NEURONAL PERIKARYA WITH DISPERSED, SINGLE RIBOSOMES IN THE VISUAL CORTEX OF *MACACA MULATTA*

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ABSTRACT

Numerous small and medium-sized neuronal perikarya in layers III and IV of the visual cortex display an unusual pattern of ribosomal distribution. Instead of being aggregated in clusters, spirals, rows, and other regular polysomal configurations, the ribosomes, whether free or attached to the endoplasmic reticulum, are randomly dispersed, with no discernible pattern. The endoplasmic reticulum in such cells is reduced to a few (perhaps only one) meandering, broad cisternae, which delimit broad fields of cytoplasmic matrix occupied almost solely by scattered, single ribosomes. The Golgi apparatus is elaborate. Mitochondria are either small and numerous or large and infrequent. The other organelles, including the nucleus and nucleolus, are not remarkable. Axonal terminals synapse in the normal fashion on the surfaces of these cells and their dendrites. Associated with these cells are more numerous intermediate cells in which a few to many polysomal clusters can be found. It is proposed that the neurons with dispersed, single ribosomes are inactive in protein synthesis and that the suspension of such an important metabolic activity is probably temporary. Thus, these cells are considered to be part of a population undergoing cyclic fluctuations in the intensity of protein synthesis that should be correlated with their specific neural behavior.

The Nissl substance of nerve cells is a composite cytoplasmic organelle consisting of granular endoplasmic reticulum and free ribosomes in polysomal array (25, 28). Although this characteristic organelle varies from large masses to minute fragments in different types of neurons and even in any individual cell, its basic architecture does not vary. The most striking constancy in its pattern is the arrangement of the attached ribosomes into rows, double rows, spirals, and other patterns, while the free ribosomes suspended in the matrix between and around cisternae are always arrayed in rosettes of five to eight members. Single ribosomes are found extremely rarely except in the initial segment of the axon, where they are much reduced in number and may be absent altogether. It is generally thought that the clustering pattern of ribosomes results from their arrangement along a strand of messenger RNA and that active protein synthesis occurs exclusively in such polysomal formations. It was therefore somewhat surprising to discover large numbers of nerve cells in the visual cortex of normal monkeys *Macaca mulatta* that contained only single, free ribosomes distrib-

uted individually and apparently randomly throughout the cytoplasm. Even the attached ribosomes were randomly dispersed without any noticeable pattern. This type of Nissl substance is so unusual that a more thorough study of the cells was carried out and is reported here.

MATERIALS AND METHODS

Blocks of tissue from the striate and peristriate cortex of eight monkeys *M. mulatta* were used for this study. The animals were all healthy young adults of both sexes, 2.5–3.0 kg in weight, obtained from the New England Regional Primate Research Center, where they had been either born or reared from an early age. Under sodium pentobarbital anesthesia (18 mg per kg of body wt), each animal was given artificial respiration with a mixture of 95% oxygen and 5% carbon dioxide through an endotracheal cannula. The rib cage was resected quickly and a large cannula was inserted through the left ventricle into the ascending aorta. The head and upper half of the body were perfused through the vessels, first with a mixture of 1% glutaraldehyde and 1% formaldehyde in 0.12 M phosphate buffer containing 0.02 mM $CaCl_2$, and second with a mixture of 3% glutaraldehyde and 3% formaldehyde in the same buffer and calcium medium. Perfusion with 500 ml of the first solution lasting about 5 min was immediately followed by 1.5–2 liters of the second solution over a period of 20 min. No washout solution was used in advance of the fixative. Further details of the perfusion procedure may be found in Palay and Chan-Palay (24).

At the end of the perfusion the brain was carefully removed from the skull, avoiding pressure on and damage to the cerebral cortex in particular. Pieces of cortex from areas 17, 18, and 19 were cut as slabs; the exact orientation and source of each block were recorded before further treatment. Pieces of area 17 in the macular projection were taken in the coronal plane, parallel to the lunate sulcus, at its intersection with the lateral calcarine sulcus. The perifoveal area 17 was represented by pieces taken in the sagittal plane on the banks of the calcarine fissure. Pieces of area 19 were taken from cortex at the crest and furrow of the anterior bank of the lunate sulcus.

The blocks were sliced so that they had faces about 10-mm square, presenting the full thickness of the cortex, and they were 3–4 mm thick. They were placed in the 3% gluataraldehyde-formaldehyde solution for about 16–18 h, postfixed for 3 h in 2% osmium tetroxide in 0.12 M phosphate buffer with 7% dextrose and 0.02 mM $CaCl_2$ was added. The blocks were rinsed briefly in the buffer and then treated for 30 min in the cold with 0.5% aqueous uranyl acetate for in-block staining. Dehydration and embedding in Epon proceeded as described by Palay and Chan-Palay (24).

Sections 1 μm thick of entire block faces were stained with toluidine blue in borax (32) and were examined in the light microscope. Thin sections for electron microscopy were obtained by the mesa technique (24), using diamond knives. They were stained with 3% uranyl acetate in 50% ethanol and 0.075% lead citrate and studied in a Phillips 300 electron microscope.

RESULTS

Cells With Dispersed Ribosomes

In most neuronal somata in the visual cortex of the monkey *M. mulatta*, the Nissl substance consists of small tangles of branching tubules and cisternae of the granular endoplasmic reticulum associated with clusters of free ribosomes suspended in the surrounding matrix. In many of these cells, especially in the larger pyramids, the Nissl substance takes the classical form (25, 28) of imbricated but communicating flat cisternae in a highly ordered array, their surfaces populated with complex polyribosomal patterns, spirals, double rows, and lines, and the intervening as well as the surrounding matrix full of rosette-like groups of ribosomes. This variety of polysomal arrangement is actually the common, banal pattern seen in nerve cells throughout the central nervous systems of various vertebrates.

Other cells of the monkey's visual cortex, however, display a Nissl substance of a completely different character (Figs. 1, 2). A large proportion of these cells completely lack the typical Nissl bodies; instead their cytoplasm is peppered with individual ribosomes, which are haphazardly arranged with varying distances between them. No clustering pattern can be discerned, even in the attached ribosomes, which in most neurons are typically the most elaborately patterned. In these cells, the ribosomes are not set out in regular rows, spirals, or rosettes, each ribosome equidistant from its neighbor in the cluster. Morphologically, the individual ribosomes display no visible abnormality (Fig. 5). They have the usual size (~250 Å), contours, density, and hollow center, whether they are attached to the surface of the endoplasmic reticulum or free in the cell matrix (22). Furthermore, the granular endoplasmic reticulum is distinctive in these cells. Instead of being distributed in small tangles or massed in imbricated layers, it is represented by a small number of broad cisternae (perhaps only one) which originate at several points from the nuclear envelope and meander loosely throughout the cytoplasm. They emit a few, recurving, subsidiary branches that join with

