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Sodium localization in the cerebellum

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INTRODUCTION

Sodium was first localized using the electron microscope by Komnick & Komnick (1963) by means of the electron-dense precipitate sodium pyroantimonate. The same method was utilized to demonstrate sodium in cornea (Kaye, Cole & Donn, 1965; Edelhauser & Siegesmund, 1968), the membranous system of skeletal muscle (Zadunaisky, 1966) and intestines (Yamada, 1967). Hartmann (1966) applied a modification of this technique to localize sodium in cells of the cerebral cortex. To verify the precipitate as sodium pyroantimonate, Hartmann utilized electron diffraction procedures. Spot patterns obtained from selected area diffraction of high concentrations of precipitate were found to be identical to those obtained from samples of pure sodium pyroantimonate.

In the present investigation sodium is demonstrated in the cerebellar cortex using a variation of Hartmann's technique. The precipitate of sodium pyroantimonate was localized specifically within various cell types and within certain cytoplasmic organelles.

MATERIALS AND METHODS

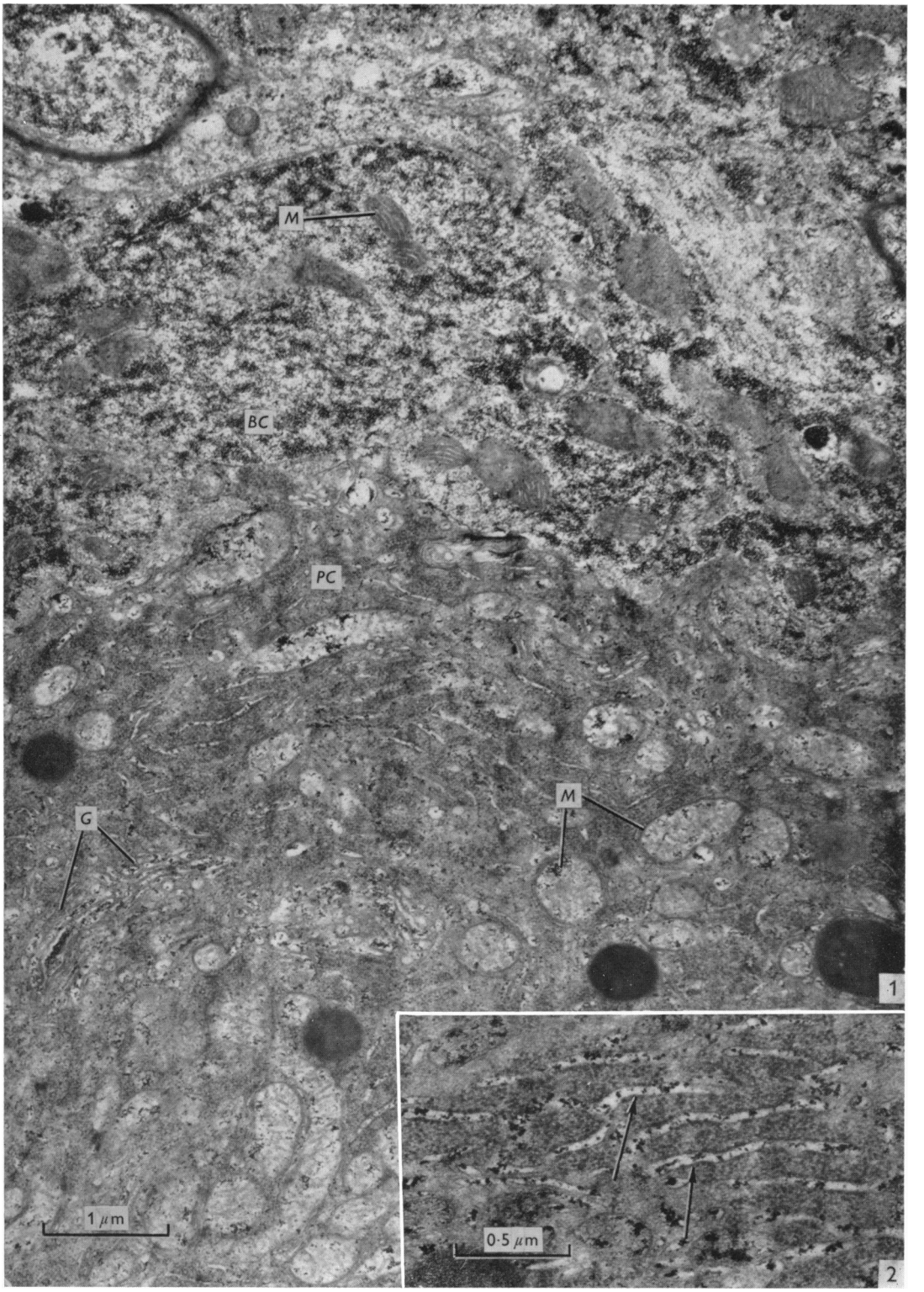
A variation of Hartmann's technique (Edelhauser & Siegesmund, 1968) for the identification of sodium in the electron microscope was used to localize sodium in the cerebellum. Tissue from cats anaesthetized with Nembutal was removed with a scalpel and quickly placed in an unbuffered glutaraldehyde-acrolein fixative. After 0·5 h of fixation the tissue was rinsed with distilled water and placed in Hartmann's (1966) mixture of 2 % potassium pyroantimonate for 1 h.

