J. Anat., Lond. (1964), 98, 1, 93–97
With 3 plates
Printed in Great Britain

The ultrastructure of the intranuclear rodlet in certain nerve cells

BY K. A. SIEGESMUND, C. R. DUTTA AND C. A. FOX
Bob Quinn Memorial Electron Microscope Laboratory, Department of Anatomy, Marquette University School of Medicine, Milwaukee, Wisconsin

INTRODUCTION

Various observers, Mann (1894), Roncoroni (1895), Lenhossék (1897), Prenant (1897,), Holmgren (1899), and Ramón y Cajal (1909, 1911), have described a small rod-shaped structure in nerve cell nuclei. Judging from a survey of the literature, this structure occurs only in some nerve cell nuclei and only in certain species of animals. Ramón y Cajal (1909, 1911), for example, illustrated it in the rabbit olfactory bulb external granule cells, cerebellar granule cells and pyramidal cells.

Recently, while studying the fine structure of the olfactory bulb of the squirrel monkey (*Saimiri sciureus*), we observed, in electron micrographs of some of the granule cell nuclei of the external granular layer, a small bundle of closely packed fibrils which we assumed might be the intranuclear rodlet described by the older anatomists. To determine the correctness of this assumption an electron microscopic search was undertaken to see if an equivalent structure could be located in the nuclei of pyramidal cells, cerebellar granule cells and olfactory bulb granule cells of the rabbit brain.

MATERIALS AND METHODS

The 2 % osmium tetroxide solution, used for fixation, was buffered to pH 7·4–7·5 (veronal-acetate) and brought to a molarity of 0·02 by the addition of calcium chloride. Small pieces of tissue from the rabbit olfactory bulb, cerebral and cerebellar cortices and from the monkey (*Saimiri sciureus*) olfactory bulb and cerebellar cortex were removed with a sharp, pointed scalpel and placed in a drop of the cold fixative on a slab of dental wax and were then diced into fragments less than 1 mm. with a fresh razor blade. These fragments were transferred to chilled osmium tetroxide in which they were fixed for 1 hr. Following fixation they were dehydrated in acetone and embedded in Vestopal W. Sections were cut on a Porter–Blum microtome and stained with 2 % uranyl acetate or with lead hydroxide according to the method of Watson (1958). The sections were examined in a RCA EMU 3b electron microscope. At times, while surveying blocks of tissue, whole sections were mounted on formvar films over large areas of copper grids from which some of the grid bars had been cut out with a sharp razor blade. This procedure permitted the unobstructed examination of relatively large areas of tissue and was useful in determining the contents and orientation of tissue blocks.

For light microscopy rabbit olfactory bulbs and pieces of rabbit cerebellum and cerebral cortices were prepared by Ramón y Cajal's reduced silver method 1B.

According to Ramón y Cajal (1910) and Greenfield & Stern (1937) this method gives an imperfect fixation but shows well the rodlet of Roncoroni.

Both male and female rabbit brains were processed for light and electron microscopic preparations.

OBSERVATIONS

The compact bundle of fine fibrils observed in the extraglomerular cells of the squirrel monkey (*Saimiri sciureus*) olfactory bulb are shown in the electron micrograph of two adjacent granule cells (Pl. 1, fig. 1, *R*). These bundles of fibrils appear to lie entirely within the nucleoplasm and are seen only occasionally in close proximity to the nuclear membrane (Pl. 1, fig. 2, *NM*). The individual fibrils comprising the bundle are 50–70 Å. in diameter in all cells examined.

Plate 2, figure 4, is a high power photomicrograph of a Cajal reduced silver preparation of the rabbit olfactory bulb. It shows a portion of two glomeruli surrounded by a number of extra-glomerular granule cells in the nuclei of which are seen the intranuclear rodlets described by Ramón y Cajal. One of these rodlets is shown in the oil immersion photomicrograph (Pl. 2, fig. 5, *R*). In the electron micrograph of a rabbit extra-glomerular granule cell the only intranuclear structure comparable in shape to the intranuclear rodlets of light microscopy is a small bundle of fibrils (Pl. 2, fig. 3, *R*). This story is repeated in the rabbit cerebellum and cerebral cortex. Plate 3, figure 6 (*R*), shows a bundle of intranuclear fibrils in a cerebellar granule cell and the oil-immersion photomicrograph (Pl. 3, fig. 7, *R*) shows two cerebellar granule cells with intranuclear rodlets. Plate 3, figures 8 and 9 (*R*), are electron micrographs of portions of a small and a medium sized pyramidal cell, respectively. The nuclei of each of these cells contain profiles of a small bundle of fibrils. An intranuclear rodlet is shown in the oil-immersion photomicrograph of a medium sized pyramidal cell (Pl. 3, fig. 10, *R*).

