ELECTRON MICROSCOPIC LOCALIZATION OF ACETYLCHOLINESTERASE AND NONSPECIFIC CHOLINESTERASE AT THE NEUROMUSCULAR JUNCTION BY THE GOLD–THIOCHOLINE AND GOLD–THIOLACETIC ACID METHODS

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ABSTRACT

By means of the gold-thiocholine (AuThCh) and gold–thiolacetic acid (AuThAc) methods, it has been demonstrated electron microscopically that acetylcholinesterase (AChE) is located at the prejunctional axoplasmic membrane and the postjunctional sarcoplasmic membrane, including the full lengths of its invaginations, at the motor end plate of mouse intercostal muscle. Nonspecific cholinesterase (ChE) is present in relatively low concentrations at the same sites, and in greater concentrations in the teloglial Schwann sheath cells. Significant amounts of reaction product appeared in the junctional cleft only after prolonged incubation with both methods. The identification of AChE and ChE was confirmed by the use of appropriate concentrations of several selective inhibitors. In confirmation of previous studies by light microscopy, the AuThCh method is more specific for AChE and ChE, whereas the AuThAc method allows greater accuracy of localization.

It has been shown recently (25, 26) that when aurous gold, as $AuNa_3(S_2O_3)_2$, is substituted for lead or copper as the capturing agent in modifications of the standard thiolacetic acid (ThAc)[1] (9) and thiocholine (19) methods for acetylcholinesterase (AChE) and nonspecific cholinesterase (ChE), primary reaction products of much finer, colloidal dimensions are obtained. At the same time, the characteristic advantages and limitations of the original procedures are retained. Thus, the gold-thiolacetic acid (AuThAc) method allows excellent penetration of the reagents into tissues, and results in an extremely fine primary reaction product, Au_2S, but its specificity is low. While the gold-thiocholine (AuThCh) method is highly specific, the cationic substrates, acetylthiocholine (AThCh) and butyrylthiocholine (BuThCh), and the free Au^+ ion penetrate tissues relatively poorly; in addition, the primary reaction product presumably gold–thiocholine phosphate, is coarser than Au_2S.

When both procedures are employed in parallel, the AuThCh method permits reliable identification of the enzymes at their approximate sites; on the basis of this information, their localization can be determined with still greater accuracy by

[1] The following abbreviations are used throughout: AuThCh, gold-thiocholine; AuThAc, gold–thiolacetic acid; AThCh, acetylthiocholine; BuThCh, butyrylthiocholine; ThAc, thiolacetic acid; AChE, acetylcholinesterase; ChE, nonspecific or pseudocholinesterase; DFP, di*iso*propyl phosphorofluoridate; BW284, 1,5-*bis*(4-allyl dimethylammoniumphenyl)-pentan-3-one diiodide; MEP, motor end plate.

the AuThAc method. In the present study, this program has been followed for the localization of AChE and ChE at the motor end plate (MEP) of mouse intercostal muscle. The results indicate that both enzymes are present at the prejunctional axoplasmic and postjunctional modified sarcoplasmic membranes, but that only ChE is present in the adjacent teloglial cells. These findings have been reported partially in abstracts (11, 24).

MATERIALS AND METHODS

The procedures followed for obtaining and fixing tissues, and for carrying out the histochemical reactions by the AuThCh and AuThAc methods were identical with those reported for light microscopy (26) with the exceptions noted below. In brief, mice were sacrificed by a blow on the head, and the entire rib cages were fixed for 2 hr in cold (4°–8°C) formaldehyde (4%)-sucrose (7.5%)-maleate buffer (0.028 M), adjusted to pH 7.40 ± 0.05, after which they were transferred to cold sucrose-maleate buffer for ½–18 hr.

For the AuThCh method, strips of muscle were sectioned at 50–100 μ in the cryostat, and the sections were carried through the preincubation (15 min), incubation (10 sec to 15 min), and postincubation (15 min) solutions at room temperature (22°–24°C). The major components of the incubation solution are 0.004 M $AuNa_3(S_2O_3)_2$, 0.004 M AThCh or BuThCh, and 4.0 M NaH_2PO_4-K_2HPO_4, adjusted to pH 5.6; the preincubation and postincubation solutions are similar, except that the former is devoid of substrate, and the latter contains no substrate or gold salt; both the incubation and postincubation solutions are saturated with the primary reaction product. Following conversion of the primary reaction product to Au_2S by immersion in acid-alcoholic $(NH_4)_2S$ solution, sections were rinsed briefly in acidic 4% formaldehyde solution, fixed for 30–60 min in cold, buffered 1% OsO_4 solution, then dehydrated and embedded in Epon.

Although cryostat sections of fixed muscle were also used with the AuThAc method, the best results were obtained when unsectioned strips of the fixed tissue were exposed to the preincubation (30 min) and incubation (1–4 hr) solutions at 2°–4°C. The incubation solution contains 0.003 M $AuNa_3(S_2O_3)_2$, 0.04 M ThAc (Eastman Kodak, Rochester, New York, practical), 0.032 M $MgCl_2$, and 0.056 sodium hydrogen maleate buffer, adjusted to pH 6.20; the preincubation solution contains 0.006 M ThAc, which is stoichiometrically equivalent to the concentration of Au^+ (15), but is otherwise identical. The tissues were then transferred to the postincubation solution (containing the same constituents as the incubation solution except for the omission of the gold-salt and thiolacetic acid, and the addition of 4% formaldehyde), and after standing for at least 15 min at 2°–4°C, they were left in the same solution while small portions containing MEP's were excised under the dissecting microscope. These were then fixed at 4°–6°C in 1–2% buffered OsO_4 for 2–4 hr prior to dehydration and embedding in Epon.

Gold sections were cut with a Porter-Blum MT-1 or MT-2 microtome. They were examined, and micrographs were taken with a Philips EM 100-B electron microscope.

