophil plaques and neurofibrillary tangles found throughout the human cortex. In 1911, T. Simchowicz (1879-1957) used the term of senile plaques and established the quantitative relationship between the number of amyloid plaques and the severity of neuropathological changes, a decisive correlation in AD diagnosis. In 1911, Max Bielschowski (1869-1940) made supposition on the amyloid nature of senile plaques, and extensively utilized silver impregnation techniques for their visibility. In 1973, H. M. Wisniewski denominated senile plaques as neuritic plaques, because of the presence of filamentous structures corresponding to altered nerve terminals and dystrophic neurites. In the previous century, amyloid plaques are characterized and neuroscientists established their internal structure and biochemical composition. At present, there are two contradictory theories about their meaning: noxious significance or protective role through lesions delimitation.

***Neurofibrillary tangles - concept progress***

Alzheimer contribution was decisive in this particular field of neuropathology:

- in 1906 he identified neurofibrillary tangles with the new Bielschowsky silver stain technique, which was an improvement on the method developed by Ramon y Cajal;
- in 1907 Alzheimer described Auguste Deter's demented brain, and found association of neurofibrillary tangles with argyrophil plaques throughout the cortex (Alzheimer, 1907).

Neurofibrillary tangles are present especially in Sommer's sector of the hippocamps, entorhinal cortex and in corpus amygdaloideum. Advances in neurobiochemistry and molecular neurobiology elucidated many unknown problems regarding tau protein - neurofibrillary pathology. Discovery in 1991 of extraction techniques for the dispersed phf (PHF, paired helical filaments of 10 nm length) allowed to establish six isoforms (ranging in size from 352 to 441 aminoacids) in the normal adult cells.

***Neurofibrillary tangles, neuropil threads and dystrophic neurites***

Neurofibrillary pathology is characteristic for AD, and it is present both intraneuronal and outside the neurons, in neuropil. Cytoskeleton damage is triple expressed: neurofibrillary tangles (intracellular), neuropil threads and dystrophic neurites (both extraneuronal). Neuropil threads,

as damaged dendritic filaments (from pyramidal cells that contain a tangle within their soma) and dystrophic neurites certify the tissual extension of AD. Thus AD becomes a chronic progressive cortical encephalopathy. ***Lipopigment deposits and AD neuropathology***

AD neuropathological changes, above analysed, always coexist with lipopigment storages. The progressive accumulation of lipofuscin in ontogenesis is the hallmark of cellular senescence (Marinescu, 1909; Riga et Riga, 1995). Ceroid, which is pathologically formed, is the stamp of external (environmental) aggression and of internal factors (cellular distresses, including genetic factors), (Riga et al., 2006a). We already described the specific correlation between aging and AD neuropathology (Riga et al., 2006b), and in the future paper, which will be published in the same journal (Riga et al., 2010 in press) we will emphasize this particular connexion.

***AD histopathological criteria***

Histopathological criteria for the post-mortem diagnostic of AD are very important, and they should be compared with ante-mortem diagnostic criteria (NINCDS-ADRDA, from ICD-10 and DSM-IV-TR). An unitary and on the whole picture of AD will contribute to a better understanding of this type of dementia and to optimization of treatment strategies in different stages of evolution. Neuropathological criteria for AD should be simple, transferable, validated and versatile, as well as associated with AD clinical diagnostic and differential diagnosis, as ante-mortem investigation (Esiri and Morris, 1997). There are three groups of histopathological criteria, elaborated by Z. S. Khatchaturian, CERAD - Consortium to Establish a Registry for Alzheimer's Disease and by E. and H. Braak. Our investigations carried on several years were in conformity with both ante- and post-mortem criteria.

***Biochemical modifications and genetic causes***

Bichemical progress in neurosciences and proteomics elucidated the composition and dynamics of amyloid plaques and neurofibrillary tangles. In this way AD is a proteinopathy (protein misfolding disease), which by amyloid cascade induces accumulation of amyloid plaques (Racchi and Govoni, 2003). Plaques are made up of beta-amyloid peptide (Aβ), peptide with 39-43 aminoacids (usually 42) in length, which is a fragment from a larger protein, amyloid precursor protein (APP). APP is a transmembrane protein, that penetrates through the neuron membrane, and it is decisive for normality to neuron growth, adaptation, survival and post-injury repair. In AD, an unknown altered APP process divides APP into smaller fragments by enzymes (γ, α, β secretases) through proteolysis. One of these fragments give rise to fibrils of Aβ. In addition, AD is a tauopathy, which causes the accumulation of neurofibrillary tangles. Tau (τ) protein, from the neuron, stabilizes the microtubules when phosphorylated, and it is therefore called a microtubule-associated protein. Tau protein is critical for the good function of microtubules from neuronal cytoskeleton. In AD, tau undergoes chemical changes, becoming hyper-phosphorylated (Goedert, 1993). Abnormal hyper-phosphorylation is an essential feature of the conversion of normal tau into phf-τ (PHF-τ, paired 10 nm helical filaments-tau), (Trojanowski et al., 1993). It then begin to pair with other threads, producing neurofibrillary tangles, and disintegrating the transport system of neurons. In the last two decades of previous century, epidemiological studies of AD and rapid progress in molecular genetics contributed to a better understanding of this dementing illness (Mayeux et al., 1985; St. George-Hyslop et al., 1987).

As autosomal dominant pathology, familial AD is early onset AD. Three chromosomes are involved:

- chromosome 1, on the long arm (q), locus 1q31-q42 is located Presenilin 2 (PSEN 2) gene;
- chromosome 14, on the long arm (q), locus 14q24.3 is located Presenilin 1 (PSEN 1) gene; and
- chromosome 21, on the long arm (q), locus 21q21.3 is located β-Amyloid Precursor Protein (APP) gene.

Sporadic late onset AD has multifactorial origin with contributions of genetic factors and environmental influences. Chromosome 19 is implicated, because on the long arm (q), locus 19q13.2 is located Apoliprotein E (APOE) gene, and ε4 allele are associated with an increase in the frequency of AD.

## CONCLUSIONS

This study is the first Romanian research which realizes a comprehensive and unitary picture of AD, from brain macroscopic level to genetic one, which synthesized the AD pathological biology (cerebral anatomy, histology and cytology) and which proves the closed connexion between AD and human and animal aging. Our investigations demonstrated high correlations between macroscopic changes (on gross, imagistic and sectional anatomy) and microscopic modifications (from microscopic anatomy, histology and cytology), both in AD and brain aging. On the other hand, we found the same macroscopic data and microscopic descriptions of subcellular lesions in AD and cerebral senescence, but in different degrees: intense and severe in AD and much more diminished in old humans and animals. Lipopigment storages (lipofuscin and ceroid), evinced both in neurons and glial cells, represent a hallmark of brain aging (human and animal), but also a constancy in AD, together with typical subcellular lesions above-described. Results and neuropathological correlations evinced by this paper open new vistas in the AD treatments, simultaneously associated with anti-aging therapies.

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