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Fibrillar Inclusions in Nerves of Dog Stomach*

(fibrillar inclusions/myenteric plexus/stomach)

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We have studied the fibrillary inclusions (including filamentous inclusions) in the nerves of dog stomach. Fibrillar inclusions, which consist of the sheaves of parallel osmiophilic fibrils (about 90 Å in width) and which are completely devoid of distinctive interconnections between the filaments, were found only in the preterminals of the vagus and not in the nerve cells of the stomach, after electric stimulation. Of 15 stomachs in which the vagus was electrically stimulated, only one stomach contained this type of inclusions. From these results, the origin of such fibrillary inclusions was not clear. Other types of fibrillar inclusions were found in the nucleus of the nerve cells : there were nuclear bodies and rod-shaped inclusions. The former was the fibrillogranular bodies, onion-like whorl bodies and "Marinesco bodies", and the latter seemed to be a variant of the former and a paracrystalline inclusion. They were frequently observed in control stomachs and after electric stimulation of the vagus and sometimes after vagotomy. In the perikaryon, both string-like inclusions and paracrystlline inclusions were found : the former was found in only one of 65 stomachs, whereas the latter was frequently found after longer electric stimulation but rarely found after vagotomy. The results suggest that neurofibrillary changes are probably one of the early degenerative changes in the myenteric plexus of the stomach. The nuclear bodies may be related to the neuronal activity, whereas paracrystalline inclusions are probably one of the early degenerative changes in the myenteric plexus of the stomach.

Fibrillar inclusions which consist of the sheaves of parallel osmiophilic filaments with a diameter of 85 to 130 Å in the neuron or neuropiles of the brain area (1—3) and 60 to 80 Å in the sympathetic ganglia (5), have been reported. These inclusions usually showed lattice-like structures. Neurofibrillary changes, so-called fibrillary tangles or Hirano bodies (8), also produced lattice-like structures. They are found primarily in the neuropiles of a particular brain region in normal or aged and pathological conditions (6), but are characteristic in human brain (7).

In sympathetic ganglion (4), fibrillar inclusions were found in perikaryon,

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axons and dendrites. In substantia nigras (2), identical inclusions were found in the cytoplasm of neurons, with the incidence of 80%. In spite of a high incidence of these inclusions, little has been reported in other materials. In this report, perhaps for the first time in the myenteric plexus (of dog), we describe identical inclusions and other types of fibrillar inclusions (including intranuclear filamentous inclusions.)

MATERIALS AND METHODS

Sixty five healthy dogs were used. Thirteen of these were vagotomized 1 to 12 weeks before and fifteen had electric stimulation (10−14V, 5 msec, 9−10 Hz) of the vagus nerve just before fixation. Stomachs were perfused through an arterial cannula with 1% glutaraldehyde in Krebs solution, then dissected out and kept in 2% glutaraldehyde-Krebs solution for 1 hour at 4°C and post-fixed for 1 hour in 2% osmium tetroxide in the phosphate buffer. After dehydration in graded ethanol, the tissues were embedded in Epon mixture. Ultrathin sections were contrasted with uranyl acetate and lead citrate.

RESULTS AND DISCUSSION

The basic fine structure of both normal and degenerated myenteric plexus have been reported (9). According to the objective of the present report, therefore, only the fibrillar inclusions will be described.

I. Axoplasmic Inclusions

Unlike those reported in the literature (1−5), fibrillar inclusions were found only in the preterminals of the vagus and not in the neurons of stomachs which had received vagal stimulation until the stomach no longer contracted. In cross section of axons (Figs. 1 and 2), the inclusions consist of sheaves of parallel osmiophilic fibrils with a width of about 90 Å. Their individual filaments varied in length, which could not be measured accurately. They also display curvilinear profiles so that some of the cored vesicles, probably undergoing intra-axonal transport, appeared to be present underneath the inclusions. Unlike those seen in the literature (1, 2, 4, 6) distinctive interconnections, or the lattice-like structures, were not recognized between the parallel filaments in the sheaves: the spaces between the filaments were about 80 to 90 Å: Where they were sectioned longitudinally, fibrillar elements appeared to be granular (Fig. 3) and possibly tubular profiles (Fig. 2). Typical twisted tubules, which consist nearly completely of bundles of beaded filaments and which resemble those observed predominantly in Alzheimer's presenile dementia (8), were not evident. In some regions, fibrillar elements were also in contact with the neurotubules. The fibrillar inclusions we have described above were virtually identical to those which occur in the sympathetic neurons (4) and in the gracile nuclei of certain old monkeys (3), except that they were observed only in the preterminal vagus nerves of a young control dog and after electrical stimulation. Of 15 stomachs in which the vagus was stimulated with electric currents, only one stomach contained this type of inclusions. None were found in the other stomachs. Therefore, the pathogenesis of the fibrillar inclusions, if any, is not clear. Some author (4) interprets these inclusions in the sympathetic neurons as a storage form of migrating proteins, because they were presumably made up of sulfer-containing protein. It is especially noteworthy that in sympathetic ganglion these inclusions occurred consistently unrelated to the sex, age or any physiological conditions of the animal. If so, it should be recognized in many regions of the sympathetic nervous system.

