ELECTRONIC MONITORING OF FEEDING FEMALE *AMBLYOMMA AMERICANUM* (L.) AND THE QUANTIFICATION OF BLOOD IMBIBED DAILY

By

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CHAPTER I

PRELIMINARY STUDY

There have been extensive research studies using electronic measurement systems (EMS) to monitor the feeding process of arthropods. Initially developed in 1964 by McLean and Kinsey, this system operates on the principle of Ohm's Law where E = I x R (voltage = current x resistance). The EMS evaluates the amount of resistance which occurs between two entities (i.e., parasite and host).

The system is supplied power by two 9V direct current (DC) batteries. The DC voltage is sent to an oscillator which converts DC to AC (alternating current) voltage and creates a frequency (100 H_z) at a voltage (millivolts) specified by the experimenter. The AC voltage is output to the host and because the tick is attached to the host the voltage passes through both the arthropod and the host. The returning signal (one of varying voltage and frequency) is input to the monitoring system and is transported through an amplifier. A filter, which removes "noise" (specific frequency bands less than 100 H_z), is the receptor for the voltage signal which then passes to the rectifier converting the AC back to DC and the voltage fluctuation is then advanced to a recording device. The recording device may be an oscilloscope, computer, or a strip chart recorder.

Such systems have been successful in recording the probing habits of plant-feeding insects (McLean and Kinsey 1964, 1965, 1967; Tjallingii 1978, 1985a,

1985b, 1986; Chang 1978; Kawabe and McLean 1978; Smith and Friend 1971; Kashin 1966; and Foster 1986). These references are only a partial listing of available research materials published concerning the use of EMS to study feeding insects. The technique and system described for the electronic monitoring of ticks is a relatively undeveloped discipline. Reports by Gregson 1969; Sweatman and Gregson 1970; Tatchell et al. 1972; Sweatman et al. 1976; and Stone et al. 1983 have been published and deal with the electronical monitoring of the tick feeding process.

Techniques and procedures used in my study have been further adapted for monitoring ticks on mammalian hosts. Electrical resistance occurs between the mouthparts of the tick and the host tissue and circuit completion transpires when there is an exchange of fluids. This interface resistance varies due to variables in the feeding process such as host tissue penetration, fluid secreted by the host in response to the tick bite on host tissue, tick salivation, or ingestion of fluids. Changes in resistance induce proportional fluctuations of input voltage to the amplifier which alters the output to the strip chart recorder, thus variations are recorded on the electrograms.

The intent of my preliminary study was to test the electronic equipment that had been set up specifically for electronically monitoring ticks and to test equipment modifications for ticks feeding on mammalian hosts. Studies were designed to gain knowledge of equipment usage and to calibrate and establish settings. Therefore, the objectives were to record feeding patterns of female ticks, Amblyomma americanum (L.), determine control settings, and to develop monitoring techniques to ascertain the best possible recordings.

Materials and Methods

Two electronic measurement systems (EMS) were set up in the laboratory located at the O.S.U. Tick Research Facilities. The primary components of the AC EMS can be seen in Figure 1. The EMS of Brown and Holbrook (1976) was modified with line amplifiers from Kendow Technologies, Perry, Oklahoma. The modified EMS used in these studies was adapted for use with mammalian hosts (Figure 2) and the line amplifiers were outputs to the hosts from the monitors. One terminal end of the line was connected to the oscillator output of the monitor while the other end had an alligator clip soldered to it that clamped onto a 20 gauge, 1.5 inch vacutainer needle that was bent into a hook-shape for subcutaneous placement into the host. The second lead, an input cable, was modified with solderless banana plugs on the free end of the cable. These plugs were inserted into insulated banana jacks that had been mounted through polyvinyl chloride (PVC) rings (Figure 3). Silver wire, [0.25 mm (Aesar, Johnson, Matthey Inc., Seabrook, New Hampshire)] ca. 10 - 15 cm. long, extended from the banana jacks to the interior portion of the PVC rings. The input cable from the tick was designed to return the electrical signal to the electronic monitor. The monitor filtered the noise and changed the current from alternating to direct. The direct current was then transported to a recording device, a Fisher Recordall Strip Chart Recorder series D-5000 (Houston Instrument Inc., Dallas, Texas).

Batteries used in conjunction with the studies were checked daily and were only used if battery voltage was 7.30 volts or greater. The specific voltage of the output gain level was 0.050 v. It was measured by a Micronta LCD Digital Multimeter (Radio Shack, Stillwater, Oklahoma) with digital settings on AC 3.

The strip chart recorders' paper speeds were set initially on 12.5 cm/min divided by 1. The recording devices were turned on 10 minutes prior to use to allow machines to warm. Baselines for recordings were adjusted to zero prior to each recording.

Amblyomma americanum paired and unpaired female ticks were acquired from the O.S.U. Tick Research laboratory. Adults used for feeding purposes were ca. 120 d post-nymphal molt.

This study used two bovine hosts and the second experiment conducted used two ovine hosts. Although the studies were conducted at different times, the experiments were replicates in terms of objectives and methods. Hosts were stanchioned, sheared and washed. Orthopedic cotton stockinette 6 inch cells (Melton Company, Oklahoma City, Oklahoma) were affixed to the dorsal-lateral portion of each experimental host with 3-M #4799 Industrial Adhesive (Adhesive, Coatings and Sealer Division/3M, St. Paul, Minnesota). Animal hosts had six cells, three cells per side on each host, and on day 0, 25 pairs of A. americanum were placed inside each cell.

Prior to infestation of the hosts, eight female ticks (two/host/experimental group) were fitted with electrode attachments. The waxy layer on the mid-dorsal regions of the ticks' scutum was removed by rubbing with acetone-treated cotton swabs. Approximately 2 μl of colloidal silver liquid was placed on the dorsum and a 0.1 mm silver wire, ca. 5 cm in length, was held in the liquid silver on the tick's back until it dried in place, thus affixing the wire to the dorsum of the tick. Aphid feedings have been monitored using colloidal liquid silver for electrode attachment to the aphids' dorsum (K. Mirkes, personal communication). Prior to

attaching the wire to the tick, the free wire end was bent into a hook-shaped form so it could be connected to the wire (0.25 mm) extending from the PVC ring for electrical connection. The circuit was thus completed. The other method used for circuit completion involved insertion of silver wire (0.25 mm) into the posterior dorsum of the tick (Figure 4).

Female ticks were monitored on a daily basis beginning 48 h post-infestation. Two female ticks from the unpaired females group and two females from the paired cells group were randomly selected daily to be monitored.

Results and Discussion

Initially, female ticks that had wire attachments prior to host infestations were monitored. However, the wires attached with liquid silver did not adhere for any appreciable amount of time post-attachment. Wire detachment was possibly due to tick movement or the waxy surface of the ticks.

Females that were attached to the hosts were rubbed with acetone-treated cotton swabs, dotted with liquid silver and affixed with silver wire leads (0.1 mm) without significant time retention of wire to ticks. Therefore, wire attachment via liquid silver was eliminated and throughout the remainder of the studies (day 4 and later) all monitoring was done by wire insertion (0.25 mm) into the female tick.

Internal damage probably occurred because some ticks detached, dehydrated, and/or died. In follow-up studies, individual ticks were used once for monitoring and then mechanically removed from the host.

The results of the preliminary studies indicated that procedures established during those experiments produced results that were satisfactory and reliable. Adaptations used for monitoring tick feeding on mammalian hosts were successful and the settings initially designated were satisfactory. Feeding patterns obtained during the studies were somewhat similar and they were comparable to patterns noted in previously published papers (Sweatman and Gregson 1970; Tatchell et al. 1971; and Stone et al. 1983).

The voltage settings for the electronic monitors were checked between individual monitoring sessions. Some female ticks needed the voltage adjusted because the feeding activity at various times or with different females was noted to be off of the recording paper. The graph paper used measured ca. 25 cm wide and the strip chart recorder was also limited in width. When the monitoring activities were recording too high (a straight line at the top edge of the recording paper), the voltage setting was adjusted to a lower setting to obtain the entire scale of the electronic recordings. After the recording period of the individual that the voltage was adjusted for, the output gain level was measured again and the adjusted voltage was noted and adjusted to 0.050 v again. The voltage setting of 0.050 v was a basic setting that encompassed the majority of female ticks that have been monitored in studies by me.

Similar feeding recordings were obtained from both bovine and ovine hosts. Due to the smaller size sheep were used in subsequent work since they were easier to manage and maintain.

CHAPTER II

ELECTRONIC MONITORING OF NORMAL FEEDING AMBLYOMMA AMERICANUM (L.) (ACARI: IXODIDAE) FEMALES

The use of an electronic measurement system (EMS) for monitoring the feeding behavior of arthropods was initially reported in work on aphids by McLean and Kinsey (1964). This system has been used for various other insects and arthropods. Gregson (1969) made use of the EMS to compare visual observations with the electrical pulses being recorded which was used to identify the sucking movements and salivation periods on the feeding patterns associated with Dermacentor andersoni (Stiles). Researchers such as Sweatman and Gregson (1970); Tatchell et al. (1972); Sweatman et al. (1976); and Stone et al. (1983) have also published reports concerning the electronic monitoring of the tick feeding process.

The importance of ticks as vectors of disease has solidified a continuing interest in the feeding behavior and pharmokinetics of ticks. Salivary glands of ticks are vital components of the feeding process (Sauer 1977). Fluid exchange between tick and host occurs, but it is not definite when, how long, or the ratio of fluid and ion exchange.

This research effort was undertaken to gain insight into the feeding behavior of female Amblyomma americanum (L.) by electronically recording feeding activities while attached to mammalian hosts. Additionally, comparisons of feeding behavior were made between unpaired and paired female ticks during their adult cycle on mammalian hosts to evaluate the effect of males have on female feeding.

Materials and Methods

Two sheep, with no known previous tick exposure, were stanchioned in the laboratory, sheared, washed and the dorso-lateral portions were closely shaven. The laboratory was cleaned twice daily to provide aseptic, sanitary, and humane conditions for the host animals and researchers.

Facilities were maintained at ca. 22° C and animals were given water and feed ad libitum. Nutritional requirements were met with a prepared sheep ration, SH-019, consisting of 60% rolled corn, 4% molasses, 10% soybean meal, 25% alfalfa pellets, 1% trace mineral salt, <1% Aureomycin 50, and <1% limestone. The host animals' health was evaluated daily and no abnormalities were noted during the conduct of this study.

Orthopedic cotton stockinette cells - eight per host and four per side - were affixed to sheep using industrial adhesive. Cells were individually lettered (A - H and I - P) for identification.

On day 0, three cells per sheep were infested with 25 female Amblyomma americanum; one cell on each host was infested with 50 male A. americanum and one with 15 pairs of A. americanum. The remaining three cells on each sheep

were infested with 35 pairs of adult A. americanum per cell. Twenty-four h post-infestation, female ticks which had not attached were removed from all cells on the hosts. Males were recorded only as to their presence within a cell and to their viability and activity as it concerned the mating of females.

Cells that contained both males and females were observed daily. The cell containing the 15 pairs was used only as an observation cell. Observations were recorded for initiation of mating from both the "observation only" cell and paired cells to ascertain the mating period of laboratory maintained ticks and the time until mating on hosts.

The electronic monitoring devices used in the course of this research were those described previously in the preliminary studies (Chapter I). Beginning on day 2 (post-infestation), three ticks per host were monitored on a daily basis. Two females from the paired cells and one unpaired female were randomly selected to be used in recording feeding patterns. Only one tick could be recorded by a machine at one time, therefore an individual tick was monitored for a 15-minute period, then the lead was switched, connected to the second tick and that individual was recorded for 15 minutes and the lead was transferred to tick #3, etc. Each tick was monitored for two - 15 minute periods and then removed and destroyed at the completion of monitoring for that day.

Individual recordings were measured for exact time. Recordings from unpaired and paired experimental groups were kept separate. Each recording was evaluated and catergorized into respective pattern types. Percentages of the pattern types were determined by calculating the total length of recordings

identified for each pattern type and dividing by the total time for all recordings for each experimental group.

Results and Discussion

Recordings were quantitiated over time for the two experimental groups. Ten different feeding pattern types were recorded and quantitated (Figures 5-14). Each pattern type depicted in the appendix represents a one-minute feeding period (chart speed = 12.5 cm/min). The Y-axis of the figures represents the magnitude, or intensity of the feeding response.

Within each individual pattern type, specific feeding activities have been noted as follows:

A ..... Resting

B ..... Sucking (or Ingestion)

C ..... Salivation

D ..... Expulsion of Saliva

The identification of specific feeding activities within the different pattern types defined within this research was based on visual comparisons between previously published feeding activity charts (Sweatman and Gregson 1970; Tatchell et al. 1971; Sweatman et al. 1976; and Stone et al. 1983). The activity noted as resting was generally characterized as a flat or straight line, occassionally with some fluctuation occurring. Feeding patterns that recorded the resting phase showed that conductivity was relatively low during the resting phase, (i.e., Figures 5 and 7).

Activity identified within the various feeding patterns as sucking (or ingestion) shows a greater voltage fluctuation. According to Tatchell and co-workers (Tatchell et al. 1972), the sucking or ingestion phase can be interpreted in terms of the opening and closing of the pharyngeal valve. When the valve is opened a significant increase in conductivity should be expected and a downwards deflection should occur with the valve closing. The feeding activity defined as sucking (or ingestion) can be seen in Figures 5-11 and Figure 14.

The salivation phases noted within the individual pattern types suggests the release of saliva and/or the ejection of water or other fluids. This activity has been visually observed and recorded by Stone et al. (1983). Visual observations by Stone and co-workers (Stone et al. 1983) were made while ticks were feeding on artificial membranes and were being electronically monitored. Salivation activity, as recorded in my study can be observed in the different patterns depicted in Figures 8, 12, and 13.

Expulsion of saliva was noted by a sharp increase in conductivity. Within the feeding pattern types a higher pointed peak occurred suddenly and then dropped rapidly, thus suggesting a burst of saliva. Similar activity was recorded by Sweatman et al. (1976). Several patterns types identified in my research expressed saliva expulsion. Those figures representative of expulsions of saliva are Figures 6, 8, 9, 11, and 14.

Percentages were calculated by day and individual pattern types to indicate the occurrence and frequency for each pattern type. Table I and Table II indicate the percent of each feeding pattern type by day for the unpaired and paired females, respectively.

