Acta Neuropathologica 1, 245—270 (1961)

Laboratory of Neuroanatomical Sciences, National Institute of Neurological Diseases and Blindness, National Institutes of Health, US Public Health Service, Department of Health, Education and Welfare, Bethesda, Maryland

The Importance of Avoiding "Dark" Neurons in Experimental Neuropathology

By
JAN CAMMERMEYER

With 9 Figures in the Text

(Received May 13, 1961)

Introduction

The interpretation of the "dark" neuron is probably one of the most controversial subjects in neuropathology. Despite the repeated demonstration that the occurrence of the "dark" neuron is caused by post-mortem traumatization of nervous tissue [33,45–47,139–141,155], this cell change has been attributed to such a genesis in relatively few neuroanatomical and neuropathological studies*. However, a review of the overwhelming literature dealing with this neuronal type, to which many names have been attached, reveals consistent exposure of the materials to post-mortem trauma [25]. If such an extraneous factor could be avoided, much of the dispute about these cells would be resolved; the current procedure of a two step perfusion followed by a delayed autopsy offers such an opportunity [18,20,21]. This will be of benefit to the entire tissue because the whole "artifact complex", including the "dark" neuron, can be avoided. When it is present, any cytological investigation is impeded, but when absent, research of subtle cytological characteristics is invited. In order to appreciate these opposed experiences, the following two series of observations are reported: one is based on material with "dark" neurons, and one without.

Material and Technique

Various normal and experimental animals have been prepared by different histological techniques, the procedure of which is indicated for each material studied because it forms an integral part of the subject under discussion. Some of the present material, as well as details of the histological technique and karyometric method, has been described elsewhere [20,21,25].

Presentation of Material

1. *Material with "Dark" Neurons*

a) Characteristics of "Dark" Neurons. The "dark" cell type discussed in this paper is noted for its shrunken cytoplasm containing compacted basophil material causing the dark hue, and for its separation from surroundings by a space of varying length and width. The shrunken nucleus usually blends with the "dark" cytoplasm, but it is sometimes surrounded by a rim of clear cytoplasm. The nucleolus is noted for its relatively large size; this nucleolus in fact is unaltered because it belongs not to the small "dark" neuron but to a much larger cell [21], the

* s. Lit. [10,12,15–18,20,21,25–27,51,86,101,102,118,121,122,144,161,162].

boundaries of which correspond to the outer limits of the perivascular space[76]. The processes, because of shrinkage, assume an irregular corkscrew-like shape (next to o in Fig. 1C; Fig. 2C).

The size and shape of "dark" neurons will vary according to the cell type involved. They are triangular in the pyramidal cell layer of the cerebral cortex or brain stem nuclei (d in Fig. 1A); elongated and irregular among the small elements in the deep layers of the cerebral cortex (Fig. 1C); polygonal or lanceform in the Purkinje cell layer (Fig. 1E); multifaceted in the sympathetic and sensory ganglia.

The number and distribution of "dark" neurons vary greatly; sometimes numerous neurons are afflicted bilaterally and at other times only a few unilaterally (Fig. 1D). Under all circumstances, there is a rather haphazard intermingling of the "dark" and "clear" cell types (Figs. 1C and 2C).

b) Other Histological Abnormalities Associated with "Dark" Neurons in the "Artifact Complex". The transformation of neurons to "dark" cells in a traumatized region depends on the amount of their basophil material; if a cell contains, in addition to the basophil material, a large amount of fat, then it will appear as a fat filled contracted neuron, "liposclerosis", but if it has no cytoplasmic material, only the nuclear shrinkage will show the evidence of damage, as so-called nuclear "pyknosis". The last type of change is noted in granule cell layers of the cerebral cortex and spinal cord. The neuroglial cells will respond in a similar manner with nuclear "pyknosis"; oligodendrocyte nuclei exhibit a characteristic "halo" (o in Fig. 1C) or what has become known as "acute swelling"[120]. Typically, blood vessels with distorted lumen and course are surrounded by wide spaces (v in Fig. 1C). Myelin sheaths and axons are of uneven diameters and no longer arranged in straight parallel bundles*; the former may undergo "pseudomarchi" degeneration. The cerebellum suffers by a loosening of the molecular layer from the granule cell layer leaving along the base of Purkinje cells an irregular cleft, so-called "lamina dissecans sive separans" (Fig. 1E).

Neurons imperfectly fixed by the perfusion of CARNOY's solution may resemble so-called "Wasserveränderung"[21]; their cytoplasm is attached only to that part of the cellular membrane nearest the blood vessel (Fig. 1B comparable to Fig. 5F in[21]).

c) Occurrence of "Dark" Neurons. The conditions under which "dark" neurons and associated changes occur, vary greatly. The most common one is at autopsy with removal of the organ for subsequent immersion in a fixative. The compression by a rongeur, or other instrument for exposure of the brain, the incision by a knife, the pull on blood vessels or nerves during the autopsy will all produce numerous "dark" neurons nearby. These cell changes develop even when the brain is removed as quickly as possible and immediately immersed in rapid fixatives like HEIDENHAIN's Susa or BOUIN's solutions, or in a slow fixative like 10% formalin. Guinea pigs (GP7, 8, 11 and 12-112957C) were narcotized and killed with chloroform, the skull removed and the brains submerged at varying intervals, as 7 or 8 min, and $3^1/_2$ hrs. After the necessary length of fixation, the tissues were embedded in paraffin. Numerous "dark" neurons are demonstrated in all preparations. Neither immediate nor delayed autopsy is of any help[21,25] (Fig. 1, A, C, D and E).

If an attempt be made to minimize the trauma, still the "dark" neurons form. Both hemispheres were exposed in an adult male cat (C1-020960C) in deep pentobarbital narcosis. Small pieces of one hemisphere were cut free and allowed to drop directly from the hanging head, one into HEIDENHAIN's Susa solution and one into BOUIN's solution. The animal was killed by an overdose of pentobarbital. The remaining head, after ligation of the neck to avoid venous congestion, was placed upside down in a jar filled with HEIDENHAIN's Susa solution for 4 hrs. Then the brain was removed and the fixation of the freed organ continued for 6 hrs. Paraffin sections of the biopsy specimens, as well as the operated brain, contain numerous "dark" neurons. None appears in the exposed non-operated hemisphere. These results indicate neurons are extremely sensitive to trauma.

In an attempt to demonstrate when the neurons acquire such sensitivity to trauma, the hemispheres of a cat were traumatized by an even and gentle stroking prior to and after inter-

* The tortuosity of fibers is most severe in material fixed by immersion in 10% formalin; it is still present in rootlets, but less developed in spinal cords after two step perfusion with 10% formalin in a gum acacia-saline solution; and fibers are of even course and thickness in both rootlets and spinal cords prepared by the current Travenol-Susa perfusion procedure (compare p. 251).

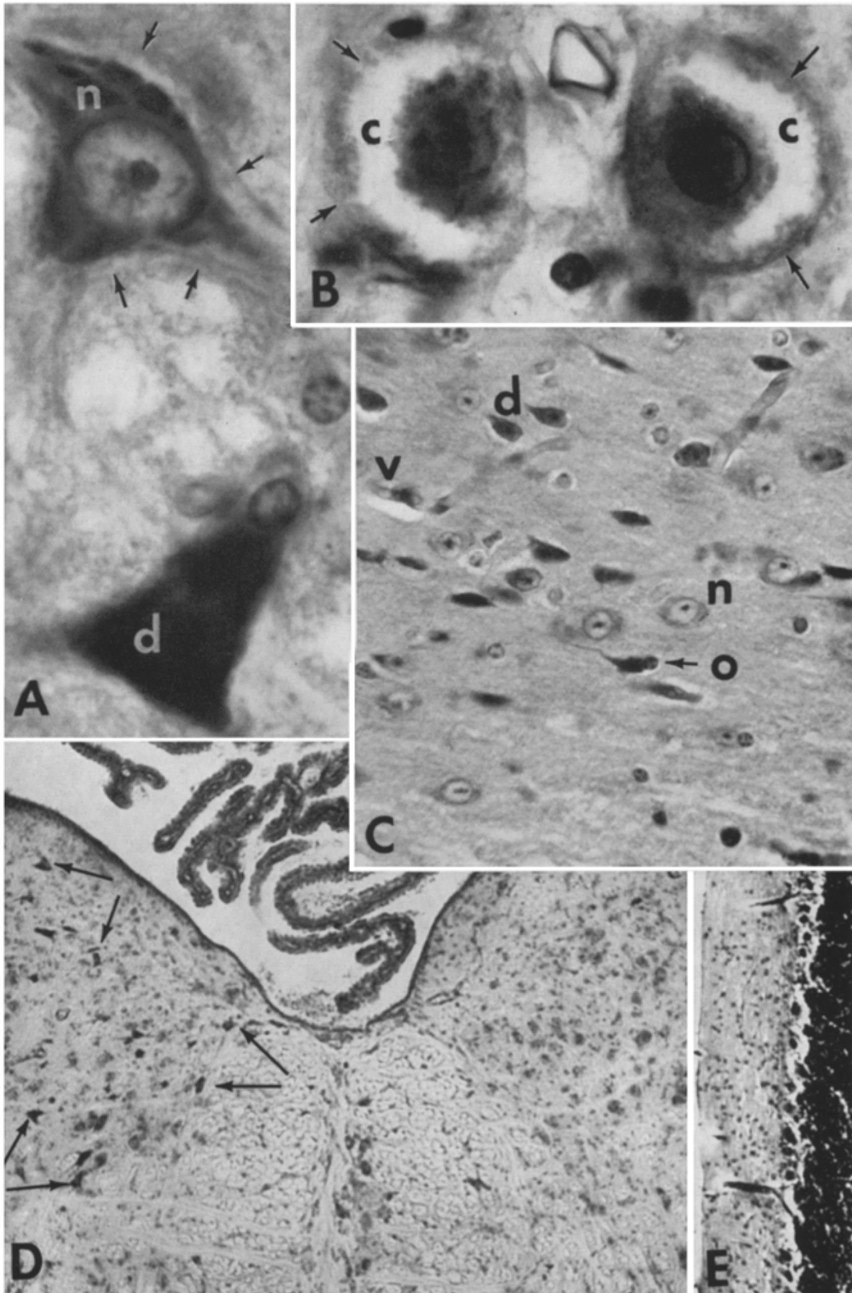

Fig. 1. A A "dark" (*d*) and "clear" neuron with a distinct "peripheral zone" (arrows). Reticular formation of guinea pig. Fixed by immersion in HEIDENHAIN's Susa solution 6 min after death. Periodic acid Schiff and gallocyanin-chromealum (PASG). GP 12-112957 C. Green filter (×1200). B Cells resembling "Wasserveränderung" with separation of perinuclear cytoplasm from membrane (arrows). Vestibular nucleus of cat. Two-step perfusion using CARNOY's solution as the fixative. PASG. C1-050958 C. Green filter (×1200). C Cortical "dark" neurons (*d*) of varying shape, normal "clear" neurons (*n*), acute "swollen" oligodendrocyte (*o*) and perivascular space (*v*). Cerebral cortex of guinea-pig. Same as Fig. 1 A (×399). D Groups of "dark" neurons between arrows on one side. Brain stem of guinea pig. Same as Fig. 1 A (×82). E Separation of molecular layer from granule cell layer. Cerebellar cortex of guinea pig. Immersion fixed in 10% formalin 7 min after death. PASG. GP11-112957 C. Green filter (×82)

rupted blood circulation. One side was touched while the animal was narcotized, 4 min prior to cannulation of the heart, and the other side 3 min thereafter. An adult male cat (C1-011261C) was kept in deep pentobarbital narcosis. Within 30 min the skull was removed on the left side, the dura opened and 2 min later a metal spatula used to stroke gently several times the cortical surface without severing blood vessels. The cat was turned on its side and the thorax opened 1 min after the left cerebral cortex had been touched. Another 1 min passed before 2 ml of 1% heparin was injected in the heart, and 2 min were required to insert a glass cannula in the ascending aorta and attach it with a ligature. Thus, the systemic blood circulation was

