# STUDIES ON AMEBOID MOTION AND SECRETION OF MOTOR END-PLATES

## III. Experimental Histopathology of Motor End-Plates Produced by Quinine, Curare, Prostigmine, Acetylcholine, Strychnine, Tetraethyl Lead and Heat *

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The pharmacologist and physiologist have presented chemical and temporal evidence that chemical substances are transmitted from nerve endings to muscle. No morphologic findings are recorded in the literature, however, which support the chemical theory of transmission of the nerve impulse.

A few observations on the histologic changes occurring in the nerve endings under the influence of curare have been made. Kühne [1] described the living nerve endings in the muscle of lizards as having more distinct outlines after deep curare poisoning, and outlines still more distinct after slight curare poisoning and prolonged electrical stimulation of the nerves. Miura [2] stated that prolonged (18 days) curare poisoning in the frog caused a dwindling in the size of hypolemmal fibers. Herzen and Odier [3] found that curare caused the hypolemmal fibers of the frog to become varicose, and the axons of the nerve outside the muscle to become covered with fine granules, the change decreasing towards the center. These early observations practically exhaust the literature of studies on the effect of curare on the histologic structure of motor nerve endings.

Langley [4] presented a theory which presupposed the presence in the cell of one or more substances (receptive substances) which were able to receive and transmit stimuli, and capable of isolated paralysis, and also of a substance or substances concerned with the main function of the cell (contraction or secretion, or, in the case of nerve cells, the discharge of nerve impulses). Langley stated that his hypothesis demanded that the stimuli passing through the nerve cannot affect the contractile molecule except by means of the radical which combines with nicotine and curare. He concluded as follows:

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"And this seems in its turn to require that the nervous impulse should not pass from nerve to muscle by an electric discharge, but by the secretion of a special substance at the end of the nerve, a theory suggested in the first instance by Du Bois-Reymond."

Du Bois-Reymond [5] considered the possibility that the excitation of a striated muscle fiber through a nerve fiber might be due to the release of a chemical stimulant when the impulse arrived at the nerve ending. He stated the following:

"Von bekannten Naturprocessen, welche nun noch die Erregung vermitteln könnten, kommen, soviel ich sehe, in Frage nur zwei. Entweder müsste an der Grenze der contractilen Substanz eine reizende Secretion, in Gestalt etwa einer dünnen Schicht von Ammoniak oder Milchsäure oder einem anderen, den Muskel heftig erregenden Stoffe stattfinden. Oder die Wirkung müsste elektrisch sein."

Loewi [6] had shown that the inhibitory action of the vagus nerve on the heart was associated with a liberation of cardiac depressor substance which had a pharmacologic action on the heart similar to that of choline. He has shown also that the stimulation of the sympathetic nerves was associated with the liberation of an augmentor substance which resembled adrenalin in its action.

Dale and Feldberg,[7] through experimental evidence, have prepared the way for a consideration of an extension of the transmission of excitation by the liberation of acetylcholine, now long familiar for its peripheral autonomic effects, to all the synaptic and neuromuscular junctions of the peripheral nervous system, whether voluntary or autonomic, with the exception of the peripheral sympathetic fibers which, however, similarly employ an epinephrine-like substance, or sympathin. Brown, Dale and Feldberg [8] demonstrated that the highly potent acetylcholine was removed immediately by choline esterase. They demonstrated that there was a delay in the disappearance of acetylcholine when the choline esterase action was depressed by eserine.

The mechanism of action of quinine and prostigmine on motor end-plates in myotonia and myasthenia has become important recently in clinical medicine through the studies of the following: Wolf,[9] Kennedy and Wolf,[10] Kolb, Harvey and Whitehill,[11] Curschmann,[12] Weiss,[13] Pritchard,[14] Lindsley and Curnen,[15] Lindsley,[16] Harvey,[17, 18] Viets and Schwab,[19] Odom, Russel and McEachern,[20] and others. Harvey [17] made the following summary of his observations:

"1. The existing evidence suggests that myasthenia gravis and myotonia are due to abnormalities of neuromuscular transmission. If these are regarded as due to changes in the excitability of the motor end-plates of the muscles involved, the excitability would be lower in myasthenia and higher in myotonia than in the normal muscle."

"2. Quinine has a curare-like action by which it decreases the excitability of the

end-plates. This effect would account for its ability to improve myotonia and to increase the severity of the myasthenic state. Physostigmine and potassium chloride, as would be expected, both produce effects on these two conditions, which are in each case the opposite of those produced by quinine."

Rosenblueth, Lindsley and Morison[21] assumed:

"That the action of curare consists either in impairing the production of the mediating acetylcholine or in preventing the action of acetylcholine on the muscle, and that the decurarizing agents (physostigmine, adrenine and acetylcholine) antagonize or overcome these possible actions of curare. Thus, if curare made the muscle relatively impermeable to the acetylcholine liberated by the nerve impulses at the nerve endings, adrenine could make it permeable, injected acetylcholine could raise the concentration outside so that sufficient penetration would occur to activate the muscle, and physostigmine could achieve the same effect by preventing its destruction. These assumptions fit the data on hand. They must await, however, further evidence to test their validity."

These assumptions of specific chemical action were in accord with the evidence summarized by Cannon[22] which allowed the conclusion that the defatiguing effects of adrenine were not exclusively due to blood pressure changes, but that adrenine also had a specific effect.

In a partially curarized muscle, the excitability of the motor end-plate is lower, so that the response to nerve stimulation is reduced. When quinine is injected under these conditions, the curarization becomes complete. This action is also, in large part, responsible for the abolition by quinine of the quick response of the muscle to injected acetylcholine. Harvey[17] stated that:

"On the whole, however, my results with quinine strengthen my belief that the disturbances in these two diseases [myotonia and myasthenia] are at the motor end-plates. Such a conception gives a reasonably satisfactory explanation of the effects of various drugs on the two conditions; but there is, as yet, no clue to the fundamental processes responsible for the suggested alterations in end-plate excitability."

The studies on the after-potential and retention of negativity in nerve (Amberson and Downing,[23] Gasser and Erlanger,[24] Amberson, Parpart and Sanders[25]) have shown: (a) That reduced polarization of the nerve membrane is associated with hyperexcitability. Gasser and Erlanger found that the phase of supernormal excitability in recovery was present under the conditions which led to the appearance of after-potential, and that the two phenomena seemed to be inseparable. (b) That the passage of impulses reduces the membrane polarization, and can even depolarize it completely in crustacean nerve. (c) That such depolarization occurs readily in the absence of oxygen. (d) That oxygen appears to be necessary for maintenance of the polarization on which transmission of impulses depends, rather than for the actual transmission. Gasser and Erlanger assumed that while the spike

and refractory period are controlled largely by a chemical reaction, the after-potential and supernormality are dependent on the state of the plasma membrane of the nerve. To explain the shortening of the after-potential by cooling, a stabilization of this plasma membrane was proposed.

Through physiologic studies with the oscillograph and amplifier, Matthews[26] found that the sensory nerve endings in mammalian muscle spindles may undergo a rapid breakdown and resynthesis under certain conditions of motor nerve stimulation and temporary occlusion of the circulation. He stated the following:

"The rapid discharges which occur in the absence of an external stimulus bear a remarkable resemblance to those recorded by Adrian (1930) from damaged nerve fibres, and suggest that as a result of muscular activity and lack of circulation, the nerve ending breaks down and allows its nerve fibre to behave as though it were cut. One of the most notable features of this phenomenon is that the whole process is reversible, and that recovery from such catastrophic changes in the nerve ending can occur quite rapidly."

Matthews[26] furthermore claimed that excitable structures like nerve endings were supposed to generate a propagated disturbance by a breakdown of a polarized surface. The breakdown was followed by a refractory phase and gradual return of the membrane to its normal polarized condition. He gave evidence that when the circulation to a muscle was occluded and the nerve stimulated a number of endings "explode," due to the increased excitability and permeability of the membrane of the nerve ending. When the response of the nerve ending finally stopped due to the restriction of blood supply and high tensions of stretch, Matthews stated this phenomenon was due to a depletion of some substance necessary for repolarization inside the membrane or its accumulation outside, leading to a slowing of the recovery process. These effects were compared by Matthews with the pain occurring in man when work was done by muscles with impeded circulation (Lewis, Pickering and Rothschild[27]). Matthews stated that pain may be evoked by the rapid discharge from stretch receptors in the muscle spindles.

Morphologic evidence of "explosive" changes in the motor end-plates stimulated by either carbon dioxide or electricity has recently been presented.[28]

No histopathologic studies have been found in the literature of the effect of myotonia and myasthenia upon the motor end-plates in skeletal muscle. Such study by biopsy should be made after the normal range of variation in the size, shape and internal structure of motor end-plates in man has been established as a morphologic norm

for comparison with any abnormal changes that may be found. The morphologic effects of the chemical action of quinine and prostigmine, as well as other drugs, on the motor end-plates are relatively unknown.

The rapid changes in the pleomorphism of the secretory mechanism of the end-plate may be appreciated by others only if an adequate number of photomicrographs of the results of crucial but simple experiments are presented for study. By this method of presentation of the objective findings others may evaluate the evidence, repeat the experiments, draw their own conclusions. Furthermore, by this method, an extensive descriptive morphology is avoided.

The purpose of this paper, therefore, is the presentation of direct and conclusive experimental morphologic evidence, in the form of easily verified and clear-cut, untouched photomicrographs, that supports the following theses: (1) That the experimental pleomorphism of the hypolemmal axons of the motor end-plates is the result of normal and abnormal functional ameboid motion; and (2) That the experimental variation in the quantity of the granules of the sole plate of Kühne is the structural expression of the differential phases in the secretion of a chemical substance, possibly acetylcholine, from the terminal axons of the motor end-plates.

## Materials and Methods

The motor end-plates in 10 different groups of muscle (pectoralis major, rectus abdominis, intercostals, erector spinae, biceps brachii, triceps, quadriceps femoris, biceps femoris, sartorius, and gastrocnemius) in the chameleon (*Anolis caroliniensis*), weighing 3 to 5 gm., were studied in 230 animals. The chameleon was selected for this experimental study because of the large size and clear-cut components of the motor end-plates. The normal range of variation of the motor end-plates was established for the biceps femoris muscle in 10 animals of the summer chameleon, from May to October (1941 and 1942). The muscles were subjected to various histologic technics such as the Bielschowsky method of silver impregnation as modified by Boeke[29] and by the *intra vitam* methylene blue method of Ehrlich as modified by Huber.[30] The best method for the study of the continuity of the epilemmal axon, hypolemmal axon, granular sole plate of Kühne and the cross striations of the teased muscle fibers is the modified Ranvier gold chloride technic previously described by me.[28] Over 10,000 slides of teased muscle preparations have been made for this study. Boeke claims that the periterminal network revealed in certain motor end-plates by the silver method is the morphologic counterpart of the

hypothetical "receptive substance" of Langley. The granules of Kühne, however, are best revealed in teased muscle fibers after the gold chloride method has been used. Both the hypolemmal axons and the granules of Kühne are profoundly affected by the chemical experiments employed and the histologic method adopted. In fact, the gold chloride method reveals better than any other the pleomorphism of the secretory mechanism of the motor end-plates and supports the statement that these granules could fulfil the function of Langley's receptive and transmitter substance, or they could be granules of acetylcholine or some related chemical substance. Although the hypothesis is advanced that these granules of Kühne may be acetylcholine, to date there is no reliable histochemical technic that has been devised to detect this substance. Pure acetylcholine, as well as choline, produces a granular precipitate with gold chloride in the test tube and a reliable histochemical technic for the detection of acetylcholine, based upon this fact, is now being sought.

The histologic effects on the motor end-plates, in 20 animals, of intocostrin (a purified form of curare), 1 mg. per Kg. of body weight injected intraperitoneally, are, within 10 minutes, those of retraction of 50 per cent of the hypolemmal axons and increased staining capacity with gold (Figs. 1, 28, 29, 31, and 46 to 56). The changes produced by the local injection of intocostrin (1 mg. per Kg. of body weight) into the biceps femoris muscle of 20 chameleons, followed within 3 minutes by the local injection, in the same site, of quinine hydrochloride (0.5 mg. per Kg.), and, after the lapse of 3 minutes subsequent to the injection of quinine, by another intraperitoneal injection of ammonium hydroxide (0.05 cc. of 1:100), are clearly evident (Fig. 2, and Figs. 73 to 82). The animals died in spastic rigidity within 2 minutes after the injection of ammonium hydroxide. The injection into the peritoneal cavity of prostigmine (1.0 mg. per Kg.) produced expansion in 70 per cent of the motor end-plates in the biceps femoris muscle in 20 chameleons within 5 minutes, at which time the animals were decapitated, the muscles excised and immediately prepared by the gold method (Figs. 3, 11, 12 and 30).

Quinine hydrochloride (0.5 mg. per Kg.) injected into the peritoneal cavity of 20 chameleons produced retraction in 60 per cent of the motor end-plates within 10 minutes, at which time the animals were decapitated, the muscles excised and immediately subjected to the gold method (Fig. 4). Acetylcholine (0.5 cc.; 1:1000) was injected into the peritoneal cavity of 20 chameleons and in 5 minutes the animals were decapitated and the biceps femoris muscle immediately prepared by the gold method (Fig. 5).

When tetraethyl lead (0.1 cc.) was injected into the peritoneal cavity of 20 chameleons the gold-staining material of the motor end-plates was augmented in amount and in staining capacity (Figs. 6, 7, and 83 to 87). Twenty living chameleons, with skin intact, were killed by placing them in Locke's solution at 55° C. for 10 seconds and then in Locke's solution at 4° C. for 1 minute. At 55° C. the animals became rigid within 3 to 10 seconds. Stimulation of the muscle is first through the sensory nerves from the skin and then through the motor nerves of the end-plates. Shortly following this nervous transmission of the heat stimulus, there is direct transmission of heat through the skin to the muscle (Figs. 14 and 15).

Curare (1.0 mg. per Kg.) injected intraperitoneally in 20 chameleons was followed within 5 minutes by the local injection into the biceps femoris muscle of quinine hydrochloride (0.5 mg. per Kg.). This double injection, consecutively timed, was repeated within 2 hours. One hour after the last injection the animals were decapitated, the muscles excised and immediately subjected to the gold method (Figs. 16 to 27). Strychnine sulfate dissolved in Ringer's solution free of $HCO_3$ and $PO_4$ was injected into the peritoneal cavities of 20 animals in concentrations of 1:1000 every 6 hours for 48 hours and in amounts of 0.05 cc. at each injection. The animals grossly manifested increased excitability to mechanical tapping throughout the period of 48 hours. They were then killed within 2 minutes by a lethal dose of strychnine injected into the peritoneal cavity (0.5 cc., 1:100), (Figs. 34 to 35).

Prostigmine (1.0 mg. per Kg.) was injected into the peritoneal cavity of 20 chameleons 1 minute after the onset of paralysis from curare (1.0 mg. per Kg.) which had been injected locally into the biceps femoris muscle (Figs. 57 to 59, and 60 to 63). The animals were decapitated 1 minute after the injection of prostigmine. Acetylcholine (0.5 cc., 1:1000) was injected into the peritoneal cavity of 20 chameleons 1 minute after the onset of paralysis from curare (1.0 mg. per Kg.) which had been injected locally into the biceps femoris muscle (Figs. 64 to 68, and 69 to 72). The animals were decapitated within 1 minute after the injection of acetylcholine when they were in a state of strong muscular spasm.