Pattern type 1 (Figure 5) was noted for the paired females on days 2 through 10 and days 2-9 for the unpaired females. Feeding activities within this specific pattern type were alternating sucking and resting phases. Type 1 accounted for the largest pattern type with 28.5% of the total chart types recorded for single females and 15.5% for the paired females.

On days 4, 6, and 7, waveform #2 (Figure 6) was noted a total of 4.5% of the time for the unpaired group while it accounted for ca. 14% of the feeding type for the paired females over a longer time period - days 2-11. Changes in pattern rhythm were much greater in type #2 when compared with the activities noted within pattern #1. During a one-minute feeding period, nine saliva expulsions were recorded. These expulsions of saliva occurred between resting and sucking activities.

Type 3 (Figure 7) accounted for the second most common pattern type (20.5%) for the unpaired female group. It was initially recorded on day 3 and was noted again on days 7, 8, 9, and 10. Pattern type #3 was also recorded for the paired female group on days 2-11 for 10% of the recorded feeding time. Approximately two-thirds of the feeding behavior in this pattern were recorded as resting phases and the remaining one-third was periods of ingestion.

Only once, on day 7, did type 4 (Figure 8) appear in the recordings for unpaired females. However, it appeared daily on days 3 through 10 in the chart waveforms for the paired females and accounted for 16% of the total patterns recorded for paired females. The majority of this pattern type was believed to be suggestive of salivation. A brief expulsion of saliva was recorded immediately following ingestion.

Figure 9 identifies feeding pattern #5. During an increase in the conductivity, resting phases were recorded. Prior to each of the resting phases recorded, a brief sucking period was noted and was immediately followed by an expulsion of saliva. Unpaired (unmated) female ticks did not display this particular feeding behavior. Paired females were noted to record pattern type #5 on days 4-7 and days 10-13. This pattern accounts for 25.0% of feeding activity and was the largest single pattern type recorded by paired females.

The electronically recorded pattern type identified as #6 (Figure 10) was similar to type 5 but with enough differences to warrant placing it as a separate pattern type. In type 5, where the higher portion of the plateau was identified primarily as a resting phase, it was suggestive of a period of salivation within pattern type 6. The resting phase as defined in type 5 was probably due to either an open, inactive pharyngeal valve or an open, resting salivarium (Tatchell et al. 1972). In pattern type #6, the magnitude of the recording was low, whereas the voltage fluctuation for type #5 was higher. Also, saliva expulsion was not recorded in pattern #6. This particular pattern (#6) was recorded for both experimental groups throughout the study. It was recorded for ca. 13% and 8.5% for the unpaired and paired females, respectively.

The seventh pattern (Figure 11) identified was a repetitive pattern with three of the feeding activities identified within. A systematic rhythmic feeding behavior was evident within this particular pattern. Initially, a period of sucking was recorded and was followed by an expulsion of saliva. A resting period was then seen. Within the one-minute feeding time depicted in Figure 11, the complete feeding patterns of sucking, saliva expulsion, and resting was repeated a

total of five times. On days 3 and 7, it was identified for paired females and accounted for 2.5% of the total patterns recorded for the paired female group. The unpaired females exhibited this particular feeding rhythm on days 4, 6, and 9 for a total of 6% of the feeding time recorded.

Pattern type #8, Figure 12, was identified for paired females in the final feeding phase just prior to detachment. There was only one feeding activity categorized with pattern #8 and that was continuous salivation. This particular pattern type was recorded only on day 13 for paired females. Type 8 was charted for a total of 4.0%.

The feeding pattern identified as #9 (Figure 13) upon initial inspection appeared to be very similar to pattern type 8 (Figure 12). However, the magnitude of the salivation phase for type 9 was very low when compared with the pattern recorded for the engorging paired females. Type 8 was recorded between lines eight and nine on the chart paper whereas pattern type #9 had a magnitude between lines two and three on the recording paper (initial baseline of 0 for both types). Feeding rhythm #9 was seen throughout the study for both experimental groups. The unpaired females exhibited this feeding pattern for 10% of the trial period as compared with 4% for paired females during the same period.

Pattern #10 (Figure 14) was seen only in the unpaired female group. It was recorded only on days 6 and 8 and accounted for ca. 6% of the total length or time of patterns recorded for the unpaired group. Specific feeding activities recorded with this pattern type were expulsions of saliva, sucking, and resting phases. The majority of the feeding activity was salivation. A sucking (or

ingestion) phase was noted prior to the saliva expulsion and again at the end of the one-minute feeding period.

The onset of mating for paired ticks was initially recorded on study day 6 when one pair was seen mating. The majority of mating occurred the following three days, 7, 8, and 9. Female ticks monitored after day 8 in the paired cells were either mating or were mated females. Electronically recorded feeding patterns from both experimental groups recorded the same feeding behavior but patterns for the groups were different in occurrence. Of the ten patterns identified, unpaired A. americanum females exhibited eight of the ten feeding pattern types. Patterns #5 and #8 were the only types not recorded during feeding for unpaired females. The paired females recorded nine of the ten pattern types. Pattern #10 was the feeding type not recorded for the paired females.

Although distinct feeding patterns with commonality for both experimental groups were apparent, there were feeding differences between unmated and mated groups. Figure 15 represents the percentage of each specific feeding pattern by the experimental groups and indicates the differences of the occurrence of pattern types recorded for each group. The feeding patterns for the unpaired and paired females were different in terms of frequency of occurrence for the majority of the feeding patterns.

Table III and Table IV indicate when a specific pattern was recorded and its' frequency (% of time) on a specific day of the study. The percentage for pattern type #2 was similar for the experimental groups but when the pattern was recorded was very different.

Although the pattern types recorded for the unpaired and paired females were similar, it was evident that feeding behavior was very divergent between the two groups. An indication of this was when and how frequent individual pattern types were recorded.

CHAPTER III

FEEDING RHYTHMS OF AMBLYOMMA AMERICANUM (L.) (ACARI: IXODIDAE) FEMALES IN RESPONSE TO PHARMACOLOGICAL AGENTS

The primary factor contributing to livestock and domestic animal losses by ticks is the transmission of disease, toxicosis, and paralysis (Strickland et al. 1976). The tenacious feeding behavior of most ticks causes bacterially infected lesions at the attachment sites or physiological toxicosis (Hair and Bowman 1986). Furthermore, tick paralysis or toxicosis in livestock has been related to saliva injection into the host by the feeding tick (Gregson 1969). Humans also suffer various diseases and adverse conditions due to tick bites.

Prior research efforts have focused on the feeding behavior of ticks and have involved the use of the EMS - electronic measurement system. Reports by Gregson 1969, Tatchell et al. 1971, and Stone et al. 1983 have focused on the feeding patterns of various ticks and those components related to tick feeding. Progress has been made on the identification of components of insect feeding processes using electronic measurement systems (Chang 1978; Kawabe and McLean 1978; and Tjallingii 1985a). Some portions of tick feeding have also been positively identified by comparing feeding activity noted visually with recordings. With the use of EMS in conjunction with pharmacological agents, the

normal feeding process may be better understood and patterns identified to a greater degree. This particular study was an extension of the previous study (Chapter II) that focused on the normal feeding behavior of female A. americanum.

The concern addressed in this chapter was to alter the normal feeding behavior of female A. americanum (L.) using pharmacological agents while electronically monitoring those ticks before and after drug application.

Materials and Methods

Two sheep, with no known previous tick exposure, were stanchioned at the O.S.U. Tick Research Facilities. The hosts were sheared, washed and the dorso-lateral portions were closely-shaven. The laboratory was cleaned twice daily to provide aesthetic, sanitary, and humane conditions for the host animals.

Adults ticks were obtained from the tick colony maintained at the O.S.U. Tick Research laboratory. Ticks used for feeding and monitoring were ca. 120 d post-nymphal molt.

Facilities were maintained at ca. 22°C and host animals were provided water and feed ad libitum. Nutritional requirements were met with a prepared sheep ration, SH-019, which consisted of 60% rolled corn, 4% molasses, 10% soybean meal, 25% alfalfa pellets, 1% trace mineral salt, <1% Aureomycin 50, and <1% limestone. The host animals' health was visually noted daily and no abnormalities were observed.

The electronic monitoring devices used in the course of this research phase were the same as those previously described for the preliminary study (Chapter I)

orthopedic cotton stockinette cells, four per side, were affixed using industrial adhesive and cells were individually lettered for identification purposes.

Cells that held males and females were observed daily. The cell containing the 15 pairs was used only as an observation cell in which observations were made for initiation of mating from both the "observation only" cell and paired cells to ascertain the mating period of ticks and the time until mating.

Female ticks used in this study were unpaired female ticks as described in Chapter II. On day 10 of the study, males were introduced into two of the three cells containing only females on each sheep. The application of pharmacological agents to the females began on day 13 of the study and were applied through day 15.

Chemical compounds administered to feeding ticks were 1) ivermectin, Ivomec®, a drug known to suppress tick feeding (Lancaster et al. 1982; Drummond 1985; and Bennett 1986); 2) pilocarpine, an agent used to induce salivation in ticks (Kaufman 1978; and Sauer et al. 1979); and 3) amitraz, Taktic®, also a drug that inhibits tick feeding (Ahrens et al 1989; and Davey et al. 1984).

Ivermectin (diluted to 0.1%) and pilocarpine (10^{-3}M) were administered using a 1 cc insulin syringe. Injections of either ivermectin or pilocarpine were administered in the posterior dorsum of the tick where the wire was inserted. The electronic monitoring was stopped, wire removed, injection of the chemical was done re-insertion of the wire, and monitoring was initiated. Needles used were 27 gauge, 1" and the needle tip was filed to a small, sharp point using a diamond nail file. Taktic® was applied topically using toothpicks with ends that

had been wrapped with a thin layer of cotton from a cotton ball to form a cotton swab. The swab was soaked with amitraz.

Initially, normal feeding female ticks were monitored for a period of 15 minutes prior to the drug applications. Females were then subjected to chemicals and monitored for an additional 15 minute period immediately following drug application.

Percentages were calculated by dividing the total time of all charts recorded into the total for the individual normal feeding patterns and for the specific chemical treatments. The percentages were expressed according to the day of the study.

Results and Discussion

Four pattern types originally recorded during the previous study (Chapter II) were observed during this portion of the study for the normal feeding females. The recurring pattern types were pattern types 1, 5, 6, and 10 (Figures 5, 9, 10, and 14), respectively. These patterns were indicative of different feeding activity combinations such as a pattern of primarily resting with a period of sucking occurring at low voltage fluctuations (type 1), or an increase in voltage with a resting period during the increase (as seen in type #5) with sucking or ingestion noted before and after the resting phases. In pattern #6, which was similar to pattern #5, feeding occurred at a lower magnitude with sucking or ingestion noted during the increase in conductivity and in recording #10 expulsion of saliva followed by ingestion and then resting periods. A different pattern, type #11 (Figure 16), was identified during this monitoring session for unaltered paired

female tick feeding. Feeding activities within this pattern include two phases of saliva expulsion with each followed by an ingestion and a resting phase.

Pattern types for the pharmacological agents were identified under their respective agents. Recordings for altered tick feeding were not similar to patterns previously recorded and generally were not comparable to patterns within the same chemical alteration group. Patterns that were noted for drug altered tick feeding were identified by the compound used to influence tick feeding.

Tracings representative of feeding patterns depict one-minute feeding periods recorded every 12.5 centimeters. The vertical Y-axis of the recordings was indicative of the magnitude of the resistance or where the actual electrical impulse was recorded on the graph paper.

Again, patterns were detailed into specific feeding activities and characterized within individual patterns. Activities within the one-minute feeding periods were categorized as follows: A = Resting; B = Sucking (or Ingestion); C = Salivation; and D = Expulsion of Saliva.

Amitraz applications altered "normal" tick feeding and induced erratic feeding behavior as depicted in Figures 17 through 21. Waveforms in figures 17-21 were those tracings which were recorded for amitraz-treated ticks. Feeding ticks that were treated with amitraz did not record one specific feeding pattern that could be considered as representative recording for amitraz-influenced tick behavior so tracings shown in Figures 17 through 21 were all the tracings recorded for amitraz-influenced ticks. Two of the amitraz patterns recorded appear to be sucking or ingesting phases. The conductivity of electrical current for Figure 18 was high because the recording was charted at line 7, but the voltage fluctuation

in Figure 17 was lower. It was recorded on line 3. Feeding pattern depicted for amitraz-altered behavior (Figure 19) recorded activities of resting, sucking, and saliva expulsion. These same feeding activities were reported for the pattern type charted in Figure 21. Again, the rhythm of the patterns was not similar and the voltage variance between the two patterns were noticeable. In Figure 19, the pattern was recorded below 3 whereas the feeding type illustrated in Figure 21 was on line 7.

The recorded pattern depicted in Figure 20 for amitraz-influenced feeding females was recorded in line 5 indicating a medium voltage occurrence. Activities categorized within the pattern were alternating periods of sucking (or ingesting) and expulsions of saliva.

Pilocarpine-influenced feeding females exhibited only one pattern type (Figure 22) and was very similar if not a replicate of normal feeding paired female ticks, pattern #8 (Figure 14). All three recordings of pilocarpine-treated females produced the same type pattern and reflected the known activity of this drug, that of induced salivation.

Ivermectin-injected ticks recorded three different feeding patterns. Figure 23 depicts a recordings (similar pattern recorded from two different female ticks) that was a replicate of the two ticks monitored and the pattern resembled to a large degree the pattern which was noted for the pilocarpine-treated ticks. The remaining two patterns, Figure 24 and Figure 25, were not typical of previously recorded pattern types nor were they similar enough to each other to be classified as the same pattern type.

Figure 24 represents ivermectin-treated females feeding behavior. Within this pattern, very little activity was recorded. Periods of resting were followed by brief sucking phases. Those feeding tendencies recorded for Figure 25 were resting, sucking, and saliva expulsion. The recording was at a low magnitude being recorded in line 1.

Table III represents the percent individual pattern types recorded on days 13, 14, and 15 for unaltered and chemically-altered feeding females. Feeding patterns for the female ticks that were subjected to amitraz or ivermectin were, in general, erratic. The patterns recorded for the pilocarpine-injected females were what was expected--continuous salivation phases. Patterns recorded for the amitraz-altered feeding ticks were divided into feeding categories within individual patterns. However, the five feeding patterns recorded were very dissimilar and feeding behavior was very erratic and atypical of normal feeding ticks. Only one pattern, Figure 20, illustrated repetitive feeding activities within the pattern. Ticks treated with amitraz continued feeding after drug application for a short time but detached ca. 30 min post-treatment.

