LAMELLAR BODIES, AN UNUSUAL ARRANGEMENT OF THE GRANULAR ENDOPLASMIC RETICULUM

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In his investigation of the fine structure of the nervous system, Fernández-Morán observed lamellar structures in the Purkinje cells of the rat cerebellum (1). The use of newer methods of preparation fail to demonstrate these bodies in the cytoplasm of neurons (6, 3).

During the course of a study of the normal Purkinje cell in rats (3), we observed lamellar bodies in two of the ten animals studied. In all of the animals fixation had been accomplished by vascular perfusion; however, in the two animals in which lamellar bodies were found the perfusing fluid was cold, whereas in the remainder of the animals the perfusion was carried out with warmed solutions using the method of Palay (6). Cold perfusion has since been carried out on two additional animals in a successful attempt to reproduce the lamellar bodies.

MATERIALS AND METHODS

Rats, weighing 200 to 300 gm, were anesthetized with pentobarbital (90 mg per kg) by the peritoneal route. In one animal a craniotomy was performed exposing the cerebellum before perfusion, while in the other three animals the skull remained closed until the perfusion was complete. The chest was opened, the anterior chest wall removed, and the descending aorta cannulated with the cannula pointed cranially. Next, the vena cava was cut and the animal perfused with 20 ml of cold normal saline followed by 40 ml of 1 or 2 per cent cold (about 4°C) buffered osmium tetroxide. The cerebellum was removed immediately, cut into 1 mm cubes, and fixed in osmium tetroxide for an additional hour. With this method of perfusion, the tissue at the time of removal was only partially blackened. The blocks were then dehydrated in ethanol, embedded in Epon (4), and sectioned on a Porter-Blum microtome. The sections were stained with alkaline lead acetate (8) or with a saturated solution of uranyl acetate in 50 per cent ethanol (2) and examined in a RCA EMU 3 E.

