HISTOCHEMISTRY OF HUMAN AUTONOMIC GANGLIA

A THESIS

by

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AUTONOMIC GANGLIA

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ABSTRACT

Human autonomic ganglia fixed in 10% formalin and embedded in paraffin were used in this study.

Several histochemical methods were applied to sections of this tissue for the investigation of the granules contained in the ganglion cells and among the fibers, as well as for the study of the other cellular inclusions.

Three types of inclusions are demonstrated in the ganglion cells. The one variety gives the reactions and the distribution of the neurosecretory substances. The other two types of granular inclusions can be differentiated by their natural color, the one being yellowish brown and the other colorless, by the fluorescence of the one type and by their reactions to the applied stains.

From the histochemical reactions of these granules it is suggested that they are related to the normal activity of the cells and they probably represent the carrier substances of the neurotransmitters or the complex of the carrier and noradrenaline itself.

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INTRODUCTION

The three upper lumbar ganglia together with the thoracic ones are included in the sympathetic trunk.

According to Kuntz (1953) the autonomic ganglia are enclosed in an outer connective tissue capsule which is continuous with the perineurium of the nerves which pass through them. The sympathetic ganglion cells are usually multipolar, but some unipolar and bipolar cells are also found. Each cell is surrounded by a nucleated membranous capsule. The cells forming the capsule, as well as the interstitial tissue cells, are regarded as being identical with oligodendroglia cells found in the central nervous system.

Binucleated nerve cells are found most commonly in young persons. These cells probably retain their capacity to undergo division in adult life without nuclear changes (Spiegel and Adolf, 1922). This idea is supported by the fact that sometimes two ganglion cells are found in a common capsule.

Different types of nerve cells are found in the autonomic ganglia. They are classified according to the morphological characters of their dendrites and their relationship to the other nearby cells.

In the lumbar sympathetic ganglia most of the cells are uniform with respect to their morphological characters.

They usually have both short and long dendrites. Most of the long ones lie in dendritic glomeruli, while the short dendrites are relatively straight. They both penetrate the capsule and terminate outside in dendritic arborizations.

The axons of the sympathetic ganglion cells are postganglionic and are unmyelinated or at most have a very thin myelin sheath. In many neurons the axons do not arise directly from the cell body but from the proximal portion of a dendrite.

The axons which pass through the ganglia and those which terminate there in synapses with the cells are large preganglionic myelinated fibers. The ramifications of the preganglionic fibers lie between the dendrites and synapse with them outside or inside the capsule. Sometimes they end directly on the surface of the cells forming ring-like boutons.

The constituents of the autonomic ganglion cells are the same as the constituents of all other nerve cells.

The Nissl substance seems to be distributed throughout the cytoplasm in the prenatal and early postnatal life, but in older ages it becomes more abundant and is found aggregated in the peripheral or perinuclear zone.

The Golgi apparatus forms a loose network distributed throughout the cytoplasm or around the nucleus. Sometimes it is composed of small spherical bodies which are either scattered in the cytoplasm or joined by threads to form a network.

The volume of the Golgi material and its distribution appears to be correlated with the functional state of the cell.

The pigments which are a cytoplasmic constituent of the sympathetic nerve cells have been the subject of numerour studies and have been related to the metabolism of the neuron. Meschede (1865) first observed in the nerve cells a fatty pigment as yellow glistening granules and called it "fatty pigmentary degeneration". Since that time many investigators have studied the pigmentation of the nerve cells. There is, however, much controversy concerning the nature and etiology of these pigments.

According to Rosin (1896), who studied the pigments of nerve cells, there are two kinds of granules, melanin and lipochrome and they both are normal constituents of the nerve cells. Hueck (1912) also found melanin and lipofuchsin as normal constituents of the nerve cells. He believes that these pigments are not restricted in any place of the nervous system and that both varieties can be found even in the same granule.

Hodge (1894) reported that pigmentation of the nerve cells is related to senility because he found that the nerve cells of older humans were filled with granules, while the nerve cells of the fetus contained almost no pigment.

According to Dolley and Guthrie (1918) the pigments, which are a melanotic and a fat holding one are not a product of normal or hypernormal activity but they are produced

by functional depression. These pigments are characteristic of senility (Sulkin and Srivanij, 1960) only because there is a depression of function in the process of aging.

However the occurrence of pigment in the cells of the sympathetic trunk has been reported in human fetuses of 6 and 7 months (Kuntz, 1953).

Marsden (1961) studying the pigmentation of the substantia nigra, which also increases with age, suggested that the presence of pigment in this nucleus is related to the motor functions of the cells, functions which become increasingly important as the relationship to man becomes closer.

Sulkin (1953) studying the histochemistry of the pigments compared the pigments found in the nerve cells of the human autonomic ganglion with the pigments found in the liver cells which are known not to contain melanin and the cells of the skin which contain melanin granules. The results of these tests present the evidence that there is no pigment of melanotic nature in the autonomic ganglion cells. According to Sulkin the pigments seem to be lipofuchsins related to ceroids.

According to Lennette and Scharrer (1946) neurosecretory granules are found in the autonomic ganglia of monkeys but these do not seem to be identical with these found in man and other mammals.

There is also some controversy in the opinion of the

investigators concerning the histogenesis of the pigments. Dolley (1917) believes that they are derived from the nucleus by transformation of nuclear chromatin. Gatenby and Moussa (1951) have reported that the pigment granules of the autonomic ganglia are derived from the Golgi apparatus by transformation of broken down pieces of Golgi.

According to Hess (1955) pigment formation in the spinal ganglion cells occurs in relation with swollen mitochondria. According to him the pigment granules accumulate and extend from one pole of the mitochondria.

The sympathetic ganglion cells are motor nerve cells and they give origin to postganglionic fibers which innervate peripheral tissues.

Elliott (1904) observed that after section of the sympathetic nerves the structures that they had innervated characteristically responded to adrenaline. This observation gave him the idea that adrenaline could be normally liberated when the impulse transmitted by the sympathetic fiber reached the peripheral organ.