## Results: Experimental Morphology

### 1. *The Pleomorphism of the Normal Motor End-Plates*

The length of the normal motor end-plates measured in the long axis of the muscle fibers in the biceps femoris muscle of the decapitated chameleon varied from 20 to 155 $\mu$. The breadth was from 25 to 65 $\mu$ and the thickness from 10 to 30 $\mu$. A statistical study of the frequency

in distribution gave the mean for the length of 1000 normal motor end-plates, 87.6 $\mu$, and the mean for the width, 32.9 $\mu$. The mean for the diameter of 1000 muscle fibers was 86.6 $\mu$. The extremes of variation in the diameter of the muscle fibers were from 30 to 170 $\mu$. The morphology regarding size and shape of the motor end-plates in the normal, therefore, was highly variable (Figs. 8, 9, 10 and 13). The amount of the granules in the sole plate of Kühne likewise changed. The granules were condensed in the normally retracted end-plates (Figs. 8 and 9) and they were in close relation to the hypolemmal axon. There was an increased staining capacity for gold. In the normally expanded hypolemmal axons (Figs. 10 and 13) the granules were less in amount and more dispersed. Under these conditions, the granular sole plate of Kühne had a decreased staining capacity for gold. The high coefficient of variation in the size of the motor end-plates in the different fibers of a single muscle and the bizarre shapes assumed by the hypolemmal axons have been explained previously on the basis of functional ameboidism.[28] The variations in the amount and staining capacity with gold of the granular sole plate of Kühne likewise were assumed to be due to different phases in the secretory activity of the motor end-plates inhibited by the death of the animals and the histologic technic employed.

*2. The Experimental Ameboid Retraction of Motor End-Plates*

Either curare or quinine produced, in many of the end-plates, an increased staining capacity for gold and an increased amount of granules in the sole plate of Kühne (Figs. 1, 4, 28, 29, 31, and 46 to 51). Quinine appeared to augment the action of curare by the increased retraction, accumulation of Kühne's granules, and staining capacity for gold, of the hypolemmal axon of the motor end-plates (Figs. 16 to 27). By the combined actions, occurring consecutively, of curare and quinine, these end-plates had a more definitely circumscribed border than normally and stood out clearly due to the retraction of the hypolemmal axons and localized condensation of the gold-staining substance. The combined action of these two chemicals appeared to form a dense, impermeable, precipitation membrane which inhibited the dispersion of the granules of Kühne into the protoplasm of the muscle fiber. Within 2 hours after the localized injection of curare followed by quinine, in repeated doses, there was a striking retraction into ball-like and oblong-shaped masses in 65 per cent of the motor end-plates. The mean for the length of 1000 of these motor end-plates was 49.5 $\mu$ (the mean for the normal length was 87.6 $\mu$) and for the

width, 25.4 $\mu$. The mean for the diameter of 1000 muscle fibers was 62.5 $\mu$. Under the combined actions, therefore, of curare and quinine there is a reduction in size of a great number of the motor end-plates which is in contrast to the average increase in size of the motor end-plates in the same muscle but in different animals under the influence of prostigmine. Certain elongated motor end-plates, under the influence of the chemical action of curare during the expansive phase of ameboid motion, had a broad, dense rim of the granules of Kühne. The external border of the granular sole plate of Kühne was in direct continuity with the dark cross striations of the muscle fiber or had a festoon shape influenced by these striations. In some of the expanded end-plates, dense islands of Kühne's granules were found within the end-plate as well as condensed streamers that extended for a considerable distance into the protoplasm of the muscle fiber (Figs. 46 to 51).

### 3. *The Experimental Ameboid Expansion of Motor End-Plates*

Within 5 minutes after the intraperitoneal injection of prostigmine, there was an expansion in over 70 per cent of the motor end-plates. The mean for the length of 1000 of these motor end-plates was 110.5 $\mu$ (whereas the normal was 87.6 $\mu$) and for the width, 54.6 $\mu$ in the biceps femoris muscle (Figs. 3, 11, 12 and 30). Fragmentation of the hypolemmal axon into discrete globules was evident in many of the end-plates expanded by the chemical action of prostigmine (Figs. 12 and 30). The morphologic effect of quinine (Fig. 4) was comparable to that of curare (Fig. 1), whereas the action of prostigmine (Fig. 3) was quite comparable to that of acetylcholine (Fig. 5) on the motor end-plates in the biceps femoris muscle. Quinine reduced the average size of the motor end-plates by ameboid retraction of the hypolemmal axons whereas acetylcholine increased the average size of the motor end-plates in the same muscle but in different animals by stimulating the expansive phase of the ameboid motion.

When the living chameleons were plunged into Locke's solution at 55° C. for 10 seconds, and the effect of this short duration of heat was suddenly stopped by plunging them into Locke's solution at 4° C., there was an expansion of 70 per cent of the motor end-plates. Most of these motor end-plates that had been expanded by heat had a diminution in the amount of immediately related Kühne's granules. These granules appeared to be dispersed into the muscle substance in relation to abnormal waves of heat rigor. Many of these heat rigor waves in the muscle substance were in direct relation to the expanded motor end-plates from which the waves appeared to radiate into the

muscle substance. The chemical action of the dispersed granules of Kühne appeared to influence the morphology of the muscle fiber by the replacement of the coarse cross striations by fine ones closely spaced. The stimulus of heat appeared to have a neoformative influence on the end-plate which underwent ameboid expansion coincident in time with the production of heat rigor in the muscle fiber. The reversible replacement of fine and coarse cross striations in muscle appeared to be the structural expression of the underlying reversible chemical changes of metabolism in normal motion. In abnormal heat rigor, these changes were irreversible.

Radiation of fine cross striations from the motor end-plates was not the result of the mere mechanical approximation of preformed coarse striations. If the motor end-plate had a constant relationship to a fixed number of preformed mechanically static membranes or units called sarcomeres, one would expect to find these striations more widely separated when the end-plate expanded and more closely approximated when the end-plate retracted. The reverse of this, however, appeared to be the case, for the coarse, widely spaced striations occurred in the small retracted end-plate, whilst fine, closely spaced striations occurred in the expanded motor end-plate. This evidence supports the statement that the coarse, widely spaced cross striations were related to the relaxed state of the muscle fiber and the fine, closely spaced ones to the contracted state of the fiber or to the condition of contracture.

### *4. The Experimental Material Exhaustion of Motor End-Plates*

When sublethal doses of strychnine sulfate were injected into the peritoneal cavity every 6 hours, for a period of 48 hours, there was a gradual decrease in size of the motor end-plates in the biceps femoris muscle (Figs. 32, 33, and 34 to 45). This prolonged stimulation and chemical fatigue resulted in a depletion of gold-staining substance in both the epilemmal and hypolemmal axons. There was, likewise, a gradual depletion to the point of complete absence of the granular sole plate of Kühne in these exhausted motor end-plates. In fact, the exhausted motor end-plates assumed the morphology of the grape-like terminals (*terminaisons en grappe*) rather than that of the plate-like terminals (*terminaisons en plaque*). The mean for the length of 1000 of these exhausted motor end-plates was 28.4 $\mu$ (whereas the normal was 87.6 $\mu$). The extremes for the length were from 9.5 $\mu$ to 88.4 $\mu$. Wilkinson[31] thought that the grape-like motor end-plates were immature ones. These grape-like terminals are, however, either the result of exhaustion or quick depletion due to extreme activity and dispersion of granules.

The ramifications and reticulations of the end-plate were highly variable. In some plates there was complete globular fragmentation of the hypolemmal axons. Many of these axonic droplets were discrete and discontinuous with the main group of branches of the hypolemmal axons (Figs. 33 and 38 to 45). The grape-like exhausted motor end-plates were found in 20 per cent of the muscle fibers (Figs. 43 to 45) whereas in the muscle fibers of the normal biceps femoris muscle these depleted end-plates were found in less than 0.25 per cent of the muscle fibers. The striking contrast in size of the epilemmal and hypolemmal axons, and granular sole plate of Kühne, in the nonexhausted motor end-plates and in the exhausted ones (Figs. 43 to 45) is evident. The substantial depletion of the motor end-plates by prolonged stimulation with strychnine was comparable to that produced by the prolonged effect of anoxia caused by carbon dioxide, sodium cyanide, and fatigue due to extensive muscular exercise of the living chameleons.

### 5. *The Experimental Formation of Acute Retention Cysts in Motor End-Plates*

When curare and quinine had produced an effective block to neuromuscular transmission, the injection of ammonium hydroxide augmented the gold-staining material in both the epilemmal and hypolemmal axons because of the production of acute retention cysts. Coincident with the formation of these dilated cysts filled with the gold-staining material, there was a diminution in the size of the pseudopod-like hypolemmal axons and in the amount of related granules of Kühne. In fact, around the cysts of the hypolemmal axons there was a complete absence of the granules of Kühne (Figs. 2, 73 to 82). There appeared to be a thickening of the membrane enclosing the dilated cysts in both the hypolemmal and epilemmal axons (Figs. 74 to 77, and 81). By these chemical means, therefore, a mechanical block appeared to be formed to the secretion of the transmitter substance. This was roughly analogous to the damming back of the flowing water in a stream resulting in the formation of a lake. These axonic lakes constitute additional evidence for the thesis that the motor end-plate is a microscopic gland of internal secretion which delivers its chemical product directly into the striated muscle fiber.

### 6. *The Experimental Massive Transmission of Material to the Motor End-Plates*

There was a massive conduction of axonic nerve substance into the motor end-plate following the intraperitoneal injection of tetraethyl lead. The animal went into a state of spastic rigidity within 10 sec-

onds. There was a deformation of the hypolemmal axons and an augmentation of gold-staining material in 25 per cent of the motor end-plates (Figs. 6, 7, and 83 to 87). There was a radiation of gold-staining material and a distortion of the cross striations of the muscle substance at the terminals of the abnormal end-plates. The effect of this chemical appeared to be to augment the amount of gold-staining material in the end-plate as well as to inhibit the normal dispersal of this substance. This experimental pathology appeared to be the result of a sudden "explosion" by the rapid transfer of abnormal amounts of the transmitter substance to the end-plate.

### 7. *The Experimental Correlation of Ameboid Motion and Secretion of Granules of Motor End-Plates*

The production of the expansive phase of ameboid motion of the hypolemmal axons by either prostigmine or acetylcholine, applied 1 minute subsequent to the failure of neuromuscular transmission induced by curare, resulted in clear-cut morphologic changes (Figs. 52 to 72).

There was an antagonism between the stimulus of expansion produced by prostigmine and acetylcholine and that of retraction produced by intocostrin, which resulted in a clear-cut demonstration of the morphology of the expanded hypolemmal axons surrounded by an abnormal increase in the quantity of the granules of Kühne. In some examples there was a more gradual dispersion of the granules of Kühne into the cross-striated substance of the muscle fiber. There was a direct transformation, in some places, of the hypolemmal axons into granules of Kühne (Figs. 55 to 59, and 62). Agglutinated streamers of the granules of Kühne were in many places cross-striated, augmented in staining capacity for gold, and found extending from the terminals of the motor end-plate into the protoplasm of the muscle fiber. The quantity of granules and the morphology of the sole plate of Kühne, therefore, were not constant, fixed and preformed structures surrounding the hypolemmal axons. The transformation of the hypolemmal axons into granules of Kühne was made evident by the stimulus of prostigmine and acetylcholine to ameboid expansion of the hypolemmal axons and by the inhibition to the dispersal of these granules of Kühne by the chemical action of intocostrin. In many places, the hypolemmal axon underwent globular fragmentation and granulation.

This relationship of ameboid motion and granular secretion of the motor end-plates was comparable to those observations made by Korschelt[32] on the ova and secreting cells of insects, by Heidenhain[33] on

the nuclei of the cells of the salivary glands, and by Huie[34] on the profound changes in the nuclei during increased activity of the secreting cells of the insect-eating marsh plant, Drosera, when the latter is fed egg albumin. The comparative histologist is familiar, therefore, with this relationship of ameboid motion and secretion.

Korschelt[32] studied chiefly the ova and secreting cells of insects. In the egg-tubes of the ovaries of *Dytiscus marginalis*, a large water-beetle, the ova are arranged in succession like a string of pearls and separated from one another by a so-called nutrient chamber. This chamber consists of cells which produce and give off nutrient material to the ova. The behavior and the position of the nuclei of the ova toward this nutrient material is very characteristic. From the chamber the nutrient material extends into the ovum in the form of a granular mass and there disposes itself in such a manner that it comes into very close contact with the nucleus. But the most interesting fact is that which makes the activity of the nucleus toward the nutrient material apparent; namely, that the former sends pointed, pseudopodium-like processes into the granular mass where the latter touches it, and only in this direction, and thus very greatly increases the surface at the place of contact with the nutrient material. If the latter completely surrounds the nucleus, the whole surface shows pseudopodium-like processes. Korschelt described a similar phenomenon, especially in regard to the nucleus, in a whole series of arthropod and coelenterate ova. The interesting behavior of the nuclei in secreting cells toward the secreted substances forms a counterpart to these phenomena of the ingestion of substance on the part of the nucleus. Here certain relations exist toward the substances produced, which are wholly analogous to those existing in ova toward ingested substances. In the eggs of certain water-bugs, Nepa and Ranatra, there occur peculiar chitinous appendages, the so-called egg-rays, which are formed by cells especially differentiated for this purpose. These cells, of which each two unite into a single cell with two nuclei, termed by Korschelt a double cell, assume a considerable size and secrete within their body a mass of chitin. The behavior of the two nuclei in this process is very characteristic. They send out toward the middle, where the secretion is taking place, numerous, frequently branched, pseudopodium-like processes, which increase the nuclear surface upon this side very considerably, while the rest of the surface remains smooth. Such enlargements of the surface of nuclei are widespread in the secreting cells of insects and show that the exchange of substance between protoplasm and nucleus in secretion must be very active.

Baum [35] found that the nuclei of resting gland cells stained much more deeply with nuclear stains than the nuclei of gland cells that had secreted strongly. This was a histologic sign that the chromatic nuclein was destroyed in secretion. In this study on motor end-plates, the differential staining capacity of the components of the motor end-plate for gold may be, likewise, a histologic sign of variations or differential phases in the secretory activity of the gold-staining granules of Kühne of the motor end-plates.

## Summary

The experimental pleomorphism of the hypolemmal axons of the motor end-plates is a result of normal and abnormal functional ameboid motion. The experimental variation in the quantity of the granules in the sole plate of Kühne is the structural expression of the differential phases in the secretion of the chemical substance, possibly acetylcholine, from the terminal axons of the motor end-plates. There is a correlation between ameboid motion and the secretion of granules which had been designated, collectively, in the past as the granular sole plate of Kühne. These aurophilic granules of Kühne may be increased in quantity by chemical reagents, such as curare and quinine, which inhibit the adequate dispersal and dissolution of the secreted granules into the protoplasm of the muscle fiber. Neuromuscular transmission is blocked by the action of curare and quinine on the motor end-plates through ameboid retraction of hypolemmal axons and the formation of a dense and circumscribed precipitation membrane composed of the granules of Kühne. The aurophilic epilemmal and hypolemmal axons undergo acute dilatations through the sudden formation of retention cysts produced by the chemical action of curare and quinine followed by that of ammonium hydroxide, which excites substantial transmission to the end-plates. The secreted granules of Kühne may be decreased in quantity to the level of complete depletion by prolonged chemical stimulation such as that produced by strychnine, sodium cyanide, carbon dioxide, and exhausting muscular exercise. Under conditions of exhaustion, in addition to the absence of the granules of Kühne, there is an abnormal decrease in the size of the epilemmal and hypolemmal axons. The terminal expansions of the hypolemmal axons of the end-plate may undergo direct transformation into the secreted granules of Kühne, without the presence of the intervening clear space, by sudden stimulation with either prostigmine or acetylcholine after the end-plate has been blocked by the local action of curare. Heat produces a sudden expansion of the end-plate and a

dispersal of the granules of Kühne that together produce perturbations in the pattern of the cross striations of the muscle fiber. These waves of contracture or rigor appear to be produced by the dispersal of some chemical substance transmitted by the motor end-plate. Tetraethyl lead produces a sudden and explosive transmission of an abnormal quantity of aggregates of aurophilic granules which results in massive radiations, distortion and increased staining capacity of the end-plates for gold. There are, likewise, abnormal distortions of the related cross striations of the muscle fiber to these abnormal end-plates produced by the chemical action of tetraethyl lead. The hypolemmal axons of the end-plate and the related granules of Kühne and cross striations of the muscle fiber are not preformed, static, and fixed in morphology. Their size, shape and internal structure are correlated with physiologic and pathologic secretory activities of neuromuscular transmission.

