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Characterization of Surface Electrical Activity Recorded from Adult Male Schistosoma Mansoni

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CHARACTERIZATION OF SURFACE ELECTRICAL ACTIVITY RECORDED FROM ADULT MALE SCHISTOSOMA MANSONI

By

David Robert Semeyn

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ABSTRACT

CHARACTERIZATION OF SURFACE ELECTRICAL ACTIVITY
RECORDED FROM ADULT MALE SCHISTOSOMA MANSONI

By

David Robert Semeyn

Spontaneous electrical activity can be recorded by means of suction electrodes placed on the dorsal surface of adult male Schistosoma mansoni. This electrical activity is a complex of bi- and triphasic potentials which range from smaller amplitude waves (0-40 μV) of high frequency (10-40/sec), to randomly occurring larger amplitude waves (40-1000+ μV) of lower frequency (0-5/sec). Many of the larger potentials (>100 μV) appear to be propagated. Regional variations of this activity exist, with the posterior region consistently exhibiting potentials greater than 120 μV, while at the anterior region activity greater than 40 μV is only rarely seen. Decreased concentrations of Ca^{2+} (0.00, 0.14, and 0.52 mM) or elimination of Ca^{2+} plus addition of $5x10^{-4}$M EGTA, increased concentrations of Mg^{2+} (3.0, 10.0, and 30.0 mM), or addition of 1 mM $CoCl_2$ significantly decreased the level of electrical activity. Drug concentrations of $1x10^{-8}$M carbachol, $1x10^{-6}$M metrifonate, $1x10^{-8}$M dopamine, $1x10^{-5}$M pentobarbital, and $1x10^{-5}$M antimony tartrate also significantly decreased electrical activity. In contrast, 5-HT ($1x10^{-7}$M) significantly increased the level of electrical activity.

To Mom and Dad

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INTRODUCTION

The blood fluke, Schistosoma mansoni, is a digenetic trematode which infects humans during the cercarial stage of its life cycle. Adult schistosomes migrate to and live in the mesenteric and portal venous system. Eggs deposited by the adult female in mesenteric capillaries are responsible for most of the pathology associated with the disease (Nobel and Nobel, 1976). A large portion of the world's population is infected by some form of schistosomiasis, making this trematode a medically important parasite.

Several approaches are possible in assessing the effects of experimental treatments on nerve or muscle in Schistosoma mansoni. Biochemical and histochemical studies have provided considerable information about putative neurotransmitters present in schistosomes (Barker et al., 1966; Bennett et al., 1969; Bennett and Bueding, 1971; Chou et al., 1972; Hillman and Senft, 1975) but give no information about the action of these compounds on nerve or muscle activity. Photoelectric and ultrasonic methods measuring overall movements of schistosomes have also been reported (Brown et al., 1973; Hillman and Senft, 1973). These methods also provide little information about activity in the nervous system or about details of locomotory behavior.

The method described by Fetter et al. (1977, 1978), which allows one to record spontaneous electrical activity by means of a suction electrode placed on the surface of the parasite, avoids some of the difficulties described above. This thesis describes studies examining the nature of the electrical activity recorded in this way from the surface of adult male schistosomes. If this activity has its genesis in the nervous system of the parasite, monitoring this activity may provide a sensitive means for examining subtle effects which experimental treatments have on the nervous system of this animal, effects which may not be overtly manifested as changes in the motor activity of the animal.

General Anatomy

Adult male Schistosoma mansoni are approximately 1 cm long, 0.2 cm wide and have a wet weight of 0.8 mg. Adult females are slightly longer (1.5 cm) and much thinner (0.2 mm) than males (Figure 1). Rostrally located ventral and anterior (oral) suckers are present in both male and female. The flat body of the male is curved to form a ventral, longitudinal groove, the gynecophoric canal, within which the adult female normally lies. Paired intestinal ceca converge and fuse at the midpoint of the adult animal, and then continue as a single gut posteriorly (Schmidt and Roberts, 1977).

The Tegument

The outer covering of Schistosoma mansoni, called the integument or tegument, is a rough surfaced structure with numberous spines which appears to be an anatomic syncytium of anuclear material (Figure 2)

Figure 1. Scanning electron micrograph of male and female Schistosoma mansoni. Female is lying within the gynecophoric canal of the male. OS, oral sucker; VS, ventral sucker; GC, gynecophoric canal; M, male; F, female; P, posterior. Calibration bar: 0.8 mm. Kindly supplied by C.S. Bricker, Michigan State University.

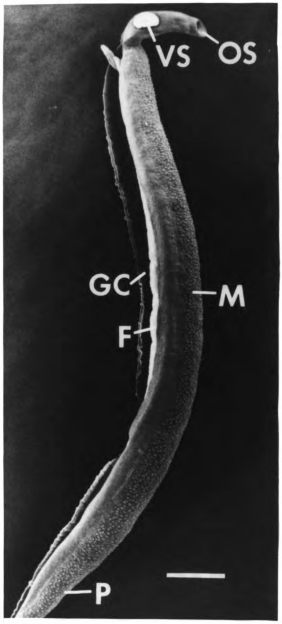

Figure 1

Figure 2. Schematic of tegument and muscle layers in Schistosoma mansoni. TEG, tegument; OM, outer membrane; IM, internal membrane of the tegument; S, tegumental spine; BL, basal lamina; IMa, interstitial matrix; CM, circular muscle; LM, longitudinal muscle; CC, cytoplasmic channel; TC, tegumental cyton; N, nucleus; JC, junctional complex; ECS, extracellular space.

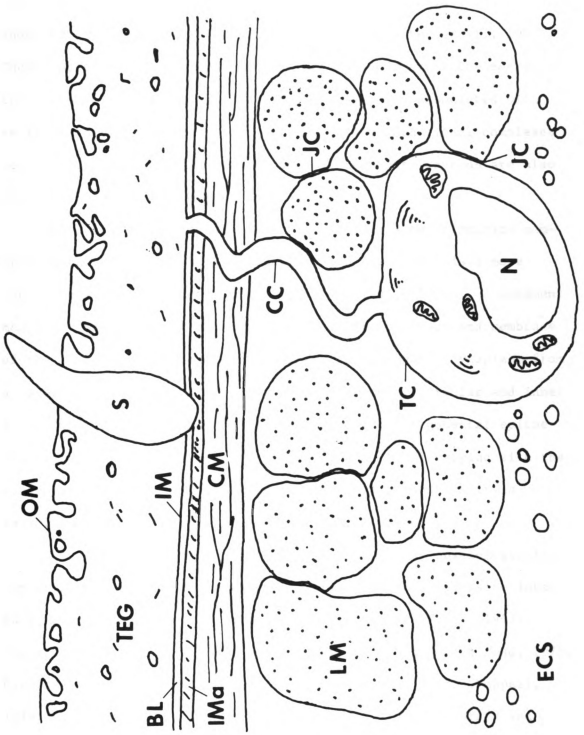

Figure 2

(Silk et al., 1969). This outer anuclear epithelium is in cytoplasmic continuity via channels with tegumental cytons situated beneath the underlying musculature (Silk and Spence, 1969; Fetter et al., 1980). These nucleus-containing cytons manufacture and secrete the matrix of the tegument and possess many organelles generally associated with secretory processes (Wilson and Barnes, 1974). Junctional complexes between muscle cells and the membranes of tegumental cytons are also observed (Silk et al., 1969).

The distal border of the outer tegument is a heptalaminate membrane with a thickness of 11 nm while the inner border is a more conventional trilaminate membrane (Hockley et al., 1975). A basement membrane (basal lamina) which lies beneath the inner plasma membrane of the tegumental epithelium, separates the tegumental cytoplasm from a layer of fibrous connective tissue, and an outer circular and inner lonngitudinal muscle layer. The thickness of the tegumental epithelium ranges from 1 to 5 μm (Wilson and Barnes, 1977) varying with the contractile state of the parasite and the region in which it is measured, in general being thicker on the dorsal surface.

The tegument has been implicated in a number of physiologically important processes. In addition to forming the host-parasite interface, carrier systems for the transport of hexoses (Fripp, 1967; Isseroff et al., 1972; Rogers and Bueding, 1975; Uglem and Read, 1975; Cornford and Oldendorf, 1979), amino acids (Senft, 1968; Chappell, 1974; Asch and Read, 1975a,b; Isseroff et al., 1976; Cornford and Oldendorf, 1979) and purine and pyrimidine compounds (Levy and Read, 1975) have been described. The outer surface of the tegumental

membrane also contains enzymes aiding in the digestion and absorption of nutrients (Pappas and Read, 1975; Lumsden, 1975).

Musculature

In general, the musculature of the schistosome resembles that of most invertebrate muscle, appearing to be of the smooth variety with no striations (Lowy and Hansen, 1962). It is located immediately beneath the basement membrane of the tegument and consists primarily of an outer, circular and an inner, longitudinal muscle layer. Radially oriented muscle fibers are also present but are well developed only in the proximal and distal areas of the acetabulum (Silk and Spence, 1969a). The female musculature is not as well developed as that of the male (Smith et al., 1969; Silk and Spence, 1969a). In the adult parasite, the longitudinal muscle layers are most prominent, especially in the dorsal surface (Smith et al., 1969).

Ultrastructural studies show that the myofibrils consist of arrays of thick (18-40 nm diameter) myofilaments surrounded by a relatively large number (8-14) of thin (5 nm diameter) filaments (Silk and Spence, 1969a). This ratio of thin to thick filaments resembles that normally observed in vertebrate smooth muscle (Perry and Grand, 1979). The thin filaments show considerable branching and cross-linking between thick and thin filaments while the thick filaments are arranged in parallel arrays (Silk and Spence, 1969a). The sarcoplasmic reticulum is poorly developed or absent but rough elements appear at scattered intervals. There is also a lack of tranverse tubules and microtubules which is characteristic of other smooth muscle preparations (Silk and Spence, 1969a).

Junctional complexes are observed between muscle cells, with the outer layers of adjoining sarcolemmas being closely apposed at a distance of 7-9 nm. These junctions are of variable length but seldom cover the total length of apposing sarcolemmas. Similar junctional complexes exist between muscle cells and the tegumental cytons (Figure 2) (Silk and Spence, 1969a).

The Nervous System

The nervous system of Schistosoma mansoni follows the same basic pattern of other trematodes as has been described by Bullock and Horridge (1965). Cholinesterase staining reveals the nervous system to consist of two pairs of anteriorly located central ganglia lying on either side of the esophagus which are joined by circumesophageal commissures. Two major pairs of nerve trunks, dorsal and ventral, extend longitudinally from the ganglia to both the anterior and posterior portions of the parasite (Fripp, 1967b). These nerve cords follow the lateral contours of the animal and are located within the parenchyma. The cords converge at the posterior end of the animal and are connected at intervals throughout their length by numerous dorsal and ventral transverse commissures. Small transverse branches of the cords project peripherally and terminate near the subtegumentary longitudinal musculature. Although no direct innervation of the tegument or the region immediately below the tegument has been observed, presumed sensory structures located within the tegument have been described (Morris and Threadgold, 1967; Silk and Spence, 1969b).

Anteriorly, a nerve cord projects from the central ganglion to innervate the oral sucker, and two branches of the ventral nerve cords innervate the ventral sucker (Fripp, 1967b).

The primary study dealing with the ultrastructure of the nervous system of the schistosome was conducted by Silk and Spence (1969b). The central ganglia contain cells with large nuclei and prominent nucleoli. These cells give rise to groups of closely packed non-myelinated axons of variable shape, size and content. Adjacent axolemmas are separated from each other and the surrounding musculature and other tissue by a 11-12.5 nm uniform layer of weakly osmiophilic cement substance. A variety of organelles are present within the predominantly electron-lucent axoplasm. Large, dense granules of 100-160 nm enclosed by a single membrane are found which may be neurosecretory in nature. These are often present in single rows or small aggregations within nerve processes investing muscle and other cells. These large granules are accompanied by large, ovoid, clear axoplasmic vesicles 50-150 nm in length. Other dense osmiophilic granules 32-90 nm in diameter found in the axoplasm appear to be similar to those containing catecholamines in other neural tissues. This type of granule is often found in association with collections of clear synaptic vesicles 20-50 nm in diameter. Other osmiophilic granules found within the axoplasm include stellate clusters resembling alpha-glycogen and individual 20 nm granules with the appearance of beta-glycogen.

