STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

VIII. Experimental Morphologic Pathology of the Chemical Transmitter of Nerve Impulses in the Course of Wallerian Degeneration *

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The effects of nerve impulses on the structure of muscle are unknown. The morphologic effects of wallerian degeneration, revealed by the gold-and-teasing method of whole muscle fibers, upon the voluntary neuromuscular apparatus, are likewise practically unknown. It is emphasized at the outset of this paper that the value of the observations to be presented depends largely upon the histologic method selected to demonstrate the structural union of nerve and muscle. Objective evidence of the morphologic transmitters of nerve impulses has not been conclusively demonstrated. Presumptive evidence has been presented that the motor end-plates influence the structure [1] of, and that they discharge granules of acetylcholine [2] into, the myoplasm of striated muscle during the early stages of poliomyelitis, certain other acute infections, rigor mortis, injection of lactic acid locally into the muscle, shock, and specific drug actions. There appears to be a metabolic destruction of the motor end-plates under certain nosologic conditions.

The whole theory of the chemical transmission of nerve impulses was the result of a simple and classical experiment by Loewi [3] on the heart of the frog. He established that, upon stimulation of the vagus nerve, acetylcholine appeared in Ringer's solution placed within the cavity of the heart. When this fluid from the donor heart was placed in the cavity of the denervated recipient heart, the inhibition was comparable to that of vagus stimulation. The accelerator nerves were stimulated, and evidence was obtained that an accelerator substance, adrenalin, appeared in the perfusion fluid. Loewi established the fact that a nerve impulse is transmitted to cardiac tissues indirectly by chemical substances which were called chemical transmitters.

When normal blood is circulating through the heart there is rapid disappearance of acetylcholine. This was proved to be due to a hydro-

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lyzing enzyme in the blood named cholinesterase. This enzyme splits acetylcholine and renders it ineffective. It was likewise proved by Loewi [3] that eserine has an inhibitory effect on cholinesterase. That eserine prolonged the effect of vagus stimulation had been known for a long time.

Dale, Feldberg, and Vogt,[4] after indirect stimulation through the nerve fibers of perfused voluntary muscles, found acetylcholine in the eserinized perfusion fluid. They stated that artificial perfusion with an eserine-containing saline fluid was necessary for success and that all that their evidence showed was that acetylcholine, which had escaped from the sites of its liberation, required protection by eserine to enable it to diffuse into fluid perfused through the vessels. Curare was demonstrated to have an effect on the receptive element by making the muscle substance more resistant to the action of acetylcholine. They likewise established the fact that direct stimulation of muscle tissue 10 days after denervation did not release a trace of acetylcholine. This substance, therefore, was released from the nerve endings and was not a product of muscle activity or of the blood supply. Brown, Dale, and Feldberg [5] then demonstrated that acetylcholine injected into the artery close to the bled muscle caused a rapid muscle twitch similar to that produced by nervous stimulation.

Nachmansohn [6] demonstrated subsequently that cholinesterase was more highly concentrated about the nerve endings in the sartorius muscle of the frog than at the ends of the muscle. Nachmansohn and John [7] found choline acetylase in the nerve axon, and stated that the presence of this enzyme is consistent with the view that the primary event responsible for the alteration of the axon membrane during the passage of the nerve impulse is the release and removal of acetylcholine, and that the energy-rich phosphate bonds during recovery are used for the synthesis of the acetylcholine removed during activity. It was found that only a part of the initial enzyme activity had been lost 3 days after nerve section when conductivity had disappeared.

The relationship of the production of acetylcholine and its destruction to the pleomorphism of the motor end-plates and the various fiber types has not been established. It is known that differences among the fibers disappear after starvation, denervation, muscle contraction, and heat rigor. The mechanism of production of dark and light fibers in the same mammalian muscle is still obscure.

Waller [8] was the brilliant initiator of the studies in the degeneration of nerves almost 100 years ago, yet wallerian degeneration of the neuromuscular apparatus in voluntary muscle has never been followed in a closely graded series of changes by the gold-and-teasing method of whole muscle fibers following the sectioning of the motor nerves. Waller

made his observations primarily on fresh tissue and on tissue treated with caustic potash. Huber [9] used methylene blue in his experimental studies and cited the limitations of this method. Cajal [10] used the silver and sectioning method which likewise has its limitations. The advantages and disadvantages of the gold method will be cited under "Materials and Methods" in this paper.

The precocious failure of transmission of nerve impulses after the third day in the course of wallerian degeneration of cholinergic nerve fibers has been attributed recently (a) to an early degeneration of nerve endings (Titeca [11]); (b) to a decrease of acetylcholine liberation (Coppée and Bacq [12]); (c) to heterochronism (Chauchard [13]); and (d) to a failure of conduction in the nerve fibers (Rogers and Parrack [14]). The contradictions of fact and of inference involved in these four interpretations prompted a restudy of the problem by Lissák, Dempsey, and Rosenblueth,[15] who came to the conclusion that the mechanism of liberation of acetylcholine at cholinergic nerve endings upon the arrival of a nerve impulse is unknown and that the failure of transmission of nerve impulses about the third day after denervation is due to a progressive decrease in acetylcholine liberation, thus substantiating the theory of Coppée and Bacq. Lissák, Dempsey, and Rosenblueth concluded that total failure of transmission about the third day after nerve section was only a terminal step in a gradual decaying process and that this failure of transmission preceded the sudden failure of conduction in the distal stump of the cut nerve.

One great gap in our knowledge of the transmission of nerve impulses through motor end-plates is due to the absence of conclusive evidence based on experimental morphology. The chemical and physiologic actions in the body are intimately related to the structural arrangement of the protoplasm. If the motor end-plates are microscopic endocrine glands that discharge, under exaggerated stimulation, neurogenic bodies or neurosomes composed of acetylcholine, then microscopic morphology must substantiate this claim.

In this paper we have occupied ourselves with the exact and clear description of those morphologic facts which are certain and easily verified. This has necessitated a large number of illustrations in the form of untouched photomicrographs made from unimpeachable preparations. This gives an atlas of the sequence of changes during the course of wallerian degeneration of the motor end-plates which may serve as a source of reference and a line of departure for additional experimental studies and for the basis of the hypothesis that ameboid motion occurs at these motor end-plates and that a granular secretion is discharged into the muscle.

The objectives of this paper, therefore, are the morphologic demon-

stration of untouched photomicrographs which substantiate the following statements: (1) that there is a morphologic as well as a chemical transmitter of normal and abnormal nerve impulses; (2) that the motor end-plates under normal conditions discharge periodically a finely granular secretion which forms a nebulous rhythmic wave of diffusion into the myoplasm, and which may be agglutinated into large neurosomes in the course of wallerian degeneration; (3) that these motor end-plates undergo a periodic retraction and expansion by ameboid motion in relation to the storage in them, and the discharge from them into the myoplasm, of neurogenic substances; (4) that the failure of transmission of nerve impulses into the muscle about the third day after nerve section parallels the disappearance of the discharge and diffusion of the fine neurogenic granular secretion and the appearance of pathologic changes in the neuromuscular apparatus; (5) that the functional, dark type of muscle fiber, which progressively disappears after denervation, is normally associated with the periodic discharge and diffusion of the fine neurogenic granules of acetylcholine from the motor end-plate into the myoplasm at the onset of contraction; (6) that the same muscle fiber may be either dark and granular or light and relatively agranular dependent upon the functional phases of either acetylcholine diffusion, at the onset of contraction, or acetylcholine destruction by cholinesterase during full contraction, respectively, when the fibers were fixed during fractional contraction; (7) that the increase of the granules around the bloated subsarcolemmal nuclei is coincident in time with the persistence of cholinesterase after denervation; (8) that the facts support the claim that denervation atrophy of muscle is associated with the loss of the normal periodic diffusion from the motor nerve endings into myoplasm of acetylcholine granules having a strong affinity for gold, and that, conversely, the hypertrophy of normal muscle due to the chronic effect of exercise may be the result of the quantitative increase, over a unit of time, of the discharge of neurogenic granules from nerve endings to muscle; and (9) that the relationship of nerve to muscle is one of periodic anatomic continuity through the confluence and compounding of neurogenic and myogenic substances. The structure of voluntary muscle, therefore, has a dual composition.

Materials and Methods

One hundred adult white rats *(Mus norvegicus)*, 10 to 12 months old, with an average weight of 255 gm., were used. All surgical procedures were carried out under ether anesthesia and aseptic precautions. Segments 1 to 3 cm. in length were excised from the right sciatic nerve at the level of the trochanter. The left sciatic nerve remained intact and the neuromuscular apparatus of the left gastrocne-

mius muscle was used as a control. Since our observations were confined largely to the first 30 days after section of the right sciatic nerve, our experiments were not complicated by regeneration, as was proved by lack of response of the muscle to electric stimulation of the distal stump of the nerve. The reaction of the gastrocnemius-soleus muscle to indirect faradic stimulation was tested through the distal stump of the cut sciatic nerve immediately after the operation and on the dates selected for excision of the muscle. There was progressive decrease in the response of the muscle to stimulation of the nerve until the third to the fifth day, after which nerve stimulation was ineffective in producing muscle contraction.

In preparing the muscle for the gold technic previously described,[1] the animals were placed under light ether anesthesia and the gastrocnemius muscles quickly excised. This procedure avoided the changes that might occur with post-mortem rigidity.

At designated times after nerve section some animals were curarized, by either intramuscular or intraperitoneal injection of approximately 1 mg. per kg. of d-tubocurarine chloride (Squibb), until neuromuscular block occurred. Under light ether anesthesia the sciatic nerve and gastrocnemius muscle were exposed on the left and right side. In some, the sciatic nerves on both sides were stimulated at the rate of 5 to 10 times per second for 30 seconds with the double electrodes composed of nickel-plated copper, of the Dumont variable frequency stimulator type 210.

After the operation, the gastrocnemius muscles from 5 rats were excised after each 24-hour interval up to 15 days. The mus es from 2 rats were excised daily from the 15th to the 25th day after nerve section, and those from the remaining 5 rats were excised on the 30th day.

The excised muscles were subjected to various histologic methods for the identification of lipoids and other substances and to visualize the nuclear and cytoplasmic constituents of the neuromuscular apparatus. The Cajal [10] silver method, as well as that of Bielschowsky modified by Boeke,[16] is a good one to identify nuclei, neurofibrils, and the perterminal network of Boeke. Since sectioning obscures the whole structure of the neuromuscular apparatus, and since the chemicals used are found to alter the real structure of the union of nerve and muscle, the silver method should be checked against results obtained by methylene blue and gold. Murray [17] stated that the heavy treatment of tissues with formalin and other chemicals in the silver method is bound to cause serious shrinkage effects and that the process is of such capricious character that tissues treated in exactly the same way show different results.

Huber,[9] in his observations on the degeneration and regeneration of

motor and sensory nerve endings in voluntary muscle, used methylene blue. He stated that, due to limitations of methylene blue, the changes in the nerve endings were not revealed beyond the second day after nerve section, and that the precariousness of the method is such that in normal tissue nerve fibers and nerve endings remain now and again wholly unstained or are only partially brought to view.

The inconstancy of Ranvier's gold method is pointed out by Galigher [18] as follows:

"Unfortunately the formation of gold deposits upon the structures is brought about by an exceedingly delicate reaction which is not well understood, and cannot be obtained with any degree of certainty. The method is notoriously unreliable, and several trials must often be made before a satisfactory impregnation is obtained. However, the results are sufficiently beautiful to justify the effort required to obtain them. It is regrettably true that for nearly fifty years no effort has been made to improve the method in this respect."

We [1,2] have succeeded in obtaining consistent results with our modification of the gold method. Since gold chloride forms *in vitro* periodic precipitates of the Liesegang type with acetylcholine, choline, lecithin, cholesterol, and certain other lipoidal substances, comparable to those cross striations of capillary chemistry previously published,[1] we are inclined to attribute a specific reaction between gold and the normal and abnormal axonic transmitters. The axonic substance is the part that dominantly reacts to gold, but under certain conditions the products of metabolism or breakdown of myelin likewise combine with gold. In the chemical detection and quantitative analysis of acetylcholine and choline by Loach,[19] gold chloride is used. The resulting compound is either acetylcholine aurichloride or choline aurichloride.

The striking neuromuscular changes demonstrated by the gold-and-teasing method will constitute the anatomic basis of this report. The one limitation is the lack of clear visibility of the nuclei. This may be revealed by the observation of neighboring tissues with nuclear stains and with silver. By the use of multiple neurologic methods, confidence is gained regarding the true structure of the union of nerve and muscle, and of the advantages of each method.

The experimental and control muscles were run through the identical fluids for the same periods of time.

The detection of the granular and agranular muscle fibers was also made by the study of fresh tissue in physiologic salt solution at 37°C., according to the method of Denny-Brown [20] and Hines.[21]

The success of our modification of the gold-and-teasing method for whole muscle fibers appeared to be due to the following factors: (1) the initial acidulation of the finely cut fresh muscle with lemon juice or

citric acid, which fixed either acetylcholine or choline: the strong bases, choline and acetylcholine, are stabilized by this initial treatment with acid; (2) the gold chloride, which appears to have a selective chemical affinity for the normal axon and its secretory discharge into muscle, and for certain abnormal products in the myelin; (3) the reduction of the gold by the use of formic acid, which stabilized the normal and abnormal nerve products discharged by the hypolemmal axons into the denervated myoplasm; and (4) the retention of the anatomic continuity of the relationship of nerve and muscle by the teasing of whole muscle fibers and nerves. The continuity of long stretches of the epilemmal axon, hypolemmal axon, sarcolemma, the granules of the sole plate of Kühne, and the cross striations of the muscle fiber are preserved and may be studied in one field of microscopic observation. This relationship is obscured by sectioning muscle fibers after silver impregnation. The granules of Kühne are not stained by methylene blue. Dark muscle fibers have a strong affinity for gold and are dark red, purple, or blue: in the light fibers these colors are decreased or absent in teased specimens.

Results: Experimental Morphology

1. *The Pleomorphism of the Normal Motor End-Plates in Relation to Dark and Light Voluntary Muscle Fibers*

The dark and light muscle fibers were clearly evident in the normal control gastrocnemius muscles after the gold technic (Figs. 1, 10, 22, 34, and 35). The dark muscle fibers had more material with an affinity for gold than the light muscle fibers. The dark anisotropic, transverse bands in the dark muscle fibers were usually broader and darker than those in the light fibers. On the other hand, the light, isotropic, transverse striations in the light muscle fibers were usually not only broader than the corresponding light spaces in the dark muscle fibers, but there was less material that had an affinity for gold in the light spaces of the light muscle fibers than in those of the dark fibers.

The width of the muscle fiber was a variable factor depending upon fixation in a state of either isometric and isotonic contraction or the termination of relaxation at the onset of contraction. Some light muscle fibers, therefore, were smaller in diameter than the dark ones. The variable capacity of the different muscle fibers for gold impregnation was just as reliable as, and more permanent than, the study of fresh teased muscle. The dark muscle fibers may be classified as hyperchrysophilous and the light ones as hypochrysophilous. The cross striations

in dark fibers are composed of neurogenic and myogenic substances. There were multiple gradations in the affinity of the fibers for gold between the two extremes. The fading of the striations in denervated muscle produced fibers that may be classified as achrysophilous.

The motor end-plates in the dark and granular muscle fibers usually possessed coarse fronds or knob-like terminals. Some of these fronds were surrounded by a light halo-like space. The retracted motor end-plates were usually surrounded by more dark granular material of the sole plate of Kühne than those of the relatively expanded end-plates in the light muscle fibers. There appeared to be a direct continuity in some places between the granules of the sole plate of Kühne and those condensed in the periodic dark cross striations of the myoplasm.

The granules of the sole plate of Kühne appeared to be derived from two sources; namely, from the granular transformation and permeability of the terminals of the hypolemmal axons of the motor end-plate, and from the nuclei of the sole plate. This granular material of the sole plate normally appeared to diffuse in a periodic manner throughout the myoplasm of the dark muscle fiber. The structure of the motor end-plate in the light muscle fiber was usually one in which there was an ameboid expansion and an attenuation of the hypolemmal axons of the motor end-plate. There was, likewise, a decreased amount of the diffusible granules of the sole plate of Kühne around these relatively expanded motor end-plates of the light muscle fiber. Because the dark muscle fiber had a strong affinity for gold and this quality was gradually lost by denervation, it was compared to the stage of periodic discharge and diffusion of acetylcholine from the motor end-plate into the myoplasm. The dark muscle fiber was correlated with the termination of relaxation, or the onset of muscle contraction, and the light one with the phase of active, full contraction, in the fractional contraction of the muscle as a whole. This fractional contraction is lost progressively by denervation and its loss is correlated with the loss of the phase of differential diffusion of acetylcholine into the muscle. It has been practically impossible to catch a muscle fiber in the state of completely unstable and physiologic relaxation by any histologic technic used to date. The dark muscle fiber probably represents the structure nearest to that of relaxation, or the onset of contraction of the myoplasm produced by the periodic diffusion of acetylcholine.

The decreased visibility of the cross striations at the onset of contraction, observed in living muscles by many investigators, may represent this stage of the periodic discharge and diffusion of acetylcholine. The periodic structures in the muscle fiber would then be obscured, altered, and realigned in a rhythmic manner by the capillary chemical changes in the metabolism of the neuromyoplasm. The shuttle-like

shift of the cross striations described by Jordan [22] could easily be explained on the above basis.

2. *The Progressive Loss of the Dark Voluntary Muscle Fibers after Denervation*

There was a progressive loss of muscle fibers of the dark type beginning 24 to 48 hours after denervation. There was an agglutination of the fine granular material that had an affinity for gold into coarse clumps, which gave a flaky appearance to the myoplasm of the muscle fiber (Figs. 6 and 7). This was a histologic sign of the beginning loss of the normal nebulous diffusion in a periodic manner of the granular substance discharged from the denervated motor end-plate. This was microscopic evidence of the beginning dissociation of the nerve and muscle substances in the muscle fiber, characterized by the initial changes in the segregation and accumulation into larger aggregates of the normally fine neurogenic granules. It was a sign of the beginning loss of substantial influence of the innervation upon the muscle. A search is now in progress to produce the chemical denervation of muscle by the injection of some substance that will combine with the chemical transmitter of nerve impulses and thereby inactivate, through segregation, the neurogenic from the myogenic substances in the muscle fiber. Preliminary experiments point to the fact that DDT can play this rôle.

There was also histologic evidence of the loss of the normal fractional contraction of the muscle fibers after denervation. All of the motor end-plates in a specific field were either abnormally retracted with an increased affinity for gold, or were expanded and decreased in their capacity to take the gold (Figs. 4 to 9). On the third day, there were certain fields within the denervated muscle that were totally devoid of muscle fibers of the dark type (Figs. 2 and 11). From the fifth to the tenth day (Figs. 12 to 16) practically all of the dark type had progressively disappeared. The dark muscle fiber had coarse, irregular, longitudinal myofibrillae, whereas those in the light muscle fiber were smaller in diameter and more regularly arranged. The arrangement of the myofibrillae appeared to be correlated with the diffusion and disappearance of the fine granular material that had an affinity for gold. The accumulation of this granular material characterized the dark muscle fiber that was usually narrow and coarsely striated in a longitudinal direction, whereas the light muscle fiber, characterized by the disappearance of the granules with an affinity for gold, was usually wide and composed of fine fibrils arranged longitudinally. The accumulation and depletion of the chrysophilous granules appeared to be correlated with the functional activities of the muscle fiber, based on the diffusion and hydrolysis of the granules of acetylcholine.

3. *The Progressive Pathologic Changes of the Motor End-Plates after Denervation*

During the first 48 hours (Figs. 4 to 9, and 36 to 49) after nerve section, there were relatively normal motor end-plates scattered among those undergoing structural changes. There were noticeable structural changes of the end-plates and adjacent epilemmal axons within the first 24 hours. These were detected by beginning enlargement of about 10 per cent of the epilemmal axons and the formation of retention cysts on the ramifications of some retracted hypolemmal axons of the motor end-plates (Figs. 5, 36, and 37). This retraction, increased affinity for gold, and cystic enlargement of the branches of the nerve endings became structural characteristics in certain microscopic fields from 24 to 48 hours after nerve section (Figs. 6, 9, 41, and 42). Around some retracted nerve endings there was evidence of the granular sole of Kühne, whereas these granules were absent around other retracted nerve endings. The granular sole was most frequently absent around the expanded endings composed of elongated and attenuated branches (Figs. 7, 40, and 45).

In other fields the nerve endings were expanded, some had decreased affinity for gold, and their ramifications were exceedingly attenuated (Figs. 40, 45, and 49). This uniformity of retraction of the nerve endings on certain trees of innervation, and the expansion of all of the nerve endings on others, were histologic evidences of loss of the normal pleomorphism usually found on each tree. They are likewise histologic signs of loss of the normal fractional contraction of the muscle fibers under the influence of normal innervation. This was evidence of uniform overstimulation and overexcitation of the motor units innervating the denervated muscle after nerve section. Between 5 and 10 per cent of the nerve endings were replaced by fine granules at 48 hours. This replacement was found in about 60 per cent of the nerve endings at 72 hours. These figures are based on a differential count of 5,000 motor end-plates.

Concomitant with the structural changes of the motor end-plates, there were associated alterations in the structure of the muscle fiber. Coincident with the loss of the normal periodic diffusion of fine chrysophilous granules transmitted from the motor end-plate, there was seen a coarse flakiness of these granules in the muscle fibers. In some trees of innervation the epilemmal axons were fragmented and had a decreased affinity for gold (Fig. 8), whereas in others there was an increased capacity to take the gold with little evidence of fragmentation (Fig. 9). Since these observations were made in the same muscle and since the technic was standardized by running the normal and abnormal

muscles simultaneously and similarly, this was considered a histologic sign of periodic decrease alternating with increase in the quantity of the gold-impregnated material in the epilemmal axons. There was a definite hyperemia of the intramuscular blood vessels and capillaries (Figs. 8 and 9), demonstrated by the increased diameter of the lumen and the packing of these vessels with red blood cells. In other locations the capillaries were collapsed and devoid of cellular elements. This was comparable to the experimental structural conditions produced by poliomyelitis, traumatic and thermal shock, and by the experimental injection of lactic and other acids locally into the muscle.

The depletion of many of the epilemmal axonic trees of their motor end-plates after the third day subsequent to the degenerative cut of the sciatic nerve was structurally associated with the progressive loss of muscle fibers of the dark type (Fig. 2). There was likewise a distinct fading of the dark anisotropic cross striations due to the loss of granules with an affinity for gold. This was due to the depletion from these striations of fine chrysophilous granules that were normally periodically discharged from the motor end-plates and that diffused into the myoplasm. It was imperative, therefore, that frequent comparisons be made of the normal reaction with gold (Figs. 1, 10, 22, 34, and 35) and the loss of this reaction in denervated muscle.

Although it was true that only the axons of the nerve fibers and their end arborizations were dominantly impregnated with gold, there was no difficulty in differentiating medullated nerve fibers with the faint outline of a sheath and the locations of the nodes of Ranvier from the varicose nonmedullated nerve fibers. In the degenerating medullated nerve fiber, the myelin was impregnated more readily than in the normal one, so that the segments of a degenerated medullated sheath may be made out during the early stages, 48 to 72 hours after nerve section. There was distinct segmentation of the myelin during this time, and the degenerated products fused with those in the central axon. It was clearly evident that the extreme distal end of a severed motor nerve and its motor end-plate degenerate before the remaining portions of the same nerve distal to the point of the degenerative cut. The degenerative changes which follow the segmentation of the myelin and the formation of cystic enlargements on the motor end-plates were clearly followed by the gold-and-teasing method applied to whole muscle fibers. Huber [9] stated definitely that it was impossible for him to follow the changes with the methylene blue technic the second day after the section of the nerve. The granular degeneration was well advanced in the motor end-plates 72 hours after denervation (Figs. 50 to 55).

Sprays of medullated nerve fibers underwent progressive degeneration by the depletion and discharge of their degenerated contents, in a centrifugal direction, from 5 to 30 days after nerve section (Figs. 12 to 21, 56, 57, 69, and 70). The discharged combined axonic and myelin substances had a strong affinity for gold and were accumulated in pleomorphic masses within and around the region of the degenerated motor end-plates. The faint, ghost-like outline of the epilemmal axons depleted of substances that had a strong affinity for gold was in striking contrast to that of the accumulated chrysophilous material discharged in a centrifugal direction into the region of the degenerated motor end-plates. Some of these pathologic masses or neurosomes were found out in the myoplasm of the muscle fiber. The muscle fibers 20 days after the degenerative cut (Fig. 19) had lost the appearance of the normally functioning dark type and were very narrow in comparison with the normal. This anatomic evidence shows conclusively that the histologic dark type of muscle fiber is dependent upon the normal functioning of the nerve supply. This functional dark type of muscle fiber, therefore, appears to be produced by the normal periodic diffusion into the myoplasm of the fine granules of acetylcholine discharged from the motor end-plate.

The axonic material which was discharged in a centrifugal direction 30 days after the degenerative cut (Figs. 20 and 21) was seen in relation to only a few of the degenerated motor end-plates. The locations of the degenerated end-plates were shown dominantly by clusters of sole plate nuclei which were surrounded by the granular material secreted by these nuclei. This granular material had an affinity for gold slightly less than that of the material discharged by the hypolemmal axons. The clusters of nuclei were detected as clear, rounded, or oval spaces surrounded by the dark granules (Fig. 20).