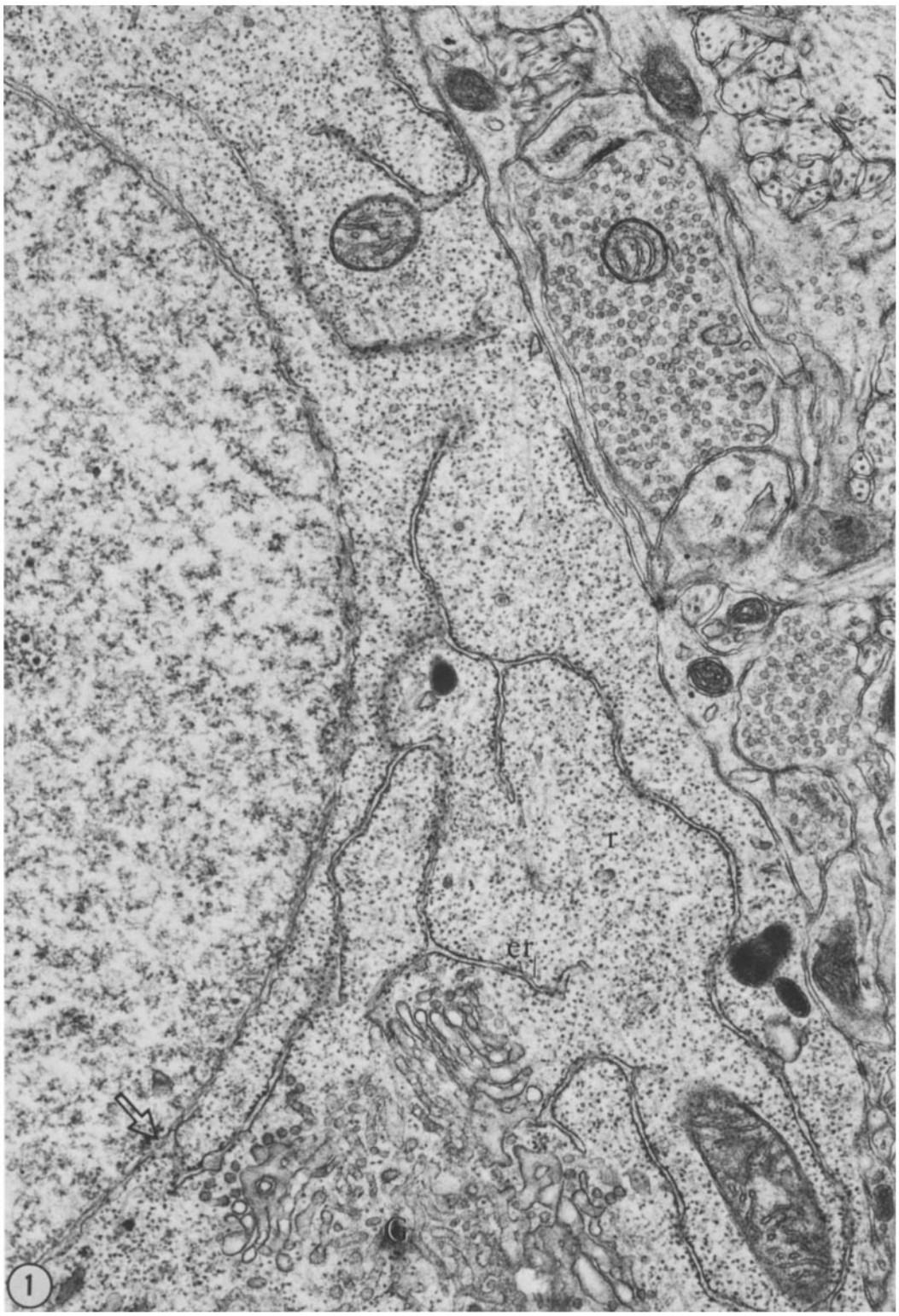


FIGURE 1 Perikaryon of a small pyramidal cell in visual cortex. The cytoplasm displays the elaborate Golgi apparatus (*G*), the meandering granular endoplasmic reticulum (*er*), and the randomly dispersed free ribosomes (*r*). A cisterna of the endoplasmic reticulum takes off from the nuclear envelope at the arrow. Layer IV, area 17 (macular region), *M. mulatta*. × 29,000.

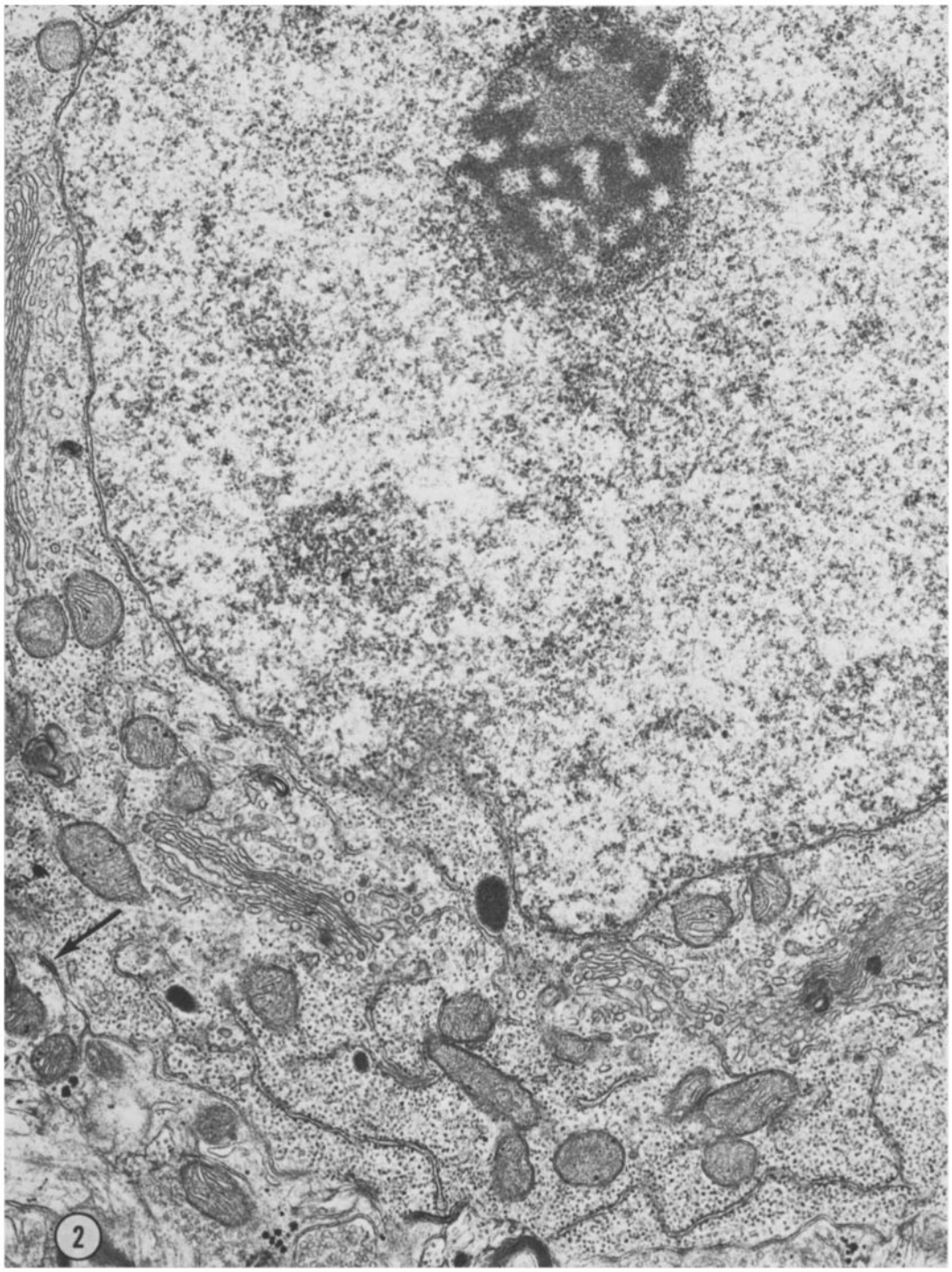


FIGURE 2 Perikaryon of a small neuron in visual cortex. The large nucleus and its conspicuous nucleolus display no unusual features, while the cytoplasm is peppered with single, free ribosomes that collect into no discernible pattern. The Golgi apparatus is extensive. At the arrow, a small synaptic junction appears on the perikaryal surface. Layer IV, area 18 (perifoveal region), *M. mulatta*. × 26,000.