II. Intranuclear Fibrillar or Filamentous Inclusions

Besides the above inclusions, two kinds of intranuclear fibrillar (or filamentous) inclusions were found : they are "nuclear bodies" first designated by Weber et al. (10) and rod-shaped inclusions commonly known as the "rodlets of Roncoroni", named by Cajal. As shown in Fig. 4 to Fig. 6, nuclear bodies had no limiting membranes, but some of them appeared to be surrounded by a dim nucleoplasmic halo. The nuclear bodies in the neurons were randomly distributed throughout the nucleus and rarely localized close to the nucleolus and to the nuclear membrane. In general, they were spherical or spheroid in shape and varied from 0.2 to 1.5 μ in diameter. There was a marked variation in their morphological profiles as reported by Bouteille et al. (14). Ultrastructurally, they are classified, in the present study, as follows : 1) granular fibrillar type, consisting of small aggregates of homogeneous granulo-fibrillar substances (Fig. 4). At high magnification, however, they appeared to consist of microtubules (Fig. 5). They were usually small and ranged from 0.2 to 0.5 μ in diameter ; 2) onion type, consisting of concentrically arranged fibrils and appearing to form an onion-like whorl, with a central granules or filaments (Fig. 6). This type of nuclear body was often multiple with two or three bodies present, in which case they were often coexisted with the granulo-fibrillar type. The onion type of nuclear bodies appeared to be increased after electric stimulation of the stomach ; 3) Besides these types, larger profiles of nuclear bodies were sometimes observed (Fig. 7). This type of the bodies appeared to be aggregate of fine granular osmiophilic materials consisting of nucleoplasm. They had a diameter of 2 to 5 μ and displayed a similar profile to those of "Marinesco bodies" (6, 11) except for the absence of lattice-like filaments. Further observations of Marinesco bodies are now in progress. Nuclear bodies with annular configulations which were devoid of central granules or fibrils were also often found after electric stimulation.

It was noted that nuclear bodies, particularly those of the onion types, were most frequently observed after electric stimulation of the vagus and sometimes after vagotomy, but were often not observed in the normal stomach. Therefore, their morphological appearences may be related to neuronal activity. This hypothesis is compatible with the findings that sympathetic ganglia showed increased numbers of nuclear bodies after a single injection of reserpine. In sympathetic neurons, however, there was also an objection to this interpretation ; Dixon (13) described that the increased protein synthesis after axon section did not affect the distribution or morphology of the nuclear bodies in sympathetic neurons. The variety of their morphological profiles presumably reflects the presence of their transitional forms depending on their neuronal activity and on different orientation of sections. This explanation is quite compatible with that of Bouteille et al. (14) ; 4) The other intranuclear fibrillar or filamentous inclusions are the "rodlets of Roncoroni". In longitudinal sections, they exhibited the profiles resembling a writing brush, with small tail ends (Fig. 8). So far there are only single rodlets in the nuclei, which sometimes lie closely associated to the nucleolus. They have neither limiting membrane nor a discernible surrounding halo in the nucleoplasm. Ultrastructurally, the rodlets were composed of a bundle of fine fibrillar elements. In contrast to the previous reports (12, 15), no regular parallel arrangement of fibrils was recognized. The thickness of fibrils varied from 70 to 90 Å. The rodlets have only rarely been found up to now in the myenteric plexus neurons and after longer electric stimulation (in three stomachs of fifteen dogs). Their cytological significance is not clear. Some authors (16), based on the morphological criteria, recently reported that Roncoroni rodlets might be a variant of the spherical bodies, depending on the different orientation of the sections. It is noted that the filaments seen in the Roncoroni rodlets were similar, in respects of the thickness and the diameter, to those of granulo-fibrillar type of the nuclear bodies (but not those of Marinesco-type).

It is also interesting that the rodlets are limited to certain nerve cells and to certain species (15).