DISCUSSION

The intranuclear rodlet, illustrated by Ramón y Cajal in reduced silver preparations of the rabbit olfactory bulb, cerebellum and cerebral cortex and shown here in photomicrographs, appears in electron micrographs of the same type of rabbit nerve cells as a bundle of closely packed fibrils. Undoubtedly, the intranuclear bundle of fibrils in the extra-glomerular granule cells of the squirrel monkey olfactory bulb is the intranuclear rodlet of the older anatomists.

It is clear from a survey of the literature and from our own material that this structure occurs only in certain nerve cells in certain species. Although we have observed it in rabbit pyramidal cells and in rabbit cerebellar granule cells and in extra-glomerular cells of the rabbit and the squirrel monkey olfactory bulbs, we have not seen it in the cerebellar granule cells of the squirrel monkey, the macaque monkey (*Macaca mulatta*) and the cat. Apparently it is not found in the cerebellar granule cells of rat or man for neither Gray (1961) nor Palay, McGee-Russell, Gordon & Grillo (1962) report it in their electron microscopic studies of the rat cerebellar cortex nor does Dahl, Olsen & Birch-Andersen (1962) mention it in their electron microscopic study of the human cerebellar cortex.

Lenhossék (1897) and Prenant (1897) found intranuclear rodlets, which they considered crystalloid in nature, in Heidenhain iron haematoxylin preparations of the hedgehog sympathetic ganglion cells. Holmgren (1899) revealed the intranuclear rodlet in iron haematoxylin and in toluidin blue, erythrosine preparations of rabbit dorsal root ganglion cells, chicken sympathetic ganglion cells and in spinal cord cells of the gull.

Ramón y Cajal (1910) refers to this structure as the rodlet of Roncoroni, but Mann (1894) appears to have been the first to observe it. In toluidin blue preparations of the pyramidal cells nuclei, he described it as a deeply stained crescent body and suggested that it might be the homologue of the centrosome, which according to him, is found in the nuclei of sympathetic nerve cells. Roncoroni (1895) described it in methylene blue preparations of medium sized pyramidal cells as a clear, intensely blue line, which passes from one pole of the nucleus to the other and which, in some cases, may be bipartite or even tripartite. He found it in the brains of guinea-pigs, rabbits, dogs and calves and in human pathological brains (paralysis, dementia, congenital criminals). He claims also to have seen it in the spinal cord. Lugaro (1896) and Prenant (1897) were of the opinion that Roncoroni had observed either a fold in the nuclear membrane or a peripheral mass of chromatin. Ramón y Cajal (1911) was able to reveal the intranuclear rodlet only in the deep, medium and small sized pyramidal cells of the rabbit brain and all his attempts to disclose it in the pyramidal cells of the human brain and other gyrencephalic mammalian brains were fruitless. Since no investigator, following Roncoroni, has found this structure in the pyramidal cells of any species, other than the rabbit, and since Roncoroni was the only observer to see bipartite and tripartite rodlets in pyramidal cells, it is quite possible that he may have been observing folds in the nuclear membrane. Infoldings of the nuclear membrane are frequently seen in electron micrographs of certain nerve cells.

The significance of the intranuclear rodlet is unknown. It appears to extend the entire length of the nucleus and is quite distinct from the chromatin material in which it is embedded. It does not appear to be sex-linked since it occurs in both male and female rabbits. All investigators have noted that there is only one rodlet per nucleus and that it is separated from the nucleolus. This is obvious in our preparations.

