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ARTICLE

New Data on the Ultrastructure of the Corpus Amylaceum (Polyglucosan Body)

Lóránt LEEL-ÔSSY

Laboratory of Neuropathology, I. Department of Neurology and Neurosurgery,
University Hospital of County Borsod-A.-Z., Miskolc, Hungary

During the semiquantitative evaluation of the occurrence of the corpus amylaceum (CA) in a large quantity of autopsy and biopsy material (1,407 cases), electromicroscopical (EM) and scanning EM examinations were carried out on 8 autopsied cases where CA was demonstrated. The EM examinations appeared to underline the astrocytic origin of the CA formation, which is initiated in the astrocytic fiber system by glycogen and other carbohydrate polymers. The biophysics of the development of the CA is indicative of the same mechanisms as for (mainly intracellular) inclusion bodies. The large amount of CA that develops at the predilection sites is a consequence of metabolic damage, a large quantity of cerebrospinal fluid and recurring disturbances in the barrier functions. The abundant CA may cause secondary blood-brain barrier disturbances. This working hypothesis demands further investigations and the continuation of research by modern immunocytochemical and ultrastructural methods recommended. (Pathology Oncology Research Vol 7, No 2, 145–150, 2001)

Keywords: Corpus amylaceum, ultrastructure, scanning EM, polyglucosan body

Introduction

The ultrastructure of the corpus amylaceum (CA) was first examined and described in detail by Ramsey.[21] She found a great quantity of CA in the Ammon's horn of the lobectomized biopsy material of a 35-year-old epileptic patient who had suffered from psychomotor seizures for 6 years. The CA appeared as a mass of randomly oriented short linear densities in stained electronmicroscopical (EM) samples. The dense bodies were situated in the cytoplasm of fibrous astrocytes, mainly in their distal processes,[2,20,25] and "they showed a marked affinity for lead". They did not have a definite limiting border or space relating to the cytoplasm, and Ramsey therefore concluded that the CA did not constitute *in sensu stricto* a real cytoplasmic inclusion body. Its central part sometimes contained an irregular dense core. Glycogen granules were present in various amounts both in the CA and in the cytoplasm. Ramsey stated that the CA developed *in situ* in the cells, because its constituents originated from the cells (metabolites of astrocytes and/or neurons). She emphasized that a distinction should be made between the CA and somewhat similar amyloid-like inclusions such as the Lewy concentric hyalin bodies and the Lafora bodies.

As the CA is characterized by definite PAS positivity, mention should be made of the EM examination of structures with similar properties. Daems and Persijn[6] performed investigations on polysaccharides, and mainly mucopolysaccharides such as glucosaminoglycan (GAG), and found in EM sections that the fibers consist of periodically bent lamina ordered helically ("crystalline") and randomly ("amorphous"). Gueft and Ghidoni[8] carried out an EM examination of the structure of amyloid and observed a different fiber system: the amyloid deposits (not the Lafora bodies) consist of extracellular fibrilla ordered in a stellate pattern, with a diameter of 50-100 Å, This accords with the findings of Seitelberger[28] and Yoshimura et al,[33] who also described the difference between the Lafora bodies and the CA. Finally Cervós-Navarro[4] summarized the characteristics and differences those formations in the central nervous system which have already proved to be of a polysaccharide nature.[23,24]

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Correspondence Dr. Lóránt LEEL-ÔSSY, Dobozy M. 14, H-2500 Esztergom, Tel: +36 33 311 199, fax: +36 33 411 950

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Their EM investigations led Schochet et al,[26] Tunon et al[31] and Hirano[10,11] to conclude that the CA is a real inclusion developing in the processes of astrocytes, and we agree with this view.[16-18] Takahashi et al,[29] also found CA in the dendrites of neurons, while Anzil et al[1] discovered it intraneuronally, though they did not rule out the astrocytic origin of the CA. There is no ultrastructural difference between the findings of the majority of authors. The expression polyglucosan (PG) has begun to replace the original term CA, and the peripheral PG bodies were recently identified with the CA characteristic of the central nervous system.[9,13,32]

Robitaille et al[22] reported the cases of 4 patients in whom PG bodies were deposited in the peripheral nervous system and in the viscera as well as in the central nervous system, which they described as an adult disease entity.[7] This disturbed the interpretation of the ubiquitous CA of the central nervous system. The similar chemical and ultrastructural characters do not necesseraly indicate complete identify.