Following this hypothesis many experiments have been made which prove the chemical mediation of the nerve impulse.

The sympathetic fibers are called also "adrenergic" because liberation of adrenaline follows their stimulation. However, the pharmacodynamic characteristics of noradrenaline seem to be more closely related to the action of adrenergic

nerves than adrenaline (Barger and Dale 1910).

Euler (1956) found that noradrenaline is the major adrenergic constituent of the sympathetic nerve and it is also present in the adrenal medulla.

Eränkö (1955) and Hillarp and Hökfelt (1953) described two kinds of cells in the adrenal medulla, one containing adrenaline and the other noradrenaline. Hillarp and Hökfelt observed also that both adrenaline and noradrenaline can be converted by oxidation into a brownish black insoluble product of a melanin like character.

Acetylcholine is known to be liberated by the parasympathetic cholinergic nerves. In some cases however also sympathetic excitation causes liberation of acetylcholine. A characteristic of neurohumoral agents is their storage in nervous tissue in a pharmacological inactive state and their release by nerve impulses. There has been a keen interest in the problems of the storage and release of neurohormones, but the nature of the mechanisms is still largely unknown. The particular locus where these mediators are formed is not clearly known. According to Blaschko and Hellman (1953) they are stored in the adrenal medullary cells in small cytoplasmic bodies which are similar, but not identical to mitochondria. Probably the same is true for the chemical mediator of the sympathetic nerves (Eliasson, Euler and Stjärne 1955).

The purpose of this study was to investigate the

morphology and cytochemistry of the cellular inclusions in sympathetic ganglia which could represent the carriers or bound forms of the neurohormones. Preliminary observations had indicated that three different types of inclusions are present in the sympathetic ganglia. Our histochemical staining methods were designed to distinguish and characterize these varieties and their relationship to intraganglionic synapses.

MATERIAL AND METHODS

The material used in this study was the lumbar sympathetic ganglionic chain removed during autopsy from a human female, 67 years old, suffering from rheumatic heart disease and an adult human adrenal. The tissues were fixed in 10% formalin and embedded in paraffin. In addition dorsal root ganglia from the dog, cat and rat were used. The dog tissue was fixed in 10% formalin, Helley's fluid and formal ammonium bromide. The cat ganglia were fixed in 95% alcohol and the rat's ganglia in Bouins fluid and 10% formalin.

Paraffin sections of 5u thickness were stained by different methods for the demonstration of myelinated and unmyelinated axons, inclusions, cellular organoids and neurosecretory substance. The following methods were used for the study of the cytoplasm.

Luxol fat blue-cresyl violet for the distribution of RNA protein and phospholipids as well as myelin. Gallocyanine alone or with carbol fuchsin and methylene blue for the demonstration of RNA and the Feulgen's reaction for the distribution of DNA.

The Gomori chrome hematoxylin the Van Giesen, Fuchsin Paraldehyde and Alloxan-Schiff methods were used for the demonstration of neurosecretory material.

For the study of the pigments which are found in

the cells, sections were stained with fuchsin paraldehyde, the Nile blue technique in sections deparaffinized and hydrated as well as extracted with methanol chloroform (1:1) over night, before hydration. Sections were also stained with methyl green for the demonstration of ceroids (Popper, György and Goldblatt, 1944) and with silver diammine (Nassar, Issidorides, Shanklin, 1960) directly or after oxidation with Gomori mixture (Potassium permanganate and sulfuric acid).

Sections were also stained according to Schmorl and Masson technique for the argentaffin cells (ferric chloride and potassium ferricyonide).

P.A.S. was used for the demonstration of 1, 2 glycols. Schiff reagent was also used after oxidation of the tissues with Gomori mixture and peracetic acid.

For the demonstration of phospholipids the Sudan black technique was followed with neutral red which shows the distribution of lipids as a counterstain.

Sections stained with the alloxan-Schiff - L.F.B. method were studied for the distribution of aminoacids.

This material was also studied with the fluorescence microscope before and after various oxidations and extractions.

References to most of the staining methods used in this study are included in a recent departmental publication (Issidorides and Shanklin, 1961) .

OBSERVATIONS

The distribution of the nuclear chromatin material was studied after the Feulgen reaction. The satellite capsule cells and interstitial neuroglia had a high concentration of DNA protein, whereas the nuclei of the nerve cells were rather lightly stained.

The chromidial substance studied in sections stained with cresyl violet and methylene blue does not always have the same distribution. In some cells it is scattered throughout the cytoplasm, while in others it is arranged in a ring around the nucleus, or at the periphery of the cytoplasm.

In sections of the human lumbar ganglia stained with fuchsin paraldehyde, a considerable amount of intensely stained purple granules is shown. These granules are usually found occupying a large part of the cytoplasm (Figs. 1,2), while in some cells the whole cell body is filled. The positive stained granules are not restricted in the cell body area only, but are also found in the interstitial tissue (Fig. 1), in the cell processes and in the capsule surrounding the cells, where they are laid down in a linear order which probably indicates that they follow an outgoing or incoming nerve fiber (Fig. 2).

When we stained sections with the LFB- cresyl violet method in order to study the distribution of the myelinated

fibers we saw that the granules were stained dark blue (Figs. 4,6,8). In this case also granules are found in the cell processes, in the interstitial tissue and along the capsules surrounding the nerve cells.

In order to determine whether the granules seen after the LFB-cresyl violet method are the same as the ones taking up fuchsin paraldehyde we used both methods on the same slide as follows.

After photographing a particular cell stained with LFB-cresyl violet we destained the section and applied fuchsin paraldehyde. We were thus able to compare the distribution of the granules in exactly the same cell. The granules have the same distribution in both methods but additional ones appear in the cells after fuchsin paraldehyde (Compare Figs. 3,4,5 and 6,7,8).