Most observers regard the rodlet as a strictly intranuclear structure having no contact with the nuclear membrane, but Holmgren shows preparations of chicken sympathetic ganglion cells in which the rodlet is partly in the nucleus and partly in the cytoplasm. In one of his figures the rodlet extends outward in the cytoplasm nearly to the plasma membrane. Holmgren suggests not only that these structures may have entered the nucleus from the cytoplasm, but also that they may be invaders from outside of the cell. The possibility, therefore, that the rodlet is the result of an infectious process should be considered, but its general morphology would seem to rule out this suggestion. Furthermore, we have viewed a large number of light and electron microscopic preparations and all of the rodlets observed have been completely intranuclear.

SUMMARY

1. The intranuclear rodlet described by various older anatomists in certain nerve cells has been identified in electron micrographs of the extraglomerular granule cells of the olfactory bulb of the squirrel monkey (*Saimiri sciureus*) and rabbit, rabbit cerebellar granule cells, and in rabbit medium and small sized pyramidal cells.

2. The intranuclear rodlet consists of a compact bundle of fibrils 50–70 Å. in diameter. There is only a single rodlet in the nuclei in which they occur.

3. The intranuclear rodlet is obviously limited to certain nerve cells and to certain species.

We are indebted to Miss Patricia Delaney for her excellent technical assistance.

REFERENCES

DAHL, V., OLSEN, S. & BIRCH-ANDERSEN, A. (1962). The fine structure in the granular layer in the human cerebellar cortex. *Acta neurol. Scand.* **38**, 81–97.

GRAY, E. G. (1961). The granule cells, mossy synapses and Purkinje spine synapses of the cerebellum. *J. Anat., Lond.*, **95**, 345–356.

GREENFIELD, J. G. & STERN, R. O. (1937). In *The Microtomist's Vade-mecum* (A. Bolles Lee), 10th edition. Ed. J. B. Gatenby and T. S. Painter. Philadelphia: Blakiston.

HOLMGREN, E. (1899). Weitere Mitteilungen über den Bau der Nervenzellen. *Anat. Anz.* **16**, 388–397.

LENHOSSÉK, M. (1897). Bieträge zur Kenntniss der Zwischenzellen des Hodens. *Arch. Anat. Physiol.* (*Anat. Abt.*), 65–84.

LUGARO, E. (1896). Su di un presunto nuovo reperto nel nucleo delle cellule nervose. *Riv. Pat. nerv. ment.* **1**, 149–150.

MANN, G. (1894). Histochemical changes induced in sympathetic motor and sensory nerve cells. *J. Anat., Lond.*, **29**, 100–108.

PALAY, S. L., MCGEE-RUSSELL, S. M., GORDON, S. & GRILLO, M. A. (1962). Fixation of neural tissues for electron microscopy by perfusion with solutions of osmium tetroxide. *J. cell. Biol.* **12**, 385–410.

PRENANT, A. (1897). Cristalloides intranucleaires des cellules nerveuses sympathiques chez les mammiferes. *Arch. Anat. micr. Morph. exp.* **1**, 366–373.

RAMÓN Y CAJAL, S. (1909). *Histologie du système nerveux de l'homme et des vertébrés.* I. Trans. L. Azoulay. Paris: Maloine.

RAMÓN Y CAJAL, S. (1910). Las fórmulas del proceder del nitrato de plata reducido y sus efectos sobre los factores integrantes de las neuronas. *Trab. Lab. Invest. biol. Univ. Madr.* **8**, 1–26.

RAMÓN Y CAJAL, S. (1911). *Histologie du système nerveux de l'homme et des vertébrés.* II. Trans. L. Azoulay. Paris: Maloine.

RONCORONI, L. (1895). Su un nuovo reperto nel nucleo delle cellule nervose. *Arch. Psichiat.* **16**, 447–450.

WATSON, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals. *J. biophys. biochem. Cytol.* **4**, 475–478.

