The History of Neuroglial Clumps

by

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Short title: Fine granular material

Abstract

The descriptions of fine granular material in the unfixed brain and spinal cord tissue by the early 19th century neuro-histologists became rare, as fixation, dehydration, staining, and mounting procedures were described, in the late 19th and early 20th centuries. Neuroglia was originally regarded as an amorphous granular material between neurons, but stains were developed early in the 20th century for astrocytes, oligodendrocytes and microglia, and the neuroglia was then regarded as being composed of the latter *cells*. However, Hydén (1959) and his collaborations isolated 'neuroglial clumps', which were found to be largely composed of mitochondria. These are illustrated.

Key Words

Fine granular material, neuroglia, brain cell structure

Early observations

Fine granular material appears to have been described in the brain first by Leeuwenhoek in 1684 (see Clarke and O'Malley, 1968, p 419). Leeuwenhoek wrote, "When I had separated this substance into very small particles, I soon observed a thin liquid or lichor issuing from it. It was composed of far smaller globules, of which, in my judgement, nor even thirty six equalled the size of those making our blood red." Leeuwenhoek was using a single lens, and one cannot be certain that it had the resolution to see such small granules. In 1827, Hodgkin and Lister using the first achromatic microscope, in a brief note about the human brain, wrote, "one sees instead of globules, a multitude of very small particles, which are most irregular in shape, and size". Neither Dr. Brian Bracegirdle nor we could find evidence that either of the latter two authors published details of these findings elsewhere. In 1836, Valentin, also in the human brain described the ground substance as a 'parenchyma', and noted that it is "mostly a grey reddish, fine granular material, which is saturated with a fluid and held together by a clear, transparent, slightly viscous mass (Blastem) which not infrequently contains filaments." The latter three authors all studied unfixed tissues although, later, Ramon y Cajal (1908) observed, in silver stained tissue, that "a granular cement or special conductivity substance serves to keep the neuronal surfaces in very intimate contact."

Neuroglia

In 1856, the Berlin pathologist, Virchow, noticed that there was a connective tissue between the neurons near the ependyma. He gave it the name 'neuroglia' or 'nerve-glue'. Originally, he thought of it as an amorphous tissue, but since then views have changed about its nature. Astrocytes were originally described morphologically as a special kind of neuroglial cells (Kölliker, 1893, Andriezen, 1893), stained by Ramon y Cajal's (1913) and Del Rio-Hortega's (1916) procedures. Oligodendrocytes were described by Robertson (1899), and stained by Del Rio-Hortega, (1921); see also, Penfield (1932). Many authors said that they could not distinguish for certain between, neurons and astrocytes, between astrocytes and oligodendrocytes, and between oligodendrocytes and microglia, respectively, on morphological grounds or by their staining properties (for summary, please see Hillman, 1986a, pp 49 – 118). Furthermore, both in respect of cultures of these kinds of cells, and tumours arising, from them, they

are often described as 'glial cells', without specification of to which of the three kinds of cells the authors were referring. The belief gradually evolved that the neuroglia was not a connective tissue, as Virchow had described it, but was actually a solid aggregate of neuroglial *cells* (Kettenmann and Ranson, 2004, p.4). The idea gained currency that if a section of brain or spinal cord could be stained simultaneously with stains characteristic for neurons, astrocytes, oligodendrocytes and microglia, virtually the whole of the sections would be stained. This was stated in a special authoritative issue about the brain for the non-specialist in the 'Scientific American', (Nauta and Feirtag, 1979). Unfortunately, those who use 'specific' stains for neuroglial cells, have not examined to see if such evident neurons as those from the cranial nuclei, the ventral horn cells or the Purkinje cells of the cerebellum, show up with the same staining systems. They do (Hillman and Deutsch, 1979).

Microdissection

During the 19th century a number of research workers teased brains and spinal cords under direct vision (Hodgkin and Lister in London, 1827; Purkinje in Prague, 1838; Hannover in Copenhagen, 1846; and Deiters in Bonn, 1865). They used needles or wires on fresh tissue which they did not stain for histology. In 1959, Hydén published the first modern paper on the isolation of neuron cell bodies under the adjacent neuroglia. His publication was well received and he soon produced a distinctive account of the microscopy of unfixed neurons, isolated in this way, (Hydén, 1960). In the earlier paper, he had not given details of his procedure, but encouraged one of us, to whom he had taught it, to publish a full description (Hillman, 1986b).