the main cisternae, or unite with those of the Golgi apparatus, and finally approach the surface membrane where they constitute the hypolemmal cisterna. These features can be seen in Figs. 1 and 2. The wandering endoplasmic reticulum delimits vast fields of cytoplasm peppered with isolated ribosomes showing no detectable order. The resulting homogeneity of the cytoplasm distinguishes these cells from ordinary nerve cells, in which the ribosomes are not only clustered in polysomal array but are also massed into small or large aggregates corresponding to the Nissl bodies of light microscopy.

The Golgi apparatus in these cells (Figs. 1 and 2) is a highly developed multilaminate organelle, which occupies the middle ground between the nuclear envelope and the plasmalemma. In many instances it almost completely surrounds the nucleus. Large numbers of vesicles and vacuoles accompany the broad cisternae of the Golgi apparatus, especially at the ends of the complexes and on their outer aspect, where many of the cisternae are apparently thoroughly perforated by regular fenestrae. Lysosomes, dense-cored vesicles, multivesicular bodies, and alveolate vesicles do not seem to be more frequent than they are in other nerve cells of comparable size and location in the same specimens.

The mitochondria, however, vary a great deal from cell to cell and from region to region in the same cell. In some cells they are infrequent and much larger than in other cells of comparable size and location; in other examples they appear as clusters of small profiles, which suggest that in three dimensions they have a complex digitate form. Both kinds of mitochondria can be found in different parts of a single perikaryon.

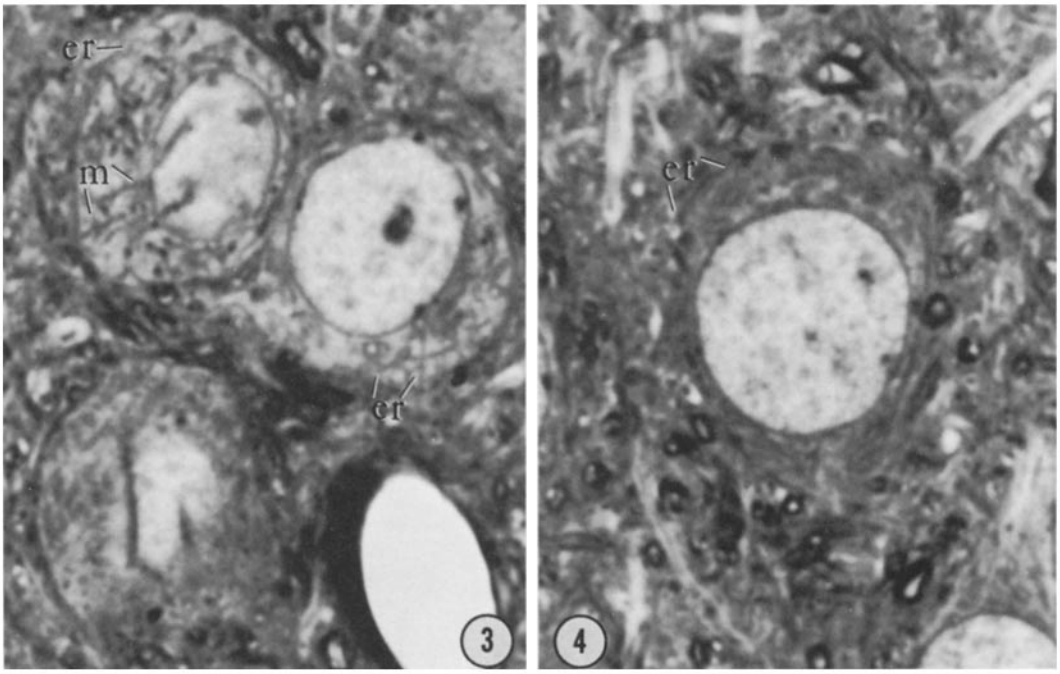
The nuclei of these cells also vary in shape. Some are smooth, round, and vesicular, whereas others are highly folded on one side or have one or two deep creases that extend almost all the way across them. As in other nerve cells, the nucleolus is large and conspicuous. The nuclear content consists of a fairly homogeneous dispersion of chromatin filaments. Small condensations of chromatin occur near the nucleus and here and there in the nucleoplasm; meager accumulations are also marginated beneath the nuclear envelope. In general, the appearance of the nucleus is that of a completely normal, ordinary nerve cell.

Synaptic terminals are infrequently encountered on the surfaces of these cells and their proximal dendritic shafts. They are usually small boutons, crowded with round and elliptical synaptic vesicles and attached to the postsynaptic surface by means of a Gray's type 2 junction (Fig. 2). The synaptic interface exhibits no unusual features and the rest of the perikaryal surface is covered by thin slips of neuroglia.

Incidence

Neurons conforming to the above description and resembling Figs. 1 and 2 are small or medium-sized cells, 12–15-μm in diameter. They tend to occur in clusters of a few members, but not all the cells in the group need be of this type. They are most numerous throughout layer IV and the deeper reaches of layer III (4) in the peristriate cortex (Brodmann area 18). They are scarcer in the striate cortex (area 17), and in area 19 they are apparently rare. Since we have not examined other parts of the cerebral cortex we cannot say whether they are restricted to the visual areas or occur generally throughout the cortex. We have not found cells of this type, however, in other regions of the nervous system, such as the cerebellar cortex and the deep cerebellar nuclei, in the same animals.

Since these cells have such a distinctive cytoplasm, we attempted to recognize them in 1- or 2-μm sections under the optical microscope in order to obtain a more accurate idea of their shape, size, and numbers than can be gained from thin sections in the electron microscope. When such semithin sections stained with toluidine blue were examined with a $\times$100 oil-immersion objective (numerical aperture 1.4) and a final magnification of about 1,000, it was often possible to distinguish the endoplasmic reticulum as gray-blue linear markings in the cytoplasm of the nerve cells. The neuronal mitochondria, which are often threadlike and sinuous, could be distinguished from these striations because they appeared much denser. In the ordinary neurons, especially the larger ones, the endoplasmic reticulum in the Nissl bodies usually appeared as clumps of parallel, gray-blue striations (Fig. 4) surrounded by a metachromatic reddish-purple haze. These clearly correspond to the imbricated cisternae of the typical Nissl body (25) and are similar to the striations seen by Palay and Wissig (26) in fresh, unstained supraoptic neurons with phase microscopy. Another cell type, however, was discernible. This exhibited a relatively clear, pale cytoplasm,



FIGURES 3 and 4 Cell types in layer IV of visual cortex. These optical micrographs show the types of cytoplasmic differentiation discernible at the light microscope level. In Fig. 3 a cluster of small neurons display a relatively clear cytoplasmic matrix in which stringy endoplasmic reticulum (*er*) can be made out. Mitochondria (*m*) appear as darker rods and granules. These cells may correspond to the perikarya with dispersed single ribosomes found in electron micrographs of the same material. In Fig. 4, a single perikaryon with typical Nissl substance, the imbricated parallel cisternae of the endoplasmic reticulum (*er*) can be seen. Layer IV, area 17 (macular region), *M. mulatta*. × 2,600.

through which looped a single, long, threadlike organelle, or several individual ones (Fig. 3). These figures correspond to the meandering endoplasmic reticulum that is characteristic of the neurons with dispersed ribosomes (Figs. 1, 2). The pallor of the cytoplasm results from the homogeneous distribution of the basophilic granules, i.e. from the dilution of the ribosomal population caused by dispersal of the polysomal clusters.