The tissue was then rinsed in distilled water and rapidly dehydrated in a graded series of acetone, embedded in Vestopal W and sections cut on a Porter–Blum microtome. The sections were stained in uranyl acetate and lead citrate and examined in an RCA–EMU 3b electron microscope.

Tissue serving as a control was fixed by a conventional technique of 0·5 h. in 2 % acrolein–3 % glutataldehyde solution buffered at pH 7·2 with phosphate buffer. Fixation in this solution was followed by 1 h in 2 % osmium tetroxide in Veronal acetate buffer at pH 7·4.

OBSERVATIONS

High concentrations of the precipitate sodium pyroantimonate were observed in the Bergmann glial cells of the cerebellum (Figs. 4, 7, 8). Processes of Bergmann cells surrounding Purkinje cell dendrites (Figs. 4, 7) had particularly large accumulations of precipitate. Very little precipitate was present within the oligodendroglial cells.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A specific localization of precipitate was found within different neurons of the cerebellar cortex. Within the Purkinje cell body almost all the precipitate was localized in the cisternae of the endoplasmic reticulum, the Golgi body and the mitochondria (Fig. 1). Although large amounts of precipitate were present within the lumen of the endoplasmic reticulum, no significant deposits were observed in the cytoplasmic matrix (Fig. 2).

Figures 3 and 4 show a comparison between primary smooth branches of Purkinje cells fixed by the conventional method and by using the potassium pyroantimonate procedure. Numerous dendritic tubules and elements of smooth endoplasmic reticulum occur in the cytoplasm of the large dendrite (Fig. 3). Precipitate was found in abundance in the endoplasmic reticulum and mitochondria (Fig. 4). Dendritic mitochondria demonstrate greater concentrations of the precipitate than the mitochondria found in the Purkinje cell body (cf. Figs. 1, 4). Occasionally multivesicular bodies were observed in the larger Purkinje dendrites (Figs. 4–6). Each of the vesicles within these structures has an electron-dense core of the precipitate (Fig. 6). Secondary and tertiary smooth branches of Purkinje cells show progressively more precipitate in the mitochondria (Fig. 8). The highest concentrations of mitochondrial precipitate were found in the spiny branchlets (Fig. 9). No precipitate was evident in the spines emerging from the branchlets (Figs. 9, 10). Precipitate was also absent from the synaptic cleft.

In all the synapses examined precipitate was found in the presynaptic process (Figs. 9–11). Synaptic vesicles contained the majority of precipitate found in the synaptic ending. Each synaptic vesicle appeared to contain a single round 'granule' of precipitate (Figs. 9–13). An examination of 500 synaptic vesicles from twenty synaptic endings (parallel fibre axons) showed that 89 % of the synaptic vesicles contained a granule of precipitate. Not infrequently a non-specific clumping of precipitate was obtained in the axoplasm (Figs. 9, 10). Axonal mitochondria, when closely apposed to the presynaptic membrane, have moderate deposits of the precipitate (Fig. 10).

Basket cell axons were found to contain the highest concentrations of sodium pyroantimonate of any cell in the cerebellum. Large deposits of the precipitate were found diffusively spread through the cytoplasm of the basket cell axons, and the concentration of precipitate was high enough in the terminal arborizations of these to conceal the synaptic vesicles present at the site of axo-somatic synapses along the Purkinje cell surface (Fig. 1). Basket cell mitochondria contained no conspicuous deposits of precipitate (Fig. 1), and in contrast to the mitochondria of other cell types had the smallest amounts of precipitate.

Mossy fibres contained large concentrations of the precipitate localized primarily in the synaptic vesicles (Figs. 11, 13). Most of the mitochondria in mossy fibres had

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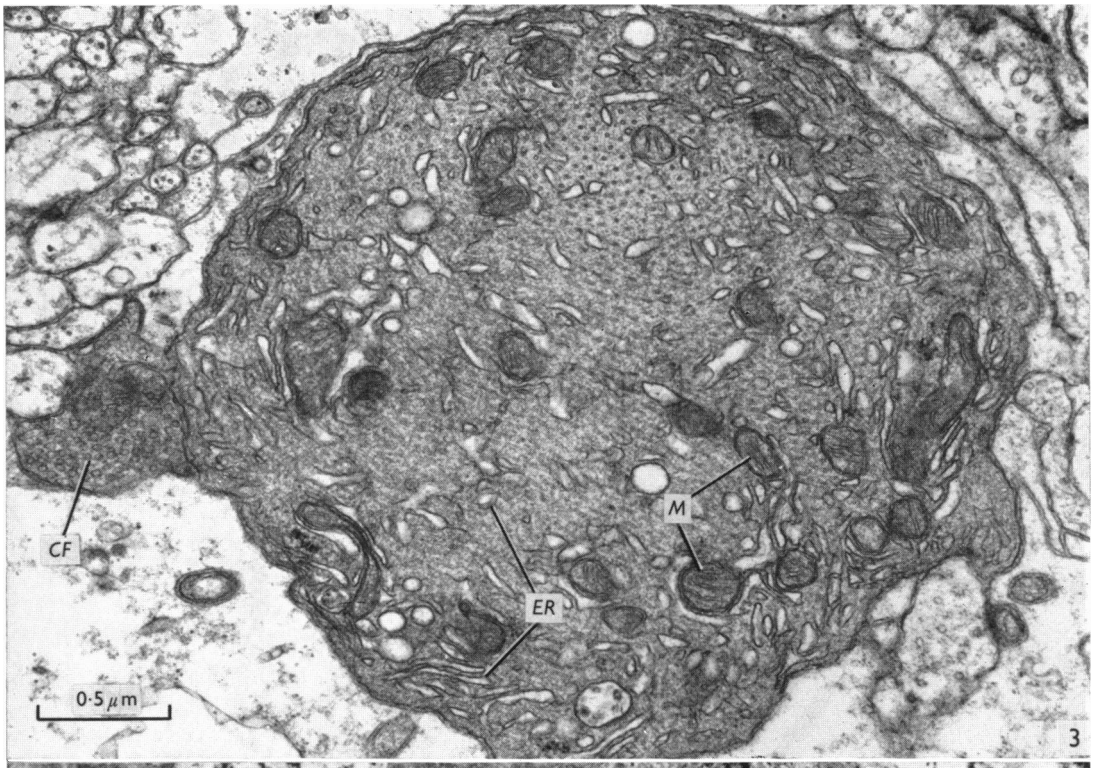
Fig. 1. Portion of Purkinje cell body (*PC*), showing concentrations of precipitate in mitochondria (*M*), the Golgi body (*G*) and the endoplasmic reticulum. The profile of a basket-cell axon (*BC*) can be seen closely apposed to the Purkinje cell. Large deposits of precipitate occur in the axoplasm of basket cells. Basket-cell mitochondria (*M*) contain very little of the precipitate. × 20000.