CA or CA-like PG bodies have been found in aged animals,[5,14,15] but their detailed EM analysis has not yet been performed.

Material and Methods

Our investigations on 1,407 autopsy and biopsy materials afforded an opportunity for EM and scanning EM (SEM) analyses of the development of CA.

The predilection sites of CA are already well known. EM and SEM examinations were carried out on 8 persons who had died of various diseases.[19] The youngest was 61, and the oldest was 88 years old. The diagnosis of the present cases were as follows: 4 cerebrovascular diseases, 2 senile type of Alzheimer disease (SDAT), 2 cerebral glioblastoma multiforme. The youngest was 61, and the oldest was 88 years old. The samples (6-8 in every cases) were obtained at obduction 6-24 hours after death from the sites where CA always occurs i.e. from the predilection sites (different subependymal and subpial regions, fornix, olfactory tract, posterior column of the spinal cord, velum medullare anterius) without the basic pathological alterations. The material was embedded in Epon (Polyscience) after glutaraldehyde fixation, and semithin slides were stained with toluidine to select the parts containing CA, which were examined in a Phillips EM200 electronmicroscope. SEM examinations were also carried out on predilection sites such as the ventricular surface, the perivascular area and in 1 case the surface of a cavity after an infarctiom. The formalin-fixed material was dehydrated in alcohol with gradually increasing concentration, desiccated at room temperature and coated with gold in an ionizing chamber. These SEM investigations of CA were the first even in the international literature18. Various types of SEM equipment (JEOL-1988, Phillips-1995) were used in the examinations.

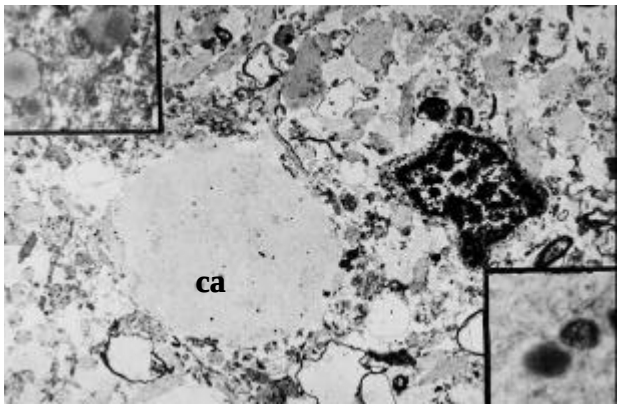


Figure 1. There is a small "mature" intracytoplasmic corpus amylaceum (CA) in the vicinity of a shrunken nucleus of an astrocyte together with tiny incipient forms of CA and with normal astrocytic fibers (x3.500). The inserts (in the upper left – arrow – resp. lower right) show the close connection between the astrocytic nucleus and CA in semi-thin EM resp. in light microscope. PAS-HE, 40 Obj.

Results

In general, only CA with larger diameters (>7 μm) was seen in the semithin sections. It was apparent that the CA was situated in the vicinity of the nuclei of the astrocytes (*Figure 1*). It was virtually impossible to establish such a connection with the structures of the cells when there was a large amount of CA. CA was always found at the predilection sites, and mainly located near the surfaces (olfactory tract, trigonal area, anterior medullar velum, fornix, pons and spinal cord).

