THE FINE STRUCTURE OF AMYLOID

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An electron-microscopic investigation was made of amyloid deposits in the spleen of mice with experimental casein amyloidosis. Sections were stained with warm solutions of phosphotungstic acid and uranyl acetate. The amyloid fibrils consist of 2 linear filaments surrounded in the photographs by a coil of electron-dense material. Structures morphologically similar to periodic rods were found.

Under low power of the electron microscope amyloid deposits in the tissues consist of a mass of irregularly arranged fibrils about 100 Å in width [4]. However, what little information is available on the fine structure of these fibrils is contradictory [2, 6, 8, 11]. Nevertheless, the determination of the fine structure of the amyloid fibrils is important so that their morphological specificity can be established. The question of the second ultrastructural component of amyloid – the periodic rods – also remains unanswered. These structures, constantly found in preparations of amyloid isolated from tissues [2, 3, 8], have not been described in amyloid deposits in the tissues. The true relations between the periodic rods and fibrils form the subject of various hypotheses [2, 8].

The object of the investigation described below was to continue the study of amyloid deposits in the tissues.

EXPERIMENTAL METHOD

Amyloidosis was induced in male BALB mice weighing 18-20 g by subcutaneous injections of 0.5 ml 5% casein solution in 0.25% NaOH solution 5 times a week. The spleen was pre-fixed by intravital perfusion with 2.3% glutaraldehyde [5]. Pieces of the spleen were fixed in 1% osmium tetroxide solution in veronal-acetate buffer, pH 7.4, for 1 h. After dehydration in acetone of increasing concentration, when the 70° acetone contained 0.5% uranyl acetate, the material was embedded in Vestopal W. Ultrathin sections 400-500 A in thickness were negatively stained by a combined method: 2% phosphotungstic acid solution in 96° ethanol for 10-13 min at 60°C [9] + 1% aqueous solution of uranyl acetate for 10-30 min at 60°C. The sections were examined in the ÉM-100 V electron microscope.

EXPERIMENTAL RESULTS

Under low power of the electron microscope (20,000-30,000 ×) amyloid appeared as a mass of single, chaotically arranged fibrils. They varied in width from 90 to 180 Å and in length from 300 to 6000 Å. Under high power of the electron microscope (90,000-100,000 × or more) the interfibrillary matrix of the amyloid had no definite ultrastructural characteristics, in agreement with other observations [4]. From time to time weakly stained structures could be seen in the mass of the amyloid fibrils, but because of deposition of a finely granular material, these structures could be seen only in small areas (400-500 Å, Fig. 1). Being about 100 Å in width, they resembled amyloid fibrils in shape. They differed from them in being divided by transverse light bands about 20 Å in length into several (possibly paired) segments each 20 Å in length. Certain structures had thinner (10-15 Å) bands running obliquely. According to the literature, these are the morphological features of periodic rods [2, 3, 8]. However, before these structures can be identified as periodic rods, further investigations are necessary.

