THE NISSL SUBSTANCE OF LIVING AND FIXED SPINAL GANGLION CELLS

I. A PHASE CONTRAST STUDY*

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INTRODUCTION

The cytoplasmic basophil substance, or Nissl material, of neurons has been shown to differ in form and distribution from one neuronal type to another and to undergo characteristic and predictable changes during differentiation and regeneration and in various pathological and physiological states. Despite these studies, Nissl bodies are regarded by some workers as being essentially artifacts of fixation. Historically, this concept stems from the oft repeated observation made first by Held (6) that when freshly isolated neurons are observed under the light microscope their cytoplasm appears optically empty with no discrete formations identifiable as Nissl bodies. Upon the addition of a histological fixative, however, dark masses which have been equated with Nissl bodies become visible. Similar bodies are demonstrated when preparations of isolated neurons are allowed to remain under the microscope for an extended period of time. Unfixed nerve cells obtained from animals some time post mortem may also exhibit bodies in their cytoplasm of similar appearance (7).

Further evidence, used to support the contention that Nissl bodies may be artifacts, has been drawn from their response to fixation. Held (7) observed that in 1 µ paraffin sections of neurons, the fine structure of the basophil bodies appeared either granular or homogeneous, depending upon the choice of fixative. Held found that by varying the concentrations of alcoholic and acidic fixatives, he could precipitate the Nissl bodies as structures containing larger or smaller granular components. Alkaline fixatives, on the other hand, either caused the basophil component of the neuronal cytoplasm to appear as homogeneous bodies (which were nonetheless distinct from...
the surrounding acidophil cytoplasm) or, with increasing alkalinity, removed most or all of the basophil material. In later researches, Hopkins (11) and Scheinin (25) reported that the size as well as the structure of the Nissl bodies was largely dependent upon the fixative procedure used. In contrast to their observations Einarson (4) observed only minimal effects of fixation upon the Nissl pattern of nerve cells. The stainability of Nissl bodies with conventional basic stains was considerably altered by varying the fixation. However, with the application of either the gallocyanin or gallamin blue techniques, he was able in all cases to demonstrate Nissl bodies in a form relatively unaltered by fixation.

Special techniques have been used in a number of attempts to discover whether the Nissl bodies preexist in the living cell.

Beams and King (1), using the ultracentrifuge at forces up to 500,000 g, were able to stratify the components of freshly isolated ganglion cells. After fixing and staining their preparations, they frequently found discrete basophil bodies lying toward the centrifugal pole of the cells. This was taken by the authors as evidence supporting the preexistence of Nissl bodies in the living state.

The ultraviolet microscope has been employed by a number of workers to examine squash preparations of isolated neurons (26, 28, 30), and discrete ultraviolet-absorbing masses were photographed which have been designated Nissl bodies. In contrast to these observations, however, Koenig (12) was not able to resolve discrete bodies in ultraviolet photographs of living neurons grown in vitro.

The phase contrast microscope has been used by Hoessley (10) and by Palay and Wissig (20) to study isolated neurons and by Hild (9) to examine neurons grown in vitro. In unfixed squash preparations of ventral horn neurons, Hoessley was able with the aid of phase contrast optics to identify cytoplasmic structures which were similar to the basophil bodies seen in the same cells after fixation and which he believed probably corresponded to Nissl bodies. Unfortunately, Hoessley was unable to study his material sooner than 4 hours after death in the case of human material or 45 minutes after death in the rabbit. Consequently he drew no conclusions as to whether the structures visible under the phase contrast microscope were present in the living cell. Palay and Wissig (20) isolated rabbit supraoptic neurons in hypertonic sucrose solutions, which they found maintained the cells in a life-like appearance for a longer time than did Tyrode’s solution or serum. In the peripheral areas of the cells, they found evidence under phase contrast of fine linear striations, which they took to be an indication of the presence of the endoplasmic reticulum of electron micrographs, and which they believed to be located in the Nissl bodies of these cells. Hild (9) made phase contrast time lapse motion pictures of ganglion cells grown in vitro and observed dark cytoplasmic masses which he found to be in constant motion and which he believed to be identical with Nissl masses. However, Hild did not support this observation with comparison to fixed and stained cells.