Fuchsin paraldehyde positive granules were also seen between the fibers which surround the cellular mass of the ganglion (Fig 9). This bundle of fibers, as seen in the LFB-cresyl violet stained section, is composed of a few large myelinated fibers, a larger number of narrow myelinated and many thin, unmyelinated fibers (Fig. 10). The nature of all these granules, found in the ganglion, was tested by the application of different histochemical methods. Almost all the reactions demonstrated two clearly distinct sets of inclusions inside the cells. The same thing was seen in completely unstained sections examined under the

microscope. Some cells, contain yellowish-brown granules, whereas the majority of cells are completely colorless. The brown granules have the tendency to retain their natural color (from light to dark brown) after the application of PAS, carbol fuchsin and methyl green (Figs. 11, 12, 13, 14). The other set of granules is positive to PAS (Fig. 11) and methyl green (Fig. 13). After carbol fuchsin more or less homogeneous acid fast material occupies the place of PAS and methyl green positive granules.

In sections stained by the Sudan black neutral red technique the granules appeared light brown in some cells and dark brown towards black in others (Fig. 15). Positive colored granules are also seen in the nerve fibers. Sometimes 2 or 3 of them, arranged one behind the other, seem to follow the course of the fibers (Fig. 16). Sudan black and neutral red negative vacuoles were observed throughout the cytoplasm of the nerve cells (Fig. 15). These appear to be identical with the red stained vacuoles after the application of the Van Giesen technique (Fig. 18).

Gomori's chrome hematoxylin demonstrated also phloxine positive vacuoles in the cytoplasm, in addition to a more or less homogeneous faint blue neurosecretory material (Fig. 17).

The Nile blue technique demonstrated positive material within the cells, which was seen in the vicinity of the nucleus, (Fig. 25) spread throughout the cytoplasm (Fig. 26),

or accumulated at the periphery of the cell (Fig. 27). The Nile blue positive material consists either of darker granules embedded in a lighter homogeneous material (Figs. 25,27), or of distinct large globules (Fig. 26). These figures are very similar to figures of neurosecretory material demonstrated in the neurons of the supraoptic nucleus.

The granules within the ganglion cells are positive to ferric chloride, whereas they remain unstained in sections stained with alloxan-Schiff. This negativity of the granules to alloxan-Schiff and the strong positive reaction to fuchsin paraldehyde resembles very much the reactions of the granules found in the Purkinje cells of the cerebellum (Issidorides and Shanklin, 1961).

In view of the fact that the adrenal medullary cells are modified sympathetic postganglionic nerve cells, we applied fuchsin paraldehyde to a section from a human adrenal. We also found in the medullary cells (Fig. 19) positive granules in a section stained with LFB-cresyl violet. We also found LFB positive granules, but this time they were much less than those stained with fuchsin paraldehyde (Fig. 20).

The observation that after fuchsin paraldehyde always more granules appeared in the sections led us to apply various histochemical methods in the sections of the ganglia after previous oxidation with the Gomori mixture. Indeed the PAS and methyl green, which gave us two different sets

of granules in the cells when they were applied without oxidation demonstrated this time positive granules in all the cells. We also applied fuchsin paraldehyde, without previous oxidation, and found that in some cells the granules remain unstained being light brown, which is their natural color, while in others there is a fuchsin paraldehyde positive material (Fig. 21). However, it is not as abundant as that seen in sections stained after oxidation (Fig. 22).

After silver diammine impregnation we got argentophilic granules but not in all the cells and not as many as those which appear after fuchsin paraldehyde (Fig. 23). However, when we oxidized the section with Gomori mixture, previous to the silver impregnation, none of the cells had stained granules. From these observations it seems to be clear that when the granules are treated with the Gomori oxidizing mixture they react identically to the various stains applied above.

Observations with the fluorescence microscope showed some cells with bright greenish yellow fluorescent granules, while the majority of cells did not fluoresce although granules can be demonstrated in them by staining procedures.

DISCUSSION

Our observations are in agreement with those of the majority of the early investigators (Dolley and Guthrie 1918) and with Kuntz (1953) who believe that the granules contained in the cells of the autonomic sympathetic ganglia are of two types. As to the nature of the two types of granules our results agree only partially with previous reports. Kuntz (1953) describes a yellow pigment of limited solubility in ether and a second pigment which is highly insoluble and of melanotic nature.

In order to determine whether any one of the two types of granules in the ganglion cells was giving the same histochemical reactions with melanin we made a comparative study of sections of the substantia nigra and of autonomic ganglia. We stained sections of both tissues with the same methods and we compared the granules found in the cells of the substantia nigra, which are known to be melanin, with the granules found in the autonomic ganglion cells.

The one set of granules found in the ganglion gave almost the same reaction with melanin except in two cases: when stained with luxol fast blue the granules of the ganglion are stained blue while the melanin granules remain unstained and are seen with their natural black color. Also with the sections stained with silver diammine after previous oxidation with the Gomori mixture the melanin granules ap-

pear black whereas all the autonomic ganglion cells are free of granules.

From these results we conclude that these granules although similar to melanin in many of their reactions are not melanin in nature. We also disagree with Sulkin (1953) who believes that there is only one type of pigment present in the cells and that this is lipofuscin related to ceroid. Lipofuscin granules in the central nervous system stain intensly black with silver diammine impregnation after oxidation with the Gomori mixture (Nassar, Issidorides and Shanklin 1960). The same oxidative procedure when applied to the autonomic ganglion cells, however, blocks completely the impregnation of all granules in all cells.

Our results indicate the presence of phospholipids in the granules, because they are both LFB and Sudan black B positive. The presence of unsaturated fats is also indicated from the positive peracetic acid-Schiff reaction. The presence of cystine is suggested by the fact that the granules are positive to fuchsin paraldehyde, which is known to stain scleroproteins even without previous oxidation.

Scleroproteins, as those found in the elastic tissue of arteries, usually contain cystine and tyrosine. This is of particular interest because tyrosine is the precursor of noradrenaline during its biosynthesis.