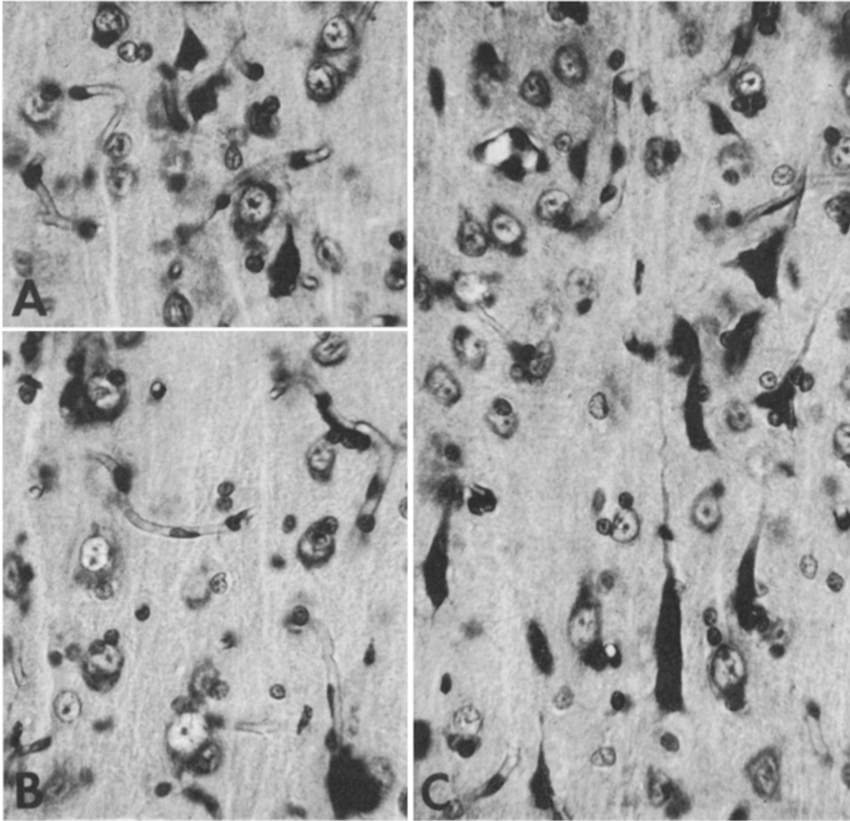

Fig. 2. A Cortical "dark" neurons subjacent to the edge of skull opening performed during narcosis. Left cerebral hemisphere of cat. Travenol-Susa perfusion (compare p. 248), autopsy delayed 11 hrs. PASG. C1-011261C (×82). B Normal neurons of cortex exposed during narcosis. Same as in Fig. 2A (×82). C "Dark" neurons of varying size throughout cortex exposed post-mortem. Same as in Fig. 2A (×82)

interrupted 4 min after compressing the left hemisphere. The skull over the right hemisphere was removed quickly, the dura opened, and 3 min after cannulation of the heart, a spatula was used to stroke the cortical surface, as on the left side. Again special care was taken to avoid rupture of blood vessels. After another minute the blood vessels were flushed with a complex salt solution (Elliott's "B" Travenol, Baxter Laboratories) with 0.5% formalin and after 2 min a modified HEIDENHAIN's Susa solution was perfused (hereinafter, this procedure is referred to by the term Travenol-Susa perfusion). Although the perfusate bottles were only 67 cm above the heart, the surface of the left hemisphere bulged slightly and abutted the medial edge of the skull opening. The fixed body was not touched for 10 hrs. The autopsy revealed perfect fixation of the brain. The microscopic study of the left hemisphere discloses that the exposed part of the cerebral cortex is entirely free of "dark" neurons (Fig. 2B), and only a few neurons subjacent to the medial skull edge are "dark" (Fig. 2A); in contrast, the cerebral

cortex of the exposed right hemisphere has a vast number of small, medium and large "dark" neurons (Fig. 2C). This experiment points out that combined trauma due to removal of skull bones and the direct touch of a spatula is tolerated by the neurons of a narcotized animal, but no longer by those in a tissue with interrupted systemic blood circulation or in the dead animal. If during fixation the brain is inadvertently touched, as when it abuts the skull edge, the neurons react abnormally. It should be remembered, when an organ is being fixed by perfusion it must not be touched until fixation is complete, which requires several hours. According to these results, the neuron assumes vulnerability to a traumatic insult at the moment of heart arrest and then transforms to a cell with "dark" perikaryon and/or "pyknotic" nucleus.

The perfusion procedure has long been adopted with the understanding that this is an ideal means of fixation for various purposes and mainly to avoid artifactual changes[5,8,9,27,32,80,88,138,142]. Unquestionably, the "dark" neurons have been ignored by many of the authors studying materials fixed by perfusion; they have been regarded as normal cell structures by some, pathological by others, and their causation by failures of the procedure has scarcely been discussed, except by COTTE[27]. If the fixative did not reach the capillaries, then neurons in the unfixed soft tissue would be just as vulnerable as in brains removed for subsequent immersion in a solution. Several factors could account for such a failure or spotty absence of tissue fixation, as e.g., air bubbles, impurities of the perfusates, coagulation of blood, low hydrostatic pressure, and short interval between death and perfusion[21,25]. If the blood vessels were torn by an operation or exhibited abnormal permeability due to an acute pathologic process with edema, the perfusates would not be properly dispersed, and there would be focal lack of fixation; again, "dark" neurons would be developed within the soft regions. Another recently discovered important factor is the time needed for the perfused fixative to complete the attachments of cellular membranes to surroundings so they no longer detach as the consequence of post-mortem traumatization during the autopsy. If in such a critical period the skull is removed, the brain frozen, or part of the surface scratched, as may occur when the brain touches the edge of a trephine opening during perfusion (Fig. 2A), then the "dark" neurons will be present within the afflicted areas. This critical period begins with the arrest of blood circulation, and its length can empirically be determined for several of the routinely used fixatives by performing the autopsies at various intervals after perfusion; for HEIDENHAIN's Susa and BOUIN's solutions, 4 and 6—8 hrs are required[20,21,25]. For 10% formalin either alone or with admixtures of gum-acacia in saline, after the lapse of 24 hrs the neurons are not yet quite fixed and many of them will react to trauma. Sulfosalicylic acid and para-toluene sulfonic acid have reportedly been used with excellent results[98].

Through these studies, it was noted that formalin was not foolproof in fixing the cellular membranes in such a manner that the neuron would no longer react to post-mortem traumatization with the formation of a "dark" cell. This failure is apparently also responsible for changes seen in tissues exposed to extreme refrigeration or frost. In a series of experiments, 23 cats were killed by chloroform and each hemisphere was placed in a "deep freeze" compartment (—15 or —20° C) for varying lengths of time (6, 12 or 24 hrs) prior to fixation and at different intervals after being either immersed in or perfused with 10% formalin. Whether the freezing took place immediately or after an interval of several days up to 2 weeks, the frozen brains are pierced by numerous holes of varying size, except for a narrow subpial zone. The septae separating the holes contain either normal neurons or compressed "dark" neurons lined up like "schools of fish". Many of the neuroglia cell nuclei are pyknotic. Also the white matter shows changes either as small holes or irregular zones of condensed tissues. These holes are often filled with a granular metachromatic substance, resembling the material of Buscaino "plaques" of ordinarily fixed brains. The holes are relatively small and often continue deep into subcortical white matter in brains frozen simultaneously with immersion in 10% formalin (Fig. 3A). If freezing was delayed, the gray matter is heavily cavitated and the white matter unaffected (Fig. 3B). Perfusion with 10% formalin in saline had no alleviating effect on the freezing damage; however, the white matter is less altered than after immersion fixation for the same length of time (Fig. 3C). If the brains were frozen immediately after removal and then allowed to thaw in 10% formalin, numerous "dark" neurons are present, however, no clefts (Fig. 3D). No vacuoles are demonstrable if the organ was frozen few or several hours after the Travenol-Susa perfusion. Such tissues show minor abnormalities in the nuclei of Purkinje cells and around astrocyte nuclei, i. e., a disarrangement of nuclear chromatin

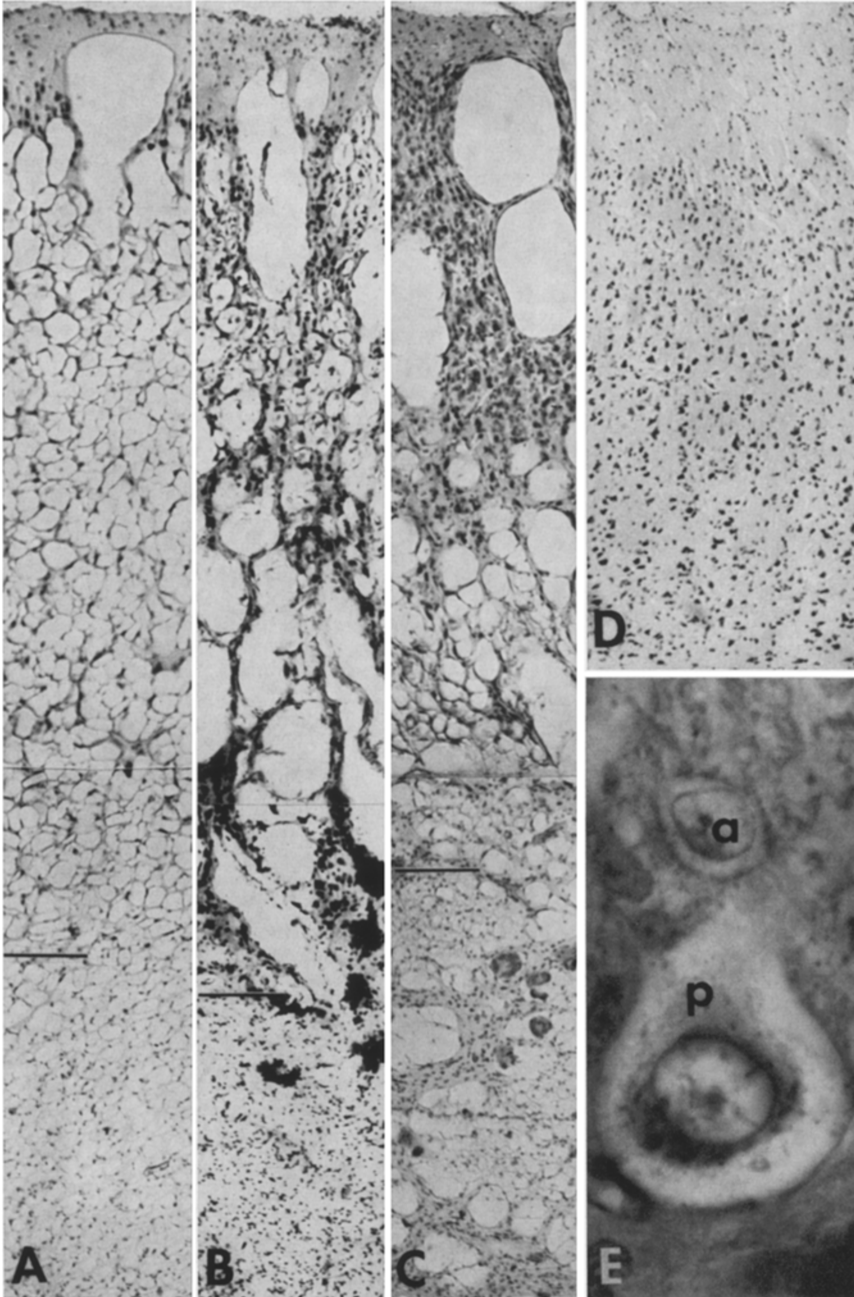

Fig. 3. A Fixed by immersion in 10% formalin and frozen simultaneously, 41 min after death. Hemisphere of cat. Gallocyanin-chrome alum. C2-110756 CII (×82). B Fixed by immersion in 10% formalin 20 min after death and frozen 4 days later for 24 hrs. Hemisphere of cat. Gallocyanin-chrome alum. C1-112056 CII (×82). C Fixed by perfusion of 10% formalin with 5.6% gum acacia in saline and frozen 1 hr later. Hemisphere of cat. Gallocyanin chrome alum. C1-113056 CI (×82). D Frozen 42 min after death for 24 hrs and then thawed in 10% formalin. Hemisphere of cat. C2-110756 CI (×82). E Purkinje cell (*p*) and astrocyte nucleus (*a*). Cerebellar molecular layer of cat. Travenol-Susa perfusion, autopsy delayed 7 hrs, and 17 hrs thereafter frozen. PASG. C3-051359 CB (×1200)

and cytoplasmic periodic acid Schiff (abbreviated PAS) stained materials, respectively (p and a in Fig. 3 E)*. Because of these changes, tissues exposed to freezing are no longer useful for histological studies. The changes are severe enough to overshadow pathological changes, but they are characteristic enough to permit an exact diagnosis of the artifactual cause of tissue vacuolization and cellular changes (compare [15,35,73,163]).

As a final test, only the skull was removed from a narcotized animal, and then all precautions taken to obtain a perfect fixation by perfusion and to delay the autopsy to avoid "dark" neurons. An adult cat (C1-021060C) was narcotized with pentobarbital. The skull was removed over the right hemisphere and the dura split. The brain surface, moistened with saline, fell in below the skull edges. 10 min later, the cat was turned carefully over on the right side and after opening of the chest given 1 ml 10% heparin intracardially. After another 2 min the first step of the Travenol-Susa perfusion was begun, and 4 min thereafter, the second step. The bottles with perfusates were placed 68 cm above the heart, sufficient to avoid bulging of the brain; it was entirely blanched within 30 sec after circulation of the fixative. The head was not touched and the autopsy was delayed 6 hrs. The microscopic sections are entirely free of "dark" neurons, again demonstrating that the living neurons can tolerate both the exposure to atmospheric pressure and a trauma, as the simple removal of skull, which in the dead would inevitably result in the formation of "dark" neurons.

2. *Material Free of "Dark" Neurons*

a) Principle of Preparation whereby the "Artifact Complex" is Avoided. After having reviewed the conditions which contributed to the formation of "dark" neurons, the ensuing problem became imminent: to ascertain the conditions under which "dark" neurons can be prevented[25]. This being achieved, the principle of what might be called perfect fixation for studies with the compound microscope was established[18,20,21,25] and thus answered the intriguing question: What is the objective of fixation?

If following the flushing of blood vessels, a rapid fixative either HEIDENHAIN's Susa or BOUIN's solution is used, and the autopsy is delayed several hours, then not only are all neurons of the same appearance, but "dark" neurons and associated changes are absent[20,21,25]. One of the striking features is the elimination of clefts or spaces around neurons and blood vessels when scrutinized with the compound microscope**. In other words, cellular and vascular membranes had become permanently affixed to surroundings and this enabled them to resist the unavoidable trauma incurred during autopsy. This then will be the criteria of perfect fixation for histological material studied with the conventional microscope.

Other unique features of the current technique will enhance the quality of the histological material. The entire organ is evenly fixed. A relatively small amount of a solution, introduced via the capillary network for a short length of time, produces rapid fixation of adjacent elements, and both the change in its concentration and the loss of substances from tissue to solution are minimized. The total amount of each perfusate in milliliters is usually determined in advance as 14% of body weight in grams, and the perfusion is usually terminated in 2—4 min or in 6—8 min after the intravenous injection of pentobarbital mixed with heparin[20,21,25].