Feeding patterns recorded for ivermectin-treated ticks were also divergent. One pattern type (Figure 25) resembled Figures 14 and 24. The other two feeding patterns recorded were different from each other and patterns previously recorded for normal feeding ticks. It is possible that ivermectin, a known tick feeding-inhibitor (Lancaster et al. 1982; Drummond 1985; and Bennett 1986), in some way prevents tick ingestion but the tick is able to continue salivation, thus a decrease in tick weight with continued tick attachment.

From this initial experiment using chemicals to alter tick feeding, very few definite conclusions can be made at this point. The feeding patterns were divergent and activities with the individual patterns, at best, were not easily identified.

CHAPTER IV

QUANTIFICATION OF BLOOD IMBIBED DAILY BY FEMALE AMBLYOMMA AMERICANUM (L.) (ACARI: IXODIDAE)

Ticks are a major concern for humans, pets, and livestock as they are capable of transmission of various diseases to them. Wildlife populations are virtually unprotected against these bloodsucking ectoparasites and serve as hosts to many different species. Ticks may transmit organisms from an array of taxonomic groups - protozoans, bacteria, viruses, and rickettsiae. Parasitism by ticks can be potentiated by tick "worry" - a combination of irritation, allergic response, and blood loss due to heavy tick infestations. Indirect damages are also caused to livestocks' carcass, fleece, or hide as a result of tick feeding and by myiasis flies invading wounds caused by ticks.

The feeding regime of adult ixodid ticks occurs over a period of 7-14 days. Males feed intermittently and females feed at a slow pace until the onset of rapid engorgement which occurs during the last 12-24 hours prior to detachment (Kaufman 1989). Females feed only once as adults and can increase their weight by 100-fold.

Salivary glands of ticks play a vital role in the feeding process (Sauer 1977). Concentration of the blood meal occurs as it is being ingested and the excess water and ions are moved across the gut of the tick into the hemocoel and back

to the host via salivary glands (Tatchell 1967, 1969; Meredith and Kaufman 1973; Sauer 1977; and Guenther et al. 1980).

It was the intention of this study to: Measure the daily blood intake of Amblyomma americanum (L.) females exposed to males compared with isolated females and to determine the blood volume and weight difference of paired females to female ticks fed without males present to estimate the effect males have on female feeding and blood intake.

Materials and Methods

Amblyomma americanum (L.) adults were obtained from the tick colony maintained at the O.S.U. Tick Research laboratory. Immature ticks were reared as described by Patrick and Hair (1975). Adults used were ca. 120 d post-nymphal molt.

Three ovine hosts used were stanchioned, washed and the dorso-lateral portions shorn. The sheep were maintained indoors at ca. 22°C and feed and water were provided ad libitum. Nutritional requirements were met with a prepared sheep ration which was made of the following: 60% rolled corn, 4% molasses, 10% soybean meal, 25% alfalfa pellets, 1% trace mineral salt, < 1% Aureomycin 50, and < 1% limestone. Housing facilities were cleaned twice daily in an effort to provide aesthetic, sanitary, and humane conditions for the hosts.

Six cotton stockinette cells per sheep - three per side - were attached with 3-M #4799 Industrial Adhesive (Adhesive, Coating, and Sealer Division/3M, St. Paul Minnesota). Cells were individually lettered for identification purposes.

On day 0, each host was infested as follows: three cells were infested with 35 female A. americanum and the other three cells were infested with 35 pairs of adult A. americanum. Twenty-four h post-infestation, female ticks that were unattached in any of the cells were removed from the hosts.

Cells containing both the males and females were checked visually daily. Males were recorded only as to their presence within a cell and to their viability and activity as it concerned the mating of females. The paired tick cells were monitored for mating activities.

Techniques used in this study were described by Sutton and Arthur (1962) and later modified by Koch and Sauer (1984). Unfed female A. americanum were used as pretreatment controls in hematin assays to estimate the quantity of blood retained from the nymphal feed. Beginning on day 2, a total of twelve female ticks were removed daily from the hosts and subjected to hematin assays. In the three cells with females only, ticks were randomly removed, weighed, and used for blood assays to determine the amount of blood imbibed by females that had not been exposed to males. Females from the paired cells were also randomly selected and removed daily to determine the volume of blood imbibed by female ticks that had been exposed to males. Hemoglobin and other hemoproteins were estimated spectrophotometrically after they had been converted to pyridine hemochromogen (Sutton and Arthur 1962).

This method consisted of homogenizing the ticks in hand held glass homogenizers. Ticks were ground in 0.1 M NaOH solutions. The volume of sodium hydroxide to be used in order to ascertain the final clear-color reaction

mixture within the range of accuracy on the spectrophotometer was calculated according to tick weight.

In accordance with individual tick weights, assays were conducted using 0.1 M NaOH diluents of 6 ml, 42 ml, 90 ml, or 360 ml. After homogenization, 0.8 g of sodium dithionite and 1 ml of pyridine per 6 ml of 0.1 M NaOH were added. The individual mixtures were allocated 10 min for chemical reaction time and then were centrifuged for 10 min at 2,000 rpm. The clear supernatants were decanted into individual test tubes and read spectrophotometrically with a Spectronic 20® colorimeter at 525 nm. These values were compared with blanks of 0.1 M NaOH, pyridine, and sodium dithionite in the same proportions as in the test solutions. Known quantities of host blood were analyzed using the procedures previously mentioned to obtain optical density readings of the host blood to establish standard curves.

Blood volumes were estimated using linear regressions. All regressions calculated had a R^2 of 98% correlation coefficient or greater. Differences were judged statistically significant ($p = 0.05$) based on a weighted analysis of variance (ANOVA); the weights being chosen were inversely proportional to the estimated variance. Weights and blood volumes were estimated using least square means. Proc GLM (SAS Institute 1985) was used to infer the coincidence of two lines.

Results and Discussion

The average weights and volumes of blood ingested by feeding female ticks, unpaired and paired, are illustrated in Figures 29 and 30, respectively. There

were feeding differences in terms of individual tick weights and quantities of blood imbibed that were not reflected in group means.

Figure 29 illustrates the relationship of average weights (mg) for unpaired and paired female groups from day 0 (unfed weight) until day 11. Feeding days 2 through 11 indicate that there were no statistically significant differences between the two experimental groups.

Points on Figure 30 represent blood volume means (μl) for both unpaired and paired female ticks. A linear relationship existed between the two groups in this parameter as it was also evident in the weights. There was statistical evidence of coincidence at the two lines i.e., that the slope and intercept were the same within the variation due to individuals ticks. Differences between quantity of blood measured for the two groups on a daily basis were not significant between days 2-11.

For days 2 through 6 slight fluctuations were recorded for the mean weights for both groups but no significant changes were noted between the groups. On day 5 blood volumes for unpaired females increased by 2.5-fold when compared with average blood volumes for the same treatment group on day 4. Weights and blood quantities indicated a decrease for both group means on day 8 although losses were not statistically significant ($p = 0.05$).

On days 10 through 13, the paired females increased average blood consumed with significant differences noted between days 11 and 12. Blood volume for the paired females on day 10 was 32.2 μl and on day 11, 40.4 μl. However, on day 12 an increase of greater than 1,000-fold was noted in the pairs experimental group. The average quantity of blood for paired A. americanum

females was 3766.5 μl. Quantity of blood imbibed by unpaired females on day 11 was 54.3 μl and was similar to that recorded for the paired females. By day 12 significant differences were apparent between the mean blood volumes of both groups. The unpaired females, on day 12, had an average blood content of 23.8 μl when compared with that of the paired females blood volume, 3766.5 μl.

The mean weight for paired females for day 11 was 41.4 mg and on day 12 the paired females average weight was significantly greater than any of the average weights previously reported for either group. Day 12 paired ticks had an average weight of 872.5 mg. For days 11 and 12, unpaired females had mean weights of 22.5 mg and 21.3 mg, respectively. Significant differences were recorded between the groups on day 12. Unpaired females on day 13 had a mean weight of 38.6 mg and an average blood content of 22.0 μl. Weight and blood volume for the paired females were 884.4 mg and 4155.4 μl, respectively on day 13. Significant differences between the two groups were noted for both parameters on day 13 also.

As it had been previously reported but not quantified, females do not imbibe significant quantities of blood nor do they increase significantly in size until they have entered the rapid engorgement phase. Females in the presence or absence of males in this study consumed a substantial volume of blood when compared with the initial blood content retained from the nymphal feed. However, when comparing the blood ingested by mated females identified in the fast-feeding phases the quantity of blood consumed by unpaired and paired females (days 2-11) were minimal. Weights did not increase significantly within the unpaired females group for the study duration when compared with female ticks that had

exposure to males. As the data in Figure 29 indicates, weights for both groups were similar until day 11.

The variation in the average volume of blood ingested by unpaired females compared with paired females could be due to the heavier tick burden within the paired cells. Initially, paired cells contained 70 ticks while the unpaired females only had 35 female ticks within a centralized area. The larger number of ticks possibly induced a definitive response by the host by reducing the blood flow to the feeding lesion. The tick then counteracts this response by releasing salivary secretions to maintain blood flow to the feeding lesion (Kaufman 1989).

Hume et al. (1985) reported that the females within their experiment had periods of temporary weight loss. Mean weights of females in both groups of this study also at some point experienced periods of temporary weight loss. Sauer and Hair (1972) observed that adult females were not consistent in their feeding habits and for this reason it is not possible to accurately predict the amount of blood consumed on a time basis after placing ticks on the host.

This study indicated that the weights and blood volumes of female A. americanum fluctuated according to individual tick differences due to the ingestion of blood, tissue, and lymph and the resting period when ticks do not actively feed as much. It is uncertain as to what the weight decrease recorded on day 8 for both experimental groups may be attributed to.

CHAPTER V

EFFECT OF IVERMECTIN ON THE VOLUME OF BLOOD INGESTED BY TWO SPECIES OF TICKS (ACARI: IXODIDAE) FEEDING ON CATTLE

An estimated $3 billion is lost annually in the United States because of parasitic infestations of livestock and more than half of this loss is attributed to arthropods (Campbell et al. 1983). A report by Drummond (1987) indicates that arthropods inflict approximately $1,545 million in damages to cattle annually. Significant among losses by arthropods are those that occur from tick feeding. Ixodid ticks attach to a host for extended periods of time and may consume a meal consisting largely of blood, but they may ingest some lymph and other host tissues. Several studies have focused on the quantity of blood imbibed by normal feeding ticks (Sutton and Arthur 1962; Snow 1970; Sauer and Hair 1972; Koch et al. 1974; Obenchain et al. 1980; and Koch and Sauer 1984).

The ability of engorging ixodid ticks to concentrate the blood meal was described by Tatchell (1967). During engorgement, large amounts of excess fluid are moved across the gut epithelium into the hemolymph and then secreted back to the host via the salivary glands. Differences in the quantity of blood ingested are not due to water loss but to variations in the proportions of non-blood to blood tissues in the imbibed meal (Snow 1970). Pathogens are obtained by

feeding ticks from various components of the blood meal (Arthur 1962), and therefore the quantity and constituents of the meal are important epidemiologically. Species which consume large amounts of blood may have a higher potential exposure to pathogens.

Ivermectin is a synthetic derivative of the avermectins which are a group of natural products derived from the soil microorganism, Streptomyces avermitilis (Campbell et al. 1983). It is one of the most potent antiparasitic agents known today and is administered in dosages of micrograms per kilogram of body weight.

Ivermectin is hypothesized to affect gamma-aminobutyric acid (GABA)-mediated neurotransmitters in susceptible arthropods (Campbell 1981) and is believed to paralyze arthropods by stimulating GABA-mediated chloride ion conductance (Bennett 1986). Treatment of a tick-infested animal with ivermectin does not cause immediate detachment of the tick but disrupts the essential processes of feeding, molting, egg production, and other necessary life functions. Paralysis and death of the tick is the usual outcome but different species of ticks respond diversely to various levels of the drug (Wilkins et al. 1980; Campbell 1981; Drummond et al. 1981; Lancaster et al. 1982a, 1982b; Campbell et al. 1983; and Drummond 1985).

Ivermectin is a candidate for inclusion in sustained-release devices such as ruminal boli or implants because of its effectiveness at low dosages (Drummond 1985). Since some engorgement of ticks attached to ivermectin-treated hosts sometimes occurs the objective of this study was to ascertain to what extent ivermectin inhibited tick feeding and the ingestion of blood by female lone star, Amblyomma americanum (L.), and American dog, Dermacentor variabilis (Say),

ticks. A secondary objective was to evaluate weight differences over time between two species of ixodid ticks feeding on bovine.

Materials and Methods

Amblyomma americanum and Dermacentor variabilis ticks were reared and maintained as described by Patrick and Hair (1975). Larval and nymphal stages were cultured on caged rabbits and/or stanchioned sheep. Adults used in this study were ca. 150 d post-nymphal molt.

Three calves (150 - 160 kg) were randomly selected for the different experimental treatment units and calf weights were recorded 48 h prior to infestation. Treatments of calves consisted of: (1) animal injected subcutaneously with propylene glycol (5 μg/kg BW) to serve as control; (2) animal injected subcutaneously with Ivomec® (1.0% ivermectin) to achieve a host animal dosage rate of 5 μg/kg BW; or (3) animal injected with Ivomec® (1.0% ivermectin) to obtain a host animal dosage rate of 15 μg/kg BW. Host injection sites were alternated to minimize dermal irritation and abscesses. Regions anterior or posterior and dorsal or ventral of the scapula were used. Injections were initiated 48 h prior to tick infestation and were continued daily to assure tick exposure to a constant level of drug in the host blood. The intent of daily injections was to simulate a drug dosage level comparable to that being emitted from a ruminal bolus.

The dorso-lateral body portions of the calves were washed, shorn, and six cotton stockinette cells per host were affixed with 3-M® #4799 Industrial Adhesive and allowed to dry for 24 h. Stockinette cells were lettered for

identification. On day 0, 30 pairs of adult A. americanum ticks per cell were introduced onto the right side of the hosts and 30 pairs of D. variabilis ticks per cell were introduced onto the left side of each host. Unattached females were removed 24 h post-infestation.