In some places in the same muscle, the myoplasm was aggregated into broad and narrow, dark, transverse bands alternating with light ones (Fig. 21). In the dark bands the cross striations were either exceedingly fine or they were completely absent because of the opacity of the dense aggregation of the myoplasm. The light bands were occupied by cross striations which were wider apart than those in the dark bands. This dark and light transverse banding of the narrow muscle fibers was produced by the slow, irregular fibrillation of the muscle fibers. This incoordinate and ceaseless fibrillation, of slow vermiform activity, of the denervated muscle fibers begins about 72 hours after the degenerative cut of the sciatic nerve. This fibrillation was correlated with the loss of the dark muscle fibers and the loss of the transmission of acetylcholine which, normally, chemically tunes the muscle

fiber to the higher pitch or frequency of fast muscle contractions and to the capacity to respond adequately to high frequency stimulation. The chemical tuning process of muscle was roughly analogous to the mechanical tuning of the strings of a piano or violin for response at a higher frequency than that of strings which are loose and sagging.

4. The Experimental Exaggeration of the Discharge of Large Neurosomes into Denervated Muscle

The progressive degeneration of peripheral nerves, clearly described by Parker,[23] was accelerated by the intraperitoneal injection of d-tubocurarine chloride. In some animals, after the muscle was curarized, the distal segment of the cut sciatic nerve was stimulated for 30 seconds, at the rate of 5 per second, with no response of the muscle. This experimental procedure caused a massive accumulation of degenerated nervous substances discharged into the muscle from 5 to 20 days after section of the nerve (Figs. 3, 23 to 33, and 58 to 68). There was complete depletion of the material in the epilemmal axons and an enormous accumulation of this neurogenic material in and around the degenerating motor end-plate, with periodic discharges of pleomorphic neurosomes into the myoplasm. This exhaustion of the axonic spray of its specific substances with strong affinity for gold, and the massive accumulation of these chrysophilous substances at the degenerating end-plate and in the muscle constitute conclusive evidence that there was a substantial transfer of some substance from the degenerating nerve to the denervated muscle. The absence of the normal dark muscle fibers was related to the loss of the normal periodic nebulous diffusion of the granules of the sole plate of Kühne. The agglutination of either the normal or abnormal transmitter substance in a relatively non-diffusible form gave rise to these morphologic changes under the conditions of the experiment. This demonstration of the pathologic structure of the abnormal transmitter substance was produced by acceleration of the discharge and prevention of the normal diffusion of granules into the myoplasm of the muscle fiber. There was great variation in the structure of this abnormal substance transmitted from nerve to muscle (Figs. 3, 23 to 33, and 54 to 68).

The discharge of the degenerated axonic and myelin materials into the peripheral terminal zone of the degenerated motor end-plates had a pleomorphic arrangement; namely, unipolar (Figs. 58 and 59), bipolar (Fig. 61), and multipolar or completely circumferential (Figs. 60 and 62). The chrysophilous material had been discharged into and around the degenerating motor end-plates in a centrifugal direction from the epilemmal axons and there was unimpeachable evidence that

this gold-impregnated material was initially in direct continuity with the structures that innervate the muscle. In one end-plate (Fig. 59) the discharged neurosome was in direct anatomic continuity with the hypolemmal axon of the motor end-plate. It formed what has been designated by some neuroanatomists as an ultraterminal nonmedullated branch of the motor end-plate, ending in the same muscle fiber in an enlarged spherical or oblong terminal. This ultraterminal branch of the motor end-plate, however, was merely the initial phase of the discharge of materials from the nerve terminal with anatomic continuity still maintained. It was the product of the abnormal discharge of substances by abnormal stimulation from the pathologic motor ending, and not a morphologic ending of specific type as claimed by some observers.

When the narrow, dark, and granular muscle fiber was observed in cross section, it was impossible to differentiate and definitely identify the neurosomes in ordinary histologic preparations. Even with lipoidal stains, it was impossible to identify with certainty acetylcholine and choline neurosomes from particles of lecithin and cholesterol. When gold preparations were imbedded in gelatin and cross sectioned with the freezing microtome, there was a striking variation in the size and staining capacity of the gold-impregnated neurogenic granules. The same normal or abnormal muscle tissue may be teased and photographed in its longitudinal projection and then imbedded, cut in transverse sections, and studied in that plane. There was no doubt, therefore, of the intramuscular location of the normal and abnormal neurosomes. At some degenerated nerve terminals, part of the neurogenic substance may be found between fibers after electrical stimulation of the distal stump of the cut nerve.

5. The Increase of Granules around the Bloated Subsarcolemmal Nuclei after Denervation

The clear, rounded, and oval spaces both within the region of the degenerating end-plate and in the granular cytoplasm of the sole plate of Kühne were occupied by nuclei. The nuclei, close to the degenerating nerve endings (the Telodendrienkern of Boeke), appeared first to enlarge and later to become smaller after nerve section (Figs. 50 and 51), whereas the nuclei in the granular sole (Sohlenkerne of Boeke) and the subsarcolemmal nuclei out in the myoplasm were increased in size, for a considerable period of time, and became surrounded by an increased quantity of granules (Figs. 50 to 53). The bloated subsarcolemmal nuclei were depleted of chromatin and showed loss of nucleoli when specifically stained. These bloated vesicular nuclei became surrounded with a greatly increased quantity of chrysophilous granules concomitant

with the loss of the normal periodic discharge of fine granules from the motor end-plates. The peripheral increase of granulation around the muscle nuclei after denervation was likewise coincident with the unneutralized and persistent activity of cholinesterase found by Nachmansohn and John [7] 3 days after nerve section, when conductivity had disappeared from the axon.

The granular material, that had the capacity to take gold, appeared to be contributed by two sources: the granular transformation of the hypolemmal axons of the motor end-plate, and the nuclei of the sole plate and the subsarcolemmal nuclei of the myoplasm. Pommé and Noël [24] stated that the telesomes or sole plate granules decreased or disappeared in progressive muscular atrophy. Tower [25] stated, however, that these granules had not been studied after experimental denervation. The increased visibility of the nuclei in denervated muscle was probably due to three factors: hypertrophy of the nucleus, accumulation of an increased quantity of granules around the nucleus, and decreased cytoplasm which resulted in crowding together the preexisting nuclei. No evidence was found of nuclear division.

The denervation atrophy caused definite shrinkage of the cytoplasm. Tower [25] defended the opinion that denervation atrophy first attacks the sarcoplasm and at a later date the myofibrillar substance. It was noted, however, that there was no discontinuity of the process of atrophy of the sarcoplasm and the myofibrils that would indicate a shift of the process from one structure to another. Tower concluded, therefore, that the sarcoplasm and myofibrils formed a structural continuum. The chemical evidence indicated that the depletion of substance was in fairly equal proportion for both the sarcoplasm and the myofibrils. There was objective evidence in our experimental study with the gold technic that atrophy of the cytoplasm of the muscle fiber was coincident with failure of discharge of the granules associated with the chemical transmission of the normal nerve impulses. The normal myoplasm, therefore, was proved to be composed of myogenic and neurogenic substances. Atrophy was coincident with the loss of one of these substances, namely, the fine neurogenic granules or neurosomes.

6 The Pleomorphism of the Neurosomes in Denervated Muscle

The discharged neurosomes (Ns., Figs. 71 to 75) found in the myoplasm were pleomorphic and hyperchromatic for gold in comparison with the faded hypochromatic cross striations of the muscle fibers in which they were found. These neurosomes may be fusiform (Figs. 71 and 72), irregularly oblong with one end tapering (Figs. 73 and 74), arrowheaded in shape, or rounded droplets that vary in size (Fig. 75).

The droplets formed either loose series of single drops widely separated or closely related series of 2 to 10 droplets. The droplets in the middle of a series were usually larger than the terminal ones. Such series of droplets formed irregular fusiform structures separated by clear spaces. The large fusiform neurosomes may have either serrated (Fig. 71) or festooned (Fig. 72) edges. The sharp projections arranged like saw teeth around the edges of the neurosomes may or may not be in direct alignment with the dark cross striations of the muscle fiber. These neurosomes undergo a granular dissolution, and the granules become incorporated into the myoplasm, and are then aligned with the cross striations of the muscle fiber. These neurosomes were periodically discharged from the motor end-plates into the muscle fiber. During degeneration they were more persistent, agglutinated, and less diffusible than when produced by the artificial overstimulation of normal muscle or when produced by neurogenic shock.

7. The Experimental Production of Giant Muscle Fibers after Denervation

Giant muscle fibers (Gmf., Fig. 76) were found in a few 10-to-14-day denervated muscles after the intraperitoneal injection of d-tubocurarine chloride followed by electrical stimulation of the distal stump of the cut nerve for a duration of 30 seconds at the rate of 5 per second. The diameter of these fibers was 3 or 4 times that of closely related muscle fibers. They were densely impregnated with gold. This represented an abnormal discharge and accumulation of increased quantities of abnormal axonic material in the fiber. The cross striations were seen only at the edges because of the opacity. There was a definite streamlined effect produced by certain large neurosomes (Ns., Fig. 77) upon the cross striations of the muscle fiber. This was detected by the altered arrangement of the cross striations. Certain large muscle fibers (Fig. 78) were not so densely packed with neurosomes as were the giant muscle fibers (Fig. 76).

In some locations the neurosomes were dense and opaque while in others they were light and cross-striated. The dense neurosomes were gradually replaced by granules which eventually became aligned with the cross striations in the muscle fiber.

8. The Intermittent and Progressive Degeneration of the Distal Stump after Section of the Motor Nerve

The degeneration of the distal stump of the sciatic nerve was tested histologically by the gold-and-teasing method of whole nerve fibers and was proved to be progressive in character and not to take place

simultaneously throughout the whole length of the nerve. The changes in the axis cylinder, medullary sheaths, and motor end-plates were unquestionably initially more advanced at the junction between nerve and muscle than in the same nerve fiber far away from the muscle. There was disappearance of the motor end-plates between the third and fifth days. The degenerated material in the trunk of the nerve advanced, in a progressive manner as well as intermittently, in a centrifugal direction. This, then, resulted in a progressive depletion of the proximal end of the distal stump of the nerve while the terminal of the nerve was discharging the degenerated materials into the muscle. One branch of the sciatic nerve was followed just before its entrance into the gastrocnemius muscle 14 days after sectioning. At this location there was complete depletion of the degenerated axonic and myelin substances that had an affinity for gold (Fig. 79). At a slightly more distal point, the gold-impregnated material was found in variable amounts in axis cylinders which were fragmented into rounded, oval, or fusiform bodies with a strong affinity for gold (Figs. 80 and 81). Just before the nerve gave rise to the branches that directly innervate the muscle, there was again a depletion of the axis cylinders of chrysophilous material (Fig. 82). Only in widely scattered areas were five fusiform bodies found that had an affinity for gold. The epilemmal axons of a cut nerve that innervate the muscle are periodically engorged and depleted of the degenerated and fragmented axonic and myelin materials until structural exhaustion occurs. This rhythmic discharge of degenerated nervous material into the muscle continues, in a progressive and periodic manner and in a centrifugal direction, until most of the degenerated material is discharged centrifugally into the muscle.

Discussion

The Mixture of Red and White Fibers in Voluntary Muscle

No attempt will be made to review the extensive histologic literature accumulated over the past 100 years on red and white muscle and on the controversial subject of the so-called mixture of red and white fibers in the same voluntary muscle. The excellent recent reviews and observations on this topic may be consulted: Tower,[25] Cobb,[26] Needham,[27] Hines,[28] Hinsey,[29] Fulton,[30] Forbes,[31] Creed, Denny-Brown, Eccles, Liddell, and Sherrington,[32] Wilkinson,[33] Roberts.[34]

Cobb [26] makes the following important statements (page 519):

"One must review the *anatomy of muscle* carefully in order to understand the possible effects of the nerve impulse upon it. Even in this field one is surprised to find indefiniteness, partly because the classification of the different types of muscle is inherently difficult on account of intergradations and transitional forms;

partly because the histology of muscle has often been overlooked in the study of its physiology";

and (page 520):

"It must be emphasized here, however, that the state of knowledge concerning the red and white muscle fibres is at present so confused, even in the field of anatomy, that much work must be done before physiological interpretations can be acceptable."

Almost 60 years ago, Grützner,[35] stated that every muscle contains two specific types of fibers, often intimately mixed: one kind narrow and dark, the other broad and light. He thought that the dark color of the narrow fibers was due to numerous granules and that all narrow muscle fibers, whether pigmented or not, corresponded to the red, slowly contracting muscles of the rabbit, and that the large, light, agranular muscle fibers corresponded to the rapidly moving white muscle. Knoll,[36] and Knoll and Hauer [36] designated the cloudy muscle fibers as "sarkoplasmareichen" and the clear ones as "sarkoplasmaarmen" or "fibrillenreichen." Krause [37] believed that the different kinds of muscle fibers seen by Grützner and Knoll were dependent upon differences in age: the narrow granular fiber was assumed to be younger than the wide light fiber. Bonhöffer,[38] however, demonstrated that the proportion and distribution of the two types of muscle fibers were the same whatever the age of the frog.

The skeletal muscle may vary in color, not only from species to species, but in the muscles of the same animal. The differences in color of the white meat of the chicken's breast muscle and the deep red of the pectoral muscle of the pigeon or wild duck is very striking. In 1865, Kühne [39] studied the cream-colored and red muscles of the rabbit by spectroscopic methods. He concluded that the deep red muscles had a higher content of myohemoglobin than the light cream-colored muscles.

The structural and functional significance, however, of the narrow, dark, and granular fibers and the wide, light, and relatively agranular fibers found in both the red and white muscles has not been solved to date. It was demonstrated by Schäffer and Licht,[40] Tower,[41] and others that denervation atrophy abolishes the morphologic distinction between the two types of muscle fibers. The mechanism, however, of abolishing this morphologic difference in the two types of muscle fibers found in so-called mixed muscles by denervation was not revealed.

Tower [41] referred to the "large pale" and "small granular" fiber types as follows (page 123):

"What such characteristics may signify for muscle function, difference in contraction rate, in the ability to store specific lipoidal or protein products, does not

lie within the province of this paper to consider. Yet the problem is of prime importance. For not until the conditions are known which gave rise to clear-cut fiber types in one cat and not in a second; not until the distribution of such diverse fibers within the muscle shall be better understood, will it be possible to evaluate small quantitative changes, the possible result of lesions of the sympathetic."

Tower [41] stated that sympathetic denervation did not alter the proportion, distribution, or constitution of the fiber types in voluntary muscle.

It was an error made years ago, that has led to endless confusion, to compare these two histologic and functional types of fibers with the distinct red and white varieties of muscle. In both red and white muscles there are in each muscle dark granular and light agranular fibers. The functional activity of the neuromuscular apparatus is associated with the release, diffusion, and subsequent destruction of acetylcholine, processes which have not been associated, heretofore, with the dark and light muscle fibers in the fractional contraction in the same muscle. In fact, the probable relationship of the Q and J granules of Holmgren,[42] certain liposomes of Albrecht [43] and Bell,[44] interstitial granules of Kölliker,[45] and fine granular neurosomes in muscle fibers has been proposed only recently by us.[46]

Tower,[47] likewise, surmised that the muscle nuclei and sarcoplasm were in some obscure way dependent upon the influence of the nervous system, as is evident in the following statement (page 25):

"In the extrafusal muscle fiber, the aggregation of nuclei around the motor endplate has always suggested a peculiarly intimate relationship of these to the nervous tissue. But nerve lesion affected not only these nuclei physically associated with the disintegrating nervous tissue, but also all the nuclei the entire length of the fiber. Do all these unconnected and seemingly inactive nuclei form, perhaps with the sarcoplasm, an organization, possibly for conduction within the fiber and for excitation of the contractile mechanism, but an organization dependent on the nervous tissue to a degree incompatible with normal existence after nerve degeneration? This is a most alluring interpretation of the reaction of muscle nuclei, in toto, to denervation."

Denny-Brown [20] stated that slowly contracting fibers were able to store lipoids in the form of liposomes with a resultant granular appearance, while few rapid fibers had this property. Histologic differences were found to disappear on emaciation, but the differences in the speed of contraction remained. Denny-Brown claimed that the dark muscle fiber was related to nutrition. He likewise stated that there were certain light muscle fibers which could not be transformed into the dark granular ones. If the light muscle fibers are the stage of full muscle contraction and hydrolysis of acetylcholine, it would not be surprising to find light fibers even in conditions of overnutrition. Regardless of the method used, a certain number of the granular muscle fibers would

be transformed into the agranular type due to contraction. There would be relatively more fibers of the dark granular type observed in muscles of slow activity in comparison to those with high rate of speed of action, because there would be a correlation between the rate of cycles of diffusion and hydrolysis of acetylcholine granules and that of relaxation and contraction of the muscle fiber. The speed of neuromuscular metabolism would, therefore, tend to determine the relative proportion of fiber types. The irreversible high rate of metabolism to the level of fixation, by heat rigor, is associated with the abnormal overproduction of the light agranular fibers in frog muscle.[1]

The mechanism of production of the atrophy of muscle by denervation has been an obscure problem. This, likewise, applies to the hypertrophy of muscle due to the chronic effects of exercise. Carlson and Johnson [48] (page 369) made the following comments:

"The muscle enlargement with correspondingly greater strength, which is developed by work or training, is a commonly observed phenomenon. There is evidence that the muscle enlargement is not due to any increased number of muscle fibers, as might be suspected, but rather to an increase in size of each fiber. The exact nature of this effect, apparently a growth phenomenon, or the mechanism of its production are not yet known. The effect seems in some way to be related to the influence of nerves and nerve impulses reaching the muscle by way of the efferents. At any rate, if the number of nerve impulses is reduced, as in disuse of the muscle, the muscle fibers become smaller, and the whole muscle shrinks in size and becomes weaker. And, if the efferent nerve of a muscle is destroyed by disease or accident, so that all nerve impulses to the muscle are cut off, the muscle shrinks in size greatly and may entirely disappear and be replaced by fibrous connective tissue, leaving no trace of the former structure."

It is our opinion, supported by experimental morphologic evidence presented in this and other papers, that some light has been shed on the obscure mechanism of the atrophy and hypertrophy of voluntary muscle by the elimination and overproduction, respectively, of a substantial transfer of materials from nerve to muscle. The degree of atrophy and hypertrophy appear to be related to the degree of the decrease and increase of the neurosomes respectively discharged from the motor end-plates into the myoplasm of the muscle fiber. There are unquestionably related vascular changes which are likewise important.

Loewi and Navratil [49] proved that atropine, and Navratil [50] that ergotamine, did not paralyze the respective nerves to the effector organs by showing that after their application nervous excitation is still effective in liberating the transmitters. This was likewise shown to hold for nicotine by Feldberg and Vartiainen,[51] and for curare by Brown and Feldberg [52] and Brinkman and Ruiter.[53] These pharmacologic experiments demonstrate that acetylcholine acts directly upon the voluntary

muscle and that chemical antagonists counteract this effect. The true point of attack of these important drugs is upon the neurogenic component of the compound neuromyoplasm. These drugs alter the structure of the motor end-plates and likewise form agglutinated masses of large neurosomes in the myoplasm. The experimental morphologic findings in this paper support the interpretations of the action of the above drugs on the myoplasm. Dale, Feldberg, and Vogt [4] concluded, likewise, that when transmission of excitation from the nerve to the perfused muscle is prevented by curarine, stimulation of the motor nerve fibers causes the usual release of acetylcholine. The effect of eserine was to aid the diffusion of acetylcholine from the site of its release in the nerve endings of voluntary muscle into the blood vessels. The experiments of Dale and his colleagues demonstrated quite definitely that the appearance of acetylcholine in the perfusion fluid is not directly or indirectly produced by the contraction of the muscle fibers.

The absence of nuclear division in denervated muscle confirms the findings of Willard and Grau.[54] The hypertrophy of the nucleus and the peripheral accumulation of granules confirm the findings of Tower.[47] Our observations confirm the statement of Hines and Knowlton [55] that the rat is a very satisfactory animal upon which to study the changes in skeletal muscle after denervation. They did not, however, study those changes in the neuromuscular apparatus, as reported in our present paper, because of the obliterating effects of the histologic methods employed by them.

Feiss and Cramer,[56] in discussing the nature of wallerian degeneration, stated that the proliferation of the neurilemmal nuclei and the fragmentation of the myelin sheath must be considered separately. The cytologic changes in the degenerating medullated nerves as correlated with the changes in the axon and myelin sheath are problems for future investigation. The changes revealed by the gold technic, however, point to the fact that the traumatic sectioning and resulting continuous overstimulation of the nerve produce an abnormal breakdown and discharge of neurogenic substances that proceed at a more rapid rate than the process of elaboration. The function of the cell body is required for the synthesis of the normal transmitter in the intact nerve fibers. That acetylcholine is formed in cholinergic nerve fibers was established by Loewi and Hellauer,[57] MacIntosh,[58] and others.

Boeke [59] stated that from a cytologic point of view the nerve endings are in many aspects one of the most interesting parts of the nervous system, and formulated several questions and statements (page 243, *l.c.*) which are a challenge to the anatomists in the study of the neuro-

muscular apparatus. The morphologic evidence on the changes in the denervated neuromuscular apparatus presented in this paper supports the prophetic challenge of Boeke and furthermore substantiates the theory of chemical transmission of nerve impulses advanced by Loewi, Dale, and others. The motor end-plates in living muscle appear to be biologic jet-pumps that discharge either normal or abnormal neurogenic substances into the myoplasm of the muscle fibers.

Summary

1. The right gastrocnemius muscles of 100 white rats (*Mus norvegicus*) were denervated by sectioning the vagus nerve at the hip. The left legs remained intact and were used as controls. The successful demonstration of the nature of the union of nerve endings and voluntary muscle depended upon the use of the gold-and-teasing method of whole muscle fibers and nerves, checked against results obtained by other histologic technics. Gold had an affinity for acetylcholine and certain degeneration products of the axon and myelin.

2. The dark muscle fibers in normal voluntary muscle, because of their strong affinity for gold, are designated as hyperchrysophilous muscle fibers. They are associated with the periodic discharge of acetylcholine from the motor end-plates and with the diffusion of this chemical transmitter in the myoplasm at the end of relaxation or the onset of contraction.

3. The light voluntary muscle fibers have a weak affinity for gold and are designated as hypochrysophilous muscle fibers. They are associated with the periodic hydrolysis of acetylcholine by cholinesterase during the period of full, active contraction.

4. After denervation there is a progressive loss of the dark hyperchrysophilous muscle fibers and motor end-plates, which involved 60 per cent of them 72 hours after denervation. This coincides with the beginning of fibrillation and with the progressive disappearance of acetylcholine. At about the 10th to 15th day after denervation dark muscle fibers are totally absent. The atrophic muscle fibers become achrysophilous. The rates of these pathologic changes vary among animals of the same species as well as among different species.

5. There is a progressive and centrifugal transport of degenerative materials from the distal segment of the sectioned nerve to the denervated muscle. This produces large pleomorphic neurosomes in the denervated myoplasm. This phenomenon is accelerated by the use of d-tubocurarine chloride, particularly when followed by electrical stimulation of the distal stump of the sciatic nerve. There is a substantial

transfer from nerve to muscle. Giant muscle fibers are produced occasionally, composed largely of neurogenic material. The giant fibers of primary muscular atrophy and dystrophy need reinvestigation.

6. The bloated subsarcolemmal nuclei of the denervated muscle become surrounded by an increased amount of granular material coincident with the persistent activity of cholinesterase, as found by Nachmansohn and John,[7] 3 days after nerve section when conductivity had disappeared in the axon.

7. The hyperchrysophilous neurosomes may be defined as ephemeral pleomorphic bodies discharged into the myoplasm of striped muscle from superpermeable terminal axons of motor end-plates. These neurosomes are composed of very fine granules, droplets, vacuoles, and elongated streamers that vary in size and staining capacity with gold, silver, and lipoidal stains.

8. Motor end-plates in living muscle appear to be biologic jet-pumps that discharge neurogenic substances into the myoplasm of the muscle fibers. Voluntary muscle has a dual composition of neurogenic and myogenic substances.

9. Muscle atrophy appears to depend upon a decrease or total inhibition of the neurogenic discharge into muscle, and hypertrophy upon an increase of this neurogenic discharge. The changes in the blood supply appear to be secondary phenomena.

10. Anatomic evidence of progressive degeneration, as contrasted with simultaneous degeneration, occurs in the distal stump of the sciatic nerve. This process may be accelerated by experimental means with resulting massive discharge of degenerated nervous material into the denervated muscle.

11. The union of nerve and muscle appears to be one of periodic anatomic continuity which results in the confluence and compounding of the neurogenic and myogenic substances. Gold chloride, after initial acidulation of the living muscle, appears to possess a selective affinity for the normal and abnormal neurogenic substances.