Besides the proper fixation of the various cellular elements, the procedure must control distortions of the organ and separations of regional areas. If the structure is fixed with a minimum of change, it follows that volume is retained. The different diameters should be unchanged, and the axis through various parts unaltered. However, the strong acidity of many fixatives would preclude such a goal[19]. In a strong acid, as well as in a strong alkaline milieu, the connective tissue fibers retract[62].

b) Application of the Principle of Perfect Fixation to Obtain Reproducible Neuroanatomical and -pathological Results. The advantage and importance of utilizing material prepared in such manner that "dark" neurons and associated changes are absent will be illustrated by the following observations.

* Since the body (C3-051359C), 12 min after the perfusion, was moved and allowed to fall on the floor from a height of 100 cm, some of the cellular changes may be due to a traumatic tearing[21].

** Occasional separation of the neuronal membranes and vascular walls from their surroundings must be blamed on incidental errors during dehydration, embedding and cutting.

If the histological techniques are to be controlled for the purpose of determining the optimal procedure, the need for an even fixation is paramount. The presence of both "dark" and

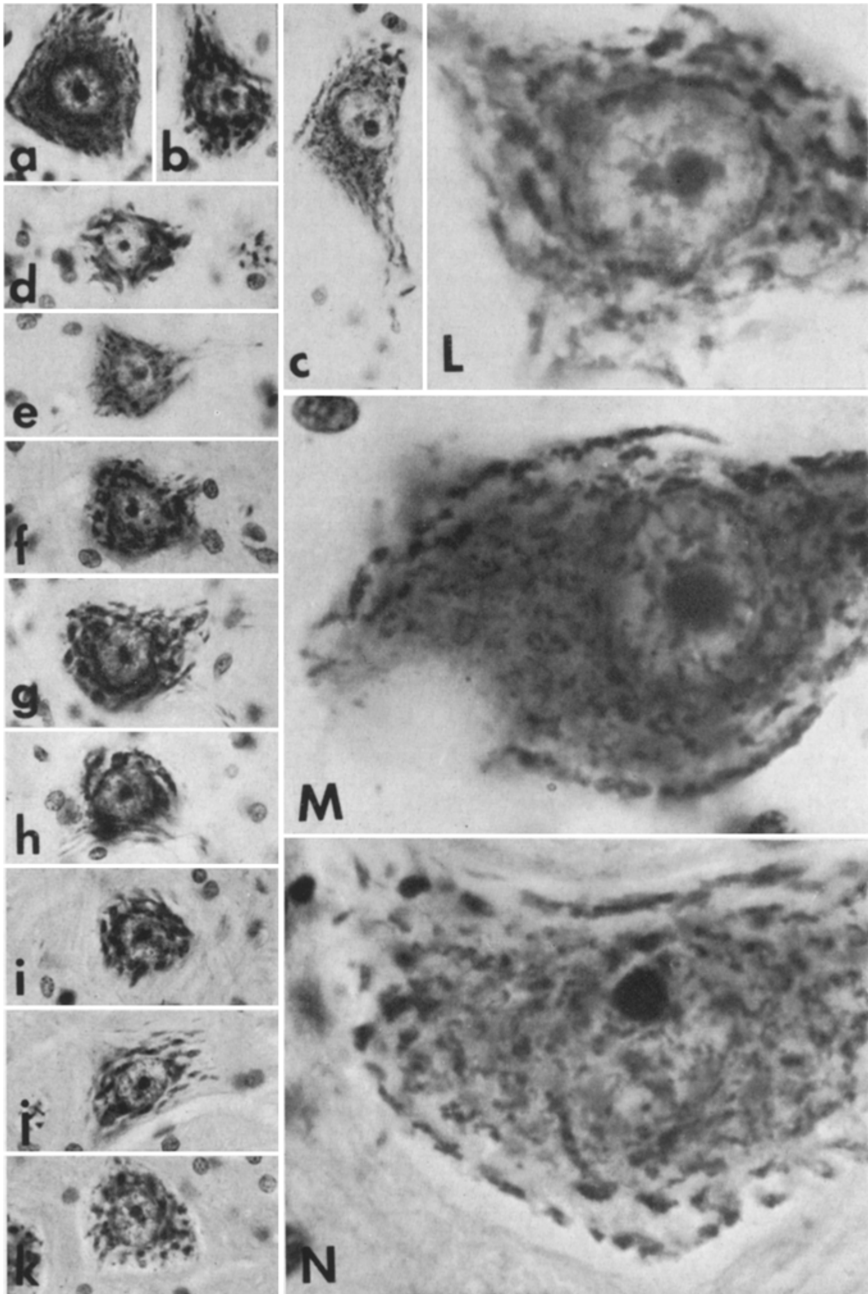

Fig. 4. Companion sections of rabbit brain stem. Travenol-Susa perfusion and autopsy delayed $4^1/_4$ hrs. Ra 2-122057 C. a, b, c Gallocyanin-chrome alum. p_H 2, at room temperature for 24 and 48 hrs, and at 42° C for 48 hrs, respectively (×399). d—k Gallocyanin-chrome alum, 48 hrs at 42° C, with various p_H, 1.5, 1.7, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5, respectively (×399). L, M, N. Gallocyanin-chrome alum, 24 hrs at room temperature, with p_H 2.0, 3.0 and 4.0, respectively (×1200)

"clear" cell types will lead to an arbitrary or biased selection of cells. This problem of selection is acute in a comparison of sections stained in solutions of different p_H. A rabbit (Ra2-122057C) was prepared by the Travenol-Susa perfusion followed by a delayed autopsy after $4^1/_4$ hrs. Then three series of paraffin sections were obtained and stained with gallocyanin-chrome alum of p_H at 1.5, 1.7, 2.0, 2.5, 3.0, 3.5, 4.0 to 4.5; one series was stained at room temperature (23—26°C) for 24 hrs and one for 48 hrs, and one in a paraffin oven (42°C) for 48 hrs. As a whole, the neurons of the two former series (Fig. 4a and b) stain just slightly more intensely

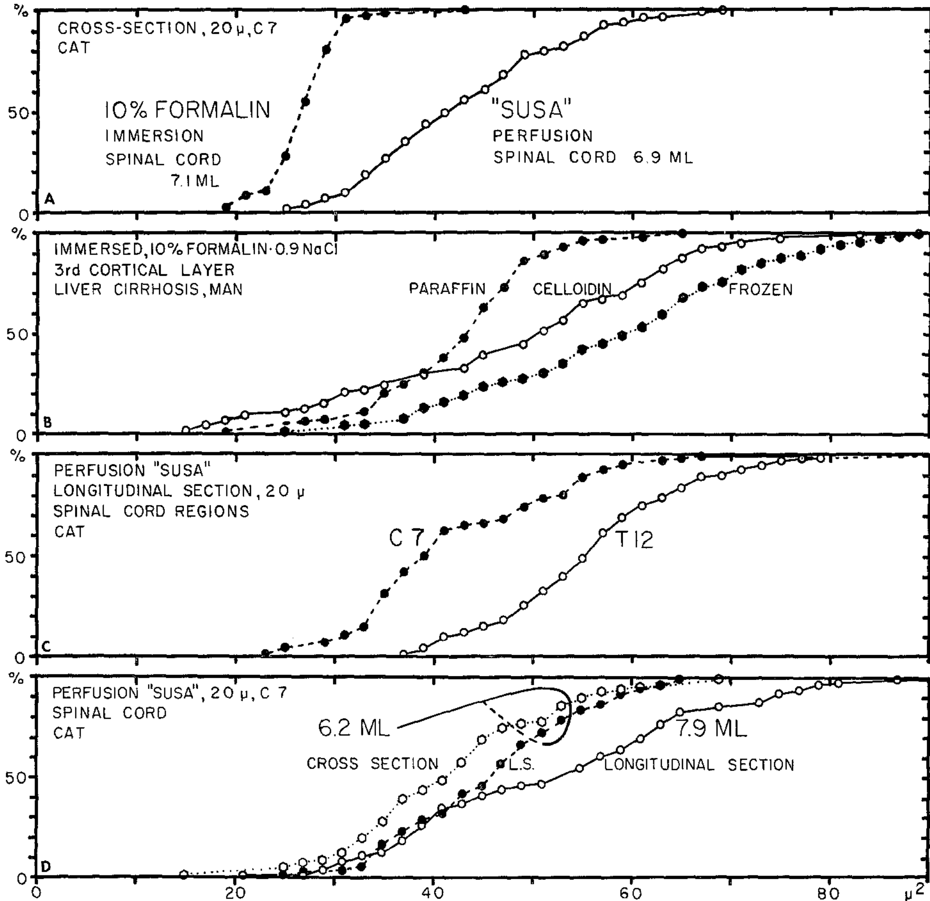

Fig. 5. Karyometric studies of astrocyte nuclei. A Comparison between materials fixed by immersion in 10% formalin (C2-022355C; 7.1 ml) and Travenol-Susa perfusion, autopsy delayed 5 hrs (C1-111557C; 6.9 ml). Cat spinal cords of almost same size (Compare [20]). B Comparison between sections of paraffin and celloidin embedded materials and frozen sections. Human 3rd cerebral cortex of liver cirrhosis. H1-011656C (Compare [15]). C Comparison between longitudinal sections of cervical and thoracic regions. Cat spinal cord. Travenol-Susa perfusion, autopsy delayed 5 hrs. C2-091658C (Compare [20]). D Comparisons between cross and longitudinal sections of same spinal cord (C2-111557C) and between longitudinal sections of small (C2-111557C; 6.2 ml) and large spinal cords (C1-071858C; 7.9 ml). Cat spinal cords. Travenol-Susa perfusion, autopsies delayed 5 hrs. (Compare [20])

than those of the third series (Fig. 4c). With change of the acidity, the basophil material of neurons appears to be equally well stained in all three series (Fig. 4d—k), in agreement with the original conclusion of Einarson[38]. The staining variations indicated in the photographs are regarded as due to differences in thickness of neurons. However, the tissue between the neurons has a distinct gray hue which is more apparent with decreasing acidity, i.e., co-staining of tissue surrounding neurons described by Einarson[38]. The interneuronal material which stains at p_H 3.5—4.5 has the same appearance as that which can be demonstrated by

the PAS treatment (Fig. 8). Staining of this material aids in identifying the cytoplasmic membrane as well as the peripheral neuronal zone poor in basophil material (compare Fig. 4d through k and L through N). This peripheral zone should not be confused with a perineuronal space; phase contrast studies reveal various structures in this zone[21,25].

Karyometric methods are often utilized in order to assess the response of nuclei to an experimental agent. There will be four major advantages in using perfused fixed material: the fixation is instantaneous, the organ is evenly fixed, the cellular or nuclear characteristics are retained to assure exact classification, and the elements attain their greatest size[20]. Indeed, the even action of the fixative is a requisite "sine qua non" for a comparison of nuclei

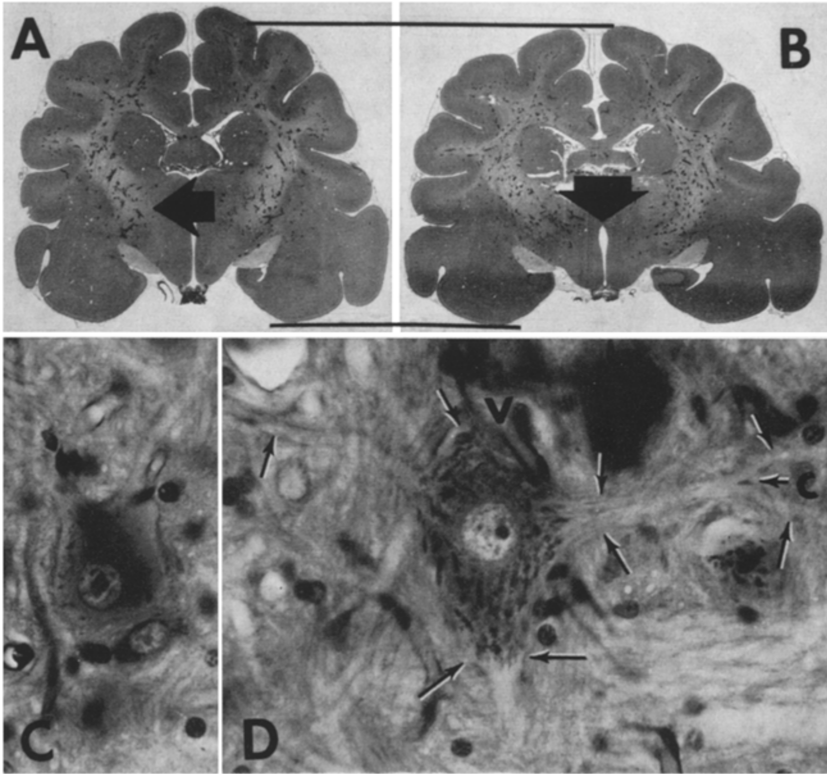

Fig. 6. A Reduction in width of brain cut from the side, indicated by arrow. Travenol-Susa perfusion, autopsy delayed $10^3/_4$ hrs. Paraffin. PASG. C1-011260C. B Reduction in height of companion section to Fig. 6A cut from top, indicated by arrow. C Motor neuron in cross section. Ventral horn of C4-segment of cynomolgus monkey. Travenol-Susa perfusion, autopsy delayed $4^1/_4$ hrs Paraffin. PASG. M1-092358C. Green filter (×399). D Motor neuron of longitudinal section with boundaries of perikaryon and processes indicated by arrows; included are adjacent blood vessel (*v*) and basophil cone at dendritic bifurcation (*c*). Ventral horn of C7-segment of cynomolgus monkey in Fig. 6C. Paraffin. PASG. Green filter (×399)

in different regions. Several cats were fixed by the Travenol-Susa perfusion and the autopsy was delayed several hours. Karyometric studies of the astrocyte nuclei disclose that, for the same animal, the nuclei are larger in longitudinal sections (central curve L.S. in Fig. 5D) than in cross sections (left curve in Fig. 5D). When longitudinal sections are studied, the nuclei are larger in the cat with the spinal cord volume of 7.9 ml than in the one of 6.2 ml (right and central curves in Fig. 5D). Also, in longitudinal sections of individual animals, the largest nuclei occur in the thoracic region (Fig. 5C). The same figure includes a comparison between perfusion and immersion fixed materials of cats of almost same size (Fig. 5A). The significance of embedding procedures is demonstrated, too (Fig. 5B). It is concluded from these results that a karyometric study requires perfectly fixed material and a homogeneous material which is obtainable by the careful selection of embedding, region, animal size, and plane of cutting.