Histology and histochemistry

Histology and histochemistry were gradually developed, mainly during the 19th and early 20th centuries, by pathologists who used them to compare the appearances of a disease tissue with those of a healthy tissue, (for history, see Sharpey – Schafer, 1885; Böhm and Davidoff, 1895; Clarke and O'Malley, 1968; Bracegirdle, 1978). There were relatively few publications on the effects of the reagents and manoeuvres on the chemistry, shapes and dimensions of the cells (Mann, 1902; Stowell, 1941; Ross, 1953; Baker, 1958; Frontera, 1958 and Kushida, 1962). Nevertheless, it would be a fair

generalisation to say that fine granular material (as opposed to synaptic knobs) is not seen in modern textbooks showing histological sections of mammalian central nervous system., (Economo, 1929; Sholl, 1956; Conel, 1939-1967; Peters and Jones, 1984; Peters, Palay and Webster, 1998).

Neuroglial clumps

Interest in neuroglia was revived when Hydén (1959) dissected out nerve cell bodies, and the adjacent neuroglia, from the Deiters' nucleus of rabbits. He added a very small quantity of methylene blue to unfixed brain in 0.25 M sucrose and could see the blue coloured neuron cell bodies. He lifted these out of a cradle of very white neuroglia, in which the cell bodies were enveloped. The neuroglia appeared to consist of a very larger number of small granules, each of 0.5 – 1 μm in diameter in a very friable mass. The mass of neuroglia was only weakly adherent to the cell bodies, and could easily be separated from them to leave 'clean' neurons upon which no granules were visible. Hydén and his colleagues assumed that the glial material consisted of oligodendrocytes or astrocytes, but they do not seem to have identified the cells by histological or immunocytochemical criteria. However, Hydén and Pigon (1960) gave the name 'neuroglial clumps' to the tissue (Fig 1) and from then on, it was regarded as a useful preparation to compare the properties of the neuron with those of the neuroglia; they judged the latter by eye to be roughly the same volume as the adjacent nerve cell bodies.

Figures 1 and 2 here

The following research workers have manipulated neuroglial clumps and most have published papers and illustrations of them (Fig 2). Hydén, H; Pigon, A; Backman, A; Augusstson, I; Hamberger, A; Hertz, L; Epstein, M.M; O'Connor, J.S; Hillman, H; Jarman, D; Deutsch, K; Hussain, T.S; Sartory, P.K; Allen J; and Chughtai, I.

Figure 3 here

Constituents of fine granular material

In order to examine the material specifically, we separated clumps adjacent to neuron cell bodies. We deliberately removed the neuron cell bodies, by agitating the steel

wires until no cell bodies were visible in our samples. We then examined and photographed the clumps in parallel-walled chambers by phase contrast microscopy, using x40 and x100 (oil immersion) objectives. We saw immediately that the granules were largely composed of mitochondria, similar in appearance to the mitochondria inside neuronal cytoplasm (Fig. 3), (Hillman et al., 1977; Hillman and Deutsch, 1978). In the 19th century, fine granular material was thought to be broken down blood vessels, cells, necrotic tissue or precipitates. Another suggestion was that neuroglial clumps were infections of the tissue by bacteria or fungi; although we did find fine granular material in human post mortem brains several days old, it was also clearly evident in the brains and spinal cords of freshly killed rabbits, rats, guinea pigs, cows and sheep. We worked in clean but not sterile conditions. Tissues would not have had time to be heavily contaminated in less than 2 hours. At a recent meeting, someone who would not give me his name, said that, in his opinion, neuroglial clumps were artifacts, but he would not tell me of what origin he believed the artifacts to be.