It would appear that the various recent attempts to demonstrate the non-artifactual nature of Nissl bodies have so far yielded only inconclusive and at times contradictory results. In general, it might be agreed that definitive
proof of the preexistence in living cells of discrete Nissl bodies cannot be based on observations made on damaged or otherwise unhealthy cells. This requirement would exclude from primary consideration studies based on dissected neurons in which the cells inevitably suffer some damage to their processes during the isolation procedure and in which additional damage may result from the usual technique of squashing the preparation under a coverglass for observation under the microscope.

The alternative procedure of growing neurons in vitro in coverglass preparations and observing them without derangement might offer a better approach to the elucidation of this problem. However, convincing evidence must be presented regarding the healthy state of cells to be examined in this manner. Further, they should be able to be maintained in vitro for considerable periods of time, during which they should show signs of progressive differentiation and maturation. Finally, it would seem most desirable to correlate the appearance of the living cells with that of the same cells after fixation and staining.

It was with these considerations in mind that we undertook to examine chick embryo spinal ganglion neurons grown in vitro for evidences of Nissl bodies in the living state. This material has been shown to be capable of an extensive and predictable in vitro differentiation (22) and normal electrical activity has been recorded in living cells cultured in this manner for as long as 7 weeks (2).

In our study of Nissl substance both phase contrast and ultraviolet microscopy have been employed. The present paper deals with observations by phase contrast, while results obtained with ultraviolet microscopy will be embodied in a subsequent report.

**Materials and Methods**

Spinal ganglia dissected from the lumbar region of 8 or 9 day chick embryos furnished the material for this study. Two or three whole ganglia, the connective tissue of which had been removed, were planted on a round 22 mm. gold seal or quartz coverglass (22). Six such coverglass cultures were stuck by coagulated plasma onto a 2 X 3 inch glass slide and were carried in a new type of culture vessel (3) and treated as a unit in all subsequent washings and feedings. With the exception of the increased efficiency of handling the cultures, there were no apparent differences between spinal ganglia grown in the new vessel and those maintained on double coverslip Maximow slides.

At the beginning of this study, the cultures were maintained on the media described by Peterson and Murray (22). Shortly afterward, a new synthetic medium, No. 858 of Healy et al. (5) became available. It was adapted for the culture of spinal ganglia and used thereafter throughout this study. It was found that a semisynthetic medium containing No. 858 encouraged more rapid spreading of the explants and a greater degree of flattening of the somas (cell bodies) of the regenerating neurons than were found in comparable cultures grown on the earlier media. The cultures grown on the new medium were maintained in excellent health for as long as 40 days in vitro (after which time they were fixed). In addition, the regenerating neurons underwent a more rapid differentiation when grown on the new medium.
The medium used in explantation consisted of 1 part chicken plasma to 1 part No. 858 adjusted to a pH of 6.9 to 7.1 with CO₂ just before sealing the culture vessel. Cultures were washed with Simms's balanced saline solution and fed twice weekly. The first feeding consisted of No. 858 alone, thereafter the feeding mixture was 3 parts No. 858 to 1 part of 50 per cent chick embryo extract. Patching was avoided when possible in order to have a thin plasma clot for best microscopy. In order to keep the cultures for more than 2 weeks, however, it was usually found necessary to patch at the end of the 1st and 3rd week. The patching mixture consisted of 4 parts plasma to 3 parts No. 858 and 1 part 50 per cent chick embryo extract.

Most of the cultures studied were prepared for observation under the phase contrast microscope by sealing the 22 mm. coverglass bearing the culture with petrolatum over an 18 mm. hole drilled in a 3 x 2 inch glass slide. The slide was then inverted, the cavity filled with feeding solution, and a coverglass sealed with petrolatum over the hole in the mount. A piece of parafilm with an 18 mm. aperture was fastened to the upper and lower surfaces of the mount. These preparations were maintained for no more than 1 to 2 hours before fixation. At that time, the under coverslip was removed without causing mechanical injury to the culture, and the preparation was washed briefly in Simms's balanced saline solution and fixed.