Fig. 2. A high magnification of Purkinje-cell cytoplasm, showing the localization of precipitate in the lumen of the endoplasmic reticulum (arrows). × 36000.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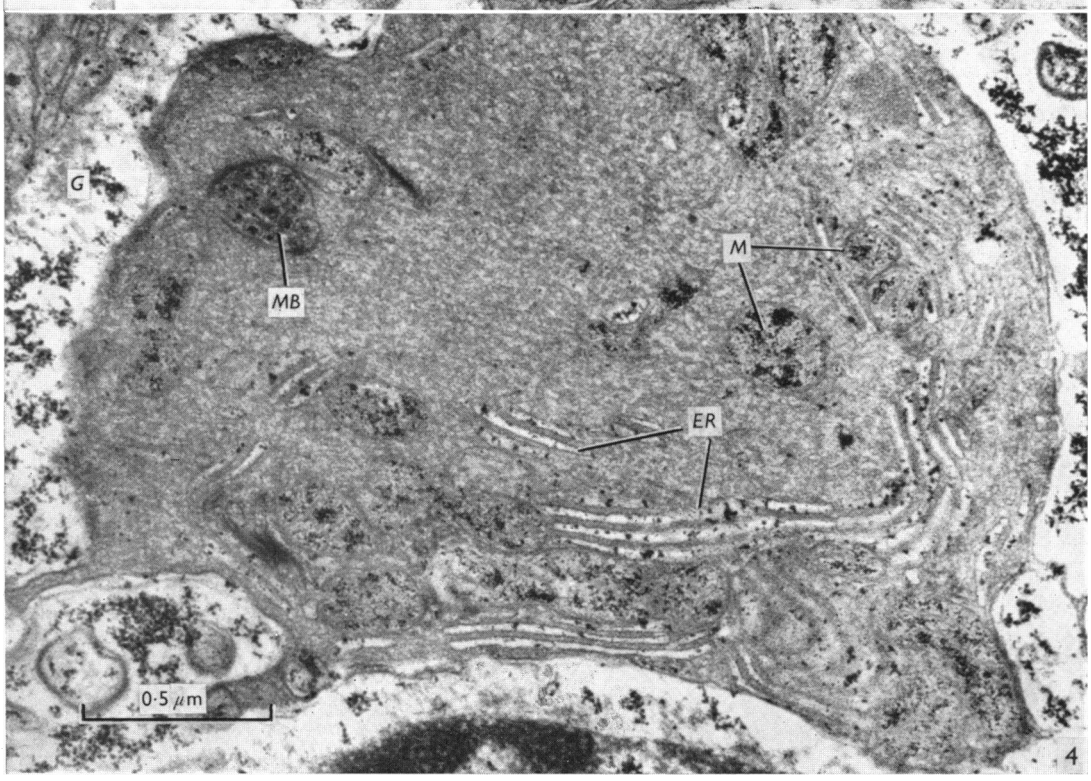
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very little of the precipitate, although occasionally large oval-shaped deposits of precipitate were present (Fig. 12).

Granular cells contained only small amounts of precipitate in their cytoplasm.

Much of the precipitate in axons was found in the mitochondria with only small amounts scattered throughout the axoplasm. In myelinated axons large clumps of precipitate were observed between dilations of the myelin lamellae (Fig. 15). This accumulation of precipitate within the myelin was common to nearly every myelinated axon.