III. Fibrillar Inclusions in Perikaryon

In perikaryon, there were two types of the fibrillar inclusions. One type displayed string-like appearances in their longitudinal section. These inclusions were randomly scattered in the somatic cytoplasm. They contained osmiophilic center lines, 20 to 30 Å thick, and present on both sides were the arrays of less osmiophilic punctate substances (Fig. 9). Their whole width measured about 110 Å. Of 65 stomachs only one contained these scattered string-like inclusions. Up to date their nature is unknown.

The other type of somatic inclusions were so-called paracrystalline inclusions rather than the fibrillar inclusions. They were frequently observed in the perikaryon, after longer electric stimulation and rarely after vagotomy. Ultrastructurally, these inclusions appeared as aggregates of parallel arrays of punctate microtubules with a width of 110—130 Å in Fig. 10. Unlike those seen in the fibrillar inclusions (2, 3), no distinctive traverse striations were found between the longitudinal arrays.

They are probably paracrystalline inclusions and, as previously presumed by Seite (4), perhaps derived from cytoplasmic protein. In perikaryon, one of the early degenerative changes following electrical long-lasted stimulation was an increase of Nissl body, later followed by its disorganization. Formation of ribosomal paracrystalline in the neurons will be described separately.

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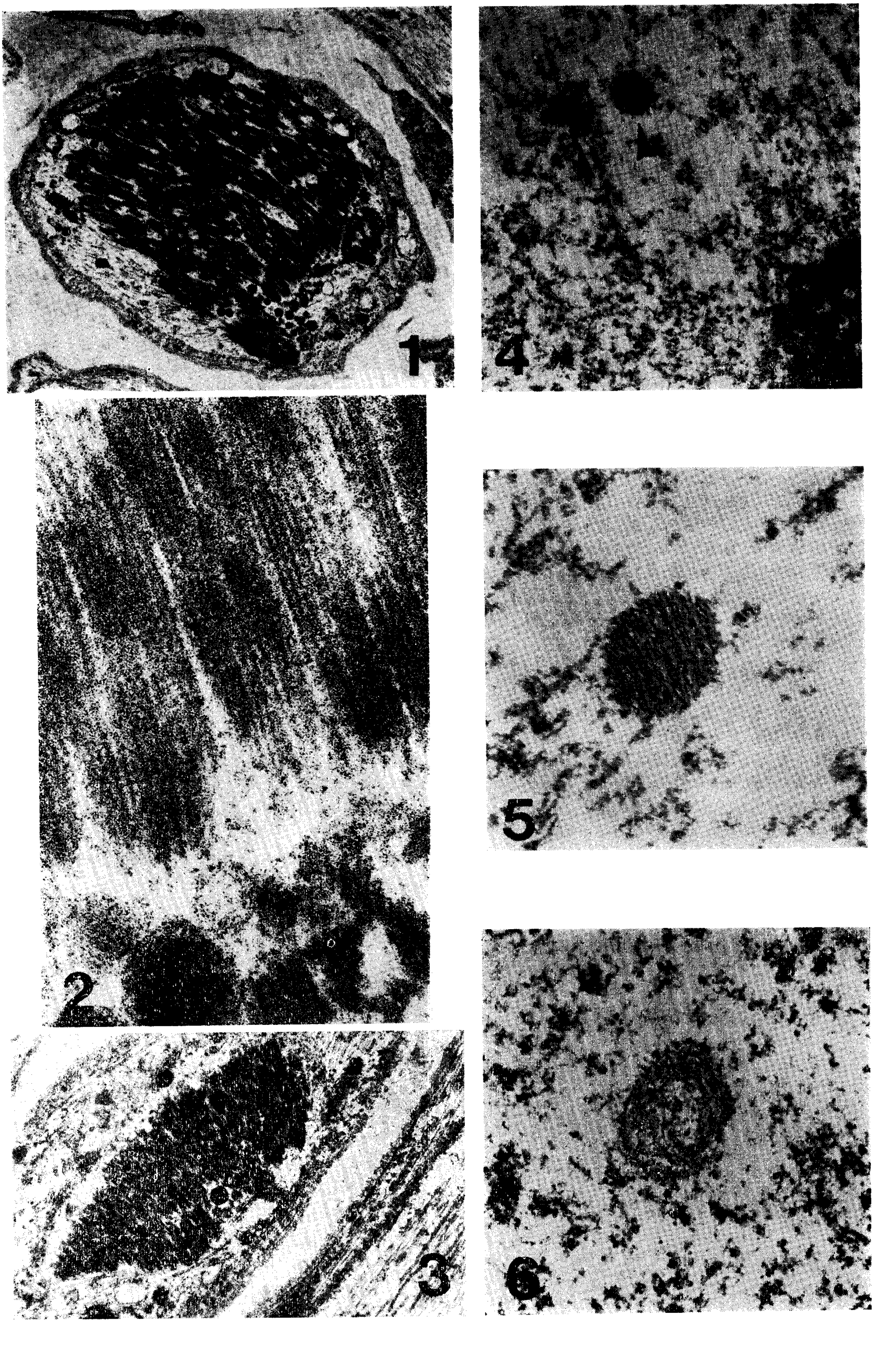
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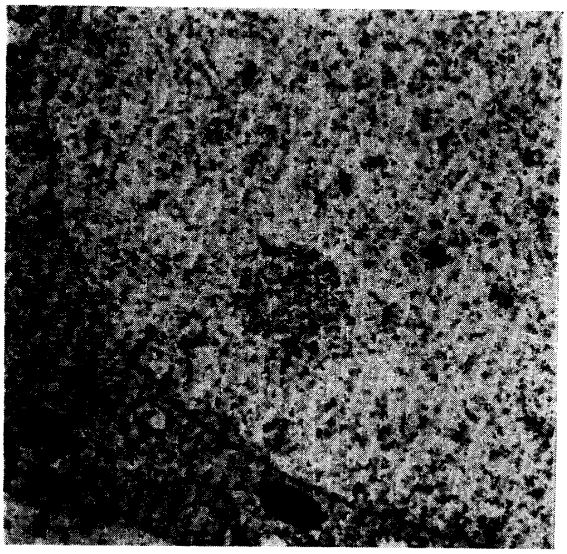