The fixation chiefly used was Palade's 1 per cent buffered osmium tetroxide at pH 7.2 or 7.4 (17). The cultures were fixed for 5 minutes and rinsed briefly with veronal buffer at the same pH. They were then postfixed with 10 per cent neutral buffered formalin, rinsed briefly in water, mounted in human placental serum, and reexamined under the phase contrast microscope. Subsequently they were dehydrated to 80 per cent alcohol, bleached in 1 per cent sodium peroxide in 80 per cent alcohol, hydrated, and stained either in 0.25 mg./cc. azure A at pH 4.0 for 20 minutes followed by tertiary butyl alcohol overnight, or by the cresyl echt violet procedure of Powers and Clark (24). Comparable results were obtained with both staining procedures. One culture was fixed for 2 hours in 10 per cent formalin in saturated HgCl₂ and washed in running water overnight before being stained with azure A.

Phase contrast microscopy was carried out using a Zeiss opton 100 X N. A. 1.30 apochromat medium dark contrast phase objective on a Zeiss lumipan stand. Photography was done with the aid of a 35 mm. Zeiss winkel camera attachment, using Kodak microfile film for phase contrast studies and adox K B-14 film for stained preparations. The refinding of explants and cells on the round coverglasses was greatly facilitated by marking fields on the coverglass with a Leitz diamond objective marker.

Substantial technical difficulties are encountered in the study of living neurons under the phase contrast microscope. Image formation with the phase contrast microscope is affected adversely by the out-of-focus parts of the object (16). Nerve cells are, of course, very large cells with long optical paths in which are suspended numerous light-retarding particles and organelles. In addition, the presence of other cells lying above and below the neuron would contribute to the deterioration of the phase contrast image. Consequently, it was necessary to find for study flattened neurons in healthy condition with no cellular and little fibrillar overlay. These conditions are met only extremely rarely in cultures maintained on the media of Peterson and Murray (22) and not too frequently in cultures grown on the semisynthetic medium used in this study. Cultures in which the appropriate amount of spreading and flattening have occurred (but which have not yet developed to the desired stages) may proceed to further lysis of the clot, resulting in disruption of the outgrowth zone and loss to view of the neurons. Consequently, large numbers of preparations need to be prepared and studied in order to select cells which combine the conditions of good health, a desired degree of maturation, and favorable position for phase contrast microscopy. Finally, another set of hazards concerns the refinding of the same cells for photography after fixation and again after staining. The very thin and delicate plasma clot may be disrupted by these procedures and the cells previously studied may become altered in position or lost to view.
OBSERVATIONS

The Appearance of the Chick Embryo Spinal Ganglion Neuron Seen with the Phase Contrast Microscope

In the cytoplasm of the living neuron three major classes of components are seen under the phase contrast microscope. The first consists of large moderately dark masses that are relatively homogeneous in appearance. They occur as flattened plaques or narrow arcuate forms (Figs. 3, 4, and 11 to 13). Frequently their peripheries are demarcated by a narrow rim of denser granular or filamentous material (Fig. 7). They are graduated in size, being largest near the cell periphery, and often bear slender terminal projections which may appear to be confluent with other major masses (Figs. 3, 4, 6, and 7).

The second class of structures is composed of masses generally of smaller size and more varied density in which dark granules are frequently to be seen embedded in a lighter matrix. They are usually located in the perinuclear area and are most prominent in younger and in chromatolytic neurons (Figs. 1, 9, 10, 14, 15, and 24). Intermediate forms between the large homogeneous and small granular masses usually exist and the distinction is somewhat arbitrary.

The third class of cytoplasmic components consists of narrow, light, spiral, or gyroform channels that are bordered by the large and small masses. These channels usually appear structureless (Figs. 3, 4, 6, and 7); occasionally, however, they are traversed by one to several central dark filaments (Fig. 13).

Comparison of the phase contrast images of the living cell with photographs of the same cell after fixation and staining for Nissl bodies reveals the identity of the larger, relatively homogeneous dark masses seen by phase contrast with the larger basophilic Nissl bodies. In favorable cases, the homology may be extended to many of the smaller bodies as well. Among these latter, it is usually possible in the phase contrast and stained pictures to establish the identity of the small Nissl bodies lying at the nuclear periphery (Figs. 1 and 2).