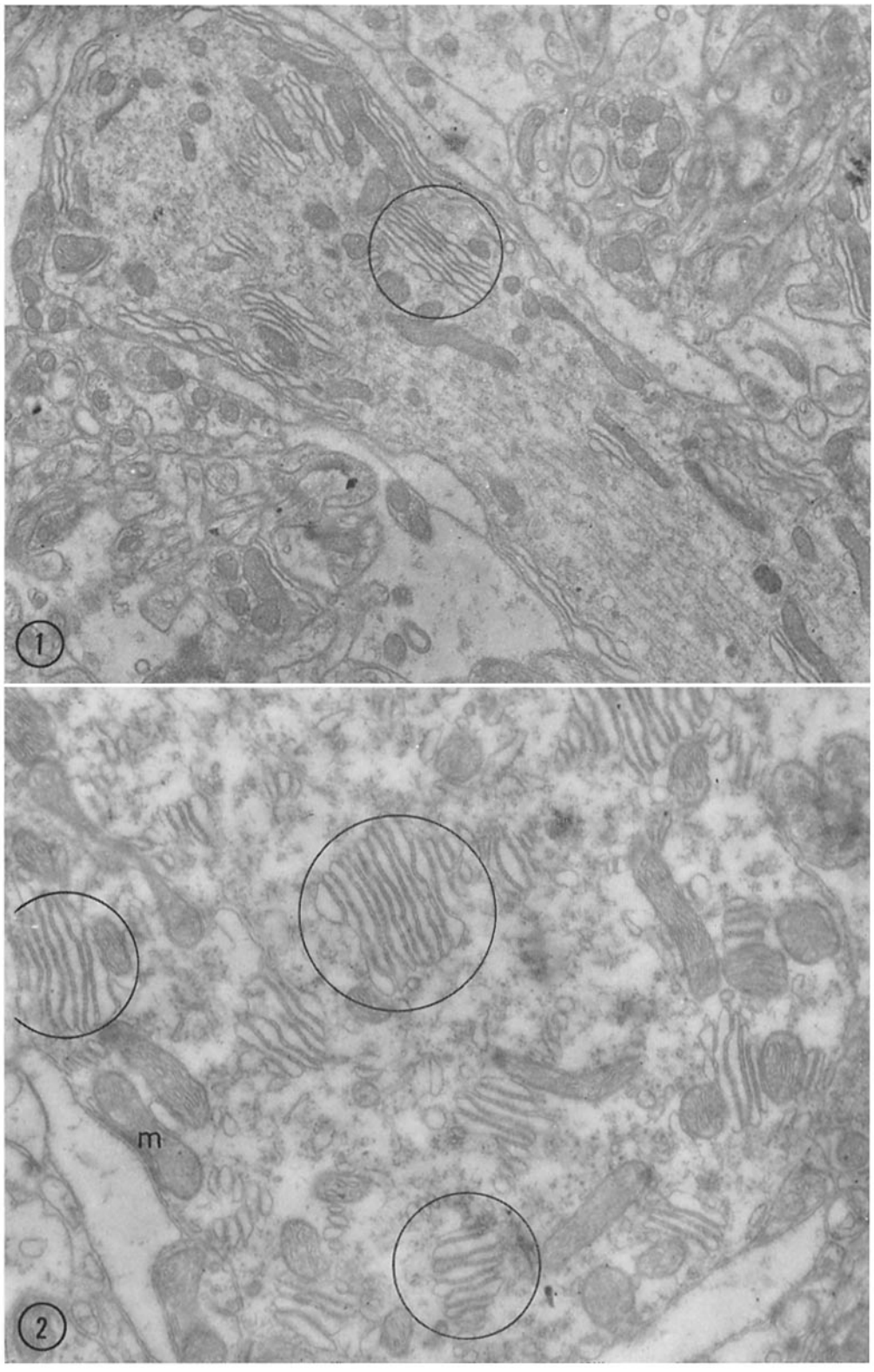
OBSERVATIONS

The Purkinje cells in each of these animals contained lamellar structures consisting of parallel membranes arranged in pairs separated by dilated cisternae. These complex structures, which we will call "lamellar bodies," vary in length from about 0.5 microns to about 3 microns. Their width varies from around 0.2 microns to 1 or occasionally 1.5 microns, and they may contain from 1 to 10 membrane pairs (Figs. 1 and 2). These bodies are seen throughout the cytoplasm and dendritic tree of the Purkinje cells. This distribution corresponds to the usual distribution of the granular endoplasmic reticulum; however, granular endoplasmic reticulum is not found or is present in markedly reduced amounts around the lamellar bodies. Virtually all of the Purkinje cells seen in these four animals contained some lamellar bodies, though the number varied from one or two to several dozen per section. The only other cytoplasmic abnormality noted was clumping of the free RNP particles into small rosettes. The amount and distribution of the remaining cytoplasmic elements appeared to be normal.

Careful examination of the lamellar bodies revealed that the membranes of some of them were continuous with the granular endoplasmic reticulum and that the lamellar array was formed by the condensation of these membranes along

FIGURE 1 This micrograph shows a Purkinje cell dendrite containing numerous lamellar bodies such as the one in the circle. Most of them have mitochondria on one side. The lamellar bodies can be seen to consist of paired membranes separated by clear cisternae. × 9,500.

FIGURE 2 In this micrograph several lamellar bodies are seen (circles). Between the apposed membranes, periodic densities can be seen. At lower left, a subsurface cistern with an overlying mitochondrion (*m*) is seen (3). × 18,500.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their outer or granular surface (Figs. 3 to 5). Such lamellar bodies can be distinguished from the Golgi apparatus by the presence of small dense particles between the aggregated membranes (Fig. 4). No formed material has been seen within the cisternae of the lamellar bodies.