EXPLANATION OF PLATES

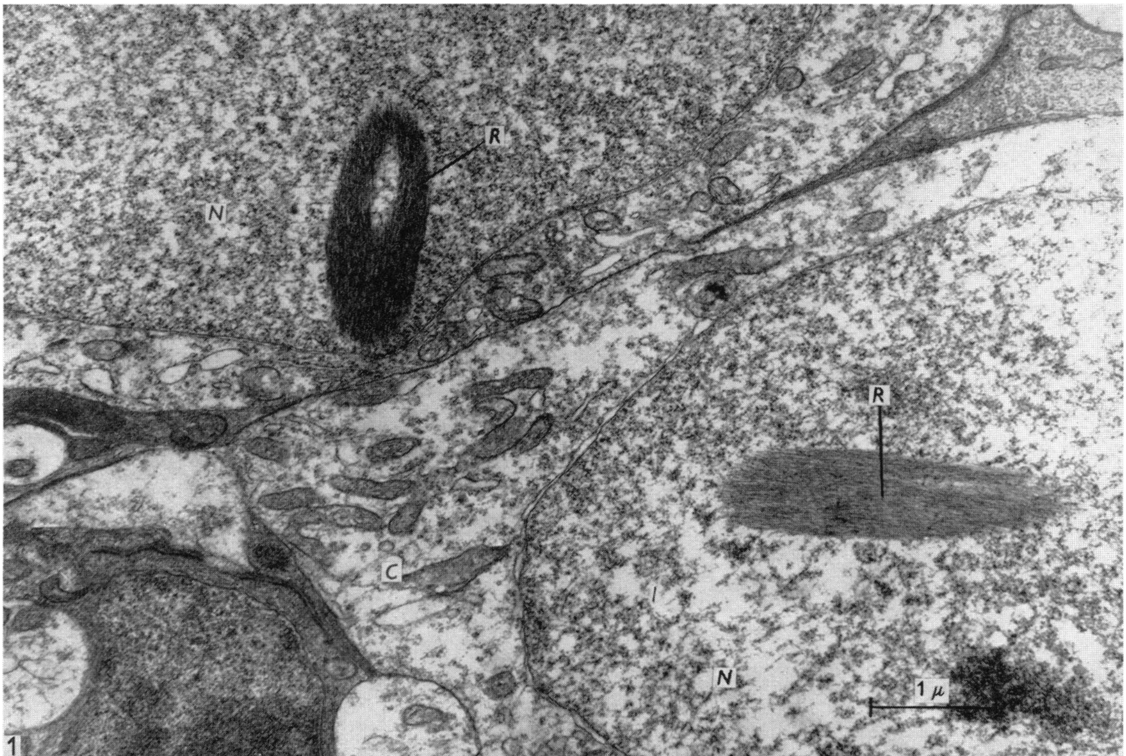
PLATE 1

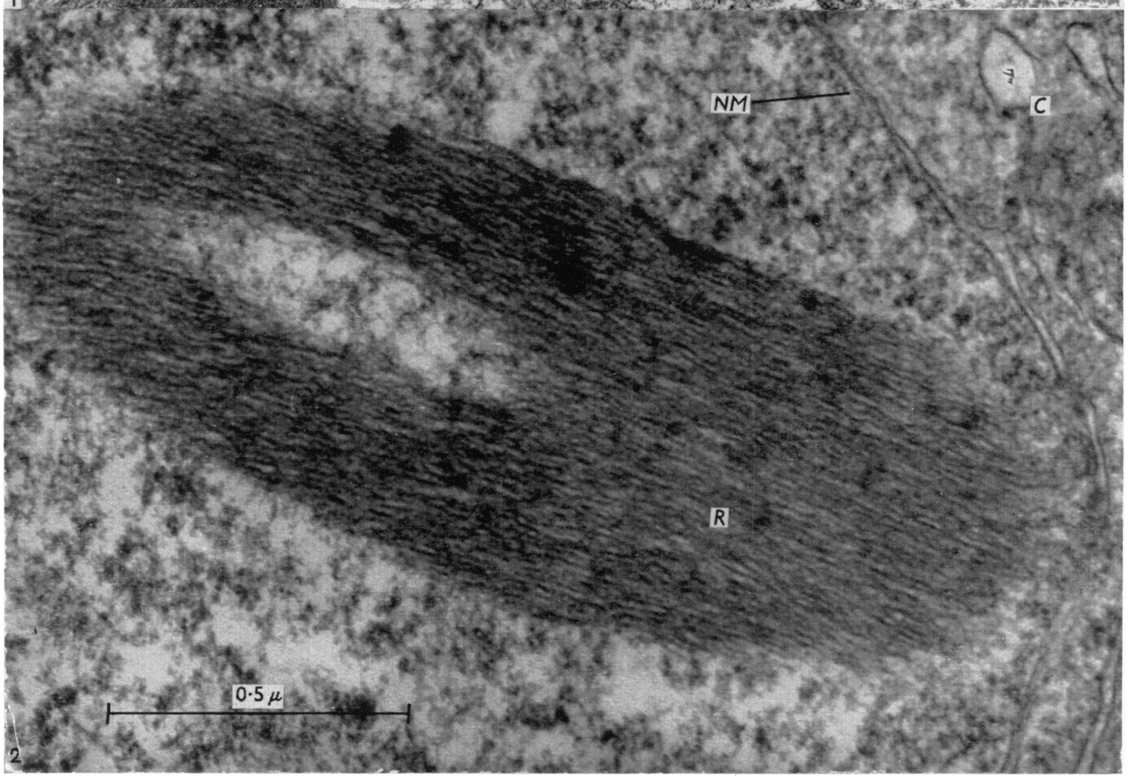
Fig. 1. An electron micrograph showing intranuclear rodlets in two adjacent extra glomerular granule cells. Squirrel monkey (*Saimiri sciureus*) olfactory bulb. × 17,000.