DISCUSSION

The presence of large accumulations of precipitate in the astrocytic process is in agreement with the electron-microscopic findings of Hartmann (1966) in rat cortex. The high sodium content of the astrocyte has been demonstrated quantitatively by a number of investigators (Koch, Banck & Newman, 1962; Reed, Woodbury & Holtzer, 1964) and is consistent with claims that glial cells may serve as compartments for exchange of fluids between blood and nerve cells (Schultz, Maynard & Pease, 1957; Wyckoff & Young, 1956) and that they may be involved in the regulation of the ionic Gerschenfeld & Wald, 1960). Increases in glial cell sodium grey matter depleted of environment of neurons (de Robertis, neurons has been reported (Koch *et al.*, 1962). Katzman (1961), studying the binding of sodium to acidic lipids, and Friede (1964), studying changes in the activity of astrocytic enzymes with changes in environmental sodium, conclude that the astrocyte represents a 'high sodium' cell involved in the active transport of sodium and the maintenance of the ionic environment. The absence of precipitate in the intercellular space and in the synaptic cleft is not necessarily inconsistent with the hypothesis that the astrocyte is functionally comparable to the extracellular space of somatic tissues (Van Harrenveld & Schadé, 1960; Katzman, 1961; Hartmann, 1966), since it is likely that mostly bound sodium is precipitated by this reaction (Hartmann, 1966). Recent studies on trout cornea in this laboratory (Edelhauser & Siegesmund, 1968; Siegesmund & Edelhauser, unpublished data) have shown that the corneas incubated for 30, 60 and 90 min in a sodium-free medium contain less pyroantimonate precipitate within the epithelial cells than their respective controls. Although most ionic sodium has probably been lost during the initial fixation some of the precipitate may represent sites where remaining sodium cations have become attached to free anionic groups.

Most of the precipitate in the Purkinje cell body and primary branches is localized in the lumen of the smooth endoplasmic reticulum. The localization of large deposits of the precipitate in the endoplasmic reticulum suggests that the organelle may be involved in the movement or metabolism of sodium.

In the Purkinje cell branchlets, where smooth endoplasmic reticulum is absent, the precipitate is almost exclusively within the mitochondria. The high concentrations

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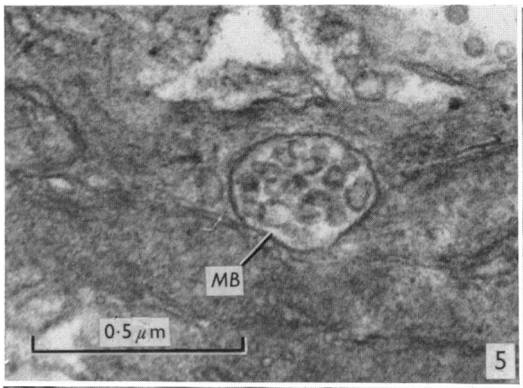
Fig. 3. Smooth branch of a Purkinje cell fixed by conventional buffered osmic acid. A climbing fibre (*CF*) synapse can be seen on the dendrite. × 36000.