Although the two-step perfusion procedure using HEIDENHAIN's Susa solution for the fixation will assure constant size of an organ, the embedding will cause considerable changes. A more important cause of distortion is the compression due to the microtome[137]. The degree of distortion is demonstrated by cutting the tissue block first in one direction (Fig. 6 A) and then after turning the block 90° (Fig. 6 B). After flattening on the slide, the height of the coronal section is maintained with the former cutting direction and the width with the latter cutting direction. The compression of paraffin embedded material in each direction amounts to approximately 10%, and this can not be adjusted by the flattening of the microscopic section; to avoid excessive flattening the sections are stretched on lukewarm water of less than 37° C (Fig. 6 A and B). This reduction could lead to an impression of cerebral atrophy[31, 160], and it also precludes measurements of paraffin embedded elements, as blood vessels and intervascular connective tissue strands[19]. Another feature which should be considered in an estimation of cell size is its variation in different directions. The astrocyte nuclei are smaller in cross than in longitudinal sections of the spinal cord (smaller spinal cord in Fig. 5 D). The spinal cord neurons are also strikingly dissimilar (Fig. 6 C and D). Not only is the perikaryon greater, but its processes are more prominent in longitudinal than in cross sections[85, 154]. The nuclei of the two illustrated neurons are of different size, but whether this is a valid difference remains unknown as long as it has not been determined whether the nucleus in each figure is seen in its maximum size.

Another abnormality frequently noted in paraffin sections of material obtained even after Travenol-Susa perfusion and the delayed autopsy, is the displacement of nucleoli. Such nucleoli in their new positions on top of nuclear membranes and cytoplasm retain their staining qualities, shape and size. They were possibly mobilized during cutting and transported a short distance while the paraffin melted, or the microtome dislodged the nucleolus for a longer distance into the cytoplasm. In many instances, the original site of the nucleolus is recognized by a clear round empty area of identical size which would discount another possibility, namely that the nucleolar expulsion occurred intra vitam[156]. The expulsion follows the direction of cutting.

Since regional subdivision of the central nervous system is based on the varying number and distribution of neurons, neuroglia and blood vessels, topographic studies must utilize material where spatial relationship of cells and arborization of vasculature have been preserved by the most critical fixation procedure. Paramount is the retention of neurons and blood vessels in inflated state (Fig. 7 A), which cannot be preserved by routine fixation (compare [126]). This particular field of the motor cortex demonstrates two capillary systems (x-x-x and y-y in Fig. 7) connected by an intervascular strand of connective tissue fibers (s in Fig. 7). The scattered astrocyte nuclei, often with a juxtanuclear PAS-stained body, are marked with arrows. More intriguing are the oligodendrocytes aggregated at points of vascular arborization, indicated by a line marker with a thickened head. Thanks to the retained relationship, not only the distribution of cells can be assessed, but also the intimate association between blood vessels and both neurons and oligodendrocytes[22,23]. Regional peculiarities are expressed by differences in these arrangements[24].

The non-equivocal classification of tissue elements is called for not only in topographic studies but also in quantitative studies on size and relative number of cells, and in the diagnosis of cell types involved in an experimental or pathologic condition. Therefore the criteria by which different cells are identified must be as precise as contemporary techniques will allow. Only the most rigorous selection of criteria can assure sampling of a homogeneous cell population for comparative studies. The development of histological techniques should aim towards the constancy of such a selection. After an examination of material fixed either by immersion or by perfusion procedures, only the latter was found to give consistent results, that is, if a coagulant fixative was introduced and the autopsy delayed several hours. The uniform appearance of cells throughout the brain and spinal cord of different species is of particular significance for studies based on appearance of nuclei for the identification of various neuroglia cells. The chinchilla would be best suited for such studies because its astrocyte nuclei are everywhere extremely pleomorphic and large in contrast to the sphere-like oligodendrocytes (Fig. 7 B). However, differences between cell types may be so insignificant in certain regions and species to elude correct identification. In the monkey, as well as the cat, the upper part of the cerebral cortex contains astrocyte nuclei which can only be distinguished from oligodendrocytes by the juxtaposition of a conspicuous PAS-stained mass (arrows in Fig. 7 A; a in Fig. 7 B).

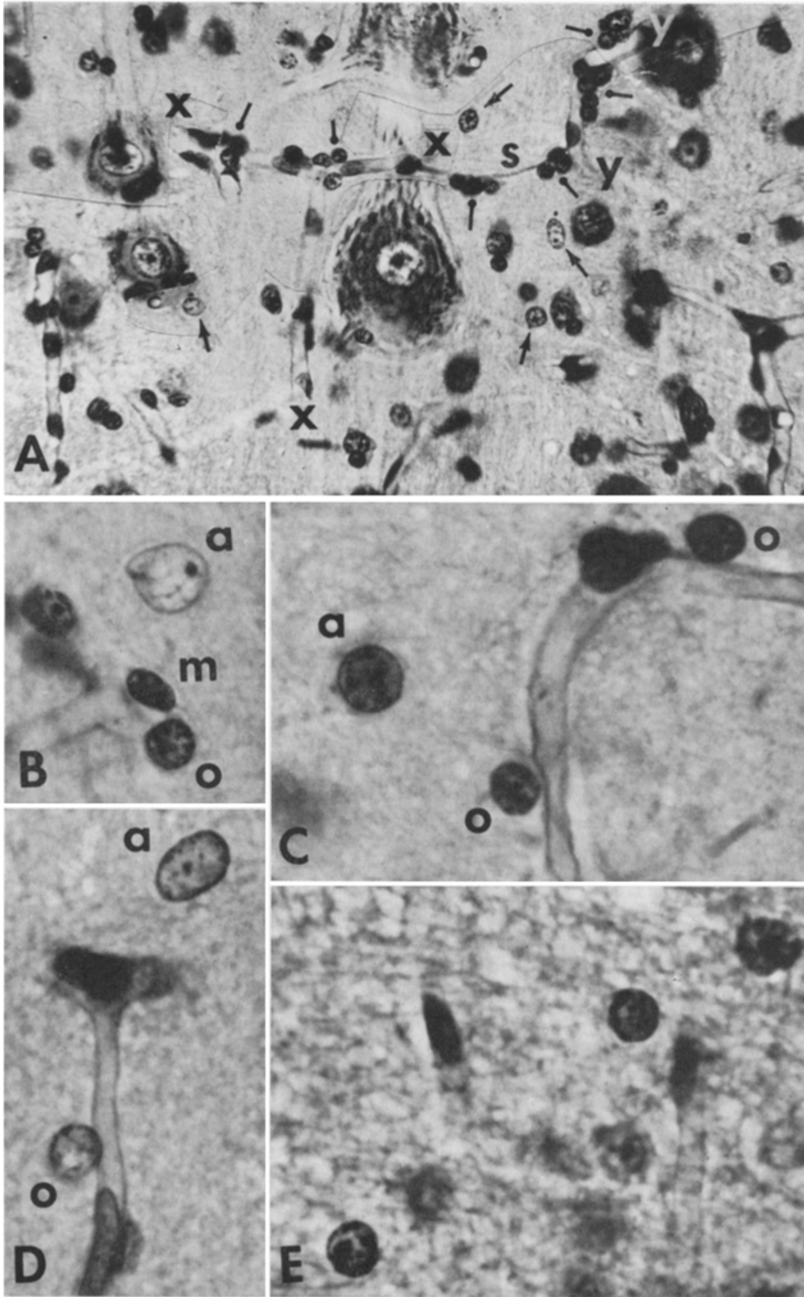

Fig. 7. A Motor region with two capillary systems (*x, x, x* and *y, y*) connected by a strand of intervascular connective tissue fibers (*s*), scattered astrocyte nuclei (arrows) with juxtaposition of PAS-stained body, and oligodendrocyte nuclei (line with pointed end) clustered near vascular arborizations. Composite of two photomicrographs taken at different focal planes from the same section of the cynomolgus monkey in Fig. 6 C. Paraffin. PASG. Green filter (×399). B Distinct difference between nuclei of astrocyte (*a*), histiocyte or microglia (*m*), and oligodendrocyte (*o*). First cortical layer of chinchilla. Travenol-Susa perfusion, autopsy delayed $4^1/_4$ hrs. Paraffin. PASG. Chin 2-092558C. Green filter (×1200). C Astrocyte nucleus (*a*) with juxtaposition of PAS-stained granule and perivascular oligodendrocyte nuclei (*o, o*). First cortical layer of cynomolgus monkey in Fig. 6 C. Paraffin. PASG. Green filter (×1200). D Large astrocyte nucleus (*a*) and perivascular oligodendrocyte nucleus (*o*). First cortical layer of rat. Travenol-Susa perfusion, autopsy delayed $4^1/_2$ hrs. Paraffin. R 1-122258 C. PASG. Green filter (×1200). E Unidentifiable neuroglia cell nuclei. First cortical layer squirrel. Travenol-Susa perfuson, autopsy delayed 5 hrs. Paraffin. Sq 1-010259 C. PASG. Green filter (×1200)

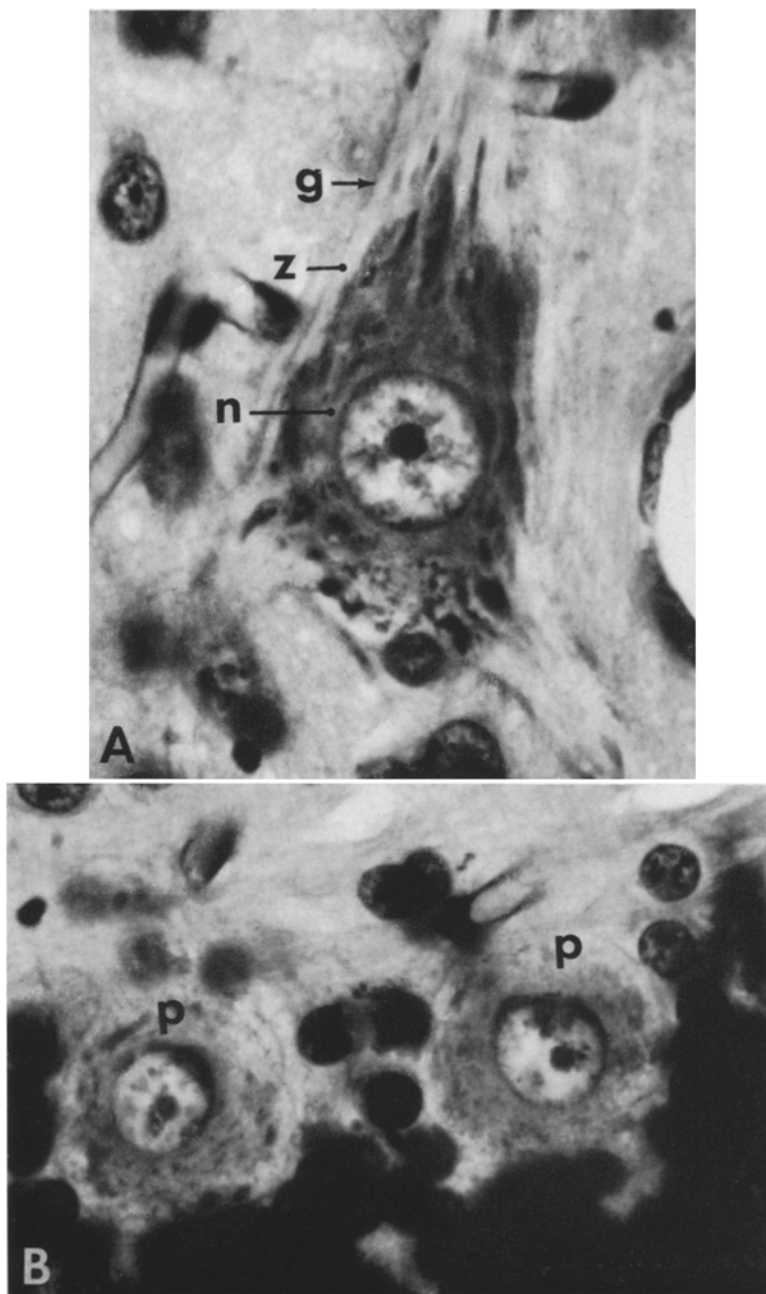

Fig. 8. A Large pyramidal cell (Betz type) with varying distribution of basophil material through perikaryon, poor around nucleus (*n*) and almost none in peripheral zone (*z*). PAS-stained granules (*g*) aggregated on cytoplasmic surface help to identify boundaries of perikaryon and processes. Blood vessels contact neuron in two places. Cerebral cortex of cynomolgus monkey in Fig. 6C. Paraffin. PASG. Green filter (×1200). B Purkinje cells (*p*) with identifiable cell membranes have relatively small amount of basophil granules except along nuclear membrane. Cerebellar cortex of cynomolgus monkey in Fig. 6C. Paraffin. PASG. Green filter (×1200)