12. The experimental anatomic evidence appears to support the claim that there are morphologic as well as chemical transmitters of normal and abnormal nerve impulses demonstrable by selective histologic methods.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

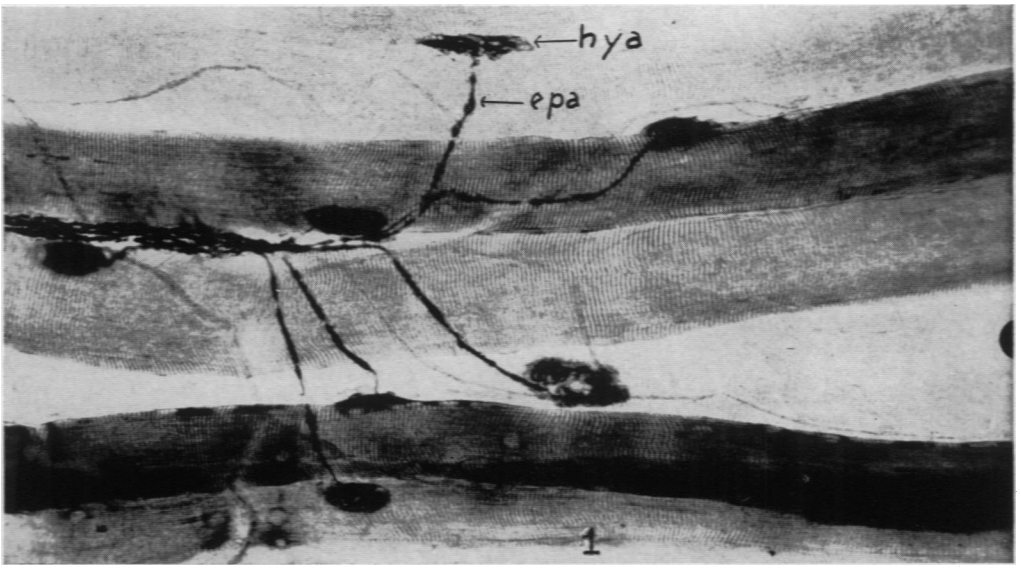
The photomicrographs of Figures 1 to 82 are from teased whole muscle fibers (gastrocnemius muscle) and motor end-plates of the white rat *(Mus norvegicus)*, previously prepared by the gold technic. The photographs were direct contact prints from negatives exposed through the microscope and not subsequently enlarged. They may, therefore, be compared with those of the white rat and the chameleon previously published.[1,2] In the plates, "epa" designates the epilemmal axon; "hya," the hypolemmal axon; "Ns," neurosomes or neurogenic particles discharged from motor end-plate jet-pumps into the myoplasm either as fine, nebulous sprays, large agglutinated clusters, or periodic series of globules; "Kg," extra-axonic Kühne's granules; "gmf," giant muscle fiber; "b.v.," blood vessel; "nuc.," nucleus; "gran.," granules. There has been no retouching of negatives or prints. The time after section of the nerve is designated in each legend.

PLATE 241

FIG. 1. Spray of medullated nerve fibers and terminal motor end-plates from a relatively normal gastrocnemius muscle of a rat killed by ether. There is pleomorphism and variation in size and impregnation capacity for gold of the motor end-plates and muscle fibers. The narrow, dark muscle fibers may be the initial phase of a nebulous and periodic diffusion of granular acetylcholine at the onset of contraction. Choline and acetylcholine react with gold chloride *in vitro* to produce either choline aurichloride or acetylcholine aurichloride. The wide, light muscle fibers may represent the phase of contraction after the diffused acetylcholine has been destroyed by the cholinesterase. This normal variation of muscle fiber types may be the morphologic expression of the fractional contraction in a muscle, and the differential diffusion alternating with the destruction of acetylcholine in the different fibers of the same muscle. The motor end-plates in the dark muscle fibers are retracted and surrounded by the granules of Kühne. In the light muscle fibers the motor end-plates are usually expanded and there is a decrease in the amount of the granules of Kühne. × 250.

FIG. 2. Spray of medullated nerve fibers depleted of motor end-plates in the gastrocnemius muscle 72 hours after the degenerative cut of the sciatic nerve. The motor end-plates are replaced by fine granules surrounding light, oval spaces occupied by the nuclei of the sole. The epilemmal axons are increased in diameter, and in some terminals there are swollen cysts. The absence of the dark type of muscle fibers in this microscopic field may possibly be related to the failure of normal transmission of acetylcholine granules or the failure of the normal nebulous diffusion of the granular transmitter substance after discharge from the end-plates into the myoplasm. × 250.

FIG. 3. Spray of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 120 hours after the degenerative cut of the sciatic nerve. The muscle was curarized by the intraperitoneal injection of d-tubocurarine chloride and the distal segment was then stimulated for 30 seconds at the rate of 5 per second with no muscular response. There is depletion of the material in the epilemmal axons and an enormous accumulation of this neurogenic material in the end-plate with periodic discharges into the muscle fiber. The exhaustion from the axonic spray of its specific substance with an affinity for gold and the accumulation of this substance at the end-plate and in the muscle constitute conclusive evidence of the substantial transfer of some substance from nerve to muscle. × 250.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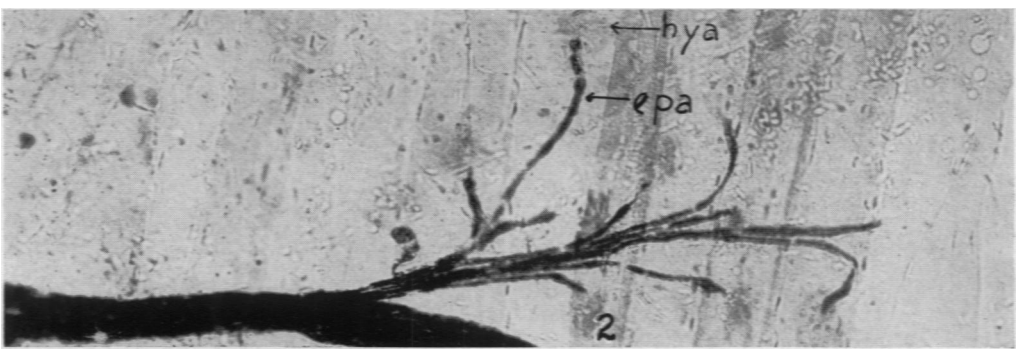
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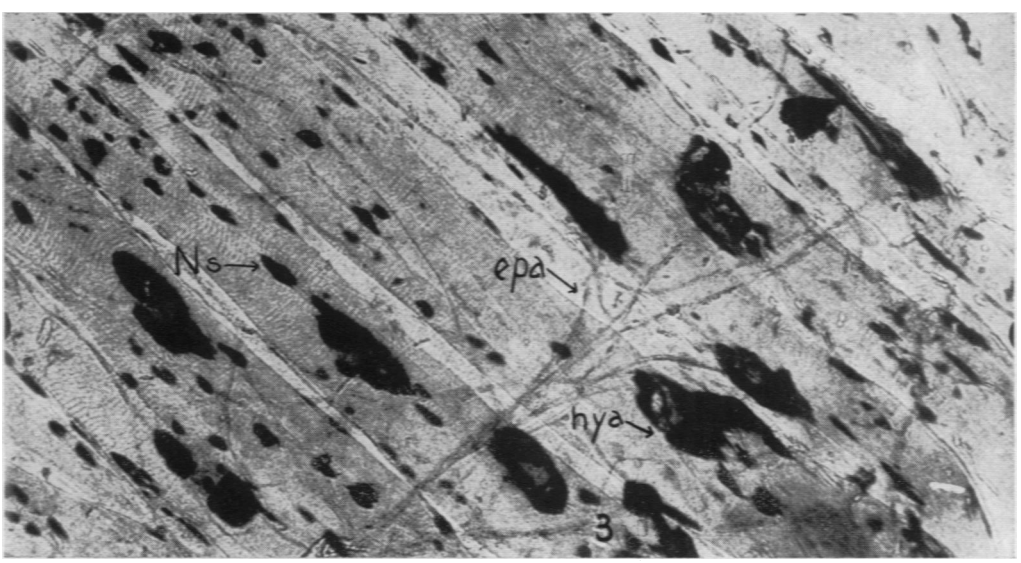
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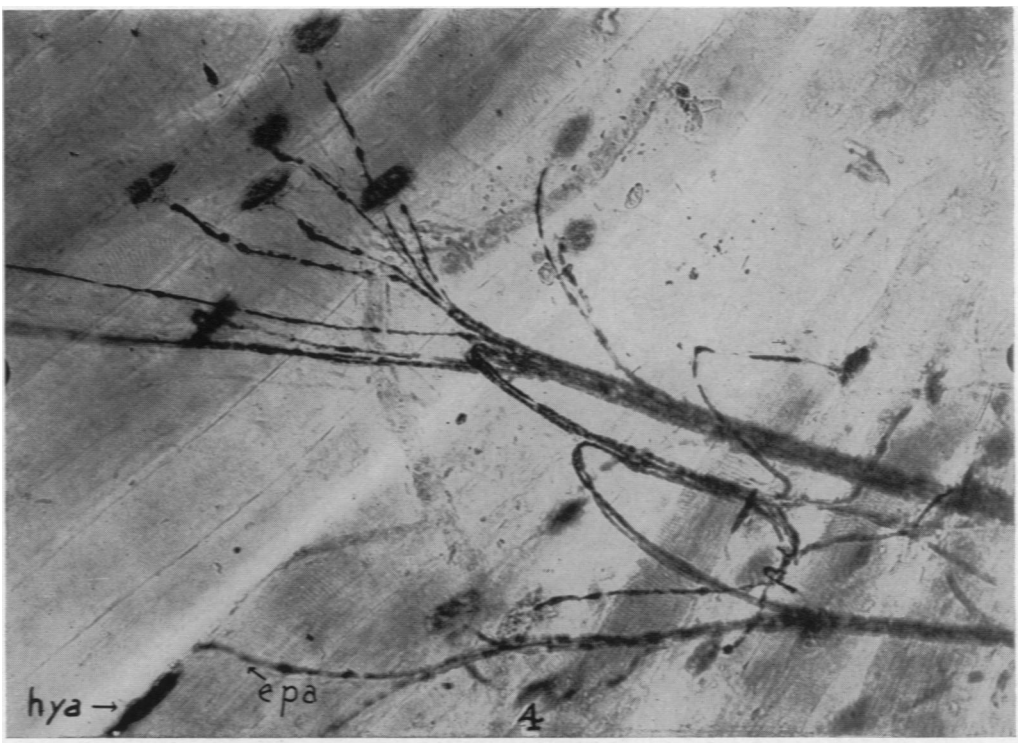
PLATE 242

FIGS. 4 and 5. Sprays of medullated nerve fibers and motor end-plates 24 hours after section of the sciatic nerve. There is a beginning loss of the dark type of muscle fiber. The epilemmal axons are definitely beaded (Fig. 4). In some places the end-plates have a diminished affinity for the gold. In other places in the same muscle all of the end-plates are retracted and have an increased affinity for gold (Fig. 5). Some hypolemmal axons have round or oval cystic enlargements. This is a histologic sign of an abnormal and uniform stimulation of the motor units of nerves and a beginning loss of the fractional contraction of the fibers in the same muscle. In other locations of the same muscle all of the end-plates in the field may be abnormally expanded. The abnormal contraction or expansion of all of the motor end-plates in a field is evidence of uniform overstimulation and overexcitation of the motor units of the muscle and loss of the fractional contraction of the muscle fibers normally controlled by the intact nervous system. × 200.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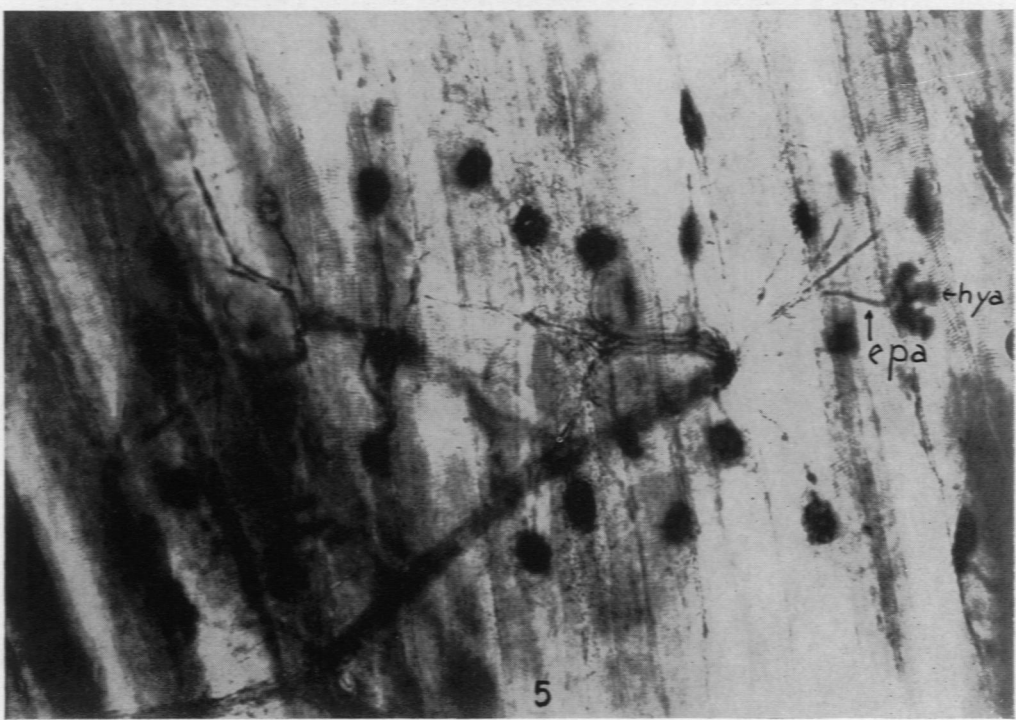
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PLATE 243

FIGS. 6 and 7. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 48 hours after the degenerative cut. There is a progressive loss of visible structural organization in muscle fibers of the dark type. In many places all of the motor end-plates in a specific field may be either retracted with increased affinity for gold (Fig. 6) or expanded with a decreased affinity for gold (Fig. 7). This is a histologic sign of an abnormal and uniform overstimulation of the motor units and a loss of the fractional contraction of the muscle fibers. There is a dark granular or flaky appearance of the muscle fiber, which is a histologic sign of the beginning loss of the normal nebulous diffusion of the granular transmitter substance discharged from the motor end-plate into the myoplasm of the muscle fiber. × 250.

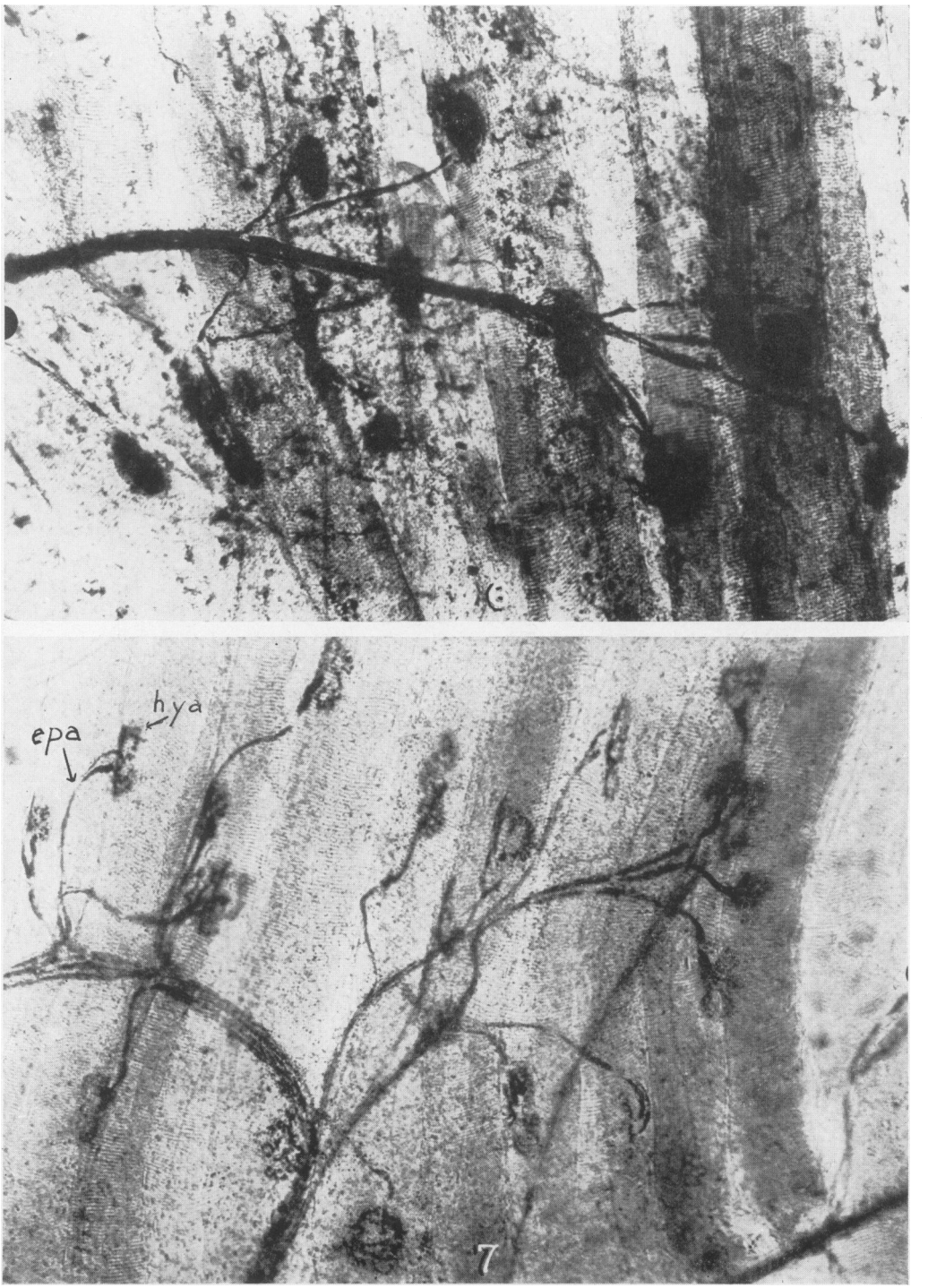
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PLATE 244

FIGS. 8 and 9. Sprays of medullated nerve fibers and motor end-plates in the gastrocnemius muscle 48 hours after the degenerative cut. There has been an advance in the loss of structure of the muscle fibers. Some of the hypolemmal axons are replaced by fine granules. In some places there is a dark, coarse, granular to flaky appearance of the muscle fibers which is a histologic sign of the beginning loss of the normal periodic diffusion of the fine granular transmitter substance. In some nerve trees the epilemmal axons are fragmented and have a decreased affinity for gold (Fig. 8), while in other places there is an increased capacity to take the gold with little evidence of fragmentation (Fig. 9). Since the technic was standardized by running the normal and abnormal muscles simultaneously through the same fluids for the same periods of time, this is considered a histologic sign of a periodic decrease alternating with an increase in the quantity of material in the epilemmal axons. There is a definite hyperemia of the intramuscular blood vessels and capillaries, demonstrated by the increased diameter of the lumen and the packing of these vessels with red blood cells. In other locations the capillaries are collapsed and devoid of cellular elements. This is comparable to the conditions produced by experimental traumatic and thermal shock and by the local injection of lactic and other acids into the muscle. × 250.

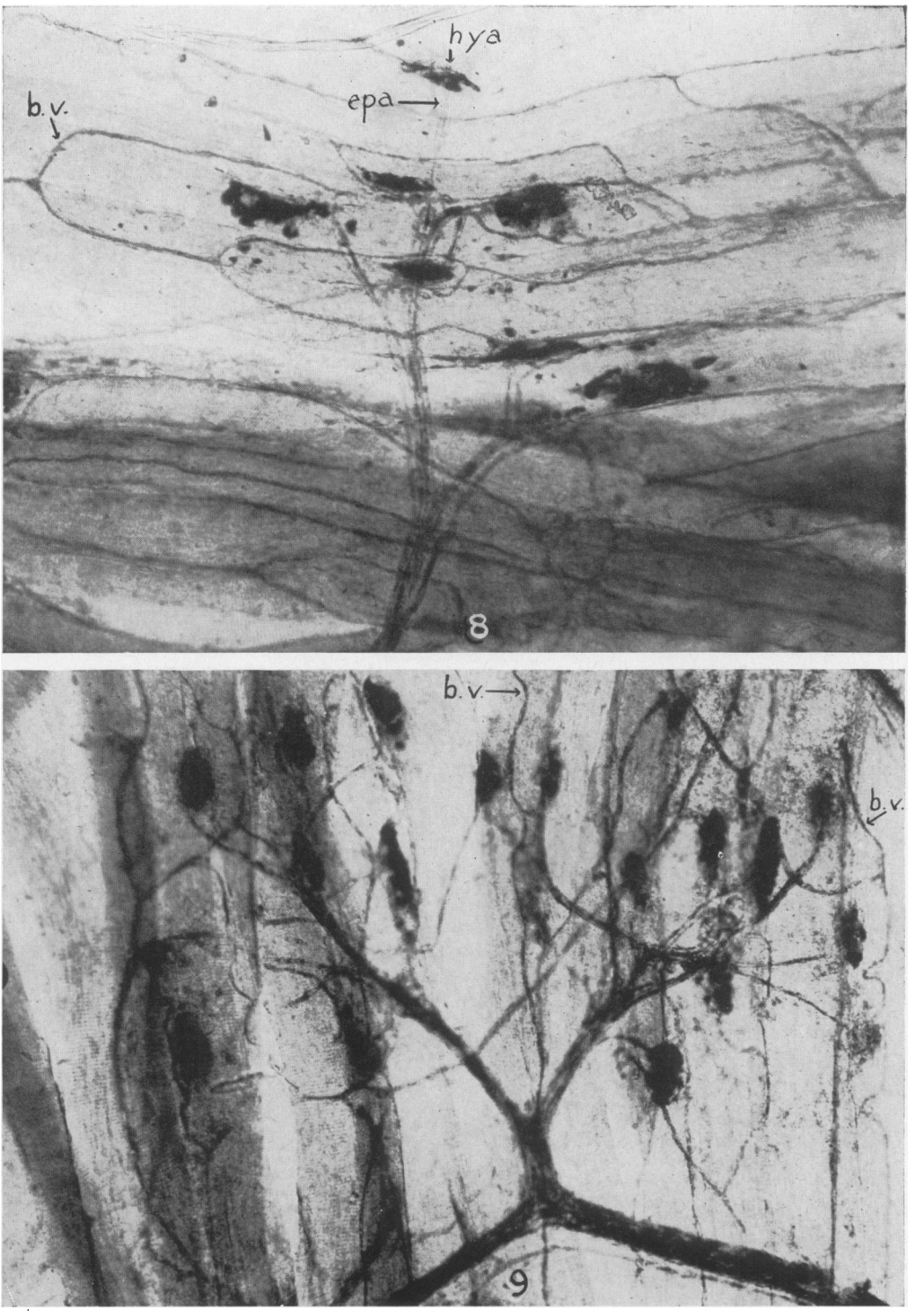
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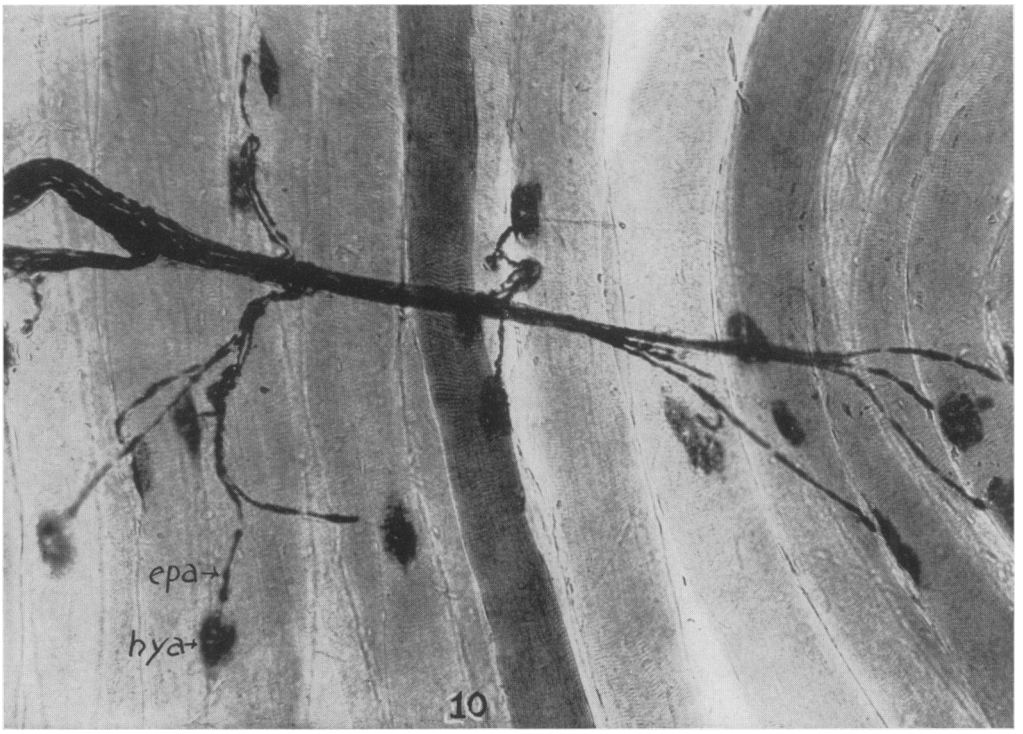
PLATE 245

FIG. 10. Spray of medullated nerve fibers and terminal motor end-plates from a relatively normal gastrocnemius muscle of a rat killed by ether. There is pleomorphism and variation in the size and impregnation capacity for gold of the motor end-plates and muscle fibers. The narrow dark muscle fiber associated with a dark retracted end-plate may represent the evanescent phase of the nebulous diffusion of the fine granular acetylcholine at the onset of the contraction of the muscle fiber. The wide light muscle fibers frequently associated with expanded end-plates with a decreased affinity for gold may represent the phase of active contraction of the muscle fiber after the diffused acetylcholine has been hydrolyzed by cholinesterase. This alternation of dark and light auriphilic fiber types may be the morphologic expression of fractional contraction in a normal muscle, and of normal periodic diffusion, alternating with destruction, of the fine granules of acetylcholine in different fibers of the same muscle. The differential capacity to take gold may be a morphologic measure of the quantity of neurogenic substances diffused into the myoplasm of the muscle fiber. The normal and abnormal muscles were concurrently subjected to the same standardized technic. × 250.