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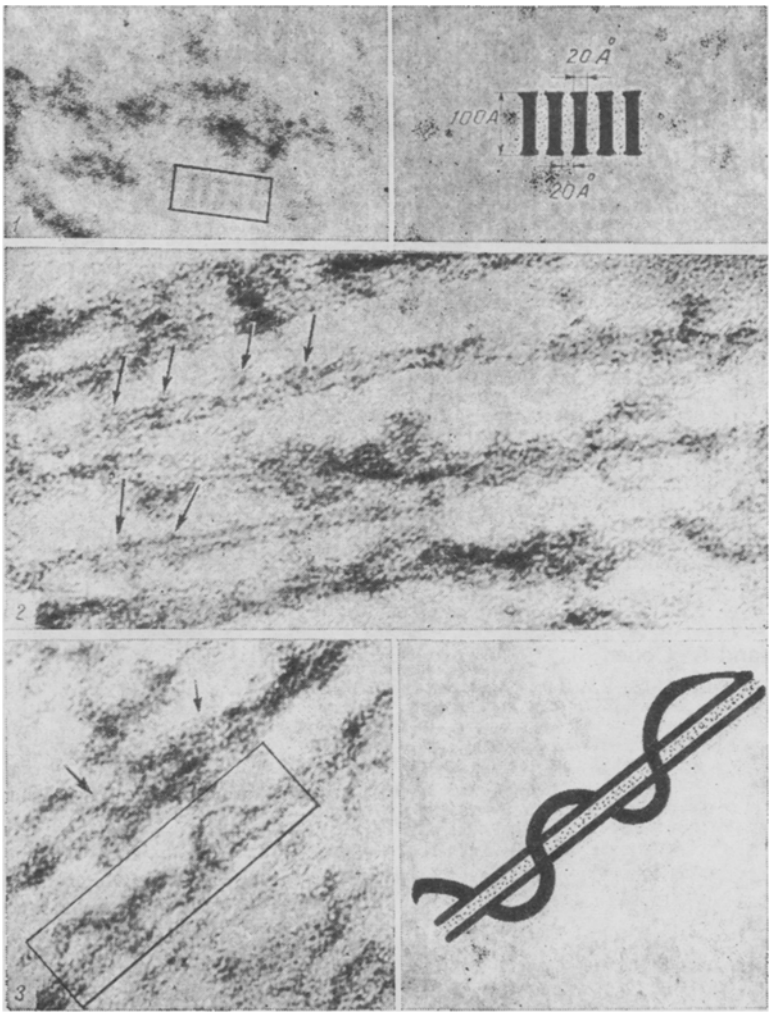

Fig. 1. Structure morphologically resembling periodic rods (450,000 ×). The same on the right, diagrammatically.

Fig. 2. Amyloid fibrils: paired filaments separated by a light zone are demonstrated; arrows point to dark "segments" (330,000 ×).

Fig. 3. Amyloid fibrils: coiled electron-dense material (the same on the right, diagrammatically); arrows point to deposits of electron-dense material (440,000 ×).

The amyloid fibrils consisted of two parallel filaments 30-35 Å in diameter (Fig. 2). The filaments were separated by a lighter zone 30-35 Å in width. In rare cases the morphological picture observed is such that the filaments may be twisted together.

These paired filaments are evidently surrounded over some of their extent by a helical coil of electron-dense material. As a rule the turns of the coil are closely applied to the filaments, giving the fibrils an apparent periodicity, in the shape of a series of dark transverse "segments" from 60 to 450-500 Å apart. The length of these "segments" varied from 40 to 100 Å, on the average about 60 Å. Their width was sometimes much greater than the diameter of the paired axial filaments (about 100 Å), namely 140-200 Å, and this is the reason for the wavy appearance of the outer contours of the fibrils (Fig. 3). This scatter of the dimensions of the "helix" is probably explained by the band-like arrangement of the electron-dense material forming it and by the unequal pitch of the "helix" along the length of the fibrils. The fine structure of the individual fibrils in general often could not be identified because of the large deposits of electron-dense material.

The question of the globular (subunit) or linear structure of the amyloid fibrils has been discussed in the literature. The globular structure of the fibrils has been observed chiefly in ultrathin sections of

amyloid in situ, when positively stained with lead hydroxide or citrate and with uranyl acetate [1, 2, 7]. This method, widely used in practical electron microscopy, is evidently unsuitable for demonstrating the filaments of amyloid fibrils [11]. The results of the present experiments, in which a new method of positive staining to increase contrast confirm both this hypothesis and also observations showing the linear character of the structure of the paired filaments in amyloid fibrils [6, 8, 10, 11].

The existence of a "helix" consisting of sulfonated mucopolysaccharides in the structure of amyloid has also been assumed in a model of the structure of the amyloid fibrils [12].

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