Six female ticks of each species were randomly selected and removed on days 4, 8, and 12 from each host animal using forceps applied to the mouthparts. Ticks were individually weighed on an Mettler AC® 100 to the nearest 0.1 mg within 1 h post-removal. Measurements of blood volume were also done initially on unfed female A. americanum and D. variabilis ticks which served as pretreatment controls to ascertain the amount of blood retained from the nymphal feed.

Hematin in the whole tick was assayed as described by Sutton and Arthur (1962) and later modified by Koch and Sauer (1984). Volumes of 0.1 M NaOH used as diluent consisted of 6, 18, 42, or 720 ml. The supernatant was used as the source of hematin measurements in the assays. The supernatant was decanted into cuvettes and read spectrophotometrically at 525 nm with a Spectronic 20® colorimeter. The spectrophotometer was calibrated with 0.1 M NaOH, pyridine, and sodium dithionite in the same proportions as the test solution. Standard curves were derived from known quantities of host blood by the same procedures used to obtain optical density readings of homogenized ticks.

Differences were judged statistically significant ($p = 0.05$) based on a weighted analysis of variance; the weights chosen were inversely proportional to the estimated variance for each dose level. Weights and blood volumes are presented as means.

Results and Discussion

The blood content and weights of unfed and feeding Amblyomma americanum females and Dermacentor variabilis females are presented in Tables IV and V, respectively. It was also recorded that lower quantities of blood were ingested by ticks feeding on treated hosts (Table IV). There were genera and treatment differences in the weights of female ticks feeding on control and experimental host animals by days 4 and 8 (Table V).

By day 12, only four A. americanum control ticks remained attached. Female A. americanum ticks had been held over from day 11 and two of those had to be used to complete the set of six replications. Six D. variabilis tick females were randomly collected from the control host on day 12.

Control D. variabilis tick females had slightly higher final engorged weights and blood volumes than those found for this tick species in other published studies (Koch et al. 1974; and Koch and Sauer 1984). It was also noted that replete A. americanum females fed on the untreated bovine weighed 17-60% more than previously reported for this species in other studies when fed on hosts other than bovine (Sauer and Hair 1972; and Koch and Sauer 1984). Surprisingly, A. americanum females ingested far more blood and concentrated the blood meal substantially more than reported previously (Sauer and Hair 1972; and Koch and Sauer 1984).

Dose rates of 5 μg/kg or 15 μg/kg of ivermectin are more impacting to the feeding cycle of the female A. americanum tick than they are to the D. variabilis. As the data in Tables IV and V indicate, weight gains and blood intake in both genera of ticks were adversely affected by the acaricide.

The amount of blood ingested by Dermacentor variabilis may be underestimated because considerable undigested reddish hematin-like material is excreted by this species while they feed. Because the assay for measuring blood in the feeding tick is a measure of heme converted to pyridine hemochromogen in whole homogenized ticks (Sutton and Arthur 1962), it is possible that this material in the fecal pellet represents another considerable unmeasured fraction of the blood meal. Amblyomma americanum, on the other hand, excrete little feces while feeding and the material that is excreted is whitish in appearance and probably guanine, the nitrogenous waste product in many species of ixodid ticks (Coons et al. 1986). It is interesting to note the differences in weights and volume of blood consumed by ticks in this study to that previously recorded for A. americanum female weights. However, the differences in estimated blood volumes ingested in this study were directly proportional to tick weight. Weights of A. americanum females in this study were 60% heavier than females documented by Koch and Sauer (1984) and a 70% increase in blood volume between ticks in the different studies was noted. The concentration factor of A. americanum ticks fed on bovine was 23 and 50% greater than ticks fed on dogs (Koch and Sauer 1984) and deer (Sauer and Hair 1972), respectively. One reason for these noted differences could be the susceptibility of young, naive calves to ticks.

At the dosage rates used in this trial male ticks of both species survived and were attached to the bovine hosts. Changes in blood intake were not measured for males of either species, nor was it confirmed that all females were mated. Matings were noted, however, in all treatments for both species.

Ivermectin is much more toxic to A. americanum ticks than D. variabilis ticks and the drug is more effective in inhibiting ingestion of the blood meal in A. americanum ticks (Table IV). Very little blood was ingested by A. americanum females at either concentration of the drug whereas D. variabilis females ingested greater amounts of whole blood at the highest concentration.

CHAPTER VI

SUMMARY

Amblyomma americanum (L.) unpaired and paired females were monitored daily. Up to a point, the feeding processes (electronically recorded feeding patterns) were similar although the percent of the types denoted for the different experimental groups varied somewhat. It was also apparent that the occurrence of the feeding patterns were diverse. As illustrated by the weights and blood volumes imbibed males have an effect on female feeding behavior but only after they have mated and thereby insuring the onset of fast feeding by mated females. The rapid engorgement stage occurred only in mated females.

Pilocarpine applications defined a typical intense salivation pattern that was similar to that electronically recorded for paired females prior to repletion/ detachment. Two of the four feeding patterns recorded post-ivermectin application were similar if not replicates of the intense salivation phases recorded for both paired females prior to detachment and pilocarpine-injected females.

Topical applications of amitraz to feeding females changed their feeding patterns soon (< 1 minute) after application. Patterns recorded for ticks treated with amitraz were not similar to each other nor to patterns previously recorded for normal ticks nor other patterns recorded for ticks altered with different drugs. Amitraz-treated ticks generally detached within 30 min post-chemical application.

Further studies are necessary using this drug in more dilute concentrations. It would be useful to dilute amitraz in an effort to affect tick feeding to a lesser degree instead of inducing complete detachment. Pharmacological applications during tick feeding will be of future use to further advance the knowledge of how chemicals effect ticks while feeding and to make comparisons between various chemicals.

The quantification of blood imbibed on a daily basis for A. americanum females, unpaired and paired, was determined using average blood volumes and mean weights from a total of 12 ticks daily. Values noted for both parameters were not significantly different between the two experimental groups until day 12 when the paired females apparently entered the rapid engorgement phase. Where differences were recorded for feeding behavior among the two experimental groups, paired and unpaired females, this data defining the daily weights and blood volume may be of some benefit in correlating the two sets of data.

Whether differences in rates of feeding, size of blood meal relative to unfed tick size, and mechanisms of excretion affect the toxicity of ivermectin in ixodid ticks has yet to be established. Differences in the mechanisms of excretion between the two species of ixodid ticks, Dermacentor variabilis and Amblyomma americanum, could provide an explanation for differences in toxicity of ivermectin to the two species. Dermacentor variabilis appear to ingest more drug than A. americanum when feeding on treated host animals but are affected to a lesser degree. Other species of ixodid ticks should be studied to see if those that have mechanisms of excretion like that found in D. variabilis are less susceptible to ivermectin than those which excrete little or no heme while feeding on the host.

It would also be of interest to see what percentage of the ingested drug is excreted by species such as D. variabilis as compared to A. americanum ticks. At the same time, the mode of action of ivermectin in ticks is little known and this should be an area of future research.

To date, only a few definite conclusions can be drawn: 1) definite and specific feeding pattern types do occur, 2) weights and blood volumes vary to a large extent and variations are due to the individual tick, and 3) males do effect female feeding but only as it relates to mating.

Further investigations are necessary to determine specific feeding behaviors over time. One such study should focus on electronically monitoring feeding ticks on a continuous basis - i.e., monitor at various times on a 24 h basis. Another area of interest would be to electronically monitor different genera of ticks--monitor unmated females, add pre-fed males so immediately upon introduction of males, mating and electronic monitoring could be recorded. Further utilization of pharmacological agents should continue and enhance our knowledge of the mechanical nature of tick feeding to perhaps one day allow us to protect man and animal from ticks and their diseases.

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Wilkins, C. A., J. Conroy, P. Ho, and W. O'Shanny. 1980. Efficacy of ivermectin against ticks on cattle. p. 18. Proc. 25th Ann. Meet. Am. Assoc. Vet. Parasitol. Washington, D.C.

APPENDIX

Table I. Occurrence of Pattern Types for Unpaired Female Amblyomma americanum (L.). Percentages of Individual Pattern Types are Expressed by Day.

	Day								
Pattern Type	2	3	4	5	6	7	8	9	10
1	3	4.5	3	6	3	3	0	6	0
2	0	0	6	0	4	3	0	0	0
3	3	0	0	0	0	3	6	3	6
4	0	0	0	0	0	3	0	0	0
5	0	0	0	0	0	0	0	0	0
6	0	1.5	0	4	0	0	3	1	3
7	0	0	3	0	1.5	0	0	2	0
8	0	0	0	0	0	0	0	0	0
9	0	6	0	1.5	0	0	0	0	3
10	0	0	0	0	3	0	3	0	0

Table II. Occurrence of Individual Pattern Types By Day of Study for Paired Amblyomma americanum (L.) Female.

	Day											
Pattern Type	2	3	4	5	6	7	8	9	10	11	12	13
1	3	2	1	3	1	1	0	3	1	0	0	0
2	1	1	1	1.5	3.5	1.5	1	1	0	2	0	0
3	1	0	2	0	1.5	0	0	2	2	1	0	0
4	0	3	2	2	2	3	2	2	1	0	0	0
5	0	0	2	0	0	0	1	0	2	4	7.5	8.5
6	1	0	1	1	0	0	1	1	1	1	1	1
7	0	1	0	0	0	1.5	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	4
9	0	0	0	1	0	1	1	0	1	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0

Table III. Percent Pattern Types for Chemically Altered Feeding Amblyomma americanum (L.) Females by Day for Days 13, 14, and 15 of Feeding.

	Day		
Pattern Type	13	14	15
1	0	0	4
5	0	4	0
6	3	0	0
10	4	0	0
11	11	13	4
Amitraz	18	4	4
Pilacarpine	4	10	0
Ivermectin	4	9	4

Table IV. Average Blood Volumes (μl) of Unfed and Engorging Female Ticks from Untreated and Ivermectin-Treated Bovine.

	Amblyomma americanum			Dermacentor variabilis		
	µg/kg ivermectin			µg/kg ivermectin		
Day	0	5	15	0	5	15
0	2.0	2.0	2.0	3.3	3.3	3.3
4	7.1a	0.2a	0.0a	128.6ab	31.2a	14.9b
8	115.5a	0.8a	0.6a	2406.0a	24.1b	13.3c
12	2617.5a	0.2b	0.0b	2057.8a	28.2b	7.4c

Lower case letters pertain to row means for each species; row means followed by the same letters are not significantly different at the 0.05 level of probability.

Table V. Comparison of Mean Weights (mg) of Unfed and Engorging Female Ticks from Untreated and Ivermectin-Treated Bovine.

	Amblyomma americanum			Dermacentor variabilis		
	µg/kg ivermectin			µg/kg ivermectin		
Day	0	5	15	0	5	15
0	5.2	5.2	5.2	5.8	5.8	5.8
4	9.8a	7.0a	5.8a	30.2a	8.6a	7.9a
8	47.9a	6.1a	6.2a	662.8a	10.4b	9.0c
12	869.3a	5.8b	5.0b	637.3a	12.9b	8.4c

Lower case letters pertain to row means for each species; row means followed by the same letters are not significantly different at the 0.05 level of probability.

Figure 1. Principle Components of the AC Electronic Measurement System (EMS).

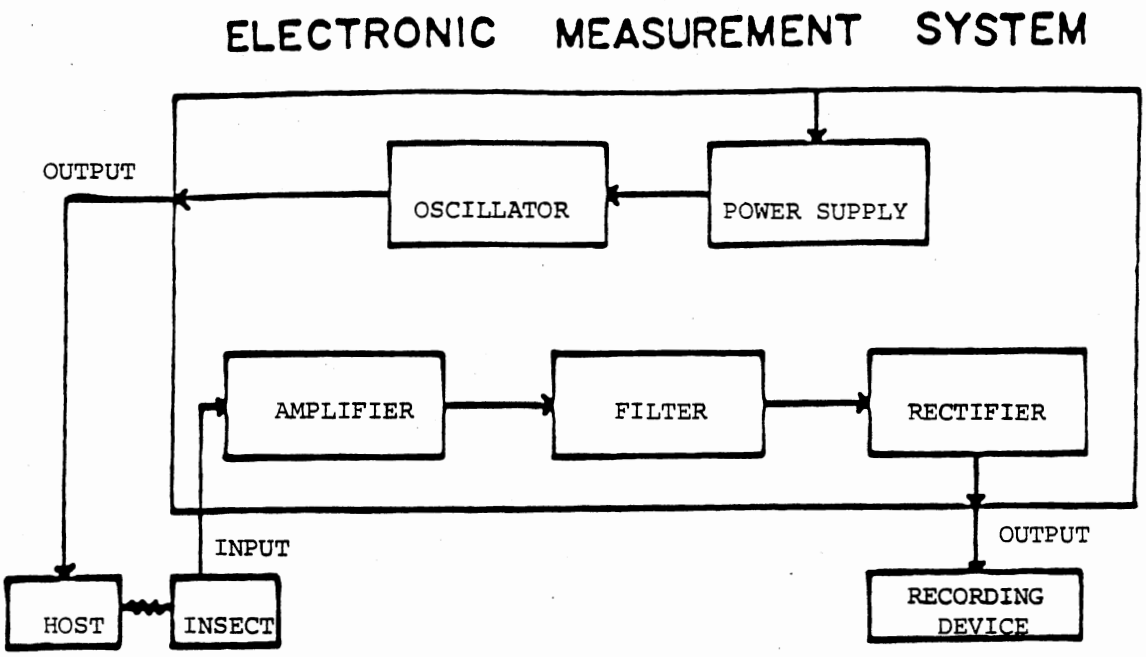

ELECTRONIC MEASUREMENT SYSTEM
OUTPUT
OSCILLATOR
POWER SUPPLY
AMPLIFIER
FILTER
RECTIFIER
INPUT
OUTPUT
HOST
INSECT
RECORDING DEVICE

Figure 2. Schematic of the Adapted EMS Technique used for Monitoring Ticks While Feeding on Mammalian Hosts.