FIG. 11. Spray of medullated nerve fibers and granular motor end-plates in the gastrocnemius muscle 72 hours after section of the sciatic nerve. The motor end-plates are replaced by fine granules surrounding the light oval spaces occupied by the nuclei of the sole. The epilemmal axons in this field have a decreased affinity for gold in comparison to the relatively normal axons (Fig. 10). There is a dark, coarse, granular or flaky appearance of the muscle fibers, which is a histologic sign of beginning loss of normal diffusion of the fine granules of the transmitter substance discharged from the motor end-plate into the myoplasm of the muscle fiber. There is likewise a definite loss of structure in the dark type of muscle fiber. This may well represent the loss of the normal initial phase of periodic diffusion of the normal neurogenic transmitter substance associated with the production of dark muscle fibers. These dark muscle fibers progressively disappear after the degenerative cut of the nerve, and their disappearance parallels the loss of transmission of acetylcholine from the motor nerve endings into muscle. × 250.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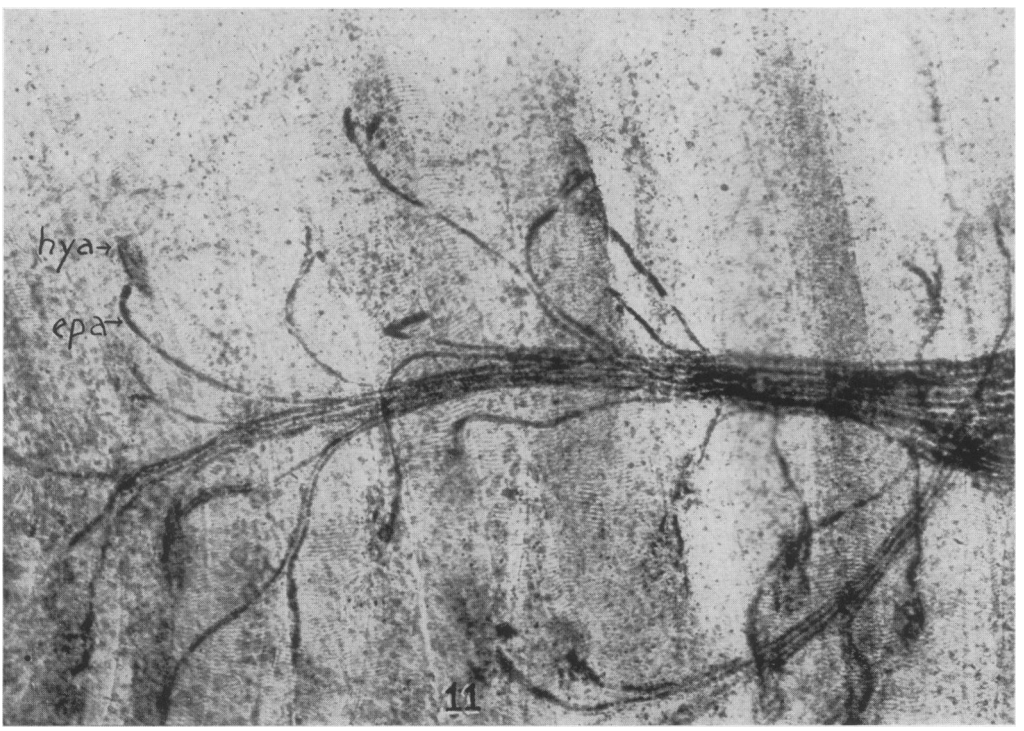
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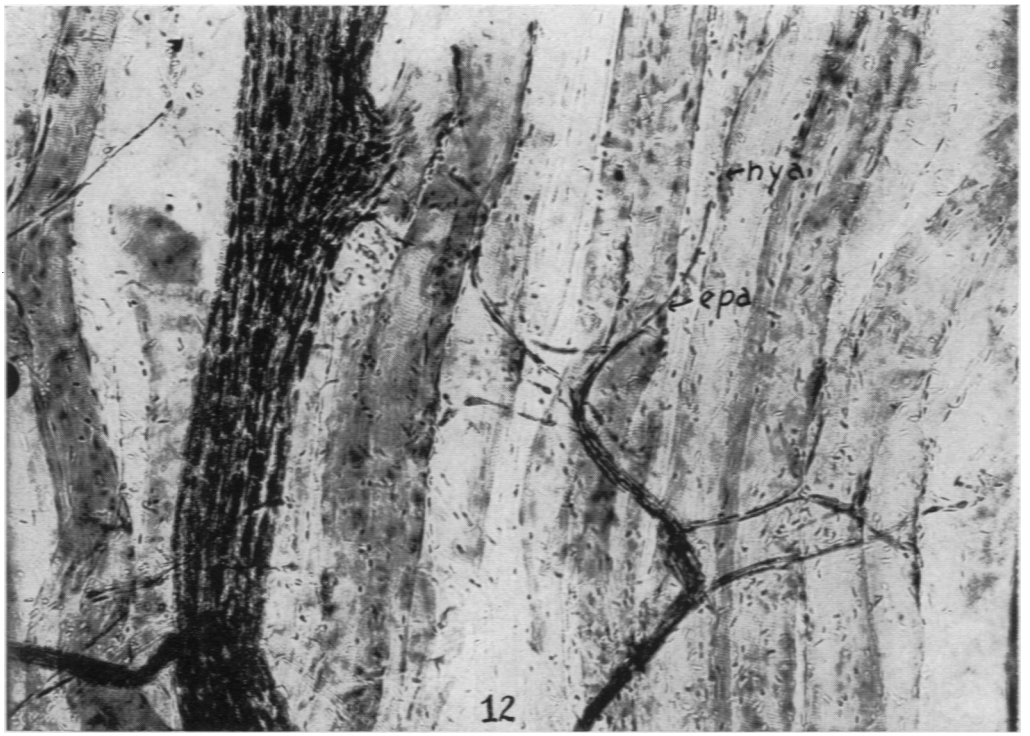
PLATE 246

FIG. 12. Spray of medullated nerve fibers and terminal granular degeneration of the motor end-plates in the gastrocnemius muscle 5 days after the degenerative cut of the sciatic nerve. These motor end-plates are replaced by fine granules surrounding the light oval spaces occupied by the nuclei of the sole. There is a loss of structure in the dark type of muscle fibers. The fibers are becoming narrower than normal and the sarcolemmal nuclei are more evident in some places than in normal muscle with the gold technic. In some places there is definite fragmentation of the epilemmal axons and in others a depletion of the axonic material. × 250.

FIG. 13. Spray of medullated nerve fibers and terminal granular degeneration of the motor end-plates in the gastrocnemius muscle 7 days after the degenerative cut of the sciatic nerve. These motor end-plates are replaced by fine granules. The nuclei of the sole plate are beginning to take the gold stain in certain places. There is a loss of structure of muscle fibers of the dark type. The fibers are becoming narrower than normal and the visibility of the sarcolemmal nuclei in some places is increased beyond the normal with gold. In certain places there is definite fragmentation of the epilemmal axons, and in others a depletion of the axonic material. The axonic material in the nerve trunk is fragmented into rounded droplets, elongated bodies with rounded ends, and fusiform structures. In following a nerve trunk throughout its course, first without and then within a muscle, this fragmented axonic material appears to travel in a periodic manner in a centrifugal direction. This material is then discharged from the distal nerve terminal into the muscle. × 250.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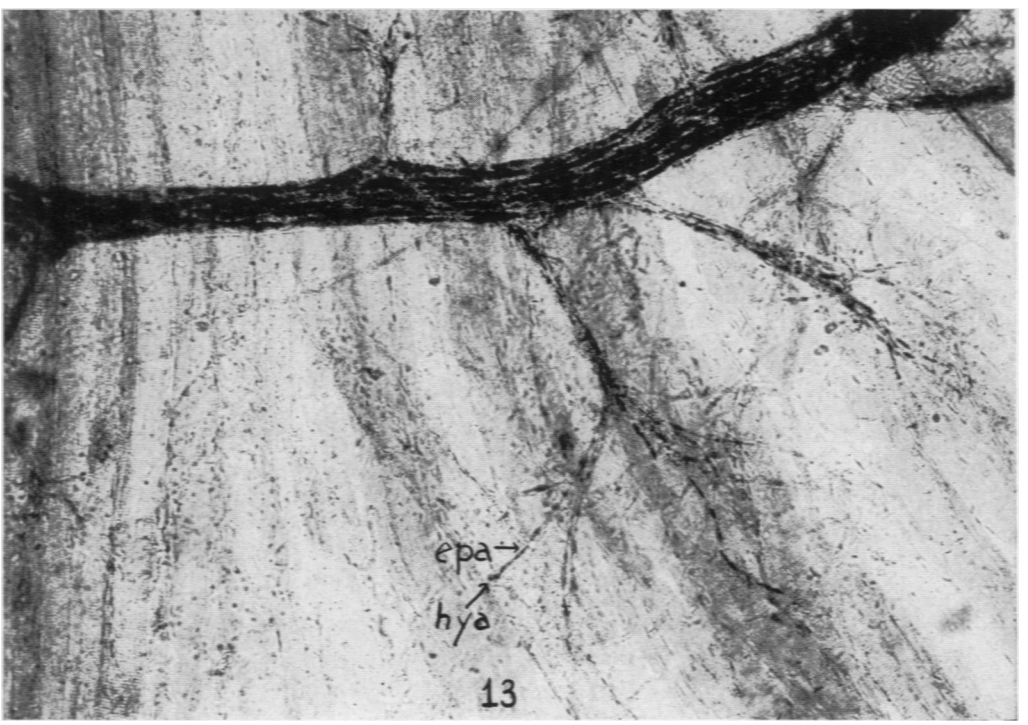
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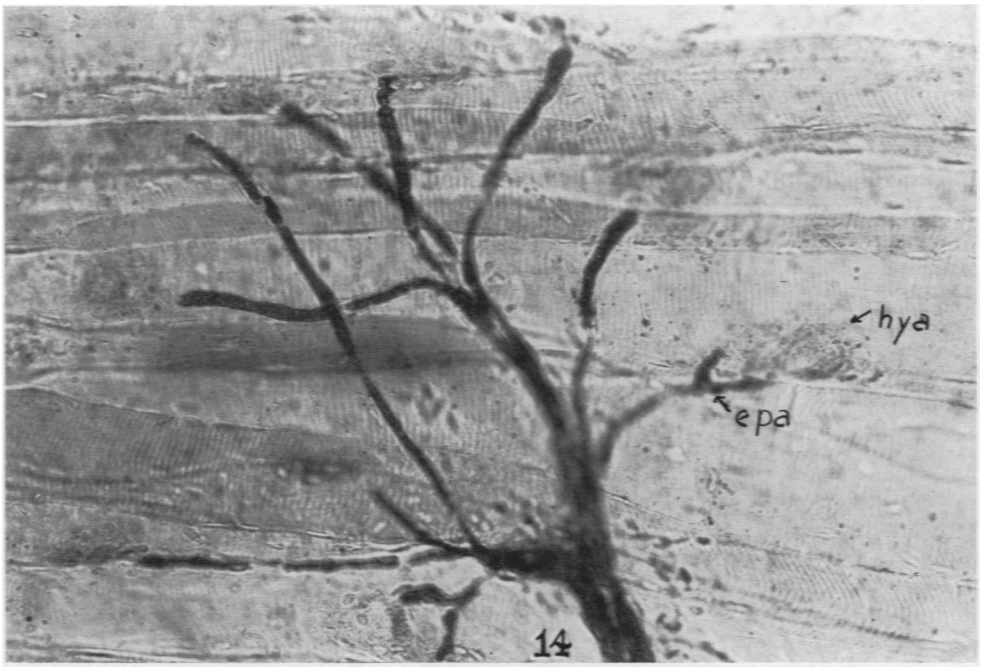
PLATE 247

FIGS. 14 and 15. Sprays of medullated nerve fibers and terminal granular degeneration of the motor end-plates in the gastrocnemius muscle 9 days after the degenerative cut of the sciatic nerve. These motor end-plates are replaced by fine granules surrounding the light oval spaces occupied by the nuclei of the sole. There is a definite loss of the dark type of muscle fiber. In these sprays the epilemmal axons are loaded with material that has a strong affinity for gold. There is very little evidence of fragmentation of the epilemmal axons. After the degenerative cut of the nerve the fragmentation of the epilemmal axon appears and disappears with the progressive and intermittent advance in a peripheral direction of the degenerated axonic material and its discharge centrifugally in the region of the degenerated motor end-plates. There is no evidence of hypolemmal axons. These have been completely replaced by rounded and oval islands of granules. Some of the epilemmal axons have dichotomous divisions. In other places the epilemmal axon is single and terminates in a rounded or frayed end which gradually disappears by confluence with the myoplasm of the muscle fiber. Figure 14, × 400; Figure 15, × 850.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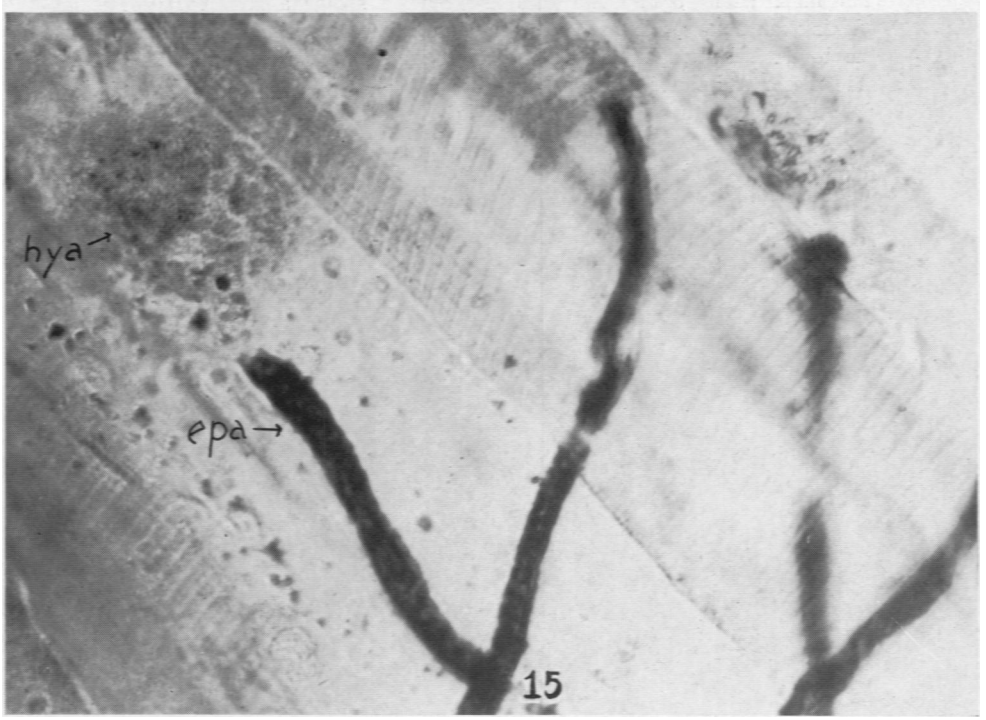
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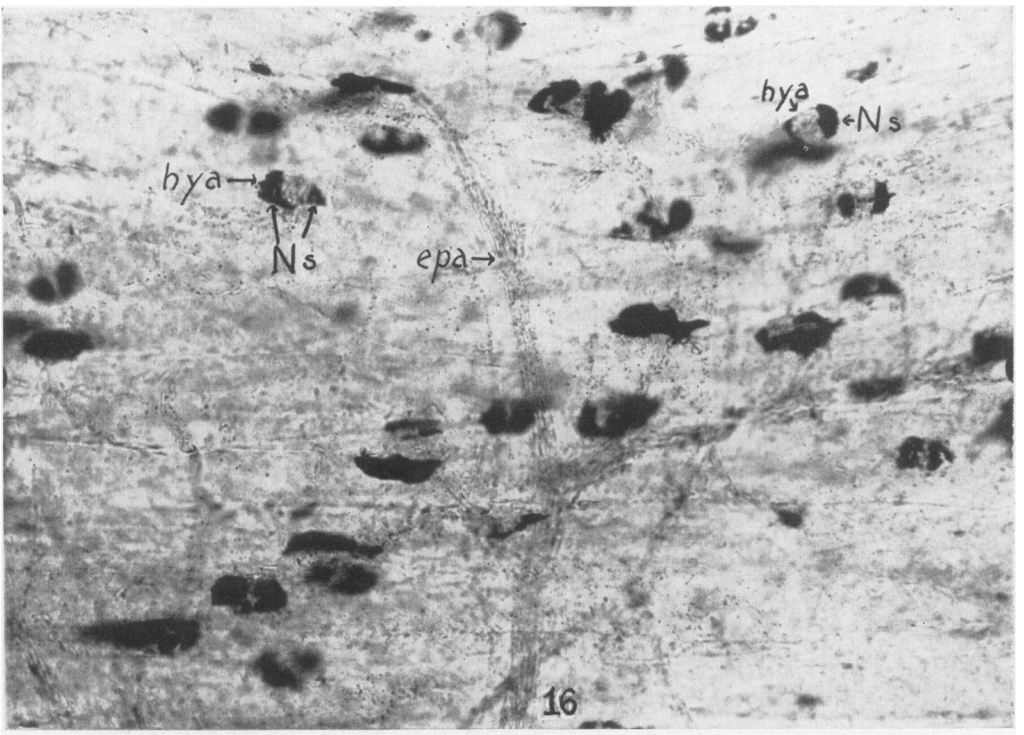
PLATE 248

FIGS. 16 and 17. Sprays of medullated nerve fibers undergoing depletion of their axonic material in a centrifugal direction in a gastrocnemius muscle 10 days (Fig. 16) and 12 days (Fig. 17) respectively after the degenerative cut of the sciatic nerve. The discharged axonic material that has a strong affinity for gold is accumulating in pleomorphic masses within and around the region of the degenerated motor end-plates. The faint, ghost-like outline of the epilemmal axons depleted of substances that have a strong affinity for gold is in striking contrast to that of the accumulated material, with the capacity to take the gold, discharged in a centrifugal direction into the region of the degenerated motor end-plates. × 250.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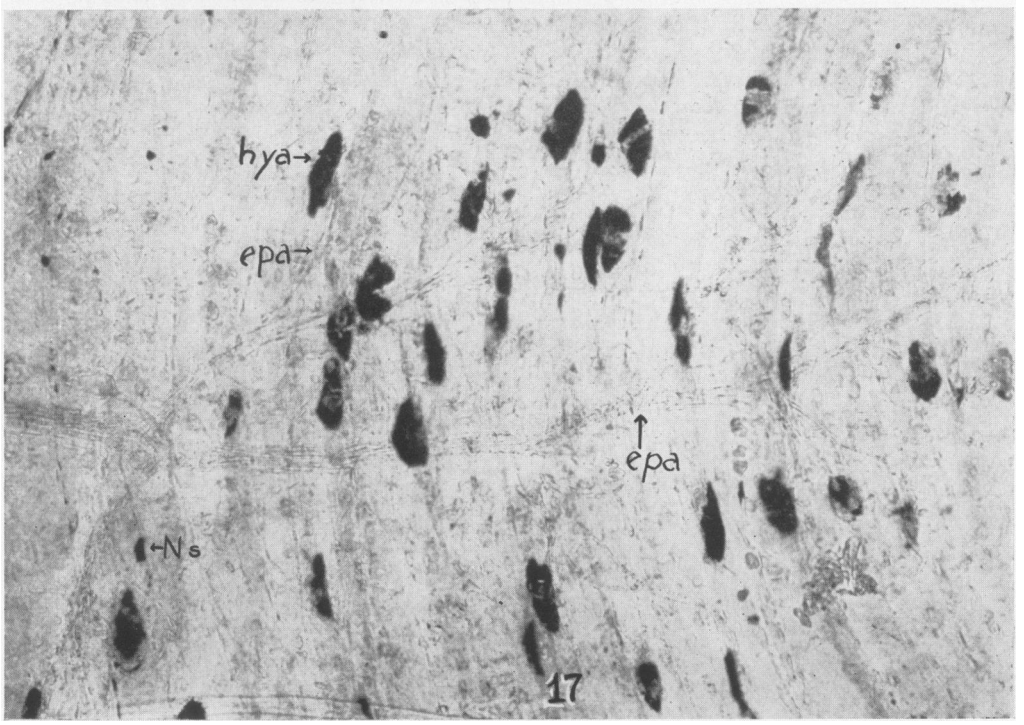
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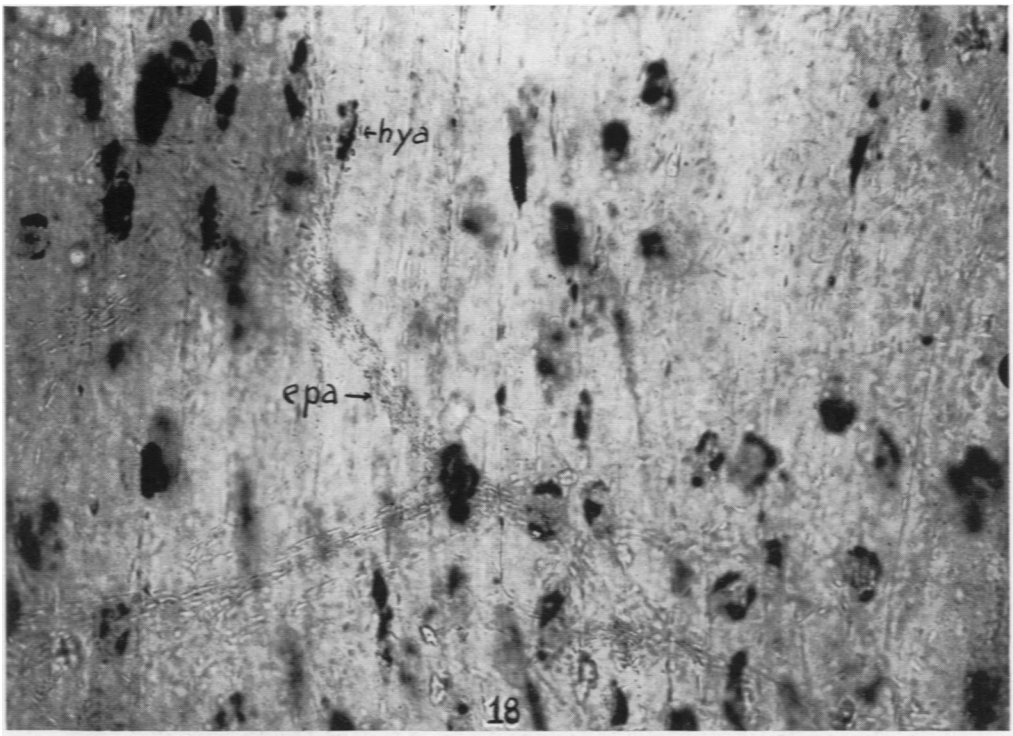
PLATE 249