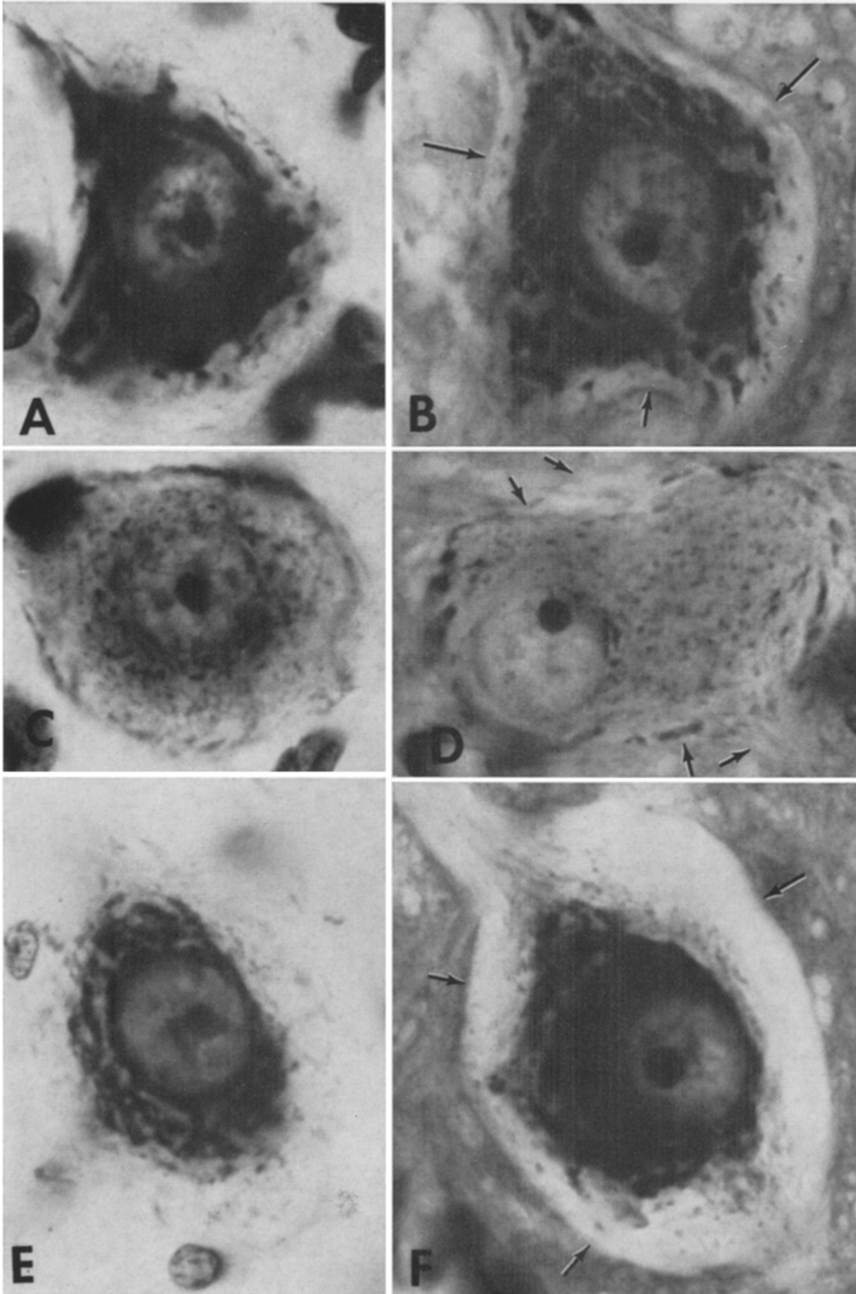

Fig. 9. Comparison between motor neurons of companion sections stained in the left row with gallocyanin-chrome alum, p_H 2.0, room temperature, 24 hrs, and in the right row with PASG. Boundaries of perikaryon indicated by arrows. Nucleus of the facial nerve in a male rabbit, 2 years 9 months of age, 14 days after severance of the left facial nerve. Travenol-Susa perfusion, autopsy delayed 4 hrs. Paraffin. Ra 12-072657 C. Green filter (×1200). A, B Normal motor neuron on non-operated side. C, D Retrograde "pale" neurons, and E, F: "activated" neurons from the nucleus of facial nerve on the operated side

In the rat (Fig. 7D) and mouse the differences are less striking than in the chinchilla, and in other rodents, as the guinea pig, hamster and glider squirrel (Fig. 7E), the nuclei are quite alike.

Should the objective of an investigation be to classify neurons according to size of perikaryon, content and distribution of basophil material, and arrangement of processes, only such methods should be used whereby all cells appear fully inflated and can be delineated correctly. The use of a staining method whereby the surroundings will stand out will help to outline the cytoplasmic membranes, as e.g., by the combined use of PAS and gallocyanin-chrome alum, p_H 1.7–2.0 (Fig. 8A). The gallocyanin-chrome alum method alone, p_H 3.5–4.5 (Fig. 4i–k, N), or the conventional hematoxylin and eosin method will be equally useful when tissues are prepared by the present two-step Travenol-Susa perfusion procedure using HEIDENHAIN's Susa solution as the fixative. In the inflated neuron, the basophil material, attached to vaguely basophil lines, is less compact than in neurons fixed by immersion (Fig. 4A, D in [20]); the distribution in the perikaryon is irregular, limited near the nucleus (n in Fig. 8) and minimal in a "peripheral zone" (z in Fig. 8). A variation in its width, ascribed to a reactive change of the neuron[93,99], is normal. This "peripheral zone" is noted over the dendrons, too (compare Fig. 1 in [111]). Another often overlooked large basophil structure is situated near the bifurcation of dendrons, "Verzweigungskegel" of NISSL[111,127] (c in Fig. 6D); it is separated from the cytoplasmic membrane by the "peripheral zone" (Fig. 23 in [23], Fig. 32 in [25]). The total amount of basophil material varies in different cell types. The normal Purkinje cell has a very scant amount of basophil material concentrically arranged around the nucleus (p in Fig. 8B). Again, in these cells the peripheral zone is very faintly stained. Such cells should not be mistaken for pathologic or affected specimens with a reduced content of basophil substance[79].

It is unavoidable, when microscopic sections are stained by any of the popular modifications of the NISSL technique, to overemphasize the neuronal perikarya because of their conspicuous content of basophil material. The damage to a brain is judged by estimating the appearance of these perikarya, their distribution and numerical concentration. Another objection is that in such preparations, because the contour of a neuron is too vague to be seen, the outer limit of the basophil material becomes equivalent to the cell boundary (Fig. 9A). This neuron, from the unaffected VIIth nucleus of a rabbit (Ra 12-072657C), prepared by the Travenol-Susa perfusion and delayed autopsy, is comparable to one with a classical polygonal shape and several concave sides. Similar neurons in a companion section, stained by the combined method of PAS and gallocyanin-chrome alum apparently have a larger perikaryon, because here the membrane is identified outside the "peripheral zone" which is almost free of basophil material (arrows in Fig. 9B). Under pathologic conditions, as e.g., 12 days after severance of the rabbit's ipsilateral facial nerve (Ra 12-072657C), most neurons display an abnormal distribution of basophil material. It may permeate the "peripheral zone" and deposit along the cytoplasmic membrane (Fig. 9C and D). The size of such a neuron, therefore, is correctly judged with both staining methods, but in comparison with normal ones stained by gallocyanin-chrome alum alone, this cell gives the impression of being enlarged. When all the microscopic sections are stained by the dual technique, no change in size is demonstrated. Another cell reaction consists of the rearrangement of basophil material, which has massively accumulated near the nucleus; the basophil material is packed closer together than ever seen in a normal neuron. Because of these peculiar arrangements, the neurons in gallocyanin-chrome alum stained sections would give the impression of being smaller than normal (Fig. 9E); however, the dual staining procedure makes it possible to estimate both the size of perikaryon and the content of basophil material (Fig. 9F). The "peripheral zone" with scattered minute basophil granules and vaguely stained lines is wider than normal. These lines are more conspicuous with phase contrast optic and resemble endoplasmic reticulum. The presence of these structures will help to rule out an artifactual perineuronal space. The large well formed nucleus with identifiable chromatin and nucleolus of normal size would be other features distinguishing these cells from "dark" neurons.

The eccentric position of nuclei, as seen in Fig. 9C and D, is typical of acute retrograde changes of the neuron[99,108]. Experiments were carried out to test whether the changes in position are influenced by extraneous factors as the terminal narcosis, the fixation procedure or the paraffin cutting. Two male, 6-month-old New Zealand rabbits, 5 days after severance of the left facial nerve 2–3 mm distal to the stylo-mastoid foramen, were deeply narcotized with an intravenous injection of pentobarbital mixed with heparin to prevent blood clotting. One

Table

Position of 400 Neuronal Nuclei 5 Days after Severance of Left Facial Nerve in Young Rabbits

Accession Number (Age)	Length of Final Narcosis min	Length of Perfusion min	Body Turned	Block Cut from	Position of Nuclei				
					Dorsal %	Lateral %	Ventral %	Medial %	Central %
Ra 1-122860 C (4 mos.)	5	2	Right (Medial)*	Ventral	8.0 (17.7)**	17.3 (38.1)	9.5 (21.0)	10.5 (23.2)	54.8
Ra 2-122860 C (4 mos.)	4	3	Left (Lateral)	Dorsal	12.3 (26.6)	12.3 (26.6)	9.5 (20.7)	12.0 (26.0)	54.0
Ra 1-021561 C (6 mos.)	(63) +37	2	Right (Medial)	Dorsal	9.3 (20.6)	14.8 (32.8)	11.5 (25.6)	9.5 (21.1)	55.0
				Average	9.8	14.8	10.2	10.7	54.6

* When animal rests on right or left side, the operated neuron's medial or lateral segment faces downward, respectively.

** The percentage distribution of eccentric nuclei, only.

animal was kept on the left side (Ra 2-122860 C) and one animal on its right side (Ra 1-122860 C). As quickly as possible without change of positions, the Travenol-Susa perfusion procedure was performed and then the animals kept on the table untouched for 12 hrs. After paraffin embedding, the brain stem of the former was cut serially from the dorsal aspect and the one of the latter from the ventral aspect. Then a third rabbit of the same age was operated in a similar manner and at the end of 5 days intravenous narcosis was induced (Ra 1-021561 C). The animal was left on its right side for 1 hr, but because it moved its head, a second injection was given; the narcotized animal was quiet for another 30 min. The Travenol-Susa perfusion procedure was performed and the animal left for 12 hrs. with its head always on the right side. The paraffin embedded brain stem was cut serially from the ventral aspect. The displacement of a nucleus towards the dorsal, lateral, ventral or medial part of the neuron was determined in 400 cells selected from every second section in the central part of the left facial nucleus. Only those cells were studied which showed a central disappearance and a peripheral accumulation of basophil material as in Fig. 9 C and D. A nucleus was classified as eccentric when it touched the basophil peripheral border or the cytoplasmic membrane; the others were assembled in one group with central position. The average number of nuclei in each position is 9.8% dorsally; 14.8% laterally; 10.2% ventrally; 10.7% medially; and 54.6% centrally. The slight differences are probably due to the over all shape of the neurons which will modify the positioning, fewer accumulating in the smallest dorsal segment, and more in the largest lateral segment. In conclusion, the direction of cutting is of no significance (Table 1); when cut from the ventral aspect 9.5% of the nuclei are seen in the ventral segment and 8.00% in the dorsal segment (Ra 1-122860 C). Regardless of whether the animal, perfused immediately after death, was kept immobile with the operated side down (Ra 2-122860 C) or up (Ra 1-122860 C), neurons with laterally placed nuclei always exceed those with medially placed nuclei by 0.3 and 6.8%, respectively (Table). Prolonged narcosis (Ra 1-021561 C does not seem to alter significantly the distribution of nuclei; the number with central position remains the same (Table). Thus, the eccentric position of nuclei during acute retrograde neuronal reaction does not appear to be influenced by factors involved in the histological preparation.

Discussion

The identical appearance of "dark" neurons in all regions and species, as described in the first part of this paper, is the outstanding feature upon which much of the discussion hinges. These cells exhibit an unusual affinity for different dyes and an abnormal distribution of histochemically activated enzymes[25,51,52]. This permits an unequivocal diagnosis. It suggests a monotony in cellular damage caused by a single factor. Traumatization of an organ after death, as stroking its

surface, instructs about the manner of action. Characteristic of the tissue so traumatized is an "all or nothing" response: the neurons react with similar changes whether a moderate or severe post-mortem trauma is applied, the cellular sensitivity becomes manifest immediately after arrest of the systemic blood circulation, and once damaged none of the commonly used fixatives can restore the original cell type. Presently there is little or no doubt about the ill effect of such a trauma and no one will deny the traumatic origin of these cells when they localize along the pathway which is in continuity with the locus of trauma[33,45–47,139–141,155]. However, it has been difficult to associate those ubiquitously occurring "dark" neurons with such a trauma because neither can a pattern of distribution be established nor can the directions of trauma transmission be predicted. Also NISSL[113], who identified the "chromophil" neuron with an artifactual factor concerned with the histological preparation of the tissue was uneasy about the classification of the diffusely developed and widespread "atrophic" cell changes. In one of the present experiments, the profuse occurrence of "dark" neurons was confined to the area being stroked. The identity of cell changes is demonstrated by the complete suppression of their genesis when the organ is fixed by the perfusion procedure followed by the delay of autopsy; during this delay, the cellular texture is transformed sufficiently so that the cell membranes can resist the effects of the inevitable trauma[18,21]*.