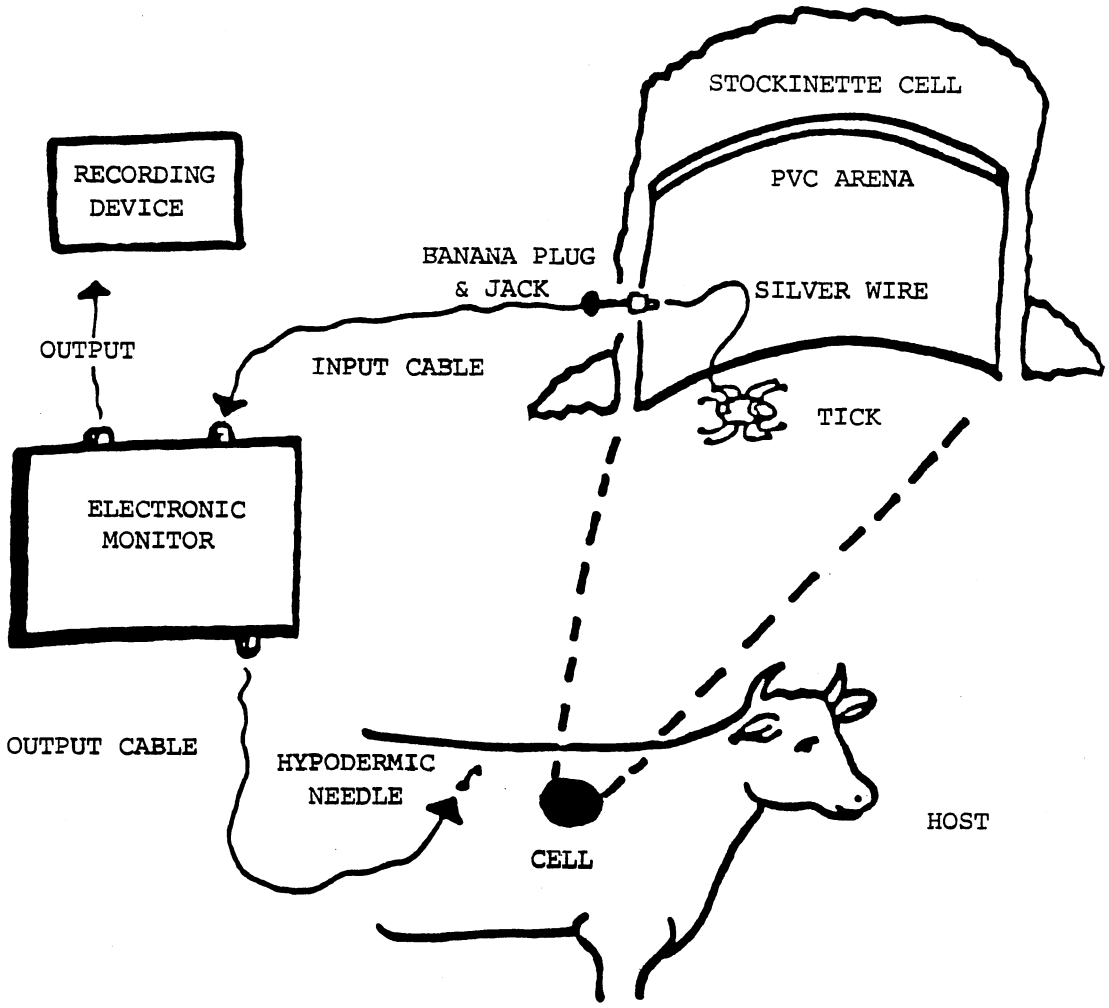
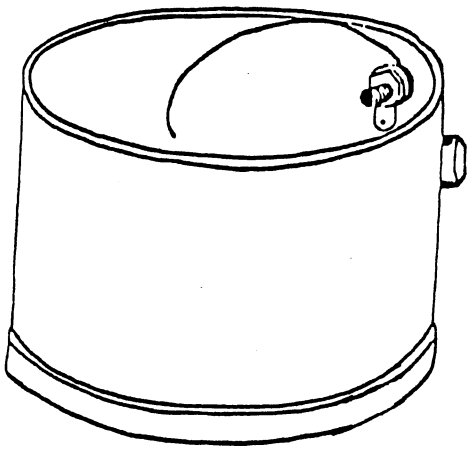
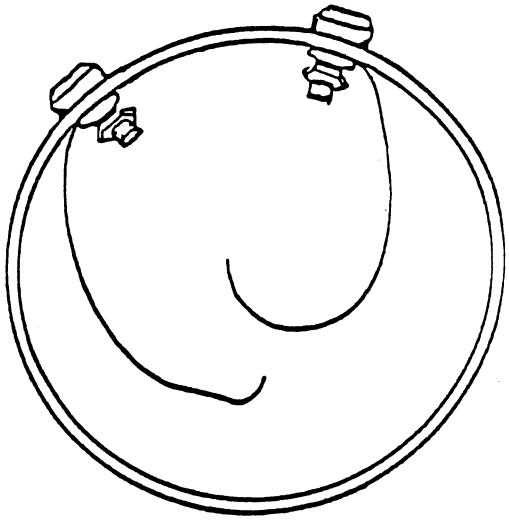

STOCKINETTE CELL
PVC ARENA
RECORDING DEVICE
BANANA PLUG & JACK
SILVER WIRE
OUTPUT
INPUT CABLE
TICK
ELECTRONIC MONITOR
OUTPUT CABLE
HYPODERMIC NEEDLE
CELL
HOST

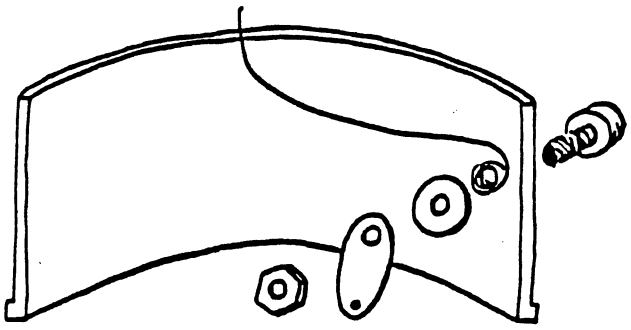
Figure 3. Modified PVC Rings Used for Connecting Ticks to the EMS.



Assembled Unit

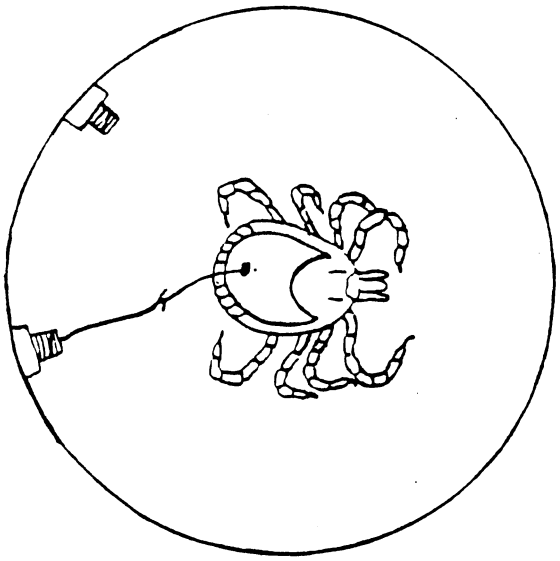


Top View

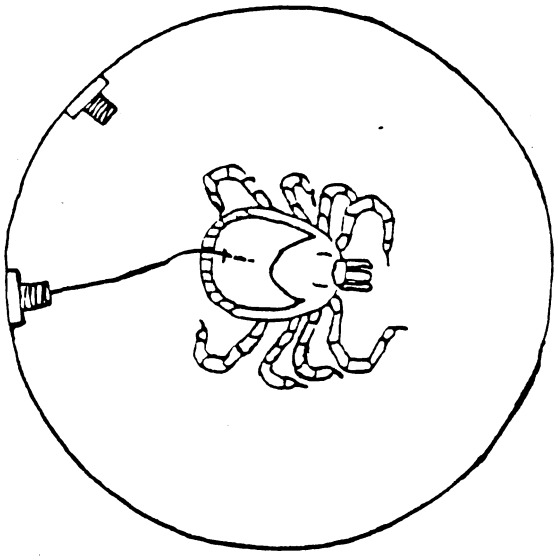


Cut-away View

Figure 4. Insertion of Silver Wire in and the Attachment of Silver Wire to Female Ticks.



Attachment of 0.1 mm silver wire to the posterior dorsum of tick using colloidal liquid silver.



Insertion of 0.25 mm silver wire into posterior dorsum of tick.

Figure 5. Pattern Type #1 Shows a Recording of Alternating Resting and Sucking Feeding Activities for Amblyomma americanum (L.) Females.

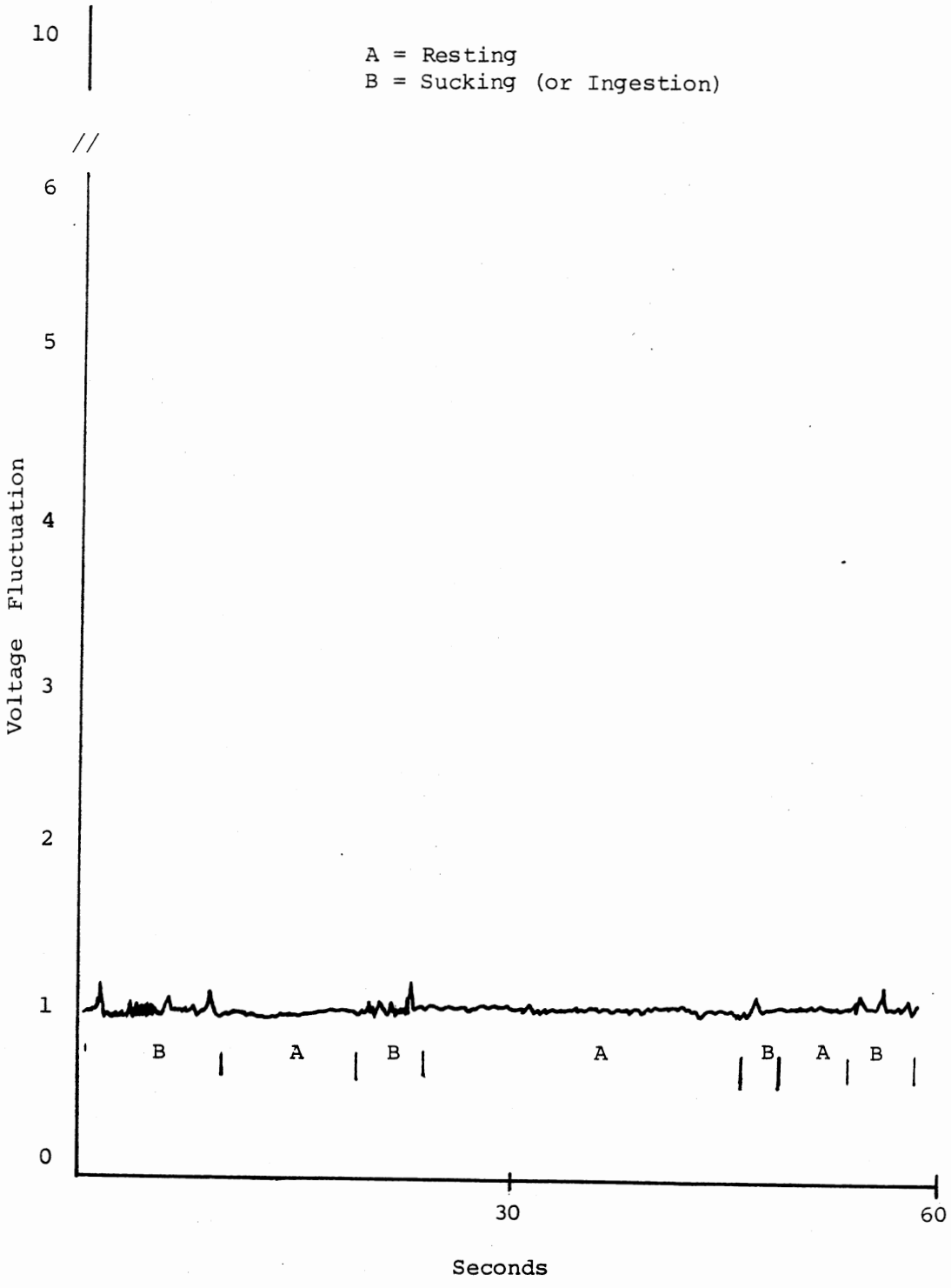

10
A = Resting
B = Sucking (or Ingestion)
//
6
5
4
3
2
1
0
Voltage Fluctuation
B
A
B
A
B
A
B
30
60
Seconds

Figure 6. Represents a Tracing for Pattern Type #2 of Electronically Monitored Female Amblyomma americanum (L.). Tracing Indicates a Repetitive Pattern of Resting, Expulsion of Saliva, and Sucking Phases.