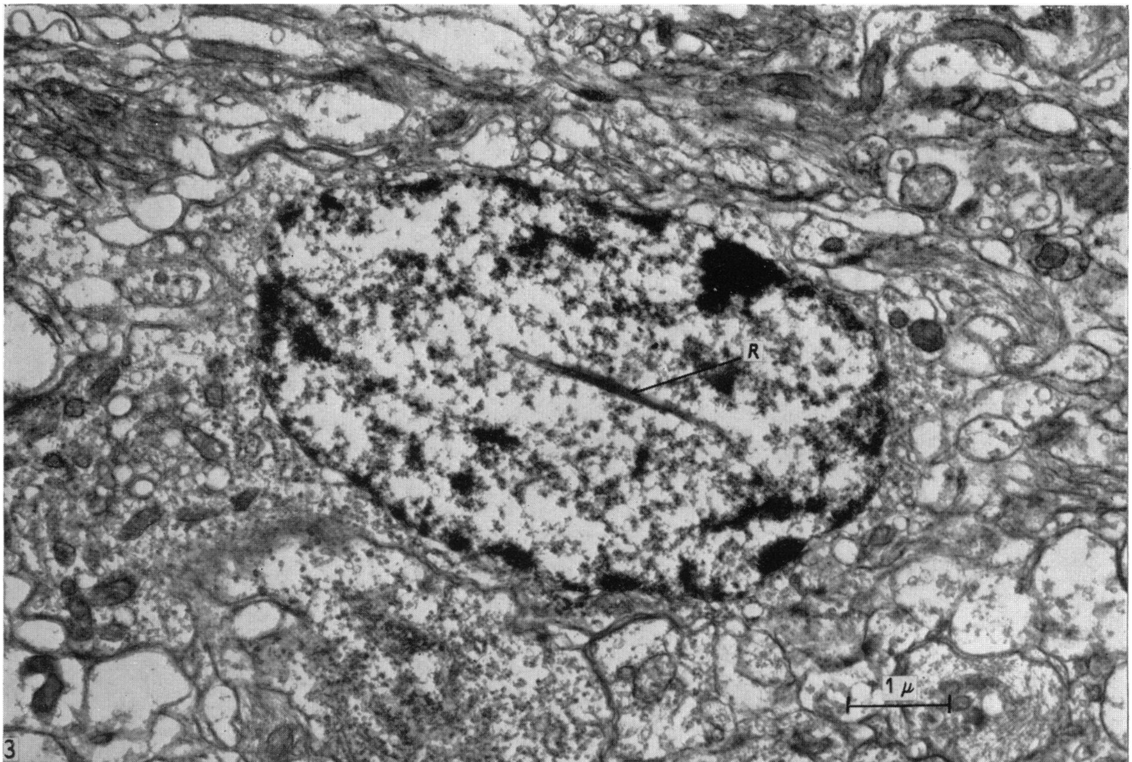
Fig. 2. A higher power electron micrograph showing one of the intranuclear rodlets in Fig. 1. Squirrel monkey (*Saimiri sciureus*) olfactory bulb. × 80,000.