Synapses between axons are observed within the central ganglia and in other regions. These synapses are characterized by accumulations

of the clear, 20-50 nm diameter type vesicles attached to the pre-synaptic membrane which is separated from the post-synaptic membrane by 9-17 nm synaptic cleft. Post-synaptic membranes are relatively devoid of cytoplasmic inclusions and appear more thickened and osmiophilic than the pre-synaptic membranes.

Neuromuscular junctions are essentially similar to the axo-axonal synapses just described. The junctional cleft is approximately 10 nm wide and is a symmetrically thickened. Post-synaptic elements consist of broadened, osmiophilic sarcolemmas of the adjoining muscle cells. In addition to the clear synaptic vesicles associated with the smaller dense granules, the large osmiophilic neurosecretory granules and their associated clear axoplasmic vesicles are also observed within the sarcoplasm of muscle cells.

Neurobiology

A number of compounds known to be neurotransmitters in other animal systems have been identified via biochemical and histochemical techniques in the schistosome. The presence and histochemical localization of acetylcholinesterases within schistosomes suggests that acetylcholine may play an important physiological role (Beuding, 1952; Fripp, 1967b). Cholinomimetic agents, e.g. carbachol, have been demonstrated to inhibit spontaneous contractions, decrease tension in the musculature, and to decrease surface electrical activity (Barker et al., 1966; Hillman and Senft, 1973; Fetterer et al., 1977). Carbachol, metrifonate and eserine have also been reported to block electrically induced muscle excitation in the schistosome (Pax et al.,

1981). These effects have led these authors to the suggestion that acetylcholine may be acting as an inhibitory transmitter in this parasite.

Monoamines have also been implicated as neurotransmitters in schistosomes. Fluorescence histochemistry and radioenzymatic assays have been used to confirm the presence of and to localize the catecholamines norepinephrine and dopamine within the nervous system of the worm (Bennett and Bueding, 1971; Chou et al., 1972; Gianutsos and Bennett, 1977). These two compounds produce a characteristic lengthening response of the parasite's musculature, an effect shown pharmacologically to be mediated via a dopamine receptor (Tomosky et al., 1974).

Fluorescence histochemistry has also revealed the distribution of 5-HT within the nervous system of the schistosome (Bennett and Bueding, 1971). Although the concentration of 5-HT in the schistosome is 10 times that of the mammalian brain (Bennett et al., 1969), synthesis of this amine via tryptophan hydroxylase could not be demonstrated (Bennett and Bueding, 1973). It appears that high affinity uptake systems for 5-HT as well as norepinephrine are present, which indicates that the worm may obtain biogenic amines from the host's plasma (Bennett and Bueding, 1973). Several studies have demonstrated 5-HT's ability to increase contractile activity in adult male schistosomes (Barker et al., 1966; Tomosky et al., 1974; Fetterer et al., 1977). Imipramine and fluoxetine, compounds known to inhibit the active transport system of 5-HT in mammalian CNS neurons, cause a marked decrease in muscle tension and contractile activity in the schistosome

(Pax et al., 1979). This same study also demonstrated fluoxetine's ability to block the excitatory effects of 5-HT on the musculature. These observations have led to the suggestion that 5-HT may be serving as an excitatory transmitter in this parasite.

It should be noted that although these putative neurotransmitters appear most concentrated in neural tissues, their distribution is not limited to the nervous system. Whether or not these substances act directly on the musculature or on structures which innervate the musculature is difficult to determine. It is possible that these substances may have functions in addition to neurotransmission. Dopamine is a good substrate for phenoloxidase (Mansour, 1958) and may be involved with egg production by the female (Seed et al., 1978).

Comparative Electrophysiology

Electrophysiology techniques employing suction electrodes have been used to study the neurobiology of a variety of invertebrate species. A number of preparations, in particular ctenophores (Horridge, 1965), coelenterate hydromedusae (Mackie and Pasano, 1968; Josephson and Schwaab, 1979), enteropneusts (Pickens, 1970) and polyclad flatworms (Koopowitz, 1973, 1975; Stone and Koopowitz, 1976; Keenan et al., 1979; Koopowitz et al., 1979b) exhibit spontaneous and evoked electrical activity that markedly resembles that recorded from the surface of S. mansoni. Amplitudes, durations and conduction velocities of potentials recorded from these preparations are comparable to those seen in the adult male schistosome by Fetterer et al. (1977).

OBJECTIVE

The present study represents an extension of the work conducted by Fetterer et al. (1977) in which a method is described which allows spontaneous electrical activity to be recorded by means of a suction electrode placed on the surface of a schistosome. The objectives of this study are 1) a more detailed characterization of the surface electrical activity recorded, 2) to explore the possible sources of the activity, and 3) to determine the applicability of this method in assessing the effects of experimental treatments on this parasite.

MATERIALS AND METHODS

Source and Maintenance of Parasitic Worms

Adult male schistosomes, Schistosoma mansoni, (St. Lucian strain) were isolated 45-60 days post-infection from the mesenteric and portal veins of infected female white mice obtained from the laboratory of Dr. J.L. Bennett, Department of Pharmacology and Toxicology, Michigan State University. Isolated worms were maintained in 1) Hank's balanced salt solution (HBS) (Grand Island Biological, New York, NY), 2) Earle's balanced salt solution (G-11; Grand Island Biological) containing 50% heat-inactivated horse serum (EBS/HS), or 3) RPMI-1640 (H-18; Grand Island Biological) containing 10% heat-inactivated fetal calf serum (RPMI-1640/FCS). All three media were buffered at pH 7.4 with 0.02 M Hepes (N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid; Sigma Chemical Co., St. Louis, MO) and contained 100 units/ml penicillin-streptomycin (Grand Island Biological). Parasites were maintained at 35°C until used and all experiments were performed within 8 hours after removal of worms from host animals.

Recording Procedures

Recordings of surface electrical activity were made by means of suction electrodes. These consisted of polyethylene tubing (PE-20; Clay-Adams, Parsippeny, NJ) pulled to a fine tip (100 μm inside

diameter). A short piece of this tubing (approximately 2 cm) was affixed to a 3/8" 26 gauge hypodermic needle connected to a 1 cc tuberculin syringe barrel containing a silver-silver chloride wire. Prior to recording, electrodes were filled with a sufficient amount of incubation medium to come in contact with the silver wire. A silver-silver chloride wire placed in the recording chamber served as a ground. The electrical signal, recorded differentially, was passed through a preamplifier (Model P-15, Grass Instrument Co., Quincy, MA; filters set at 0.3 Hz and 0.3 kHz), displayed on an oscilloscope (Model 5113, Tektronix, Inc., Beaverton, OR) and a chart recorder (Physiograph Four, Narco Biosystems Inc., Houston, TX), and then stored on magnetic tape (Model B, Vetter Instrument Co., Rebersburg, PA; recording speed 3 3/4 i.p.s.) for later analysis. In cases where mechanical activity was also monitored, the method described by Fetterer et al. (1977, 1978) was used with the recording electrode simultaneously serving as the stationary electrode.

The recording chamber consisted of a polyethylene dish containing 2.5 ml of incubation medium. The temperature of the bathing medium was maintained at 37°C with a thermoelectric heater placed under the recording chamber. A thermistor was used to monitor the temperature of the bathing medium.

Surface electrical activity was recorded by means of the suction electrode placed mid-dorsally on the male schistosome either a) at the anterior end above the ventral sucker, b) at the midbody 0.1 mm posterior to the gut bifurcation, or c) at the posterior end 0.25 mm

from the tip of the tail. After placement of the electrode, 10 minutes equilibration time was allowed before data collection was begun. All results presented are from adult male schistosomes still paired with females.

The recorded electrical activity was quantified by replaying tapes (at 3 3/4 i.p.s.) and counting all negative potentials in excess of 10 μV over 40 second intervals. Potentials less than 10 μV were not counted since they were often indistinguishable from the background electrical noise of the system. The counts were determined by passing the output of the tape recorder through a window discriminator (Model 120, WPI, Inc., Hamden, CN) connected to a digital counter. Starting with the upper limit of the window discriminator set at 10 μV, "above window" counts were determined. The upper limit was then raised in 10 μV increments up to a value of 120 μV, with "above window" counts being made at each setting. In some instances "within window" counts over the same range of 10 μV increments were also determined. From these data, amplitude histograms of the electrical activity were constructed.

Ion Substitution Experiments

The effects of altered external ion concentrations on surface electrical activity were determined by first monitoring the electrical activity from animals bathed in normal HBS for 10 minutes and then exchanging the HBS for a modified saline containing the altered ion concentration desired. Electrical activity was then monitored for a minimum of 15 minutes with one minute samples of the activity being

recorded on tape at 5 minute intervals. Control responses for these experiments were obtained in a separate group of worms in the same manner except that after 10 minutes the HBS in the recording chamber was exchanged for fresh HBS. Electrical activity was then again monitored for a minimum of 15 minutes with one minute samples of activity being recorded on tape at 5 minute intervals.

Calcium. Ca^{2+} concentrations of 0.52, 0.14 and 0.00 mM were tested. These solutions were obtained by altering or eliminating the $CaCl_2$ concentration in HBS. The effect of 0 Ca^{2+} HBS containing $5x10^{-4}$M EGTA was also tested.

Magnesium. Mg^{2+} concentrations of 30.0, 10.0 and 3.0 mM were tested. These solutions were obtained by altering the $MgSO_4$ concentration of HBS.

Cobalt. A Co^{2+} concentration of 1 mM was tested. This solution was obtained by adding the appropriate amount of $CoCl_2$ to HBS.

Pharmacological Agents

Compounds tested were carbachol (Carb) (carbamylcholine chloride), dopamine (DA) (3-hydroxytyramine hydrochloride), pentobarbital (PB) (pentobarbitone sodium), 5-hydroxytryptamine creatinine sulfate (5-HT), all from Sigma Chemical Co., St. Louis, MO; cobalt chloride ($CoCl_2$), Mallinckrodt, St. Louis, MO; metrifonate (MT), kindly supplied by Drs. P. Andrews and H. Thomas of the Bayer A.G., and potassium antimony tartrate, kindly supplied by Dr. E. Bueding of the Johns Hopkins University, Baltimore, MD. All compounds were dissolved in double

distilled water at the necessary concentrations immediately prior to use.

In these experiments, control activity was monitored for 10 minutes prior to drug addition. Twenty-five microliters of the desired solution were then added to the recording chamber to give the final concentration wanted. Electrical activity was then monitored for a minimum of 10 minutes with one minute samples of activity being recorded at 5 minute intervals during the test period. Control responses for these studies were obtained from animals which received twenty-five microliters of distilled water instead of a solution containing one of the compounds.

Statistical Procedures

All values presented in histograms and tables represent the mean $\pm$ 1 standard error of the mean (SEM) for a minimum of 6 animals unless otherwise noted. All tests for significance of difference between means were performed using Student's *t*-test (non-paired).

RESULTS

Electrical Activity Recorded

Characteristics of Spontaneous Electrical Activity

The electrical activity recordable from the adult male schistosome by way of a suction electrode is a complex of bi- and triphasic potentials occurring at a rate of about 60-70/sec with amplitudes ranging from greater than 1 mV down to values indistinguishable from the background electrical noise of the system (<10 μV) (Figures 3 and 4). Activity varies from a more or less tonic firing of mixed amplitude potentials in some preparations to more phasic and irregular bursts of potentials in others. In general, smaller potentials have durations of 4 to 20 msec with mean rise times of 7 μV/msec while larger potentials have durations ranging from 25 msec to 1.2 sec and rise times which vary from 2.1 μV/msec to 7.3 μV/msec. This electrical activity has its source within the living parasite since exposure of the worm to a high concentration of ethanol abolished all activity within 30 to 60 seconds (Figure 4). Exposure of worms to high temperature (>42°C) or overnight refrigeration also abolished all electrical activity.