Intermediate Neurons

When we attempted to estimate the number of cells comprised in this type, it became clear that there are many cells of similar size that display both a rambling form of the endoplasmic reticulum and a few small Nissl bodies. This observation stimulated us to review our electron micrographs with the aim of recognizing cells that might be intermediates between the ordinary nerve cell with Nissl bodies and the one with dispersed ribosomes. Upon assembling and comparing large numbers of micrographs, we discovered, not unexpectedly, that a spectrum of cytoplasmic types could be constructed, leading from the diffuse, monoribosomal arrangement to the typical Nissl pattern. Sample specimens of the intermediates are shown in Figs. 6–10.

The smallest sign of a difference from random monosomal distribution is the rare appearance of a short row of ribosomes attached at regular intervals to the endoplasmic reticulum (Figs. 6, 7). In Fig. 7 the row is actually attached to the outer surface of the nuclear envelope. The other ribosomes in the field are randomly arranged. A more advanced intermediate is indicated in Fig. 8, in which a few clusters or rosettes appear among the free ribosomes suspended in the matrix in addition to regular patterning among some of the attached ribosomes. Although large numbers of individual ribosomes remain peppered throughout the cytoplasm in this stage there is a noticeable patchy

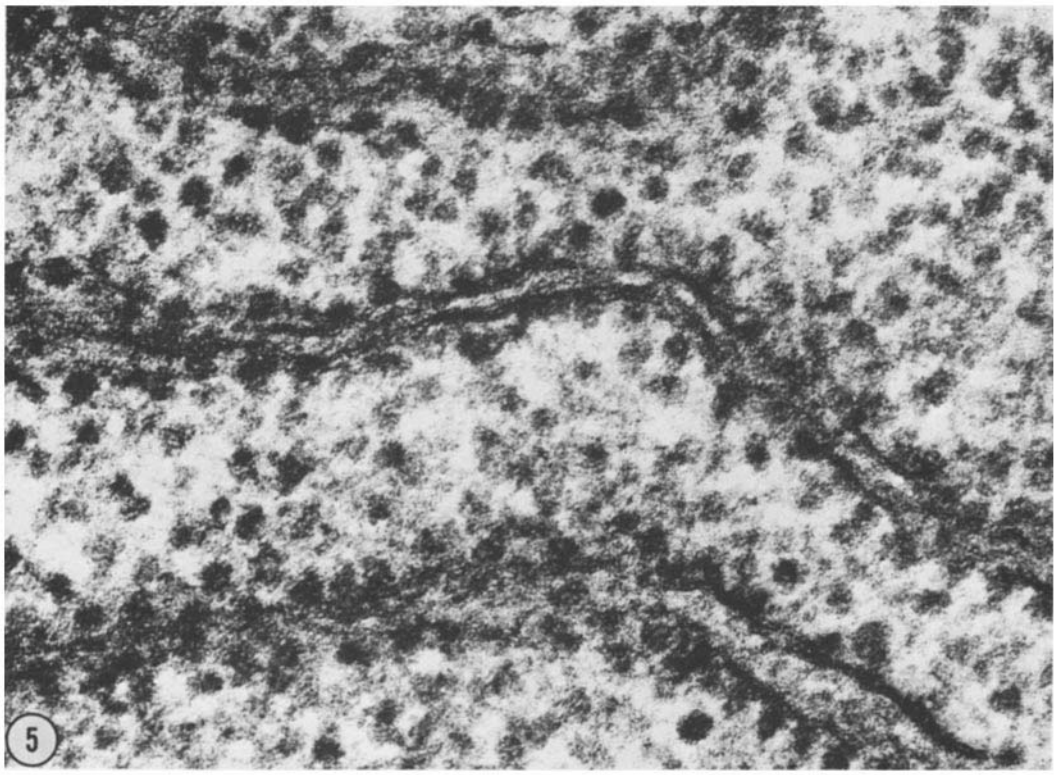
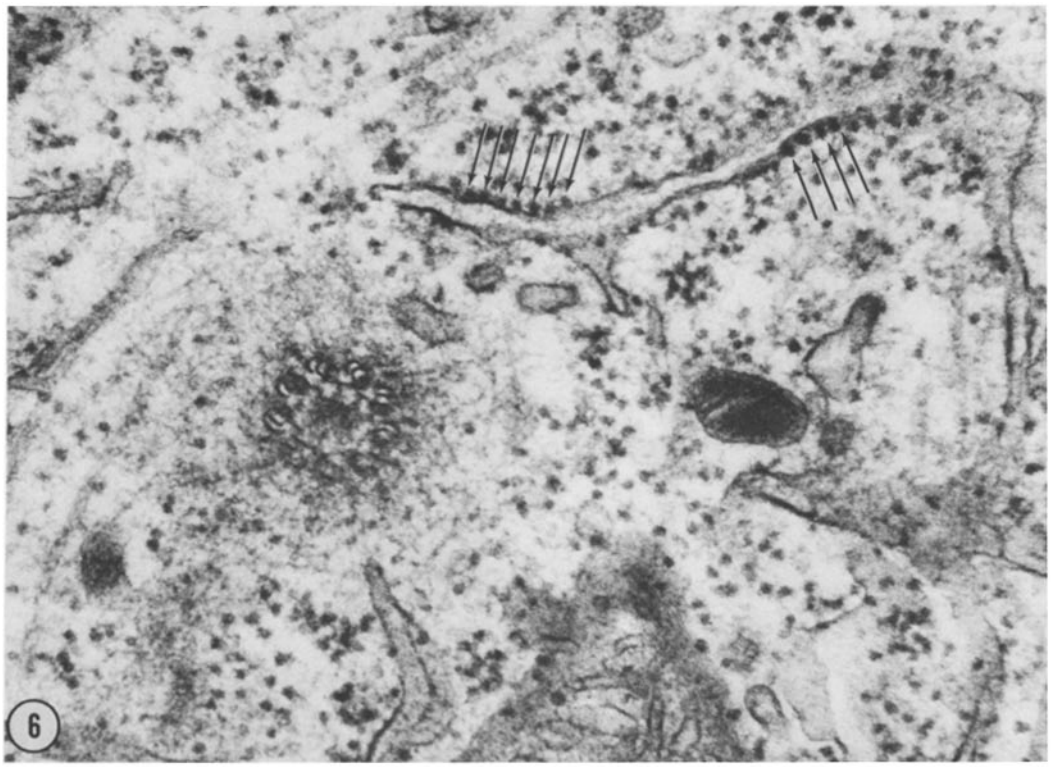


FIGURE 5 Ribosomes suspended in the matrix and attached to the endoplasmic reticulum of a small pyramidal cell in visual cortex. The usual clustering pattern is lacking, but the individual ribosomes show no abnormality in size or morphology. Layer IV, area 17 (macular region), *M. mulatta*. × 210,000.

clearing in the cytoplasm, as if the drawing together of ribosomes into polysomal arrays necessarily leaves some of the matrix depleted. At this stage, the endoplasmic reticulum is still poorly developed and probably some of these cells present an ambiguous image in the light microscope. The next intermediate, represented by Fig. 9, in which the polysomal configuration is more common, may also be difficult to classify in the light microscope because although distinct Nissl bodies are evident, there are still many monosomes in the intervening clear areas. With progress toward the more typical end of the spectrum, the endoplasmic reticulum becomes more extensive, more elabo-

FIGURE 6 Centriole and endoplasmic reticulum in a small pyramidal cell in visual cortex. Although most of the free ribosomes are randomly dispersed, some of the ribosomes attached to the upper cisterna are aligned in regular intervals (arrows). These are the only examples of arrayed ribosomes that could be found in this cell, which thus has an intermediate ribosomal distribution between random and ordered. Layer IV, area 17 (macular region), *M. mulatta*. × 76,000.