As in the previously published procedures (26), selective inhibition of ChE was obtained by incubating the tissue strips or sections with 10^{-7} to $3 \cdot 10^{-7}$ M di*iso*propyl phosphorofluoridate (DFP) in sucrose-maleate buffer for 30 min at room temperature immediately prior to their being placed in the preincubation solution. With the AuThCh method, AChE was inhibited selectively by incorporating

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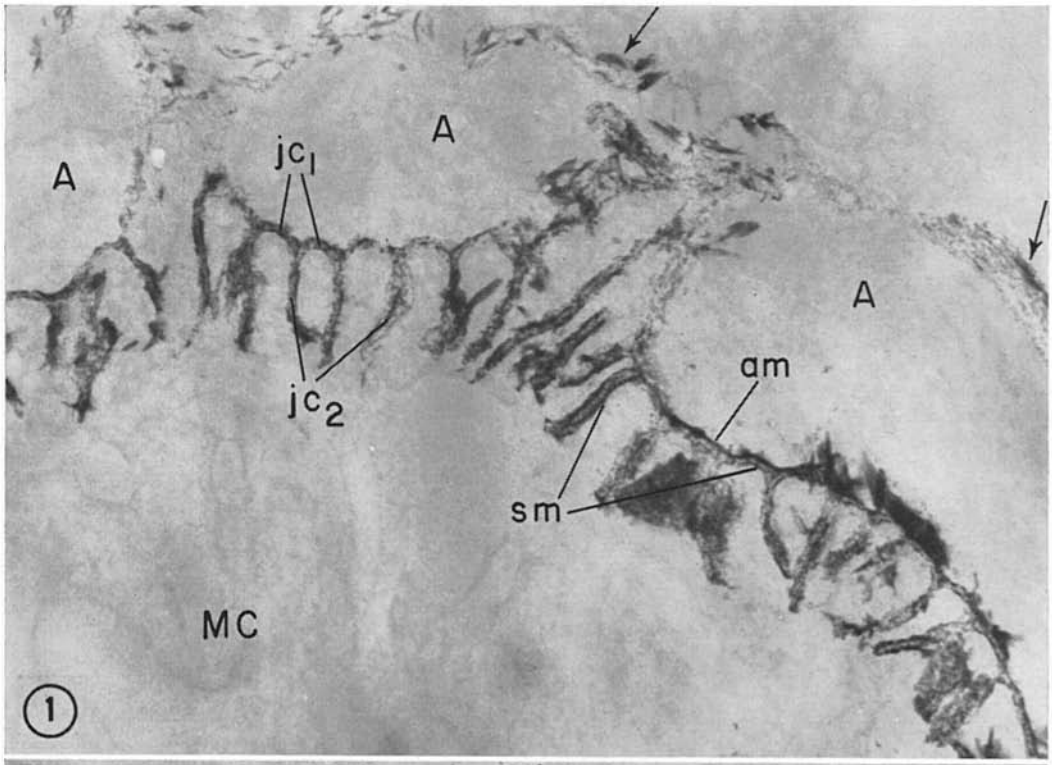
All electron micrographs show sections of MEP's of mouse intercostal muscle stained for AChE, ChE, or both, by the AuThCh or AuThAc method, followed by brief fixation in OsO_4, as described in the text. Enzyme localized, method, substrate, inhibitor, incubation time, and magnification are indicated for each figure.

FIGURE 1 Three portions of the axonal terminal (*A*) make junctional contact with the muscle cell (*MC*). Enzymatic reaction product is present principally at the prejunctional axonal membrane (*am*) and postjunctional sarcoplasmic membrane (*sm*) of the muscle cell. Moderate amounts of deposit are also in the primary (jc_1) and secondary (jc_2) junctional clefts. Deposit at the external surface (arrows) of the axonal terminal is less well localized, but probably represents axon-to-Schwann cell or Schwann cell–to–Schwann cell apposition. AChE, AuThCh method. AThCh; DFP; 3-min incubation; × 31,000.

FIGURE 2 Reaction product much less dense than in Fig. 1, but showing localization at prejunctional (*am*) and postjunctional (*sm*) membranes, with minimal deposit accumulated within the primary (jc_1) and secondary (jc_2) junctional clefts. Labeled as in Fig. 1. AChE; AuThCh method. AThCh; DFP; 10-sec incubation; × 31,000.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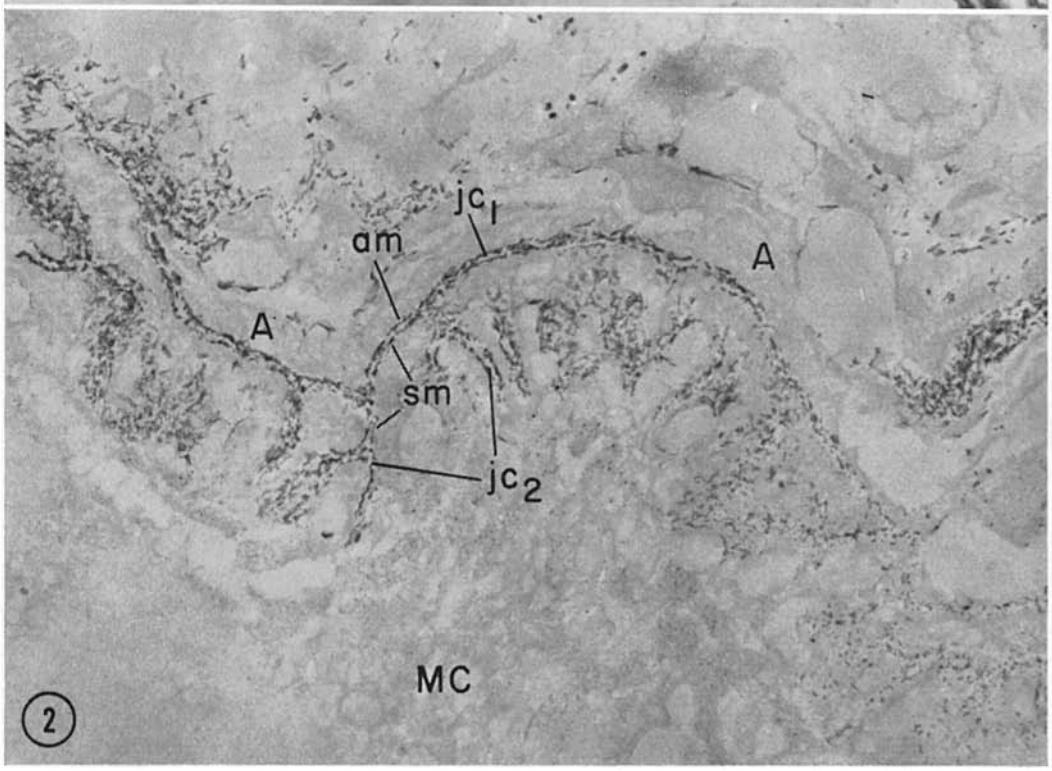
jc1
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10^{-5}M 1,5-*bis*(4-allyl dimethylammoniumphenyl)-pentan-3-one diiodide (BW284) in the preincubation and incubation solutions. Accordingly, with this method sections were treated by four modifications, as in the earlier light microscopic studies: (*a*) incubation with AThCh, for the demonstration of AChE and ChE; (*b*) incubation with AThCh following inhibition of ChE by DFP for the demonstration of AChE; (*c*) incubation with BuThCh plus BW284 for the demonstration of ChE; and (*d*) incubation with BuThCh following DFP as a control.