I wish to express deep gratitude to Mr. Leo Massopust, Director of the Department of Art and Photography, for aid with the photomicrographs; to Dr. G. Kasten Tallmadge, Assistant Professor of Anatomy, for reading the manuscript; to Messrs. John Schmitz, James Keyes, Robert Jeub, Joseph Hamel and Eugene Haushalter for technical aid in the teasing of muscle and nerve plates; and finally to Dr. H. Sidney Newcomer, Medical Department, E. R. Squibb and Sons, for the intocostrin used in these experiments.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

The photomicrographs of Plates 58 to 75 are from the teased whole muscle fibers (biceps femoris) and motor end-plates of the summer (May to October) chameleon (*Anolis caroliniensis*). These teased preparations of motor end-plates in skeletal muscle were previously prepared by the gold chloride technic. The photographs were prepared as direct contact prints from the negatives which were photographed under the microscope and not subjected to subsequent enlargement. In this manner, these photographs are easily comparable with those of the white rat previously published.[28] In the plates, "epa." means epilemmal axon and "hya.," hypolemmal axon. There has been no retouching of either negatives or prints.

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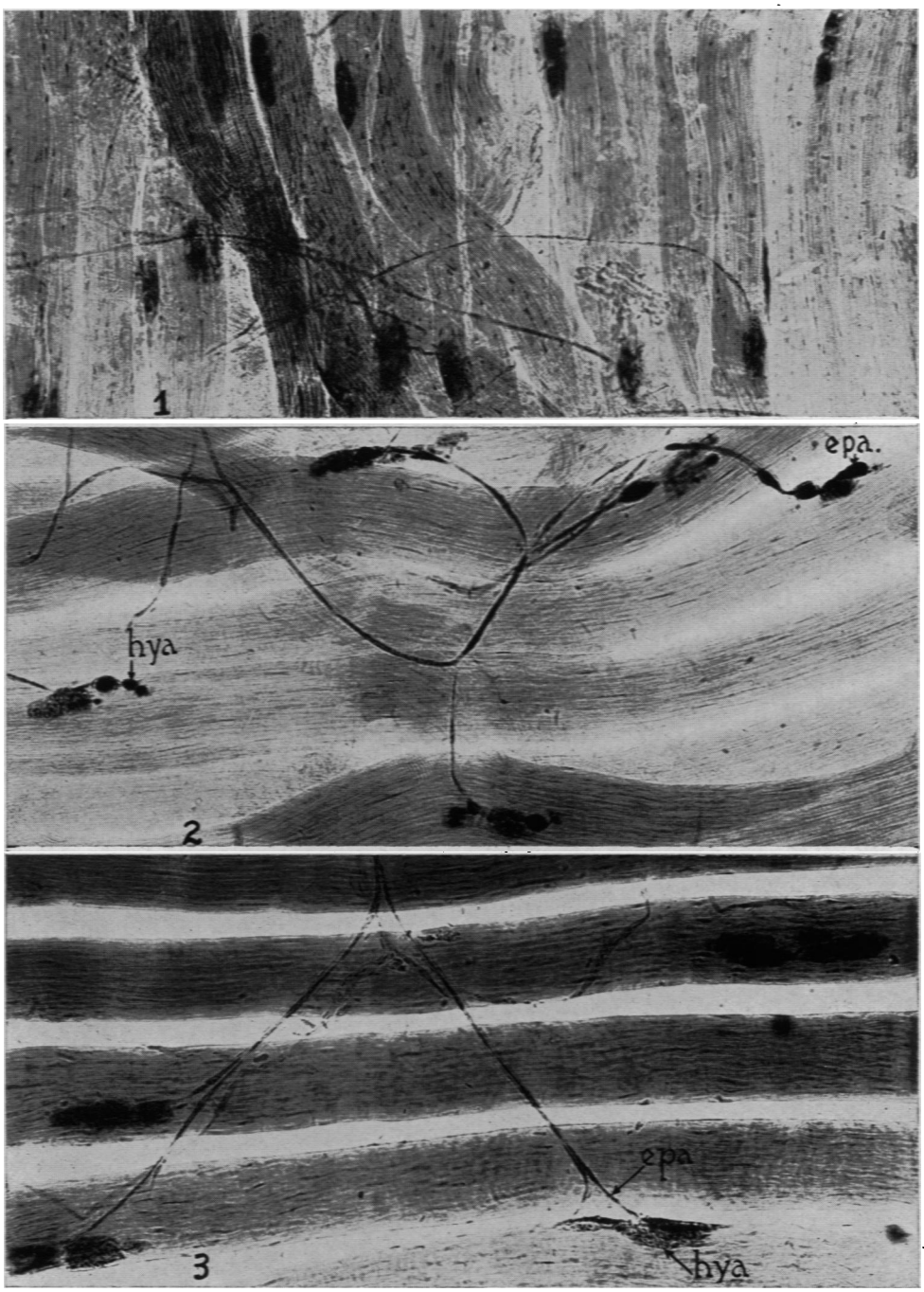
### PLATE 58

FIG. 1. Sprays of medullated nerve fibers and motor end-plates which are retracted as a result of the intraperitoneal injection of intocostrin (curare). By centripetal retraction of ameboid motion the end-plates vary in size from 24 to 85 $\mu$ measured in the long axis of the muscle fiber. The axis cylinders vary from 1 to 12 $\mu$ in diameter. × 125.

FIG. 2. Sprays of medullated nerve fibers and motor end-plates with acute cystic retention of the gold-staining axonic substance in both the epilemmal (epa.) and in parts of the hypolemmal (hya.) axons. This acute cystic retention was produced by first injecting intocostrin locally into the biceps femoris muscle and 3 minutes later injecting locally quinine hydrochloride. Three minutes after the last injection, ammonium hydroxide was injected into the peritoneal cavity. These relatively simultaneous actions of a chemical block to the secretion of the transmitter substance and the stimulating action centrally by the ammonium hydroxide resulted in the sudden accumulation of the axonic liquid substance into dilated cysts, 10 to 40 $\mu$ in diameter, of the axis cylinder both in the epilemmal and hypolemmal axons. The axis cylinder varies from 1 to 40 $\mu$ in diameter. × 125.

FIG. 3. Sprays of medullated nerve fibers and motor end-plates expanded by the action of prostigmine injected into the peritoneal cavity. By the centrifugal expansion of ameboid motion the surface area of the motor end-plates under the action of prostigmine is greatly increased. This morphologic change aids in the dissemination of the transmitter substance from its point of origin in the end-plate and its dispersion throughout the muscle fiber. The axis cylinder varies from 1 to 18 $\mu$ in diameter. The motor end-plates vary from 40 to 185 $\mu$ in length measured in the long axis of the muscle fiber. The value of the gold chloride technic in preserving the anatomic continuity of the epilemmal axon, hypolemmal axon, ramifications of the terminal axons, the granules of Kühne and the muscle striations is demonstrated not only in Plate 58 but in all of the subsequent illustrations. × 125.

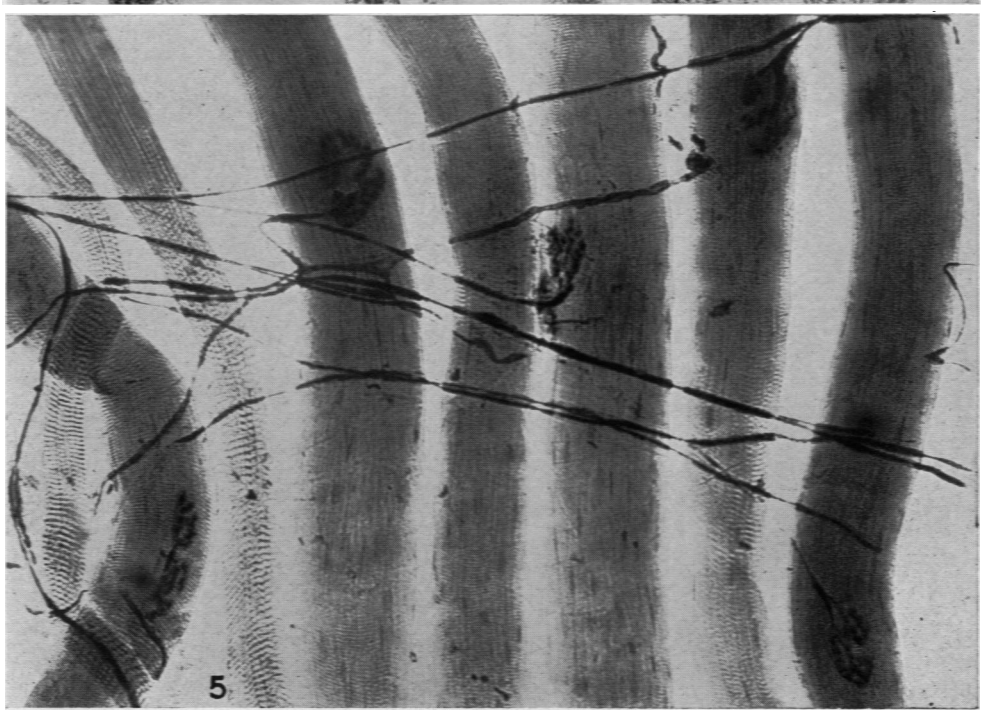



Carey Motion and Secretion of Motor End-Plates

PLATE 59

FIG. 4. Retraction of motor end-plates under the influence of quinine sulfate injected intraperitoneally. The motor end-plates vary from 35 to 85 $\mu$ in length. measured in the long axis of the muscle fiber. These end-plates have an intense affinity for gold and have in many places sharply defined, well circumscribed borders. Many of the muscle fibers are narrow in diameter, granular and coarsely cross-striated. The axis cylinders vary from 1 to 18 $\mu$ in diameter. $\times$ 125.

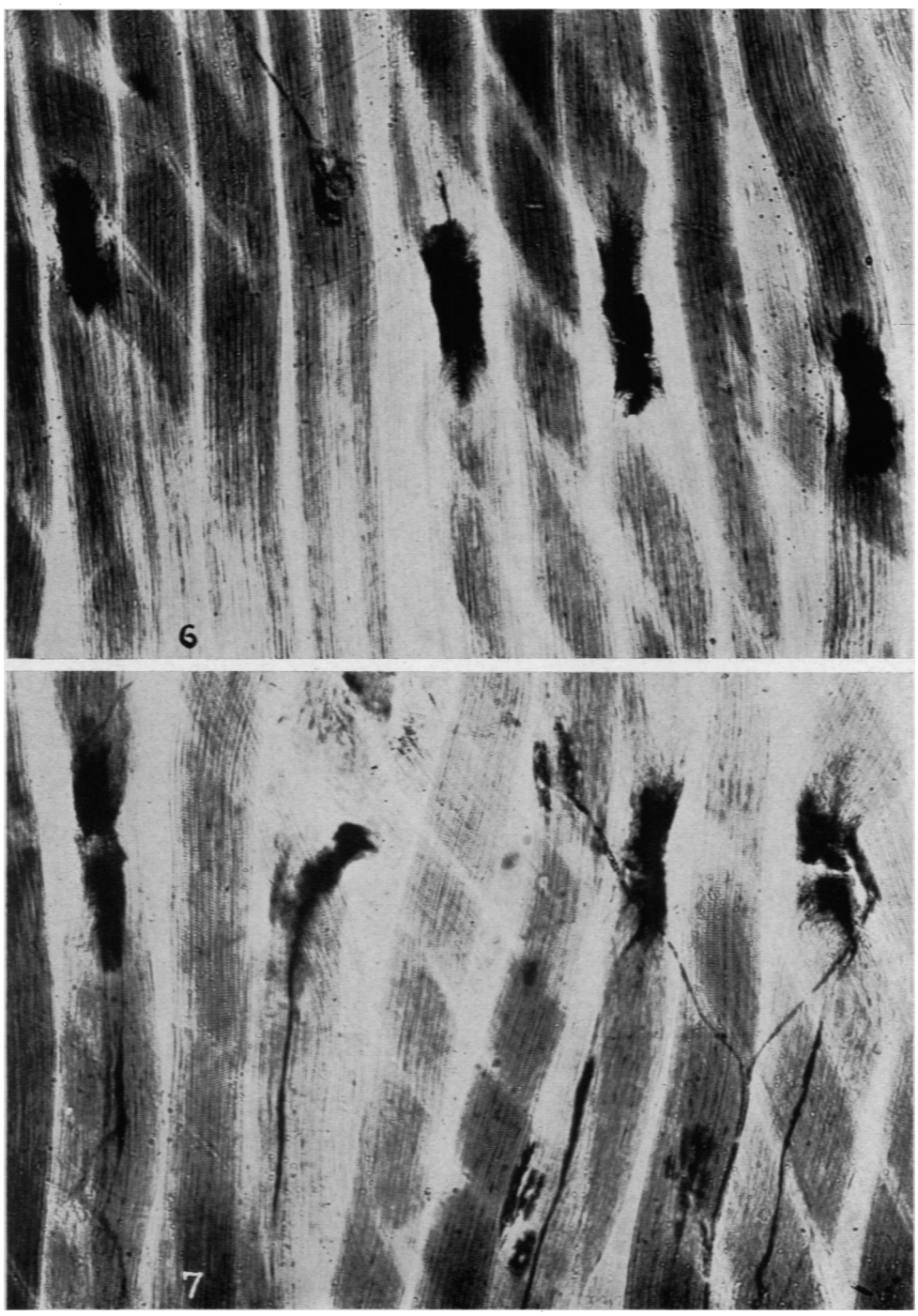
FIG. 5. Sprays of medullated nerve fibers and motor end-plates in the state of centrifugal ameboid expansion produced by the intraperitoneal injection of acetylcholine. The axis cylinders vary from 1 to 22 $\mu$ in diameter. The motor end-plates with multiple ameboid ramifications vary from 50 to 205 $\mu$ in length, measured in the longitudinal axis of the muscle fiber. Most of the muscle fibers are wide in diameter and have fine, closely spaced cross striations. Two of the fibers toward the left (Fig. 5) are narrow and coarsely cross striated, with a zone of transition into fine cross striations at the upper end of each fiber. These stimulated motor end-plates have a greater number of terminal dichotomous divisions than the retracted ones under the influence of either intocostrin or quinine (Figs. 1 and 4). Acetylcholine excites neurocladism or the production of new ameboid projections of the end-plate. $\times$ 125.


Carey Motion and Secretion of Motor End-Plates

## PLATE 60

FIGS. 6 and 7. The motor end-plates are distorted with enormous accumulations of the axonic substance which has an intense affinity for gold. This effect is produced by the intraperitoneal injection of tetraethyl lead. There are radiation rays extending from the terminals of these distorted end-plates, which plates vary in length from 80 to 285 $\mu$. In the right half of Figure 7, one axis cylinder has three branches, two of which terminate in expanded plates and one in a distorted end-plate intensely stained with gold chloride. The epilemmal axons vary from 1 to 25 $\mu$ in diameter. $\times$ 125.