FIGURE 7 Perinuclear cytoplasm of a medium-sized pyramidal cell in visual cortex. Most of the ribosomes, whether attached or free, are randomly distributed as single particles. Several *en face* views of the endoplasmic reticulum (*er*) demonstrate the lack of order in attached ribosomes. A fragment of the nuclear envelope (*n*), sectioned tangentially, lies in the middle of the field. In addition to the *en face* views of several nuclear pores and of ribosomes randomly arranged on its outer surface, the envelope also exhibits a single chain of attached ribosomes (arrow), which may represent the beginning of recovery from a pause in protein synthesis. Layer IV, area 18 (macular region), *M. mulatta*. × 38,000.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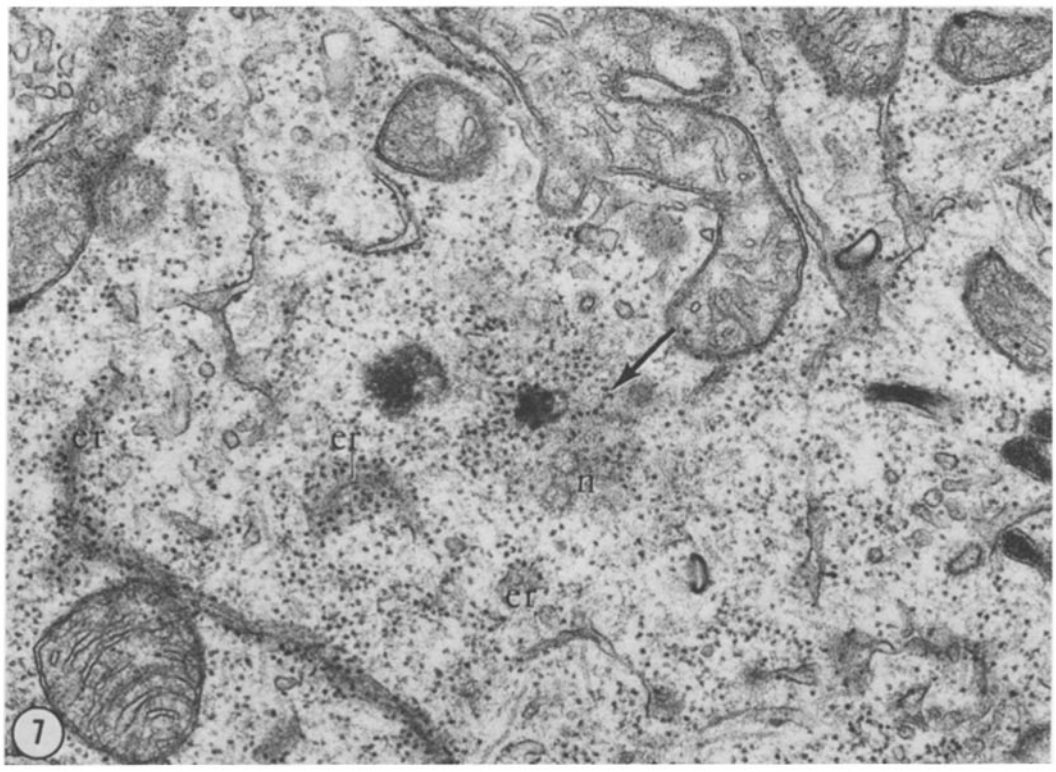
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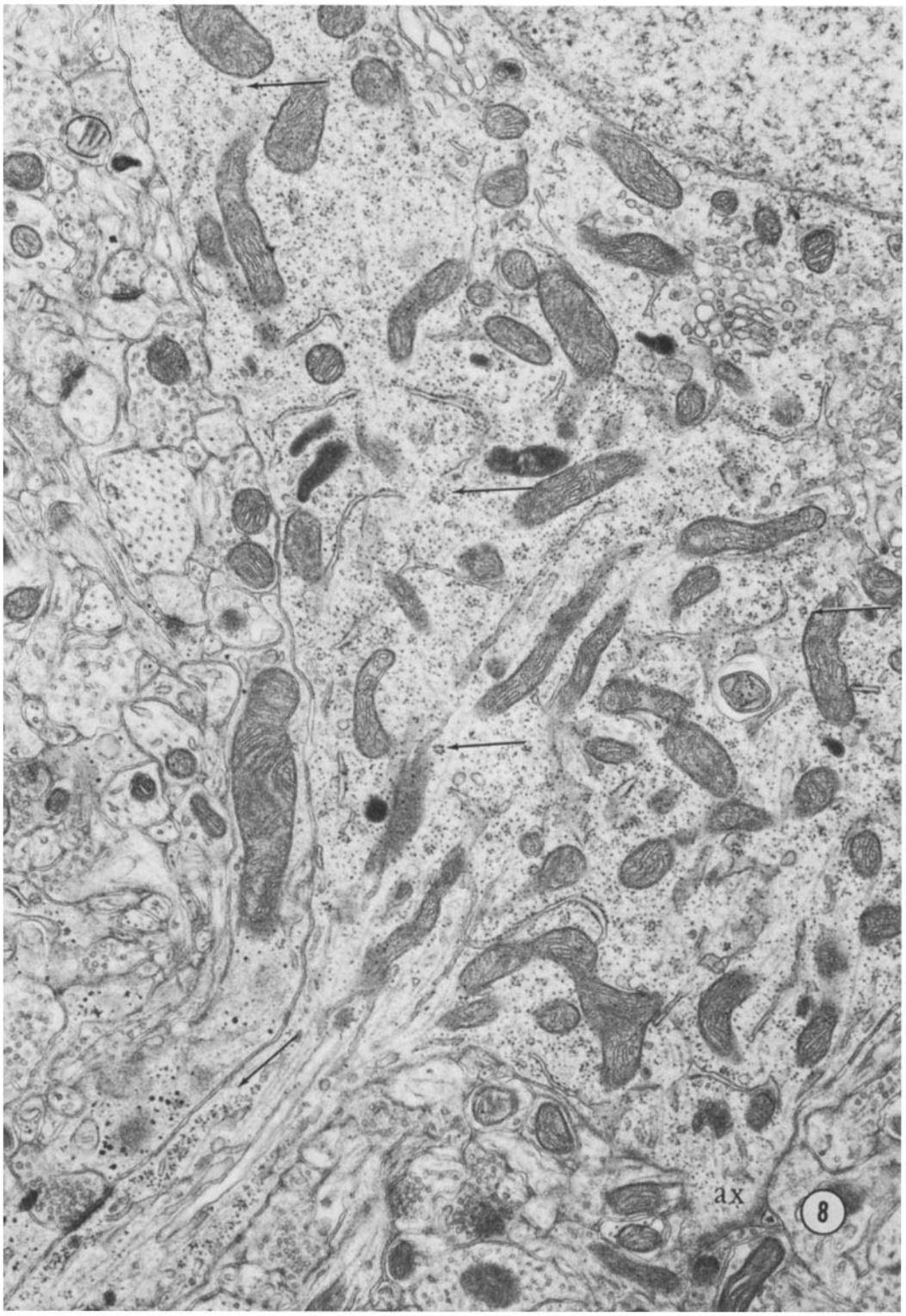