FIGS. 18 and 19. Sprays of medullated nerve fibers depleted of their axonic auriphilic material which is discharged in a centrifugal direction into the gastrocnemius muscle 14 days (Fig. 18) and 20 days (Fig. 19) respectively after the degenerative cut of the sciatic nerve. The discharged axonic material with a strong capacity to combine with gold is accumulated in pleomorphic masses within and around the region of the degenerated motor end-plates. Some of these pathologic masses or neurosomes are found scattered in the myoplasm of the muscle fiber. The faint, ghost-like outline of the epilemmal axons depleted of their substance that has an affinity for gold is again in striking contrast to the accumulated gold-impregnated material discharged in a centrifugal direction. The muscle fibers 20 days after the degenerative cut have lost the appearance of the normally functioning dark type, and they are considerably narrower in comparison with the normal. The anatomic evidence is incontrovertible that the histologic dark type of muscle fiber is related to a normally functioning nerve supply. This functional dark type of muscle fiber appears to be produced by the normal diffusion into the myoplasm of the fine granules of acetylcholine discharged from the motor end-plate. (For comparison with Figs. 1, 10, and 22). × 250.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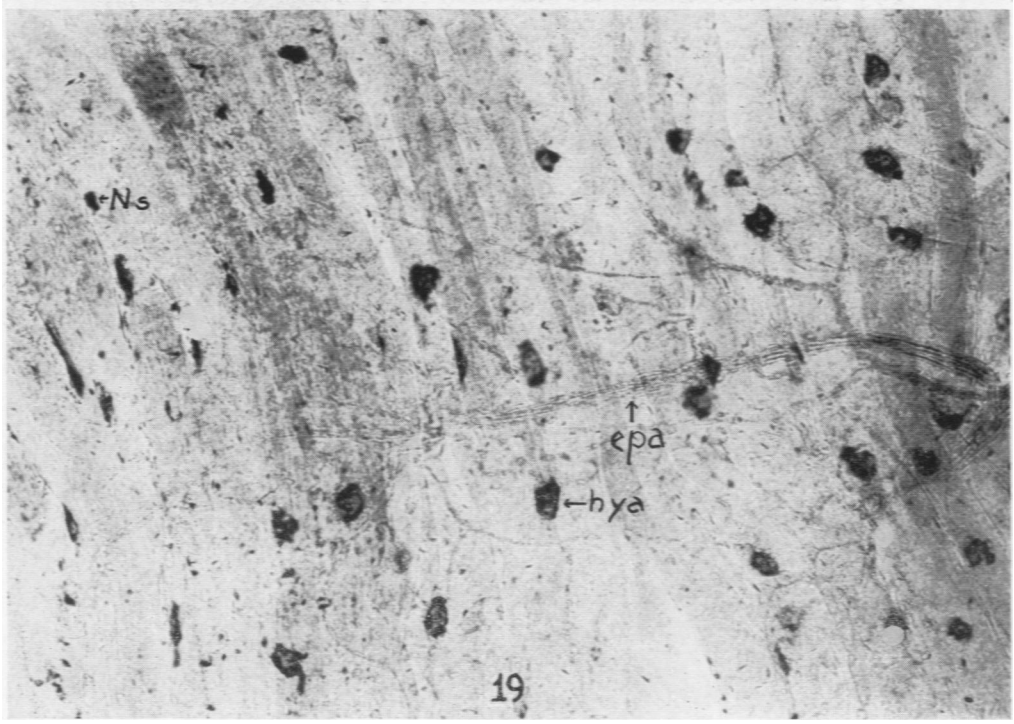
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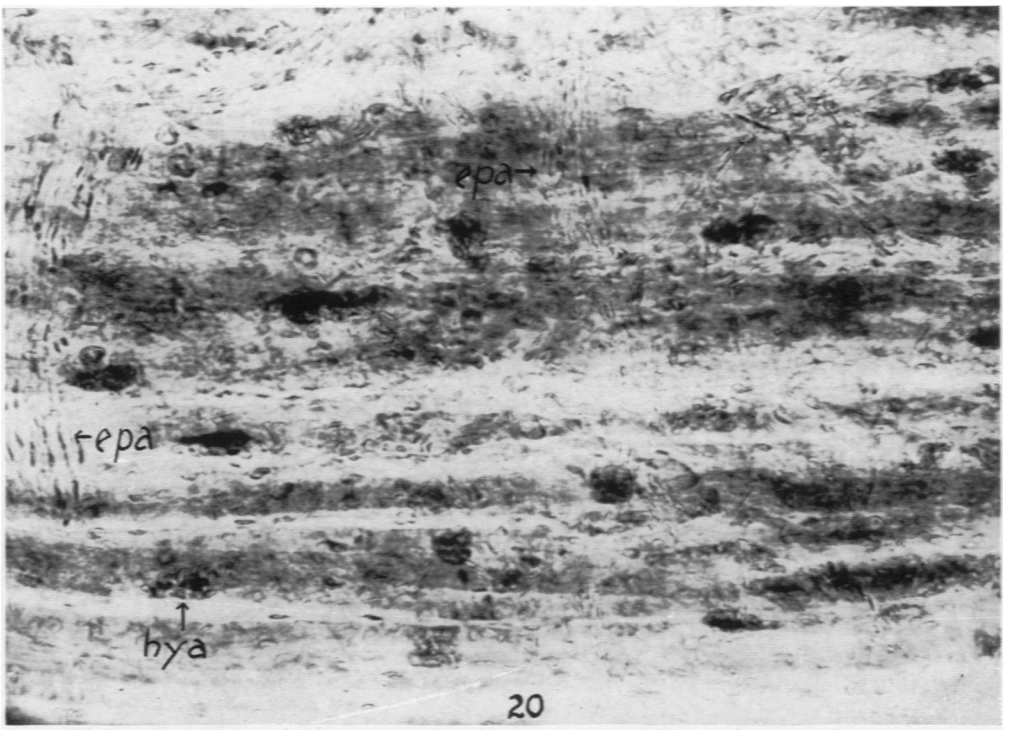
PLATE 250

FIGS. 20 and 21. Sprays of medullated nerve fibers depleted of their axonic material in the gastrocnemius muscle 30 days after the degenerative cut of the sciatic nerve. The axonic material, which was discharged in a centrifugal direction, is seen only in relation to a few of the degenerated end-plates. The locations of the degenerated end-plates are indicated principally by clusters of sole-plate nuclei which are surrounded by the granular material secreted by these nuclei. This granular material has an affinity for gold slightly less than that of the material discharged by the hypolemmal axons. The clusters of nuclei are detected as clear, rounded, or oval spaces surrounded by dark granules (Fig. 20). In some places, in the same muscle, the myoplasm is aggregated into broad or narrow, dark, transverse bands alternating with light ones (Fig. 21). In the dark bands the cross striations are either exceedingly fine or they are completely absent because of the opacity of the dense aggregation of the myoplasm. The light bands are occupied by cross striations which are farther apart than those in the dark bands. This dark and light transverse banding of the narrow muscle fibers is produced by the slow, irregular fibrillation of the muscle fibers. This incoordinate, ceaseless, slow, vermiform fibrillation of the denervated muscle fibers begins about 72 hours after the degenerative cut of the sciatic nerve, and is correlated with loss of transmission of acetylcholine, which, normally, chemically tunes the muscle fiber to the higher pitch or frequency of fast muscle contractions and maintains its capacity to respond adequately to high-frequency stimulation. This chemical tuning process is roughly analogous to the mechanical tuning of the strings of a piano or violin for normal response at a higher frequency than is possible in strings sagging between their supports. × 250.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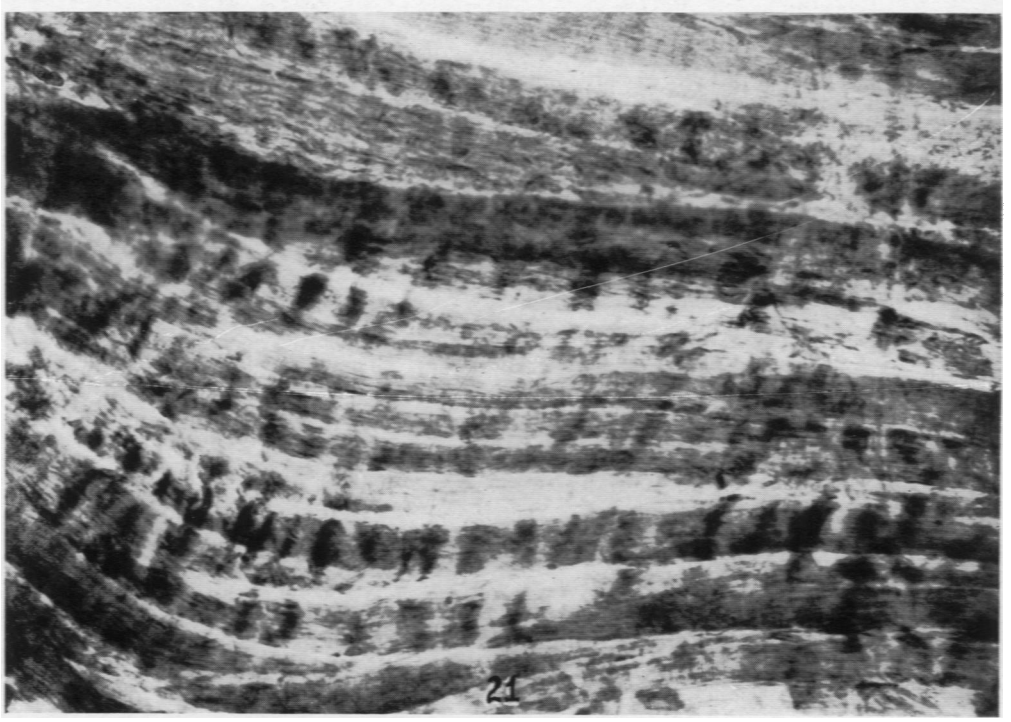
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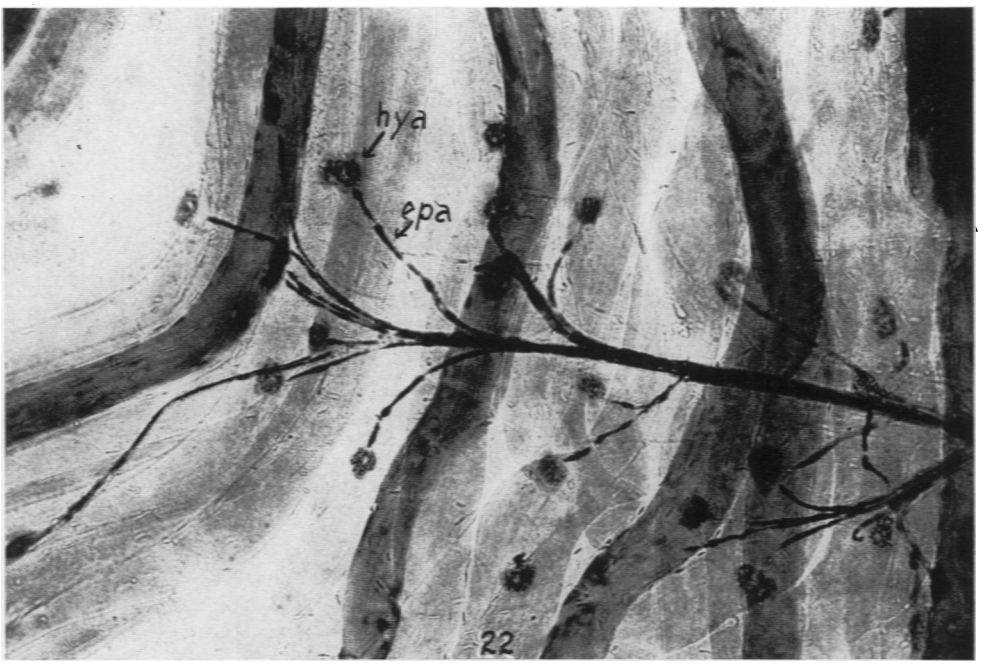
PLATE 251

FIG. 22. Spray of medullated nerve fibers and terminal motor end-plates from a relatively normal gastrocnemius muscle of the rat killed by ether. There is definite pleomorphism with variation in the size, shape, and impregnation capacity for gold of the motor end-plates and muscle fibers. The narrow, dark muscle fibers may represent the initial phase of the fine, nebulous diffusion of granular acetylcholine at the beginning of contraction of certain muscle fibers. The wide, light muscle fibers may represent the phase of active contraction of some muscle fibers after the diffused acetylcholine has been destroyed by hydrolysis through the action of the cholinesterase. This variation and alteration of fiber types in the same muscle fiber may be the morphologic expression of the fractional contraction of the fibers in a muscle and the active periodic diffusion, alternating with the destruction by hydrolysis, of acetylcholine in the different muscle fibers of the same muscle related to a normal innervation. The same muscle fiber at different periods of time may be either dark or light by gold impregnation, dependent upon the phase of active diffusion or hydrolytic destruction respectively of acetylcholine granules. The dark and light muscle fibers, according to this concept, are morphologic variations that parallel the different periods of functional activity. The dark muscle fiber loses that character when the normal secretion of acetylcholine is abolished by denervation. × 150.

FIG. 23. Spray of medullated nerve fibers depleted of their axonic and degenerated myelin materials in the gastrocnemius muscle 10 days after the degenerative cut of the sciatic nerve. There is a definite loss of structure of the different types of muscle fibers. The dark type of muscle fiber has been eliminated. The epilemmal axons are definitely depleted of the fragmented axonic and myelinic materials. This depletion of the epilemmal axons of their gold-impregnated materials is due to the centrifugal discharge of the degenerated axonic substance into and around the degenerated motor end-plates. There is, likewise, a discharge of neurosomes throughout the muscle fibers. This morphologic change was produced by the intraperitoneal injection of d-tubocurarine chloride followed by electrical stimulation of the degenerating peripheral stump of the cut sciatic nerve. The demonstration of the pathologic structure of the normal or abnormal transmitter substance may be demonstrated best experimentally by accelerating the discharge of the axonic material into the muscle and by preventing or delaying its normal diffusion into the myoplasm of the muscle fiber. × 150.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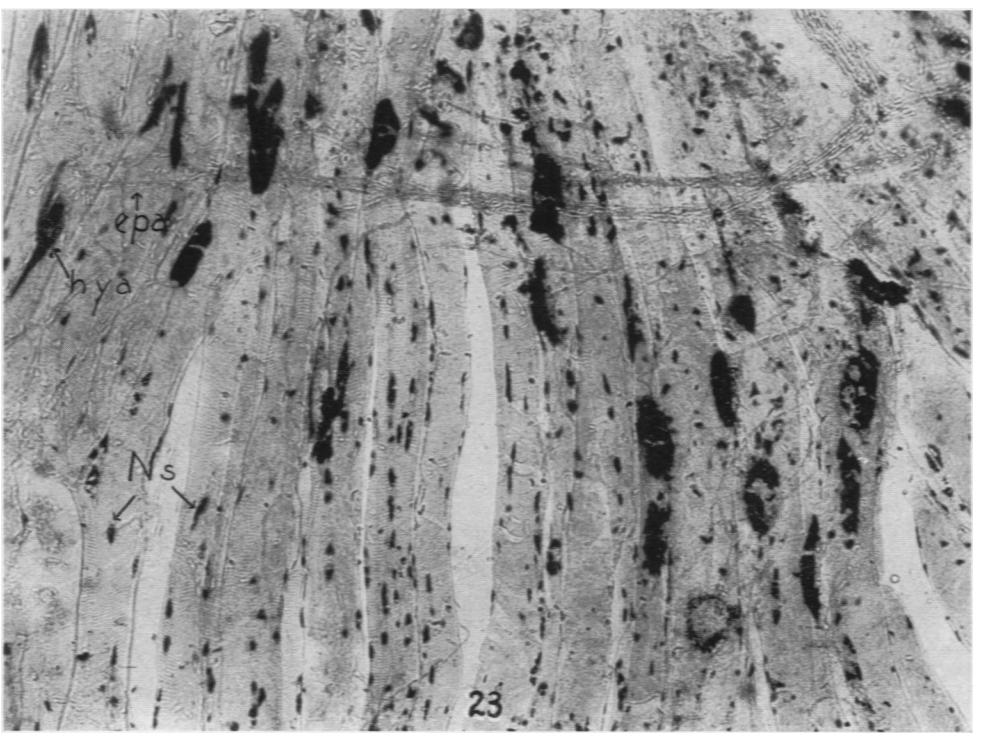
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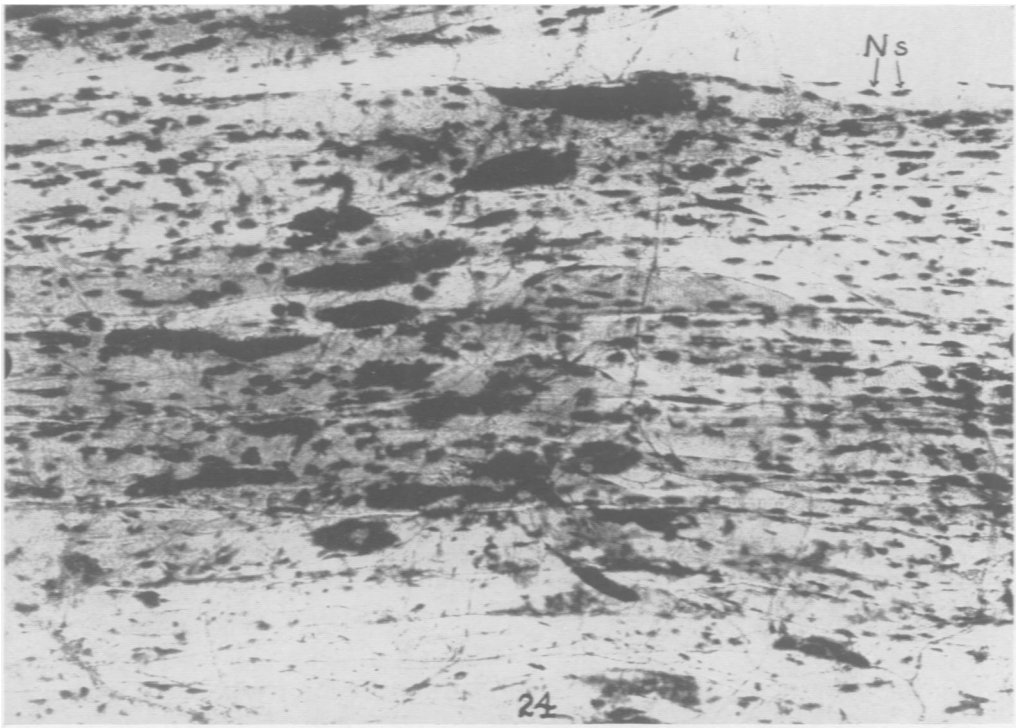
PLATE 252

FIGS. 24 and 25. Sprays of medullated nerve fibers depleted of their axonic and myelin material with the degenerated motor end-plates surrounded by augmented material with a strong affinity for gold, from the gastrocnemius muscles of rats, 12 (Fig. 24) and 14 (Fig. 25) days, respectively, after section of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. There is practically complete depletion of the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has been projected in a very profuse manner throughout the muscle fibers. There is a definite loss of the dark type of muscle fiber as a result of denervation (for comparison with Fig. 22, normal innervation of gastrocnemius muscle). The pathologic structure of this abnormal transmitter substance is due to the experimental acceleration of the discharge and the lack of normal diffusion of this abnormal transmitter substance into the myoplasm of the muscle fiber. The irregular discharge of the nondiffusible nervous substances into the muscle apparently prevents the appearance of the functional dark type of muscle fiber, which appears to be the product of the discharge and uniform nebulous diffusion in a periodic manner of fine granules from a normal innervation. These fine neurogenic granules may be interstitial granules of Kölliker found in normal muscle. The large neurosomes may represent not only an agglutination of the granules of Kölliker, but also the discharge of abnormal substances into the muscle. × 150.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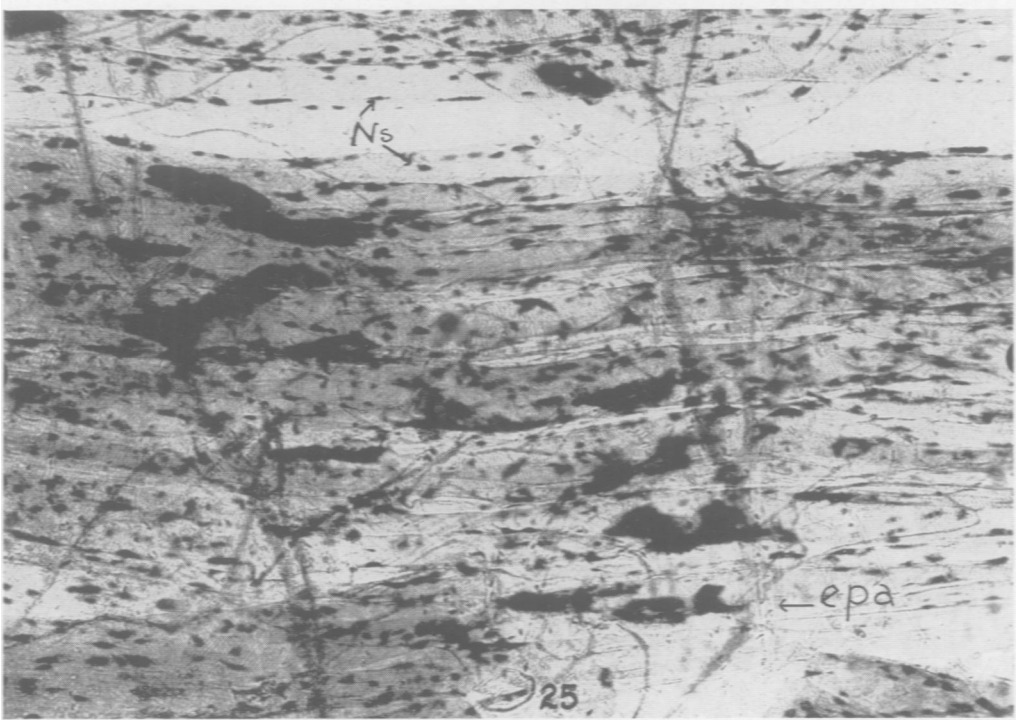
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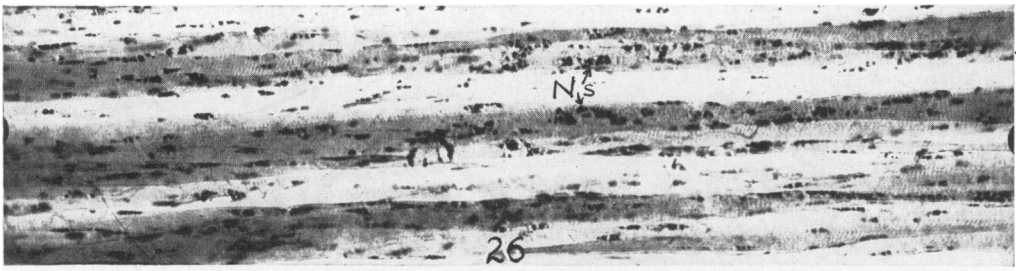
PLATE 253

FIGS. 26 to 29. Sprays of medullated nerve fibers depleted of their axonic and myelin materials, and the degenerated motor end-plates surrounded by an augmented amount of material with a strong affinity for gold in the gastrocnemius muscle of the rat 15 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for a duration of 30 seconds at the rate of 5 per second. There is practically complete depletion from the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has likewise occurred as multiple series of small, round droplets with a strong affinity for gold. Two to ten little droplets that have a strong affinity for gold are found in each series. The beginning and the end of each series is composed of droplets smaller than those at the middle of the series. Some muscle fibers do not contain these droplets of neurogenic material. This pleomorphism of the material discharged by the end-plate is related to the different phases in the abnormal secretion from the motor nerve terminal into the myoplasm of the muscle fibers. × 150.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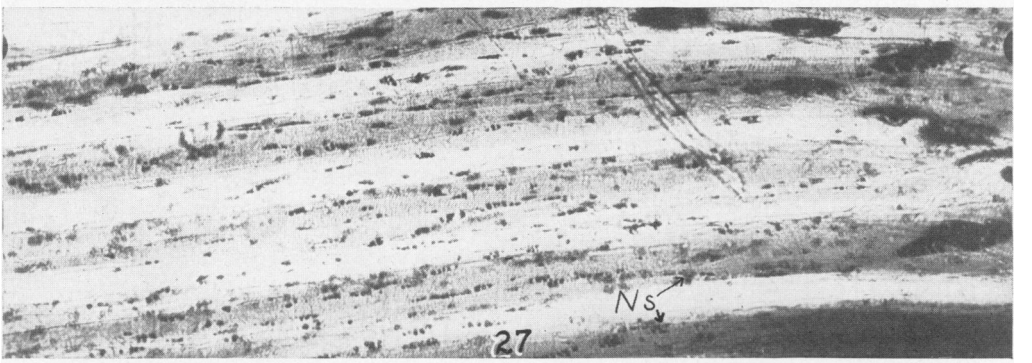
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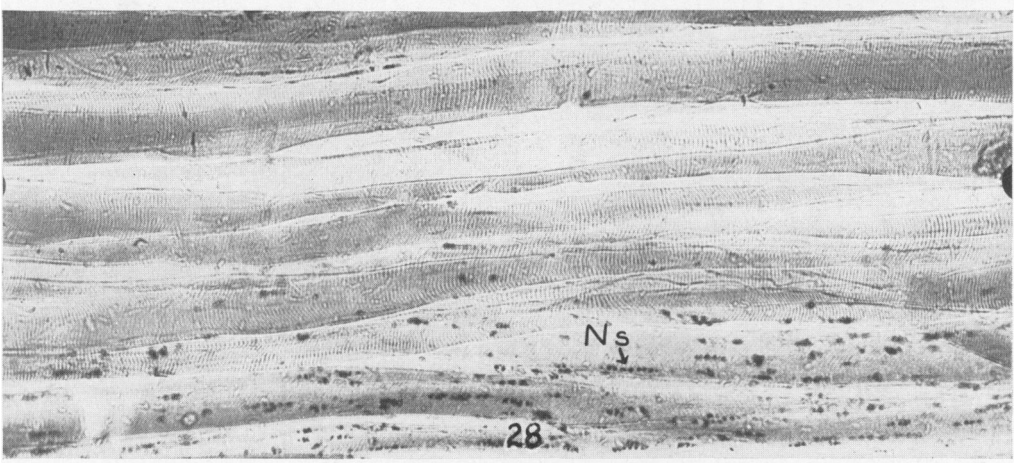
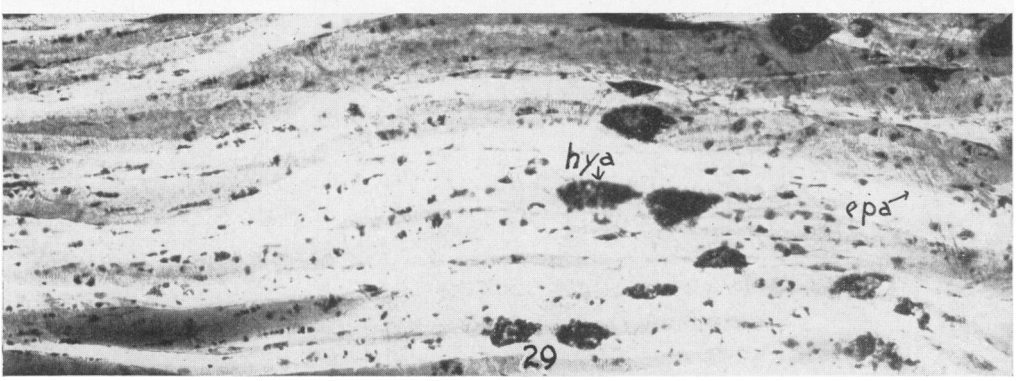

Ns
28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29

PLATE 254

FIGS. 30 and 31. Sprays of medullated nerve fibers depleted of their degenerated axonic and myelin materials, and degenerated motor end-plates surrounded by an augmented amount of substances with a strong affinity for gold in the gastrocnemius muscles of rats, 5 (Fig. 30) and 10 (Fig. 31) days, respectively, after the degenerative cut of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for a duration of 30 seconds at the rate of 5 per second. There is practically complete depletion from the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has, likewise, occurred in a very profuse manner through the myoplasm. There is a definite loss of the functional dark type of muscle fiber (for comparison with Fig. 22, normal innervation of gastrocnemius muscle). The pathologic structure of this abnormal transmitter substance is due to the experimental acceleration of the abnormal discharge and the lack of the normal nebulous and periodic diffusion of the fine, granular transmitter substance into the myoplasm. The profuse discharge of nondiffusible and abnormal nervous particles into the muscle apparently prevents the appearance of the functional dark type. Three experimental factors are working concurrently to produce the large amount of neurogenic material in muscle: the curare-like effect on muscle of nerve sectioning, the active effect of relatively pure curare upon the myoplasm, and the accelerated discharge of relatively nondiffusible or delayed-diffusible degenerated axonic and myelin materials produced by the electric stimulation of the distal stump of the cut sciatic nerve. With the discharge of great masses of the abnormal axonic material into the muscle there is a concomitant disappearance of this material in both the epilemmal and hypolemmal axons of the motor end-plate. × 350.