The nucleus of the living neuron seen with phase contrast is a relatively clear, rounded body containing no prominent structures other than the nucleolus and small heterochromatin bodies. One or 2 nucleoli are found per cell. Generally they have a nearly spherical, mulberry-like form, but in some cells they may appear greatly elongated. The heterochromatin bodies vary in number and position. They may be absent altogether, or one may be present in close association with the nucleolus (Fig. 2), or there may be as many as 3 or 4 small heterochromatin granules lying in the nuclear sap.

Changes in the Phase Contrast Image of the Living Neuron during Differentiation

When 8 or 9 day chick embryo spinal ganglia are explanted, they are initially too opaque to permit microscopic examination. During the 1st week
after explantation, large numbers of fibroblast-like cells migrate from the zone of outgrowth leaving behind the regenerating neurons, which do not migrate, and the Schwann and satellite cells. By the end of the 1st week in vitro, the explant may have thinned sufficiently for microscopic study. The nuclei of regenerating neurons which have been 1 week in vitro usually lie eccentrically and may project outward at one pole of an ovoid cell (Figs. 9 and 10). In fixed and stained preparations of this stage, the Nissl material is seen to extend as a relatively homogeneous, basophilic peripheral ring or horseshoe around the cell (see, for example, Fig. 17 of Peterson and Murray (22)). In comparable living neurons seen under the phase contrast microscope, this peripheral region is seen to consist of a smoothly contoured homogeneous material of moderate darkness which is quite sharply demarcated from the granular and fibrillar central cytoplasm (Figs. 9 and 10). In cells of older cultures which have undergone regression to a chromatolytic state, much the same appearance is shown and almost complete equivalence exists between the peripheral homogeneous cytoplasm of the living cell and the basophilic Nissl material of the same cell when fixed and stained (Figs. 24 to 26). The large central juxtanuclear area of granular and fibrillar cytoplasm is almost, if not entirely, devoid of basophilic material (Fig. 25).

As differentiation proceeds in vitro, the nucleus comes to lie in a more central position. The neuron grows in size and the peripheral homogeneous material increases in extent and becomes broken by fine light channels into larger and smaller masses. The smaller bodies which lie in the perinuclear cytoplasm assume a concentric arrangement about the nucleus and they too become separated from one another by fine light channels (Figs. 14 and 15).

In subsequent differentiation, long, narrow, arcuate offshoots of the homogeneous peripheral masses project into the central cytoplasm. The latter becomes broken into small elongated bodies which differ somewhat from the more peripheral bodies in their more abruptly varied densities. The diameters and lengths of the light channels are seen to be increased at this stage (Figs. 3, 4, 6, 7, 11, and 12).

In the more mature stages of development (generally reached in about 4 weeks in vitro) the neuronal soma is seen to have grown extensively. The nucleus lies centrally and the homogeneous masses have become separated by interlying channels into flattened plaques and flakes. While there is often still some suggestion of a concentric array in this material, a more complex mosaic pattern is often seen, and the distinction between the perinuclear and peripheral cytoplasm with regard to degree of granularity has disappeared (Fig. 13).

The rate of these changes in the size, form, and distribution of the cytoplasmic components is variable and highly dependent on cultural conditions. Cultures maintained on the medium containing No. 858 develop faster.
by several days than those grown in the medium of Peterson and Murray. In any one culture at a given time there is a range of maturation, with the largest neurons usually being the most advanced. In addition, the state of differentiation of these cultured cells is highly labile, and at times regression to earlier states may be noted.

The Effect of Fixation with Osmium Tetroxide

If cultures fixed by osmium tetroxide and postfixed by formalin are examined mounted in serum without having been subjected to dehydration, remarkably little cytoplasmic change can be noted as compared with the living state. There is no shrinkage or relative distortion in these preparations (Figs. 14 to 22). In general, contrast is only slightly improved and the occasional small cytoplasmic lipid droplets of these cells are blackened by osmium. The most conspicuous change relates to the nucleoli which upon fixation frequently appear to lose density, becoming almost invisible under the phase contrast microscope. This is especially evident if the fixed preparations are allowed to remain mounted in serum for several days.