Properties of fine granular material

Since Hydén named the tissue 'glial clumps'; the following experiments and observations have been published:

(i) most of the volume of the brain and spinal cord is composed of fine granular material (Hillman and Jarman, 1991). Neurons are also present in them. The neuroglia also has nuclei floating about with it, in a syncytium (Hillman, 1986a; Hillman and Jarman, 1991). The mass of cerebral parenchyma is tranversed by axons, dendrites, fibrils, and small blood vessels;

(ii) the clumps take up oxygen and produce carbon dioxide (Hydén, 1959, 1960; Hydén and Pigon, 1960; Epstein and O'Connor, 1965; Hertz, 1966). The oxygen uptake of the clumps increases if a high potassium ion concentration is added to the incubation fluid unlike the oxygen uptake of the neuron cell bodies;

(iii) the clumps demonstrate autofluorescence (Hillman, Hussain and Sartory, 1973);

(iv) they also show cytochrome oxidase and succinic dehydrogenase activity (Hamberger,1963).

The nature of fine granular material

Evidence has been brought that fine granular material was seen in the mammalian brain and spinal cord in the 19th century, mainly before histological and histochemical procedures were developed. After that, they gradually disappeared from the histological, histochemical and electron microscopic textbooks. They reappeared when Hydén and his school dissected out fresh unstained neuroglial clumps adjacent to neuron cell bodies. They have featured in publications by several authors, and micrographs of them have appeared, but they are generally not illustrated in books on the histology of the brain, other than our own (Hillman and Jarman, 1991). The simplest explanation is that processing for histology, histochemistry and electron microscopy washes away the fine granular material or neuroglial clumps. Mr Ven Dodge, Dr Iffat Chughtai and the two authors of this paper made a film to show the effects of haematoxylin and eosin, Palmgren's and osmium tetroxide procedures on single neuron cell bodies (Chughtai, Hillman and Jarman, 1987). We photographed the cell bodies under phase contrast microscopy, and then after they were stained, by bright field. When the cell was being perfused by xylene, we noticed a neuroglial clump swimming across the field. The clumps are friable and easily dislodged. During the flow of 10-20 reagents and several manoeuvres during procedures for histology, histochemistry and electron microscopy, it seems likely that the very small granules are washed away, while the insoluble membranes, nuclei, mitochondria and precipitates remain in situ. This would explain why the clumps are seen in unfixed nervous tissue but not in stained sections.

The general philosophy of modern cell biologists seems to be that the information obtained from stained histological or electron microscopic sections is more valid that that obtained from unfixed unstained tissue. This paper concludes that fine granular materal aggregates as neuroglial clumps. It suggests that the latter be restored to the pantheon of neurobiology, where they merit further examination.

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References

Andriezen, W. (1893). The neuroglial elements of the human brain. *Brit. Med. J.* **2**, 227-235.

Baker, J.R. (1958). *Principles of Biological Microtechnique.* Methuen, London.

Böhm, A.A. and Davidoff, M. (1895). *Lehrbuch der Histologie des Menschen einschliesslich der Microscopischen Technik*. Oldenbourg, Wiesbaden.

Bracegirdle, B. (1978). *A History of Microtechnique.* Cornell University Press, Ithaca.

Chughtai, I, Hillman, H. and Jarman, D. (1987). The effect of haematoylin and eosin, Palmgren's and osmic acid procedures, on the dimensions and appearances of isolated rabbit medullary neurons. *Microscopy*, **35**, 625-629.

Clarke, E. and O'Malley, C.D. (1968). *The Human Brain and Spinal Cord*. University of California Press, Berkeley, pp. 17-138.

Conel, J. LeRoy (1939-1963). *The Post Natal Development of the Human Cerebral Cortex*. 6 volumes. Harvard University Press, Cambridge, Mass.

Deiters, O.F.C. (1865). *Untersuchungen űber das Gehirn und die Ruckenmark des Menschen und der Saugethiere.* Vieweg. Braunschweig, pp 1-3.

Del Rio-Hortega, P. (1916). Estruttura fibrillar del protoplasmo neuroglico y origine de las gliofibrillas. *Trab. Lab. Invest. Biol., Universided de Madrid.* **14,** 269-278.

Del Rio-Hortega, P. (1921). Estudios sobre la neuroglia la glia de essasas radiciones. *Biol. Real. Soc. Esp. Hist. Nat., January.*

Economo, C. von. (1929). *The Cytoarchitecture of Human Cerebral Cortex.* Oxford University Press, London.