The two varieties of granules described in this study can be differentiated by the tendency of the one variety to

retain its natural brownish color when stained by the different methods except after Nile blue. This tendency disappears after oxidation with acidified potassium permanganate. As we have noticed earlier, Nile blue stains a fairly homogeneous ground substance and globules in the cytoplasm. The distribution of these substances is similar to neurosecretory material as seen in the hypothalamic nuclei. Furthermore the chrome hematoxylin and Van Giesen methods also, to some extent, reveal globular positive inclusions. On the basis of these reactions we consider this to be a third variety of ganglionic inclusion related also to the metabolism and production of neurohormones.

The catechol amines (adrenaline and noradrenaline) found in the chromaffin cells of the adrenal medulla are stored in different cells and in specific granules (Hillarp and Hökfelt 1953). Eränkö (1955) differentiated the cells containing adrenaline from those containing noradrenaline by their fluorescent properties, noradrenaline with formalin giving bright greenish fluorescence while adrenaline not being not fluorescent.

Reversible bindings have being shown to occur between noradrenaline and lecithin. This complex is broken up by the action of acids (Euler, 1956). Hillarp and Nilson (1954) also found that about one half of the adrenal medullary phospholipids are concentrated in the granules containing the hormone.

Noradrenaline in sympathetic cells is produced inside granules which contain ATP 15 percent, proteins 35 percent, and lipids 22 percent in a complex with which the noradrenaline becomes bound and in which it is stored away from the destructive enzymes of the cytoplasm. All this information has been obtained from physiological experiments and biochemical determinations from homogenized sympathetic ganglia. Although these granules have been histochemically demonstrated in the adrenal medulla they have never been described in the sympathetic ganglia.

In this study we have obtained information about the pigments or inclusions of the sympathetic neurons which indicates that they may possibly be the structures carrying noradrenaline in a bound form in the same way as in the adrenal medullary cells. Noradrenaline can be released from this bound form, by the action of acids, by heating, or by detergents.

We made the hypothesis that these granules which produce and store the neurotransmitters according to Euler (1958) could be the granules observed in our preparations. These granules occupy not only the cell body but they are also found in the cell processes and in the axons.

The bodies which manufacture and store the neurotransmitters can be formed according to Euler (1958) in the area around the nucleus and then spread towards the periphery. In a section stained with Nile blue sulphate we saw

such a course of the granules (Fig. 25, 26, and 27).

Elliott (1904) was the first who made the hypothesis that stimulation of the sympathetic nerves could normally cause liberation of adrenaline from their nerve endings. Indeed after many experimental studies the fact of the adrenergic mediation is established. However noradrenaline is found to be the specific neurohumor of the adrenergic nerves. Extractions from sympathetic nerves contain only a small amount of adrenaline (Euler, 1956). However adrenaline is known to potentiate the action of acetylcholine and furthermore has been demonstrated in the autonomic ganglion cells as a result of preganglionic stimulation (Burn, 1945). This amount of adrenaline liberated in the postganglionic neurone modifies the transmition of the impulse.

The presence of noradrenaline in the axons as well as in the cell body of the sympathetic nerves is indicated from extracts of thoracic sympathetic ganglia. Euler and Hillarp (1956) homogenised spleen tissue and splenic nerves and subjected the suspension to fractional centrifugation. Noradrenaline was present in the obtained sediment in a bound form.

The fact that in sections stained with fuchsin paraldehyde after oxidation more granules were always present in the cells lead us to the hypothesis that probably in our preparations we were seeing both the carrier substance and the neurohormone. According to Euler (1958) the granules do

not leave the axon during stimulation, but they only discharge their contents of hormone.

The acidic environment of the Gomori mixture could liberate the catechol amines from their carrier and this may result in a uniform reaction of the granules to the applied stains.

The presence of noradrenaline is indicated in the cells which contain the greenish yellow fluorescent bodies. However further investigation is needed for the identification of the exact nature of these two types of granules and the globules which are found in the cells, after special treatment of the tissues for the demonstration of adrenaline and noradrenaline.

Kuntz (1953) believes that excessive pigmentation is always related to pathological conditions and that extracellular pigment is found only when the cells are heavily pigmented.

We never found necrotic figures between the cells of the human autonomic ganglia although most of them were heavily pigmented. In addition in the dog and cat ganglia where only slight amount of pigment was found inside the cells, granules were also seen between the nerve fibers.

That increased granulation is an indication of depression (Dolley and Guthrie 1918) is apparently not true because even melanin in the phylogenetic scale of animals increases per cell and the cells increase in number as the

animals get closer to man and the motor functions depending on the substantia nigra nucleus become more important (Marsden 1961).

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PLATE 1

Explanation of Figures

All figures are photomicrographs of human autonomic ganglia fixed in formalin.

Fig. 1 Ganglion cell stained by fuchsin paraldehyde demonstrating well stained granules in the cytoplasm and in the interstitial tissue. X.630

Fig. 2 Ganglion cell stained with fuchsin paraldehyde showing granules in the cell body and in the capsule of the ganglion cell. X.630

Figs. 3 and 4 the same ganglion cell stained first (fig. 4) by luxol fast blue - cresyl violet, then destained and restained (fig. 3) by fuchsin paraldehyde. X.1080

Figs. 5 and 6. A binucleated ganglion cell first stained with luxol fast blue (fig. 6), destained and restained (fig. 5) with fuchsin paraldehyde. X.1080

Figs. 7 and 8. A ganglion cell first stained with luxol fast blue (fig. 8), destained and restained (fig. 7) with fuchsin paraldehyde. X.1080

In the above 6 figures more abundant granules appear after fuchsin paraldehyde than after luxol fast blue.