The Effect of Dehydration and Staining

Upon dehydration, a considerable shrinkage of the neuronal soma occurs. This may amount to about one-third of the area of these cells. However, when the photographs of the stained neurons are enlarged to compensate for this shrinkage (i.e., to the same size as photographs of living or fixed, undehydrated neurons) excellent correspondence can be made between the relatively homogeneous phase-dark masses of the living neuron and the Nissl bodies of the stained preparation (Figs. 1 to 8). The shrinkage due to dehydration appears to be rather uniform throughout the cell, with the exception of the cell processes which usually show the greatest effects. Phase contrast studies of these fixed and stained preparations reveal little discernible coagulation that might be considered to be due to dehydration, and in general, the phase-dark areas of the stained preparations correspond to the basophil or Nissl bodies (Fig. 23).

The Effect of Mercuric Chloride-Formalin Fixation

The correspondence between the medium dark homogeneous areas and the Nissl material is almost as good after fixation with formalin–mercuric chloride as it is after osmium fixation (Figs. 24 to 32). However, phase contrast examination of the stained neurons fixed with formalin–mercuric chloride reveals an increased granularity especially noticeable in the nucleus. It is of interest that the karyosome, or acidophilic nucleolus, is made visible with phase contrast after this treatment, but not after osmium fixation (Figs. 26, 29, and 32).
Neurofibrils.—In a number of living neurons in healthy preparations, filamentous structures have been observed which appear to correspond to neurofibrils. They are most readily seen in the narrow channels separating the peripheral Nissl masses near the cell processes in cells of intermediate stages of development (Figs. 15, 21 and 22). They may also be seen traversing considerable areas of the cell in the youngest and in the most mature neurons (Figs. 9, 10, and 13). The appearance of the neurofibrils does not seem to be affected by osmium fixation.

The youngest neurons differ from the older stages in lacking the narrow, clear channels separating the phase-dense masses. In the 7 day old preparation pictured here (Figs. 9 and 10), a number of neurofibrils are seen to traverse the proximal region of the axon and to surround both the central granular mass of cytoplasm and the outer nuclear periphery. Other neurofibrils with an intermittently beaded appearance are seen to penetrate the central cytoplasmic mass (Fig. 9).

Observation of living neurons containing several fine parallel neurofibrils lying in the channels between Nissl masses which are adjacent to the axon hillock demonstrates that the neurofibrils are labile in form. If such cells are observed for 15 to 30 minutes under the phase contrast microscope, the number of visible neurofibrils is seen to decrease, while the thickness of the fibrils that do remain shows corresponding increase. Neurons in unhealthy preparations may also show many prominent neurofibrils. Such preparations, however, usually also show other signs of cell damage (such as cytoplasmic blebbing) which are absent from the preparations shown in this report.

DISCUSSION

The use of osmium tetroxide as a fixative dates back to 1865 (14), and a number of workers have pointed out its suitability for life-like preservation of cells (27, 23, and 17). Long osmium fixation, however, is known to prevent uptake of basic dyes by cellular structures. Therefore, the procedure adopted in this study was a short osmium fixation followed by a longer postfixation in formalin which has also been recommended as a method yielding life-like preparations (16).

The phase contrast comparison of the osmium-formalin-fixed neurons with the living state demonstrates that the appearance of these cells is practically unchanged after this fixation, the most notable change relating to the loss of density of the fixed nucleolus. This may be due to extraction of a basophilic nucleolar component by the serum in which the preparations were mounted for observation after fixation. Apart from this, and the expected blackening of small lipid droplets by the osmium procedure, there is remarkable similarity of appearance between all parts of the living and fixed, hydrated neurons (Figs. 14 to 22). The large, homogeneous moderately dark cytoplasmic

1 With the exception of this change, there are no other indications of alteration or damage to the preparations while they are being studied under the phase contrast microscope. The densities, sizes, shapes, and distribution of the cellular components appear unchanged throughout the 1 to 2 hour period in which they are studied and photographed prior to fixation.
areas, in particular, are seen to occur in the same locations and to be of the same sizes and shapes before and after fixation. Changes in the appearance of the smaller cytoplasmic masses are most probably due to the technical difficulty of achieving exactly the same focal level in the photographs of the fixed and living cells.