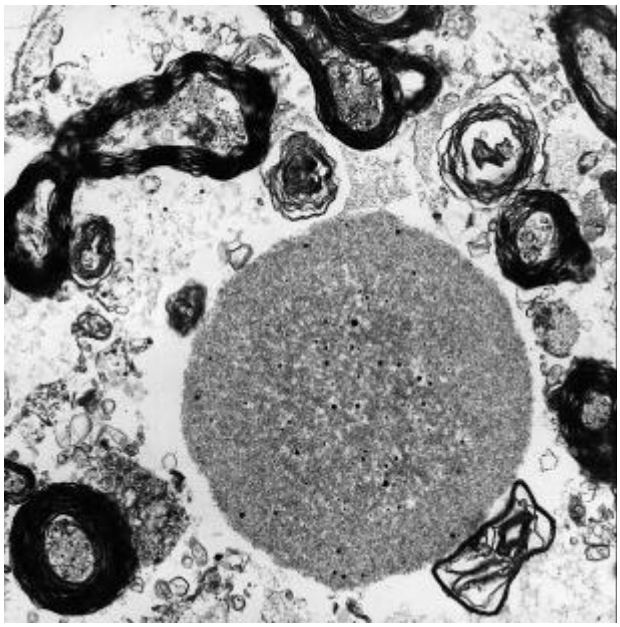


Figure 2. CA among the myelin sheaths of the olfactory tract. x3.600

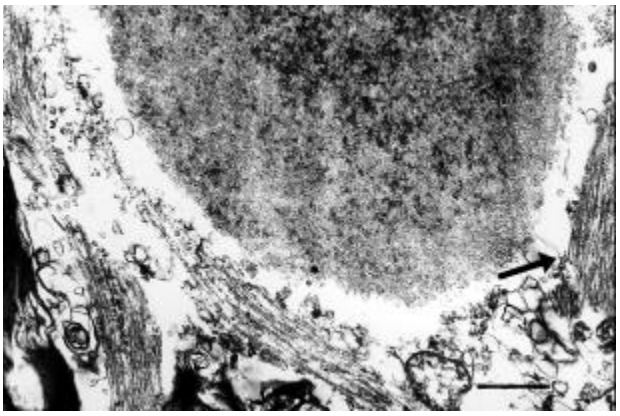


Figure 3. The remnants of normal astrocytic fibers may be seen around a "mature" CA (arrow). x7.500. Bar: 1 μ

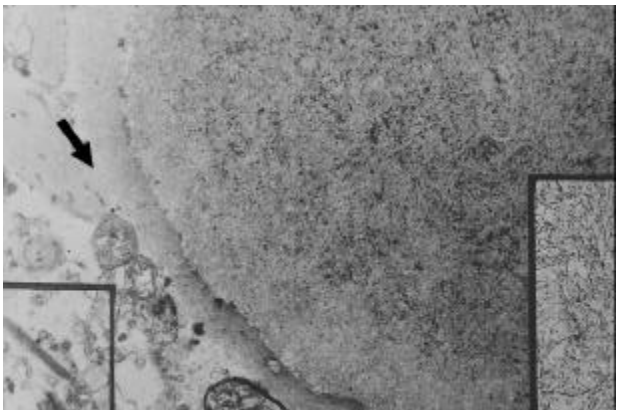


Figure 4. A close relation between the normal fibers of an astrocyte and CA is demonstrated (arrow). 7.000 X. The inserts show the remnant of normal astrocytic fibre (lower left) and the fibre system of a CA with great magnification (lower right). x7.200/x40.000

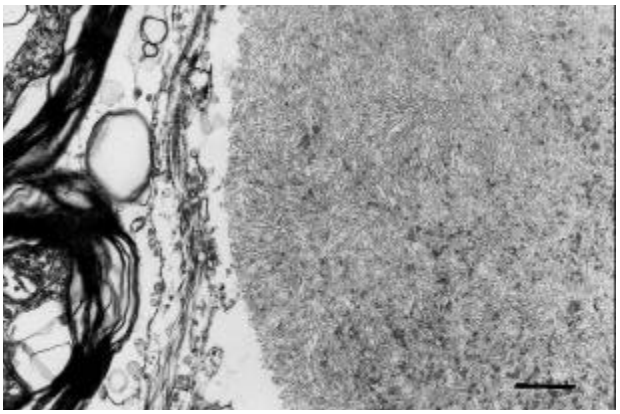


Figure 5. There are coarser grains beside the fibrous-granular structure in and around the CA. x10.000 Bar: 500 nm