Conduction of Potentials

In several preparations, simultaneous recordings were made by means of two electrodes located at varying distances apart on the

Figure 3. Chart recording showing electrical activity recorded simultaneously at two positions from worms incubated in HBS. A: electrical activity recorded from the dorsal midbody position; B: simultaneous electrical activity recorded from a point 2.0 mm posterior to A. Voltage calibration is the same for all traces. Lower sets of traces are expanded sections of upper traces over times indicated by bars. Note many but not all larger potentials recorded in one trace are correlated with similar events in the other trace.

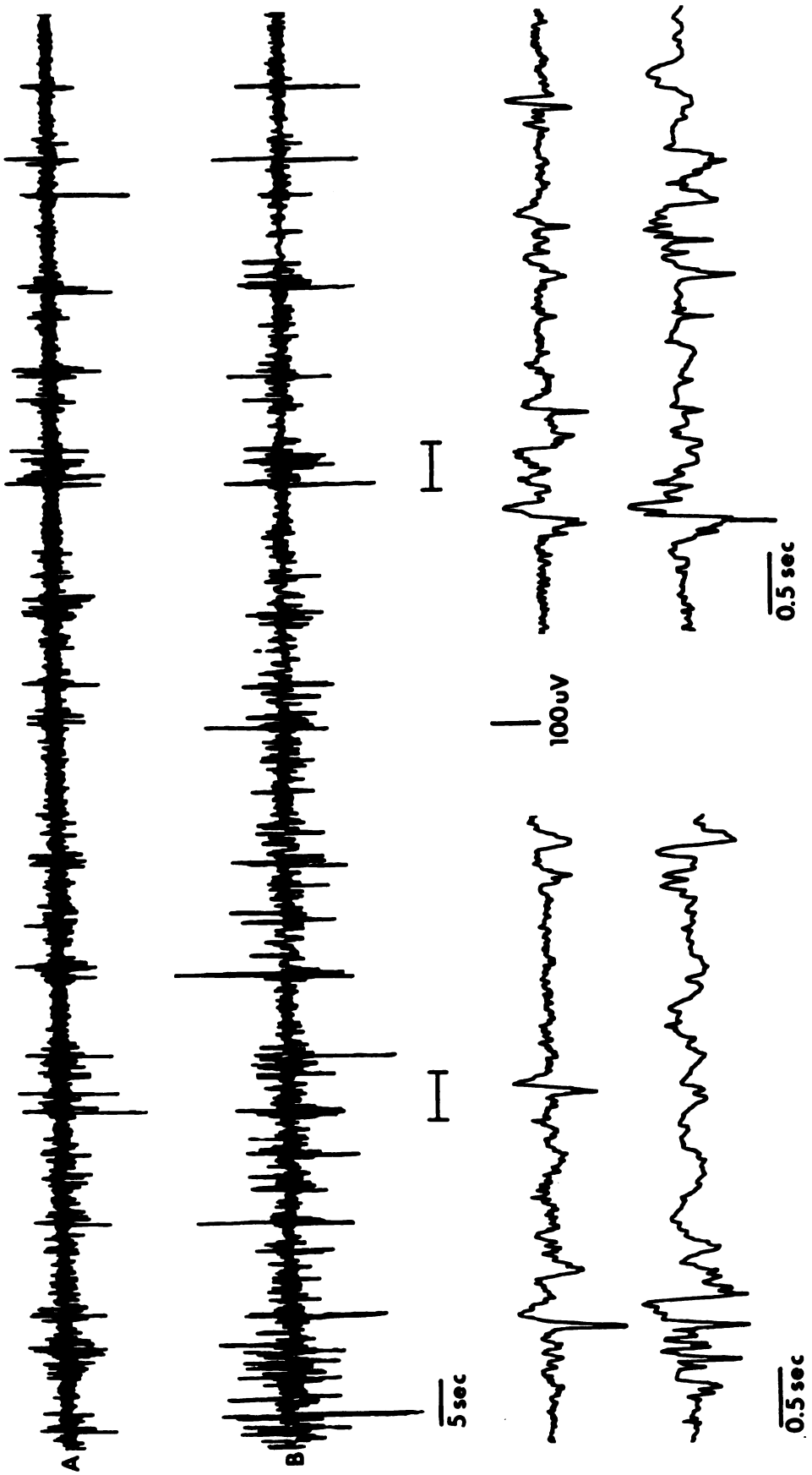

Figure 3

Figure 4. Spontaneous electrical activity recorded at three different sites on the dorsal surface of worms incubated in HBS. At the arrow ethanol was added to the recording chamber (Final concentration of ethanol approximately 50%).

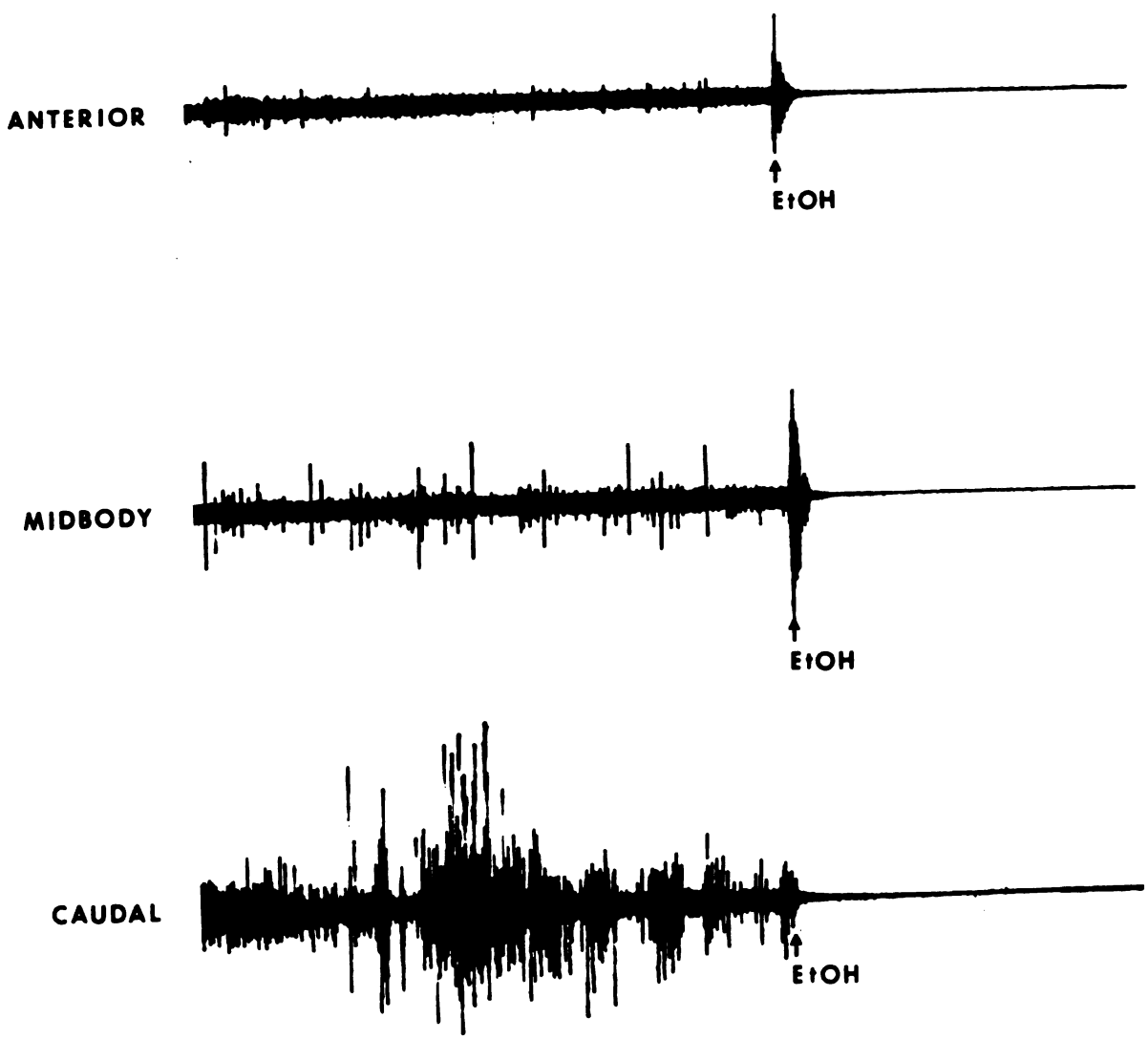

Figure 4

dorsal surface of worms incubated in HBS. The small size, frequency and absence of distinguishing characteristics made it impossible to determine whether the smaller amplitude potentials were propagated. However, many of the larger (>100 μV) potentials did appear to be propagated (Figure 3). Conduction of these potentials was almost always from anterior to posterior, occurring a small percentage of the time in the opposite direction. The conduction velocity of these potentials in HBS averaged 40 $\pm$ 4 cm/sec (N=11) for distances from 0.5 to 3.0 mm.

Activity in Sectioned Worms

In some experiments worms were cut into three equal length pieces. The level and pattern of electrical activity recorded from each of the pieces was indistinguishable from that recorded from the intact animals. From this it appears the activity recorded at any particular region of the worm is independent of adjacent regions and that the activity arises locally and is not generated by some single central pacemaker.

Regional Variation of Electrical Activity

The exact pattern and level of electrical activity recorded depends on the area on the worm from which the recordings are taken (Figures 4 and 5). At the midbody position potentials ranged in amplitude from less than 10 μV to greater than 120 μV with approximately 13% being greater than 40 μV and 1% being greater than 120 μV. At the anterior position the frequency of low amplitude signals was

Figure 5. Amplitude histograms (representing electrical activity recorded at three different sites) from worms incubated in HBS. (A) anterior; (B) midbody, and (C) posterior recording positions. Counts/sec for each bin were determined over 10 second periods for at least six different animals.

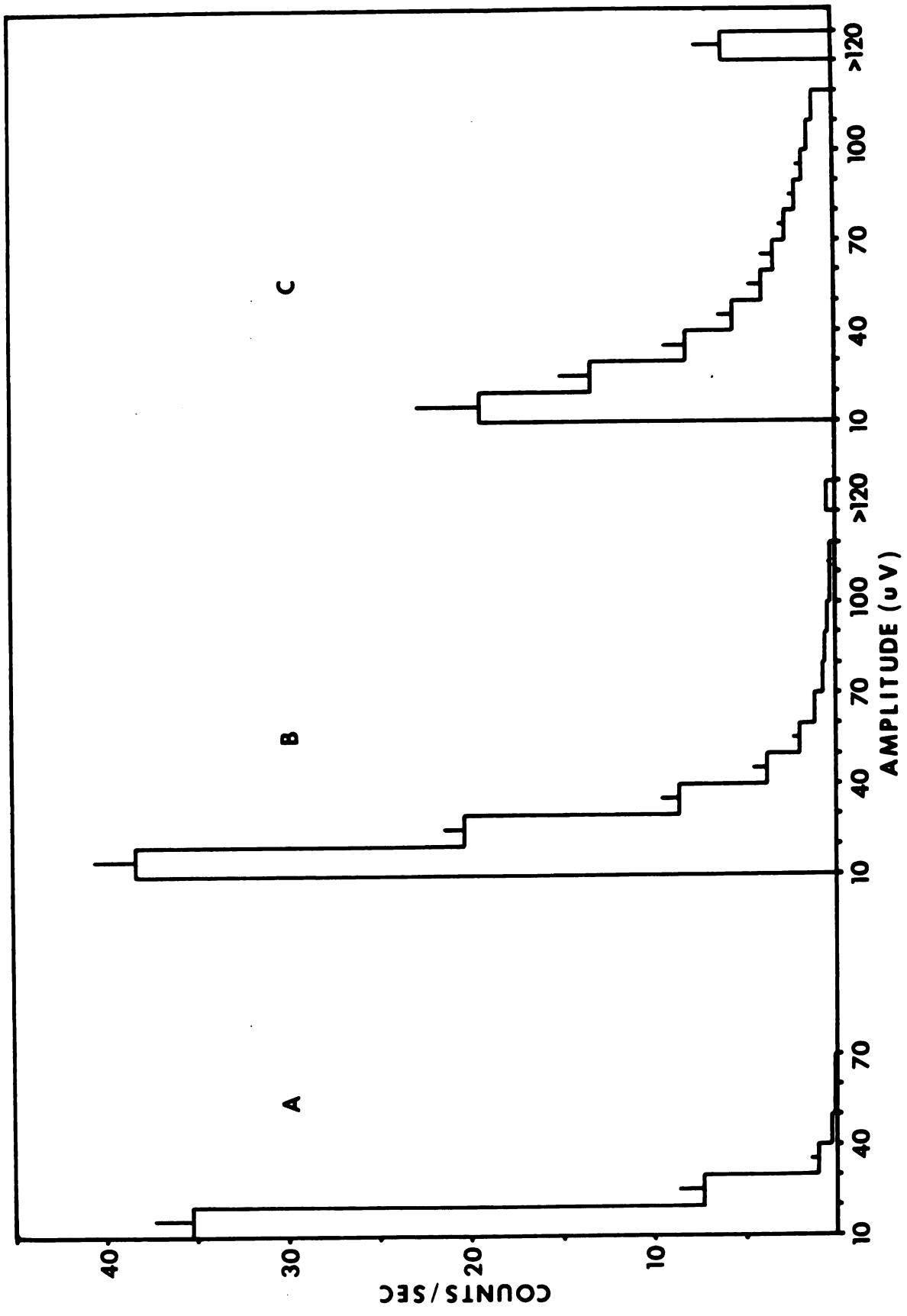

Figure 5

only slightly less than at the midbody, but in contrast to the midbody, signals greater than 40 μV were very rare and no potentials greater than 120 μV were ever recorded. At the posterior, larger potentials were much more prevalent with nearly 33% of the recorded potentials exceeding 40 μV; nearly 6% exceeded 120 μV and in some instances potentials as large as 1 mV were recorded. By contrast, the apparent frequency of potentials in the 10 to 30 μV range was only about one-half that seen at the midbody or anterior region. This apparent lower frequency of the smaller amplitude potentials at the posterior may be an artifact introduced by our method of counting these potentials. With the presence of a significant number of large potentials with comparatively long time courses, many of the smaller, more rapidly occurring potentials may have been obscured and thus not counted by the window discriminator.