With dehydration of this material, some shrinkages occur. To date, no adequate procedure has been developed to eliminate this artifact which may result in a decrease of 10 to 20 per cent of the somal diameters. However, enlargement of the fixed and stained pictures of the neuron to the same size as those of the living preparation demonstrates that the shrinkages consequent on dehydration are relatively uniform throughout the cell (Figs. 1 to 8 and Figs. 18 to 23).

The accurate comparisons that can be made between the homogeneous phase-dark regions of the living and fixed neurons on the one hand, and the stained Nissl bodies on the other, would seem to make it irrefutable that Nissl bodies preexist in the living cell and are not substantially changed in form or distribution by the osmium-formalin fixation and subsequent staining procedures. Nor is there much significant change in the presence, distribution, and form of these structures after fixation in formalin-mercuric chloride (Figs. 24 to 32), a mixture which has previously been used extensively in the study of cultured ganglion material (22).

The Nissl bodies, then, are not artifacts of fixation. This view is in direct support of the observations of Einarson (4) who found them unchanged in general form after a considerable variety of fixation procedures, and is in disagreement with the previous findings of Hopkins (11) and Scheinin (25). In this connection, the technical advantage gained by following the effects of these procedures on individual cells which are studied alive and after fixation can hardly be overemphasized.

The recent electron microscope study of Palay and Palade (19) on the fine structure of the osmium-fixed neuron demonstrated that the Nissl substance is composed of flattened plate-like masses of endoplasmic reticulum, which are more or less longitudinally oriented with respect to each other, and between which lie clusters of fine granules 10 to 30 μ in diameter. In this respect, it is noteworthy that the basophilic substance of the neuron does not differ fundamentally from the highly basophilic substance of other cells, such as glandular epithelial cells, which also have a well developed system of fine granules and endoplasmic reticulum (18).

The mitochondria of the neuron are smaller than those of many other cell types (8), and differ in distribution in different stages of in vitro neuronal development. Peterson reports that in the early stages of differentiation when the nucleus lies eccentrically, they are localized in the central mass of cytoplasm. During maturation of the neuron, they gradually become dispersed throughout the cytoplasm (21). Their small size and large numbers make phase contrast demonstration of individual mitochondria uncertain. The presence of aggregates of mitochondria, however, undoubtedly contributes
to the granular appearance of the central cytoplasmic mass of the youngest and chromatolytic stages and to the small perinuclear masses of the intermediate stages (Figs. 9, 10, 14, and 24).

In their electron micrographs of neurons, Palay and Palade (19) demonstrated groups of fine filaments, each filament about 100 Å in diameter and of indefinite length, which traverse the soma between the Nissl bodies. No evidence of filaments of larger size was found in the type of neurons studied by them. The authors propose that if the filaments they described were caused to aggregate side by side by conventional histological fixation and were subsequently encrusted with silver, the resulting structures might be of sufficient diameter to correspond to the usual picture of fixed neurofibrils. In witnessing the formation of one or two thickened fibrils from several finer ones, we may have observed the last stages in a series of similar aggregative changes of submicroscopic units. Whether visible neurofibrils such as presented here (Figs. 9, 10, 13, 15, 21, and 22) are to be considered artifacts or representative of injurious treatment to the neurons, on the one hand, or whether they ought to be considered as part of the normal mechanism of response to changing conditions of the neuron cannot be determined from our observations. It would seem that this area might be a fruitful one for investigation with the electron microscope.

Weiss and Wang (29) and Levi and Meyer (13) claim to have demonstrated the presence of neurofibrils in living cultured nerve cells. Examination of their pictures, however, makes it at least as likely that in some of their preparations they were observing only the narrow gyroform channels which traverse the soma, and in which the visible neurofibrils lie. These channels can be readily demonstrated under the light microscope without the use of phase contrast. (See, for example, Figs. 8, 9, and 11 of Murnaghan (15) and Figs. 4 and 12 of Peterson and Murray (22).) Because these channels are bounded by the Nissl masses, it also becomes possible to gain some indication of the distribution of the major Nissl masses of the neuron without phase contrast.