As noted previously (26), BW284 cannot be used for the selective inhibition of AChE by the AuThAc method, presumably because it combines with the enzyme only at the anionic site, whereas ThAc reacts only at the esteratic site (34). However, it was discovered that ambenonium chloride, another *bis*-quaternary ammonium compound which inhibits AChE selectively, is effective for this purpose, probably because a portion of its *bis*carbamate group blocks the access of ThAc to the esteratic site. In preliminary light microscopic studies, pretreatment with $3 \cdot 10^{-7}$M DFP and incorporation of 10^{-6}M ambenonium in the preincubation and incubation solutions blocked completely staining of the MEP's by the AuThAc method in 10 μ cryostat sections. However, with thicker sections (20–100 μ) treated identically, there was distinct residual staining, apparently as a result of the limited penetration of the tissue by ambenonium. The same limitation applied to strips of fixed, unsectioned muscle as employed in preliminary electron microscopic studies. It was found that this factor could be overcome by the following procedure. Mice were injected subcutaneously with atropine sulfate (10.0 mg per kg, subcutaneously), followed 10 min later with ambenonium (20 μmoles per kg, interperitoneally); death, following characteristic convulsions, occurred usually 5–10 min after the latter injection. The rib cage was removed and fixed as usual, but 10^{-6}M ambenonium was incorporated in the fixative and rinse solutions, as well as in the preincubation and incubation solutions. When 100-μ cryostat sections for light microscopy, or muscle strips for electron microscopy (see Results), so treated were also incubated with $3 \cdot 10^{-7}$M DFP (containing 10^{-6}M ambenonium) between the rinse and preincubation solutions, staining of MEP's by the AuThAc method was blocked completely, but was still obtained when DFP treatment was omitted. In the light microscopic studies, Pb^{++} was also used in place of Au^{+} to confirm these results because of the much greater intensity of staining obtained with the former metal.

The following combination of inhibitors was employed for electron microscopic studies with the AuThAc method: (*a*) no inhibitor, (*b*) $3 \cdot 10^{-7}$M DFP, for selective inhibition of ChE, (*c*) 10^{-6}M ambenonium, under the conditions described above, for the selective inhibition of AChE, (*d*) DFP plus ambenonium, and (*e*) 10^{-5}M eserine for inhibition of both ChE and AChE.

RESULTS

AuThCh Method

After the inactivation of ChE with $3 \cdot 10^{-7}$ M DFP, optimal staining for AChE at the MEP was obtained by incubating sections with AThCh for approximately 3 min. Under these conditions, structural staining was restricted to the axonal membrane and the postjunctional sarcoplasmic membrane, including the full length of the infoldings of the latter; irregular and varying amounts of the reaction product were present within the junctional cleft and its prolongations within the infoldings (Fig. 1). Interrupted, irregular staining at the same sites was obtained after incubation periods as short as 10 sec (Fig. 2). With longer incubation periods (10 or 15 min), the amount of deposit at all these sites was progressively increased, and the cleft was essentially filled. Traces of deposit were noted at the vicinity of the teloglial Schwann cells, but its localization at their membranes or at that of the adjacent axolemma was uncertain. No staining was noted at the synaptic vesicles or mitochondria, or in the immediately adjacent aneural portions of the muscle fiber.

The distribution of ChE, as revealed by incubation with BuThCh in the absence (Fig. 3) or presence (Fig. 4) of 10^{-5} M BW284 for approximately the same time course, was similar to that of AChE, but in addition there was intense staining of the superficial teloglial cells. The relative activity at the latter site was considerably greater than at the pre- and postjunctional membranes, as illustrated in Fig. 3, where there is intense, diffuse staining of the teloglial elements but the junctional cleft is mostly unstained. As with AChE, there was no evidence of ChE activity in the nearby adjacent regions of the muscle fiber at the incubation periods employed.

The identities of AChE and ChE at the sites described above were confirmed by the absence of detectable staining which resulted when both DFP and BW284 were used in the same concentrations, or when 10^{-5} M eserine was included during incubation with either substrate over the same time periods.