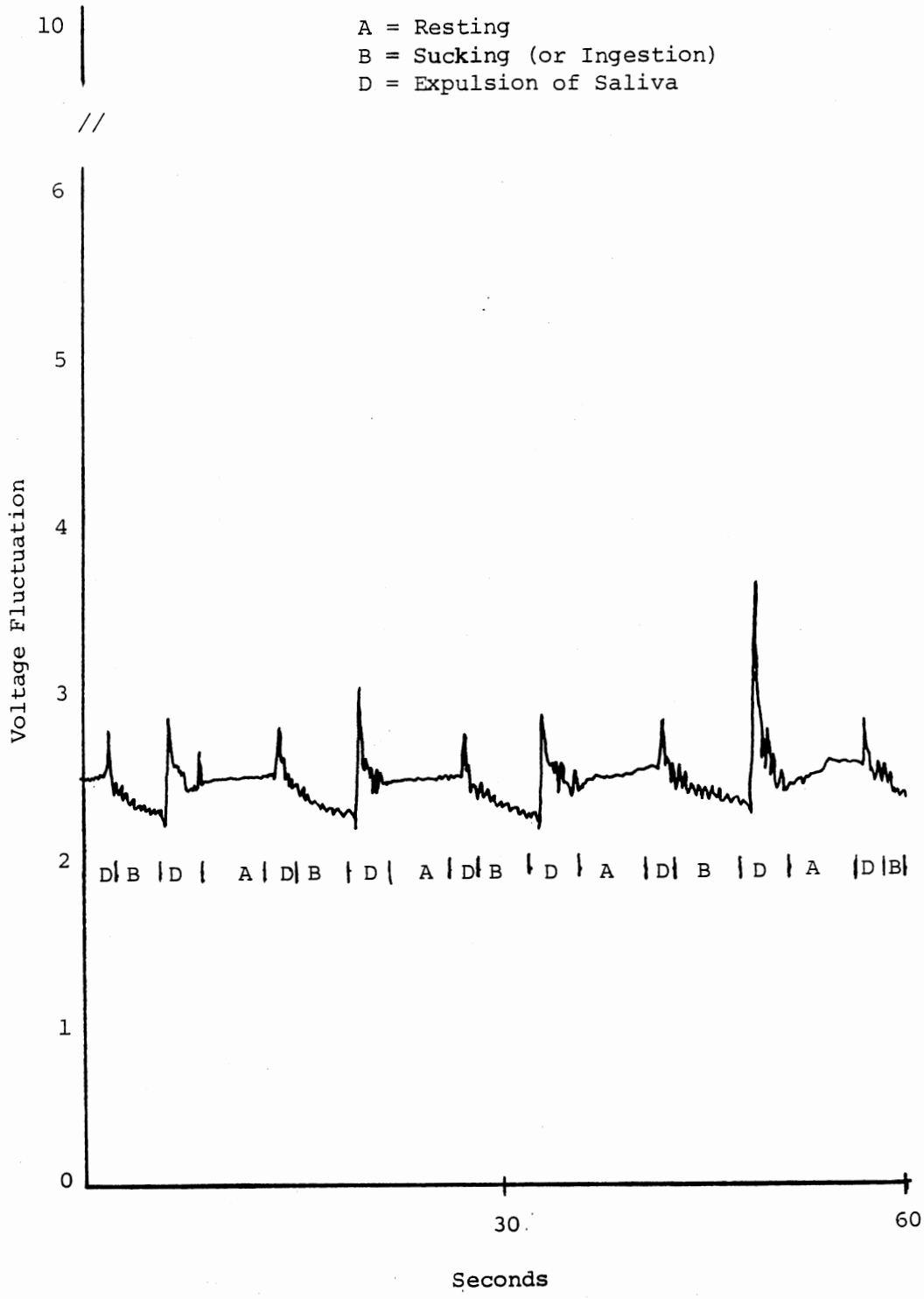

A = Resting
B = Sucking (or Ingestion)
D = Expulsion of Saliva
10
//
6
5
4
3
2
1
0
Voltage Fluctuation
D B D A D B D A D B D A D B D A D B
30
60
Seconds

Figure 7. Pattern Type #3 Showing Feeding Activities of Two-Thirds Resting and One-Third Sucking for Feeding Female Amblyomma americanum (L.).

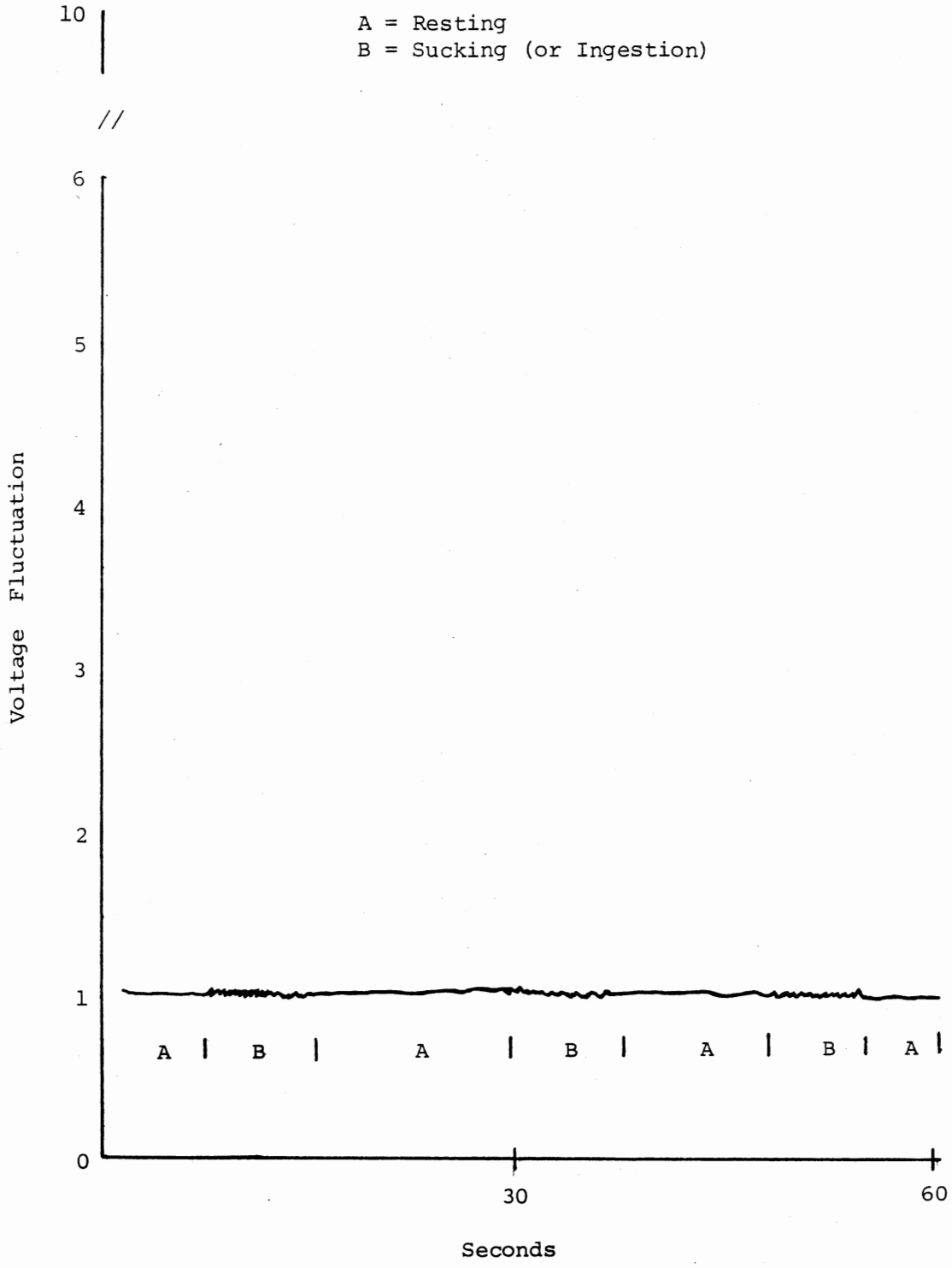

A = Resting
B = Sucking (or Ingestion)
10
//
6
5
4
3
2
1
0
Voltage Fluctuation
A
B
A
B
A
B
A
30
60
Seconds

Figure 8. Tracing of Pattern Type #4 Recorded for Amblyomma americanum (L.) Females. The Majority of the Feeding Activities was Salivation but Sucking and an Expulsion of Saliva were also Recorded.

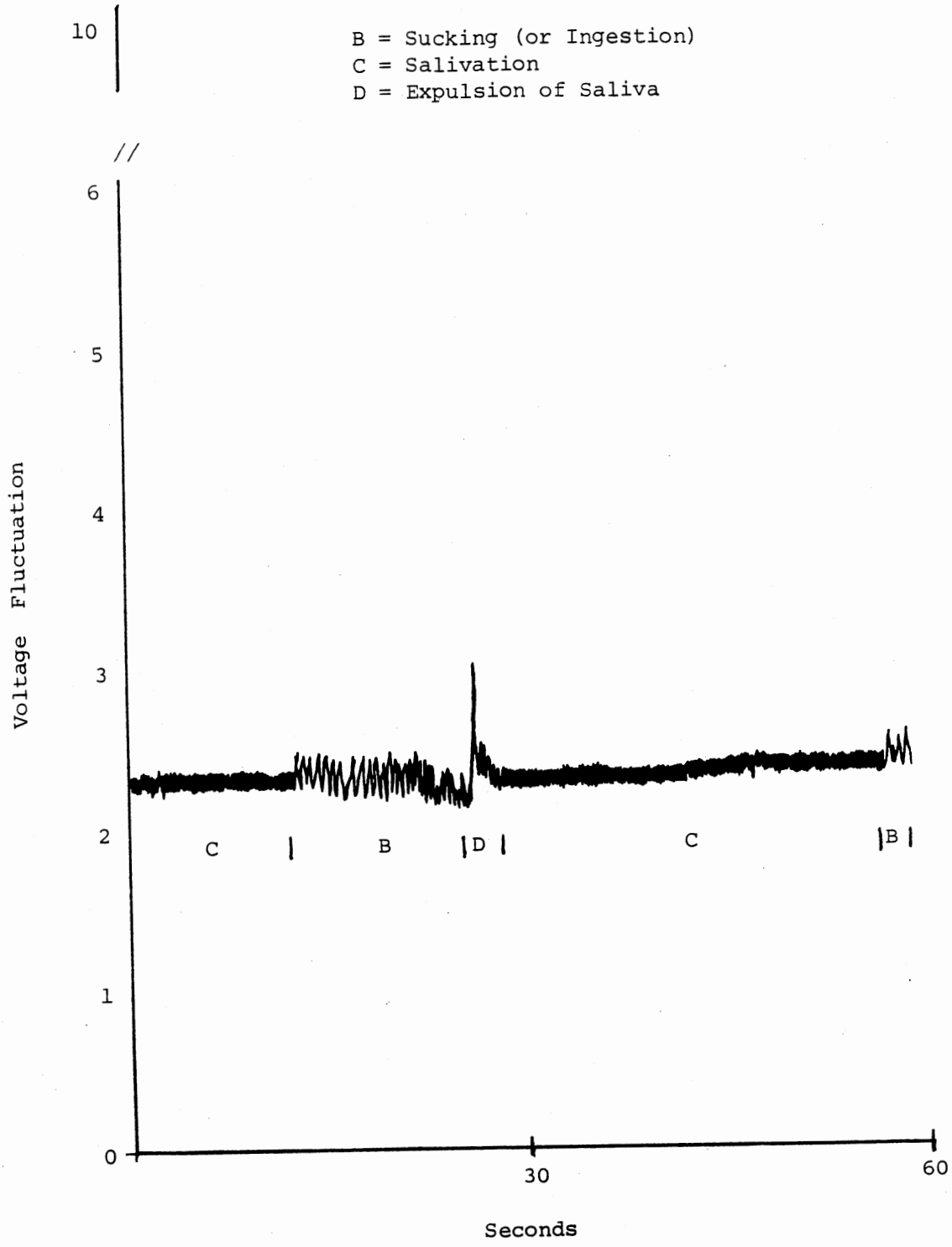

10
B = Sucking (or Ingestion)
C = Salivation
D = Expulsion of Saliva
//
6
5
4
3
2
1
0
Voltage Fluctuation
C
B
D
C
B
30
60
Seconds

Figure 9. This Pattern, Type #5, Represents an Increase in Attenuated Voltage Fluctuation with Resting Phases Occurring During the Voltage Increase. A Minimal Amount of Sucking and Saliva Expulsion was also Recorded for Amblyomma americanum (L.) Females.

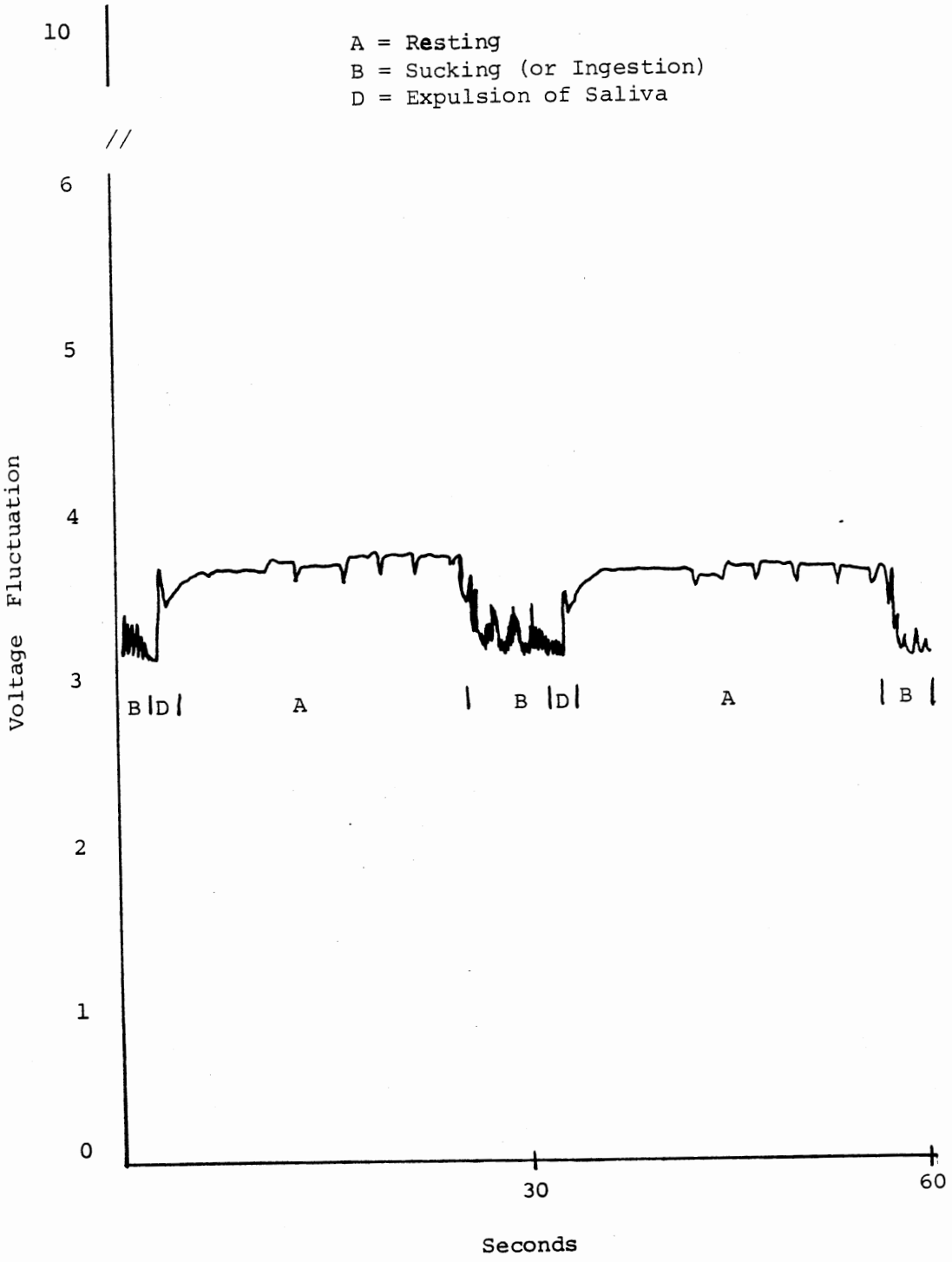

10
A = Resting
B = Sucking (or Ingestion)
D = Expulsion of Saliva
//
6
5
4
3
2
1
0
Voltage Fluctuation
B D
A
B D
A
B
30
60
Seconds

Figure 10. Pattern Type #6 Depicts Feeding Activities of Alternating Sucking and Resting Phases for Feeding Amblyomma americanum (L.) Females.