Fig. 4. Smooth branch of a Purkinje cell fixed by the pyroantimonate method. Precipitate is localized heavily in the glia (*G*) surrounding the dendrite. Precipitate can also be observed in the endoplasmic reticulum (*ER*), the mitochondria (*M*) and the multivesicular body (*MB*) of the smooth branch. × 50000.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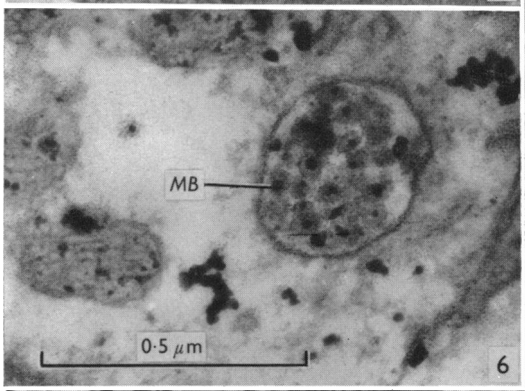
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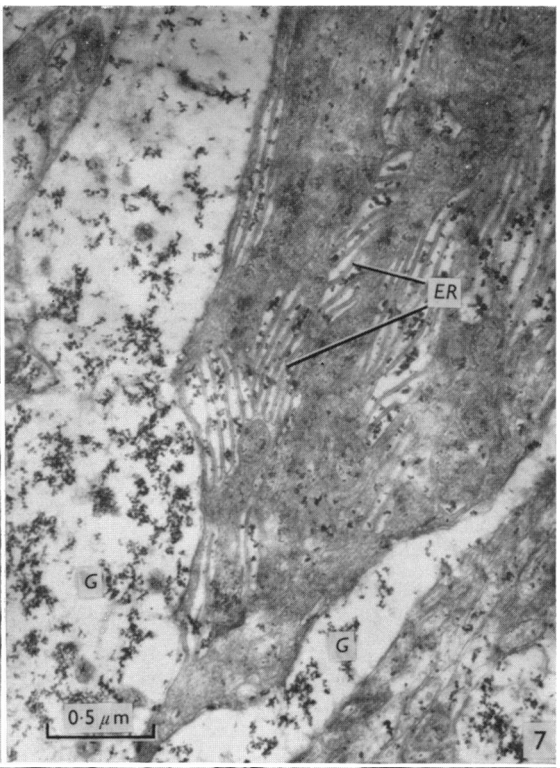
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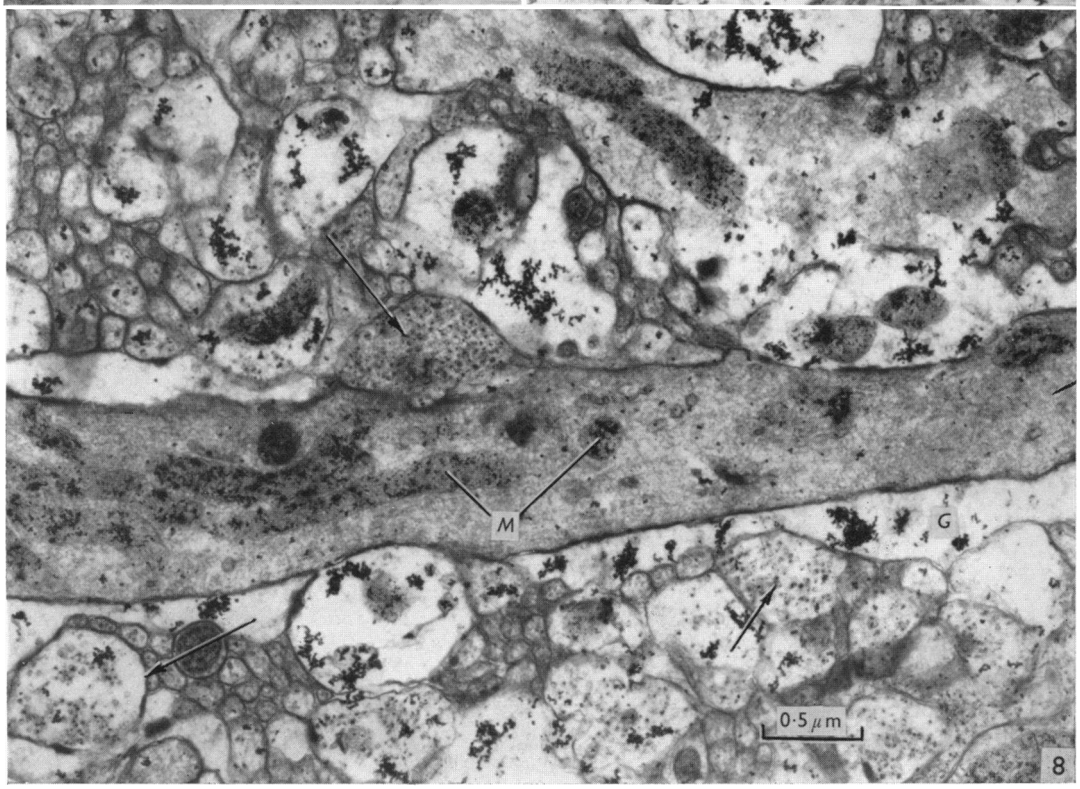
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of precipitate within these branchlet mitochondria are in marked contrast to the very low concentrations found in mitochondria of the cell body. A progressive increase in mitochondrial precipitate can be observed from the large smooth branches of the Purkinje cell up to the small spine-laden branchlets. This variation in the amount of sodium pyroantimonate in mitochondria of the same cell may indicate a basic difference in the metabolic machinery of the mitochondria. The Purkinje-cell mitochondria located near the nerve ending may be more actively involved in sodium metabolism. The physiological role of sodium in the depolarization of the post-synaptic membrane during excitation is well established. The absence of precipitate from the synaptic cleft and from regions adjacent to the post-synaptic membrane, where ionic sodium might be expected, could be explained if the sodium precipitated by this technique was in a bound state. This would be consistent with Hartmann's (1966) finding that only a small percentage of the total cell sodium is demonstrated by the pyroantimonate method.

The vesicles within multivesicular bodies resemble synaptic vesicles both in size and in the presence of an inclusion granule of precipitate (Figs. 5, 6). The inclusion granules of multivesicular bodies and synaptic vesicles are remarkably uniform in size and are less irregular than granules of precipitate found in other areas of the cytoplasm.

The precipitate in basket-cell axons occurs in even higher concentrations than that found in glial cells. This would suggest a major role for the basket-cell axon in physiological processes involving sodium in the vicinity of the Purkinje cell body. This is interesting in view of a recent suggestion by Palay (1967) that the basket-cell axons surrounding the Purkinje cell axon may represent a new type of synapse in which inhibition is produced by a 'gross alteration in the ionic environment surrounding the post-synaptic membrane' rather than by the action of a chemical transmitter.

The deposits of precipitate seen between myelin lamellae in most of the myelinated axons are probably located in the cytoplasmic islands described by Hirano & Dem-

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Fig. 5. Multivesicular body (*MB*) in Purkinje cell smooth branch. Fixed by conventional buffered osmic acid. × 55000.

Fig. 6. Multivesicular body (*MB*) in Purkinje-cell smooth branch. Each vesicle within the multivesicular body has a core of precipitate. × 70000.

Fig. 7. Smooth branch of Purkinje cell, showing precipitate in dilations of the smooth endoplasmic reticulum (*ER*). The precipitate is similar in appearance to that found in the surrounding glia (*G*). × 28000.