Effect of Incubation Media on Electrical Activity

HBS, RPMI-1640/FCS and EBS/HS were tested for their effect on the electrical activity recorded from the various positions. The frequency and pattern of electrical activity appeared to be affected to only a minor degree by the incubation medium (Table 1). The most notable effect was that the frequency of potentials, both smaller and larger, was greater at the anterior region of worms incubated in EBS/HS. In this medium potentials greater than 100 μV were recorded, while no potentials exceeding 70 μV were recorded from the anterior of worms incubated in the other two media.

TABLE 1

Regional Variation and Effect of Incubation Medium on Surface Electrical Activity

Medium	Anterior		Midbody		Posterior	
	10 μV<Amp≤40 μV	Amp>40 μV	10 μV<Amp≤40 μV	Amp>40 μV	10 μV<Amp≤40 μV	Amp>40 μV
HBS	424 ± 23	4 ± 2	648 ± 23	93 ± 15	408 ± 51	274 ± 51
RPMI-1640	534 ± 72[c]	7 ± 3[c]	568 ± 28[b]	94 ± 14[d]	494 ± 37[c]	172 ± 37[c]
EBS/HS	603 ± 19[a]	11 ± 8[d]	600 ± 24[d]	79 ± 17[d]	487 ± 28[c]	211 ± 27[d]

[a] $p<.001$; [b] $.05<p<.10$; [c] $.10<p<.20$; [d] $p>.20$

Values represent the frequency in counts/10 sec of potentials in the designated amplitude range and are given as the mean ± 1 SEM for a minimum of six animals.

Ionic Alteration Experiments

In an effort to gain some insight into the nature and origins of the electrical activity recorded, the effects of ionic alterations in the bathing medium on the activity were determined. Quantitative data are presented for the midbody position only but similar responses were seen at the anterior and posterior.

Calcium. Decreasing or eliminating Ca^{2+} from the bathing medium (HBS) decreased the level of electrical activity (Figures 6 and 7, Table 2). Reducing the $CaCl_2$ concentration to 0.52 mM from its usual value of 1.40 mM in HBS eliminated all potentials greater than 100 μV within 10 minutes and significantly decreased the frequency of potentials less than 100 μV. Further reduction of the $CaCl_2$ concentration to 0.14 mM eliminated all potentials greater than 60 μV within 10 minutes and decreased the frequency of potentials less than 60 μV by 60%. Complete elimination of $CaCl_2$ from the bathing medium abolished all potentials greater than 50 μV within 10 minutes. Few potentials exceeding 30 μV were recorded and the frequency of potentials less than 50 μV was decreased by half. Complete elimination of $CaCl_2$ from the bathing medium plus addition of EGTA ($5x10^{-4}$M) further decreased electrical activity to the extent that no potentials greater than 30 μV were recorded and the frequency of potentials less than 30 μV was reduced by 89%.

Magnesium. Increasing the concentration of Mg^{2+} in HBS from its normal value of 1.0 mM to 30.0 mM resulted within 5 minutes in an overall decrease in the level of electrical activity, but after a

Figure 6. The effects of altered ionic concentrations on surface electrical activity recorded from the midbody position of worms first incubated in HBS. The break in the record indicates the time during which the incubation medium was exchanged. Ion concentrations are given as mM.

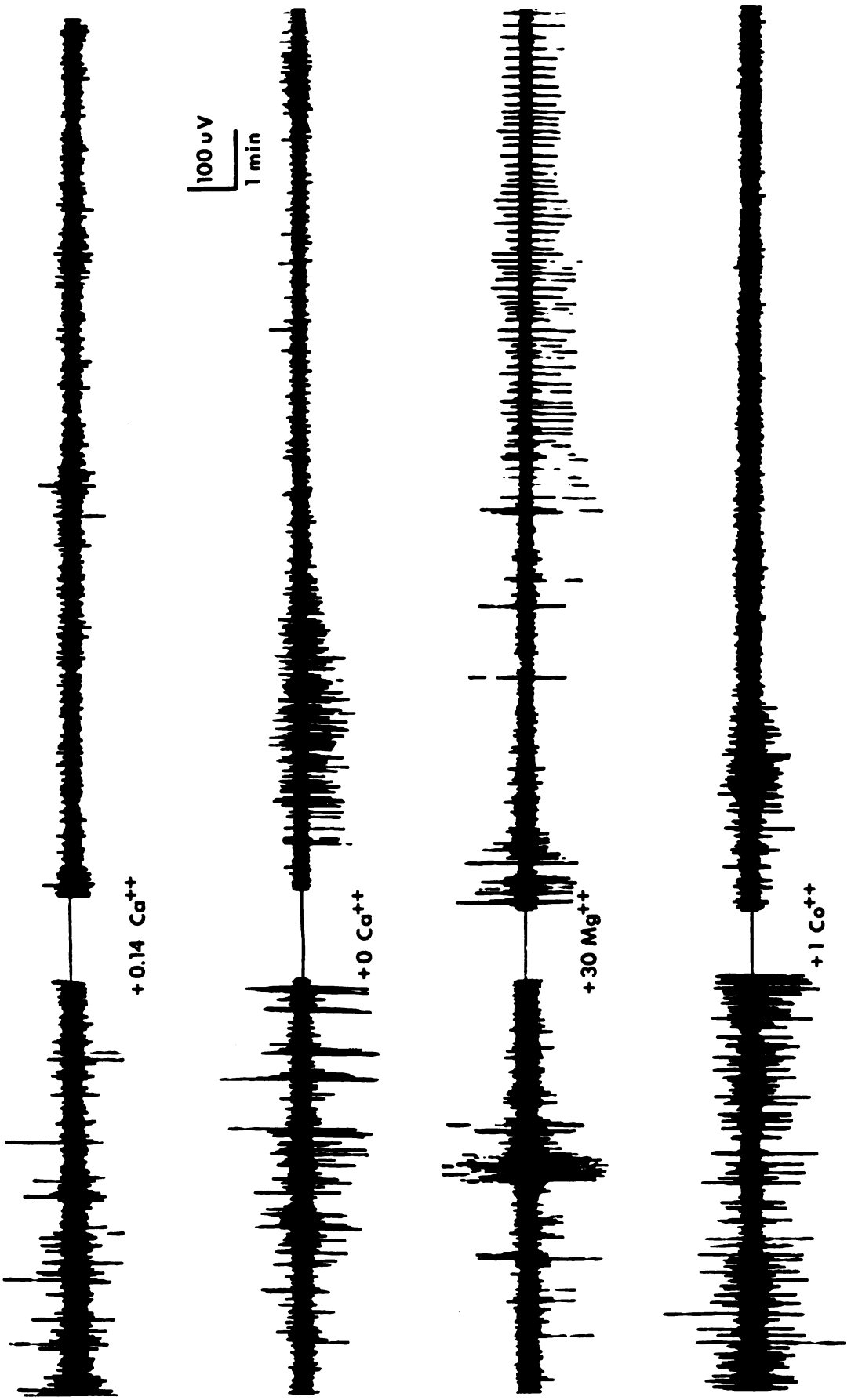

Figure 6

Figure 7. Amplitude histograms representing the change from control in frequency of potentials in response to various ionic alterations. Bins are in 10 μV increments beginning on the left of each histogram with potentials in the 10-20 μV range. The bin on the far right of each histogram represents potentials greater than 120 μV.

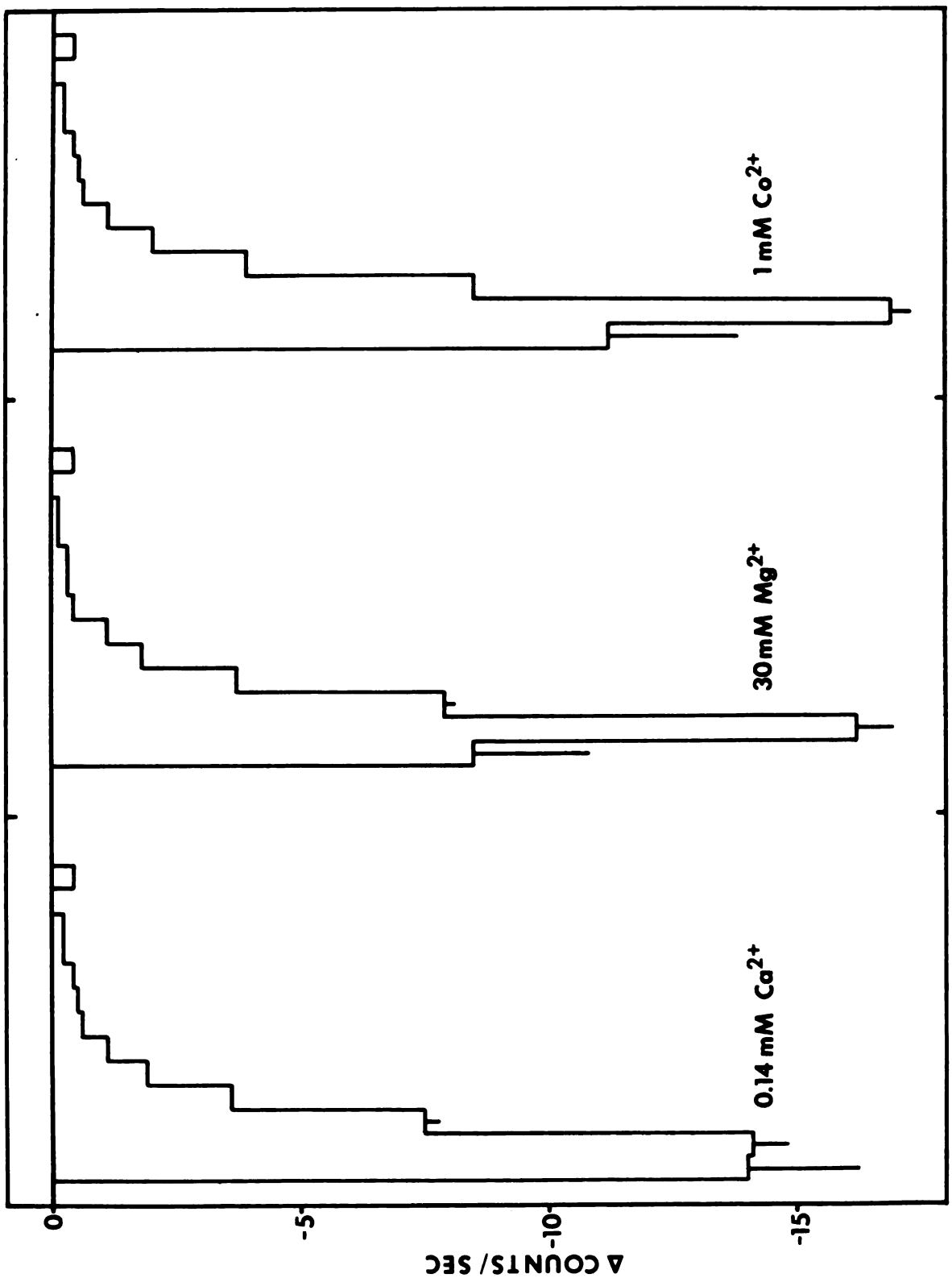

Figure 7

TABLE 2

Effects of Altered Ionic Concentrations on Surface Electrical Activity Recorded from the Midbody Position at 10 Minutes Following Medium Exchange