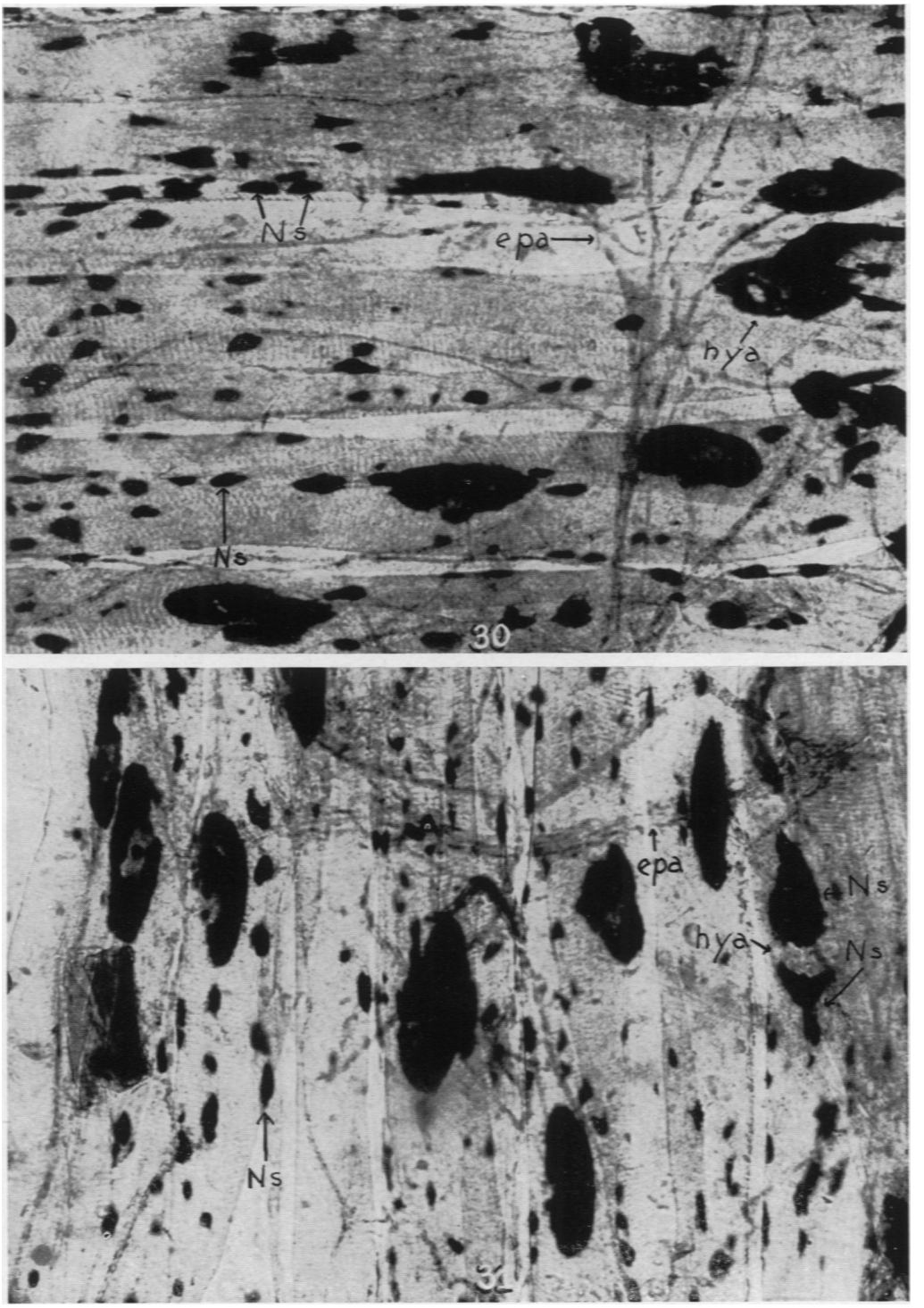

Ns
epa
hya
Ns
30
epa
Ns
hya
Ns
Ns

PLATE 255

FIGS. 32 and 33. Sprays of medullated nerve fibers depleted of their degenerated axonic and myelin materials, and degenerated motor end-plates surrounded by an augmented amount of substances with a strong affinity for gold in the gastrocnemius muscles of rats, 15 (Fig. 32) and 20 (Fig. 33) days, respectively, after degenerative section of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. There is practically complete depletion from the epilemmal axons of the substances with an affinity for gold. This gold-impregnated material has been discharged in a centrifugal direction into and around the degenerated motor end-plates. This discharge has, likewise, occurred in a very profuse manner throughout the muscle fibers. There is a definite loss of the dark type of muscle fiber (for comparison with Fig. 22, normal innervation of gastrocnemius muscle). The demonstration of the pathologic structure of this abnormal transmitter substance is due to the experimental acceleration of the discharge and the lack of normal diffusion of this abnormal transmitter substance into the myoplasm of the muscle fiber. The irregular discharge of nondiffusible nervous substances into the muscle apparently prevents the appearance of the functional dark type of muscle fiber. There is a definite elongated and fusiform structure assumed by the gold-impregnated material that accumulates around the degenerated motor end-plates about the 20th day after the degenerative cut. This is a mechanical resultant of the atrophy and decrease in diameter of the denervated gastrocnemius muscle fibers. This atrophy of the muscle fibers mechanically molds the liquid or semiliquid neurogenic materials discharged at the terminals of the degenerated motor nerve, into the muscle fibers. The demonstration of the pathologic structure of the normal or abnormal transmitter substance may be accomplished best, experimentally, by accelerating the discharge of the axonic material into the muscle and by preventing or delaying its normal diffusion into the myoplasm. × 350.

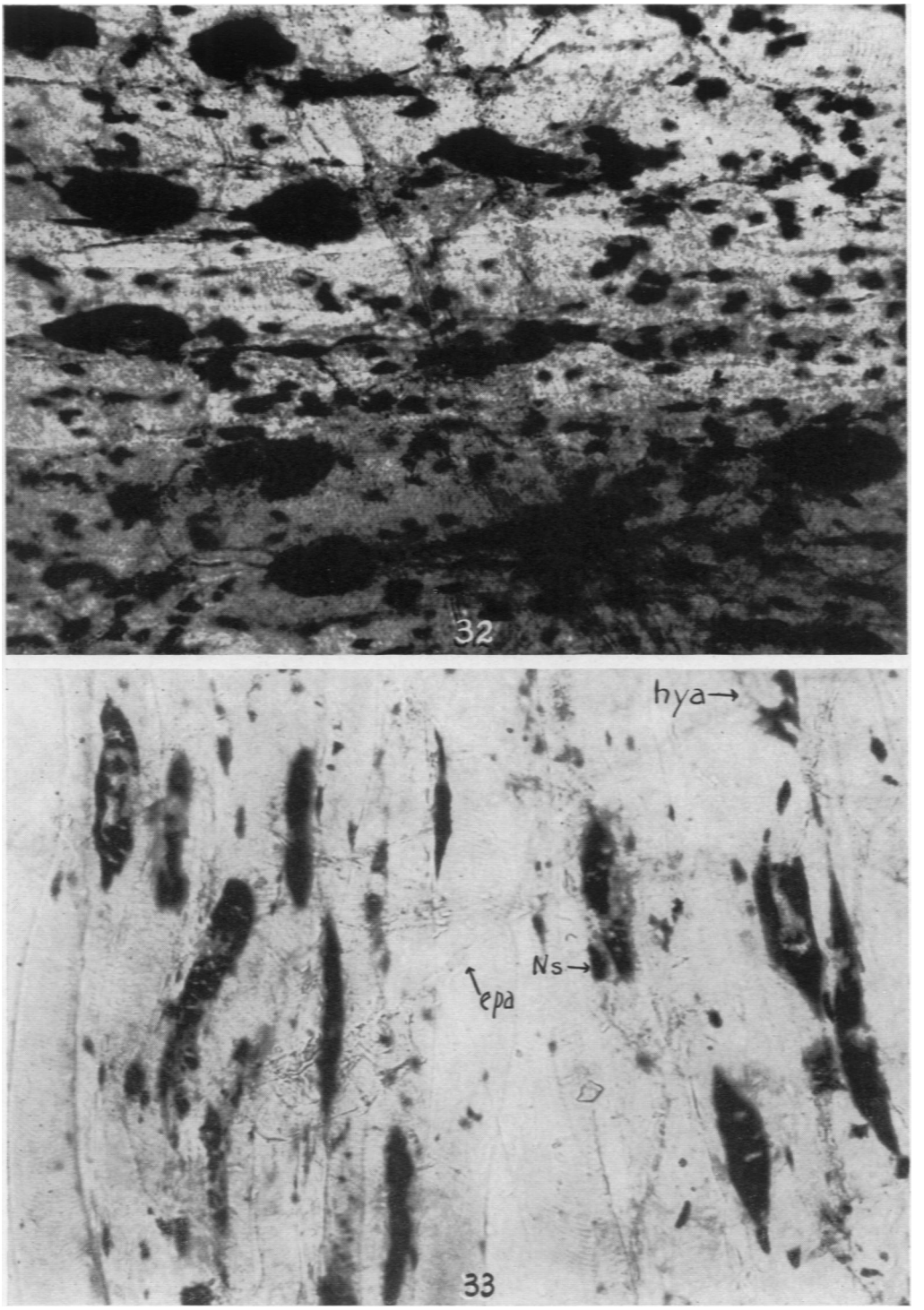

32
hya→
Ns→
epa
33

PLATE 256

FIGS. 34 and 35. Sprays of medullated nerve fibers and terminal motor end-plates from the relatively normal gastrocnemius muscle of a rat killed by ether. The dark and light muscle fibers are evident. The dark muscle fibers contain more material that has an affinity for gold than the light muscle fibers. The dark, anisotropic, transverse bands in the dark muscle fibers are usually broader and darker than those in the light fibers. On the other hand, the light, isotropic, transverse striations in the light muscle fibers are usually not only broader than the light spaces in the dark muscle fibers, but there is less material that has an affinity for gold in the light spaces of the light muscle fibers than in corresponding cross striations of dark fibers. There are multiple gradations in the width and capacity to take gold of the cross striations. The motor end-plates in the dark, narrow muscle fibers usually possess coarse fronds or knob-like terminals. These fronds may or may not be surrounded by light halo-like spaces, but they are usually surrounded by more dark, granular material of the sole plate of Kühne than in the sole of the expanded end-plates in light muscle fibers. The granules of the sole plate are derived from two sources: from the granular transformation and permeability of the terminals of the hypolemmal axons, and from the nuclei of the sole plate. This granular material of the sole plate normally diffuses in a periodic manner throughout the myoplasm of the dark fiber. The motor end-plate in the light muscle fiber usually exhibits an ameboid expansion and an attenuation of the hypolemmal axons. There is, likewise, a decreased amount of the diffusible granules of Kühne around the motor end-plates of the light muscle fibers. The dark fiber may well represent the stage at the onset of muscle contraction and the light muscle fiber the phase of active contraction. It is practically impossible to catch a muscle fiber in a state of completely unstable relaxation by any histologic technic used to date. The dark muscle fiber probably represents the state nearest to that of relaxation or at the beginning of the stage of contraction of the myoplasm produced by the periodic diffusion of acetylcholine. The dark muscle fibers in the teased specimens have a strong affinity for gold and are stained deep red, blue, or purple. The light muscle fibers have a decreased affinity for gold or are entirely devoid of this staining property, probably because of the temporary absence of chemicals that have an affinity for gold. There are multiple gradations between the hyperchrysophilous and the achrysophilous fibers. × 850.

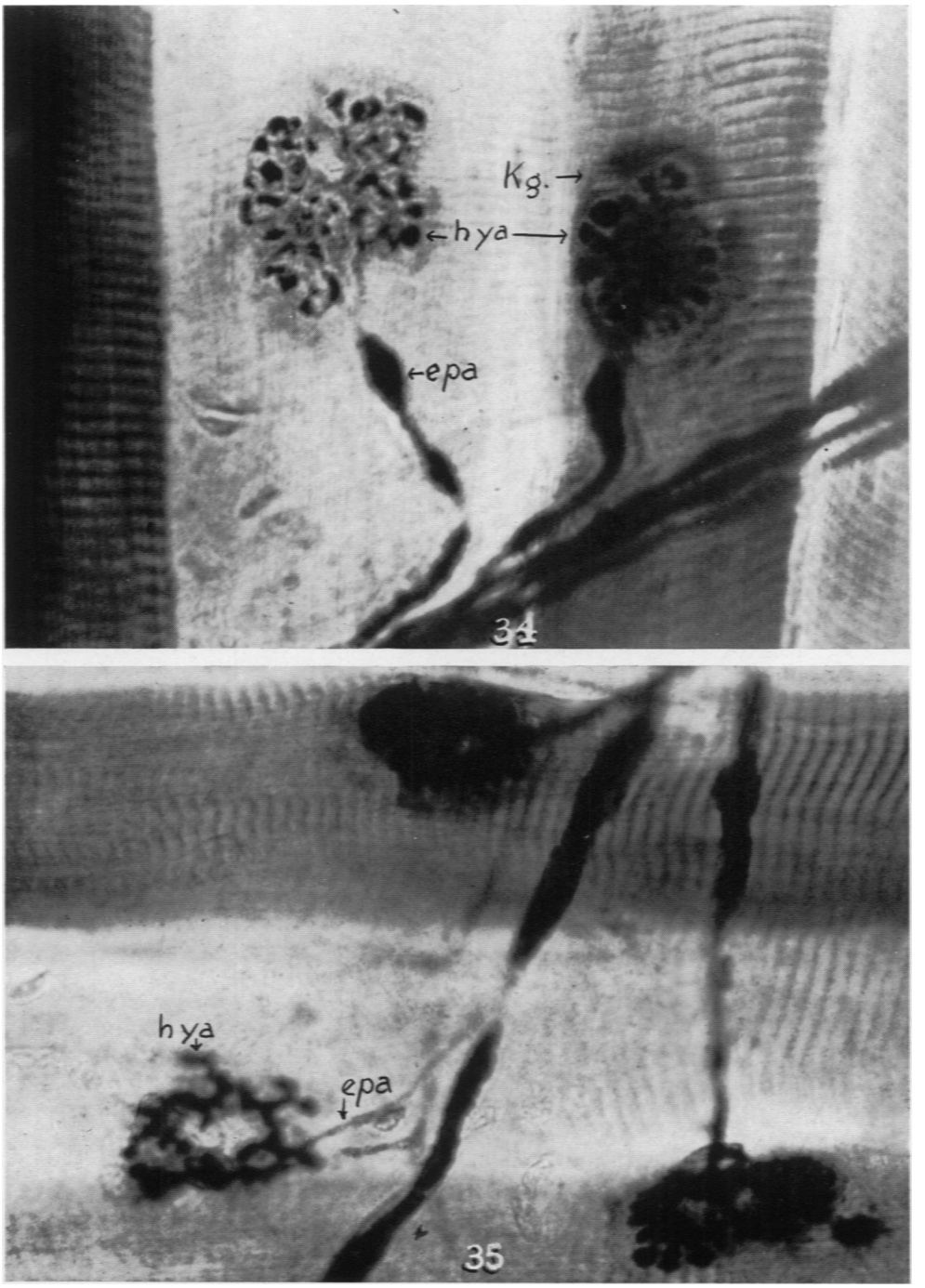

Kg.
hya
epa
34
hya
epa
35

PLATE 257

FIGS. 36 to 40. Motor end-plates from the gastrocnemius muscle of a rat 24 hours after the degenerative cut of the sciatic nerve. Some retracted end-plates (Figs. 36 and 37) are associated with very few Kühne's granules but are in continuity with epilemmal axons that are enlarged and form cysts. There appears to be a temporary block of transmission of substance from the epilemmal axon to the hypolemmal ramifications in the nerve ending. Some of the end-plates are relatively normal, but in places there are relatively large, rounded, or oval thickenings, varying in number, size, and shape, which appear on the arborizations of the motor endings. These retention cysts contain materials that have a strong affinity for gold (Figs. 36, 37, and 38). Other end-plates are relatively expanded with ameboid branchings of their terminal arborizations (Figs. 39 and 40). These terminal branchings are more elongated, more narrow, and have a decreased affinity for gold as compared to the relatively retracted endings. The expanded endings, likewise, have a decreased amount of the Kühne granules of the sole plate. The direct transformation of the hypolemmal axons into granules appears to be one origin of the granular sole plate of Kühne. The retracted and expanded nerve endings, therefore, are functional types that alternate in the same muscle fiber at different times and do not represent a specific definitive type of nerve ending that is always constant in its structure. × 850.

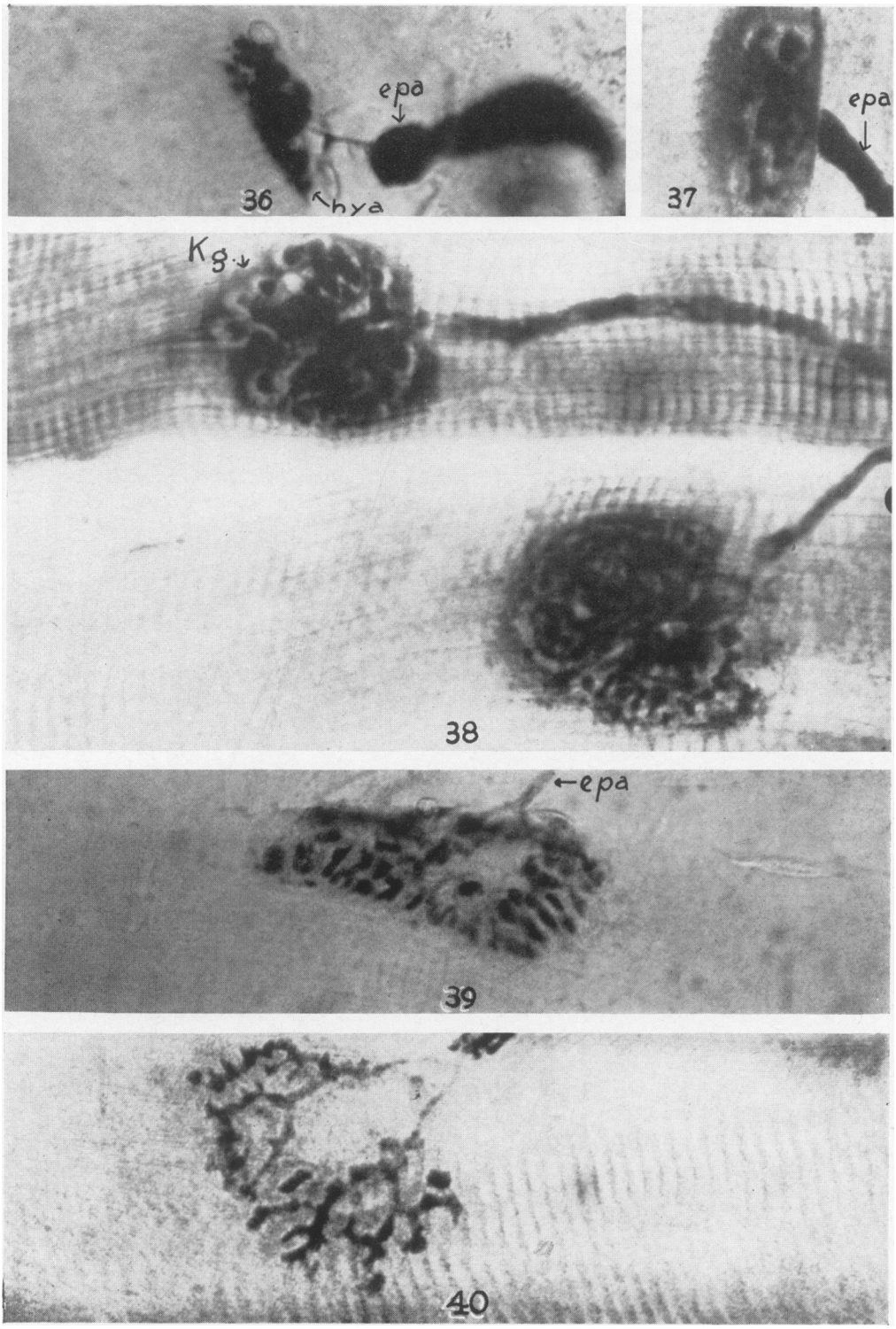

epa
36
hya
epa
37
Kg.
38
epa
39
40

PLATE 258

FIGS. 41 to 45. Motor end-plates from the gastrocnemius muscle of a rat 48 hours after the degenerative cut of the sciatic nerve. Some retracted end-plates (Fig. 41) are associated with very few Kühne's granules whereas others (Fig. 42) possess a large amount of surrounding Kühne's granules. There appears to be a cystic enlargement of both the hypolemmal (Fig. 41) and epilemmal (Fig. 42) axons in some places. These cystic enlargements contain materials that have a strong affinity for gold. In other locations of the same muscle (Figs. 43, 44, and 45) there is an expansion of the hypolemmal axons of the motor end-plate. This appears to be accomplished by an ameboid extension of the hypolemmal axons into the myoplasm. In some of these expanded motor endings (Fig. 43) there is found a peripheral disposition of Kühne's granules separated from the axons in some places by a clear, halo-like area, while in others the granules are in direct continuity with the hypolemmal axons. Certain expanded motor end-plates (Fig. 44) have an increased quantity of materials with an affinity for gold. In other locations of the same muscle (Fig. 45) the extremely attenuated and narrowed ramifications of the motor end-plate are undergoing a transformation into granules. They possess a greatly decreased quantity of materials with an affinity for gold. This morphologic change appears to be due to the direct transformation of the motor nerve endings into granules, and the discharge and periodic diffusion of these granules into the myoplasm. Under abnormal overstimulation of a continuous type due to the degenerative cut of the sciatic nerve, this results in a progressive exhaustion of the motor end-plates of their materials that have an affinity for gold. There are 15 single dark cross striations related to the retracted motor end-plate (Fig. 41) and 33 duplex striations related to the expanded end-plate (Fig. 44). It is clearly evident, therefore, that the cross striations do not form constant and fixed relations with the motor end-plate. If they did, they would be mechanically greatly separated during the period of the expansion of the motor end-plate. The cross striations periodically appear and disappear depending upon capillary chemical changes in composition and concentration at the neuromuscular apparatus. This morphologic evidence is not consistent with the theory that there is a constant number of units of sarcomeres in a voluntary muscle fiber or that the *sine qua non* of striated muscle is the continuous existence of a series of so-called sarcomeres composed of ZJQJZ cross bands. These cross striations are produced by the appropriate microcapillary chemical conditions of periodic condensation and normal diffusion of substances, involving the Liesegang phenomenon. They do not depend upon constant and rigid architectural design of a static structure as taught in histology. × 850.