PLATE 2

Explanation of Figures

Fig. 9 Bundle of nerve fibers passing through one of the autonomic ganglia. Note the fuchsin paraldehyde positive granule among the nerve fibers. X.1080

Fig. 10 Bundle of nerve fibers passing through one of the autonomic ganglia. Large ones showing incisures of Schmidt-Lantermann are myelinated fibers, while the small ones in the center of the field are unmyalinated. Luxol fast blue - cresyl violet. X.1080

Fig. 11 Ganglion cells stained with PAS - luxol fast blue showing two varieties of cellular inclusions. X.1080

Fig. 12 Cells stained by carbol fuchsin - luxol fast blue showing two types of inclusions. X.1080

Fig. 13 Ganglion cells stained with methyl green for demonstration of cerroid - like substances. Note positively stained cell upper left and other cells with granules retaining their natural brown color. X.108

Fig. 14 Ganglion cells stained with carbol fuchsin for acid fast material and methylene blue for Nissl. bodies. The acid fast material is stained red. X.1080

Fig. 15 Two ganglion cells stained with Sudan Black B - neutral red. The Sudan Black B positive granules are stained black, while the neutral red stained material is globular. X.1080

Fig. 16 Bundle of nerve fibers stained with Sudan Black B - neutral red. Note the Sudan Black B positive granules among the fibers. X.1080

PLATE 3

Explanation of Figures

Fig. 17 Ganglion cells stained with Gomori chrome hematoxylin-phloxin showing blue stained neurosecretory material. X.1080.

Fig. 18 Ganglion cells stained by van Giesen method showing globular inclusions in the cell. X.1080.

Fig. 19 Adrenal medullary cells stained by fuchsin paraldehyde showing positively stained granules. X.1080.

Fig. 20 Adrenal medullary cells stained by luxol fast blue - cresyl violet showing luxol fast blue stained granules. X.1600.

Fig. 21 Two ganglion cells stained with fuchsin paraldehyde without oxidation. Note positive stained granules in cell on the left, and natural color of pigment in cell on the right. X.1080.

Fig. 22 Two ganglion cells stained with fuchsin paraldehyde following oxidation. After oxidation all granules are fuchsin paraldehyde positive. X.1080.

Fig. 23 Two ganglion cells after silver impregnation without oxidation showing argentophilic granules. X.1080.

Fig. 24 Two ganglion cells after silver impregnation and oxidation. Note all argentophilic granules are negative. X.1080.

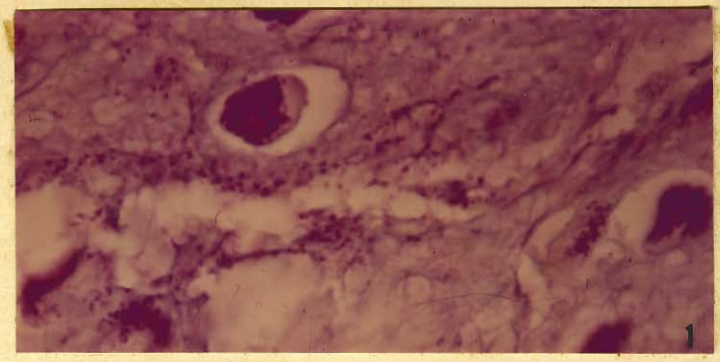
PLATE 4

Explanation of Figures

Fig 25 Ganglion cell stained with Nile blue. X.1080.

Fig 26 Two ganglion cells stained with Nile blue. Note the large positively stained globules. X.1080.