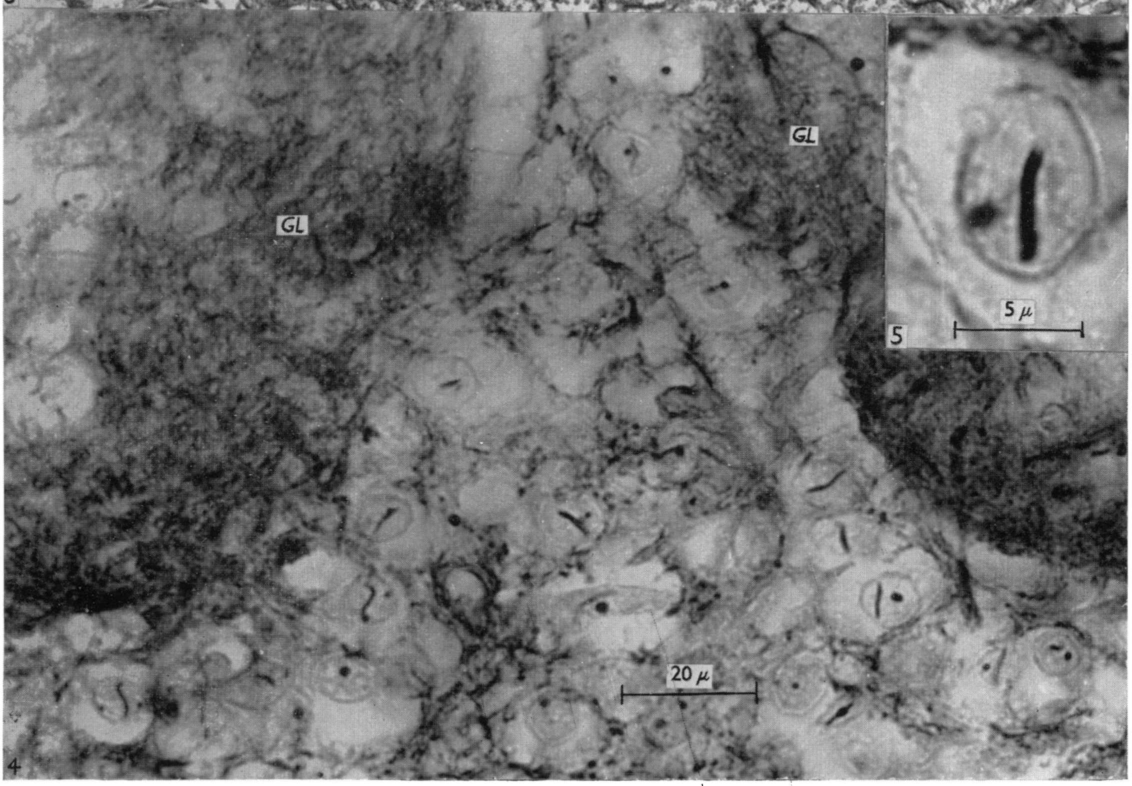
PLATE 2

Fig. 3. An electron micrograph of an extra-glomerular granule cell containing an intranuclear rodlet. Rabbit olfactory bulb. × 12,000.