Epstein, H.M. and O'Connor, J.S. (1965). Respiration of single cortical neurons and surrounding neuropil. *J. Neurochem.,* **12,** 385-395.

Frontera, J.G. (1958). Evaluation of the immediate effects of some fixatives upon the measurements of the brain of macaques. *J. Comp. Neurol.,* **109,** 417-438.

Hamberger, A. (1963). Differences between isolated neuronal and vascular glia with respect to respiratory activity. *Acta Physiol. Scand.,* **58,** suppl **203,** 1-58.

Hannover, A. (1846). Medical Thesis. *Investigations into Brain.* University of Copenhagen, Phillipson, Copenhagen.

Hertz, L. (1966). Neuroglial localization of potassium and sodium effects on respiration in brain. *J. Neurochem.,* **13,** 1373-1387.

Hillman, H. and Deutsch, K. (1978). Mitochondria in isolated neuroglial clumps and neuronal cell bodies of rabbit. *Microscopy,* **33,** 308-309.

Hillman, H. and Deutsch, K. (1979). Staining of isolated rabbit neurons and neuroglial clumps. *Experientia,* **35,** 771-772.

Hillman, H. (1986a). *The Cellular Structure of the Mammalian Nervous System.* M.T.P. Press, Lancaster, pages 431-480.

Hillman, H. (1986b). Hydén's technique of isolating mammalian cerebral neurons by hand dissection. *Microscopy*, **35,** 382-389.

Hillman, H., Deutsch, K., Allen, J. and Sartory, P. (1977). The histology of neuroglial clumps in rabbit brain. *Microscopy,* **33,** 175-180.

Hillman, H., Hussain, T.S. and Sartory, P.K. (1973). Autofluorescence of isolated unfixed rabbit Deiters' neurons and surrounding neuroglial clumps. *Experientia,* **29,** 113-115.

Hillman, H. and Jarman, D. (1991). *Atlas of Cellular Structure of the Human Brain.* Academic Press, London, pps 44-127.

Hodgkin, T. and Lister, J.J. (1827). Notice on some microscopic observations of the blood and animal tissue. *Phil. Mag.* **2** (8), 130-138.

Hydén, H. (1959). Quantative assay of compounds in isolated fresh nerve cells and glial cells from control and stimulated animals. *Nature,* **184,** 433-435.

Hydén, H. (1960). The neuron, in, *The Cell,* edited by Brachet, J. and Mirsky, A.E. Vol. **4,** Academic Press, New York, pp 215-323.

Hydén, H. and Pigon, A. (1960). A cytophysiological study of the functional relation between oligodendroglial cells and cells of Deiters' nucleus. *J. Neurochem.,* **6,** 57-72.

Kettenmann, H. and Ranson, B.R. (2004). *Neuroglia,* third edition, Oxford University Press, New York.

Kölliker, A. (1893). *Handbuch der Geweblehrer des Menschen,* second edition. Engelmann, Leipzig.

Kushida, H. (1962). A study of cellular swelling and shrinkage during fixation, dehydration and embedding in various standard media. *J. Electron Microsc. Japan.* **11**, 135-141.

Leeuwenhoek, A. van. (1684). Opera omnia, seu arcana naturae (1772). De structura cerebri diversorum animalium **I,** 29-41 in *The Human Brain and Spinal Cord,* edited by Clarke E. and O'Malley, C.D. (1968). University of California Press, Berkeley, p. 419.

Mann, G. (1902). *Physiological Histology. Methods and Theory.* Clarendon Press, Oxford, pp 103-133, 180-306.

Nauta, W.J.H. and Feirtag, M. (1979). The organisation of the brain. *Scientific American,* September, pp 88-111.

Penfield, W. (1932). Neuroglia, normal and pathological, in, *Cytology and Cell Pathology of the Nervous System,* Vol. **2.** (edited by Penfield, W. Hoeber, New York, pp. 423-479.

Peters, A. and Jones, E.G. (eds) (1994). *Cellular Compounds of the Cerebral Cortex.* Vol. A. Plenum, New York.