The well-known perivascular, white matter and spinal septal localizations were typical. The CA was normally found between the myelin sheaths in the olfactory and optic tracts and the posterior columns of the spinal cord (*Figure 2*). CA never occurred intraneuronally or near a neuron in semi-thin slides. CA was rarely observed in the vicinity of the neurons of the anterior horn of the spinal cord, but light microscopy with PAS and haematoxylin-eosin stainings never revealed its intraneuronal or intraaxonal localization.

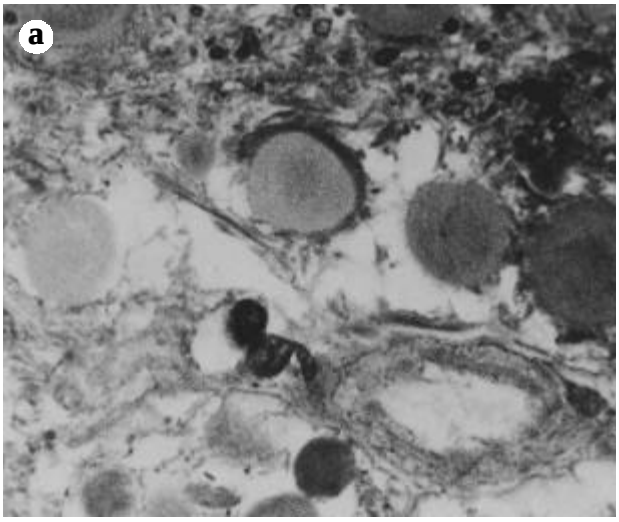
In ultrathin slides, a close correlation was observed between the CA and the astrocytes, and particularly their fibers. The progressive development of the CA could be followed by EM (*Figure 3*). A tiny irregular lamellar fiber mass initially formed in the cytoplasm of the astrocyte (*Figure 1*). The cytoplasmic localization of CA could be proved only by the vicinity of the shrunken nucleus of an astrocyte. Where a large amount of CA developed it was practically impossible to discern the original neuronal structures. As the CA increased in size the normal fiber pattern of the astrocyte gradually disappeared or remained only at the edges (*Figure 4*). Merely, a large mass of CA was sometimes seen at the predilection sites and on the surfaces, together with the remnants of some ultrastructural parts of the normal tissue.

The inner structure of the CA corresponded with the previously published findings, i.e. a jumble of fibers 50-100 nm in diameter, which were the same as the normal fibers of the astrocytes. Coarse amorphous granules or a little finer granulation was often found in the CA. The arrangement of the coarse granules did not exhibit any regularity, but the finer ones were usually localized in the central part of the CA this appeared clearly in the EM pictures (*Figure 5*).

The CA was not usually encapsulated but was closely connected with different parts of the myelin and other nervous structures. The CA was often surrounded by granules similar to those found inside the CA these were reported by Ramsey[21] and others to be glycogen granules.

The perivascular CA sometimes adhered very closely to the adventitia and the pial surfaces (*Figure 6*). When the surface of the cortex was completely covered with CA, it was surrounded by the fibers of the membrana limitans gliae. These normal fibers always displayed GFAP-positivity in contrast with the negativity of the CA. The GFAP positivity was observed only in the border zone or "capsule" of the CA. The CA sometimes separated from the membrana limitans gliae and was situated free in the subarachnoideal space, or in the case of ependyma, in the ventricle. The CA sometimes appeared to adhere to the pia mater or to lie in it, similarly as experienced in the septum of the spinal cord, which indicates a very close relation to the connective tissue at some structural predilection sites (perivascular, subpial and septal (*Figures. 7,8*).