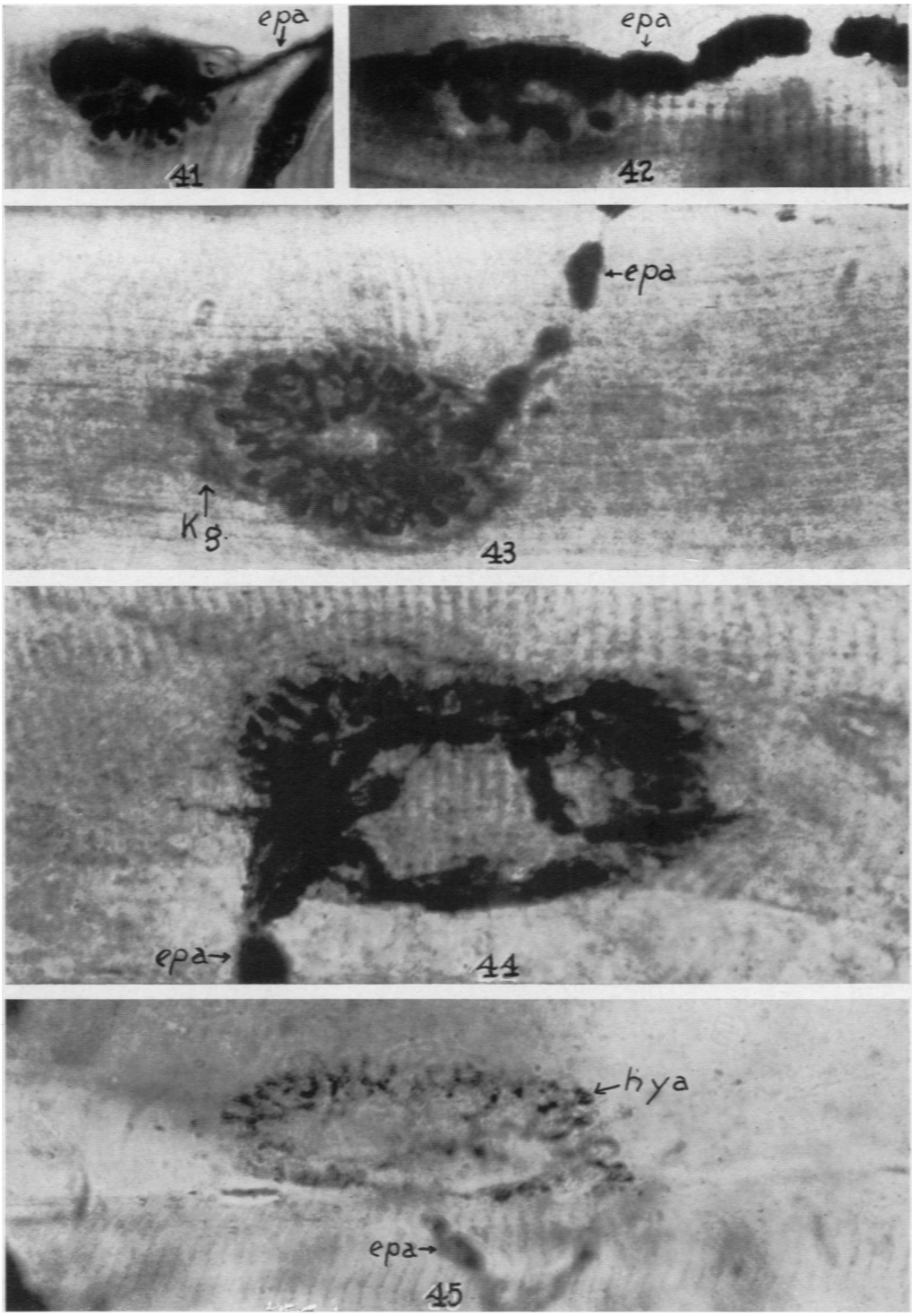
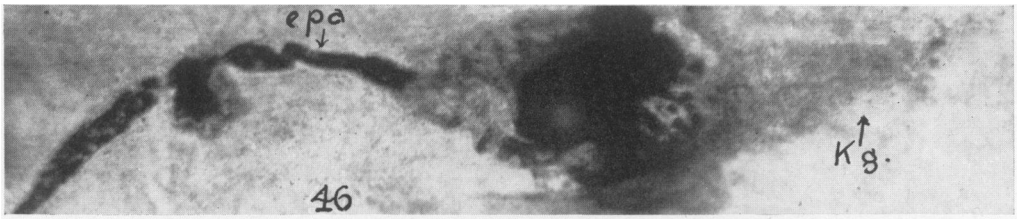

epa
41
epa
42
epa
Kg
43
epa
44
hya
epa
45

PLATE 259

FIGS. 46 to 49. Motor end-plates from the gastrocnemius muscles of rats 24 hours (Figs. 48 and 49) and 48 hours (Figs. 46 and 47), respectively, after the degenerative cut of the sciatic nerves. Some retracted end-plates (Fig. 46) contain a large central island composed of materials that have a strong affinity for gold. Some of the hypolemmal axons have undergone granular transformation and are continuous with the streaming of granules from the sole plate of Kühne out into the myoplasm. Other arborizations of the motor end-plates are enlarged and form cysts containing material with a strong affinity for gold (Fig. 46). In other locations of the same muscle practically all of the hypolemmal axons have undergone a granular transformation. These granules are directly continuous with, and are discharged into, the myoplasm (Fig. 47). Among the granules of the transformed motor end-plates will be found clear, rounded, and oval spaces which contain the nuclei of the sole plate. The epilemmal axons have an irregular contour. In other places there is an enormous enlargement of the motor end-plates by ameboid expansion (Figs. 48 and 49). These expanded terminals have relatively large, rounded, or oval thickenings, which vary in number, size, and shape. These enlargements which appear on the arborizations of the motor endings have a strong affinity for gold. There is no definite granular sole plate but there is evidence, in certain places, of the direct transformation of the end arborizations into granules which diffuse into the myoplasm. In some retracted (Fig. 46) and expanded (Figs. 48 and 49) end-plates the related cross striations of the muscle fibers are replaced by granules. It is clearly evident that more cross striations would be related to the expanded than to the retracted nerve ending if the inconstant cross striations were present. × 850.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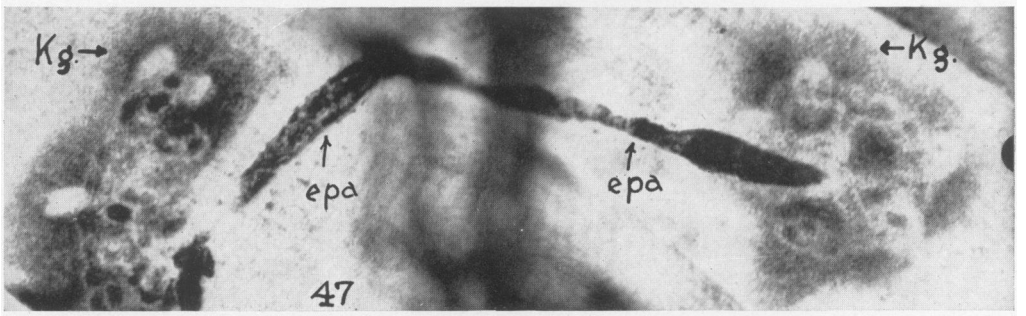
46


Kg.
Kg.
epa
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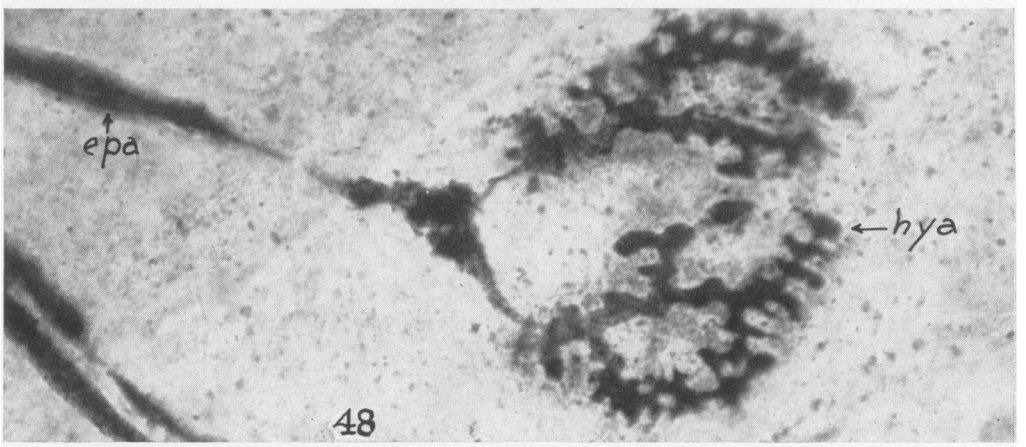
47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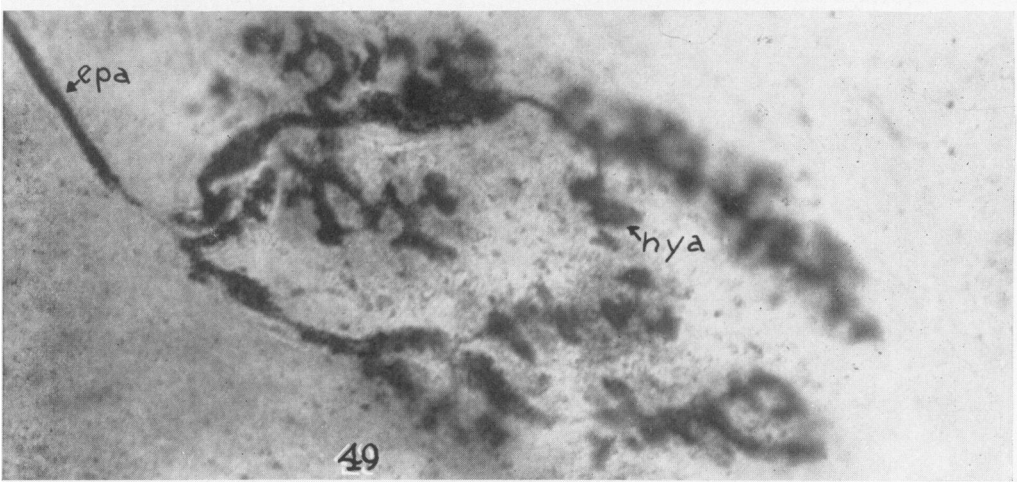
hya
48


epa
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49

PLATE 260

FIGS. 50 to 53. Motor end-plates from the gastrocnemius muscle of the rat 72 hours after the degenerative cut of the sciatic nerve. Progressive stages are demonstrated in the direct transformation of the hypolemmal axons of the motor end-plates into granules (Figs. 50 and 51). Practically all of the end arborizations of the motor end-plate (Fig. 51) have undergone granular transformation. The dark islands with a strong affinity for gold in this end-plate represent aggregations of granules discharged from the hypolemmal axons by their direct granular transformation. The clear, rounded, and oval spaces both within the region of the end-plate and out in the myoplasm are occupied by nuclei. Each nucleus has a clear, oval, or rounded space, and is surrounded by a rim of granules that varies in thickness and density. The subsarcolemmal nuclei have become larger than those within the granular sole plate. The increase in granules around the bloated nuclei of the myoplasm may be correlated with a temporary increase of the enzyme, cholinesterase, because of failure of neutralization due to diminution of nondiffusible acetylcholine at this time. The nuclei of the sole plate appear atrophic in comparison with those in the myoplasm of the same fiber. They are either fusiform, rounded, or oval, and with the gold technic form clear spaces surrounded by granules. The granular material both in the sole plate of Kühne and out into the myoplasm is contributed from two sources: the granular transformation of the hypolemmal axons of the motor end-plate, and the nuclei of the sole plate and the sarcolemmal nuclei of the muscle (Figs. 52 and 53). × 850.

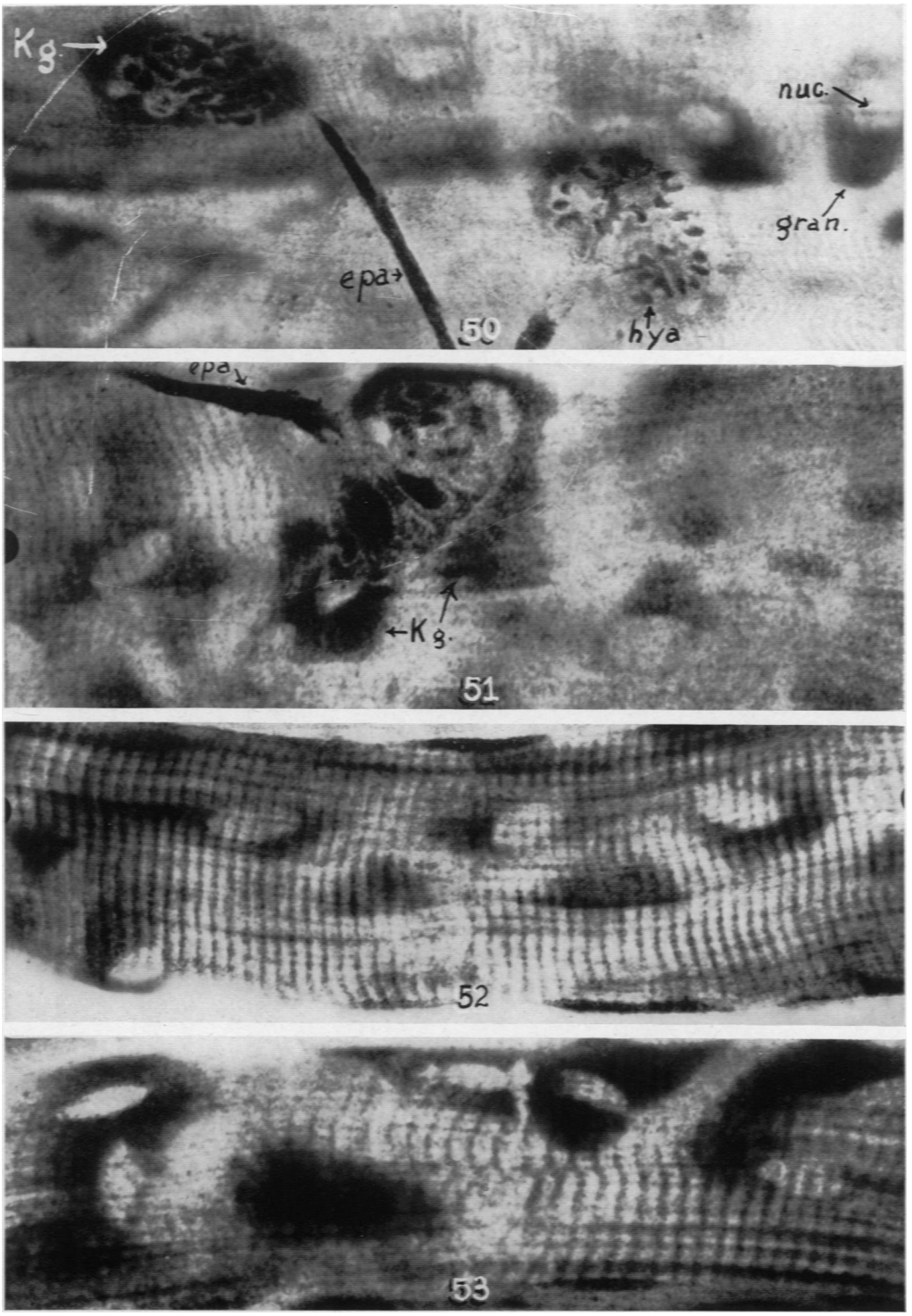

Kg.
nuc.
gran.
epa
50
hya
epa
Kg.
51
52
53

PLATE 261

FIGS. 54 to 57. Motor end-plates in the gastrocnemius muscles of rats 72 hours (Figs. 54 and 55), 5 days (Fig. 56), and 20 days (Fig. 57), respectively, after the degenerative cut of the sciatic nerves. Progressive stages are demonstrated of the direct transformation of the hypolemmal axons of the motor end-plates into granules (Figs. 54 and 55). At the fifth day, in many places, the granules of the hypolemmal axons and of the sole plate of Kühne have disappeared by diffusion into the myoplasm (Fig. 56). This depletion of the epilemmal axons of their motor end-plates does not remain constant during the process of wallerian degeneration of the neuromuscular apparatus. At periodic intervals the epilemmal axon discharges the degenerated material into the region of the degenerated motor end-plates. The nuclei of the motor end-plate and sole plate of Kühne become increasingly visible and aggregated due to the accumulation of unused granules around their periphery. Some of these granules are the products of nuclear activity and other granules in this region are discharged from the degenerating epilemmal axon into the region of the degenerated hypolemmal axons. With complete exhaustion of the degenerated material in the epilemmal axons the nuclei still remain visible by the gold technic as clear, rounded, or oval spaces surrounded by granules discharged by the nuclear activity. × 850.

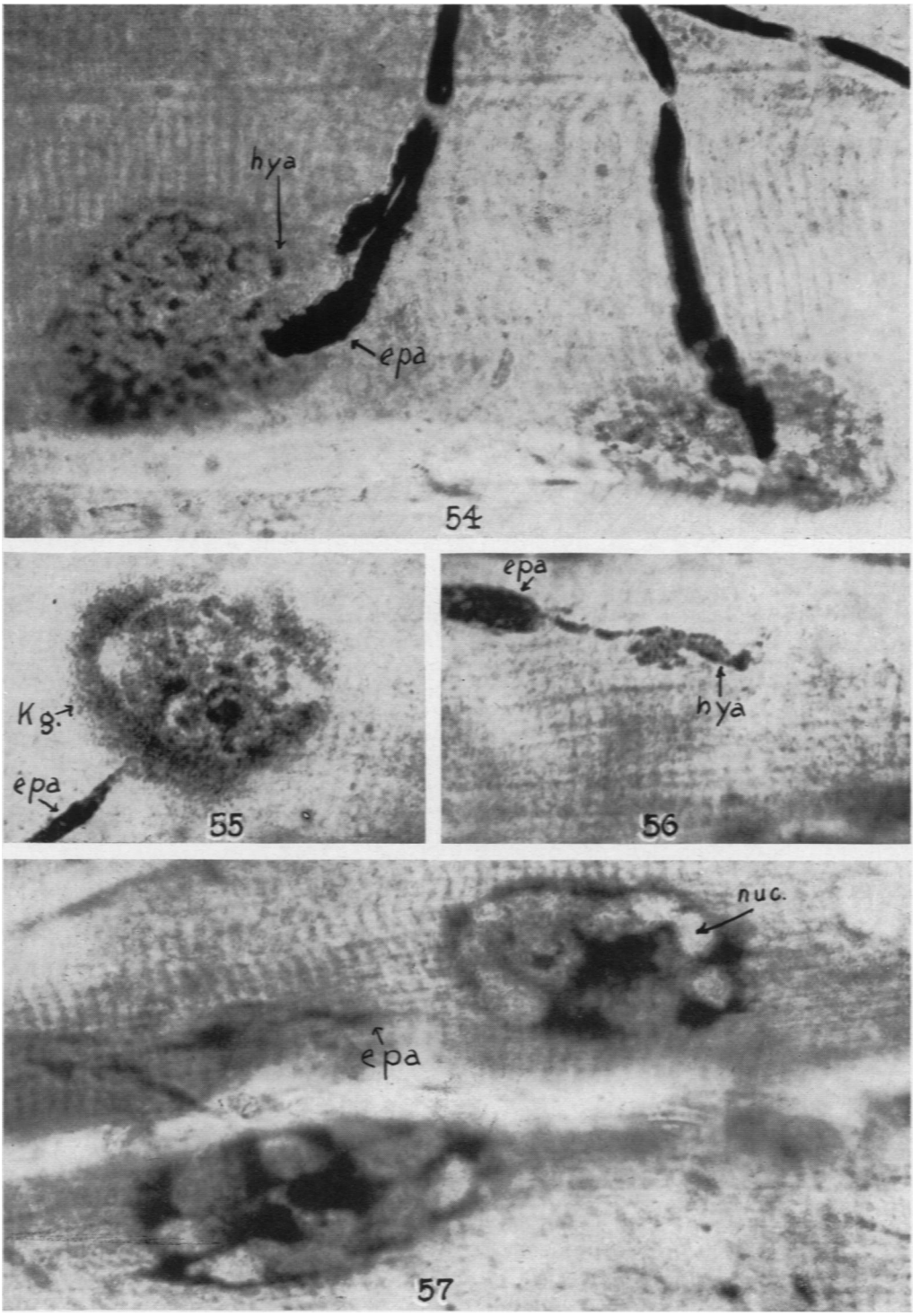
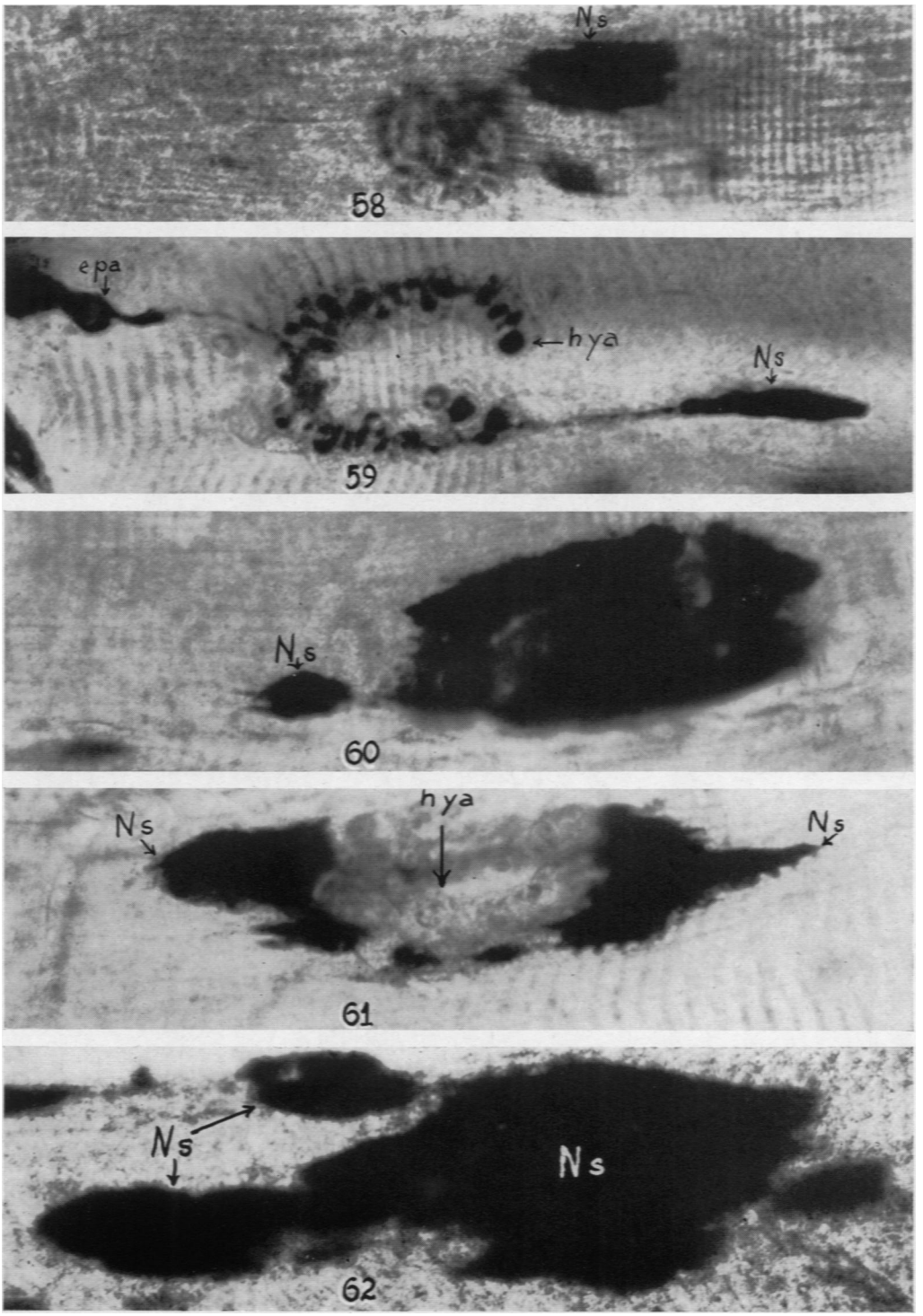

hya
epa
54
Kg.
epa
55
epa
hya
56
nuc.
epa
57

PLATE 262

FIGS. 58 to 62. Motor end-plates in the gastrocnemius muscles of rats 5 days (Figs. 58 and 59) and 10 days (Figs. 60, 61, and 62), respectively, after the degenerative cut of the sciatic nerves. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut sciatic nerve for 30 seconds at the rate of 5 per second. The degenerated axonic and myelin materials discharged into the zone of the degenerated motor end-plates have a pleomorphic arrangement: unipolar (Figs. 58 and 59), bipolar (Fig. 61), and multipolar or completely circumferential (Figs. 60 and 62). This gold-impregnated material has been discharged into and around the degenerated motor end-plates in a centrifugal direction from the epilemmal axons. There is a progressive depletion of the material with an affinity for gold from the epilemmal axons during this period of discharge into the region of the degenerated motor end-plates. There is unimpeachable evidence that the gold-impregnated material is initially in direct anatomic continuity with the structures that innervate the muscle. In one end-plate (Fig. 59) the discharged neurosome is in direct anatomic continuity with the hypolemmal axon of the motor end-plate. It forms what has been designated by some neuroanatomists as an ultraterminal nonmedullated branch of the motor end-plate that ends in the same muscle fiber in an enlarged spherical or oblong terminal. This ultraterminal branch of the motor end-plate, however, is merely the initial phase of the discharge of materials from the nerve terminal when anatomic continuity is still maintained. It is the product of the abnormal discharge of substances by abnormal stimulation from the motor ending and not a specific type of morphologic ending as claimed by some observers. × 850.