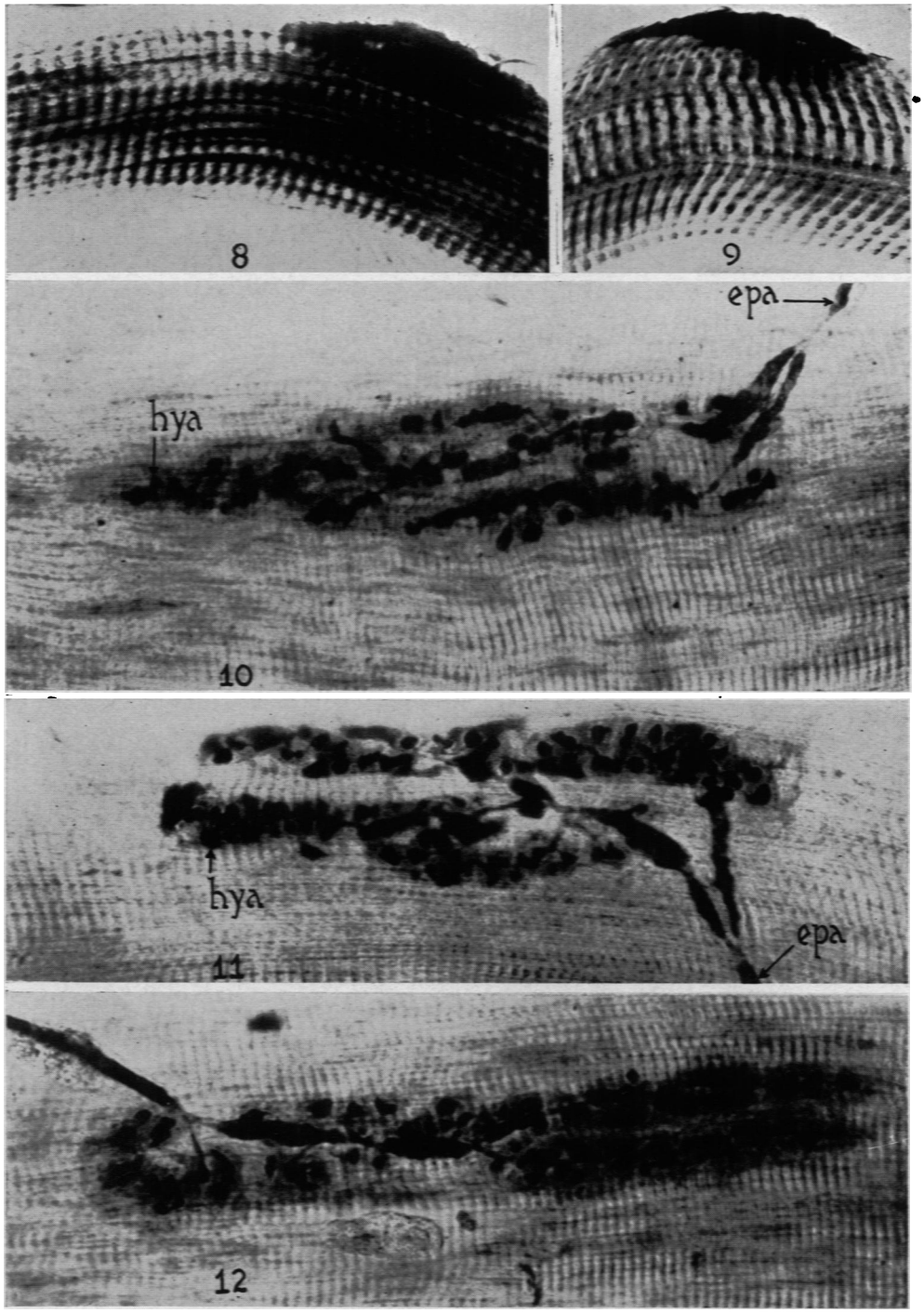



Carey Motion and Secretion of Motor End-Plates

## PLATE 61

FIGS. 8 to 10. Normal teased biceps femoris muscle fibers of the chameleon. The narrow muscle fibers with coarse cross striations (Figs. 8 and 9) have retracted motor end-plates with strong affinity for gold chloride. The wider muscle fiber in the same muscle (Fig. 10) has an expanded end-plate with multiple ramifications to which are related fine, closely spaced, cross striations. The expanded nerve plates in the active muscle fiber have variable degrees of ameboid extensions of the terminal arborizations of the axon. The hypolemmal (hya.) axons in some of the end-plates terminate in rounded or oblong swellings peripherad to which there may be a light halo-like space around which are found the granules of Kühne. The quantity of the granules in the sole plate of Kühne is highly variable, from the point of complete absence of granules to that of the accumulation of a considerable quantity of the granular material. The granules of Kühne, therefore, are a highly inconstant part of the morphology of the end-plate even in relatively normal muscle. The granules of Kühne are in direct continuity with the dark, anisotropic cross striations of the muscle fiber. The retracted motor end-plates (Figs. 8 and 9) vary from 50 to 60 $\mu$ in length and have, respectively, 18 and 16 related dark bands of the cross striations. The elongated end-plate (Fig. 10) is 150 $\mu$ in length and has 71 related dark bands of the cross striations. $\times$ 750.

FIGS. 11 and 12. Expanded motor end-plates influenced by the intraperitoneal injection of prostigmine. Another type of expanded end-plate (Fig. 30) is likewise characteristic of the effect of prostigmine, which appears to influence the centrifugal phase of expansion of ameboid motion as well as fragmentation of the hypolemmal axons into droplets of gold-staining globules. Although the granular sole of Kühne is present in many places, it appears to be undergoing rapid dispersal throughout the muscle fiber. The granules are not accumulated into densely stained islands and membranes as they are under the influence of intocostrin (curare) and quinine. $\times$ 750.

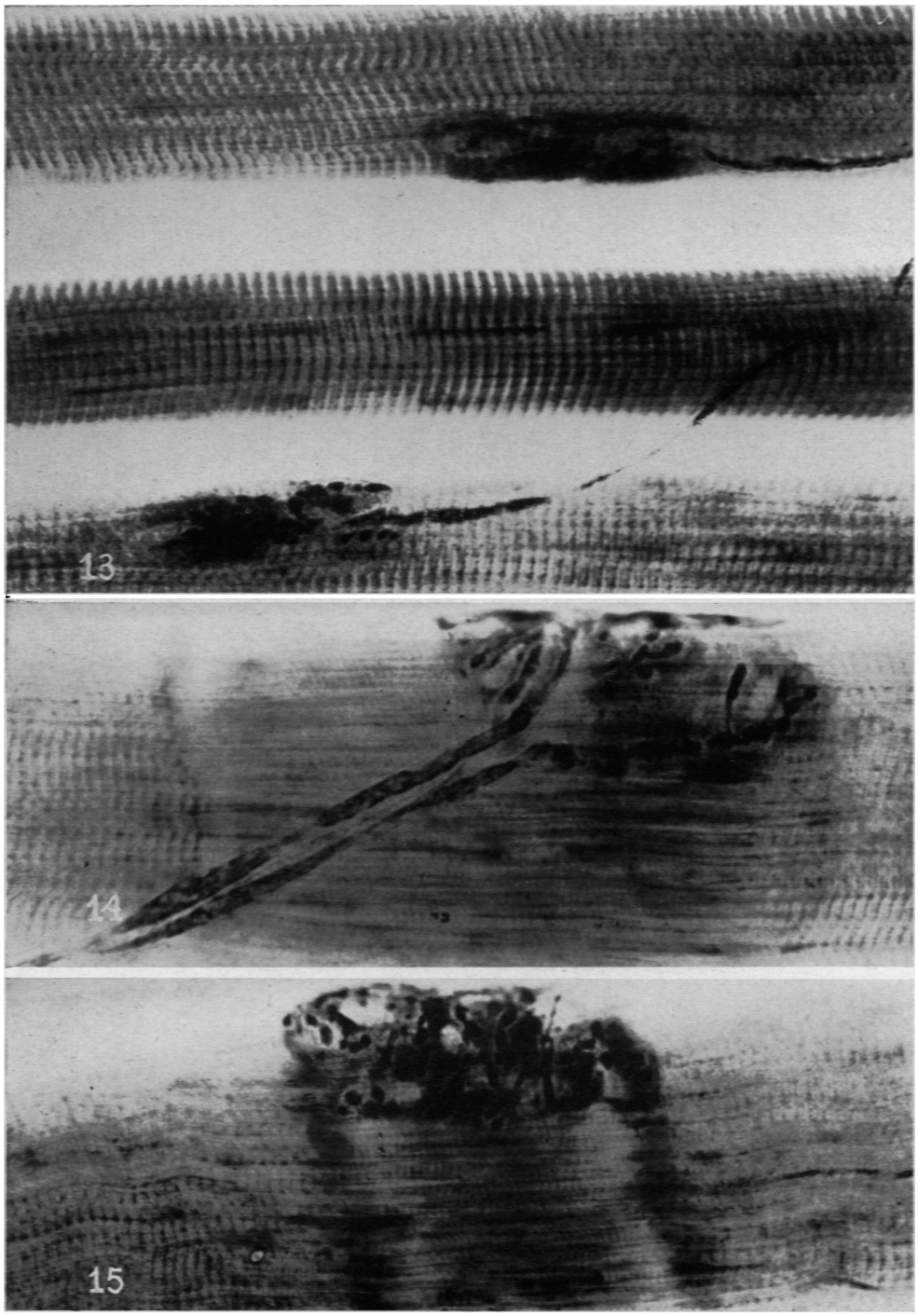



Carey Motion and Secretion of Motor End-Plates

PLATE 62

FIG. 13. Two normal motor end-plates in narrow, coarsely striated fibers of the biceps femoris muscle. There are found variable degrees of splitting of the coarse striations into finer ones in these fixed muscle fibers. The axis cylinders vary from 1 to 15 $\mu$ in diameter. $\times$ 750.

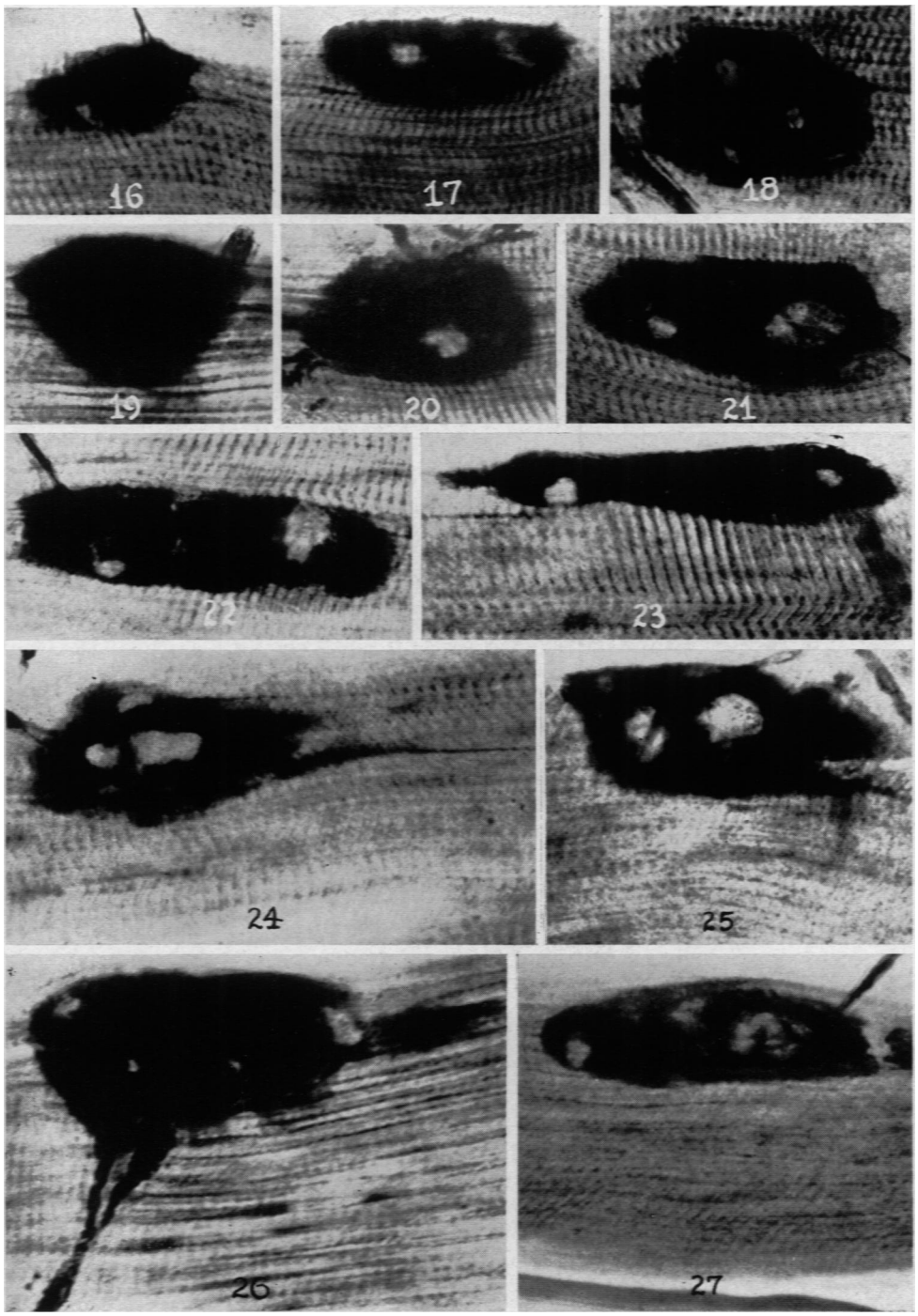
FIGS. 14 and 15. Radiations of fine, closely spaced cross striations from motor end-plates quickly fixed after the living animal, with skin intact, was subjected to 55°C. for 10 seconds and was then immediately plunged in Locke's solution at 4°C. The motor end-plates, expanded by sudden changes in thermal energy, have the surrounding granules of Kühne undergoing dispersal and in direct continuity with the wave of fine cross striations radiating from the expanded end-plates. The chemical action of the dispersed granules of Kühne appears to influence the morphology of the muscle fiber by the replacement of the coarse by the fine cross striations. The transmitter substance secreted from the end-plates appears to have a profound effect on the morphology of the cross striations. This Leisegang phenomenon in a capillary space, such as that of a muscle fiber, is dependent upon temperature and on the concentration and composition of the chemical reactions. If the cross striations were preformed, constant in number and fixed in structure, and in relation to the motor end-plate, one would expect that when the so-called sarcomere is shortened during contraction the motor end-plates likewise would be shortened mechanically. The fact is that the stimulus of heat has a neoformative influence on the end-plate which undergoes ameboid expansion during the time when the muscle fiber contracts. There are a great number of fine, closely spaced striations in relation to the expanded motor end-plate. This evidence points to the fact that the fine striations of contraction and heat rigor rapidly replace the coarse ones of relaxation, and, furthermore, that the fine striations are not the mere mechanical approximation of the coarser ones. This reversible replacement of fine and coarse cross striations of contraction and relaxation respectively, giving the appearance of a shuttle-like shift, is the structural expression of the reversible chemical changes of metabolism. This occurs with flash-like rapidity and may easily mislead the observer to the conclusion that during contraction there is a mere mechanical approximation of constantly fixed and preformed membranes. $\times$ 750.