MEDIUM	10 μV<AMP≤40 μV	AMP>40 μV
HBS	648 ± 23	93 ± 15
0.52 mM Ca^{2+}	348 ± 28[b]	16 ± 3[c]
0.14 mM Ca^{2+}	292 ± 29[a]	5 ± 2[c]
0 Ca^{2+}/No EGTA	344 ± 25[b]	1 ± 0[b]
0 Ca^{2+} with EGTA	84 ± 29[a]	0[a]
3 mM Mg^{2+}	316 ± 30[a]	21 ± 7[c]
10 mM Mg^{2+}	266 ± 30[a]	8 ± 4[b]
30 mM Mg^{2+}	322 ± 29[a]	13 ± 2[b]
1 mM Co^{2+}	254 ± 26[a]	1 ± 0[b]

[a] $p<.001$; [b] $.001<p<.01$; [c] $.01<p<.02$

Values represent the frequency in counts/10 sec of potentials in the designated amplitude range and are given as the mean ± 1 SEM for a minimum of six animals.

time, single 70 to 120 μV potentials superimposed upon the smaller amplitude background activity appeared. Visual observation showed that each of these potentials was associated with a twitch-like contraction of the whole body of the worm. These single potentials were of relatively constant amplitude and frequency for a particular animal, and tended to decrease in frequency with time (Figures 6 and 7, Table 2). With Mg^{2+} concentrations of 3.0 and 10.0 mM there was also an overall decrease in the level of electrical activity within 5 minutes. However, the single large potentials seen with the 30.0 mM concentration were not as consistently produced by these two lower Mg^{2+} concentrations. With all three Mg^{2+} concentrations tested, the frequency of potentials in the 10 to 40 μV range was reduced by approximately 50%. The frequency of potentials greater than 40 μV was also significantly decreased by the three concentrations of Mg^{2+} tested.

Cobalt. Cobalt chloride (1 mM) also produced a significant decrease in the level of electrical activity. Within 10 minutes all potentials greater than 50 μV were eliminated and the frequency of potentials less than 50 μV was decreased by 60% (Figures 6 and 7, Table 2).

Responses to Pharmacological Agents

A number of compounds known to affect mechanical activity, several of which are described as putative neurotransmitters in schistosomes, were also examined for their effects on electrical activity. Data presented represent recordings from only the midbody position of

parasites incubated in HBS, but in all cases similar responses were observed at the anterior and posterior regions. In these studies, control responses were established in a group of 12 animals, and experimental data are presented as the differences from these controls.

Carbachol. Carbachol at a concentration as low as $1x10^{-8}$M, had significant depressive effects on electrical activity (Figures 8 and 10). Within 10 minutes the total frequency of potentials was reduced by 15% ($.01<p<.02$). Potentials at all amplitudes appeared similarly affected. At higher concentrations of carbachol, progressively larger depressive effects were seen. At $1x10^{-6}$M the total frequency of potentials was reduced by 38%, and few potentials exceeding 60 μV were seen. At $1x10^{-4}$M the total frequency of potentials was reduced by 76% and no potentials exceeding 40 μV were present.

Metrifonate. The organophosphorus compound metrifonate produced effects similar to those of carbachol except for a longer onset of action (Figures 9 and 10). Approximately 30 minutes were required for the full depressive effects on electrical and mechanical activity to be seen with $1x10^{-6}$M MT, and roughly 20 minutes were needed for $1x10^{-5}$M MT to show its full effect. By 30 minutes after addition of $1x10^{-6}$M MT, few potentials exceeding 50 μV were recorded and all potentials greater than 80 μV were abolished. The frequency of potentials less than 80 μV was also decreased to 76% of control ($p<.001$). Within 20 minutes after addition of $1x10^{-5}$M MT, an extreme depression of electrical and mechanical activity was produced. Worms treated with this concentration of metrifonate appeared to go through a short period of excitation before gradually becoming less active. No potentials

Figure 8. Amplitude histograms representing the change from control in frequency of potentials in response to various concentrations of carbachol. Bins are in 10 μV increments beginning on the left of each histogram with potentials in the 10-20 μV range. The bin on the far right of each histogram represents potentials greater than 120 μV.

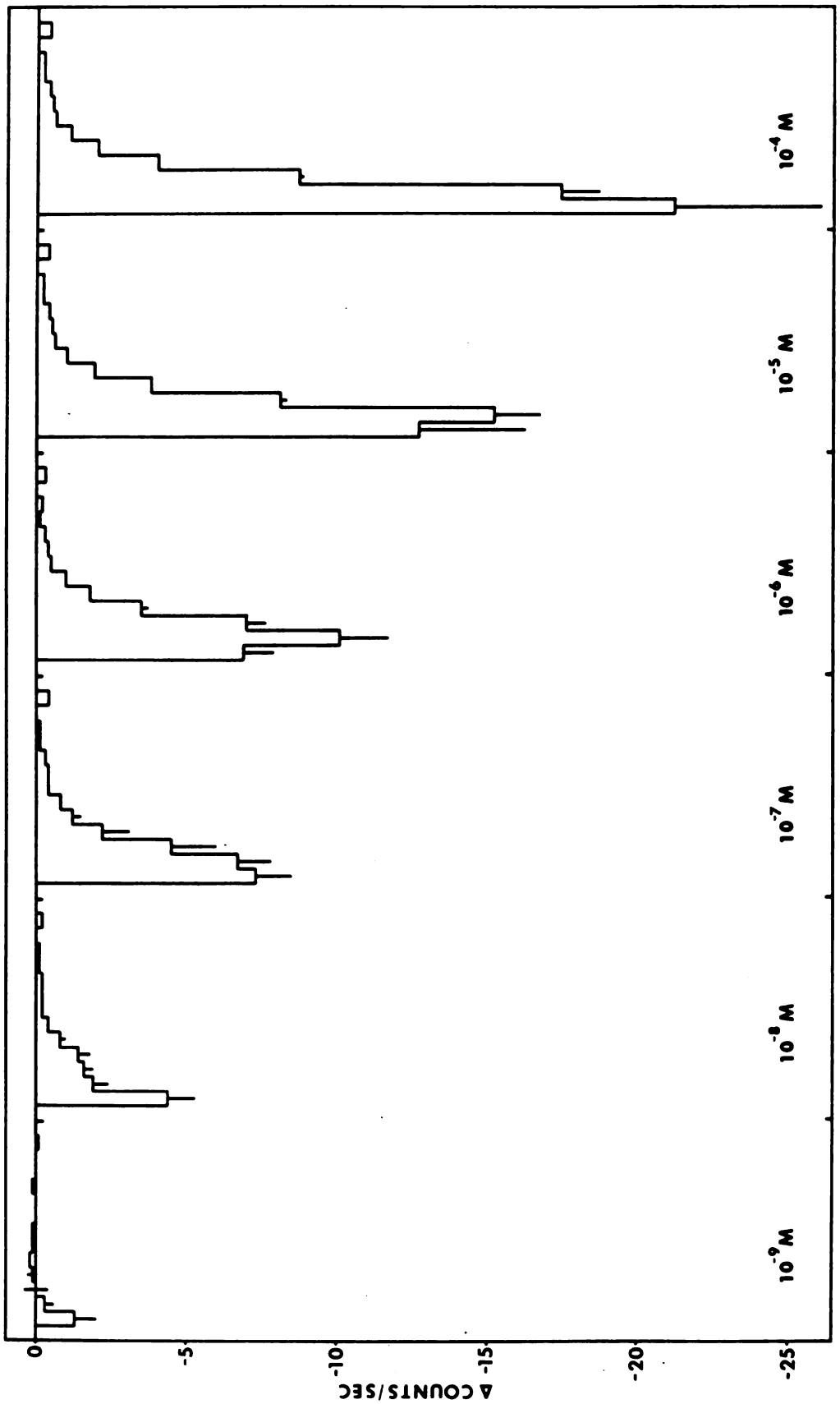

Figure 8

Figure 9. Amplitude histogram representing the change from control in frequency of potentials in response to two concentrations of metrifonate. Bins are in 10 μV increments beginning on the left of each histogram with potentials in the 10-20 μV range. The bin on the far right of each histogram represents potentials greater than 120 μV.

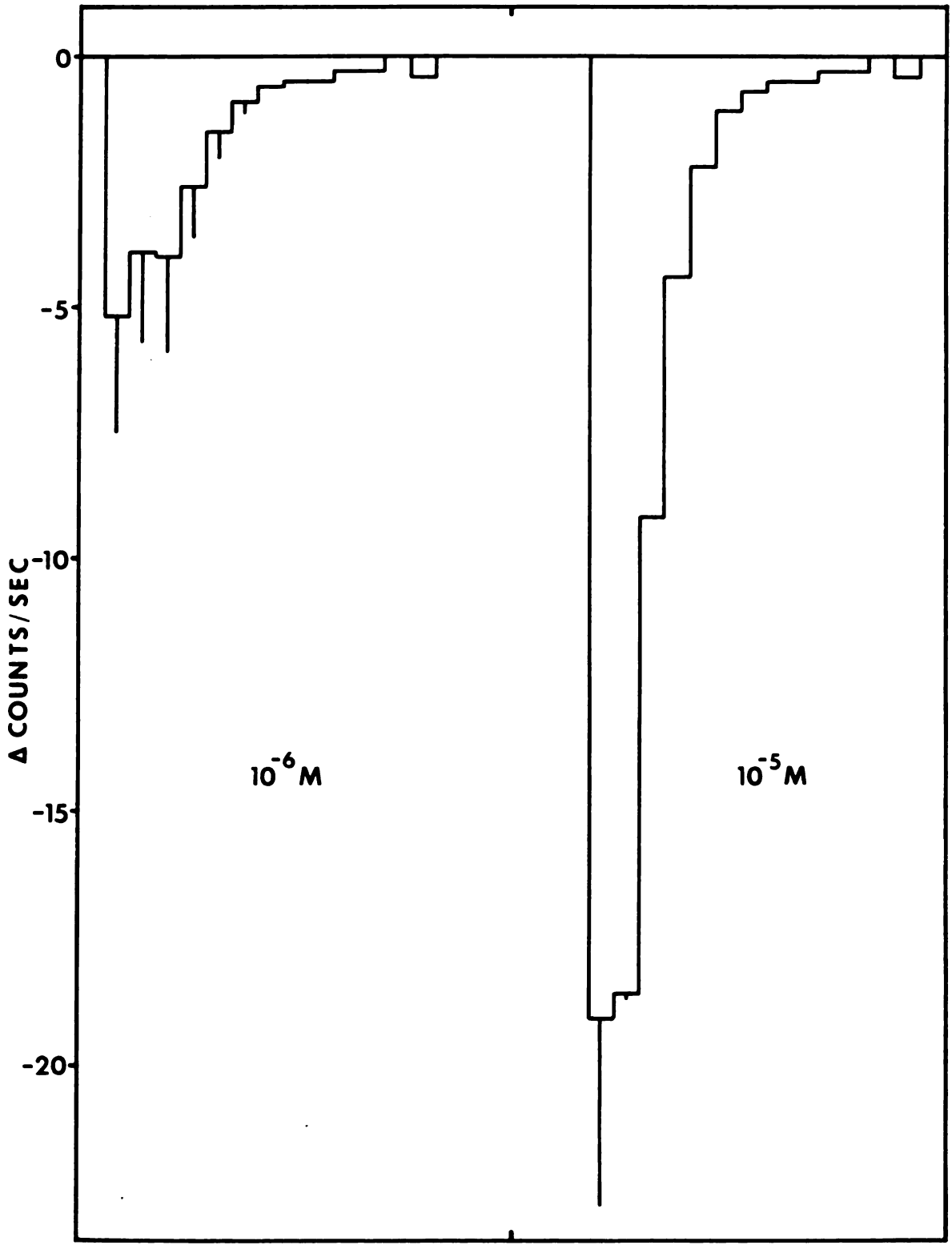

Figure 9

Figure 10. Chart recordings showing electrical activity recorded from the midbody position of worms incubated in HBS in response to carbachol and metrifonate. At the arrow, $1x10^{-4}$M carbachol (upper trace) or $1x10^{-5}$M metrifonate (lower trace) was added to the recording chamber. Time and voltage calibrations are the same for both traces.