FIGURE 8 Perikaryon of an intermediate small pyramid in visual cortex. The form of the endoplasmic reticulum is stringy and large fields of free ribosomes lie between the cisternae. Although most of these particles are randomly disposed, clusters appear in various places (arrows), notably in the dendrite going off into the left lower corner of the picture. This dendrite receives a small axonal bouton synapsing on its surface. The section just grazes an initial axon segment (*ax*) emerging in the right lower corner. Layer III, area 17 (macular region), *M. mulatta*. × 21,000.

rate, and more regularly arrayed, while nearly all of the ribosomes adopt a polysomal disposition (Figs. 10, 11). The sequence of this description is, of course, arbitrary and interpretative. The intermediates could proceed in the opposite direction, or they could be regarded not as transition stages but as permanent states with no relation to one another.

Such intermediates were very frequent in layers III and IV of all of the visual cortical areas, 17, 18, and 19. Like the cells with completely dispersed ribosomes, the intermediates are much more frequent in area 18 than in area 17. In area 19 they are uncommon but more widespread, for they were encountered not only in layers III and IV but also in the deeper parts of layer II. The ambiguity introduced by the existence of the intermediate neurons made it impossible for us to obtain any meaningful counts of the cells with wholly dispersed ribosomes or any estimates of their proportions in the different areas of the visual cortex. Rigid criteria for recognizing them at the light microscope level could not be established. Counts at the electron microscope level were also considered unreliable since the samples were necessarily too small at the magnifications that were necessary for correct diagnosis.

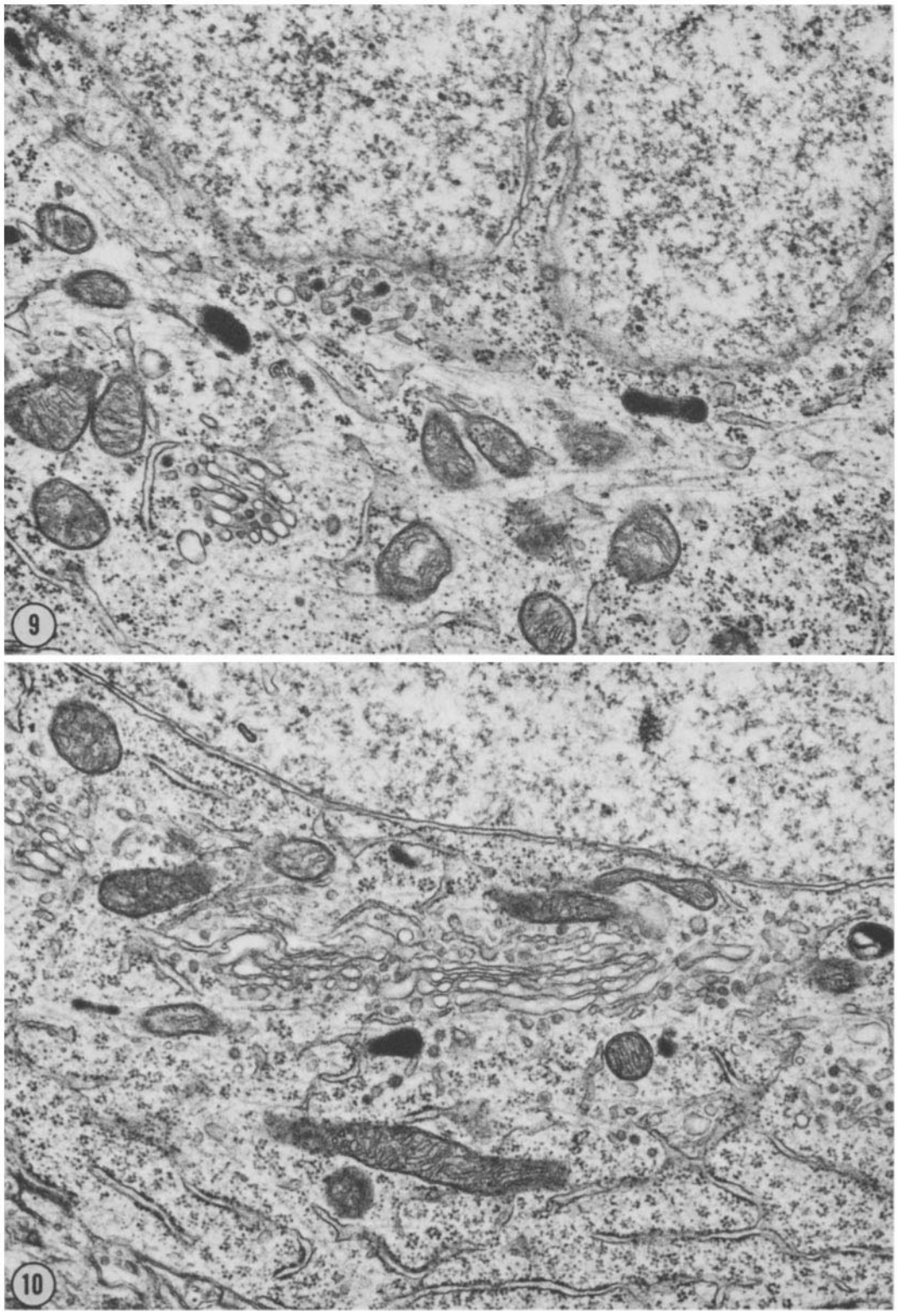
DISCUSSION

The unusual disposition of the ribosomes in the neuronal perikarya described in the foregoing observations raises the question of its reliability. Since there are no signs of inadequate cytological fixation it is unlikely to be an artifact of the preparative procedures. Neighboring cells, of the same size and depth in the cortex, can have the typical polysomal pattern or dispersed ribosomes. The neurons in question display no other features that could be considered abnormal, such as swollen mitochondria, pyknotic or extracted nuclei, expanded nucleoli, or shrunken or exploded endoplasmic reticulum. Similar ribosomal patterns are absent from other cortical structures in the same animals, e.g. the cerebellar cortex, which is notoriously susceptible to fixation artifacts. These cells do not have either the disrupted and extracted or the condensed and dark appearance of poor fixation. Furthermore, it seems highly unlikely that such cells would appear with the regularity and distribution that they have in different animals if they were the uncontrolled results of fixation artifacts.

It is less easy to rule out the possibility that the ribosomal pattern is the expression of some disease. All of the animals used in this study appeared to be vigorous and healthy. They had been born and reared in the colony of the New England Regional Primate Research Center or purchased from dealers and held in that colony for several months to assure that they had no disease.