Different CA formations were revealed by SEM examination on the ventricular surface, in the perivascular space and sometimes on the inner surface of the cavity that



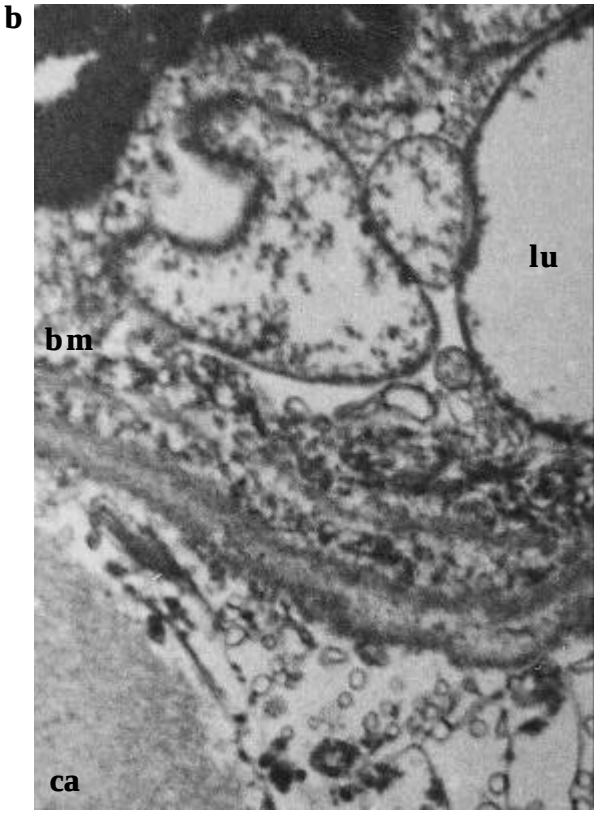


Figure 6. a) The perivascular accumulation of CA may be seen together with some nuclei of astrocytes in semi-thin slide. A hyperdense core appears definitely in some CA. – b) CA localizes beside the basal membrane of a vessel (bm). Above the lumen of a capillary (lu) lies a part of an endothelial cell. x3.700

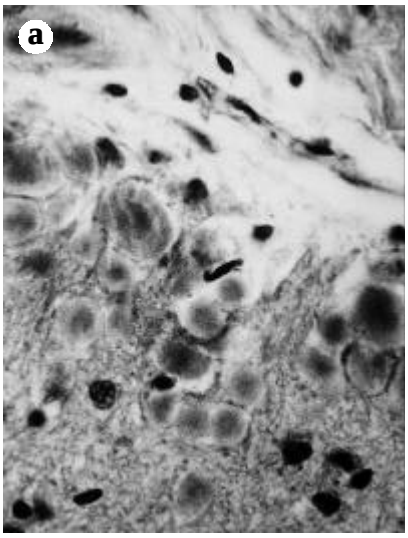
developed after an infarction. Fibrous forms joined by a bridging fiber occurred as did single fibrous, pedunculated and regular round and other forms of CA (*Figure 9*). The fibrous surface may be indicative of an astrocytic origin, but SEM has not yet furnished other and more exact findings relating to the formation of the CA.

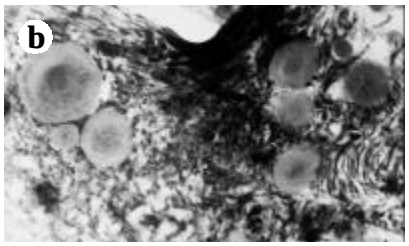
Discussion

We agree with the authors cited in the introduction[10,21,23,26,31] as concerns the astrocytic origin of the CA.[16,17,20,25] Light is shed on the mechanism of development the CA by the following findings:

1. The normal fibers of an astrocyte become "entangled" in response to some stimulus, and this results intracellularly in an increasingly spherical formation. Above a certain size of the CA the nucleus of the astrocyte shrinks and finally disappears, in parallel with the destruction of the other intracellular structure.