None of the other proposals concerning the genesis of the artifactual "dark" neurons can be adopted because they are inadequate both in explaining the varied conditions under which these cells occur and in formulating a principle for avoiding them. Since these theories have been discussed elsewhere[25], merely a listing of pertinent theories will be given. The dual origin of a "dark" cell type has had the greatest appeal[58,59,105,107,144,148,158]**, although the preparatory factor was emphasized repeatedly by NISSL[109–116]. Others have paid more attention to post-mortem autolysis[11,89], the combined effect of hypoxia and autolysis[65]***, [92]+, the labile condition of protein molecules[27,119], the unwanted effect of the fixative[54,72,77,85,90,94,106], the hypertonic action of fixatives[7,46]++, [47]+++, [80,81,87,136,146,159], the different functional conditions of cells at the moment of fixation[3,6,82–84,104], or the histochemical substrates used for incubation[50]. To dismiss "dark" neurons

* Under certain conditions an extraordinary cell change takes place, namely one resembling the so-called "Wasserveränderung"[21], which has been identified with a pathologic cell change[53].

** Influenced by contemporary opinions, SCHARRER in 1933[139], the strongest proponent of the post-mortem traumatic origin, could not entirely reject the possibility of a pathologic cell process. The weakness in the original argumentation compelled EINARSON, also in 1933[39], to express dogmatically that his "chromophily" and "chromophoby" represent different functional stages ". . . . keeping well in mind that these conditions have nothing to do with the phenomena of artificial shrinking and swelling" (loc. cit., p. 145), a statement repeated on several occasions[40–42]. The uncertain classification is reflected in the inconsistent conclusions that various authors have arrived at in the course of investigations[65] and over periods of few[49–52] or many years[78,79].

*** "Morphotropic necrobiosis" is regarded as a function of prolonged premortem hypoxemia and post-mortem autolysis by HAYMAKER et al. ([65], p. 25), and "pyknotic" and "hyperchromatic cells" both "are regarded as an expression of morphotropic necrobiosis occurring *post mortem*" (loc. cit., p. 26) and are identified with "damaged cells" (loc. cit., p. 155–156).

+ "Morphotropic necrobiosis" of LINDENBERG[92].

++ "Cellule non-gonflée" of FORTUYN [46].

+++ "Non-inflated cell" and "wound phenomenon" of FORTUYN[47].

because of their widespread occurrence in both control and experimental or clinical materials[36,61,71,76,97,164] can not help solve the problem of genesis.

Proposals that cell types identical with the "dark" neurons are due to physiologic and/or pathologic processes are not relevant, because the present study was made on normal animals at rest, and are invalidated by the current experience of the effect of post-mortem trauma on the staining qualities of cells*. Because of the misleading assumption that rapid removal of an organ is essential for obtaining optimal histological preparations, "dark" neurons were classified as specific cell types in material that had been carefully removed shortly after death, followed by the rapid submersion in a fixative[153–155], or removed immediately after perfusion of a fixative[56,67–70,78]. If the presumption were correct that the "dark" cells are normal representatives of the neuronal population of a region[1,74,100,117,124,134,135,149], then precautions to prevent them would be preposterous.

The "dark" neuron as a non-equivocal sign of post-mortem trauma to an organ acquires greater significance as a representative of the "artifact complex" which the tissue harbors as a sequelae of the trauma[20,21]. This is constituted of "dark" neurons, "pyknotic" nerve cell and neuroglia cell nuclei, "liposclerosis" of neurons filled with lipid materials, "atypical" small nerve cell forms, irregular axons, abnormal myelin sheaths, perivascular spaces, and divided cerebellar cortex. The usefulness of such a tissue is limited because of the presence of these changes, but their random distribution makes it still possible to scrutinize the remaining neurons, nerve fibers and neuroglia cells**. The many successful clinical neuropathological studies will bear this out, and carefully performed experimental investigations have achieved far reaching results. On the other hand, similar material with irregular preservation of both the histological elements and their spatial relationship will lend itself poorly to such critical studies as those concerned

* Identical "dark" neurons have been labelled differently, but these names, given in the order of publication date, should be regarded as synonymous, as e.g., "dunkle" and "chromophile Zellen" of KONEFF[82,83], "chromophile", "osmophile" and "argyrophile Zellen" of KOTLAREWSKY[84], "pyknomorphe Zelle" of NISSL[113], "cellule retractée" and "cellule obscure" of RAMÓN Y CAJAL[128], "Zellschrumpfung", "einfache Schrumpfung" and "sklerotische Ganglienzelle" of SPIELMEYER[148] and others[34,143], "hyperchromatic cell" of MISKOLCZY[105], "specialized" cortical neurons of VON ECONOMO[37,130], "contracted cortical cell" and "retracted cell of sensory ganglion" of RAMÓN Y CAJAL[129], "shrunken homogeneous cell" of GILDEA and COBB[56,57], "sympathetic cells of cranial and spinal ganglia" of KISS[75], "Fischzelle" of STERN[149] "Purkinje cell type II" of HYDÉN[72], "chromophilia", "hyperchromasia", "extreme chromophily", "chromophily of initially increased activity" and "chromophily of depressed activity" of EINARSON[41,43], "multiangular formalin sensitive ganglion cells" of BACSICH and WYBURN[3], "siderophile Zelle" of HÄGGQVIST[63], and "primary axonal reaction" of GRÜNTHAL and WALTHER-BÜEL[60]. The "shrunken homogeneous cell" of GILDEA and COBB[56,57] should not be confused with "ischämische Zellerkrankung" of SPIELMEYER[148].

** Tissues are evenly fixed only by the perfusion procedure followed by the delayed autopsy. Any other routine procedures whereby the neurons do suffer from post-mortem traumatization, result in the uneven appearance of neurons; this exposes the inefficiency of the principle of "equivalent cell pictures" proposed by NISSL[112,115,116] and the fallacious claim that the procedure is standardized by having used the same fixative for submerging the organ[40,91,134,135,145,148,157]. Few have heeded the warning words of experienced investigators that even if the working principle of "equivalent cell pictures" were followed, not all histological aberrations are specific or pathologic[123]. In order to avoid inconsistencies it should be dismissed as a guide in neuropathological research[15,20,21].

with concentrations of cytoplasmic compounds[72], the shape of cortical neuron[4,150], the quantitative characteristics of cerebral regions[64], the submicroscopical composition of nervous tissue[95,96,147], or the distribution of neurosecretory material[133]. An unawareness of the profuse admixture of artifactual changes caused by post-mortem trauma has led to many controversial interpretations, as e.g., the acute swelling of oligodendrocytes[44,95,96,120], the optimum p_H of gallocyanin-chrome alum solutions[42], the separation of neurons from blood vessels[48], the susceptibility of the cerebellar Purkinje cell layer to an edematous separation[2,28,103,151,165], and the perineuronal vacuolization or laminary microcavitation[29,30,152]. These conditions were selected because they can be checked by a recapitulation of *Presentation of Material* with and without the histological abnormalities.

When tissues are free of "dark" neurons or the "artifact complex", as a whole, two important conclusions are drawn, namely that the fixation is perfect and the effect of a post-mortem trauma is eliminated*. If the fixative is rapid in action, the tissue has these additional qualifications, the cellular elements are preserved identically and instantaneously. Such a tissue lends itself to sophisticated cytological studies, but because of its exclusive appearance, normal cytological details must be investigated anew and experimental results re-evaluated by the comparison of tissues prepared in like manner. The second group of material gives examples of tissues prepared according to specified conditions of perfusion and autopsy, and discusses the need for utilizing such procedures in studies of the regional topography, the identification of cells, and the estimation or measurement both of size of neurons and of their amounts of basophil material. A staining procedure should be chosen whereby the boundaries of neurons are readily visible. The preliminary use of such material has revised the concepts about the spatial relationship between blood vessels and neurons as well as oligodendrocyte nuclei[22–24], and it has provoked a renewed interest in the classification of normal neurons and in the reactive changes of pathological neurons as "swelling" and "atrophy".

Summary

The significance of "dark" neurons has been discussed from two points of view:

a) When present, they unequivocally signify that the tissue has suffered from post-mortem traumatization and that a number of other cellular elements are severely damaged, too. Because the effect of trauma is widespread, such material is of limited value for detailed microscopical studies.

b) When absent, the tissue has usually been perfectly fixed by the perfusion of a coagulant fixative followed by the delayed autopsy. Because of the strictly standardized conditions of preparations, only such material should be used for studies of regional characteristics and the appearance of normal and pathologic cell types.

* The perfusion procedure will not hamper the post-mortem examination except in cases of embolism. At the moment of heart arrest, a complete shift in the distribution of blood takes place and an ensuing increased pressure in the superior vena cava[66,125,131,132] will have a retrograde action on the intracranial veins[13,14]. The degree of vascular dilation or blood filling in the brain becomes, therefore, a rather unreliable post-mortem observation. Whereas measurements of the blood vessels in immersed fixed brains are obsolete, in perfused fixed brains, such measurements will provide information about the differences in width along the vascular "tree". The use of perfused fixed material for a study of the capillaries has been recommended because they all appear inflated[55].

Résumé

Deux séries microscopiques, l'une comprenant les cellules sombres et l'autre dépourvue de ces cellules, ont été étudiées parallèlement pour mieux souligner la nécessité de préparer les matériaux anatomiques de telle façon qu'on en évite la formation au moment du prélèvement de l'organe.

Les matériaux contenant ces cellules sombres doivent être étudiés avec précaution, parce que ce ne sont pas seulement les neurones mais aussi les autres cellules de la neuroglie et des vaisseaux qui sont traumatisés. Le cytoplasme et le noyau rétractés montrent aux colorants histologiques une affinité extrême, réaction qui se manifeste dès l'arrêt de la circulation.

Au contraire, si l'autopsie est effectuée plusieurs heures après une fixation par perfusion on ne trouve plus ni cellules sombres ni autres déformations artificielles. Cette méthode apporte immédiatement après la mort une fixation parfaite des cellules dans l'organe tout entier. Aussi l'émploie d'une quantité la plus minime de cette solution empêchera la perte de substance. A cause de l'action uniforme du fixateur, les cellules des organes nerveux conservent leur forme, leur taille et leur coloration ce qui est indispensable aux études de cytologie comparée et aux études cytométriques et topographiques.

References

[1] ANDY, O. J., and H. STEPHAN: The nuclear configuration of the septum of Galago demidovii. J. comp. Neurol. **111**, 503—545 (1959).

[2] ARCHAMBAULT, L.: Parenchymatous atrophy of the cerebellum. J. nerv. ment. Dis. **48**, 273—312 (1918).

[3] BACSICH, P., and G. M. WYBURN: Formalin-sensitive cells in spinal ganglia. Quart. J. micr. Sci. **94**, 89—92 (1953).

[4] BARASA, A.: Forma, grandezza e densitá dei neuroni della corteccia cerebrale in mammiferi di grandezza corporea differente. Z. Zellforsch. **53**, 69—89 (1960).

[5] BARTELMEZ, G. W., and N. L. HOERR: The vestibular club endings in ameiurus. J. comp. Neurol. **57**, 401—428 (1933).

[6] BEEVOR, C.: Die Kleinhirnrinde. Arch. Anat. Physiol., anat. Abt. 365—388 (1883).

[7] BLAIR, D. M., P. BACSICH and F. DAVIES: The nerve cells in the spinal ganglia. J. Anat. (Lond.) **70**, 1—9 (1935).

[8] BODIAN, D.: A new method for staining nerve fibers and nerve endings in mounted paraffin sections. Anat. Rec. **65**, 89—97 (1936).

[9] —, The virus, the nerve cell, and paralysis. A study of experimental poliomyelitis of the spinal cord. Bull. Johns Hopk. Hosp. **83**, 1—107 (1948).

[10] BRODAL, A.: Experimentelle Untersuchungen über retrograde Zellveränderungen in der unteren Olive nach Läsionen des Kleinhirns. Z. ges. Neurol. Psychiat. **166**, 646—704 (1939).

[11] CAMERER, J.: Untersuchungen über die postmortalen Veränderungen am Zentralnervensystem, insbesondere an den Ganglienzellen. Z. ges. Neurol. Psychiat. **176**, 596—635 (1943).

[12] CAMMERMEYER, J.: Recherches anatomo-pathologiques sur les lésions cérébrales dans la thérapeutique convulsivante de la démence précoce. J. belge Neurol. Psychiat. **40**, 169—229 (1940).

[13] —, The agonal nature of cerebral red softening: re-evaluation of morphological findings. Acta psychiat. scand. **28**, 9—25 (1953).

[14] —, Agonal nature of the cerebral ring hemorrhages. A. M. A. Arch. Neurol. Psychiat. (Chicago) **70**, 54—63 (1953).

[15] —, Difficulties in interpreting neuropathology of neuroglia cells; in WINDLE's Biology of Neuroglia. Springfield: Thomas 1958.