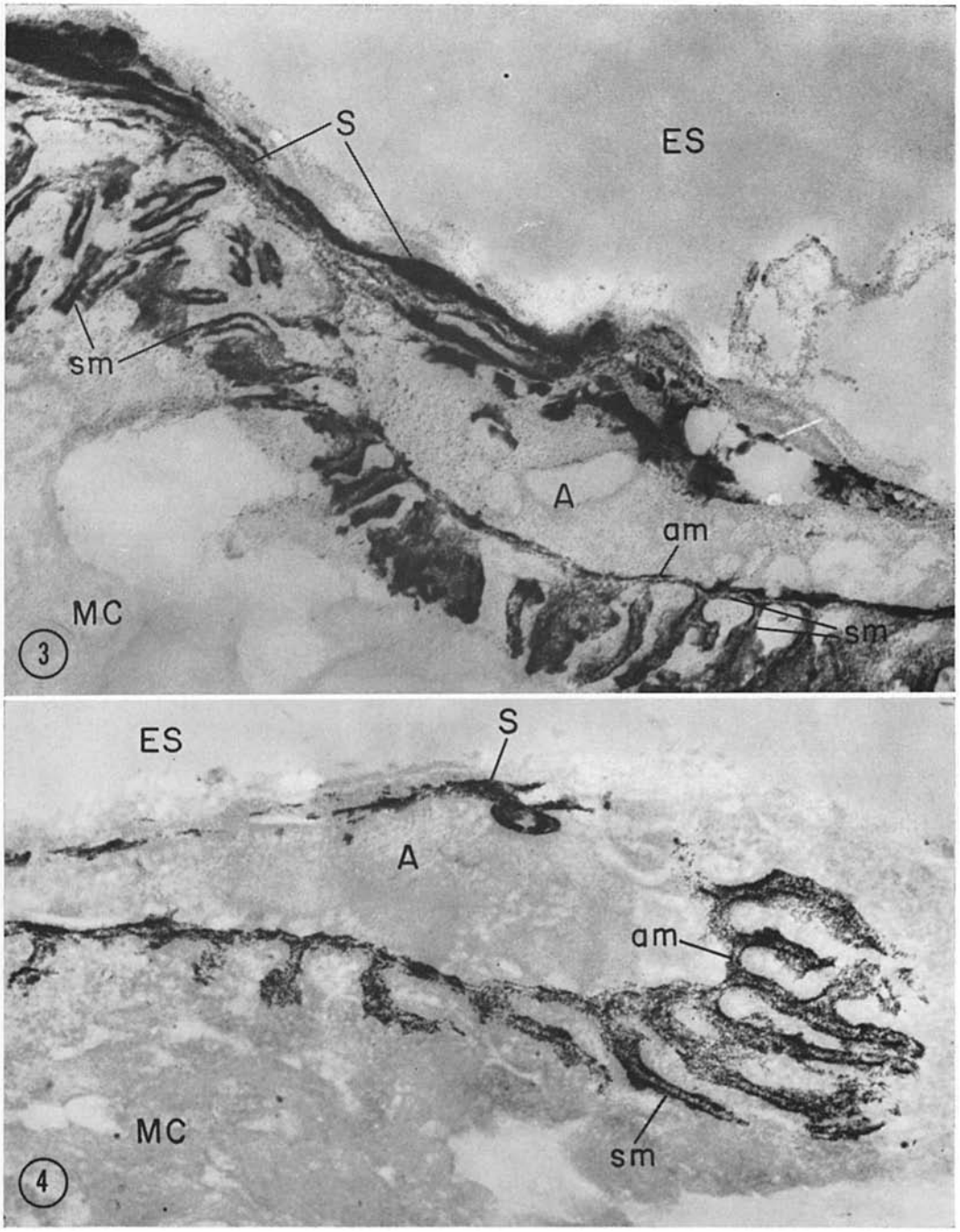


FIGURE 3 Heavy deposit seen in folds of the postjunctional membrane (*sm*), at prejunctional membrane (*am*) and in the area of the teloglial Schwann cell sheath (*S*). Other items labeled: *A*, axonal terminal; *MC*, muscle cell; *ES*, extracellular space. ChE; AuThCh method. BuThCh; 3-min incubation; × 31,000.

FIGURE 4 Same distribution of precipitate as in Fig. 3, but less dense. Labeled as in Fig. 3. ChE; AuThCh method. BuThCh; BW284; 2-min incubation; × 41,000.

AuThAc Method

There were two major differences between the results obtained with the AuThCh method, described above, and the AuThAc method: the much sharper localization obtained with the latter, and the considerably longer incubation periods required with it for equivalent intensity of staining. Both these factors are consistent with previous light microscopic observations (26). The difference in sharpness of localization is due chiefly to the relative dimensions of the primary reaction products. The slower development of staining with the AuThAc method results both from the much lower velocity of the reaction of the enzyme with the substrate, and from the lower incubation temperature (1°–3°C vs. 22°–24°C) employed.

Following incubation with ThAc for 1 hr in the absence of inhibitors, staining occurred along the pre- and postjunctional membranes and in the teloglial cells, as illustrated in Figs. 5 and 6. When incubation was prolonged to 2 hr, there was in addition considerable accumulation of reaction product within the junctional cleft (Fig. 7); in this micrograph, staining of the superficial teloglial elements is more evident. As with the AuThCh method, the remaining structures in the immediate vicinity of the MEP remained unstained.

Pretreatment with $3 \cdot 10^{-7}$ M DFP for the selective inactivation of ChE eliminated staining of the teloglial cells, and increased the time required for staining the junctional membranes at various degrees of intensity (Figs. 8 and 9). In individual sections, the intensity of staining of the prejunctional axonal terminal membrane for AChE showed some variation, probably depending upon its state of preservation, but in general it was somewhat lighter than the postjunctional membrane, including the infoldings of the latter.

Following treatment with ambenonium in vivo and in vitro, as described under Methods, staining for ChE occurred at the teloglial cells at a considerably greater relative intensity than at the pre- and postjunctional membranes (Fig. 10). It was also noted occasionally that staining of the sarcoplasmic membrane extended for some distance beyond its immediate postjunctional region; this is seen in Fig. 11, where the membrane is stained between two portions of the postjunctional folds.

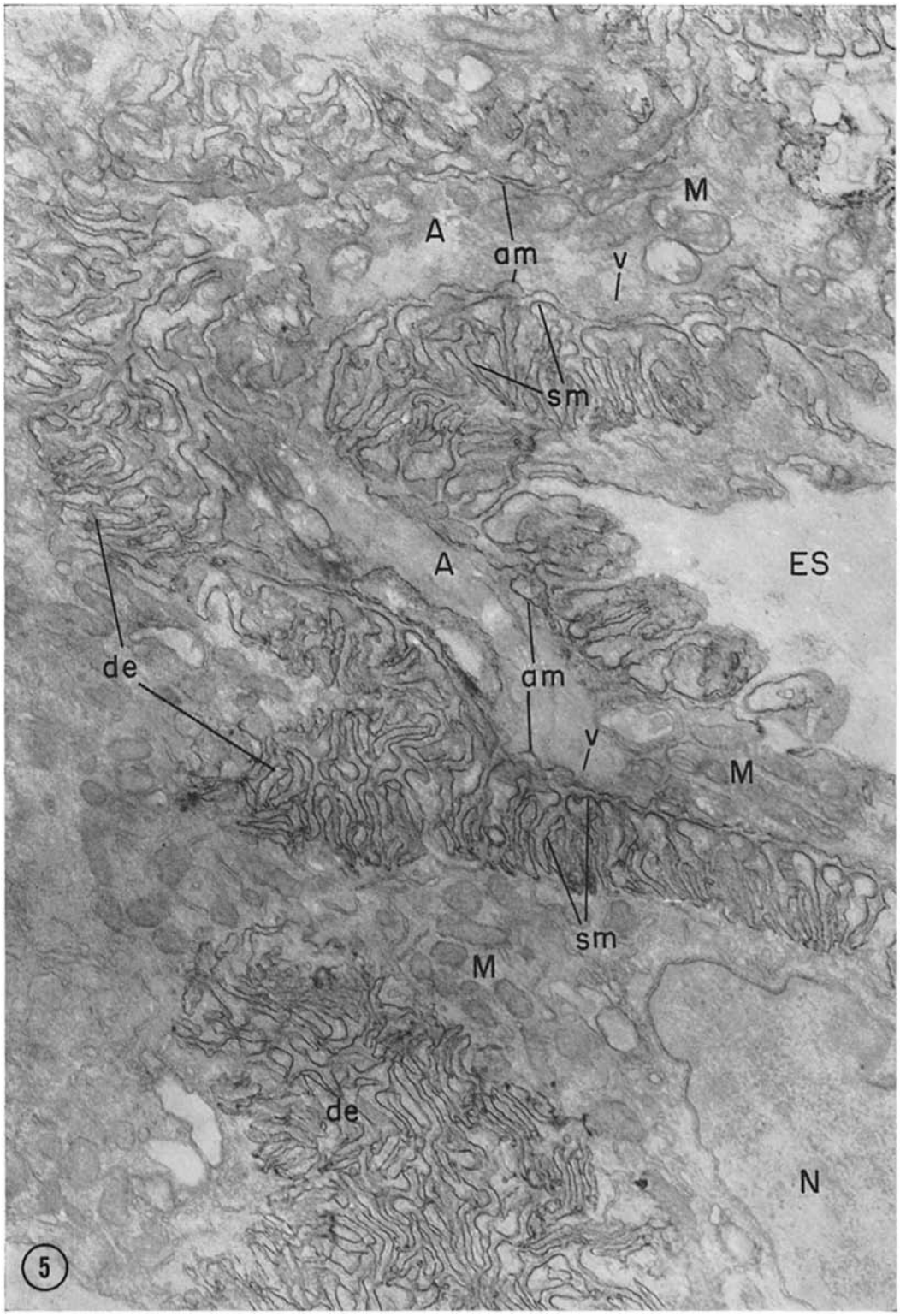
Treatment with $3 \cdot 10^{-7}$ M DFP plus 10^{-6} M ambenonium (Fig. 12) or with 10^{-5} M eserine (Fig. 13) eliminated completely the staining of the foregoing structures, thus confirming the identities of AChE and ChE at the sites mentioned.