2. The majority of authors including ourselves agree that the initial stimulus may be superfluous (unused) glycogen or a large amount of some other carbohydrate polymer.[2,21,27]





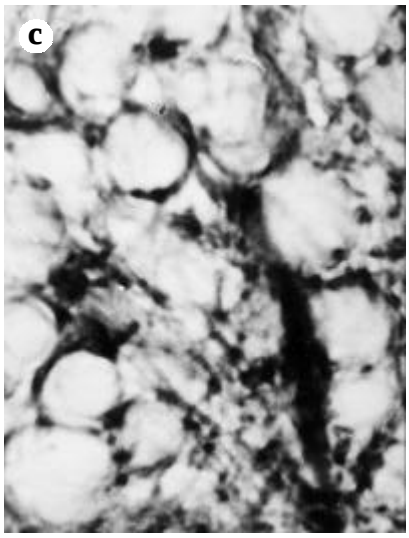


Figure 7. a) CA are on the pial surface of the spinal cord (HE) and b) they are embedded in the thickened connective tissue of the pia-arachnoidea in semi-thin section (toluidine blue). – c) The collagen fibers are stained only around the CA by Masson-trichrome.

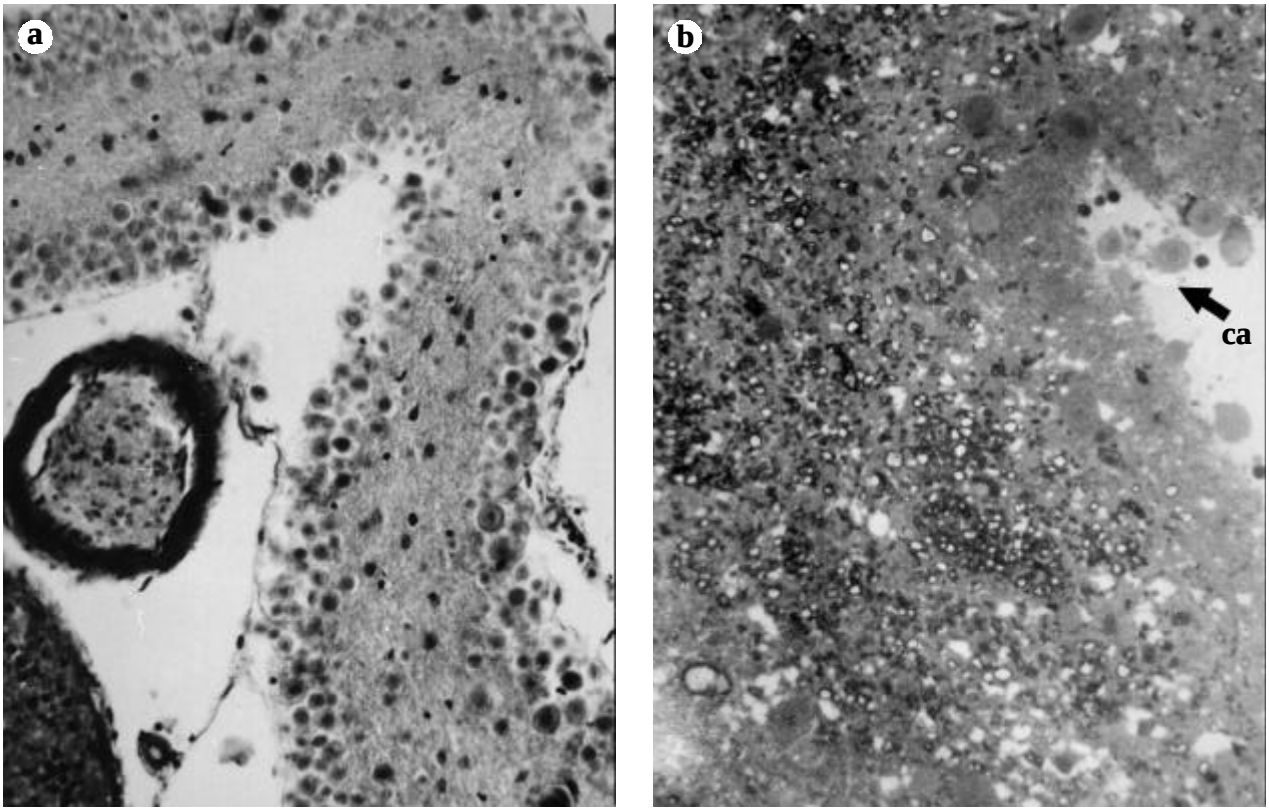


Figure 8. a) Large amount of CA may be seen in the velum medullare anterius. (HE) – b) Some CA enters in the ventricular cavity as the semi-thin picture demonstrates.

This could be the tiny granules which are commonly found in and around the CA. The granules may form the "germen" initiating the formation of a biocrystalloid structure. The initial stimulus may originate in other chemical constituents which arise from the metabolites of the decomposed cell, myelin or axon in the case of a degenerative process (amyotrophic lateral sclerosis, multiple sclerosis, etc.). This mechanism may also presumed to be involved in the development of other intracellular inclusions, where protein, polysaccharide or some other compound connected with an enzyme disturbance accumulates in a diseased cell. The result will be a specific morphological formation which in most cases remains in an intracellular localization. The extreme accumulation of harmful material associated with other pathological processes (a barrier disturbance, aging, etc.) aggravates the disease of the cells this finally destroys the cells, and the CA or other inclusion may then be found extracellularly. The well-known predilection sites, the large amount of CA and the extracellular enlargement of the body may also suggest the participation of constituents originating from the blood, connective tissue or cerebrospinal fluid in the development of this formation.