[16] —, Discussion. J. Neuropath. exp. Neurol. **18**, 352 (1959).
[17] —, Discussion. J. Neuropath. exp. Neurol. **19**, 139 (1960).
[18] —, A critique of neuronal hyperchromatosis. J. Neuropath. exp. Neurol. **19**, 141—142 (1960).
[19] —, A comparative study of intervascular connective tissue strands in the central nervous system. J. comp. Neurol. **114**, 189—208 (1960).
[20] —, Differences in shape and size of neuroglial nuclei in the spinal cord due to individual, regional and technical variations. Acta anat. (Basel) **40**, 149—177 (1960).
[21] —, The post-mortem origin and mechanism of neuronal hyperchromatosis and nuclear pyknosis. Exp. Neurol. **2**, 379—405 (1960).
[22] —, Is the perivascular oligodendrocyte another element controlling the blood supply to neurons? Angiology **11**, 508—517 (1960).
[23] —, Reappraisal of the perivascular distribution of oligodendrocytes. Amer. J. Anat. **106**, 197—231 (1960).
[24] —, The distribution of oligodendrocytes in cerebral gray and white matter of several mammals. Amer. J. Anat. **107**, 107—127 (1960).
[25] —, An evaluation of the significance of the "dark" neuron. Ergebn. Anat. Entwickl.-Gesch. **36**, 1—61 (1961).
[26] —, and R. L. Swank: Acute cerebral changes in experimental canine fat embolism. Exp. Neurol. **1**, 214—232 (1959).
[27] Cotte, G.: Étude critique de la signification de l'état hyperchromophile des cellules nerveuses. Arch. Biol. **68**, 297—380 (1957).
[28] Courville, C. B.: Case studies in cerebral anoxia. Bull. Los Angeles neurol. Soc. **20**, 138—144 (1955).
[29] —, Case studies in cerebral anoxia. XII. Residual cortical and striatal changes consequent to exposure to a simple asphyxiant gas (Butane "poisoning"). Bull. Los Angeles neurol. Soc. **21**, 192—198 (1956).
[30] —, Etiology and pathogenesis of laminar cortical necrosis. A. M. A. Arch. Neurol. Psychiat. (Chicago) **79**, 7—30 (1958).
[31] —, Antenatal and paranatal circulatory disorders as a cause of cerebral damage in early life. J. Neuropath. exp. Neurol. **18**, 115—140 (1959).
[32] Cowdry, E. V.: The structure of chromophile cells of the nervous system. Contr. Embryol. Carneg. Instn. (No. 11) **4**, 27—43 (1916).
[33] Cox, A.: Ganglienzellschrumpfung im tierischen Gehirn. Beitr. path. Anat. **98**, 399—409 (1937).
[34] Creutzfeldt, H. G.: Histologische Besonderheiten und funktionelle und pathologische Veränderungen der nervösen Zentralorgane; in Bethe-Bergmann-Embden-Ellingers Hb. d. norm. und path. Physiol. Bd. 9, S. 461—514. Berlin: Springer 1929.
[35] Dickson, W. E. C.: Accidental electrocution: with direct shock to the brain itself. J. Path. Bact. **59**, 359—365 (1947).
[36] Dunlap, C. B.: Dementia praecox. Amer. J. Psychiat. (3) **80**, 403—421 (1924).
[37] Economo, C. von: Eine neue Art Spezialzellen des Lobus cinguli und Lobus insulae. Z. ges. Neurol. Psychiat. **100**, 706—712 (1926).
[38] Einarson, L.: A method for progressive selective staining of Nissl and nuclear substances in nerve cell. Amer. J. Path. **8**, 295—308 (1932).
[39] —, Notes on the morphology of the chromophil material of the nerve cells and its relation to nuclear substances. Amer. J. Anat. **53**, 141—176 (1933).
[40] —, Histological analysis of the Nissl-pattern and -substance of nerve cells. J. comp. Neurol. **61**, 101—127 (1935).
[41] —, Om nervecellernes indre struktur og deres histologiske tilstandsaendringer ved experimentelt fremkaldte funktionelle aktivitetsstadier. Acta jutland. **17**, 1—150 (1945).
[42] —, On the theory of gallocyanin-chromalum staining and its application for quantitative estimation of basophilia. A selective staining of exquisite progressivity. Acta path. microbiol. scand. **28**, 82—102 (1951).
[43] —, og K. Aa. Lorentzen: Om nervecellernes indre struktur og deres tilstandsaendringer under irritation, inaktivitet og degeneration. Acta jutland. **18**, 1—16 (1946).

[44] ELVIDGE, A. R., and G. E. REED: Biopsy studies of cerebral pathologic changes in schizophrenia and manic-depressive psychosis. Arch. Neurol. Psychiat. (Chicago) **40**, 227—268 (1938).

[45] FISHER, C., and S. W. RANSON: On the so-called sympathetic cells in the spinal ganglia. J. Anat. (Lond.) **68**, 1—10 (1933).

[46] FORTUYN, A. B. D.: Changements histologiques dans l'écorce cérébrale de quelques rongeurs. Trab. Inst. Cajal Invest. biol. **22**, 67—98 (1924).

[47] —, Histological experiments with the brain of some rodents. J. comp. Neurol. **42**, 349—391 (1927).

[48] FRIEDE, R. L.: Über die trophische Funktion der Glia. Virchows Arch. path. Anat. **324**, 15—26 (1953).

[49] —, Eine histochemische Reaktion zum Nachweis von Phosphorylase mit besonderer Berücksichtigung des Nervensystems. Arch. Psychiat. Nervenkr. **195**, 325—336 (1956).

[50] —, Histochemical demonstration of phosphorylase in brain tissue: association of post mortal neuron changes with phosphorylase activity. J. Histochem. Cytochem. **7**, 34—38 (1959).

[51] —, Histochemical distribution of phosphorylase in the brain of the guinea-pig. J. Neurol. Neurosurg. Psychiat. **22**, 325—329 (1959).

[52] —, Specific cord damage at the atlas level as a pathogenic mechanism in cerebral concussion. J. Neuropath. exp. Neurol. **19**, 266—279 (1960).

[53] FÜNFGELD, E. W.: Zur Entwicklung der vesiculären Nervenzellveränderung (Wasserveränderung Nissls) unter besonderer Berücksichtigung einer Fettfärbung nach Paraffineinbettung. J. Hirnforsch. **1**, 421—441 (1955).

[54] GERHARD, L., H. MEESEN and G. VEITH: Nervensystem. A. Normale Anatomie und allgemeine Pathologie; in COHRS-JAFFÉ-MEESENS Pathologie der Laboratoriumstiere, Bd. 1, S. 698—736. Berlin, Göttingen, Heidelberg: Springer 1958.

[55] GEROTA, D.: Contribution à l'étude du formol dans la technique anatomique. Int. Mschr. Anat. Physiol. **13**, 108—139 (1896).

[56] GILDEA, E. F., and S. COBB: The effects of anemia on the cerebral cortex of the cat. Arch. Neurol. Psychiat. (Chicago) **23**, 876—903 (1930)

[57] —, — The effects of anemia on the cerebral cortex of the cat. Res. Publ. Ass. nerv. ment. Dis. **7**, 231—268 (1931).

[58] GREENFIELD, J. G.: Recent studies of the morphology of the neurone in health and disease. J. Neurol. Psychiat. **1**, 306—328 (1938).

[59] —, W. BLACKWOOD, W. H. McMENEMEY, A. MEYER and R. M. NORMAN: Neuropathology. London: Arnold 1958.

[60] GRÜNTHAL, E., u. H. WALTHER-BÜEL: Über Schädigung der Oliva inferior durch Chlorperphenazin. Psychiat. Neurol. (Basel) **140**, 249—257 (1960).

[61] GUREWITSCH, M., u. G. BYCHOWSKY: Zur Architektonik der Hirnrinde (Isocortex) des Hundes. J. Psychol. Neurol. (Lpz.) **35**, 283—300 (1928).

[62] GUSTAVSON, K. H.: The Chemistry and Reactivity of Collagen. New York: Academic Press 1956.

[63] HÄGGQVIST, G.: Siderophile und siderophobe Zellen in der Gehirnrinde. Z. mikr.-anat. Forsch. **64**, 121—128 (1958).

[64] HAUG, H.: Über die Beziehungen des Hirngewichtes zum Grauzellkoeffizienten der Sehrinde bei den Primaten und einigen primitiven Säugern. J. Hirnforsch. **4**, 189—204 (1958).

[65] HAYMAKER, W., C. MARGOLES, A. PENTSCHEW, H. JACOB, R. LINDENBERG, L. SAENZ ARROYO, O. STOCHDORPH and D. STOWENS: Pathology of Kernicterus and posticteric encephalopathy; in Amer. Acad. cerebral Palsy's Kernicterus and its Importance in Cerebral Palsy, pp. 21—228. Springfield: Thomas 1961.

[66] HENDERSON, Y., A. W. OUGHTERSON, L. A. GREENBERG and C. P. SEARLE: Muscle tonus, intramuscular pressure and the venopressor mechanism. Amer. J. Physiol. **114**, 261—268 (1935/36).

[67] HEYCK, H., u. W. HÖPKER: Hirnveränderungen bei der Ratte durch Ultraschall. Mschr. Psychiat. Neurol. **123**, 42—64 (1952).

[68] HÖPKER, W.: Über Hirnveränderungen nach Glukosemangel. Verh. dtsch. Ges. Kreisl.-Forsch. **19**, 241—246 (1953).

[69] —, Die Wirkung des Glucosemangels auf das Gehirn. Leipzig: Thieme 1954.

[70] —, Über die Struktur der großen Nervenzellen der Formatio reticularis der Ratte in Beziehung zur Zellfunktion. Acta neuroveg. (Wien) **15**, 197—214 (1957).

[71] HOFF, H., u. F. SEITELBERGER: Die Altersveränderungen des menschlichen Gehirns. Z. Alternsforsch. **10**, 307—318 (1957).

[72] HYDÉN, H.: Protein metabolism in the nerve cell during growth and function. Acta physiol. scand. (Suppl. 17) **6**, 1—136 (1943).

[73] JANSEN, J.: Über Hirnveränderungen bei Holzgeistvergiftung. Acta path. microbiol. scand. **26**, 146—153 (1936).

[74] KENNARD, D. W.: The anatomical organization of neurons in the lumbar regions of the spinal cord of the frog (Rana temporaria). J. comp. Neurol. **111**, 447—467 (1959).

[75] KISS, F.: Sympathetic elements in the cranial and spinal ganglia. J. Anat. (Lond.) **61**, 488—498 (1932).

[76] KLAUE, R.: Parkinsonsche Krankheit (Paralysis agitans) und postencephalitischer Parkinsonismus. Arch. Psychiat. Nervenkr. **111**, 250—321 (1940).

[77] KÖLLIKER, A.: Handbuch der Gewebelehre. Leipzig 1889.

[78] KOENIG, H.: An autoradiographic study of nucleic acid and protein turnover in the mammalian neuraxis. J. biophys. biochem. Cytol. **4**, 785—792 (1958).

[79] —, Production of injury to feline central nervous system with nucleic acid antimetabolite. Science **127**, 1238—1239 (1958).

[80] KOENIG, H., R. A. GROAT and W. F. WINDLE: A physiological approach to perfusion-fixation of tissues with formalin. Stain Technol. **20**, 13—22 (1945).

[81] KOENIG, R. S., and H. KOENIG: An experimental study of post mortem alterations in neurons of the central nervous system. J. Neuropath. exp. Neurol. **11**, 69—78 (1952).

[82] KONEFF, H.: Beiträge zur Kenntnis der Nervenzellen in den peripheren Ganglien. Inn. Dissert. Bern 1886.

[83] —, Beiträge zur Kenntnis der Nervenzellen. Mitt. naturforsch. Ges. Bern No. 1143—1163, 13—44 (1887).

[84] KOTLAREWSKY, A.: Physiologische und mikrochemische Beiträge zur Kenntnis der Nervenzellen in den peripheren Ganglien. Inn. Dissert. Bern 1887.

[85] KREYSSIG, F.: Über die Beschaffenheit des Rückenmarks bei Kaninchen und Hunden nach Phosphor- und Arsenikversorgung nebst Untersuchungen über die normale Struktur desselben. Virchows Arch. path. Anat. **102**, 286—298 (1885).

[86] KRIEG, W. J. S.: Connections of the cerebral cortex. J. comp. Neurol. **91**, 467—506 (1949).

[87] LAVELLE, A., and F. W. LAVELLE: Neuronal swelling and chromatolysis as influenced by the state of cell development. Amer. J. Anat. **102**, 219—241 (1958).

[88] —, C.-N. LIU and F. LAVELLE: Acid phosphatase activity as related to nucleic acid sites in the nerve cell. Anat. Rec. **119**, 305—323 (1954).

[89] LEVI, G.: Alterazioni cadaveriche della cellula nervosa studiate col metodo di Nissl. Riv. Pat. nerv. ment. **3**, 18—20 (1898).

[90] —, Über das mutmaßliche Bestehen von sympathischen Zellen in den kranialen und spinalen Ganglien. Anat. Anz. **75**, 187—190 (1932/33).

[91] LEWY, F. H.: Nervensystem; in KRAUSES Enzyklopädie der mikroskopischen Technik 3. Aufl. S. 1636—1676. Berlin, Wien: Urban & Schwarzenberg 1926/27.

[92] LINDENBERG, R.: Morphotropic and morphostatic necrobiosis. Amer. J. Path. **32**, 1147 bis 1177 (1956).

[93] LIU, C.-N., and W. F. WINDLE: Effects of inanition on the central nervous system. Arch. Neurol. Psychiat. (Chicago) **63**, 918—927 (1950).

[94] LUGARO, E.: Nuovi dati e nuovi problemi nella patologia della cellula nervosa. Riv. Pat. nerv. ment. **1**, 303—322 (1896).

[95] LUSE, S. A.: The ultrastructure of normal and abnormal oligodendroglia. Anat. Rec. **138**, 461—492 (1960).