Ns
58
epa
hya
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Ns
60
Ns
hya
Ns
61
Ns
Ns
62

PLATE 263

FIGS. 63, 64, and 65. Motor end-plates in the gastrocnemius muscle of the rat 5 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. This gold-impregnated material has been discharged into and around the degenerated motor end-plates in a centrifugal direction from the epilemmal axons. The central region of the degenerated motor end-plates may be clear, surrounded by a thick rim of material that has a strong affinity for gold (Fig. 65), or there may be a dark center with radiating dark streaks like a wheel with hub and spokes, constituting the arrangement of the material discharged from the terminal of the depleted epilemmal axon (Fig. 63). To the right of one end-plate (Fig. 64) there is direct anatomic continuity between the gold-impregnated material surrounding the degenerating end-plate and the discharge of a fusiform neurosome out into the myoplasm of the muscle fiber. × 850.

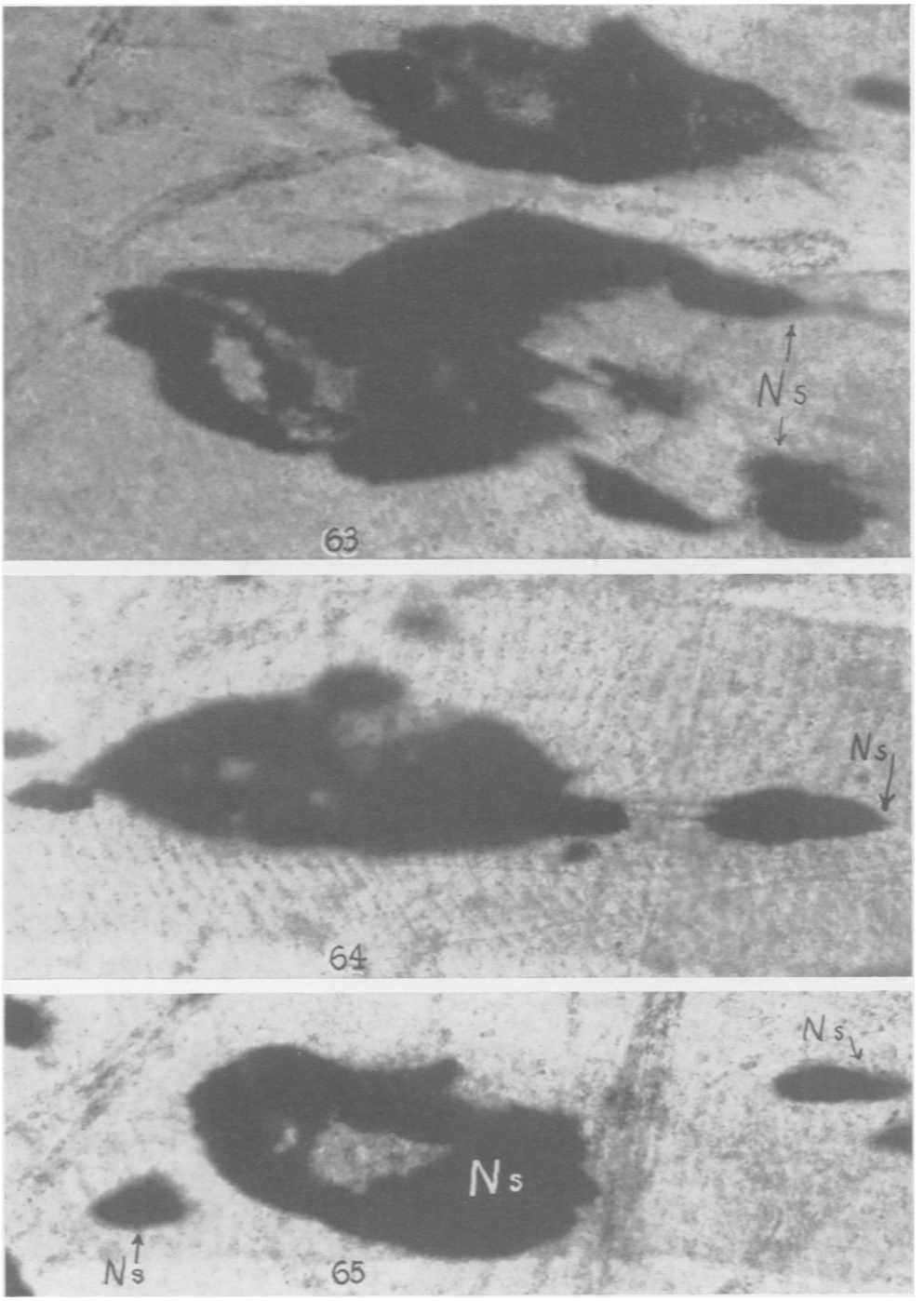
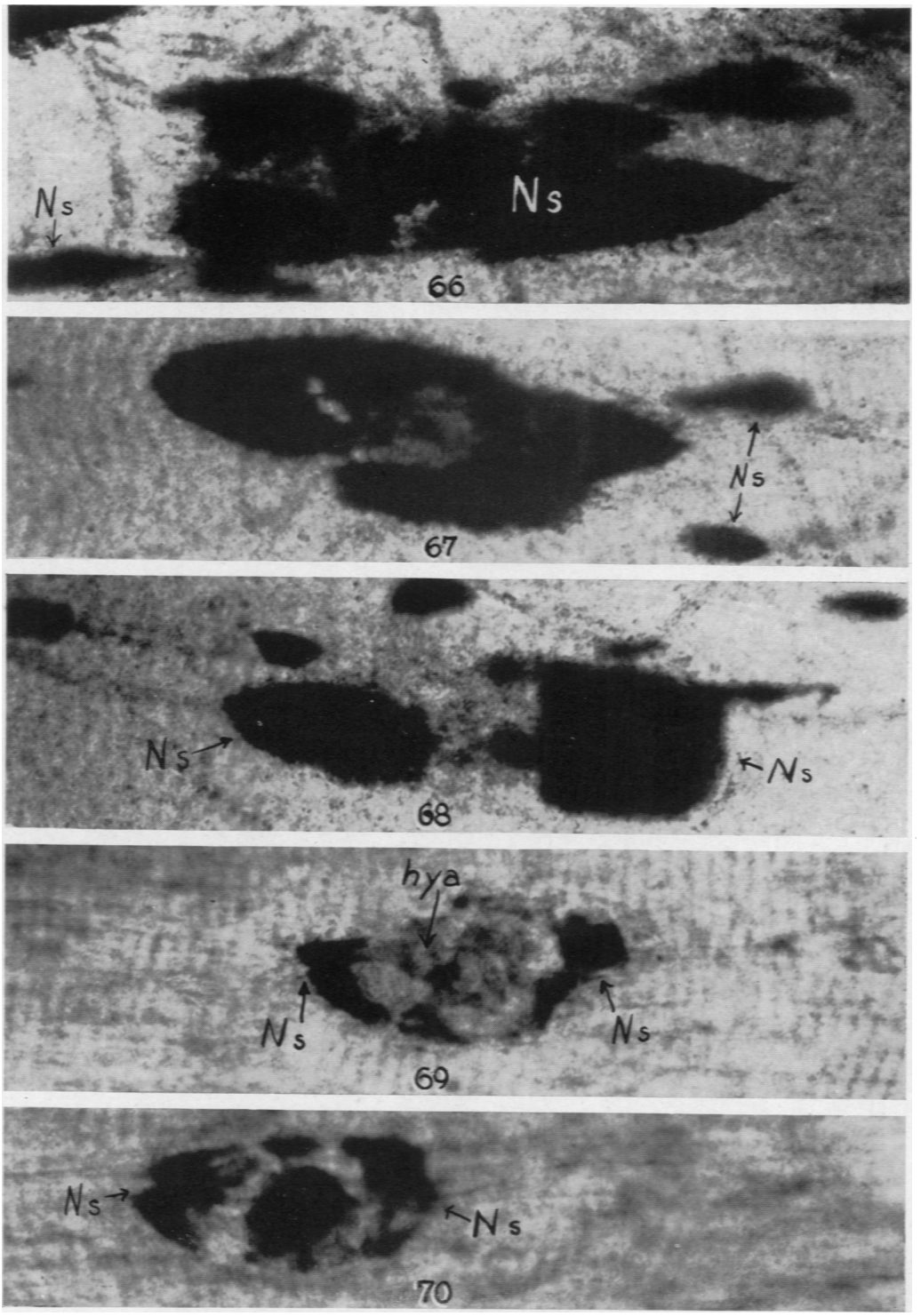

Ns
63
Ns
64
Ns
Ns
Ns
65

PLATE 264

FIGS. 66 to 70. Degenerated motor end-plates in the gastrocnemius muscle of the rat 15 days (Figs. 66 to 68) after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by a short electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. Curare and electrical stimulation were not used in the animal from which the motor end-plates were obtained (Figs. 69 and 70) 15 days after the degenerative cut of the sciatic nerve. It is clearly evident that under the conditions of the experiment there was an accelerated discharge of increased quantities of substances with an affinity for gold from the epilemmal axons into and about the regions of the degenerated motor end-plates (Figs. 66 to 68) in comparison with the condition in the end-plates after simple section of the sciatic nerve (Figs. 69 and 70). Thus, by experimental means, there may be an acceleration of the discharge of abnormal neurogenic substances from the motor nerve endings into the muscle fiber. × 850.


Ns
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hya
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Ns
70

PLATE 265

FIGS. 71 to 75. Gastrocnemius muscle fibers from an etherized rat 15 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. The discharged neurosomes (Ns) found in the myoplasm are pleomorphic and hyperchromatic for gold in comparison with the cross striations of the related muscle fibers in which they are found. These neurosomes may be fusiform (Figs. 71 and 72), irregularly oblong with one end tapering (Figs. 73 and 74), arrow-headed in shape, or like rounded droplets that vary in size (Fig. 75). The droplets form series of either single drops widely separated from one another or a closely related series composed of 2 to 10 droplets. The droplets in the middle of the series are usually larger than the terminal ones. Such series of droplets form an irregular fusiform structure separated by clear spaces. The large fusiform neurosomes may have edges that are either serrated (Fig. 71) or festooned (Fig. 72). The sharp projections arranged like saw teeth around the edges of the neurosome may or may not be in direct alignment with the dark cross striations of the muscle fiber. These neurosomes undergo a granular dissolution, and the granules become incorporated into the myoplasm, and are then aligned with the cross striations of the muscle fiber. These neurosomes are periodically discharged from the motor endplates into the muscle fiber. During degeneration they are more persistent than those produced by the artificial overstimulation of normal muscle or those produced by neurogenic shock. × 850.

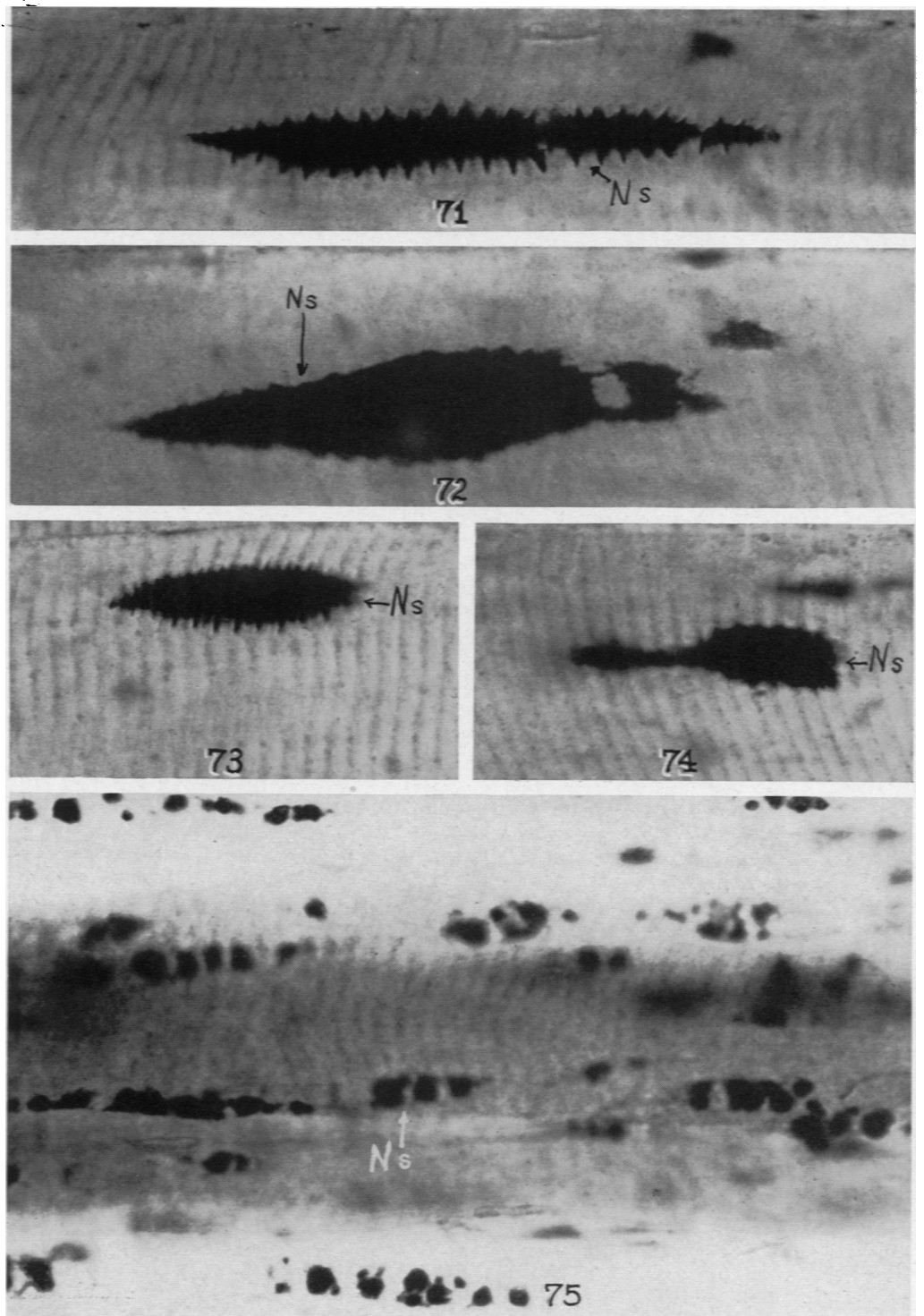
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PLATE 266

FIGS. 76, 77, and 78. Gastrocnemius muscle fibers from an etherized rat 14 days after the degenerative cut of the sciatic nerve. The intraperitoneal injection of d-tubocurarine chloride was followed by electrical stimulation of the distal segment of the cut nerve for 30 seconds at the rate of 5 per second. In a few locations giant muscle fibers (Gmf., Fig. 76) are found. Their diameter is increased three to four times above that of the closely related muscle fibers. These giant muscle fibers are densely impregnated with gold, indicating an abnormal discharge and accumulation of increased quantities of abnormal axonic material in the fiber under the conditions of the experiment. The cross striations of the giant muscle fiber are seen only at its edges because of the dense opacity produced by the discharged nervous material. There is a definite streamlined effect produced by certain large neurosomes (Ns) upon the cross striations of the muscle fiber. This streaming effect of the neurosomes discharged into the myoplasm is detected by the altered arrangement of the cross striations in the muscle fiber (Fig. 77). Certain large muscle fibers (Fig. 78) are not so densely packed with neurosomes as those of the giant muscle fibers (Fig. 76). The neurosomes are clearly evident as gold-impregnated bodies intermingled with the cross striations. In some locations these neurosomes are dense and opaque, while in other places, where they are undergoing granular liquefaction, they are light and cross-striated in appearance. Some of the muscle fibers in close proximity to those containing neurosomes do not possess these bodies in their myoplasm (Fig. 78). The muscle fibers without discharged neurosomes are more lightly impregnated with gold than those that possess them. Figure 76, × 200; Figure 77, ×400; Figure 78, × 850.

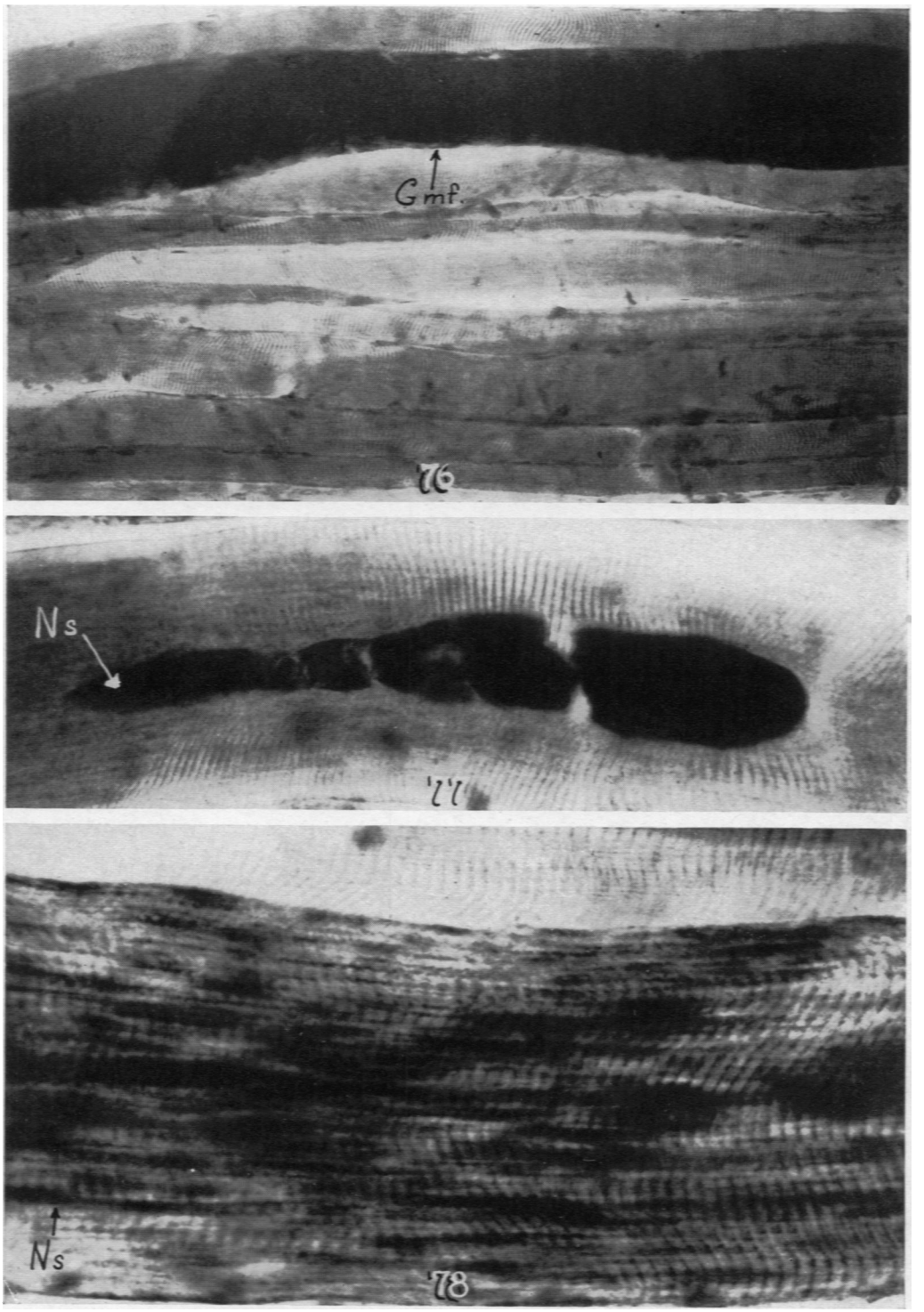
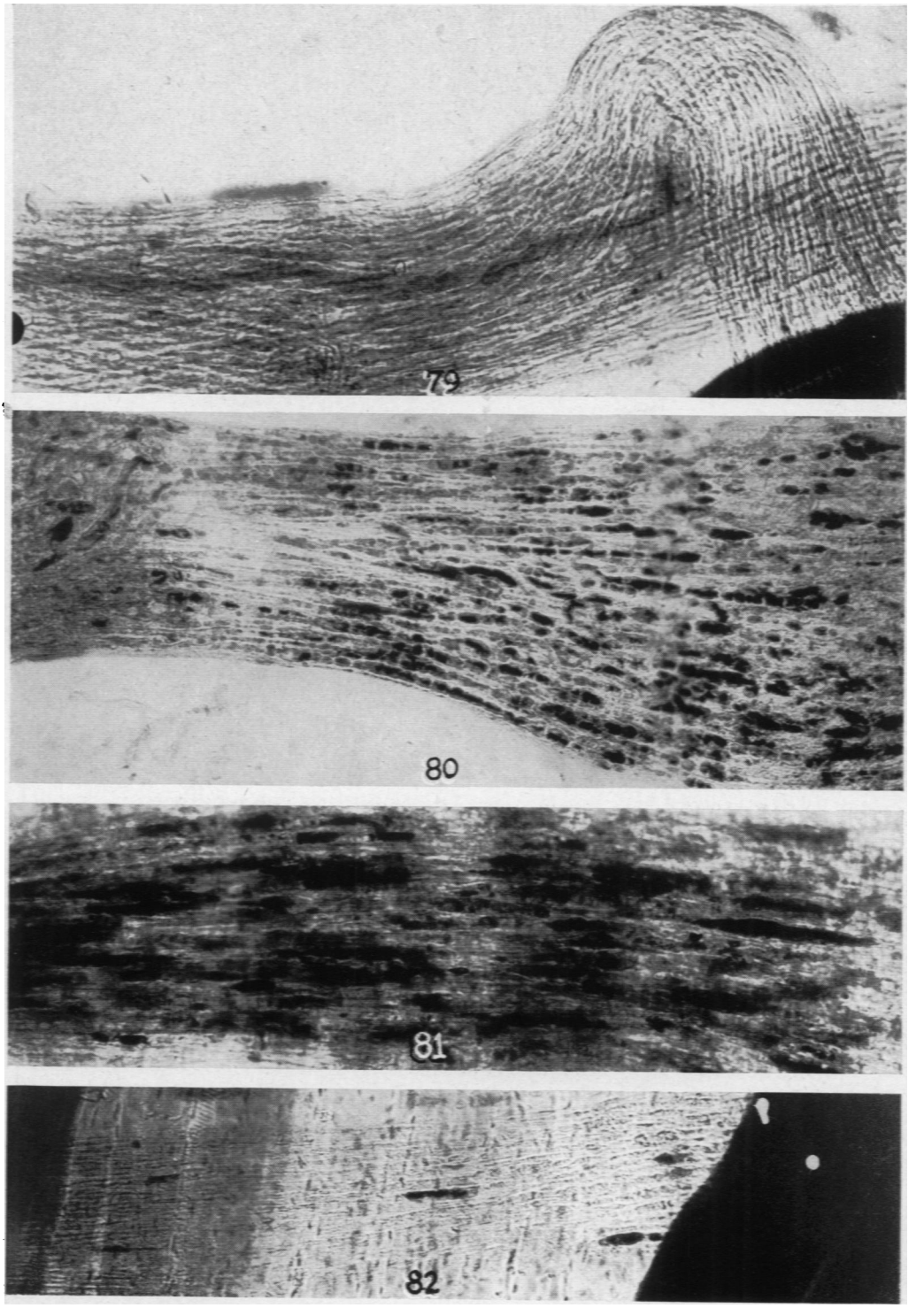

Gmf.
76
Ns
77
Ns
78

PLATE 267

FIGS. 79 to 82. Branches of the distal stump of the sciatic nerve innervating the gastrocnemius muscle 14 days after the degenerative cut of the nerve. The progressive degeneration of the nerve from its proximal to its distal end is evident. The branch of the nerve just before entrance into the gastrocnemius muscle is completely depleted of the degenerated axonic and myelin substances that have an affinity for gold (Fig. 79). At a slightly more distal point in the same nerve, the gold-impregnated material is found in variable amounts in different axis-cylinders which are fragmented into rounded, oval, or fusiform bodies with a strong affinity for gold (Figs. 80 and 81). Just before the nerve gives rise to branches that directly innervate the muscle, there is again a depletion from the axis-cylinders of material that has a strong affinity for gold (Fig. 82). Only in widely scattered areas are five bodies found that have an affinity for gold. The epilemmal axons of a cut nerve that innervate the muscle are periodically engorged and depleted of the degenerated and fragmented axonic and myelin materials. This rhythmic discharge of degenerated nervous material into the muscle continues in a progressive and periodic manner and in a centrifugal direction until all of the degenerated material is discharged centrifugally into the muscle. × 250.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