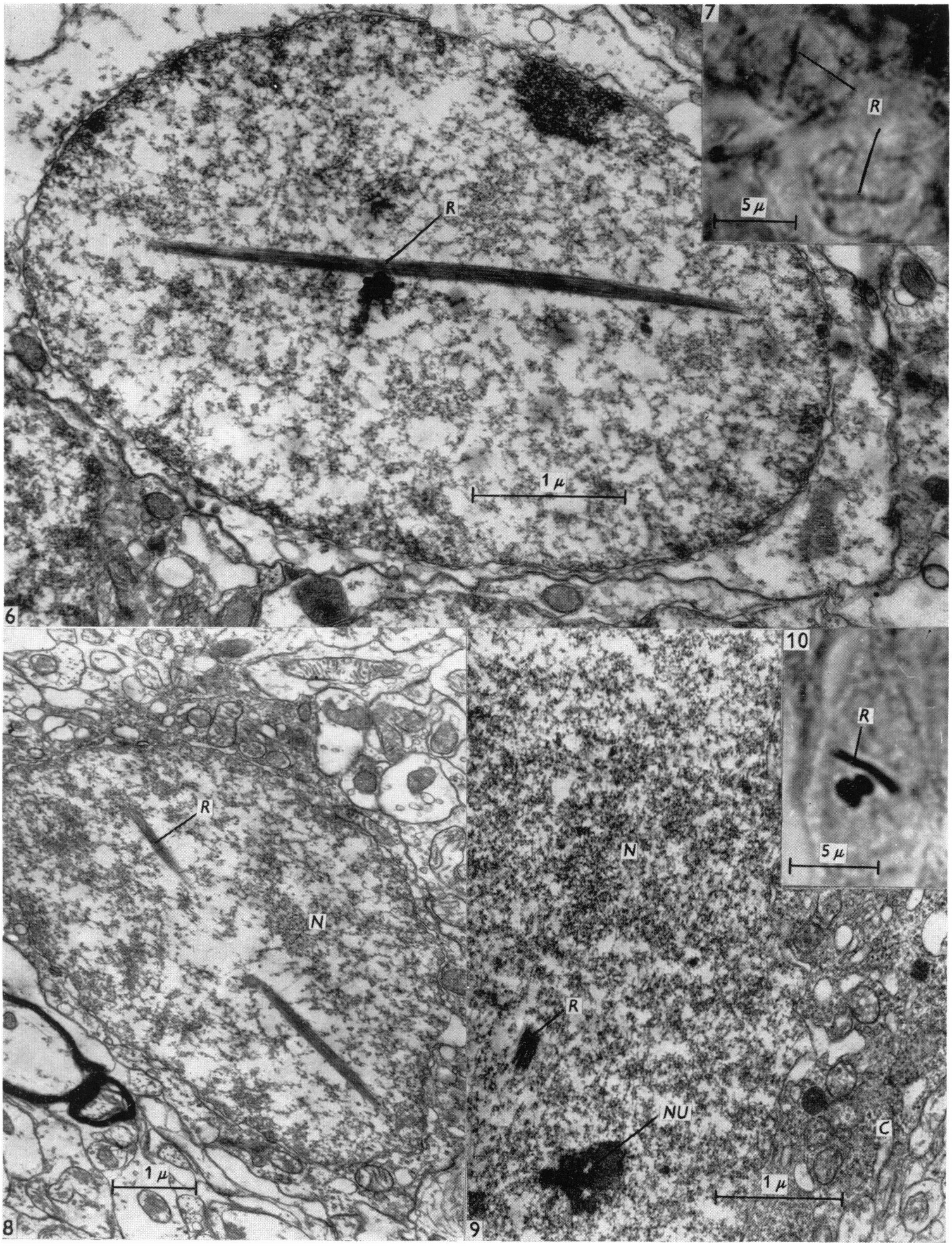

R
N
R
C
N
1 μ
1


NM
C
R
0·5 μ
2





K. A. SIEGESMUND, C. R. DUTTA AND C. A. FOX



K. A. SIEGESMUND, C. R. DUTTA AND C. A. FOX

Fig. 4. Photomicrograph showing two glomeruli and a number of extra glomerular granule cells with intranuclear rodlets. Cajal reduced silver preparations. Rabbit olfactory bulb.

Fig. 5. An oil immersion photomicrograph of an extra glomerular granule cell containing an intranuclear rodlet. Cajal reduced silver preparation. Rabbit olfactory bulb.

PLATE 3

Fig. 6. An electron micrograph of a rabbit cerebellar granule cell showing an intranuclear rodlet. $\times 25{,}000$.

Fig. 7. An oil immersion photomicrograph showing two rabbit cerebellar granule cells with intranuclear rodlets. Cajal reduced silver preparations.

Fig. 8. An electron micrograph showing a horizontally sectioned profile of a small pyramidal cell with an intranuclear rodlet sectioned at two different places. Rabbit cerebral cortex. $\times 14{,}000$.

Fig. 9. An electron micrograph showing a small portion of an intranuclear rodlet sectioned in a medium sized pyramidal cell. Rabbit cerebral cortex. $\times 19{,}000$.

Fig. 10. An oil immersion photomicrograph of a small sized pyramidal cell with an intranuclear rodlet. Cajal reduced silver preparation. Rabbit cerebral cortex.

LIST OF ABBREVIATIONS

C Cytoplasm
N Nucleus
R Intranuclear rodlet
NM Nuclear membrane
GL Glomerulus
NU Nucleolus