[96] —, Electron microscopic observations of the central nervous system; in ROBERTS' Inhibition in the Nervous System and Gamma-Aminobutyric Acid. Oxford, London, New York, Paris: Pergamon 1960.

[97] LUTTRELL, C. N., and K. E. MASON: Vitamin E deficiency, dietary fat and spinal cord lesions in the rat. Ann. N. Y. Acad. Sci. **52**, 113—120 (1949).

[98] MALM, M.: Introduction of p-toluenesulfonic acid for perfusion-fixation of the central nervous system. Anat. Rec. **136**, 237 (1960).

[99] MARINESCO, G.: La cellule nerveuse. Paris: Doin 1909.

[100] MEESEN, H., u. J. OLSZEWSKI: Cytoarchitektonischer Atlas des Rautenhirns des Kaninchens. A Cytoarchitektonik Atlas of the Rhombencephalon of the Rabbit. Basel, New York: Karger 1949.

[101] MEYER, A.: Critical evaluation of histopathological findings in schizophrenia. Atti 1° Congr. int. Istopat. Sistema Nerv., Roma, Tom 1, pp. 649—666. Torino: Rosenberg & Sellier 1952.

[102] —, and M. MEYER: Nucleoprotein in the nerve cells of mental patients: a critical remark. J. ment. Sci. **95**, 180—181 (1949).

[103] MEYER, J.-E.: Über mechanische Lageveränderungen der Purkinjezellen der Kleinhirnrinde. Arch. Psychiat. Nervenkr. **181**, 736—747 (1949).

[104] MILLER, R. A.: A morphological and experimental study of chromophilic neurons in the cerebral cortex. Amer. J. Anat. **84**, 201—229 (1949).

[105] MISKOLCZY, D.: Über die Frühveränderungen der Pyramidenzellen nach experimentellen Rindenverletzungen. Trab. Inst. Cajal Invest. biol. **23**, 135—150 (1925).

[106] MÜLLER, E.: Untersuchungen über den Bau der Spinalganglien. Nord. med. Ark. (N.F. Bd. 1) **23**, (No 26) 1—55 (1891).

[107] MÜLLER, G.: Zur Frage der Altersbestimmung histologischer Veränderungen im menschlichen Gehirn unter Berücksichtigung der örtlichen Verteilung. Z. ges. Neurol. Psychiat. **124**, 1—112 (1930).

[108] NISSL, F.: Über die Veränderungen der Ganglienzellen am Facialiskern des Kaninchens nach Ausreißung der Nerven. Allg. Z. Psychiat. **48**, 197—198 (1891).

[109] —, Über experimentelle erzeugte Veränderungen an den Vorderhornzellen des Rückenmarks bei Kaninchen mit Demonstration mikroskopischer Präparate. Allg. Z. Psychiat. **48**, 675—682 (1892).

[110] —, Mitteilungen zur Anatomie der Nervenzellen. Allg. Z. Psychiat. **50**, 370—376 (1893).

[111] —, Über die sogenannten Granula der Nervenzellen. Neurol. Cbl. **13**, 676—688, 781—789, 810—814 (1894).

[112] —, Über die Nomenklatur in der Nervenzellenanatomie und ihre nächsten Ziele. Neurol. Cbl. **14**, 66—75, 104—110 (1895).

[113] —, Der gegenwärtige Stand der Nervenzellen-Anatomie und -Pathologie. Cbl. Nervenhl. Psychiat. **18**, 1—21 (1895).

[114] —, Die Beziehungen der Nervenzellensubstanz zu den tätigen, ruhenden und ermüdeten Zellzuständen. Allg. Z. Psychiat. **52**, 1147—1154 (1896).

[115] —, Über die Veränderungen der Nervenzellen nach experimentell erzeugter Vergiftung. Neurol. Cbl. **15**, 947—949 (1896).

[116] —, Nervensystem; in EHRLICH-KRAUSE-MOSSE-ROSIN-WEIGERT's Enzyklopädie der Mikroskopischen Technik. 2. Aufl. S. 243—287. Berlin, Wien: Urban & Schwarzenberg 1910.

[117] OLSZEWSKI, J., and D. BAXTER: Cytoarchitecture of the Human Brain Stem. Philadelphia, Montreal: Lippincott 1954.

[118] ORSÓS, F.: Die vitalen Reaktionen und ihre gerichtsmedizinische Bedeutung. Beitr. path. Anat. **95**, 163—237 (1935).

[119] PAPADIMITRIOU, D. G.: Morphologische Untersuchungen am Zentralnervensystem über die stabilisierende Wirkung von Kaliumcitrat. Beitr. path. Anat. **120**, 371—381 (1959).

[120] PENFIELD, W., and W. CONE: Acute swelling of oligodendroglia. A specific type of neuroglia change. Arch. Neurol. Psychiat. (Chicago) **16**, 131—153 (1926).

[121] PETERS, G.: Zur Frage der pathologischen Anatomie der Schizophrenie. Z. ges. Neurol. Psychiat. **160**, 361—380 (1937).

[122] —, Schizophrenia. Atti 1° Congr. int. Istopat. Sistema nerv., Roma Tom 2, pp. 624—628. Torino: Rosenberg & Sellier 1952.

[123] —, Möglichkeiten und Grenzen der Hirnforschung in der Neurologie und Psychiatrie. Dtsch. med. Wschr. **80**, 433—437 (1955).

[124] RAHN, J.: Über besondere Zellbefunde in der Großhirnrinde des normalen Kaninchens. Z. mikr.-anat. Forsch. **62**, 70—84 (1956).

[125] RALSTON, H. J., W. D. COLLINGS, A. N. TAYLOR and E. OGDEN: Venous return in the absence of cardiac drive. Amer. J. Physiol. **145**, 441—445 (1945).

[126] RAMÓN Y CAJAL, S.: Über die Beziehungen der Nervenzellen zu den Neurogliazellen anläßlich des Auffindens einer besonderen Zellform des Kleinhirns. Mschr. Psychiat. Neurol. **1**, 62—66 (1897).

[127] —, Die Struktur des nervösen Protoplasma. Mschr. Psychiat. Neurol. **1**, 156—167, 210—229 (1897).

[128] —, Histologie du système nerveux de l'homme et des vertébrés. Trsl. from Spanish ed. 1909 by L. AZOULAY. Madrid: Inst. Ramon y Cajal, reprint 1952.

[129] —, Degeneration and Regeneration of the Nervous System. Trsl. from Spanish ed. 1913 by R. M. MAY. London: Oxford University 1928, and New York: Hafner, reprint 1959.

[130] REISER, K. A.: Die Nervenzelle; in v. MÖLLENDORFF-BARGMANN's Hb. d. mikr. Anat. d. Menschen, Bd. 4, 4. Teil, Ergänz. 4, S. 185—514. Berlin, Göttingen, Heidelberg: Springer 1959.

[131] RIML, O.: Über das Verhalten des Blutdrucks in der Vena cava bei plötzlichem Zirkulationsstillstande. Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. **139**, 231—239 (1929).

[132] —, Über das Verhalten des Kreislaufes bei Blutverlusten, operativer Schädigung, nach Halsmarkdurchschneidung, bei der Carotisabklemmung und der Kohlensäurevergiftung. Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. **139**, 240—256 (1929).

[133] RINNE, U. K.: Neurosecretory material around the hypophysial portal vessels in the median eminence of the rat. Acta endocr. (Kbh.) (Suppl. 57) **35**, 1—108 (1960).

[134] ROSE, M.: Über den Einfluß der Fixierung auf das Zellbild der Großhirnrinde. J. Psychol. Neurol. (Lpz.) **38**, 155—167 (1929).

[135] —, Cytoarchitektonik und Myeloarchitektonik der Großhirnrinde; in BUMKE-FOERSTERS Hb. d. Neurol., Bd. 1, S. 588—778. Berlin, Göttingen, Heidelberg: Springer 1935.

[136] SAMUEL, E. P.: Chromidial studies on the superior cervical ganglion of the rabbit. J. comp. Neurol. **98**, 93—111 (1953).

[137] SARKISSOW, S. A.: Über die Schrumpfung des Gehirns bei Paraffineinbettung. J. Psychol. Neurol. (Lpz.) **41**, 76—95 (1930).

[138] SCHABADASCH, A. L.: Morphology of glycogen distribution and transformations. I. Principles of fixation and staining of glycogen for micro- and macroscopic study. Bull. Biol. méd. expér., URSS, Moskva **4**, 13—16 (1937).— Abstr. Ber. ges. Physiol. **105**, 180—181 (1938).

[139] SCHARRER, E.: Bemerkungen zur Frage der „sklerotischen" Zellen im Tiergehirn. Z. ges. Neurol. Psychiat. **148**, 773—777 (1933).

[140] —, Über die Ganglienzellschrumpfung im tierischen Gehirn. Beitr. path. Anat. **100**, 13—18 (1937).

[141] —, On dark and light cells in the brain and in the liver. Anat. Rec. **72**, 53—65 (1938).

[142] SCHIROKOGOROFF, J. J.: Die Mitochondrien in den erwachsenen Nervenzellen des Nervensystems. Anat. Anz. **43**, 522—525 (1913).

[143] SCHOLZ, W.: Über den Einfluß chronischen Sauerstoffmangels auf das menschliche Gehirn. Z. ges. Neurol. Psychiat. **171**, 426—450 (1941).

[144] —, Erkrankungen des zentralen Nervensystems; in SCHOLZ' Nervensystem, LUBARSCH-HENKE-RÖSSLES Hb. d. spez. path. Anat. und Histol. Bd. 13, 1. Teil, Bandteil B, S. 1—265. Berlin, Göttingen, Heidelberg: Springer 1957.

[145] SHEININ, J. J.: On changes in the size, shape and distribution of the basophil substance in the neurocytosome after fixation in different fluids. Anat. Rec. **52**, 83—95 (1932).

[146] SMITH, A. G., and G. MARGOLIS: Camphor poisoning. Amer. J. Path. **30**, 857—869 (1954).

[147] SMITH, S. W.: "Reticular" and "areticular" Nissl bodies in sympathetic neurons of a lizard. J. biophys. biochem. Cytol. **6**, 77—84 (1959).

[148] SPIELMEYER, W.: Histopathologie des Nervensystems. Berlin: Springer 1922.

[149] STERN, K.: Der Zellaufbau des menschlichen Mittelhirns. Mit einem histopathologischen Anhang. Z. ges. Neurol. Psychiat. **154**, 521—598 (1936).

[150] SYRING, A.: Die Verbreitung von Spezialzellen in der Großhirnrinde verschiedener Säugetiergruppen. Z. Zellforsch. **45**, 399—434 (1957).

[151] TANS, J. M. J.: On the axonal swellings of the Purkinje cells (Torpedoes); in BIEMOND's Recent Neurological Research, pp. 223—244. Amsterdam, London, New York, Princeton: Elsevier 1959.

[152] TERLECKI, S., and L. M. MARKSON: Cerebrocortical necrosis in cattle and sheep. Vet. Rec. **73**, 23—27 (1961).
[153] TUREEN, L. L.: Effect of experimental temporary vascular occlusion on the spinal cord. Arch. Neurol. Psychiat. (Chicago) **35**, 789—807 (1936).
[154] —, Effect of experimental temporary vascular occlusion on the spinal cord. Arch. Neurol. Psychiat. (Chicago) **39**, 455—466 (1938).
[155] TURNER, J.: An account of the nerve-cells in thirty-three cases of insanity with special reference to those of the spinal ganglia. Brain **26**, 27—70 (1903).
[156] WEBER, A.: Expulsions de nucléoles hors des noyaux, dans des cellules pyramidales du cortex préfrontal, chez des malades atteints de troubles mentaux. Acta neuroveg. (Wien) **13**, 5—17 (1956).
[157] WEIL, A.: The form of the anterior horn cells of vertebrates. Trans. Amer. neurol. Ass. **52**, 547—558 (1926).
[158] —, Textbook of Neuropathology. 2nd ed. New York: Grune & Stratton 1945.
[159] WINDLE, W. F.: Discussion. Res. Publ. Ass. Res. nerv. ment. Dis. **35**, 165—166 (1956).
[160] WINDLE, W. F., R. F. BECKER and A. WEIL: Alteration in brain structure after asphyxiation at birth. J. Neuropath. exp. Neurol. **3**, 224—238 (1944).
[161] WOLF, A., and D. COWEN: Pathology; in METTLER's Selective Partial Ablation of the Frontal Cortex. New York: Hoeber 1949.
[162] —, — Histopathology of schizophrenia and other psychoses of unknown origin; in Milbank Memorial Fund's 27th Annual Conference, The Biology of Mental Health and Disease, pp. 469—486. New York: Hoeber 1952.
[163] ZEMAN, W.: Electrische Schädigungen und Veränderungen durch ionisierende Strahlen; in SCHOLZ' Nevensystem, LUBARSCH-HENKE-RÖSSLES Hb. d. spez. path. Anat. und Histol. Bd. 13, 3. Teil, S. 327—362. Berlin, Göttingen, Heidelberg: Springer 1955.
[164] ZIMMERMANN, H. M., and E. BURACK: Lesions of the nervous system resulting from deficiency of the vitamin B complex. Arch. Path. (Chicago) **13**, 207—232 (1932).
[165] ZURHEIN, G. M., P. L. EICHMAN and F. PULETTI: Familial idiocy with spongy degeneration of the central nervous system of van Bogaert-Bertrand type. Neurology (Minneap.) **10**, 998—1006 (1960).

Dr. JAN CAMMERMEYER,
Room 113, Building 9, National Institutes of Health, Bethesda, Maryland