Peters, A., Palay, S.L. and Webster, H de F (1998). *The Fine Structure of the Nervous System.* Oxford University Press, fourth edition, New York.

Purkinje, J.E. (1838). Neueste Unterscuhungen aus den Nerven und Hirnanatomie, in, *Bericht Uber die Versammlung Deutscher Naturforscher und Aerzte in Prag, September, 1838.*

Ramon y Cajal, S. (1908). Structure et connections des neurones. *Les Prix Nobel en 1906.* Norstedt, Stockholm, pp 1-25.

Ramon y Cajal, S. (1913). El proceder del oro-sublimado para la coloracion de la neuroglia. *Trab Lab. Invest. Universidad de Madrid.* **11**, 255-259.

Robertson, W.F. (1899). Normal and pathological histology of the nerve cell. *Brain,* **22,** 208-327.

Ross, R.F.A. (1953). Cell shrinkage caused by fixatives and paraffin wax embedding in ordinary cytological preparations. *Quart. J Microscopy,* **24,** 125-139.

Sharpey – Schafer, E. (1885). *Essentials of Histology.* Longmans, London.

Sholl, D. (1956). *The Organisation of the Cerebral Cortex.* Methuen, London.

Stowell, R.E. (1941). Effects of tissue volume of various methods of fixation, dehydration and embedding. *Stain Technol.* **16**, 67-75.

Valentin, G.G. (1836). Ueber der Verlauf und die letzten Ende der Nerven. *Nova Acta Phys. Med. Acad. Caes. Leopold. Carol, Nat Curiosorum, Breslau*, **18,** 51-240.

Virchow, R. von (1856). *Gesammelte Abhandlungen zur wissenschaftlicher Medizin.* Meidinger. Frankfurt am Main, p. 890.

Legends

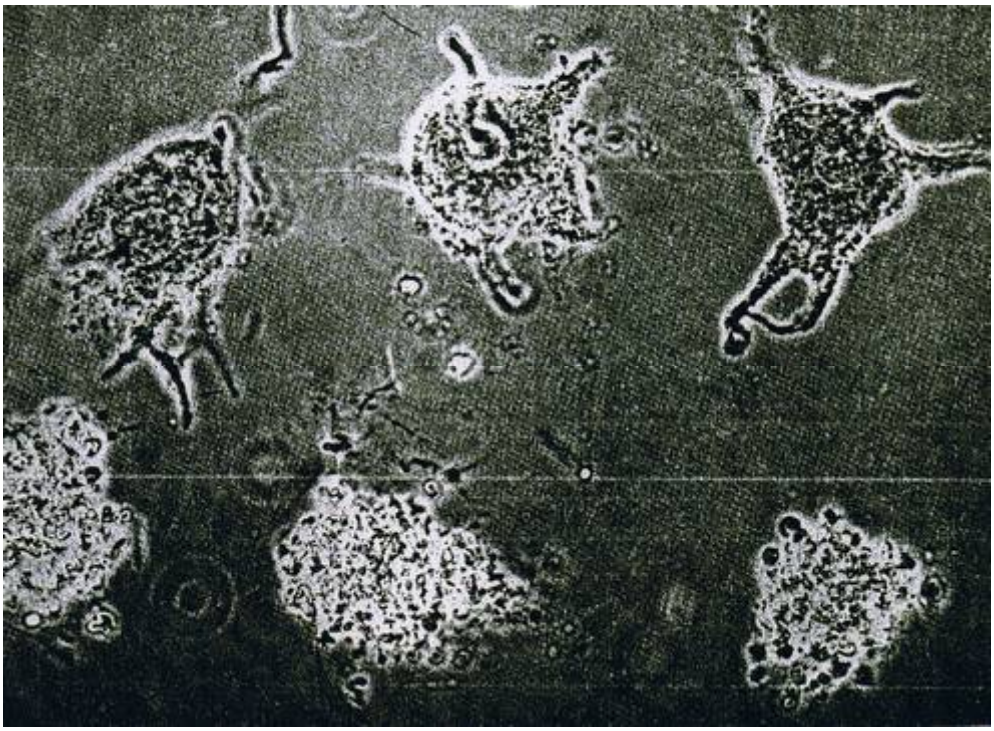

Figure 1. *Upper;* three Deiters nerve cells, dissected out free hand, 'cleaned' from the surrounding glia, and photographed under phase contrast microscopy. *Lower;* three collections of glial cells each of which originally surrounded the nerve cell situated above it. The glial samples were trimmed by freehand dissection to approximately the same volume as that of the nerve cell. In the photograph they are pressed lightly against the glass, and hence have a larger area than when taken out, x170. This figure is produced from Hydén and Pigon, (1960), by kind permission of Elsevier Publications.