**SUMMARY**

Living chick embryo spinal ganglion neurons grown from 1 to 4 weeks in vitro were studied under the phase contrast microscope. In the peripheral cytoplasm of the earliest stages studied, a homogeneous, phase-dense material is seen which corresponds in location to the cytoplasmic basophil material of the same stages. As maturation proceeds, this material increases in extent, and becomes separated by lighter channels into discrete bodies.

Short fixation by 1 per cent buffered osmium tetroxide followed by post-fixation with neutral buffered formalin does not significantly alter the size, shape, or distribution of any of the cytoplasmic components, and the fixed, hydrated cell is almost indistinguishable from the living cell. Dehydration causes some shrinkage of the fixed preparations, but if the photographs of the stained preparations are enlarged to correspond with those of the living cell, excellent correspondence can be made between at least the larger basophil masses and the larger dark masses seen with phase contrast. Fixation by a
formalin-mercuric chloride procedure also results in satisfactory correspondence between the stained Nissl bodies and the phase-dark homogeneous areas. It is concluded that discrete Nissl bodies preexist in the living neuron and are essentially unchanged after good cytological fixation.

Evidence is also presented of the presence of neurofibrils in the living state.

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BIBLIOGRAPHY

EXPLANATION OF PLATES

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FIG. 1. Phase contrast photomicrograph of a living chick embryo spinal ganglion neuron, 13 days in vitro. The nucleus is clear except for the nucleolus and adjacent heterochromatin (H). Within the cytoplasm, there are large, relatively homogeneous phase-dense masses (L) and smaller, more granular masses (S and G). Satellite cells (SC) surround the neuronal soma. × 1,800.

FIG. 2. The same cell as in Fig. 1, after osmium tetroxide fixation and staining with cresyl echt violet. The large phase-dense masses of Fig. 1 are seen to correspond to the large basophilic Nissl bodies (L) of the stained preparation. Some of the smaller masses (S) forming a nuclear cap are seen to be basophilic, while the more granular masses (G) are less so. The heterochromatin (H) is basophilic and the satellite cell nuclei (SC) show clearly after this stain. × 2,000.
Figs. 3 and 4. Phase contrast photomicrographs of a living neuron, 13 days in vitro. Two focal levels 2 μ apart are shown. In the nucleus, two nucleoli are present; while in the cytoplasm, the homogeneous, phase-dark masses are seen to be cut by narrow spiral channels (C) into large peripheral masses (L) and smaller arcuate bodies (A), × 1,340.

Fig. 5. The same cell after osmium fixation and staining with cresyl echt violet. The large peripheral masses (L) and arcuate bodies (A) are seen to correspond to basophilic Nissl bodies. × 1,630.

Figs. 6 and 7. Phase contrast photomicrographs of a second living neuron from the same preparation as Figs. 3 to 5. The major homogeneous phase-dark masses are bordered by a narrow rim (R) of denser granular and filamentous material. × 1,340.

Fig. 8. The same neuron as in Figs. 6 and 7 after osmium fixation and staining with cresyl echt violet. Most of the larger homogeneous phase-dark masses and some of the smaller ones can be seen to correspond to Nissl bodies. × 1,630.
(Deitch and Murray: Nissl substance of spinal ganglion cells, I)
Phase contrast photomicrographs of living spinal ganglion neurons in different stages of maturation.

Figs. 9 and 10. 7 days *in vitro*. The nuclei of these cells lie eccentrically. The homogeneous appearing substance (H) is limited to the periphery of the cell. The central cytoplasmic mass (C) is very granular. Fine neurofibrils (N) traverse the soma. X 1,040.

Fig. 11. 13 days *in vitro*. The nucleus is more central in position, while the homogeneous peripheral cytoplasm (H) has increased in extent and the central granular cytoplasm (C) has become concentrically arranged and extends in arcuate masses (A) into the more central cytoplasm. Fine light channels separate the cytoplasmic masses. X 1,250.