3. After study of the light and EM figures, the question may arise of whether the large mass of CA demonstrated may cause a secondary disturbance of the function of the given area.[17-19] It is difficult to answer this question because it can scarcely be proved that the CA masses in the Ammon's horn and in the fornix (memory) or in the olfactory tract (smell) cause the disturbance of the functions when these structures have already been damaged by a disease process. The absorption of the cerebrospinal fluid probably changes, because the *membrana limitans gliae* may disappear in the event of the presence of dense CA, and the function of the blood-brain barrier may also be

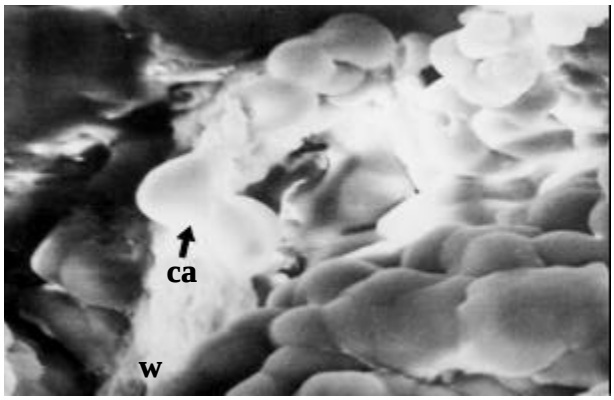


Figure 9. Many CA situated near a capillary wall in close connection with its adventitia. SEM 2.450 X

impaired in such a case. However there may also be an improved protective effect, because the barrier is better able to inhibit the entry of noxious material. These findings demonstrate that it is necessary to continue investigat of the formation and occurrence of the CA in order to further our understanding of the mechanisms of the chronic barrier disturbance and aging. From a survey of the literature it seems that the pessimistic view of Tomlinson[30] and the simplification of Hirano[12] ("the CA is an astrocytic inclusion of polyglucosan") have not encouraged the progress of the investigations into the nature of human CA as Cavanagh[3] concluded in his full-length review on CA in 1999. He says: "If in normal health their main role is protection against potentially harmful products of metabolic activity and the effects of aging of long lived protein, then the numbers of CA present may be a quantitative and certain reflection of the individual's own aging process. Clearly there is a lot to be learnt about these enigmatic bodies."

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