It has been shown (36) in rats that malnutrition during the neonatal period results in reduced total cellular RNA and decreased protein synthesis in the brain. The normal polysomal pattern is said to be disrupted. The dispersal of ribosomes described in the present paper might be attributable to lack of adequate nutrition. However, our animals were all in a good nutritional state, judging from their plentiful subcutaneous and omental fat. Since they had been kept in our small animal facility with free access to food and water only overnight or a few hours before perfusion, it seems unlikely that they could have consistently developed an acute nutritional deficiency in this short time. Furthermore, the selective distribution of the affected nerve cells seems hardly compatible with a factor so general as undernutrition. These animals were, however, kept in confined quarters (compared with their normal free range) for indeterminate lengths of time before death. They may have suffered from the limitations of this environment. Although the effects of prolonged confinement in cages on the structure of the central nervous system are unknown, there is some evidence that enrichment of the environment and experience of young animals has a positive effect on the thickness of the cortex and the protein metabolism of the brain (8, 9). It has also been reported that training adult rats to a specific task results in a significant increase in the ratio of polysomes to single ribosomes extractable from whole brain by density gradient centrifugation (7). Studies on the cytological effects of limited environments might be informative.

It is also possible to consider that these cells are specifically and selectively diseased. The healthy appearance of the nuclei and of all the cytoplasmic organelles except for the ribosomal arrays militates against such an interpretation. So does the presence of normal synaptic terminals attached to the surfaces of their perikarya and dendrites. It is instructive to compare these cells with neuronal perikarya undergoing chromatolysis after interruption of their axons. This injury results in one of the gravest disturbances known in the morphology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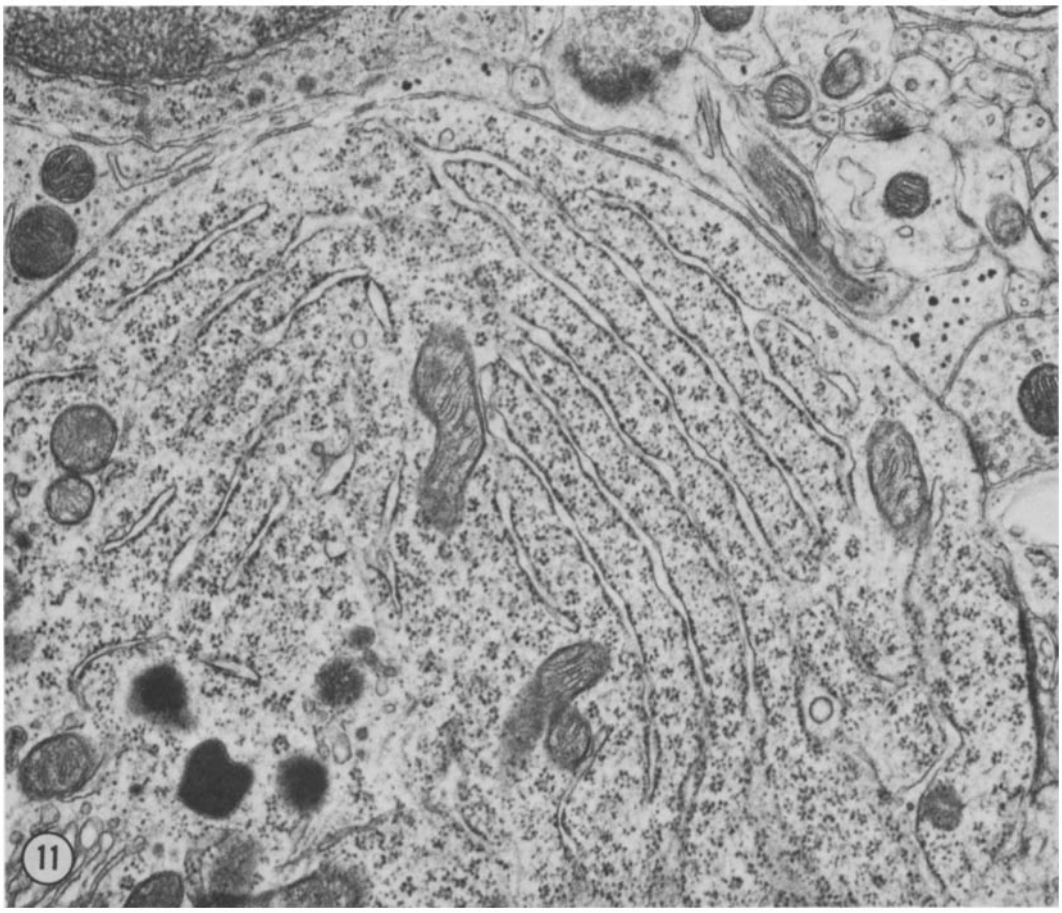

FIGURE 11 Typical Nissl substance in a medium-sized pyramidal neuron in visual cortex. This type of Nissl body produces the multistriate appearance seen in light micrographs (Fig. 4). Nearly all ribosomes are confined to polysomes. Layer IV, area 17 (macular region), *M. mulatta*. × 27,000.

of the perikaryon. The nucleus swells and moves eccentrically, the cytoplasm swells and becomes pale. At the fine structural level, the great majority of the ribosomes detach from the endoplasmic reticulum, which fragments into small islands. Synaptic terminals separate from the surface of the cell and their places are taken by neuroglial processes. Despite this devastation of the normal architecture, the free ribosomes remain aggregated in polysomal clusters (5, 6, 19, 27, 30). Embryonic cells and early differentiating cells also have more dispersed ribosomal arrays than their more mature

FIGURE 9 Perinuclear area of a small pyramidal cell in visual cortex. This field shows an intermediate stage in the distribution of ribosomes. The formation of Nissl bodies is exhibited in the right and left lower corners and adjacent to the nucleus. Here the ribosomes tend to clump into the usual patterns. Elsewhere the cytoplasm is strewn with single, random particles, and clearings have appeared through which the microtubules and neurofilaments stream. Layer IV, area 17 (macular region), *M. mulatta*. × 27,000.

FIGURE 10 Perinuclear area of a small neuron in visual cortex. This field shows completely typical neuronal cytoplasm with an immediately perinuclear rim of Nissl substance and a broad peripheral Nissl mass separated from each other by the Golgi apparatus. Few ribosomes appear to be distinct from clustered patterns. Layer III, area 17 (macular region), *M. mulatta*. × 26,000.

forms in the adult animal. But here, too, the ribosomes, although crowded and diffusely distributed, are present in polysomal aggregates. Disaggregation appears in certain conditions when cells die slowly, but then serious alterations in the nucleus, nucleolus, mitochondria, and Golgi apparatus also occur, usually accompanied by an increased number or size of lysosomes and autophagic vacuoles (23). Thus the conclusion seems inescapable that the cells under consideration are in a peculiar but physiological state.