Carey Motion and Secretion of Motor End-Plates

## PLATE 63

FIGS. 16 to 27. Greatly retracted motor end-plates in the biceps femoris muscle after the injection of intocostrin locally into the muscle and of quinine sulfate into the peritoneal cavity. These retracted end-plates have a well defined circumscribed border and an intense affinity for the gold chloride. This is morphologic evidence of a localized increase in concentration of the aurophilic substance both within the terminal axons and in the region of the granular sole plate of Kühne. These granules form a condensed precipitation-membrane which gives the clearly defined border of the motor end-plate. This failure of dispersal of the secreted granules of Kühne by the chemical combination with both intocostrin and quinine, which have a strong astringent action, leads to a condensed membrane formation. The light vacuolar spaces are occupied by nuclei of the granular sole plate of Kühne. Streamers of condensed granules of Kühne are found to the right in Figures 24, 25 and 26. There appear to be conclusive findings of a morphologic nature correlated with the physiologic block in neuromuscular transmission produced by both curare and quinine. × 750.




Carey Motion and Secretion of Motor End-Plates

## PLATE 64

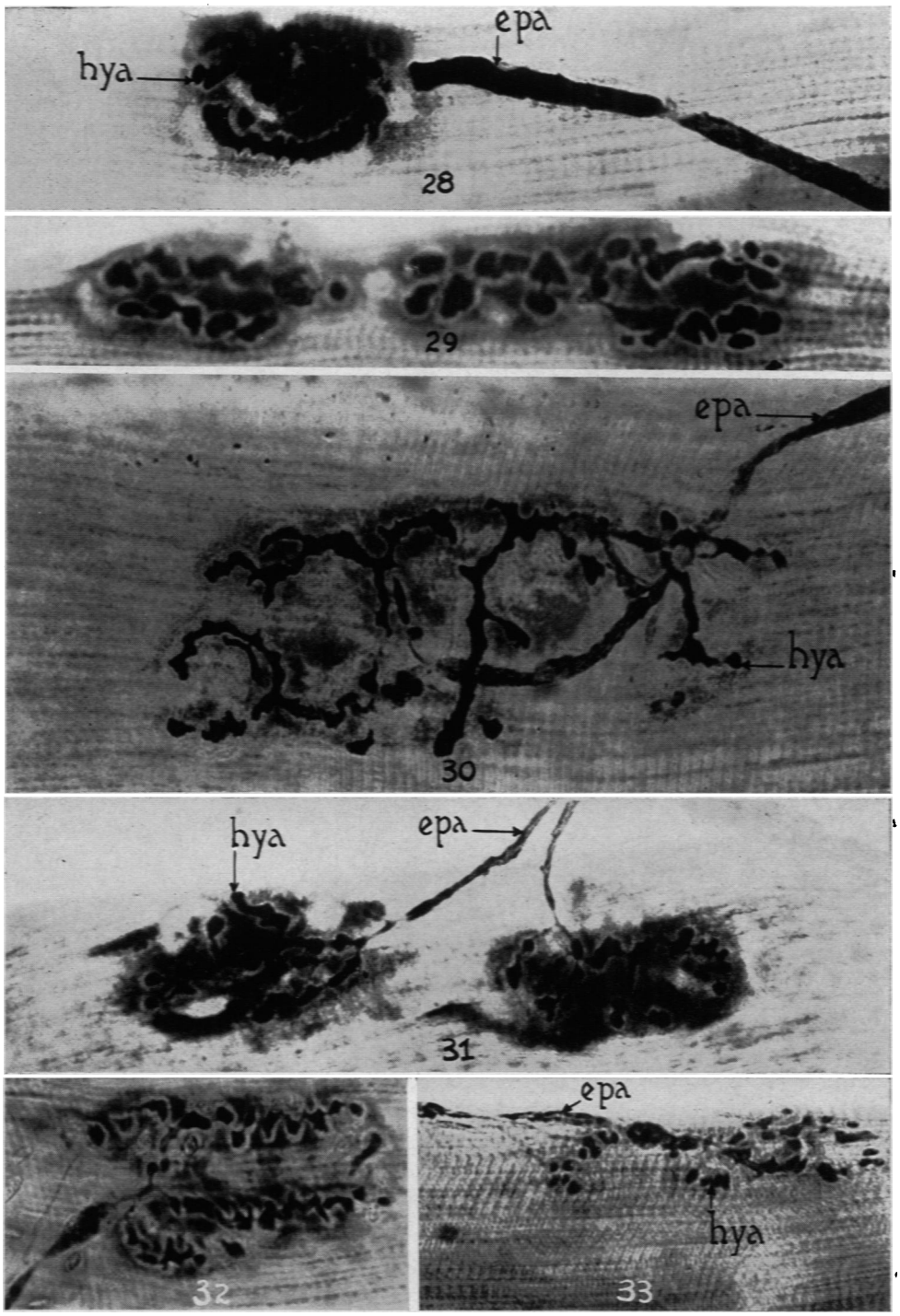
FIG. 28. Retracted motor end-plate in the biceps femoris muscle of the chameleon after the intraperitoneal injection of intocostrin. Both the epilemmal (epa.) and the hypolemmal (hya.) axons are increased in diameter from 1 to 20 $\mu$. In the crotch of the divided hypolemmal axon there are dense islands of the granules of Kühne. × 750.

FIG. 29. Expanded motor end-plate in the biceps femoris muscle with large fragmented globules of the hypolemmal axon after the intraperitoneal injection of intocostrin. There is a clear space between the expanded terminals of the hypolemmal axon and the granular sole plate of Kühne. × 750.

FIG. 30. Expanded motor end-plate with centrifugal projection of ameboid processes and globular fragmentation of the hypolemmal axon after the intraperitoneal injection of prostigmine. In some locations there are isolated droplets of the hypolemmal axon which are completely disconnected from the main axonic network. The hypolemmal axons are thinner and have relatively less accumulation of the granules of Kühne than those under the influence of either curare or quinine. × 750.

FIG. 31. Duplex motor end-plate in a biceps femoris muscle that was first under the influence of curare injected locally into the muscle, and, 2 minutes later, excited by prostigmine injected into the peri oneal cavity. In the hypolemmal axon which appears to be secreting granules of Kühne, the axon becomes more attenuated and less in diameter than in those in which there appears to be (Figs. 28 and 29) either a failure of secretion or of dispersion of the granules of Kühne. × 750.

FIGS. 32 and 33. Motor end-plates of the biceps femoris muscle in which there is a gradual depletion of gold-staining substance in both the hypolemmal axons and granules of Kühne. These granules are practically absent (Fig. 33) after prolonged stimulation with repeated injections of strychnine sulfate over a period of 48 hours. These two end-plates were taken from neighboring muscle fibers in the same muscle. There is progressive decrease in size in both the epilemmal (epa.) and hypolemmal axons (hya.) which is morphologic evidence of exhaustion of the transmitter substance by prolonged chemical stimulation. There is complete fragmentation of the hypolemmal axons in many places into droplets (Fig. 33), around which there is either a great reduction or a complete absence of the granular sole plate of Kühne. × 750.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epa
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29
epa
hya
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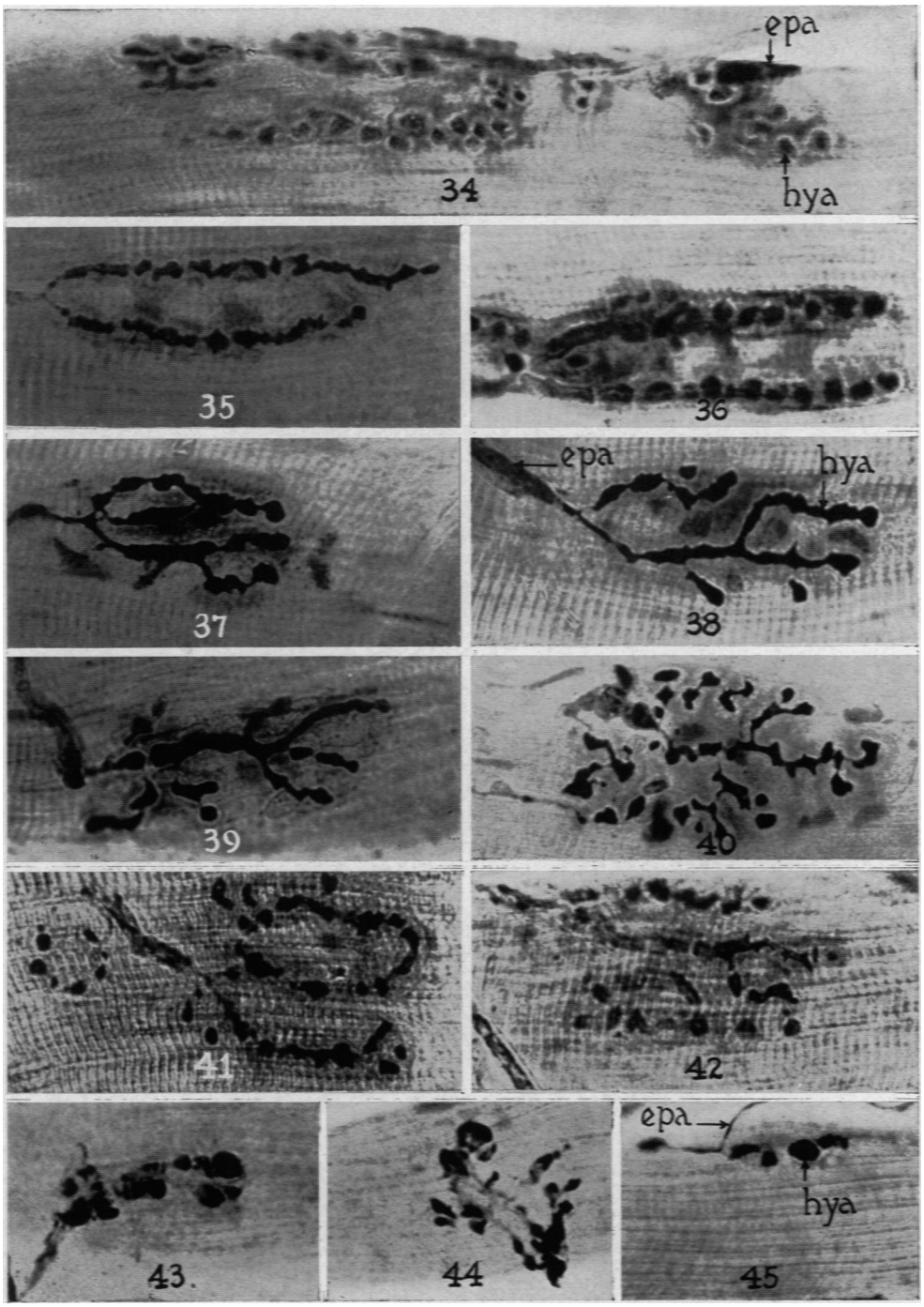
hya
epa
31
32
epa
hya
33

## PLATE 65

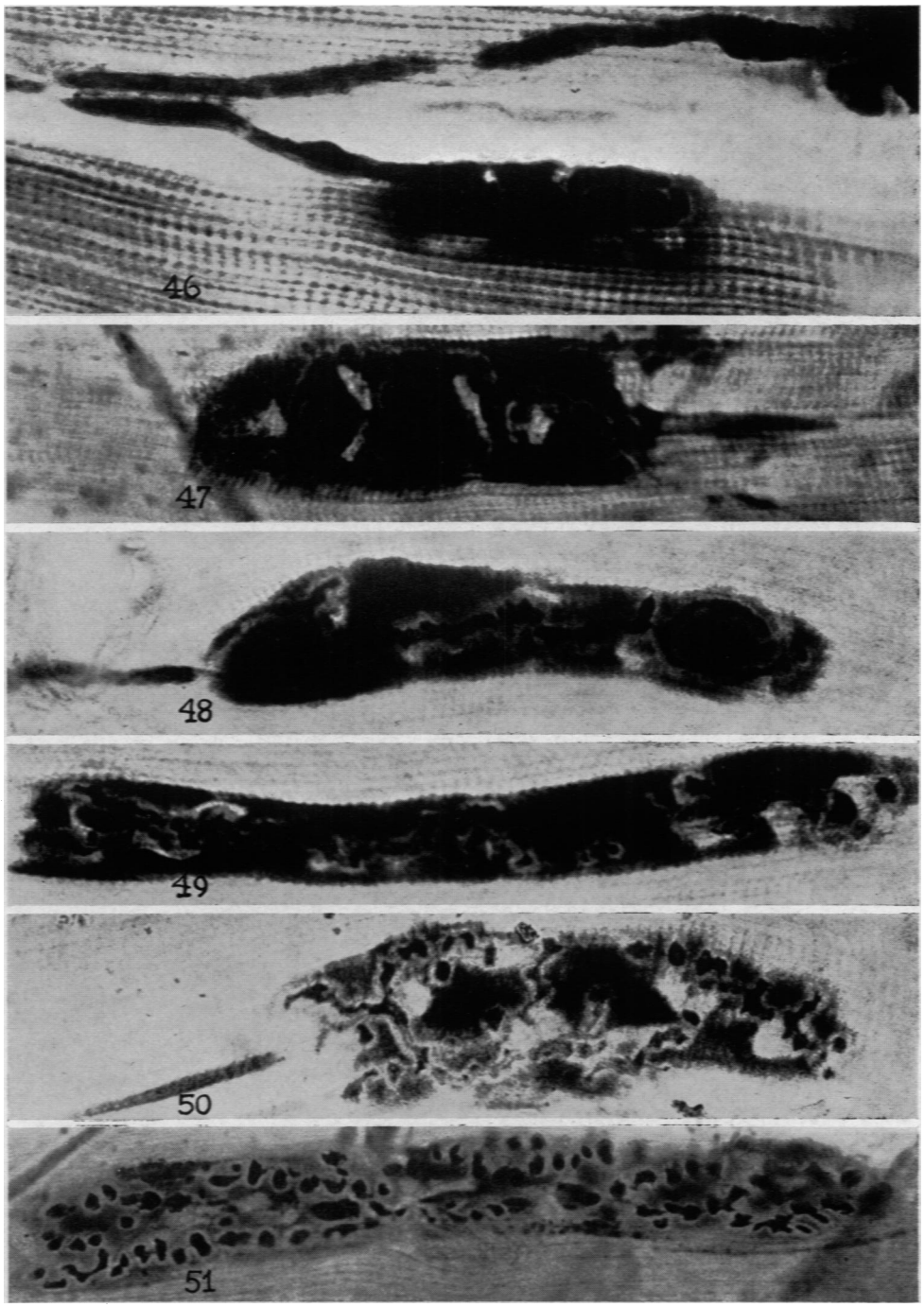
FIGS. 34 to 45. Gradual depletion in the amount of the aurophilic substance in the epilemmal axons (epa.), hypolemmal axons (hya.) and granular sole of Kühne, in the biceps femoris muscle after repeated injections of sublethal doses of strychnine sulfate over a period of 48 hours, terminating in a lethal dose. These motor end-plates appear to be formed by ameboidism during superfunctional stimulation. The motor end-plates undergo protoplasmic streaming in a centrifugal direction into the muscle substance. The pleomorphism is as variable as that of the pseudopods of an ameba. There are dichotomous branchings and anastomotic reticulations of the terminals of the hypolemmal axons in the end-plate. In many places, the globoid and oblong terminals are completely pinched off from the main trunk of the hypolemmal axon. This progressive decrease in the amount of the gold-staining substance in the motor end-plates to the point of practically complete absence of Kühne's granules is morphologic evidence of a substantial depletion of the transmitter substance leading to exhaustion by the prolonged abnormal stimulation with strychnine. The droplet endings with an absence of Kühne's granules (Figs. 41 to 45) give a morphologic appearance of one type of motor end-plate classified by morphologists as the grape-like ending (*terminaisons en grappe*) in contrast to the plate-like ending (*terminaisons en plaque*). These endings of axonic droplets, however, were produced by exhaustion through prolonged chemical stimulation. They represent depletion of both the axonic and granular substances by abnormal stimulation. Certain endings in normal muscle that are devoid of the granules of Kühne may represent a stage in which the granules are quickly dispersed. × 750.