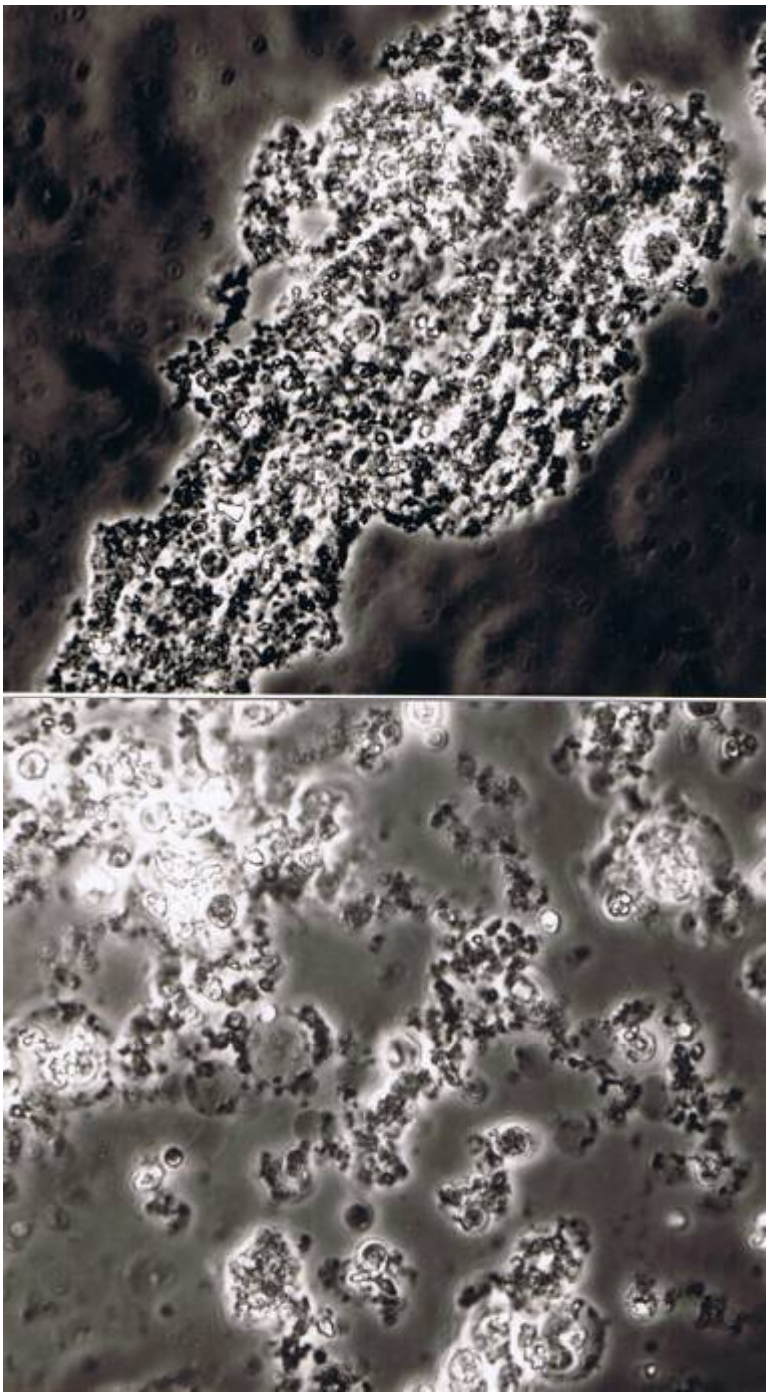

Figure 2. *Upper;* neuroglial clump from human putamen dissected out in normal saline. *Lower;* clump from human putamen, showing individual elements of fine granular material, also in saline, both phase contrast, x700.