DISCUSSION

The present results bear out the preliminary indications from light (26) and electron (11, 25) microscopic studies of the comparison of the AuThCh and AuThAc methods with respect to several criteria: (*a*) fineness of localization; (*b*) penetration of reagents; (*c*) velocity of the staining reactions; and, insofar as applicable here, (*d*) specificity.

While the deposit obtained with the AuThCh method is not nearly as fine as that seen with the AuThAc procedure, early comparisons in our laboratory showed the former to be far superior to the standard copper-thiocholine method with respect to both the dimensions and electron opacity of the precipitate. The appearance of the precipitate at the pre- and postjunctional membranes of the motor end plate with the AuThCh method appears comparable with that obtained at dorsal root ganglia (30) and sciatic nerve (32) by the ferrocyanide modification of the copper-thiocholine method (17). On the other hand, the substitution of Au^{+} for Pb^{++} in the ThAc method has resulted in much finer localization of enzymatic activity at the membranes of the motor end plate, with practically no deposit within the clefts ex-

FIGURE 5 Two large portions of the innervating axon (*A*) are visible. The prejunctional axonal membrane (*am*) shows activity over its entire length. The postjunctional membrane (*sm*) is also well stained with the reaction product, both where it is immediately apposed to the axon and throughout the junctional folds. In a few areas the plane of section passes through the depths of the junctional folds (*de*), so that their relationship to the innervating axon is not depicted. Nucleus (*N*), mitochondria (*M*), and cytoplasmic membranes are visible in the muscle cell. Synaptic vesicles (*v*) and mitochondria (*M*) are present in the axoplasm. *ES*, extracellular space. AChE and ChE; AuThAc method. ThAc; 1-hr incubation; × 16,000.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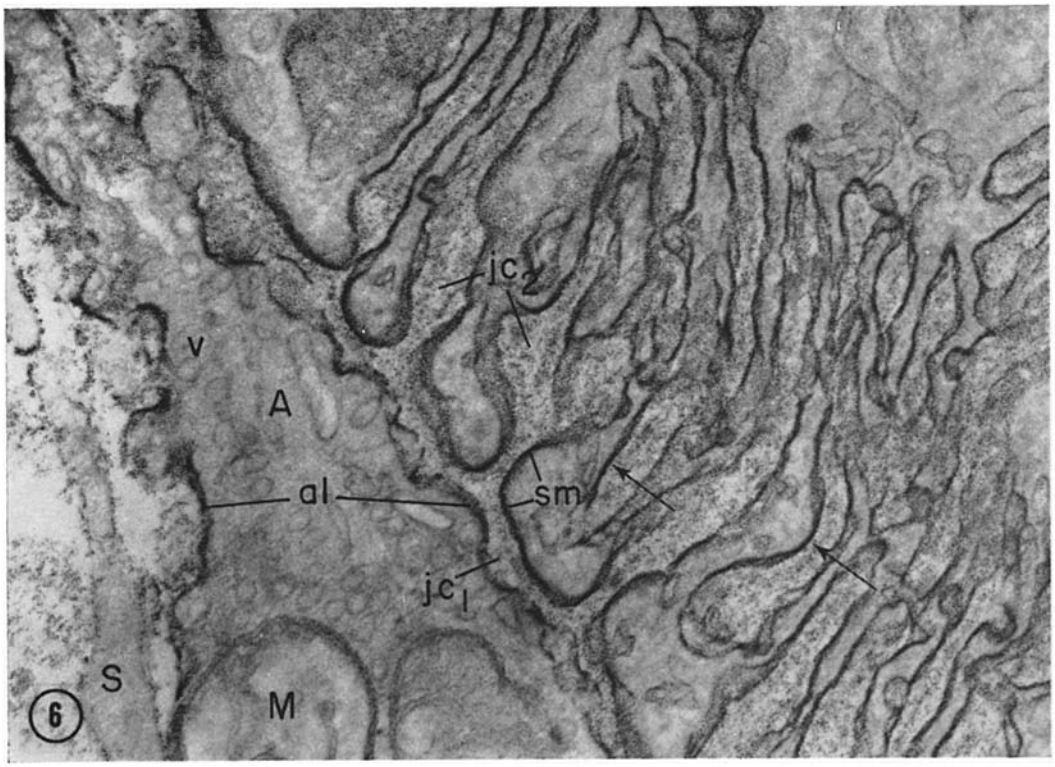

FIGURE 6 A higher magnification view of the junctional complex, showing the axonal terminal (*A*) containing mitochondria (*M*) and numerous synaptic vesicles (*v*), the junctional cleft, and junctional folds of the sarcolemma (*sm*). The axolemma (*al*) exhibits marked activity both on the surface facing the primary junctional cleft (jc_1) and at the surface facing the teloglial Schwann cell sheath (*S*) (the axonal terminal is somewhat separated from the Schwann cell in this micrograph). At this magnification, the Au_2S deposit appears as electron-opaque particles about 40–50 A in diameter. Where the plane of section is perpendicular to the sarcolemma (arrows), the particles form a dense line about 120–140 A in thickness. A few particles are also present in the primary (jc_1) and secondary (jc_2) junctional clefts, possibly indicating some diffusion of the reaction product. AChE and ChE; AuThAc method. ThAc; 1-hr incubation; × 63,000.

cepting after prolonged incubation (see reference 1).