Carey Motion and Secretion of Motor End-Plates

## PLATE 66

FIGS. 46 to 51. Types of retracted and expanded motor end-plates produced by the intraperitoneal injection of intocostrin. The physiologic block to neuromuscular transmission is correlated with a failure of dispersion of the transmitter substance, or the granules of Kühne, from the motor end-plate to the substance of the biceps femoris muscle fiber. There is an abnormal accumulation of the condensed granules of Kühne into a thickened precipitation-membrane surrounding the hypolemmal axons of the end-plate. The accumulated granules of Kühne in the majority of the end-plates have a well defined, circumscribed border from which radiate the dark cross striations. This is evidence of failure of dispersion of the granules of Kühne into the muscle substance. In some of the end-plates (Figs. 47, 48 and 49) the definite border of the sole plate of Kühne is scalloped by the continuous relationship of the dark cross striations. Physiologic block by curare, therefore, appears to be due to an accumulation through failure of dispersal of the granules of Kühne secreted from the terminals of the hypolemmal axons. In some end-plates (Figs. 47 and 50) there are elongated and agglutinated streamers of the granules which appear to be condensed, *in situ*, by the chemical combination with curare. Curare appears to form a precipitation-membrane of Kühne's granules. This inhibits the normal transmission by diffusion and dispersion of this fulminate-like nervous substance that is secreted from the hypolemmal axons and that normally excites the muscle substance by this chemical transmission of nerve impulses. × 750.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47
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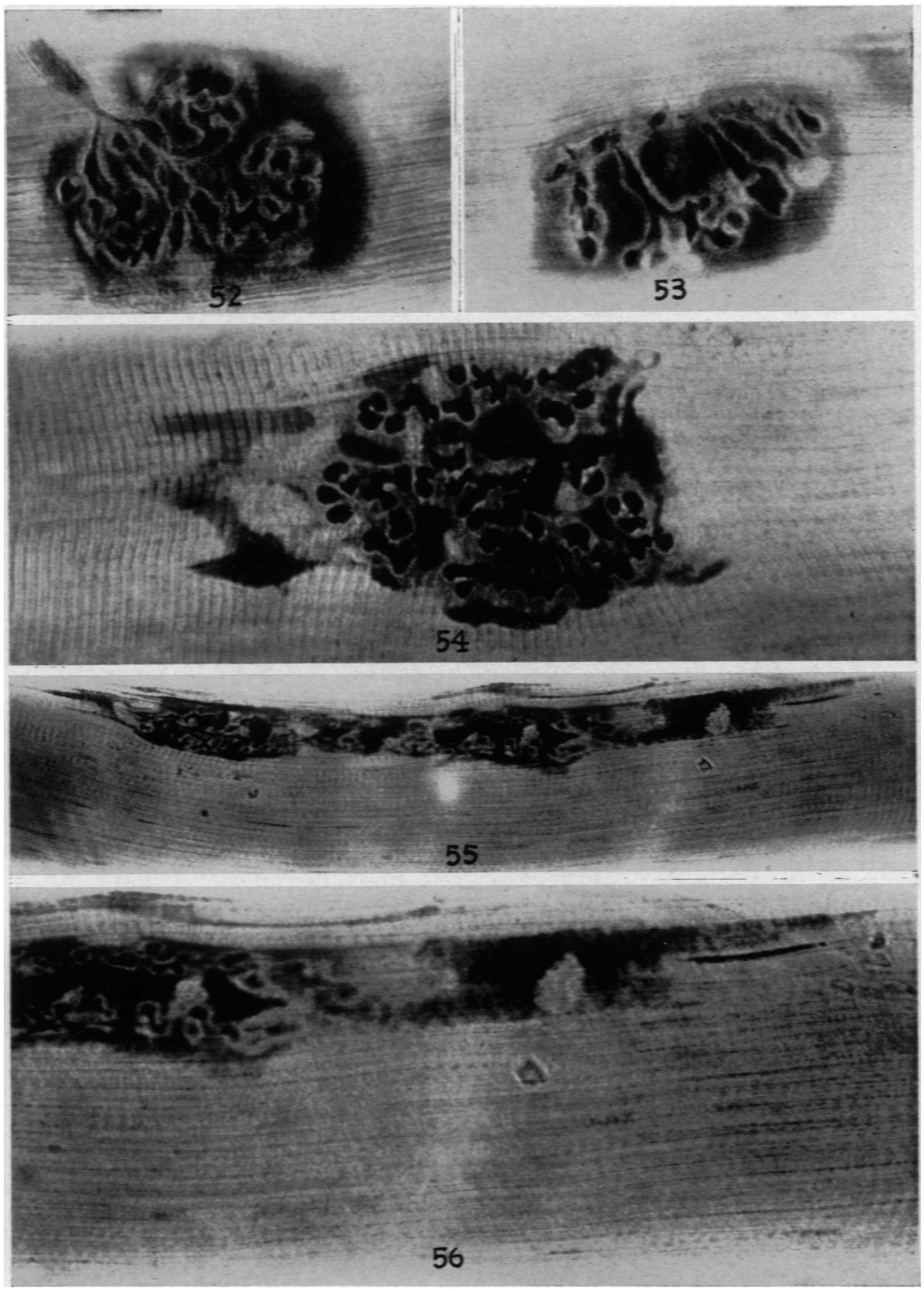
51

## PLATE 67

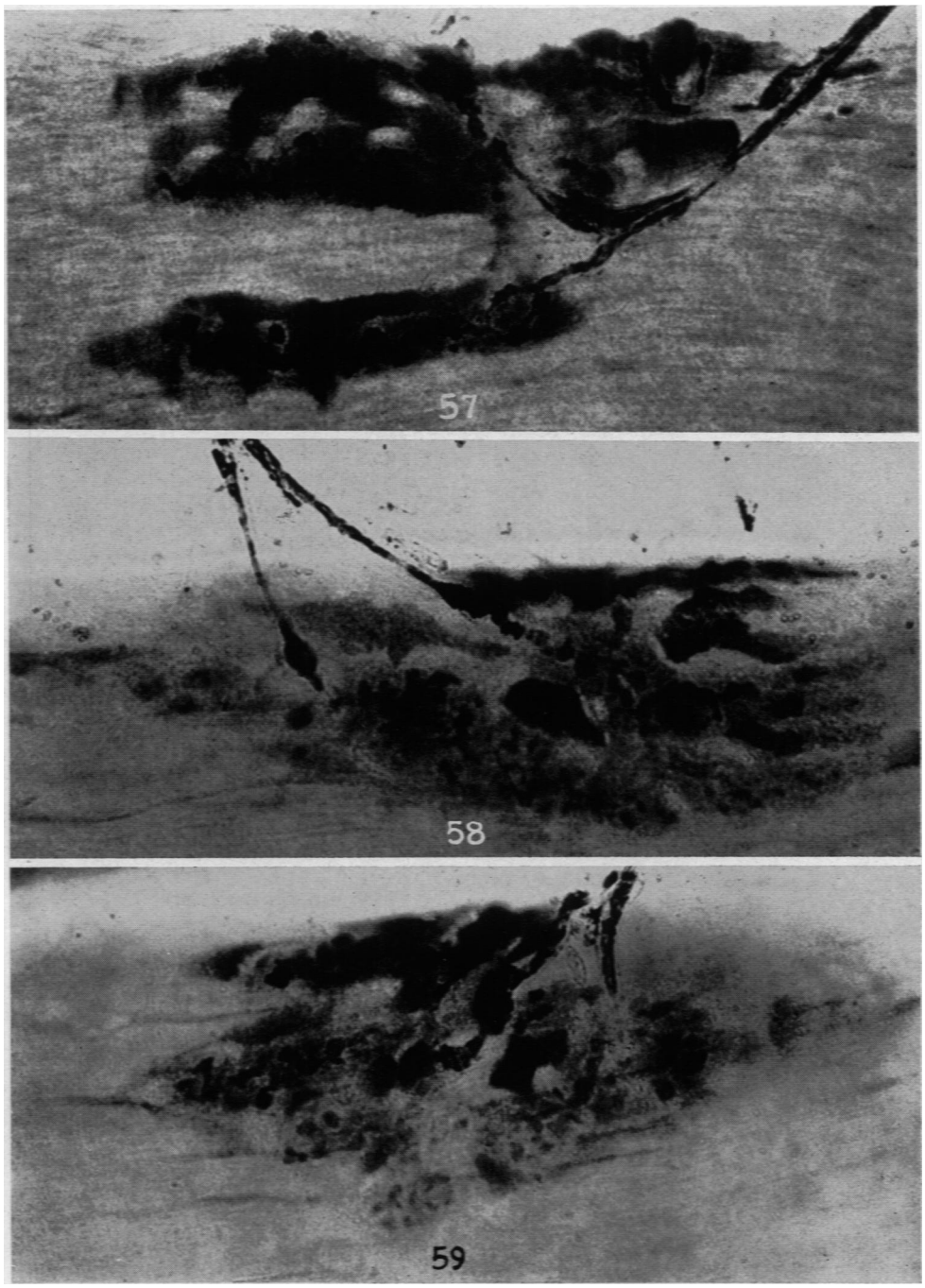
FIGS. 52 to 56. Pleomorphism of motor end-plates in the biceps femoris muscle. Intocostrin was injected into the muscle locally and 1 minute later prostigmine was injected into the peritoneal cavity. There is an abnormal accumulation of the secreted granules of Kühne around the hypolemmal axons. Streamers of the secreted granules of Kühne which failed to disperse normally are produced by the chemical combination with curare (Figs 54 to 56). The gradual transformation of the globular terminals of the hypolemmal axons into Kühne's granules is evident in the right half of the illustrations (Figs. 55 and 56). There is a gradual transition from left to right of clear-cut hypolemmal axons surrounded by light spaces into the granular material of the elongated sole plate of Kühne without the intervening clear halo-like spaces. At the left (Figs. 55 and 56) the islands of concentrated Kühne's granules take an intense stain with gold comparable to that of the terminals of the axon. There is an antagonism between the stimulus of expansion produced by prostigmine and the stimulus of retraction produced by intocostrin which results in the clear-cut morphology of the hypolemmal axons and abnormal accumulation of the granules of Kühne. This is morphologic evidence that the terminal axons in the end-plate are microscopic endocrine glands. Their secretion motivates the muscle fibers by a transmitter substance—the granules of Kühne. A precipitate comparable to that of Kühne's granules is produced, *in vitro,* by the chemical interaction of either acetylcholine or choline with gold chloride. At the present time there is no good histologic test for either acetylcholine or choline at the myoneural junction except possibly that of gold chloride. Most other histologic methods destroy the granular sole plate of Kühne. The motor end-plates (Figs. 52 to 72) were obtained from the same biceps femoris muscle after the local injection of intocostrin into the muscle and prostigmine into the peritoneal cavity. (Fig. 55 magnified 300 ×; Figs. 52, 53, 54 and 56 are 750 ×).




Carey Motion and Secretion of Motor End-Plates

## PLATE 68

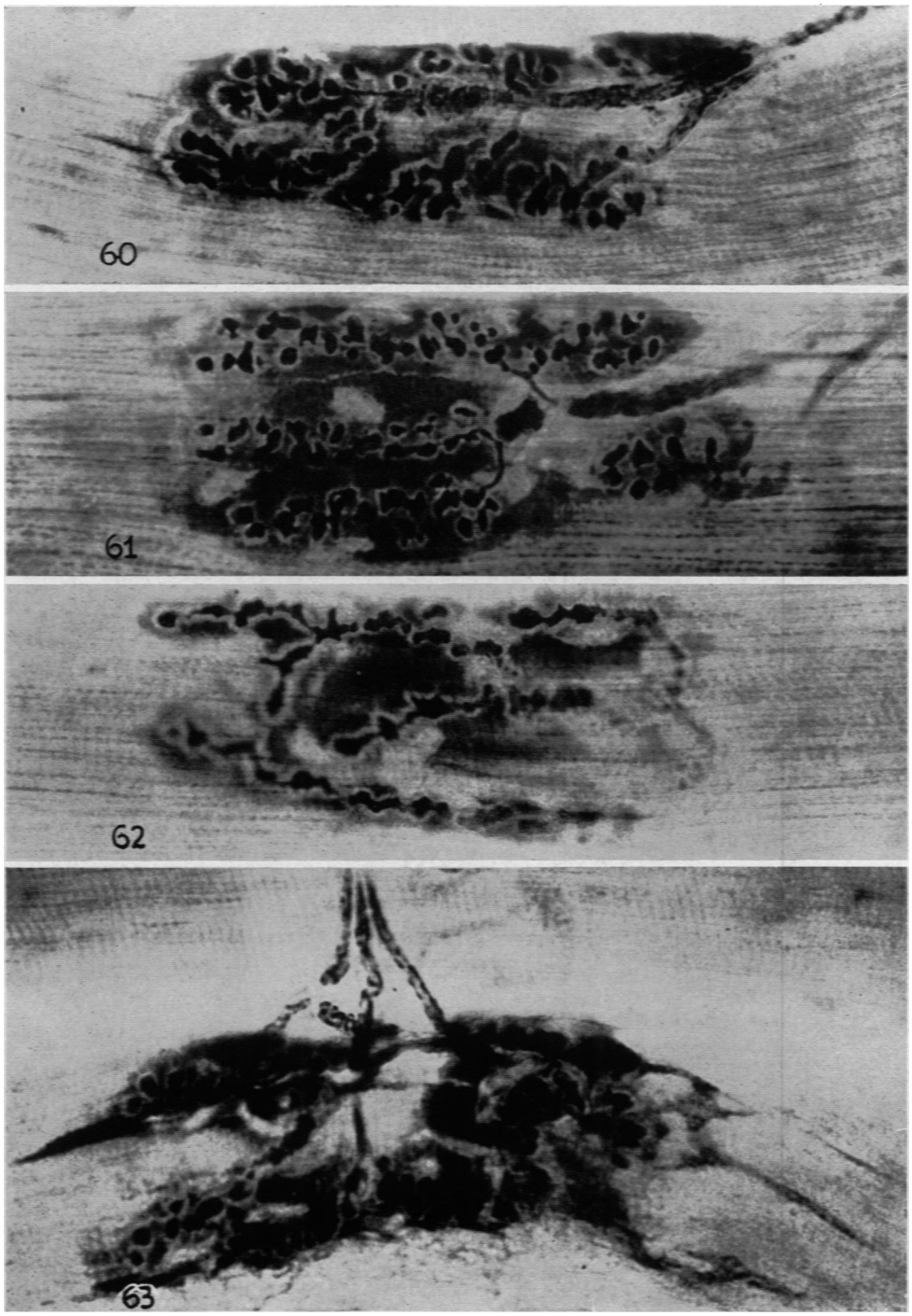
FIGS. 57 to 59. Greatly expanded motor end-plates in the biceps femoris muscle. This pleomorphism followed the injection into the muscle of intocostrin and later the injection of prostigmine into the peritoneal cavity. There is an abnormal accumulation of the granules of Kühne (Fig. 57) and a gradual transition of the hypolemmal axons (Figs. 58 and 59) into the granules of Kühne without the intervention of the clear halo-like space. There appears to be a direct transformation of the terminals of the hypolemmal axons into the secreted granules of Kühne through the violent chemical excitation produced by prostigmine and of inhibition by curare. There is a more gradual dispersion of the granules of Kühne into the substance of the cross-striated muscle fiber than that produced by the chemical action of either quinine or curare. The expansion effect on the motor end-plate produced by prostigmine appears to neutralize partially the microscopic changes in the motor end-plate produced by either curare or quinine acting alone. These antagonistic chemical actions give favorable evidence of the transformation of the axon into the specific secreted transmitter substance. The granular sole plate of Kühne, therefore, is not a constant, fixed and preformed structure. × 750.