Fig 27 A ganglion cell stained with Nile blue showing positively stained granules concentrated at the periphery of the cell. X.1080.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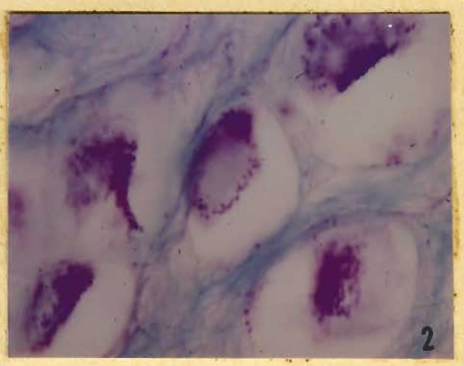
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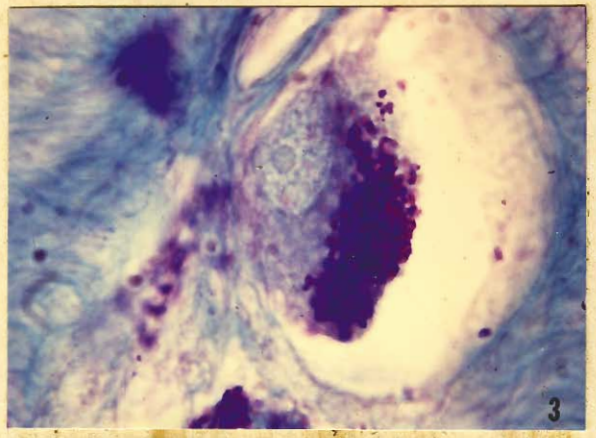
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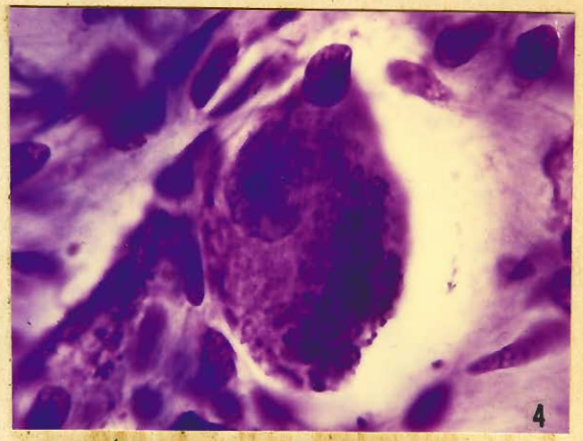
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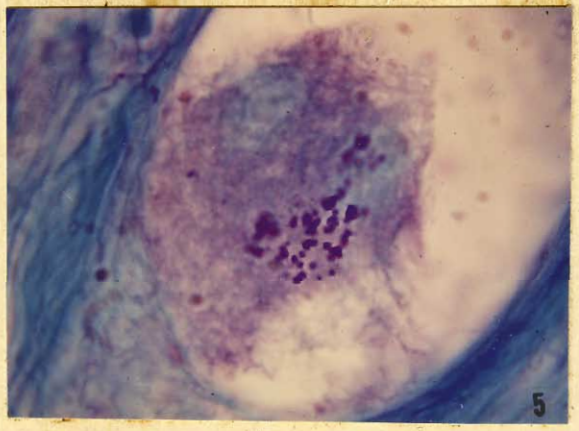
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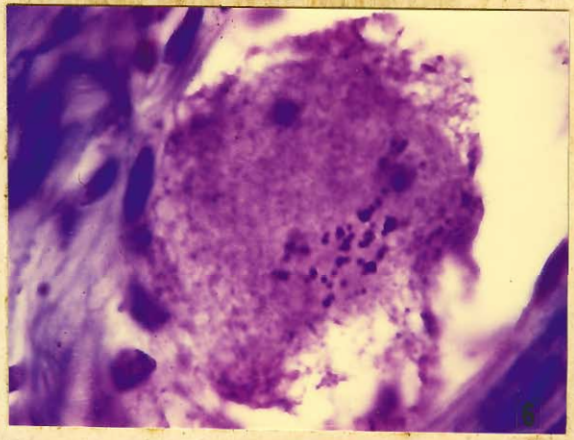
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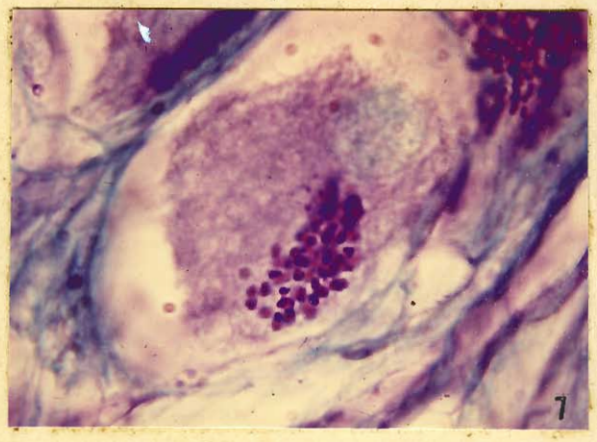
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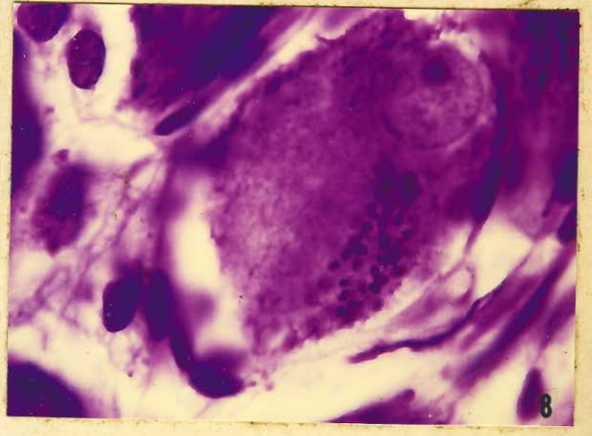
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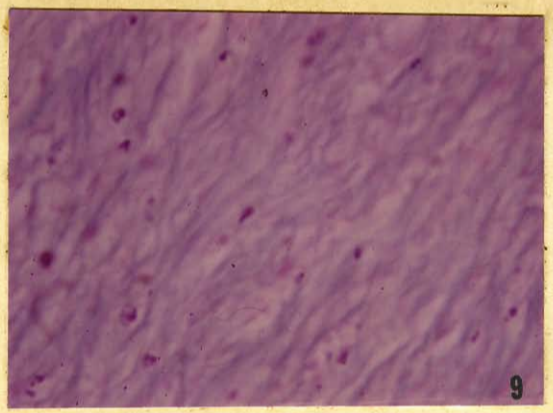