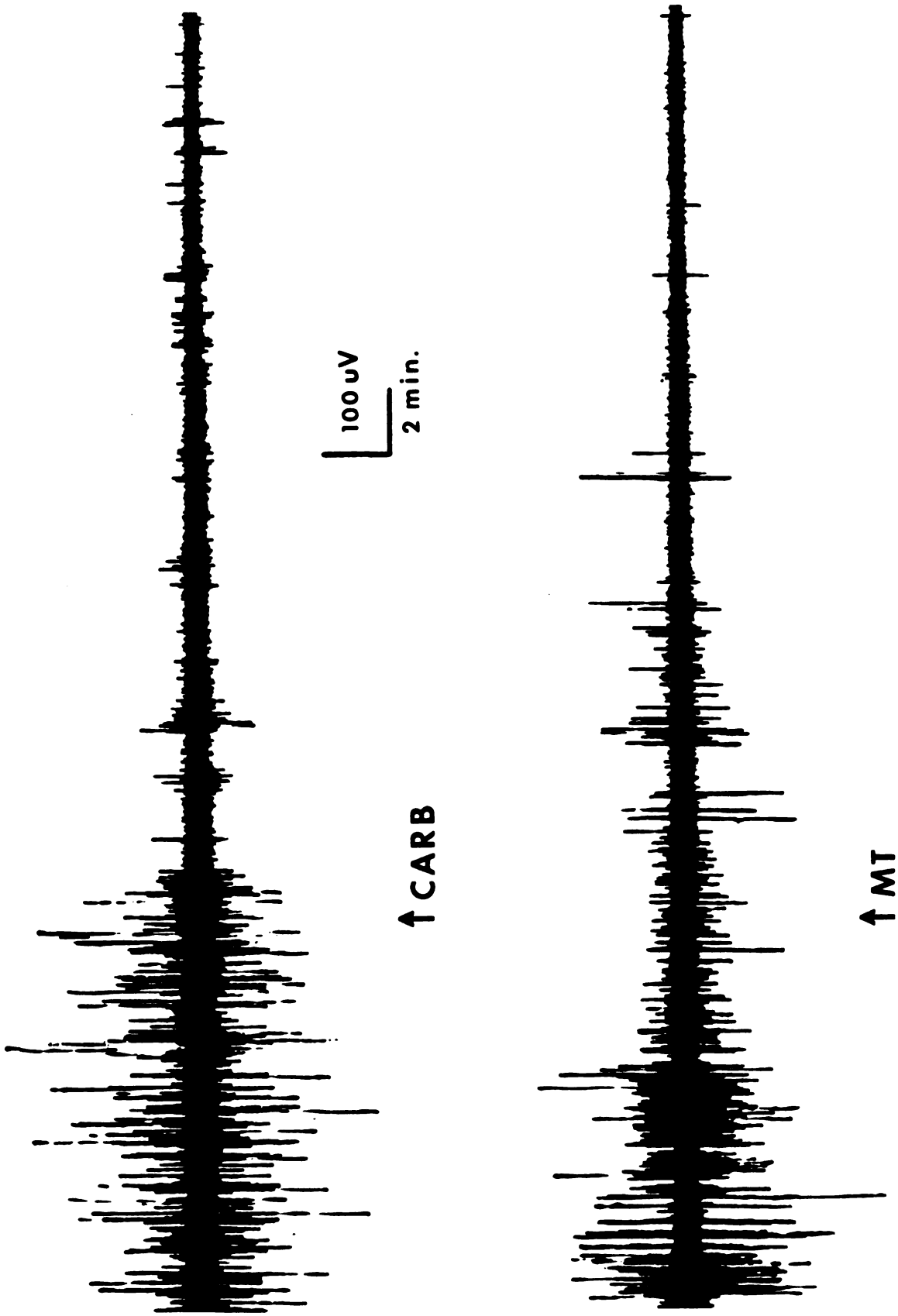

Figure 10

greater than 30 μV were recorded by 20 minutes, and the frequency of potentials less than 30 μV was decreased by 76%.

5-HT. The effect of 5-HT on the electrical activity appeared complex (Figures 11 and 13). One effect appears to be an increase in the frequency of potentials greater than 40 μV, an increase detectable at a concentration of 5-HT as low as $1x10^{-7}$M. Along with the increased frequency of larger potentials, however, there was a depression in the frequency of potentials counted in the 10 to 30 μV range. This depression is detectable even at the lowest concentration of 5-HT tested ($1x10^{-8}$M). However, as mentioned previously, the counted frequency of small potentials may be artificially decreased in the presence of many larger potentials. Since 5-HT does increase the frequency of larger potentials, it is not possible to conclude with certainty whether the depression of lower amplitude potentials is real or is simply an artifact of this method of recording them.

Dopamine. A concentration of dopamine of $1x10^{-8}$M, though it had no observable effect on motility, had a significant depressive effect on electrical activity (Figures 12 and 13). With higher concentrations of dopamine, $1x10^{-6}$M or greater, the activity corresponded with a decreased mechanical activity and a characteristic lengthening response of the schistosome's musculature. $1x10^{-6}$M dopamine produced its greatest depression of activity within 5 minutes, eliminating all potentials greater than 60 μV and significantly decreasing the number of potentials less than 60 μV. Partial recovery of electrical activity was seen with this concentration, with the frequency of potentials

Figure 11. Amplitude histograms representing the change from control in frequency of potentials in response to various concentrations of 5-HT. Bins are in 10 μV increments beginning on the left of each histogram with potentials in the 10-20 μV range. The bin on the far right of each histogram represents potentials greater than 120 μV.

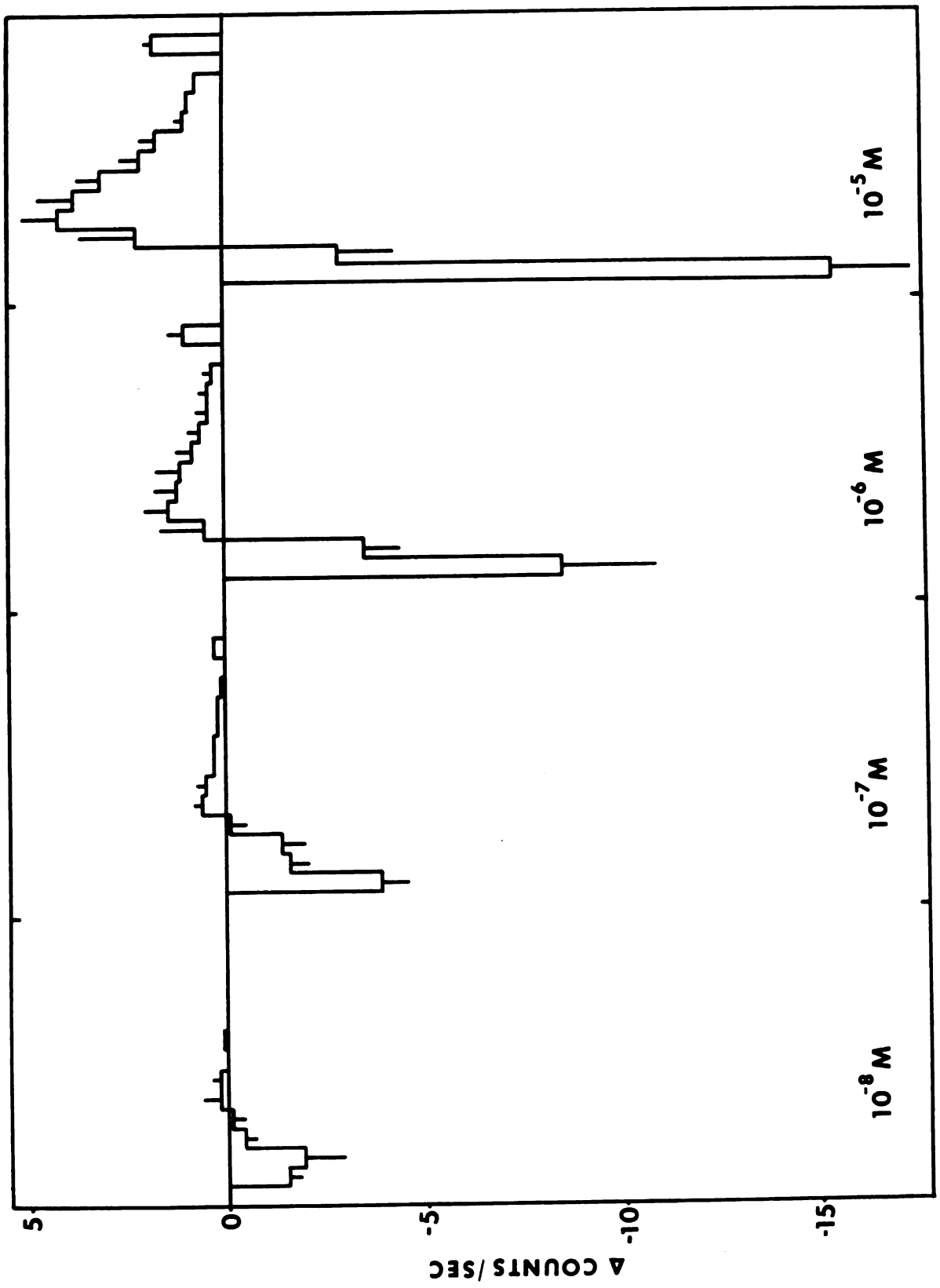

Figure 11

Figure 12. Amplitude histograms representing the change from control in frequency of potentials in response to various concentrations of dopamine. Bins are in 10 μV increments beginning on the left of each histogram with potentials in the 10-20 μV range. The bin on the far right of each histogram represents potentials greater than 120 μV.

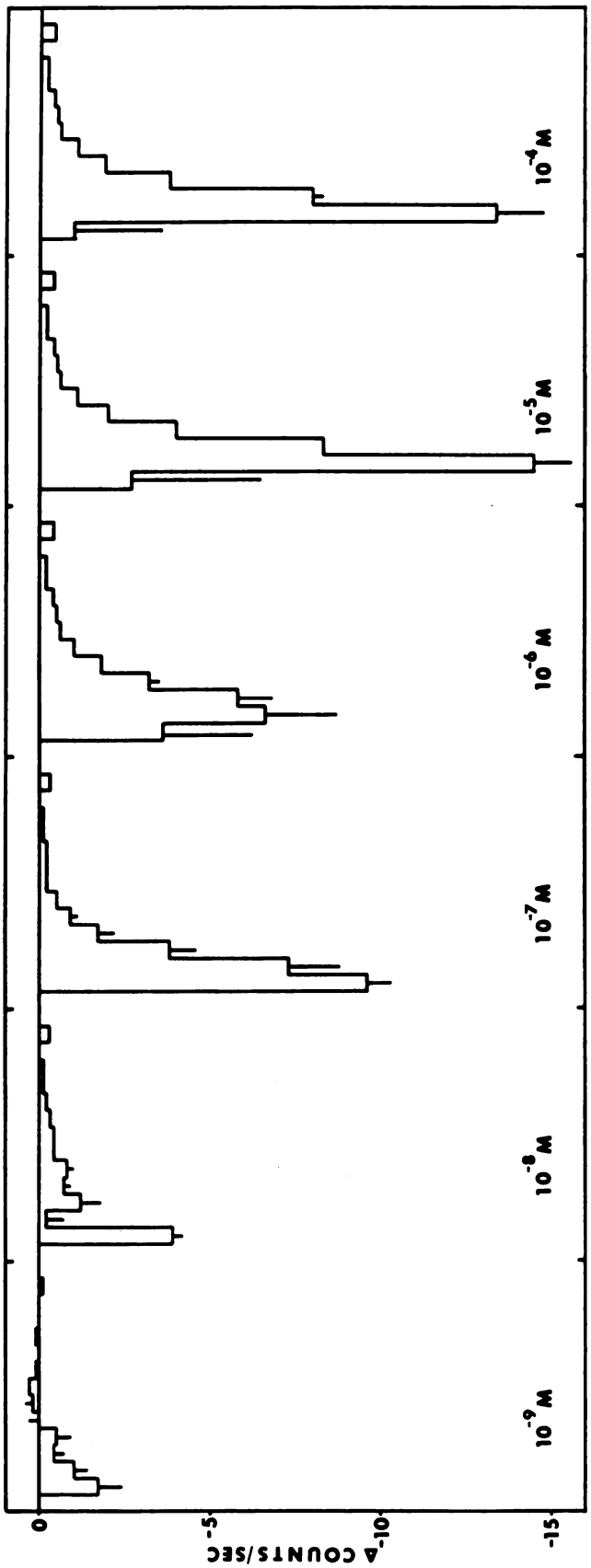

Figure 12

Figure 13. Chart recordings showing electrical activity recorded from the midbody position of worms incubated in HBS in response to 5-HT and dopamine. At the arrow, $1x10^{-6}$M 5-HT (upper trace) or $1x10^{-4}$M dopamine (lower trace) was added to the recording chamber. Time and voltage calibrations are the same for both traces.