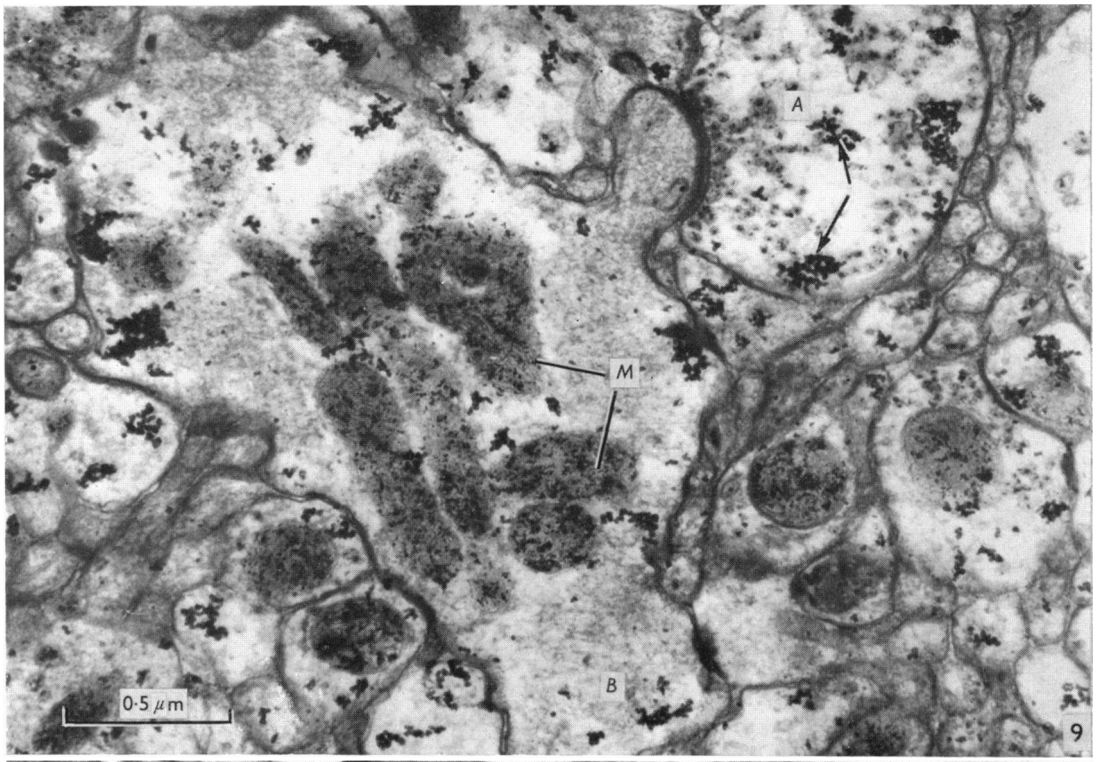
Fig. 8. Tertiary branch of a Purkinje cell, showing larger amounts of mitochondrial (*M*) precipitate than found in the larger branches. Numerous synaptic endings (arrows) can be observed in which the synaptic vesicles contain a single granule of precipitate. × 26000.

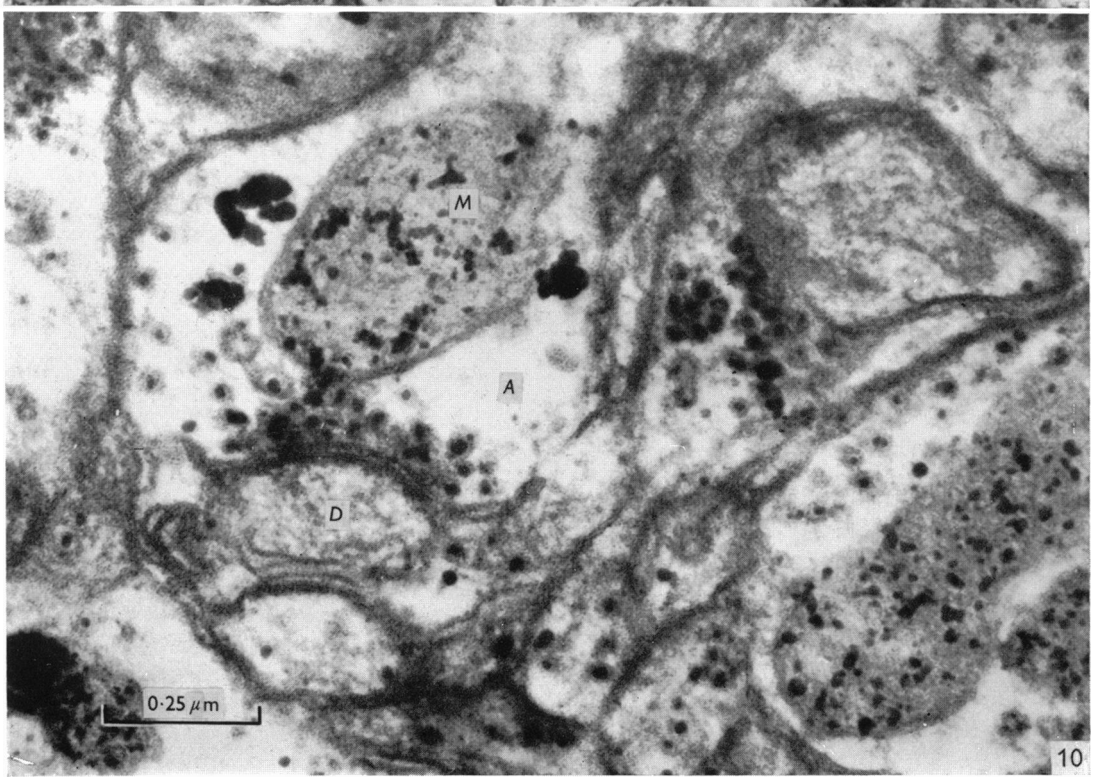
Fig. 9. Purkinje cell branchlet (*B*) with a large cluster of mitochondria (*M*) containing heavy deposits of precipitate. No precipitate can be seen in the dendritic spine. A granule of precipitate can be observed in the synaptic vesicles of the axon (*A*) in synaptic contact with the spine. Irregular clumps of precipitate (arrows) also occur throughout the axon. × 44000.