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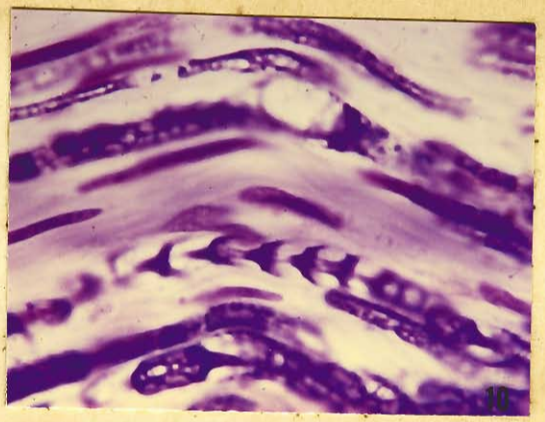
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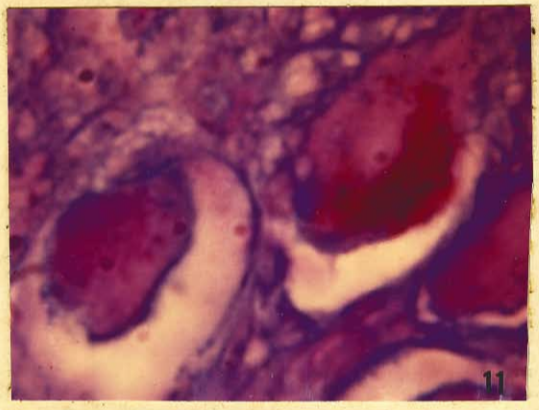
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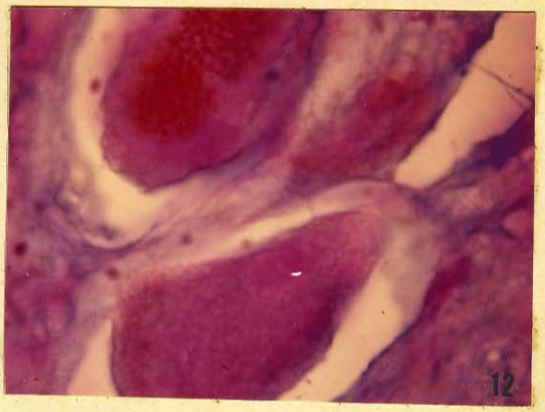
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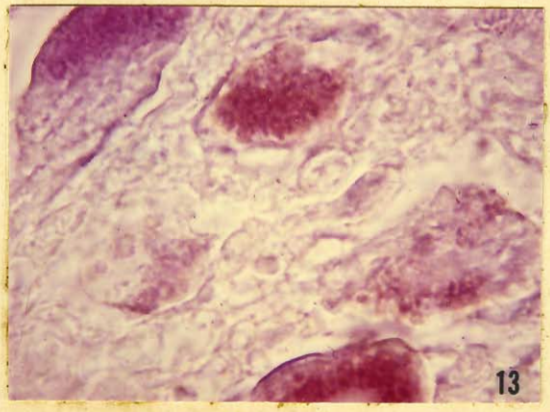
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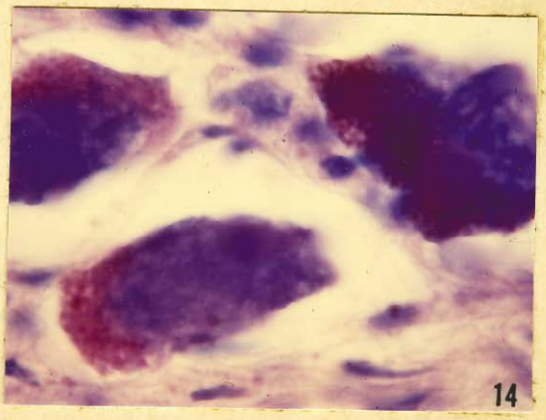
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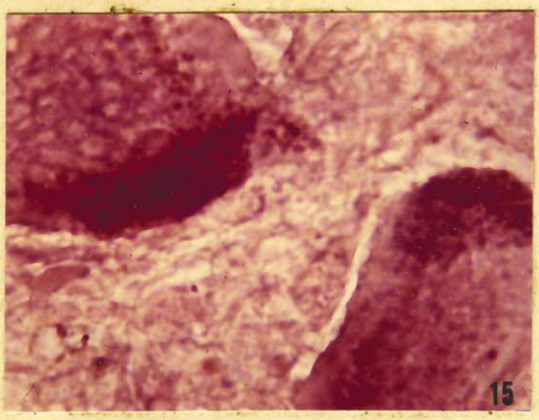
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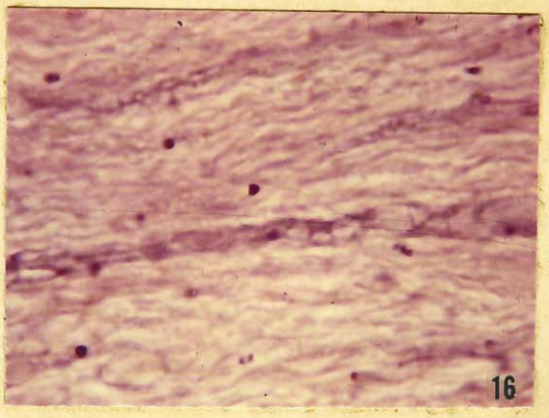
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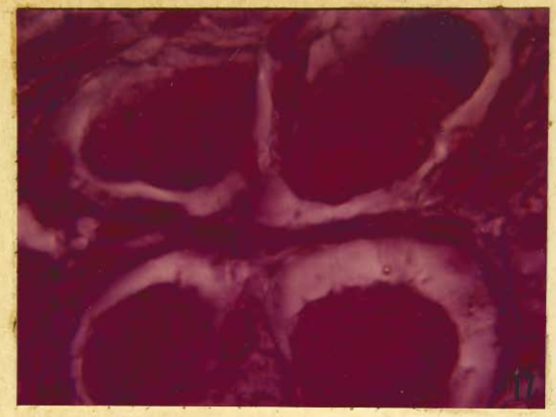
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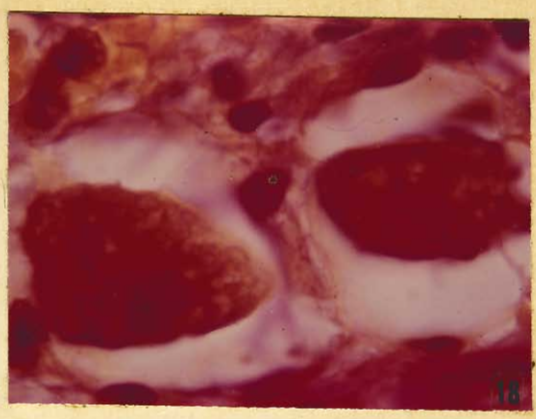