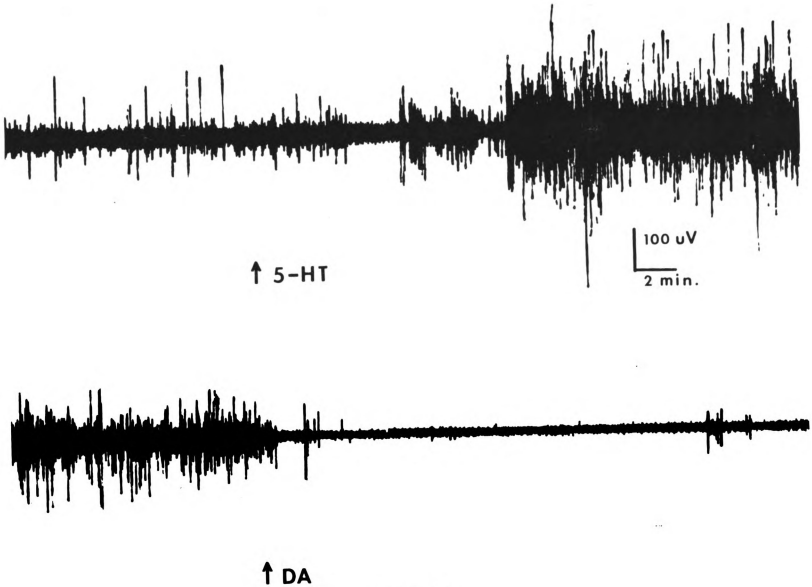

Figure 13

increasing from 66% of control at 5 minutes, to 75% of control at 15 minutes following drug addition. A more pronounced decrease in electrical activity was produced by $1x10^{-5}$M dopamine. All potentials greater than 40 μV were abolished and the number of potentials less than 40 μV reduced to 53% of control within 10 minutes. $1x10^{-4}$M dopamine produced a more rapid effect, but the response was otherwise not significantly different from that of $1x10^{-5}$M ($.20<p$). It is of interest to note that in contrast to carbachol, the higher concentrations of dopamine had a much smaller effect on potentials in the 10 to 20 μV range than those of larger amplitudes.

Pentobarbital. Pentobarbital, both $1x10^{-4}$M and $1x10^{-5}$M, produced a decrease in electrical activity corresponding with its suppression of contractile activity over the 15 minute test period (Figures 14 and 16). Within 10 minutes after addition of $1x10^{-5}$M pentobarbital, few potentials greater than 60 μV were recorded and no potentials exceeding 100 μV were seen. The frequency of potentials less than 100 μV was also substantially decreased by 10 minutes ($p<.001$). By 15 minutes all potentials exceeding 70 μV were abolished and the frequency of potentials less than 70 μV was significantly reduced to 64% of control. Ten minutes after addition of $1x10^{-4}$M pentobarbital, all potentials greater than 40 μV were abolished and the number of potentials less than 40 μV was reduced to 39% of control.

Antimony tartrate. The two concentrations of antimony tested ($1x10^{-4}$M and $1x10^{-5}$M) also produced depression of electrical activity over the test period (Figures 15 and 16). No potentials greater than 90 μV were recorded 10 minutes following addition of $1x10^{-4}$M antimony

Figure 14. Amplitude histogram representing the change from control in frequency of potentials in response to two concentrations of pentobarbital. Bins are in 10 μV increments beginning on the left of each histogram with potentials in the 10-20 μV range. The bin on the far right of each histogram represents potentials greater than 120 μV.

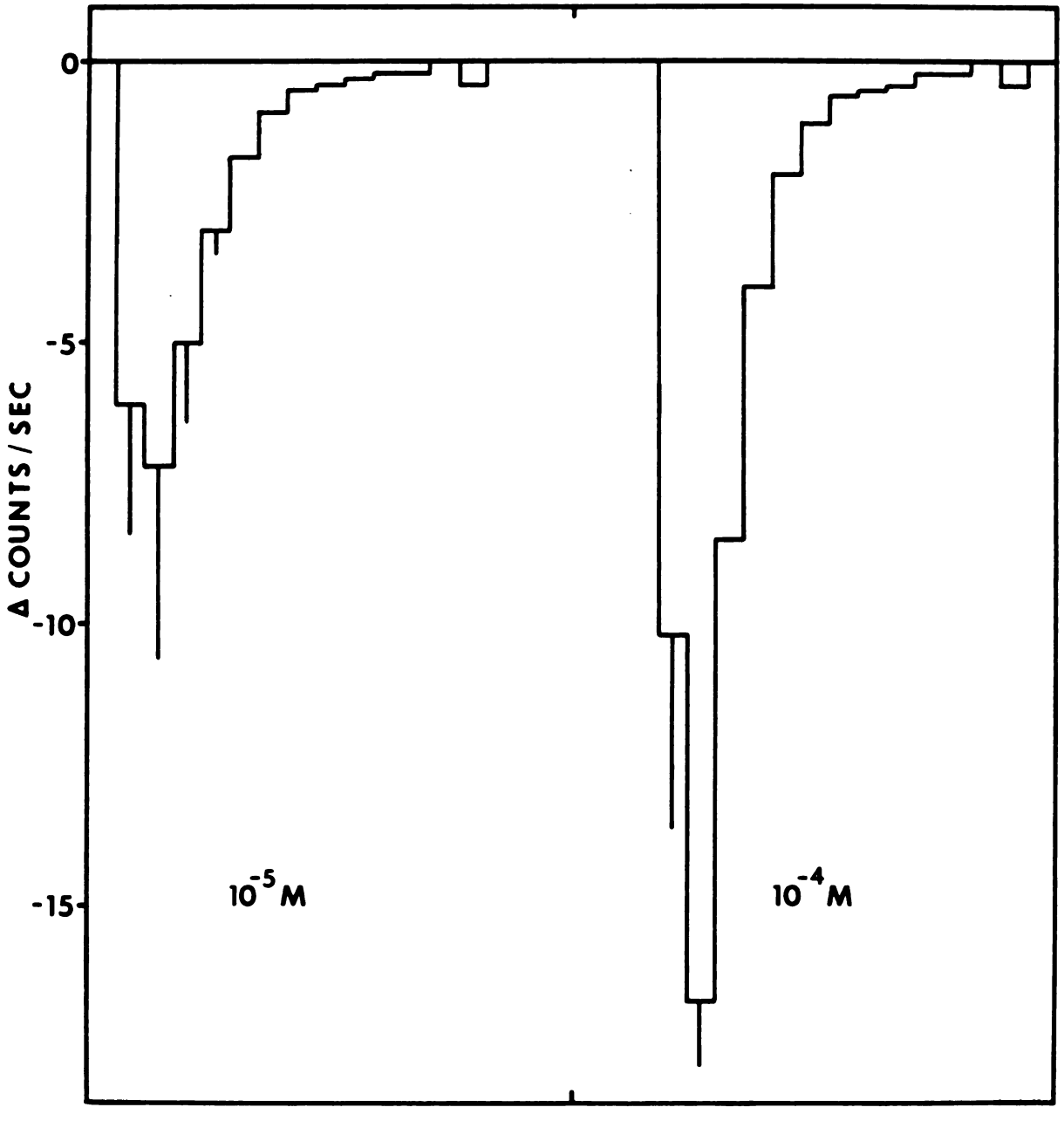

Figure 14

Figure 15. Amplitude histogram representing the change from control in frequency of potentials in response to two concentrations of antimony tartrate. Bins are in 10 μV increments beginning on the left of each histogram with potentials in the 10-20 μV range. The bin on the far right of each histogram represents potentials greater than 120 μV.

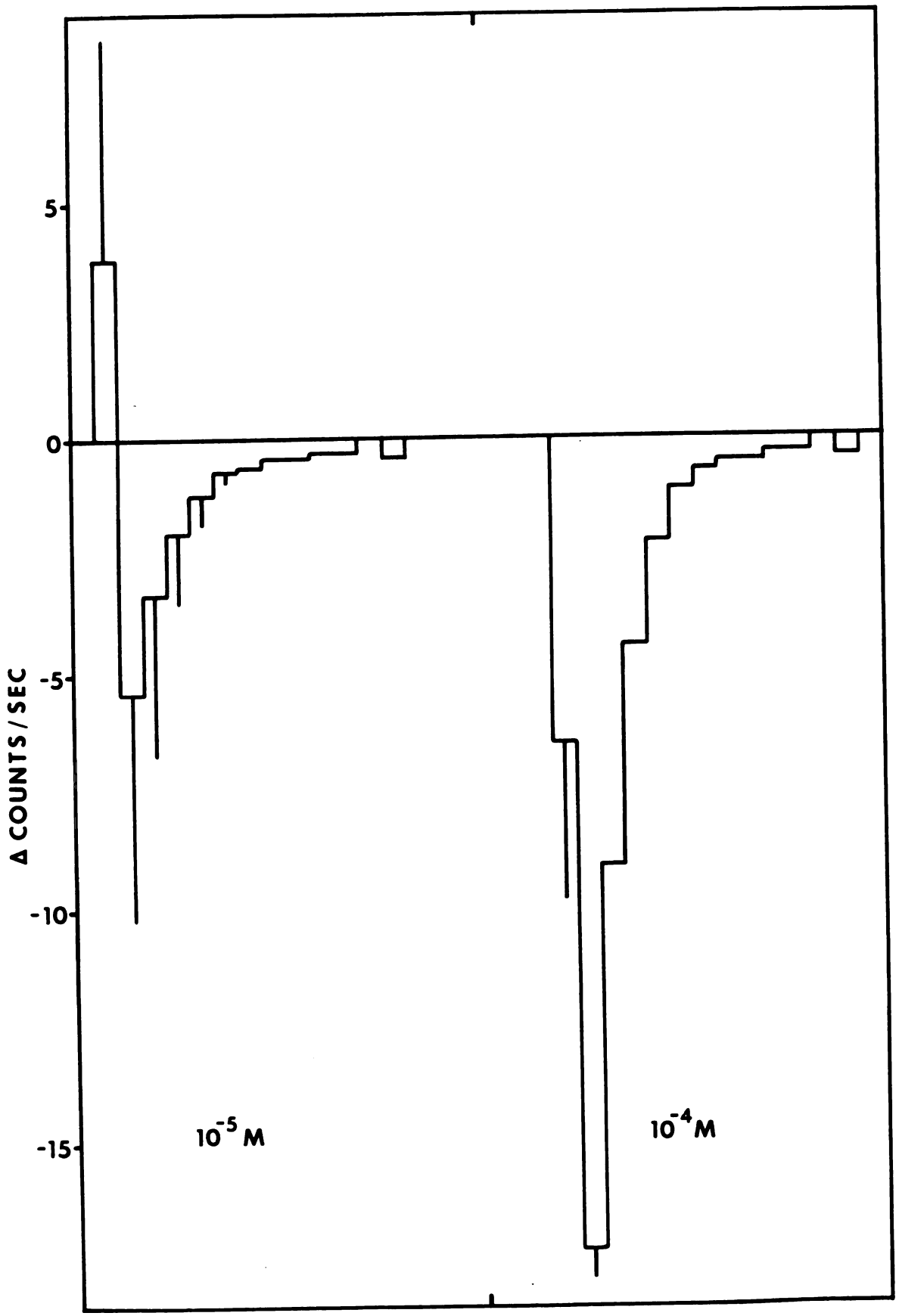

Figure 15

Figure 16. Chart recordings showing electrical activity recorded from the midbody position of worms incubated in HBS in response to pentobarbital and antimony tartrate. At the arrow, $1x10^{-4}$M pentobarbital (upper trace) or $1x10^{-4}$M antimony tartrate (lower trace) was added to the recording chamber. Time and voltage calibrations are the same for both traces.