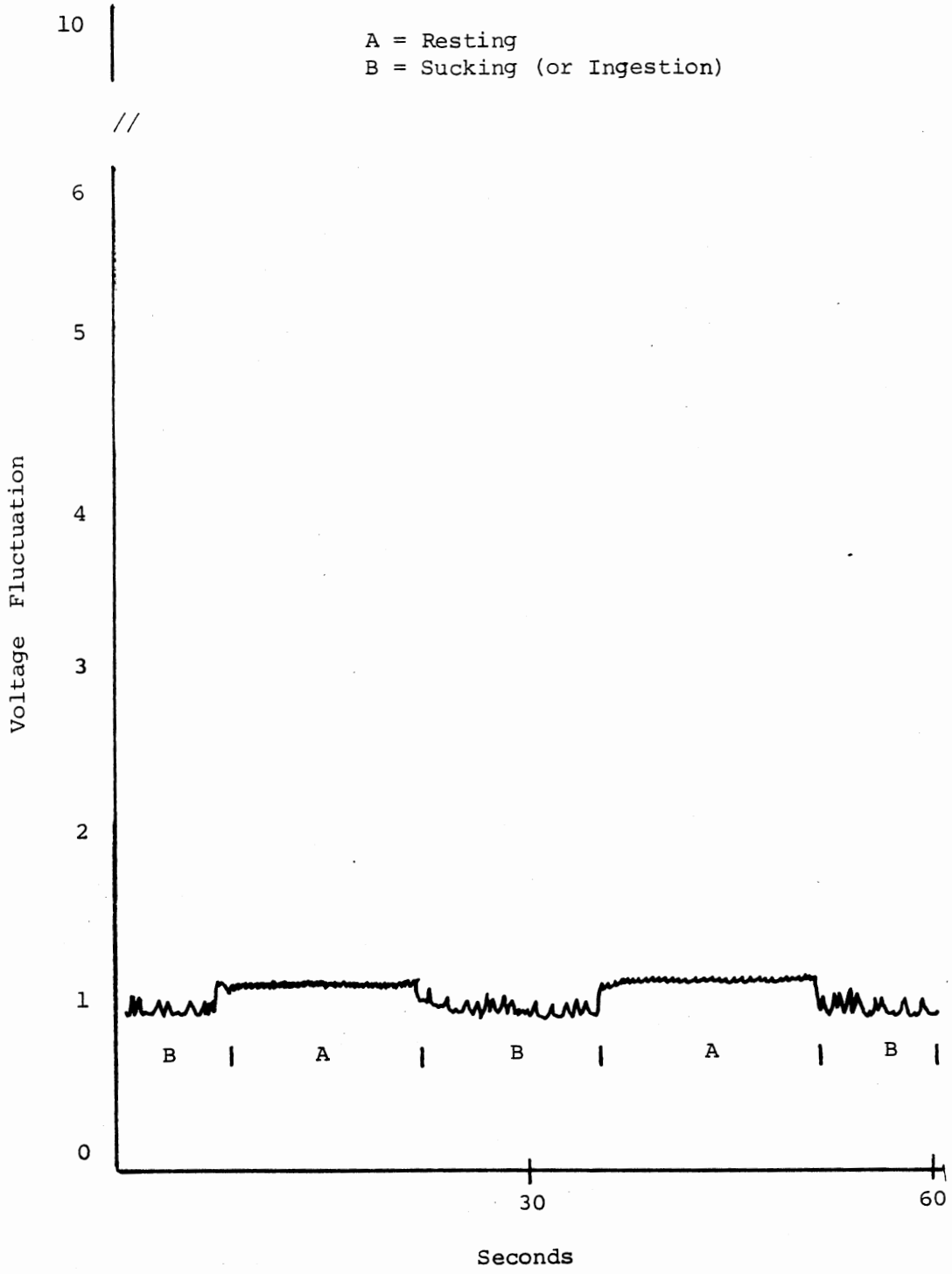

10
A = Resting
B = Sucking (or Ingestion)
//
6
5
4
3
2
1
0
Voltage Fluctuation
B
A
B
A
B
30
60
Seconds

Figure 11. Electronically Recorded Feeding Pattern for Amblyomma americanum (L.) Females, Pattern Type #7. The Pattern Indicates Recurring Feeding Activities of Sucking, Saliva Expulsion, and Resting.

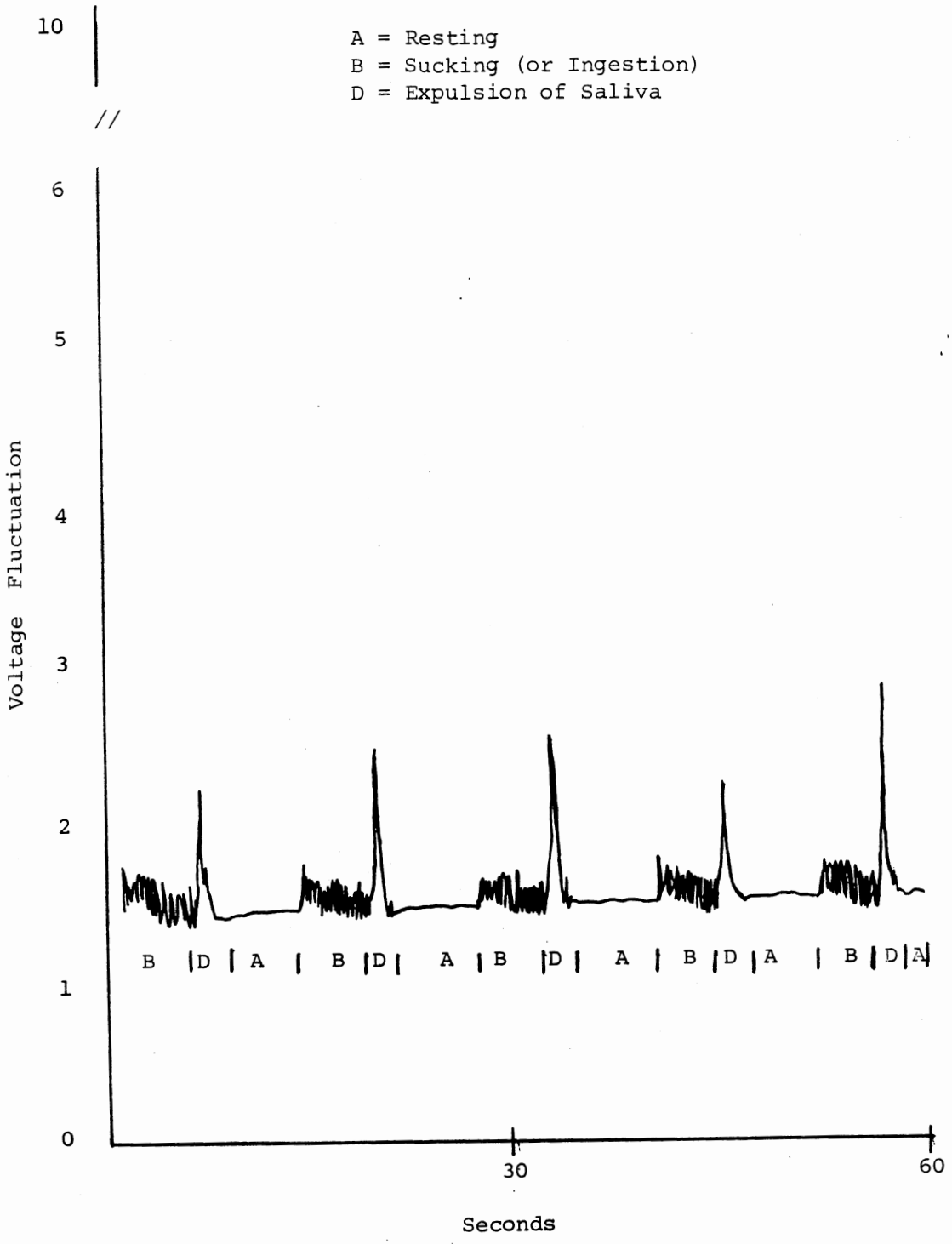

10
A = Resting
B = Sucking (or Ingestion)
D = Expulsion of Saliva
6
5
4
3
2
1
0
Voltage Fluctuation
B D A B D A B D A B D A B D A
30
60
Seconds

Figure 12. Feeding Activity Indicated in Pattern Type #8 was Continuous Salivation. It was Recorded on Day 13 for Paired Amblyomma americanum (L.) Females Prior to Repletion.

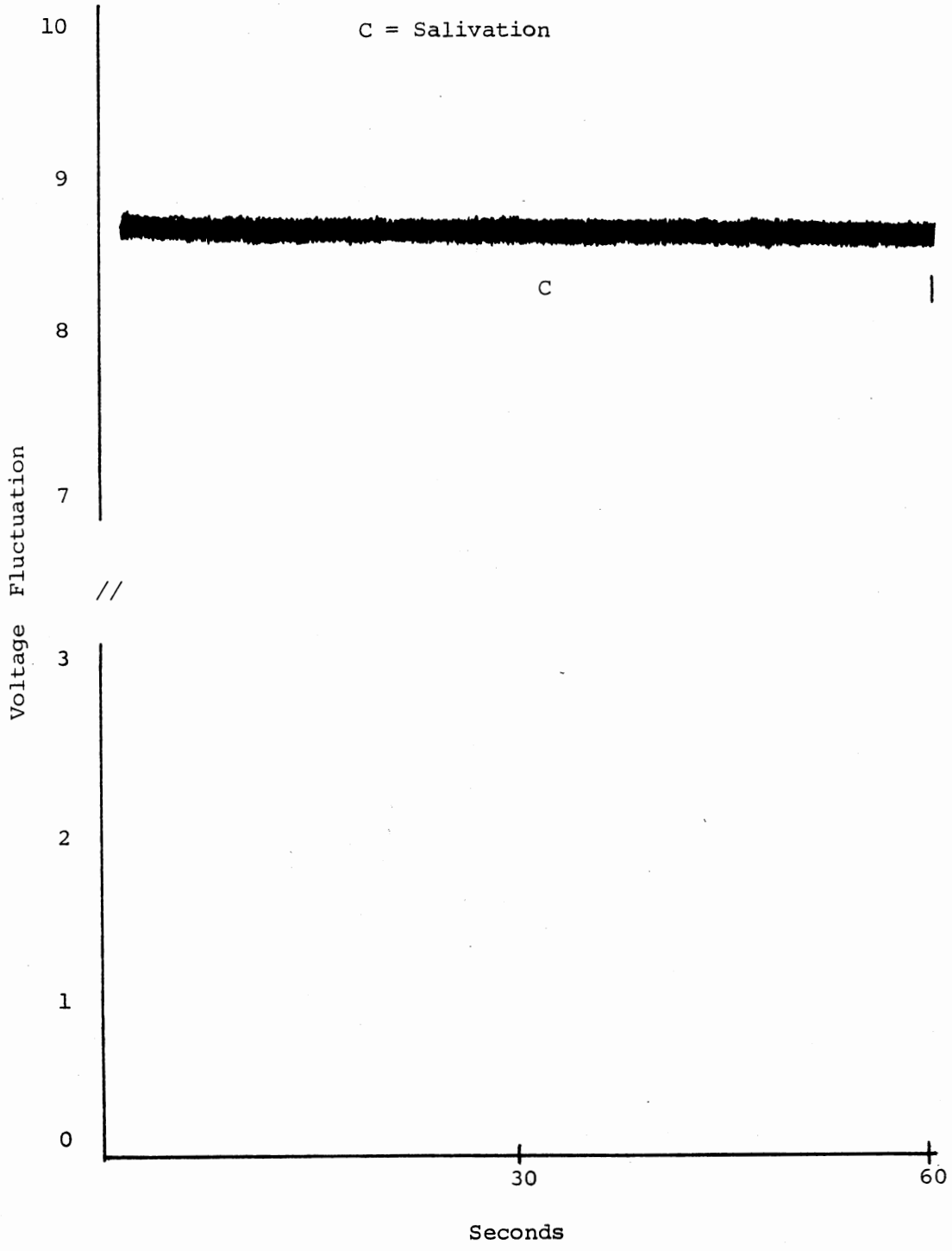

C = Salivation
10
9
8
7
C
//
3
2
1
0
Voltage Fluctuation
30
60
Seconds

Figure 13. Electronically Recorded Feeding Pattern for Amblyomma americanum (L.) Females, Pattern Type #9. Type #9 Recording Shows a Feeding Rhythm of Constant Salivation That was Similar to Pattern #8.