It is pertinent to consider here the conversion of the mercaptide salts to the corresponding sulfides by treatment with $(NH_4)_2S$, as employed in both the standard copper-thiocholine and the present AuThCh methods. As was pointed out in conjunction with light microscopic studies some years ago, this step entails some risk of translocation of the precipitate (35); to avoid this possibility, a modification was introduced in which the final conversion was omitted (16). However, it is extremely hazardous to employ this modification for electron microscopy without assurance that the mercaptide salt remains unaltered during the subsequent steps of fixation in OsO_4, dehydration, embedding, and sectioning, as demonstrated recently (4). The thiocholine salts of both copper and gold are significantly soluble in aqueous-alcoholic solutions; the main purpose of the high concentrations of $SO_4^{=}$ and $PO_4^{\equiv}$ included in the respective incubation media is to effect precipitation of the minute amounts of the mercaptides as rapidly as possible after their formation, by exceeding the solubility products of their corresponding sulfate and phosphate salts. As described previously (26), in the present AuThCh method the conversion of the mercaptide salt to highly insoluble Au_2S is

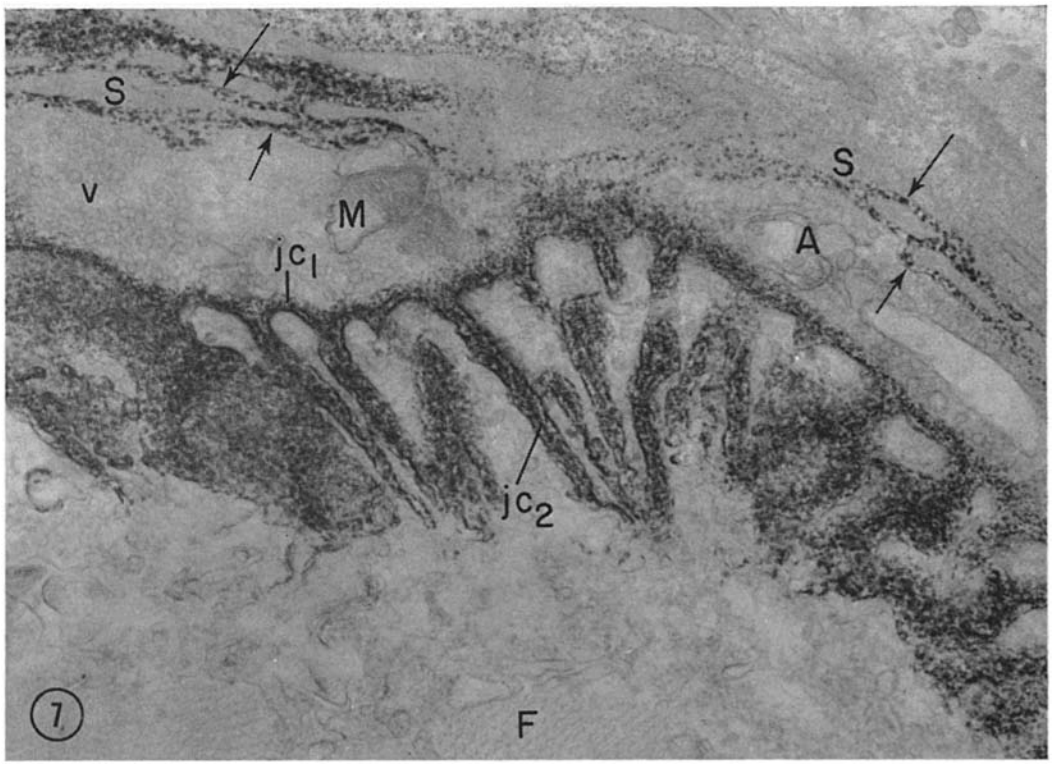

FIGURE 7 Primary (jc_1) and secondary (jc_2) junctional clefts are practically filled with end product after prolonged incubation. Plasma membranes of the axon and teloglial Schwann cell (*S*) process as well as the space between (short arrows) are coarsely stained. Where cell processes of Schwann cell are in close contact (long arrows), plasma membranes and intervening spaces are also stained. *M*, mitochondria; *v*, vesicles in the axon; *F*, myofilaments in the sarcoplasm; *A*, axonal terminal. AChE and ChE; AuThAc method. ThAc; 2-hr incubation; × 31,000.

achieved by immersion of the sections in an acid-alcoholic solution of $(NH_4)_2S$, modified from that suggested by Bull et al. (5) for the copper-thiocholine method. Insofar as could be determined by observation with light and phase-contrast microscopy at 1000 × magnification, this effected no change in the dimensions or location of the precipitate.

The relative effectiveness with which ThAc, its gold complex, and DFP penetrate formaldehyde-fixed tissue permitted the use of unfrozen strips of muscle with the AuThAc method, which probably accounts in part for the superior preservation of structural integrity seen in most of the electron micrographs, in comparison with those obtained with the AuThCh method. Nevertheless, satisfactory localization was obtained generally at the MEP's of only the most superficial one to three layers of musle fibers; at deeper levels, staining was irregular or absent. Adequate penetration of ambenonium, the *bis*quaternary inhibitor of AChE, required the elaborate steps described under Methods. Previous reports have emphasized the importance of adequate prior exposure of the sections or strips of tissue to a preincubation solution, containing essentially all constitutents of the incubation solution excepting the substrate, in order to insure the immediate capture of the initial enzymatic reaction product following introduction of the substrate (7, 19). With the AuThAc (or lead–thiolacetic acid) method, the preincubation solution should include also ThAc in a concentration equivalent stoichiometrically to that of the capturing agent (but considerably below its final concentration as the substrate), since the heavy

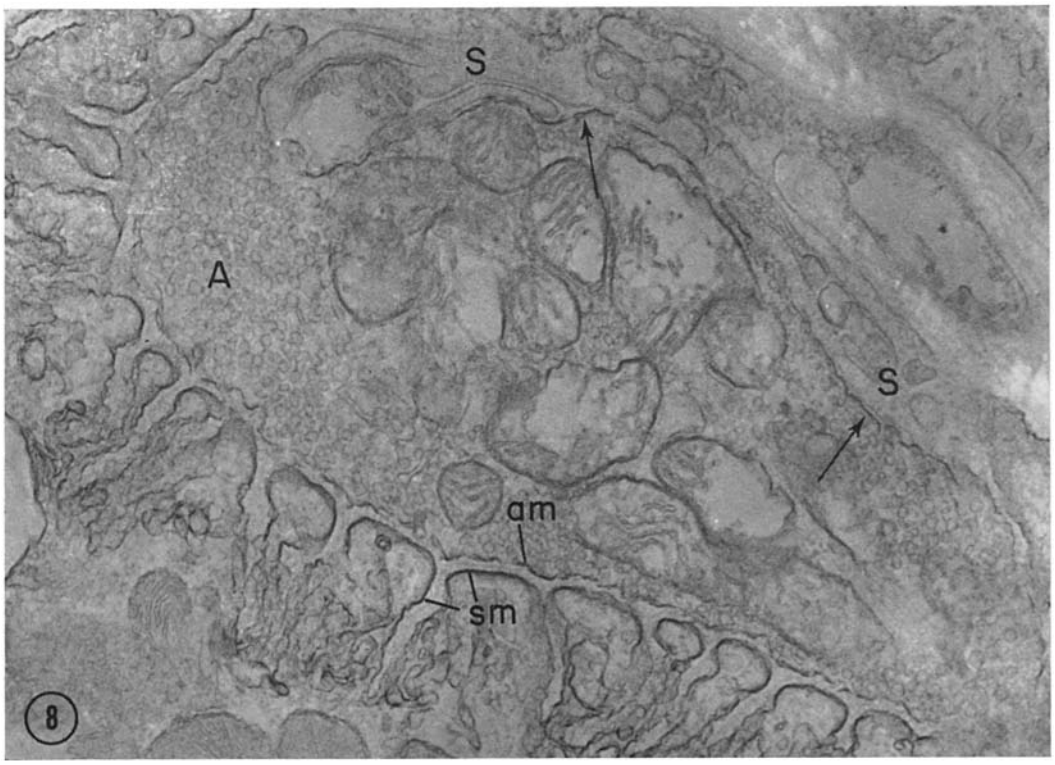

FIGURE 8 The remaining AChE activity is represented by distinct staining of the postjunctional membrane (*sm*). The prejunctional membrane (*am*) appears somewhat more lightly stained. In areas where the axolemma is facing (arrows) a Schwann cell (*S*), the axolemma is lightly stained, but the plasma membrane of the Schwann cell is unstained. *A*, axon. AChE; AuThAc method. ThAc; DFP; 100-min incubation; × 31,000.

metal probably penetrates far more readily as the thiolacetate complex (15).