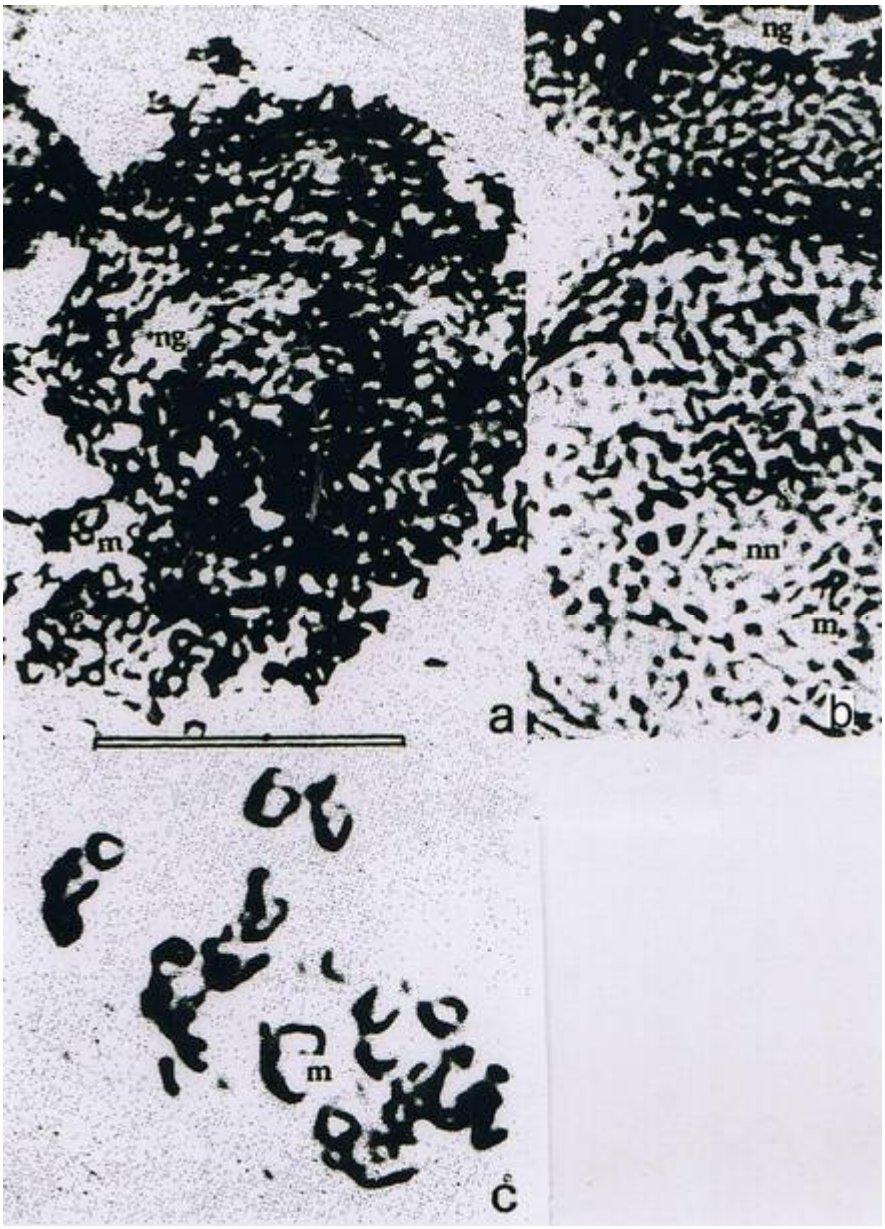

Figure 3. Fine granular material stained with Janus green-neutral red, viewed by phase contrast microscopy. *m* is mitochondria. The bar is 20 μm for *a* and *b* and 50 μm for *c*. *a* is an isolated neuroglial clump; *b* has a neuroglial clump in the *upper* part of the picture, and a neuron cell body in the *lower,* and *c* shows the clump separated to show that the fine granular material consists of mitochondria. This illustration comes from Hillman, Deutsch, Allen and Sartory, (1977) by kind permission of the Quekett Microscopical Club.