Carey Motion and Secretion of Motor End-Plates

## PLATE 69

FIGS. 60 to 63. Pleomorphism by ameboid motion of the axonic branches in the motor end-plate in the biceps femoris muscle of the chameleon. Intocostrin was injected into the muscle locally and 1 minute later prostigmine was injected intraperitoneally. The chameleon died in violent spasm 2 minutes after prostigmine was injected. There is an accumulation of the granules of Kühne around the hypolemmal axons which have undergone, in many instances, globular fragmentation. These droplets vary from 4 to 15 $\mu$ in diameter. The clear oval spaces in the granules of Kühne are occupied by nuclei. This blocking of the dispersal of Kühne's granules by curare, followed relatively soon by chemical excitation with prostigmine, results in neurocladism or dichotomous division of the branches of the axon in the end-plate. This centrifugal extension and separation of the terminal branches in the end-plate and the simultaneous block to the dispersal of the granules of Kühne result in the abnormal accumulation of these granules around the related axon. The gradual replacement of the axon directly into the granules of Kühne is observed in the lower and right aspect of the end-plate in Figure 62. To the left and right of the end-plate (Fig. 63) there are elongated streamers of the granules of Kühne that have been agglutinated by the chemical retraction action of intocostrin and projection action of prostigmine. × 750.




Carey Motion and Secretion of Motor End-Plates

## PLATE 70

FIGS. 64 to 68. Various stages in the expansion and globular fragmentation of the hypolemmal axons and the accumulation of the surrounding granular sole plate of Kühne. Intocostrin was injected into the muscle locally and 1 minute later acetylcholine was injected intraperitoneally. The chameleons were decapitated in violent spasm 1 minute after acetylcholine was injected. The pleomorphic changes in these motor end-plates are the results of mutual antagonism of curare and acetylcholine. The curare partially blocks the transmission of the granules of Kühne into the muscle substance resulting in abnormal accumulations around the branches of the axons and agglutinated streamers of these granules in the muscle substance. The acetylcholine excites the expansive phase of the ameboid motion of the hypolemmal axons. The granules of Kühne (Figs. 65, 67 and 68) have a more intense affinity for the gold chloride than the dark bands of the cross striations in the muscle substance. × 750.

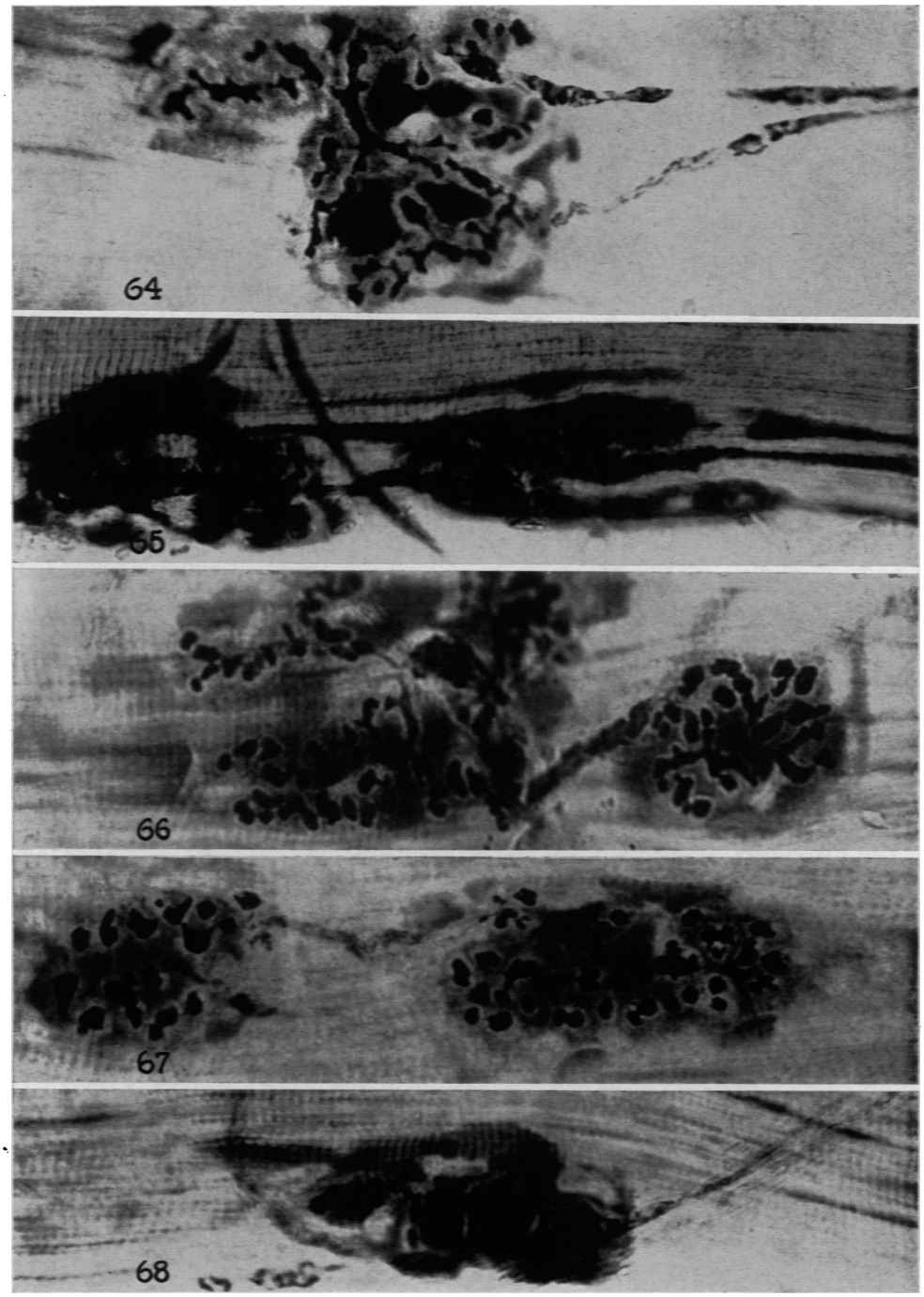
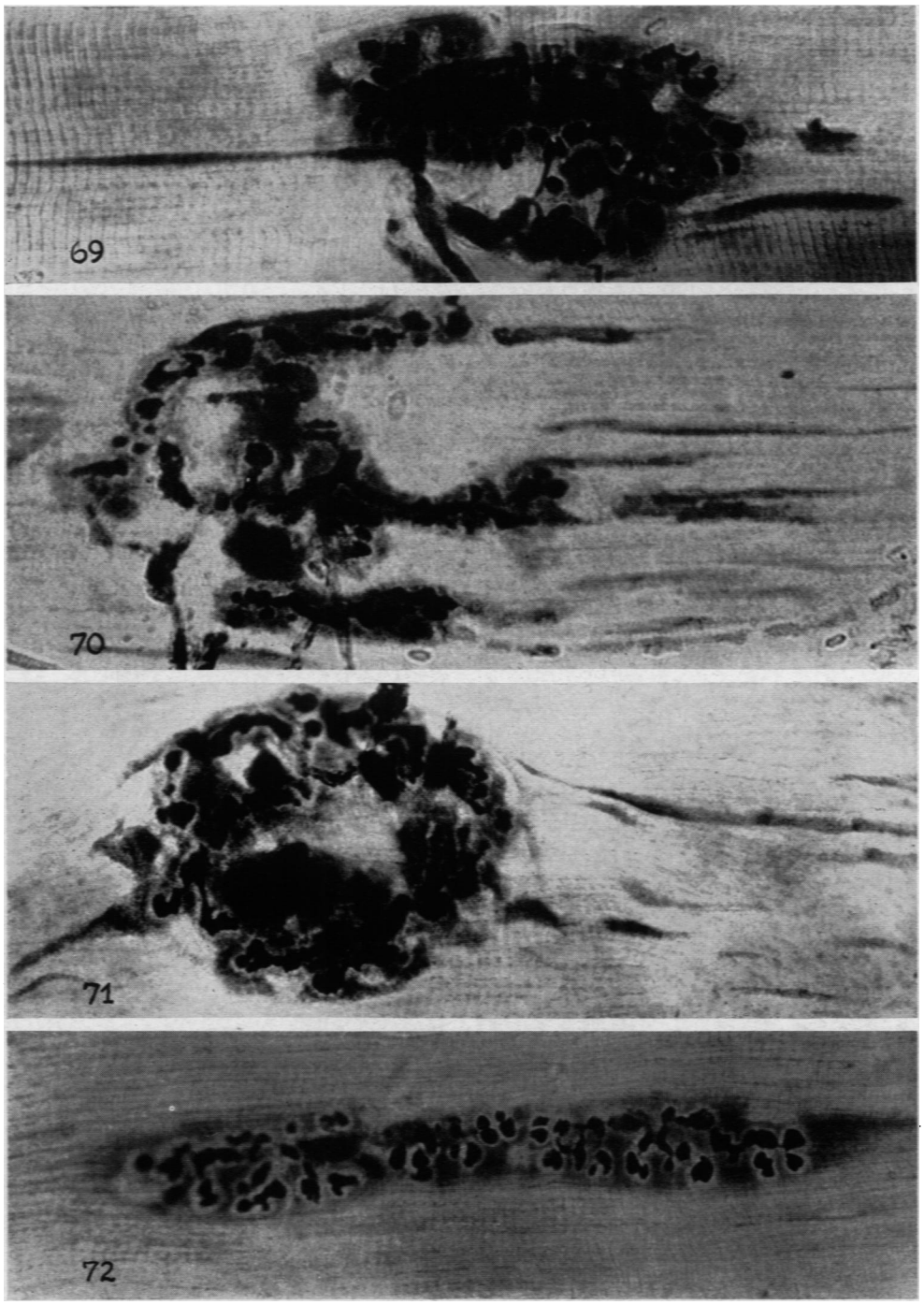

64
65
66
67
68

PLATE 71

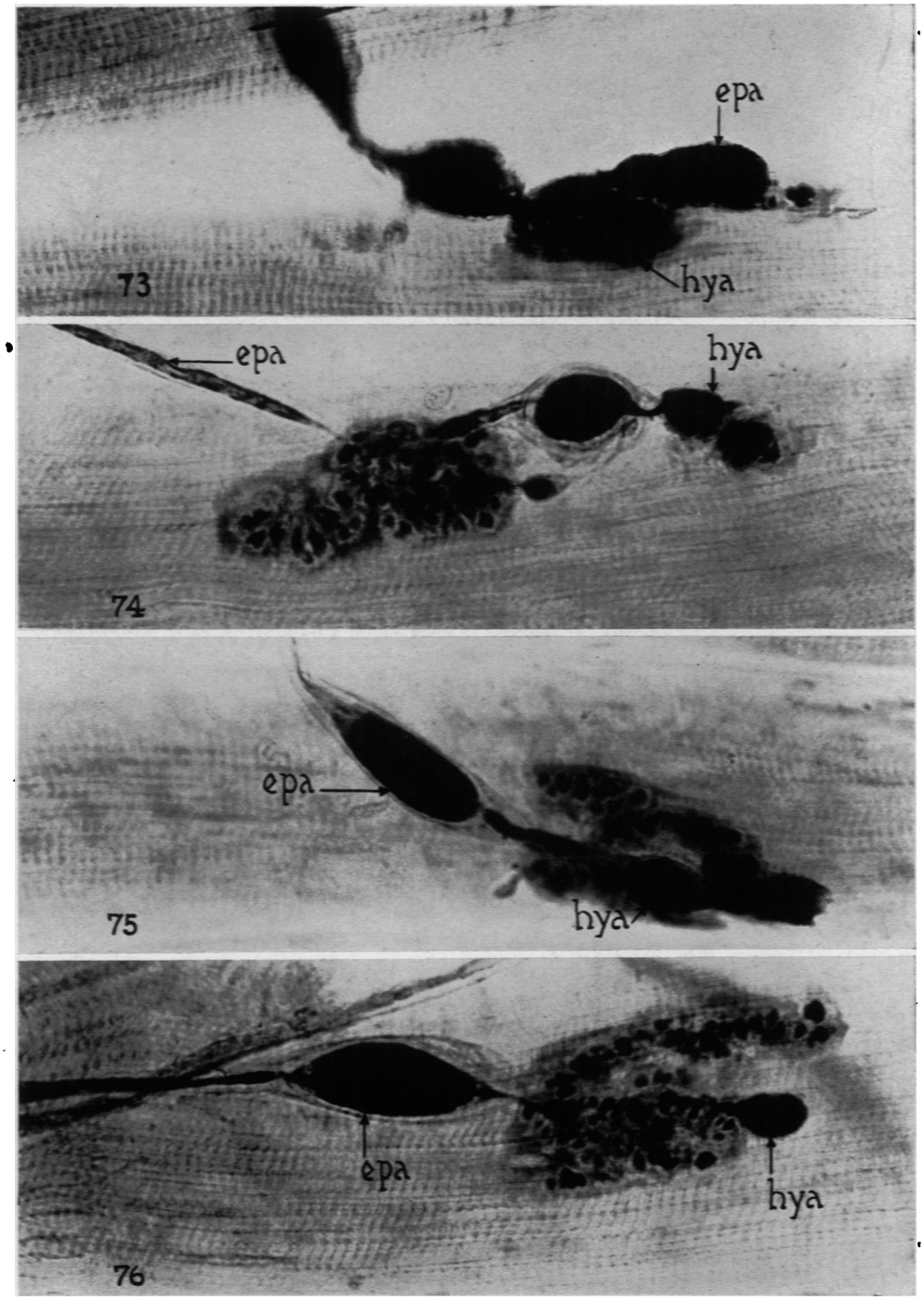
FIGS. 69 to 72. Pleomorphism of the hypolemmal axons of the motor end-plates produced by the chemical action of intocostrin followed by acetylcholine. Intocostrin was injected locally in the biceps femoris muscle of the chameleon and 1 minute later acetylcholine was injected in the intraperitoneal cavity. These end-plates were from the same muscle as those fibers illustrated in Plate 69. There is an abnormal accumulation of the granules of Kühne into insular masses found between the axonic branches as well as streamers of these granules projected away from the end-plate (Figs. 69 to 71). These granular streamers represent inadequate dispersal of the transmitter substance which is secreted from the hypolemmal axons into the muscle fiber. Globular fragmentation of the hypolemmal axon is clearly evident in the elongated end-plate (Fig. 72). × 750.