DISCUSSION

The significance of this arrangement of the endoplasmic reticulum is not clear. Several different modes of formation of lamellar bodies are possible. It could be due to the effect on the endoplasmic reticulum of very severe anoxia of short duration. No attempt has been made to give artificial respiration to these animals during the dissection and, since the tissue seemed incompletely fixed at the time of removal, anoxia was present. Examination of the electron micrographs of lamellar bodies published in 1957 by Fernández-Morán (1) reveals clumped chromatin and swollen glia which indicate the presence of anoxia in his preparations. Another possible explanation is that the bodies are due to the effect of pressure from the knife used in cutting the blocks of partially fixed tissue. A third possible cause could be the effect of lowered temperature. Fernández-Morán used immersion fixation in osmium tetroxide solution at 4°C. and this was also used for perfusion in the present study. It is unlikely that the lamellar bodies are the result of some unknown disease process, as all four animals appeared healthy and no histological evidence of disease was found.

These profound changes in the endoplasmic reticulum appear to involve a redistribution of the intracellular fluid and may be considered as evidence of the plastic nature of the cellular membrane systems. Evidence of this plasticity in normal animals has been presented by Palade (5), who demonstrated dilatation of the cisternae of the granular endoplasmic reticulum to accommodate intracisternal granules in the pancreas, and by Sheldon and Kimball (7) who described rapid dilatation and hypertrophy of the Golgi apparatus of stimulated cartilage cells with intracisternal accumulation of collagen. The endoplasmic reticulum of nerve cells has long been known to be sensitive to certain pathological processes which produce changes of central or peripheral chromatolysis. In addition, the light and dark cell artifacts which so commonly result from immersion fixation of nervous tissue are probably due largely to alteration in the granular endoplasmic reticulum.

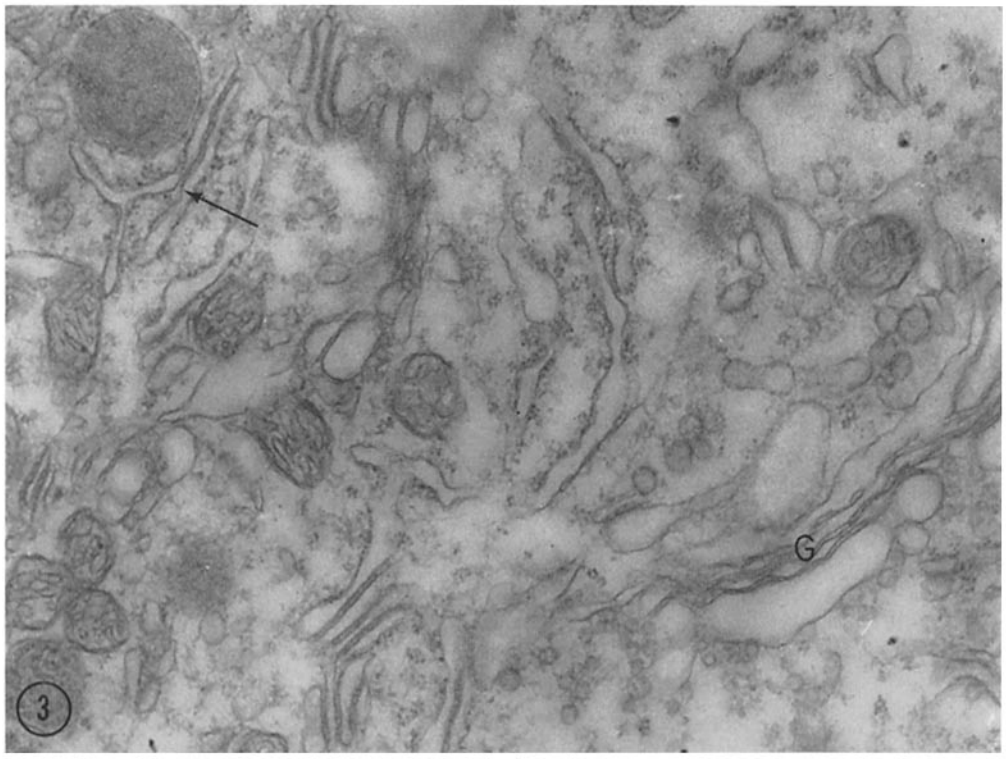
We do not regard lamellar bodies as normal, as they were not found in animals which were given artificial respiration up to the time of perfusion and which were perfused with warmed (37°C) osmium tetroxide solutions before removal of the cerebellum. (For a discussion of criteria of adequate fixation, see Palay, 1962) (5).