In order to understand the significance of the ribosomal patterns found in these cortical neurons, it would be necessary to have data on their protein synthetic capacity and their nucleic acid metabolism. Lacking such information, however, we must draw on experimental work in other systems for suggestions. Biochemical studies of the past decade have made it abundantly clear that in both prokaryotic and eukaryotic cells polysomal arrays and not single ribosomes are the active sites of protein synthesis (*inter alios*, 1, 3, 10, 12, 18, 20, 33, 34). This central conclusion derives from elucidation of the successive steps in mRNA-directed protein synthesis. During active protein synthesis a cyclic interchange occurs between the members of the polysomal arrays and a pool of ribosomal subunits. Single ribosomes appear to lie on a side track that is not an essential part of this cycle. According to the widely accepted formulation of the events in protein synthesis (10) the cycle begins with complexing of a small (30s) ribosomal subunit with a molecule of initiation factor IF-3. This is followed by the formation of an "initiation complex," including messenger RNA, aminoacyl transfer RNA, GTP, and other initiation factors. Initiation factor IF-3 is eliminated as a large (50s) ribosomal subunit is added on, thus making a complete 70s ribosome, bound to a strand of messenger RNA and active in the assembly of polypeptides. Several monosomes can be successively attached to a single messenger RNA, the number depending upon the length of the mRNA strand (31, 34), and such a group of active ribosomes constitutes a polysome. As translation proceeds, each ribosome shifts one triplet at a time along the messenger RNA until it reaches a specific termination codon, whereupon polypeptide assembly ceases, and the ribosome and its product are released.

Kaempfer (13–18) has shown convincingly that the separation of a ribosome from the polysomal array at the end of the polypeptide synthesis involves its dissociation into its 30s and 50s subunits. When the conditions for active protein synthesis prevail, the 30s subunit is immediately complexed once more with initiation factor IF-3, joined with mRNA in an initiation complex, and passed through the protein-synthesizing ribosome cycle again. Thus it is effectively prevented from joining with a 50s subunit to form a single, free ribosome. The evidence for these conclusions was presented in a series of papers describing ingenious experiments analyzing the kinetics of ribosomal subunit exchange in *Escherichia coli* (13–18). When the conditions for protein synthesis are not favorable, e.g. exhaustion of substrates, reduced temperature, presence of certain inhibitors like puromycin, single ribosomes accumulate (29). Furthermore, the properties of single ribosomes indicate that their composition is different from that of the monomers of the polysomes (14), e.g. they require a higher Mg^{++} ion concentration to prevent their dissociation into subunits, they sediment more slowly than monosomes, and they are dissociated more readily by Na^{+} ions and by ribonuclease. All of these differences in properties and behavior lead to the conclusion that single ribosomes "constitute side-track products of the ribosome cycle which are bypassed by ribosomes (polysomes) and subunits active in protein synthesis" (14, p. 537).

Studies on eukaryotes also indicate that single ribosomes do not actively engage in protein synthesis. In the first paper to propose that polysomal arrays have a specific role in protein synthesis and to suggest that the monosomes are held together by mRNA, Warner, Knopf, and Rich (34) demonstrated that the single ribosomes of reticulocytes were not active in polypeptide assembly. According to Marks, Rifkind, and Danon (20) 80–90% of the ribosomes in immature reticulocytes are aggregated into polysomes. As the reticulocytes mature there is a decrease not only in the capacity of the polysomes to synthesize protein, but also in the size and number of the polysome clusters, and there is an increase in the proportion of single ribosomes. In growing HeLa cells forming protein at nearly maximal rates, newly synthesized ribosomal subunits equilibrate very rapidly with polysomes but much more slowly with the pool of single ribosomes (11). Similarly in the skeletal muscle of chick embryos, Kabat and Rich (12) showed that newly synthesized subunits pass rapidly into the fraction of polysomes but very slowly

into the single ribosomes. They concluded that only 10% or less of the single ribosomes could be involved in the ribosomal cycle that is a necessary part of protein synthesis. Furthermore, in a cell-free system reconstituted from reticulocytes, Adamson, Howard, and Herbert (1) demonstrated that hardly any exchange takes place between ribosomal subunits and the single ribosome pool during the time it takes for at least six to eight rounds of translation in an actively synthesizing incubation mixture.

Becker and Rich (3) analyzed a system that is perhaps closer to our concern in the present paper. They studied alterations in the polysome distribution of lymphocytes in immunized rabbits. They found that 25–35% of the ribosomes in the cytoplasmic extracts from lymph nodes of immunized rabbits were in polysomal aggregates, in contrast to 15% or less in nonimmunized animals. This increase parallels the increase in specific protein synthesis associated with antibody production, but is considerably less than the percentage of ribosomes in the polysomal mode found in bacterial extracts, chick embryos, or mammalian cells in tissue culture. The difference is probably attributable to the dilution of the specific antibody-producing cells by the other, nonreacting cells in the lymph nodes.

Still closer to our present concern are recently reported experiments showing that a single injection of L-phenylalanine into immature rats produces a rapid disaggregation of the ribosomes extracted from cerebral cortex (but not from liver) and that this effect is accompanied by depletion of brain tryptophan and by markedly impaired protein synthetic capacity (2). Injections of L-dopa into immature rats, with larger doses in older rats, also produce disaggregation of brain polysomes, but although the effect is accompanied by reduced protein synthetic capacity, there is an increase in the concentration of tryptophan in the brain (21, 35). The explanations for these effects are not clear at present, but the correlation between disassembly of the polyribosomal arrays and the reduction in protein synthetic activity is consistent with the results in other systems cited above.

It would be very difficult to obtain biochemical evidence regarding the protein synthetic activity of the cortical neurons described in the present paper. Because of their location and their relative numbers, they would be highly diluted in any extraction procedure designed to separate their ribosomes, but unlike the antibody-producing cells in the lymph nodes studied by Becker and Rich (3), these cells would be overwhelmed by cells yielding large amounts of active polysomes. Furthermore the other cell types, neuroglial and vascular cells, may contribute an indeterminant amount of inactive or active ribosomal material. Nevertheless, fractionation on gradients coupled with autoradiography of sections for identifying the source might give some interesting results.

In view of the biochemical evidence in other systems, both prokaryote and eukaryote, it may be permissible to predict that these cortical nerve cells with highly dispersed, randomly arrayed, single ribosomes are probably inactive in protein synthesis or at most carry it on at a low level. But how can a nerve cell survive such an interruption or lag in protein synthesis? The typical nerve cell is thought to sustain a high rate of protein synthesis, enough to replace one-third of its protein each day, at least enough to keep up with the amounts lost through somatofugal axoplasmic transport. An interruption of synthetic activity, if prolonged, should ordinarily lead to ruin. But the healthy nuclei, well-granulated nucleoli, and otherwise normal cytoplasmic organelles suggest that the dispersed state of the ribosomes reflects only a pause in the protein synthetic activity of the cells under consideration here. Perhaps the axoplasmic flow has been temporarily stemmed, perhaps a preceding surge in synthesis has exhausted the supply of substrates, mRNA, or initiation factors. Kaempfer (15) suggested that the balance between a critical initiation factor like IF-3, which is usually in short supply, and the ribosomal subunits could provide a mechanism for the control of the number of active ribosomes and thus of protein synthesis. Whatever the reason, it would appear likely that the suspension of protein synthesis is a phase in the cyclic function of these cells. The return towards full activity, or alternatively the last stage before complete cessation, may be signalled by the few polysomes—small clusters and strands of ribosomes—that appear in the intermediate cells. These may be cells that are passing into or out of the lull phase in synthesis, when all ribosomes are completely dispersed. If such cycles in protein synthetic activity exist in these nerve cells, they might coordinate with some aspect of cellular behavior, for example, with such variables as the changing rhythm of spontaneous discharges, or fluctuations in the level and specificity of

stimulus thresholds, and these in turn might reflect the response of the cell to such variables in its environment as the diurnal rhythm of the organism.

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