Fig. 12. 19 days *in vitro*. The peripheral cytoplasm has further increased in extent and has begun to be divided into small plaques (P). X 1,250.

Fig. 13. 32 days *in vitro*. The cytoplasm is a mosaic of plaques and flakes of homogeneous material between which run narrow channels containing neurofibrils (N). X 990.
(Deitch and Murray: Nissl substance of spinal ganglion cells. 1)
Phase contrast photomicrographs of one neuron, 11 days in vitro, living and after osmium fixation.

Fig. 14. Living; photographed at the nucleolar level. \( \times 1,440 \).

Fig. 15. Living; there is 2 \( \mu \) difference in focus between Figs. 14 and 15. A delicate neurofibril (N) is to be seen in the clear channel separating the peripheral homogeneous masses. \( \times 1,440 \).

Fig. 16. The same neuron fixed by osmium tetroxide and mounted in serum without being dehydrated. The focal level corresponds rather closely with Fig. 14, but the nucleolus can hardly be seen in the fixed cell. \( \times 1,440 \).

Fig. 17. The fixed neuron at a focal level corresponding to Fig. 15. \( \times 1,440 \).
(Deitch and Murray: Nissl substance of spinal ganglion cells. I)
Photomicrographs of one spinal ganglion neuron, 11 days in vitro, living, osmium-fixed, and stained.

Figs. 18 and 19. Phase contrast, living. Two focal levels about 3 μ apart, ×1,280.

Fig. 20. Osmium-fixed, azure A stained. The nucleolus appears to have taken up less dye than the surrounding nucleus or Nissl material, ×1,470.

Figs. 21 and 22. Phase contrast, osmium-fixed, mounted in serum. Two focal levels corresponding to Figs. 18 and 19. A slight tilt in the position of the fixed cell brings into focus a neurofibril (N) which was not visible at this focal level of the living preparation, ×1,280.

Fig. 23. Osmium-fixed, azure A stained, seen with phase contrast. The phase-dark areas are seen to correspond to the basophilic areas. ×1,470.
(Deitch and Murray: Nissl substance of spinal ganglion cells. I)
Three cells from a spinal ganglion culture 32 days in vitro, photographed alive and after fixation with a formalin-mercuric chloride mixture and staining with azure A.

Fig. 24. Phase contrast photomicrograph of a living chromatolytic cell. The homogeneous, phase-dark substance (H) is almost entirely limited to a horseshoe-shaped area of the periphery of the soma. The central cytoplasmic mass (C) is highly granular in appearance. × 960.

Fig. 25. The same cell after fixation and staining. The Nissl masses are seen to correspond to the homogeneous phase-dark peripheral material (H), while the central granular cytoplasm (C) shows very little basophilia. × 1,220.

Fig. 26. The same cell after fixation and staining, seen with phase contrast. The nucleus is more granular than in osmium-fixed preparations (cf. Fig. 23). The karyosome (K) shows as a prominent phase-dark granule. There are evidences of unequal shrinkages in the cytoplasm. × 1,220.

Fig. 27. Phase contrast photomicrograph of a living bipolar neuron from the same culture. × 960.

Fig. 28. The same cell after fixation and staining. The correspondence between the homogeneous masses and the basophilic Nissl bodies is clear. × 1,200.

Fig. 29. The same cell after fixation and staining, seen with phase contrast. Note the granularity of the nucleus and the prominent karyosome (K). × 1,200.

Fig. 30. Photomicrograph of a more mature living neuron from the same culture. The homogeneous phase dense material is aggregated in the somal periphery but extends inward as arcuate offshoots. × 960.

Fig. 31. The same cell after fixation and staining. The small basophilic Nissl bodies appear to have suffered somewhat more shrinkage than the surrounding cytoplasm, but correspondence is good between the large phase-dark masses and the large peripheral Nissl accumulations. × 1,080.

Fig. 32. The same cell after fixation and staining, seen with phase contrast. The karyosome (K) is apparent with phase contrast. × 1,080.
(Deitch and Murray: Nissl substance of spinal ganglion cells. I)