Whether the lamellar arrangement of the endoplasmic reticulum occurs before death and might be of some physiological significance, or whether it occurs after cell death due to an interaction of a number of factors related to fixation, remains to be determined. Though this change has been looked for only in cerebellum, it seems likely that the endoplasmic reticulum of cells other than the

FIGURE 3 In this micrograph of Purkinje cell cytoplasm, portions of granular endoplasmic reticulum can be seen coming together along their outer surface (arrow) to form the paired membranes seen in the lamellar bodies. Periodic densities can be seen between the paired membranes. This is contrasted with the parallel membranes in the Golgi apparatus (*G*) at lower right which do not have these particles. The periodic densities probably are altered RNP particles. × 28,000.

FIGURE 4 In this micrograph of a lamellar body, RNP particles are clearly seen (arrows) between the apposed membranes. Note also the clumped appearance of the unattached RNP particles. × 49,000.

FIGURE 5 This micrograph shows two membranes of endoplasmic reticulum coming together (arrows) to form a pair of closely apposed membranes similar to those seen in the lamellar body. A subsurface cistern with a mitochondrion (*m*) overlying it is seen at the bottom of the micrograph, and a small lamellar body is seen just above the mitochondrion. × 29,000.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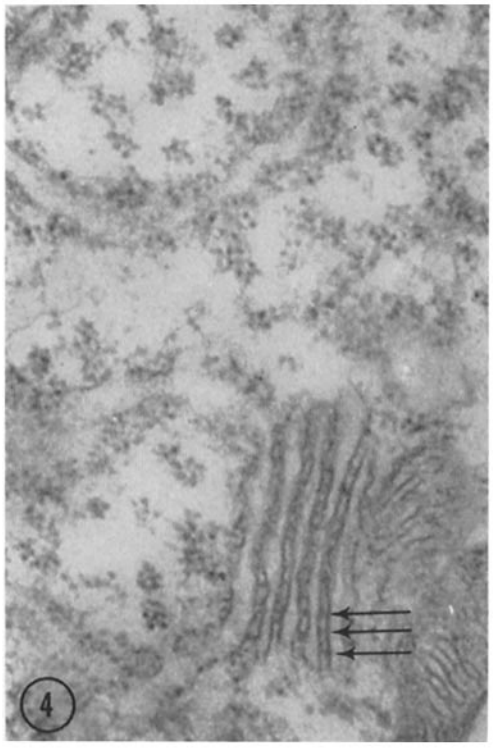
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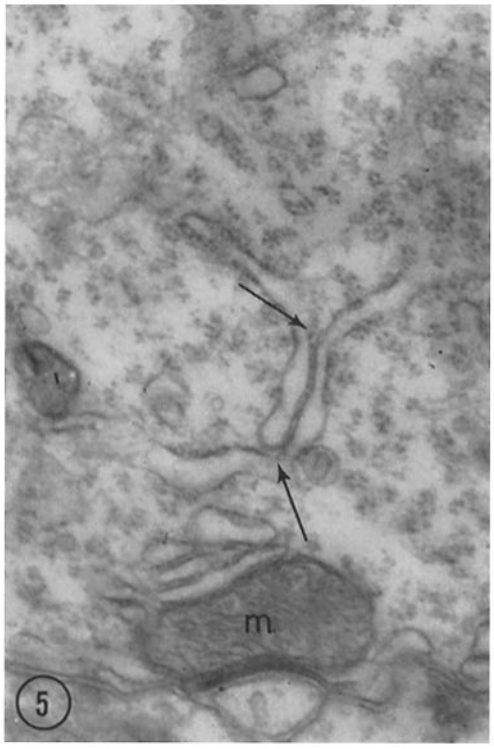
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Purkinje cell, particularly that of other large neurons, may undergo similar changes.

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