In the AuThCh method, the penetration of frozen sections by Au^+ and the *bis*quaternary AChE-inhibitor, BW284, during the preincubation period, and of the thiocholine substrates immediately following immersion in the incubation solution appeared to be satisfactory. Thus, staining for AChE was detectable after incubation periods as short as 10 sec, whereas in sections treated with BW284 following DFP, staining was prevented with either AThCh or BuThCh as substrate. The reagents used in the standard copper-thiocholine method have been reported to penetrate to the endoplasmic reticulum and nuclear envelope of peripherally located neuroblasts in blocks of fixed embryonic rabbit neural tube (33) and neurons of fixed frog ganglia (4). In the ferricyanide modification (17), the ferricyanide ion itself appears to have the most limited penetrating ability, which could be overcome by osmotic shock or brief freezing with sections of spinal cord (2), but apparently not with cerebellar sections (31).

The relative incubation periods required for staining for AChE and ChE by the AuThCh and AuThAc methods are consistent with the corresponding reaction velocities for the enzymatic hydrolysis of their substrates, as determined quantitatively. The enzymes have extremely high turnover-numbers for the thio-analogs of the choline esters, exceeding those for the choline esters themselves (18), whereas ThAc is hydrolyzed by AChE relatively slowly (34). Furthermore, the AuThAc method was conducted at a temperature approximately 20°C lower than that used with the AuThCh method; if it is assumed that the Q_{10} values are approximately three, this would account for a further difference of approximately

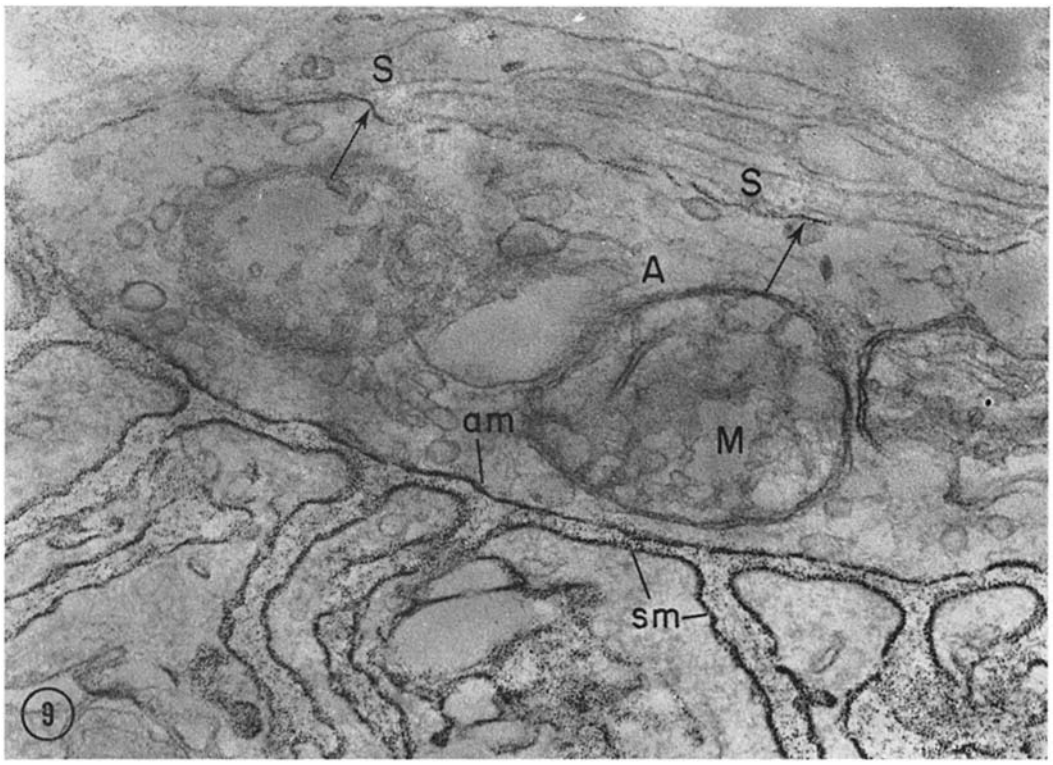

FIGURE 9 The prejunctional (*am*) and postjunctional (*sm*) membranes are distinctly stained. Moderate staining is also present in the axonal plasma membrane (arrows) facing the teloglial Schwann cell (*S*) covering. *M*, mitochondria; *A*, axon. AChE; AuThAc method. ThAc; DFP; 2 hr incubation; × 63,500.

tenfold. Accordingly, it is not surprising that optimal results were obtained with the former method at 1–3 min, and with the latter at 1–3 hr. A report of results to the contrary (3) may have been owing to the loss of much of the unconverted mercaptide salt in the thiocholine method during dehydration and embedding, as discussed above.

The relative specificities of the AuThCh and AuThAc methods are not readily apparent from the present study, since AChE and ChE are the only esterases that are selectively concentrated at the MEP (12). Accordingly, with the selective inhibition of either enzyme, only the other would be expected to cause staining in this region with the limited incubation periods employed. In autonomic ganglia, in contrast with the MEP, considerable residual staining occurred after treatment with inhibitors of both AChE and ChE with the AuThAc but not with the AuThCh method (26). It is at such sites that the combined use of the two methods should prove particularly useful.

Although the presence of significant amounts of ChE at the MEP has been noted previously (12, 14, 16, 26), it was of considerable interest to find that it is present in the pre- and postjunctional membranes as well as in the teloglial cells. In the sympathetic ganglia of the cat, ChE is present only in the Schwann sheath and satellite cells, which are the analogs of the teloglial cells, but in the rat the enzyme occurs in some ganglion cells also (20, 21). It is generally assumed that ChE is not involved physiologically in the hydrolysis of acetylcholine; however, no other function has yet been demonstrated for it (23).