Fig. 10. Axodendritic synapse in the molecular layer of cerebellum. The dendrite (*D*) has no visible precipitate. A discrete localization of precipitate can be seen in the synaptic vesicles and mitochondria (*M*) of the axon. × 85000.

Fig. 11. Mossy fibre (*MF*) in granular layer of cerebellum. Most of the precipitate occurs in the synaptic vesicles. × 57000.

Fig. 12. Mossy fibre with mitochondria containing large deposits of precipitate (arrows). × 48000.





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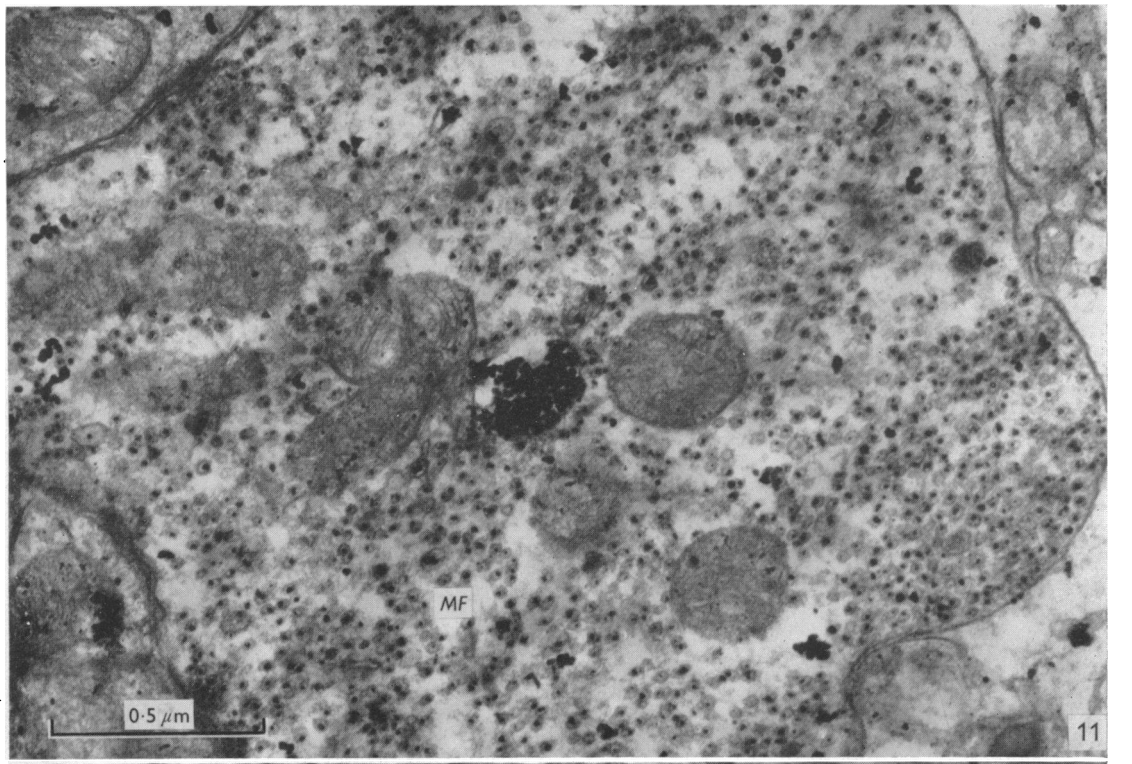