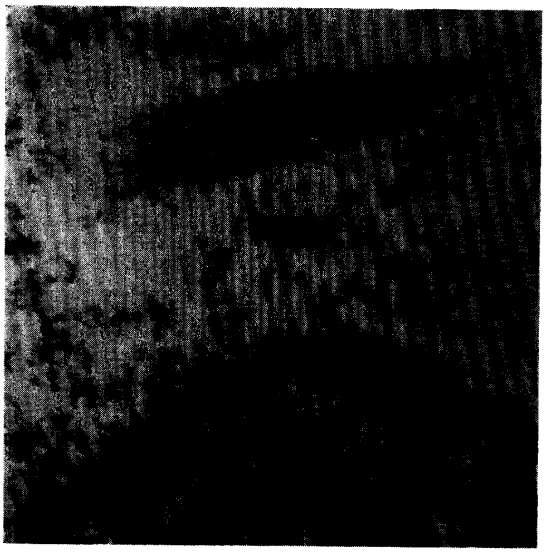

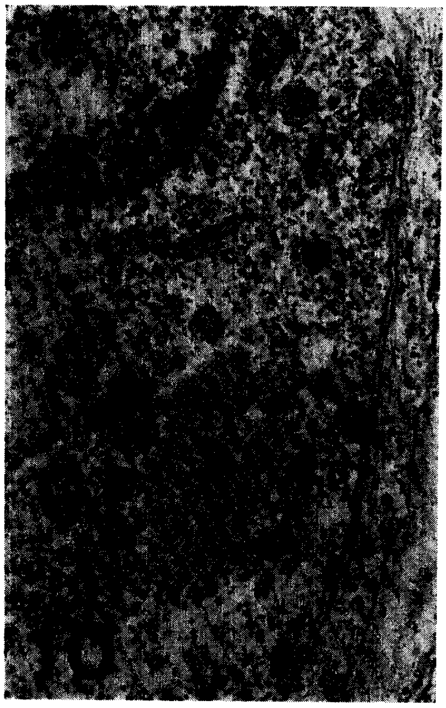

LEGENDS TO FIGURES

Fig. 1. Axoplasmic inclusion in preterminal vagal axon of dog stomach. (× 6,750)

Fig. 2. High power view of inclusion in figures. (× 76,000)

Fig. 3. Axon as in Figure 1, but cut more longitudinally. (× 12,000)

Fig. 4. Nuclear body in neuron of myenteric plexus of dog stomach. (× 16,000)

Fig. 5. Higher power view of inclusion like that in Figure 4. (× 38,000)

Fig. 6. Another (onion) type of nuclear body in neuron of myenteric plexus of dog stomach. (× 12,000)

Fig. 7. Nuclear inclusion resembling "Marinesco body" in myenteric neuron of dog stomach. (× 4,800)

Fig. 8. Rodlet of Roncoroni in nucleus of myenteric neuron of dog stomach. (× 22,500)

Fig. 9. Inclusion in perikaryon of myenteric neuron. (× 50,000)

Fig. 10. Paracrystalline inclusion in perikaryon of myenteric neurons. (× 28,500)