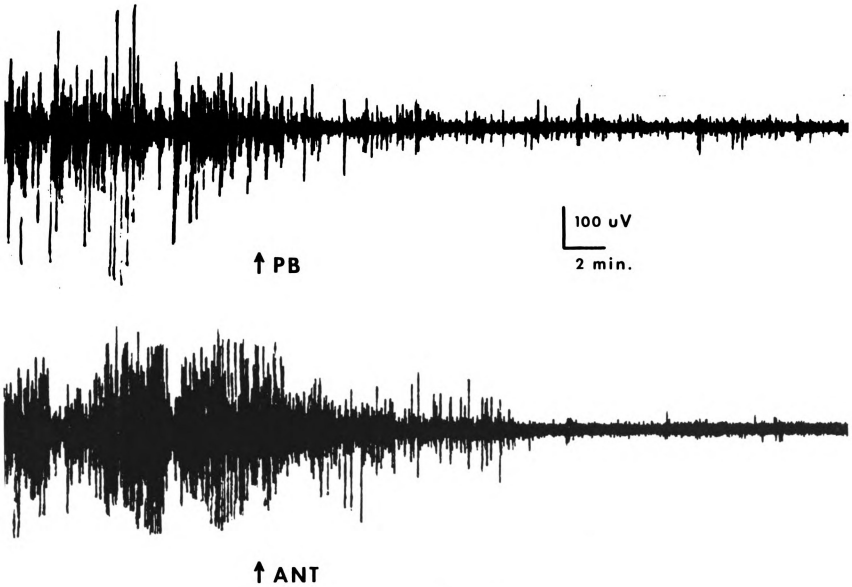

Figure 16

tartrate, and the number of potentials less than 90 μV was somewhat reduced, but not significantly ($p>.20$). By 20 minutes few potentials greater than 50 μV were recorded and all potentials exceeding 80 μV were eliminated. The frequency of potentials less than 80 μV was reduced by 15% ($.05<p<.10$). The effect of $1x10^{-4}$M antimony followed a time-course similar to that of $1x10^{-5}$M, but produced a greater depression of electrical activity. By 20 minutes following addition of $1x10^{-4}$M antimony, no potentials greater than 40 μV were recorded and the frequency of potentials less than 40 μV was decreased to 44% of control.

DISCUSSION

Possible Sources of Electrical Activity

There are a number of possible sources for the electrical activity one records from the schistosome by way of suction electrodes. The potentials may originate at the tegumental surface itself, or they may be volume conducted potentials originating from structures deeper within the animal, such as nerve or muscle. The existence of spontaneous depolarizations 20-100 msec in duration and of variable amplitude, recorded intracellularly from the tegument of schistosomes, have been reported (Fetterer et al., 1980; Thompson et al., 1981). Fetterer et al. (1980) report that these spontaneous depolarizations were recorded only from animals displaying contractile activity. Because of the junctional complexes existing between the tegument and musculature (Silk and Spence, 1969a), it is possible that the electrotonic spread of current from the muscle layer to the tegument makes it feasible to record muscle cell action potentials extracellularly via suction electrodes.

The potentials probably originate from more than one source since the range of amplitudes, rise times and durations is so large. Dopamine and antimony tartrate have less impact on the smallest potentials than the other treatments do, but cause major reductions in the

number of larger potentials. This also indicates the potentials may have multiple sources.

Since the electrical activity differs only in detail at different regions of the body, this activity probably has its genesis in structures which are more or less homogeneous along the length of the worm, and not in discrete local structures, such as the anterior and ventral suckers or the central ganglia, etc. Results obtained monitoring electrical activity in sectioned worms further supports this idea. Tegument, muscle, nerve trunks and the peripheral nerve net are all structures which are found throughout the length of the worm, so any or all of these structures are potential sources for the electrical activity.

Many of the larger potentials, but not all, appear to be propagated significant distances, primarily in the anterior to posterior direction. This again would indicate that at least the structures involved in production of the larger potentials are of considerable length. The mean conduction velocity of these potentials had a value of 40 cm/sec. This value compares favorably with the velocity of potentials conducted along the ventral nerve cords of enteropneusts (Pickens, 1970) and the marine flatworm Notoplana acticola (Koopowitz et al., 1979). This value also falls within the range of epithelial conduction velocities observed in ctenophores (Horridge, 1965) and coelenterate hydromedusae (Mackie and Pasano, 1968; Josephson and Schwab, 1979).

Effects of Ionic Alterations

The lack of information about the basic physiology of tegument, muscle and nerve in the schistosome makes it extremely difficult to unequivocally assign an origin to any of the potentials recorded. In a variety of other tissues, lowered external Ca^{2+}, increased Mg^{2+} or the presence of Co^{2+} interferes specifically with synaptic transmission, both centrally in the nervous system and at the neuromuscular junction (Harvey and MacIntosh, 1940; Stanbury, 1948; Hutter and Kostial, 1954; Katz and Miledi, 1965; Guerrero and Riker, 1973). In the present experiments each of these treatments markedly decreased the level of electrical activity, with all amplitudes of potentials appearing to be equally affected. If the assumption is made that these treatments act specifically at synapses in the schistosome, it appears that much of the activity is dependent on synaptic transmission. This, however, does not necessarily mean that the activity recorded by this method has its origin in nervous tissue, since blocking synaptic function would also be expected to eliminate activity in post-synaptic structures dependent on synaptic activation, e.g. muscle or other neurons.

In a variety of invertebrate preparations, e.g. arthropod muscle (Fatt and Ginsborg, 1958; Werman and Grundfest, 1961), barnacle muscle (Hagiwara and Naka, 1964), and molluscan neurons (Kerkut and Gardner, 1967; Geduldig and Junge, 1968; Meves, 1968; Jerelova, Krases, and Veprinsten, 1971), action potentials are Ca^{2+} dependent rather than Na^{+} dependent. In tissues where such "Ca^{2+}-spikes" are present, the action potentials are also readily blocked by decreased external Ca^{2+},

increased Mg^{2+}, or the presence of Co^{2+} (Hagiwara and Nakajima, 1966; Hagiwara and Takahashi, 1967; Hagiwara et al., 1969; Wald, 1972; Standen, 1974; Eckert and Lux, 1976).

Nothing is known about the nature of active propagated responses in schistosomes. If propagated responses are Ca^{2+} dependent, then the decreased electrical activity in response to these ionic alterations may reflect a block of action potentials in tegument, muscle or nerve in addition to a block of synaptic transmission.

A decreased Ca^{2+} concentration in the recording medium could also be interfering specifically with potentials originating at the tegumental surface and/or in the musculature by depolarizing these structures. Decreasing the external Ca^{2+} concentration below its usual value of 1.40 mM causes a depolarization of the tegumental membrane in schistosomes (Fetterer et al., 1980). This alone could be responsible for the decreased electrical activity observed. However, because a depolarization of the tegumental compartment occurs simultaneously with a depolarization of the musculature (Bricker et al., 1981), probably due to the existence of junctional complexes between muscle cells and tegumental cytons (Silk and Spence, 1969a), lowering external Ca^{2+} could be expected to also suppress potentials of muscular origin, with a resultant decrease in electrical activity.

Effect of Pentobarbital

Barbiturates are known to decrease contractile activity in schistosomes (Hillman and Senft, 1973). These authors report that complete "narcosis" is seen in worms exposed to $1x10^{-2}$M pentobarbital,

and a partial suppression of contractile activity is achieved in worms bathed in $1x10^{-4}$M pentobarbital. The suppression of contractile activity induced by pentobarbital was found in the present experiments to be associated with significant decreases in the level of electrical activity. Barbiturates may be acting by causing depression of nerve activity. Sodium pentobarbital and sodium thiopental have been reported to interfere with sodium and potassium conductance changes in lobster axons (Blaustein, 1968), by a direct action on the axon membrane. This may partially explain pentobarbital's effect in schistosomes if the electrical activity has its genesis within the nervous system. Pentobarbital ($1x10^{-4}$M) has also recently been shown to inhibit spontaneous mechanical activity in a vascular smooth muscle preparation (Altura and Altura, 1975), possibly due in part to a barbiturate-induced inhibition of calcium movement across smooth muscle cell membranes (Altura et al., 1980). Because the musculature of schistosomes most closely resembles smooth muscle morphologically (Silk and Spence, 1969a), pentobarbital may be acting directly on muscle cell membranes in a similar manner to suppress contractile activity, with a resultant decrease in surface electrical activity.

Sensitivity of Method

Though the lack of knowledge about the basic physiology of tegument, muscle and nerve in schistosomes makes it impossible to assign specific origins to any of the potentials recorded, this method I have described here for recording these potentials represents a sensitive, quantifiable method for detecting subtle changes in the neurobiology

of the adult male schistosome. With carbachol, significant effects are detectable at a concentration as low as $1x10^{-8}M$, compared to concentrations of $1x10^{-4}M$ (Barker et al., 1966; Hillman and Senft, 1973; Fetterer et al., 1977), or $1x10^{-5}M$ (Tomosky et al., 1974) seen with previous methods. The sensitivity of previous methods for the effects of 5-HT were concentrations of $2x10^{-5}M$ (Barker et al., 1966), $5x10^{-5}M$ (Tomosky et al., 1974), or $1x10^{-6}M$ (Fetterer et al., 1977), whereas with this method one is able to detect measurable effects with a concentration of $1x10^{-7}M$. Tomosky et al. (1974) detected changes in motor activity of schistosomes following their exposure to $1x10^{-6}M$ dopamine, while with this method significant decreases in electrical activity are detectable with a concentration as low as $1x10^{-8}M$, a concentration that has no measurable effects on mechanical activity. Since this method is both sensitive and quantifiable, it should prove to be a valuable tool for the in vitro assessment of potential anti-schistosomal agents.

SUMMARY

1. Spontaneous electrical activity can be recorded from the dorsal surface of adult male Schistosoma mansoni by means of suction electrodes.
2. This electrical activity is a complex of bi- and triphasic potentials occurring at a rate of about 60/sec with amplitudes ranging from greater than 1 mV down to values indistinguishable from the background electrical noise of the recording system (<10 μV).
3. Many of the larger potentials (>100 μV) appear to be propagated significant distances.
4. Regional variations in the level of electrical activity exist, with the posterior region consistently exhibiting potentials greater than 120 μV, some of which exceeded 1 mV. At the anterior region, activity greater than 40 μV is only rarely seen.
5. Decreased concentrations of Ca^{2+} (0.00, 0.14 and 0.52 mM) or elimination of Ca^{2+} plus addition of $5x10^{-4}$M EGTA, increased concentrations of Mg^{2+} (3.0, 10.0 and 30.0 mM), or addition of 1 mM $CoCl_2$ significantly decreased the level of electrical activity.
6. Drug concentrations of $1x10^{-8}$M carbachol, $1x10^{-6}$M metrifonate, $1x10^{-8}$M dopamine, $1x10^{-5}$M pentobarbital, and $1x10^{-5}$M antimony tartrate also significantly decreased electrical activity. In contrast, 5-HT ($1x10^{-7}$M) significantly increased the level of electrical activity.

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