Carey Motion and Secretion of Motor End-Plates

## PLATE 72

FIGS. 73 to 76. Pleomorphism of the epilemmal (epa.) and hypolemmal (hya.) axons of motor end-plates in the biceps femoris muscle of the chameleon. Intocostrin was injected locally into the biceps femoris muscle and 3 minutes later quinine sulfate was injected into the same location. Three minutes after the injection of the quinine sulfate locally, ammonium hydroxide was injected into the peritoneal cavity. The animal died in 2 minutes after injection of ammonium hydroxide in a state of violent spasm. One per cent of the end-plates had acute retention cysts which contained a substance that had strong affinity for gold chloride. These acute retention cysts were found either in the epilemmal (Fig. 73) or hypolemmal (Fig. 74) axons or they were found in both locations (Figs. 75 and 76). The morphologic accompaniment of these acute retention cysts which contained aurophilic substance was the diminution in the projection of the hypolemmal axons. The chemical block to the secretion of the granules by the hypolemmal axons together with excitation in the transmission of the nerve substance to the end-plate may be compared roughly to the production of a lake behind the dam erected in the course of a flowing river. This is experimental evidence that a liquid nerve substance is secreted normally from the motor end-plates into the muscle substance. Intocostrin and quinine apparently form a dense precipitation-membrane, by astringent action, around the periphery of the naked hypolemmal axons. This impermeable precipitation-membrane would then inhibit the normal transference of the axonic substance into the secretion granules of Kühne. Where this mechanical block by chemical action has become adequate, the circumscribed dilatations of the hypolemmal axons appear to possess a thickened membrane around which there is a diminution or complete absence of the granules of Kühne (Figs. 74 and 76). In other locations of the same end-plates, where the block has not become complete, there are hypolemmal axons surrounded by granules of Kühne. The teasing technic of muscle fibers impregnated previously with gold chloride preserves the anatomic continuity of the epilemmal axon, hypolemmal axon, ramifications of the terminal axons, the granules of Kühne and the muscle striations. × 750.


epa
hya
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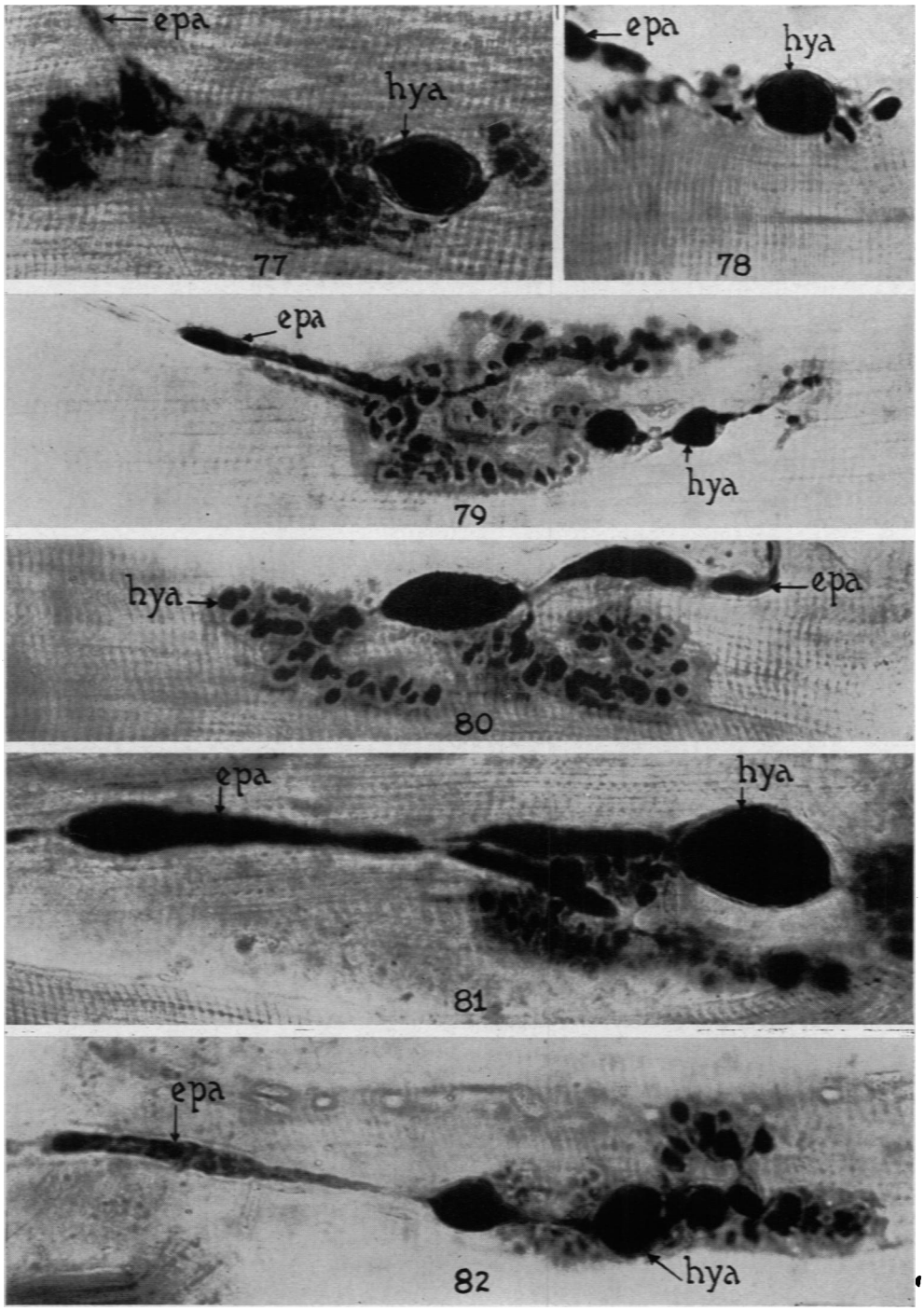
epa
hya
74
epa
hya
75
epa
hya
76

## PLATE 73

FIGS. 77 to 82. Pleomorphism of the epilemmal (epa.) and hypolemmal (hya.) axons of motor end-plates in the biceps femoris muscle of the chameleon. Intocostrin was injected locally into the biceps femoris muscle and 3 minutes later quinine sulfate was injected into the same location. Three minutes after the injection of the quinine sulfate locally, ammonium hydroxide was injected into the peritoneal cavity. The animal died in 2 minutes following injection of ammonium hydroxide in a state of violent spasm. One per cent of the end-plates had acute retention cysts which contained a substance that had strong affinity for gold chloride. These acute retention cysts were found either in the epilemmal (Figs. 80, 81 and 82) or hypolemmal axon or in both locations (Figs. 77, 78, 79, 80, 81 and 82). The morphologic accompaniment of these acute retention cysts which contained aurophilic substance was the diminution in the projection of the hypolemmal axons. These acute retention cysts are surrounded by a definite, circumscribed membrane which apparently has been thickened and precipitated by the chemical actions of intocostrin and quinine. There are no secretion granules of Kühne surrounding these cystic dilatations of the hypolemmal axon. Apparently an effective block has been produced to the secretion of Kühne's granules around the retention cysts which contain a substance having a very strong affinity for gold chloride. × 750.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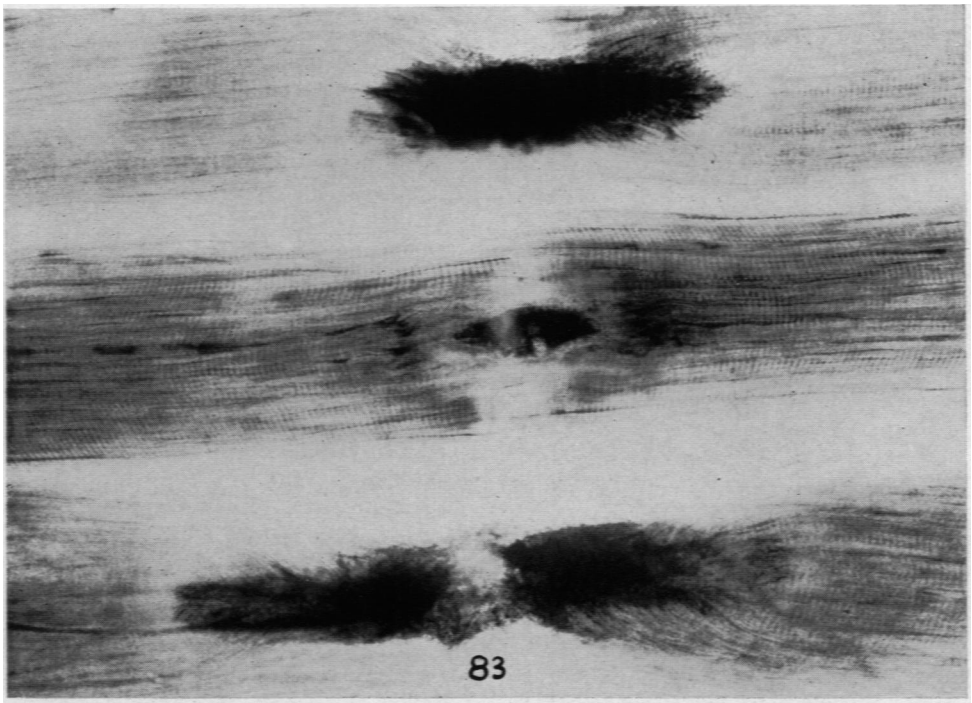
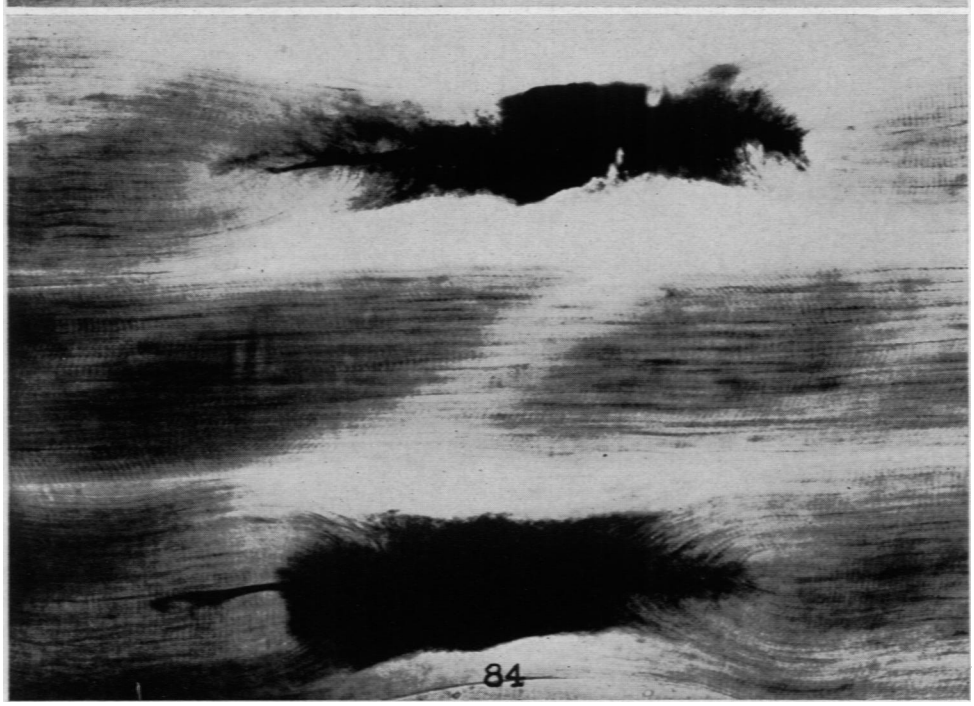
## PLATE 74

FIGS. 83 and 84. Sudden conduction of masses of nerve substance into the motor end-plates of the biceps femoris muscle of the chameleon by the action of tetraethyl lead injected into the peritoneal cavity. This acute conduction of nerve substance into the end-plate results in a complete distortion by the explosive action of this substance which disrupts the end-plates. There are radiations of gold-staining substance extending from the terminals of the distorted end-plates as though a violent explosion had destroyed the normal morphology of the end-plate. There is an abnormal accumulation of gold-staining substance in the disrupted plates. This likewise applies to the morphology of the cross-striated muscle substance in close proximity to these end-plates. × 300.

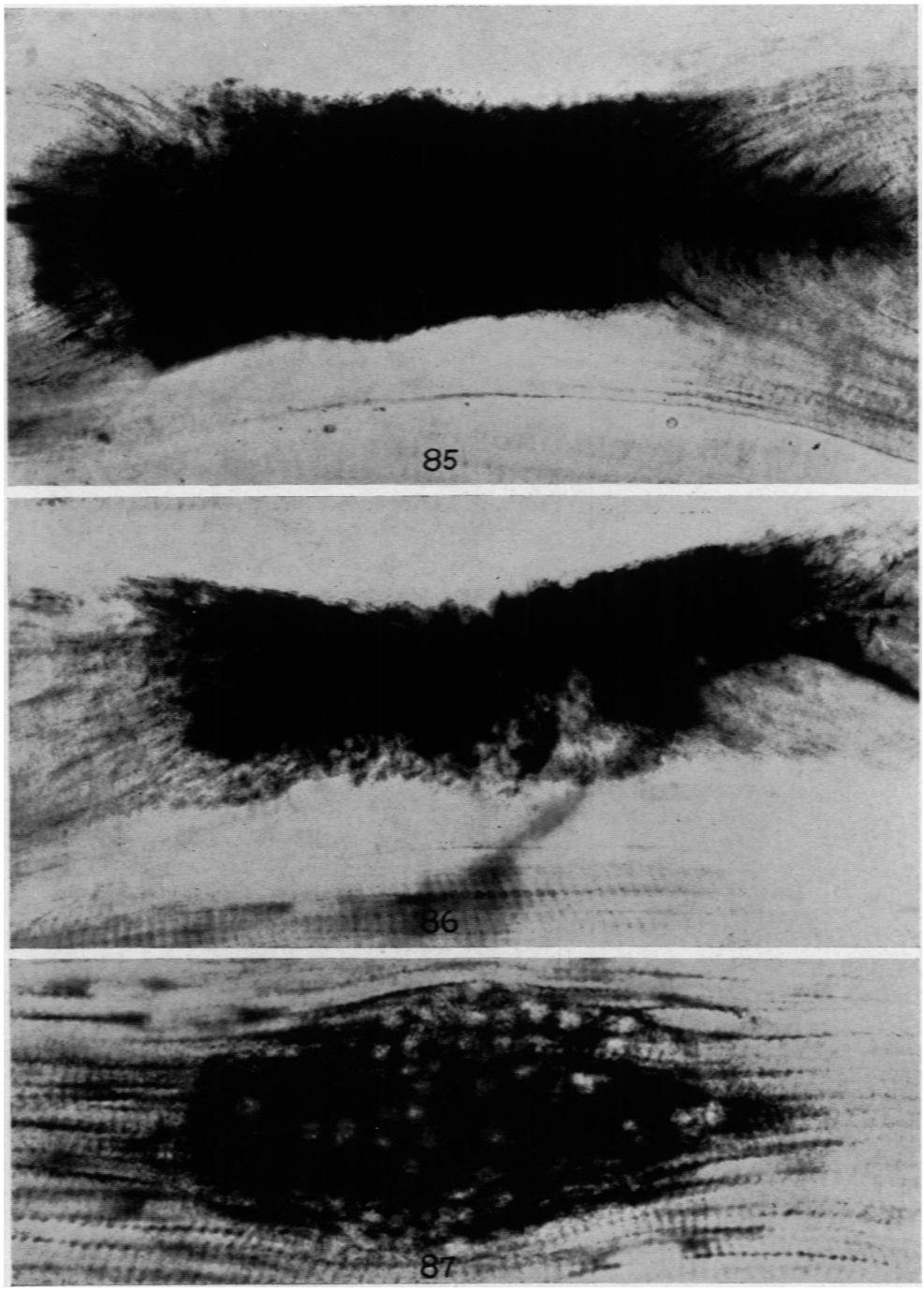



Carey Motion and Secretion of Motor End-Plates

## PLATE 75

FIGS. 85 to 87. Motor end-plates in the biceps femoris muscle of the chameleon disrupted by the explosive chemical action of tetraethyl lead injected into the peritoneal cavity. There is a massive conduction of an increased amount of gold-staining substance into the motor end-plates, which massive conduction appears to have a violent, explosive effect by destroying the normal morphology of the end-plate. Radiations of gold-staining substance extend from the terminals of the destroyed end-plate. These radiations appear to be the effect of explosive violence which radiates out into the striated muscle substance and destroys the normal morphology of the cross striations in many places. The chemical action of tetraethyl lead apparently delivers suddenly abnormal amounts of the gold-staining axonal substance to the end-plate. This supernormal transmission of nerve substance destroys the myoneural junction with explosive violence and shatters the normal morphology by a real intramuscular chemical explosion of some of the motor end-plates. × 750.




Carey Motion and Secretion of Motor End-Plates