The extension of staining of the sarcoplasmic membrane beyond the immediate subneural region obtained with the AuThAc method in the presence of ambenonium (Fig. 11) should be regarded only as a chance observation, since it was not noted consistently under similar conditions and no systematic search has yet been made for staining at this region with any of the staining modifications

employed here. In most types of striated muscle studied, the concentration of AChE at the non-innervated portion of the sarcoplasmic membrane is extremely low in comparison with that of the innervated area (13, 23, 29); certain exceptions have been noted, such as intrafusal fibers (6) and the swimming muscles of some species of fish (28). The absence of staining from sites in the immediate vicinity of the MEP other than those described in the present study does not preclude the possible presence there of AChE or ChE in concentrations below those detectable at the incubation periods employed.

With both the AuThCh and AuThAc methods, the concentrations of AChE and ChE appeared in general to be somewhat higher at the post- than at the prejunctional membrane. With the much greater surface area of the postjunctional membrane, based on its extensive infoldings as the secondary clefts, the absolute amount of AChE present postjunctionally is far greater, as indicated previously by extensive light microscopy studies (8, 10). This raises the interesting question of what role the infoldings play in the process of transmission: are receptors, in addition to AChE and ChE, located along their full length, or do they merely provide a reserve of AChE, or a sufficient extracellular volume to serve as a source of ions for the current that flows through the postsynaptic membrane during depolarization? The physiological implications of the relative concentrations of AChE at the pre- and postjunctional membranes of the motor end plate in comparison with various synaptic sites have been discussed in detail in previous publications (22, 23, 27). Further consideration of this matter awaits the electron microscopic histochemical investigation of mammalian autonomic ganglia, which is now in progress.

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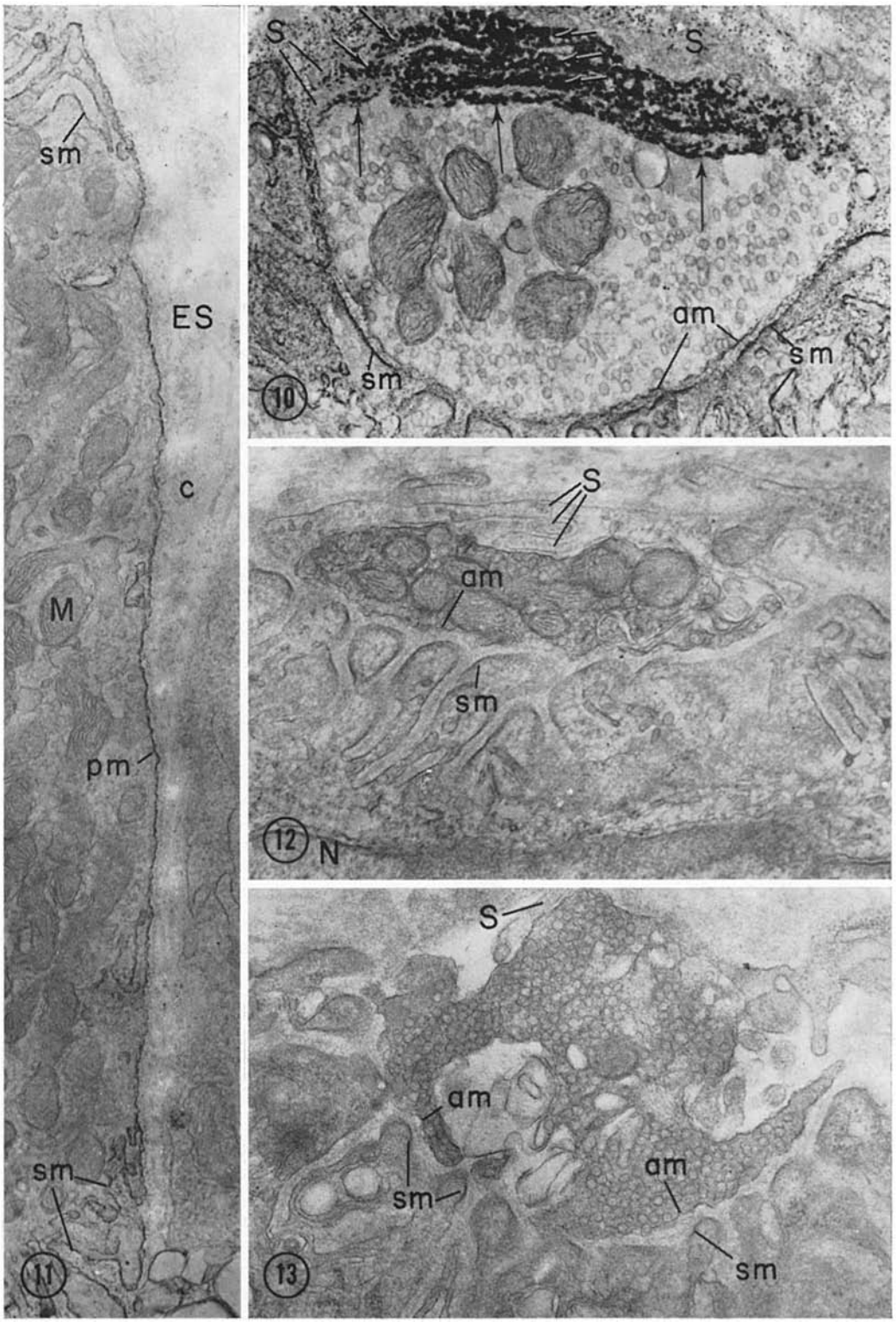
FIGURE 10 Moderate amounts of deposit are present at the prejunctional membrane (*am*) and throughout the junctional folds (*sm*). Irregular lines of very heavy deposit are present at the apposition (long arrows) of axon to teloglial Schwann cell (*S*) membranes, and between (short arrows) Schwann cell processes. ChE; AuThAc method. ThAc; ambenonium; 4-hr incubation; × 31,000.

FIGURE 11 This micrograph demonstrates ChE activity of the muscle plasma membrane (*pm*) between two areas of junctional contact. Junctional folds (*sm*) are distinguishable in upper and lower extremes of the picture. Numerous mitochondria (*M*) are present in the sarcoplasm. Collagen fibers (*c*) are present in the extracellular space (*ES*). ChE; AuThAc method. ThAc; ambenonium; 4-hr incubation; × 31,000.

FIGURE 12 Plasma membranes of the muscle cell (*sm*), the axonal terminal (*am*), and of Schwann cell processes (*S*) are without deposit. *N*, muscle cell nucleus. Control; AuThAc method. ThAc; DFP plus ambenonium; 4-hr incubation; × 31,000.

FIGURE 13 All ThAc-hydrolyzing activity is inhibited at prejunctional (*am*), postjunctional (*sm*), and Schwann cell (*S*) membranes. Control; AuThAc method. ThAc; eserine; 1-hr incubation; × 31,000.


sm
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