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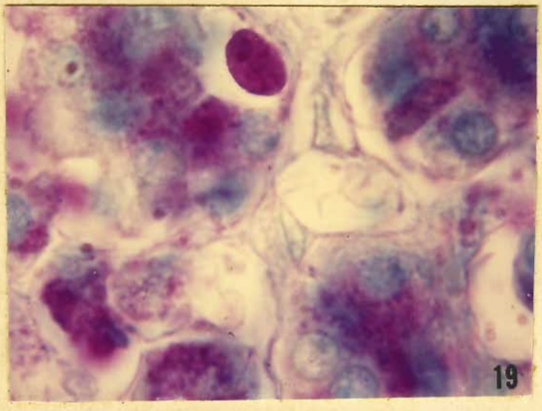
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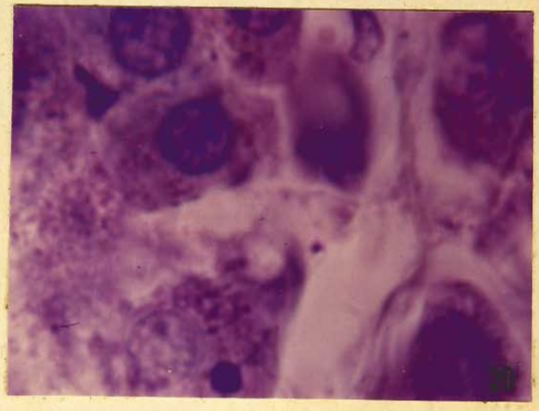
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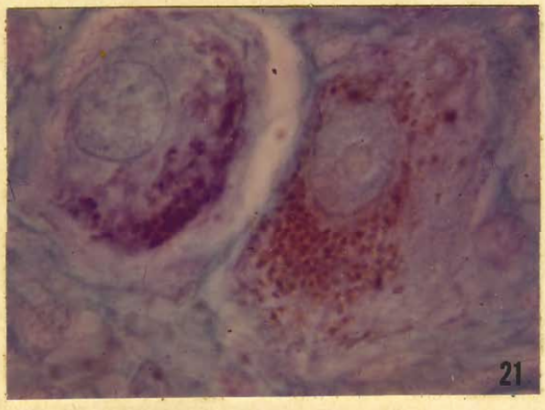
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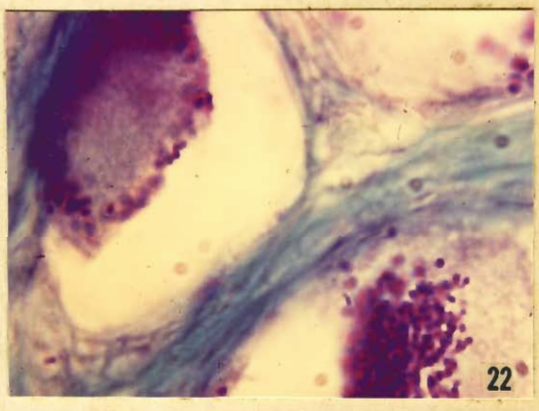
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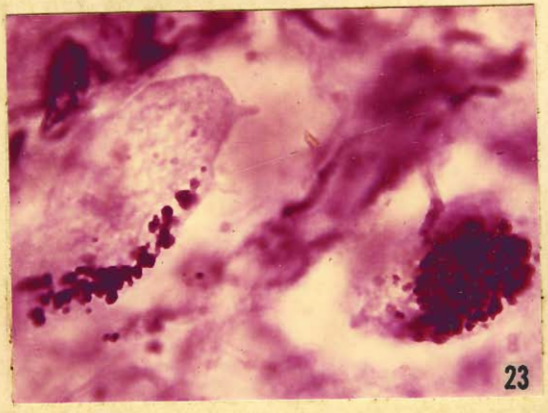
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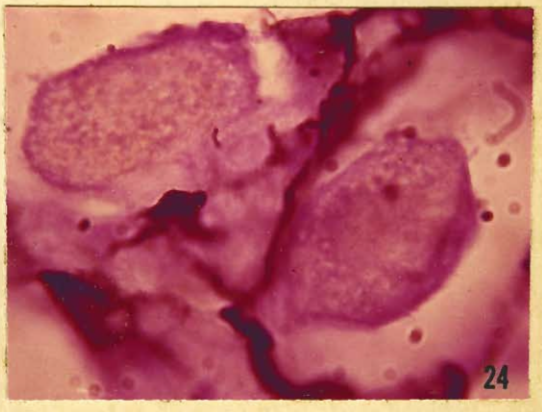
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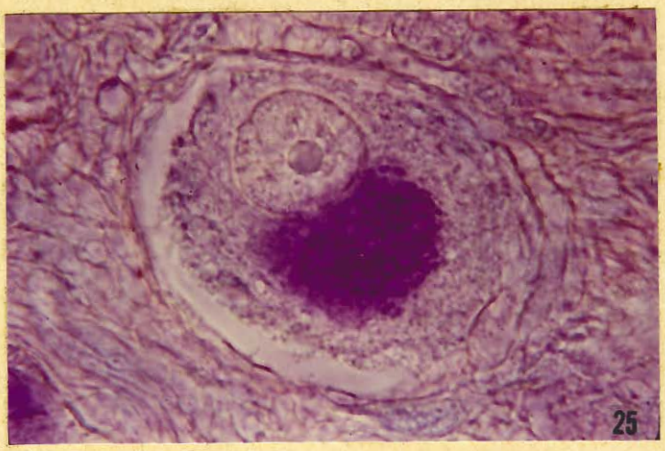
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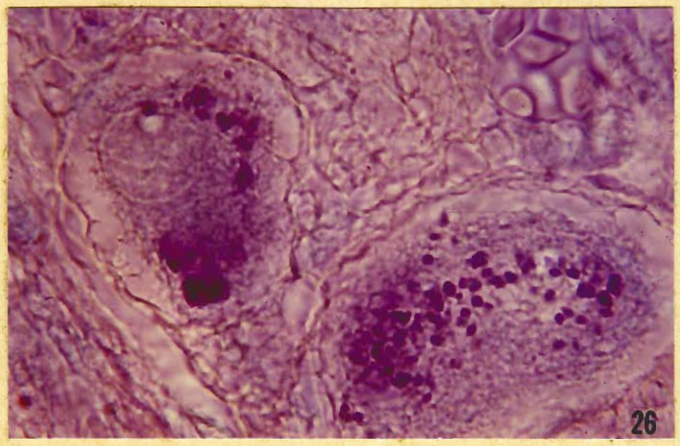
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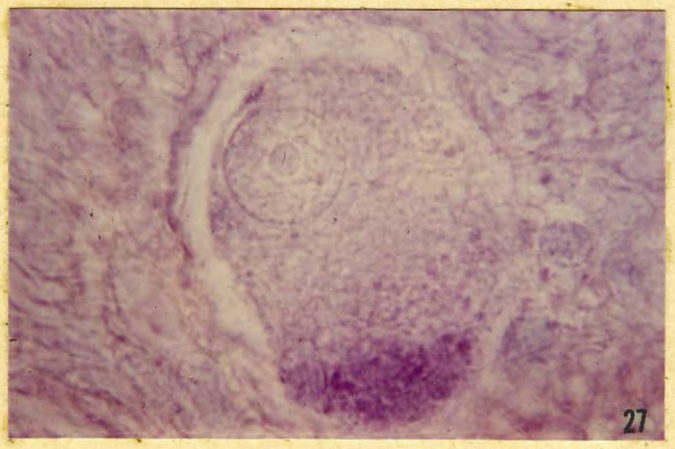
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