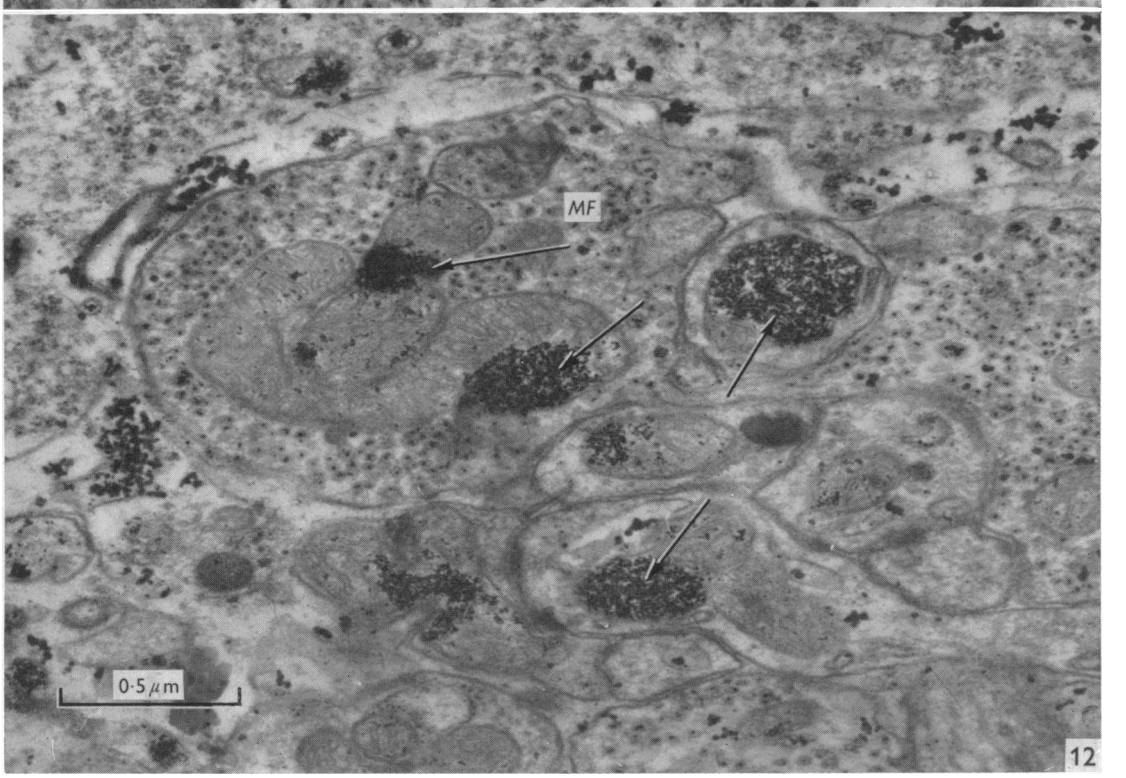

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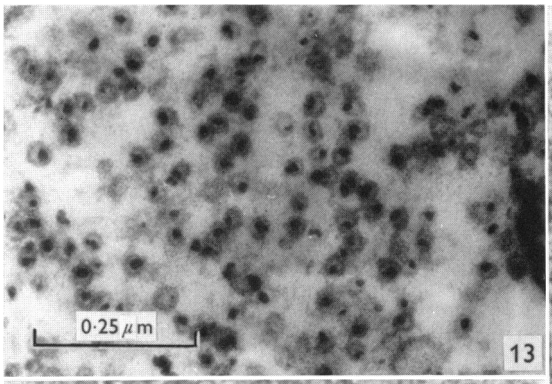
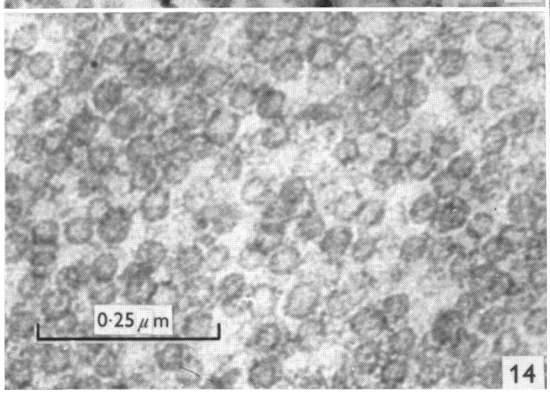
bitzer (1967). These cytoplasmic islands, which occur where adjacent lamellae have not fused, may represent areas where the precipitate has become trapped between isolated deposits of glial cytoplasm. The islands may be an artifact of fixation since they are not nearly as abundant in material fixed with osmic acid alone.





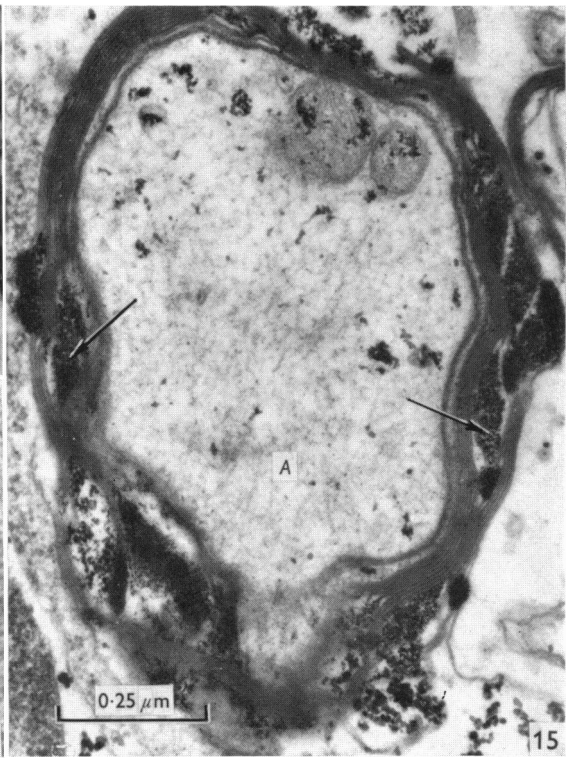


Fig. 13. A high magnification of synaptic vesicles in a mossy fibre. Most synaptic vesicles contain a single granule of precipitate. × 85000.

Fig. 14. Synaptic vesicles in a mossy fibre from tissue fixed by conventional buffered osmic acid. × 95000.

Fig. 15. A myelinated axon (*A*) from the granular layer of cerebellum. Large clumps of precipitate (arrows) appear between separated lamellae. × 80000.

SUMMARY

1. Sodium was localized in the cerebellum by means of the precipitate sodium pyroantimonate. Sodium pyroantimonate was localized specifically in certain cells and within cytoplasmic organelles as a fine electron-dense precipitate.

2. Specific deposits of precipitate appeared in mitochondria, endoplasmic reticulum and synaptic vesicles whereas glial cells and basket cells contained precipitate spread diffusely throughout the cytoplasm.

3. A progressive increase in mitochondrial precipitate was observed from the Purkinje cell body to the spiny branchlets. No precipitate was observed in the synaptic cleft or in regions directly adjacent to the cleft.

4. The basket-cell axons contained the highest concentrations of precipitate of any cell in the cerebellum. It is suggested that the terminal axons of basket cell may participate in physiological processes involving sodium in the vicinity of the Purkinje cell body.

The author wishes to express his appreciation to Miss Patricia Delaney and Mrs Constance Klippel for their valuable technical assistance.

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