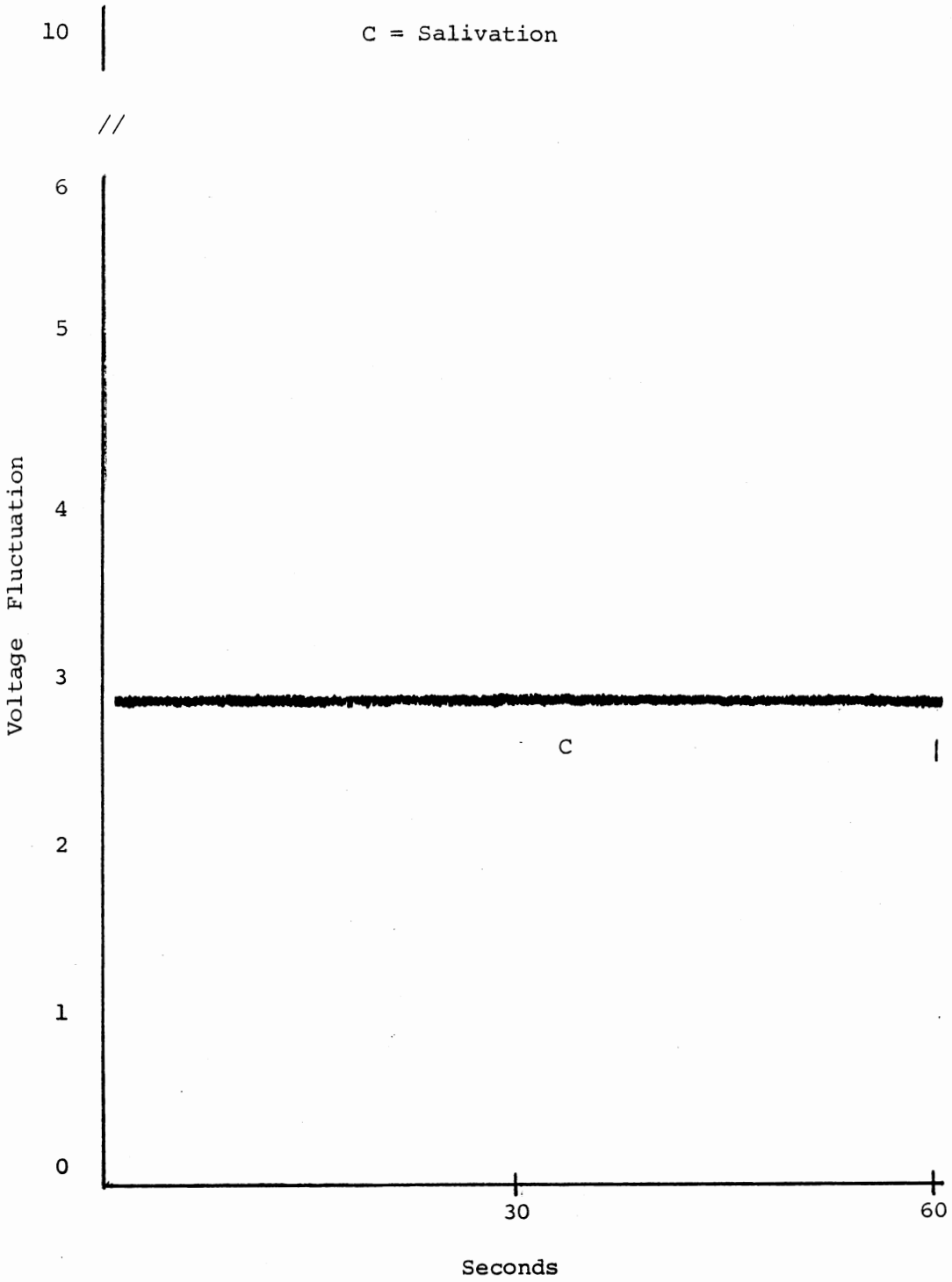

10
C = Salivation
//
6
5
4
3
2
1
0
Voltage Fluctuation
C
30
60
Seconds

Figure 14. On Days 6 and 8, Feeding Pattern #10 was Only Recorded for Unpaired Amblyomma americanum (L.) Females. Feeding Activities that Occurred within Type #10 were Expulsion of Saliva, Sucking (or Ingestion), and Resting.

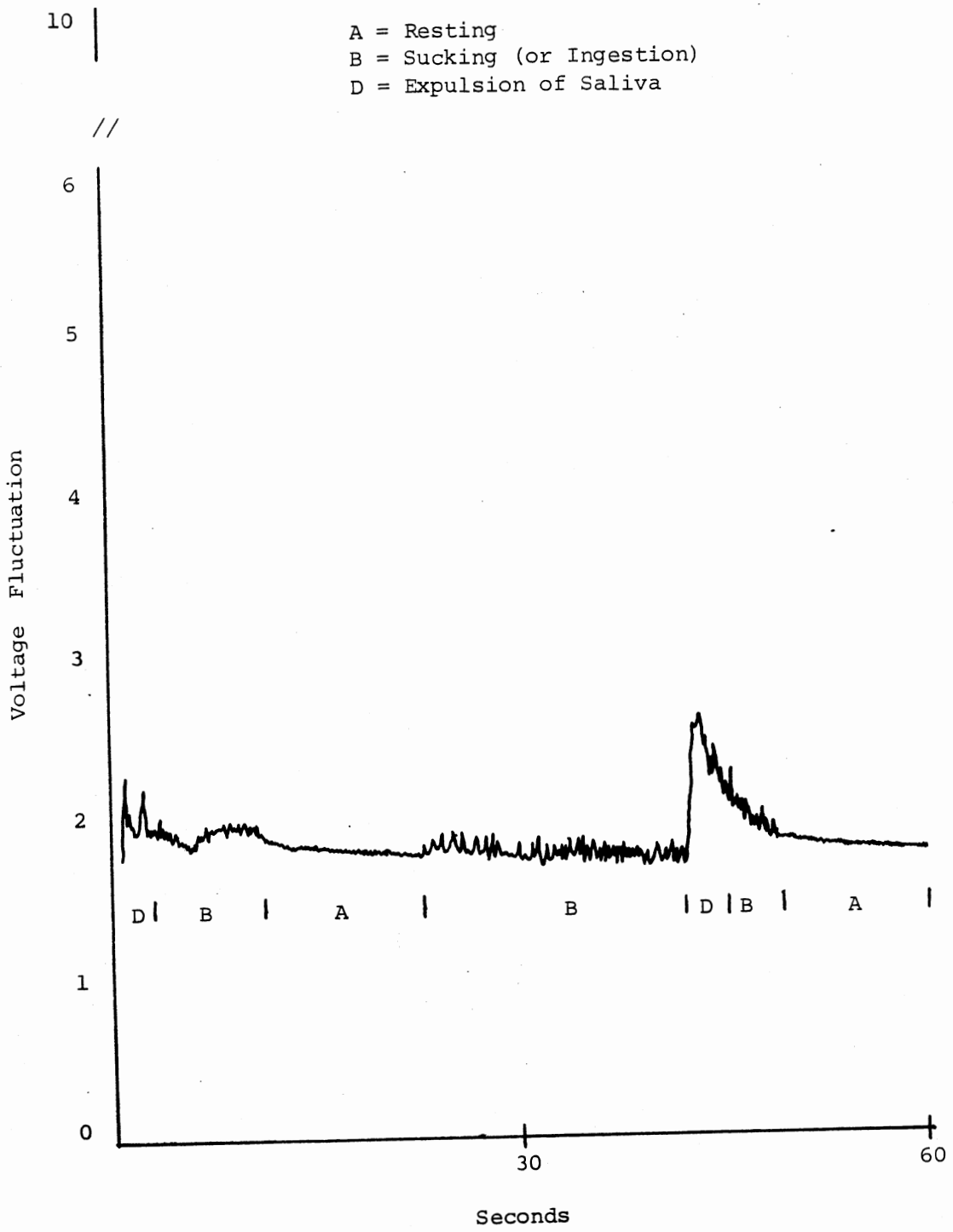

10
A = Resting
B = Sucking (or Ingestion)
D = Expulsion of Saliva
6
5
4
3
2
1
0
Voltage Fluctuation
D
B
A
B
D
B
A
30
60
Seconds

Figure 15. Total Percentile of Pattern by Types for Unpaired and Paired Amblyomma americanum (L.) Females. Pattern Types Identified #1-10 were Calculated for Total Time Recorded for the Duration of the Study and Percentages of Each Pattern was Estimated.

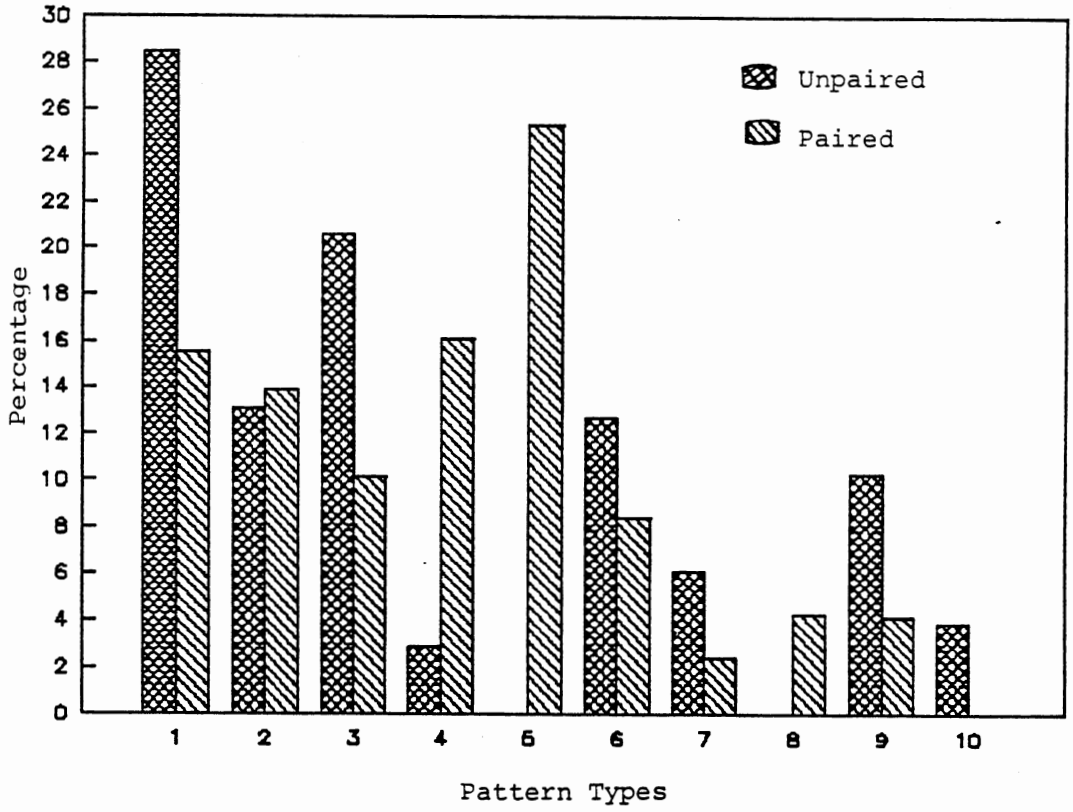

30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
Percentage
Unpaired
Paired
1
2
3
4
5
6
7
8
9
10
Pattern Types

Figure 16. Pattern Type #11 was Electronically Recorded for Normal Feeding Female Amblyomma americanum (L.) on Days 13, 14, and 15. This Pattern was not Recorded Prior to Day 13 for Paired Females Monitored Previously. Feeding Activities within Type #11 were Alternating Resting and Sucking (or Ingestion) Phases.

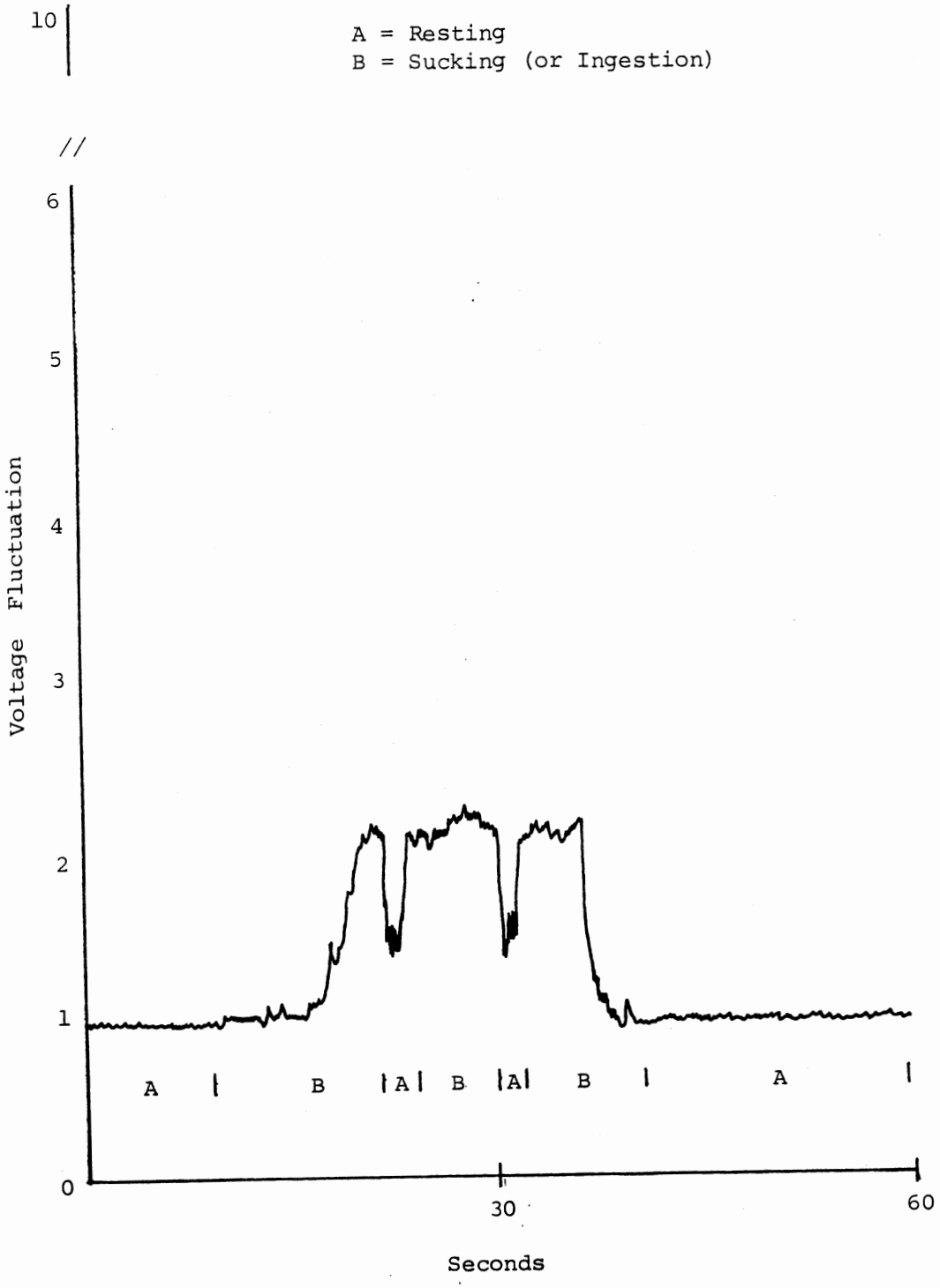

10
A = Resting
B = Sucking (or Ingestion)
//
6
5
4
3
2
1
0
Voltage Fluctuation
A
B
A
B
A
B
A
30
60
Seconds

Figure 17. Pattern Recorded for Amitraz-Treated Amblyomma americanum (L.) Females. Sucking was the Only Type Feeding Activity Recorded for This Pattern.

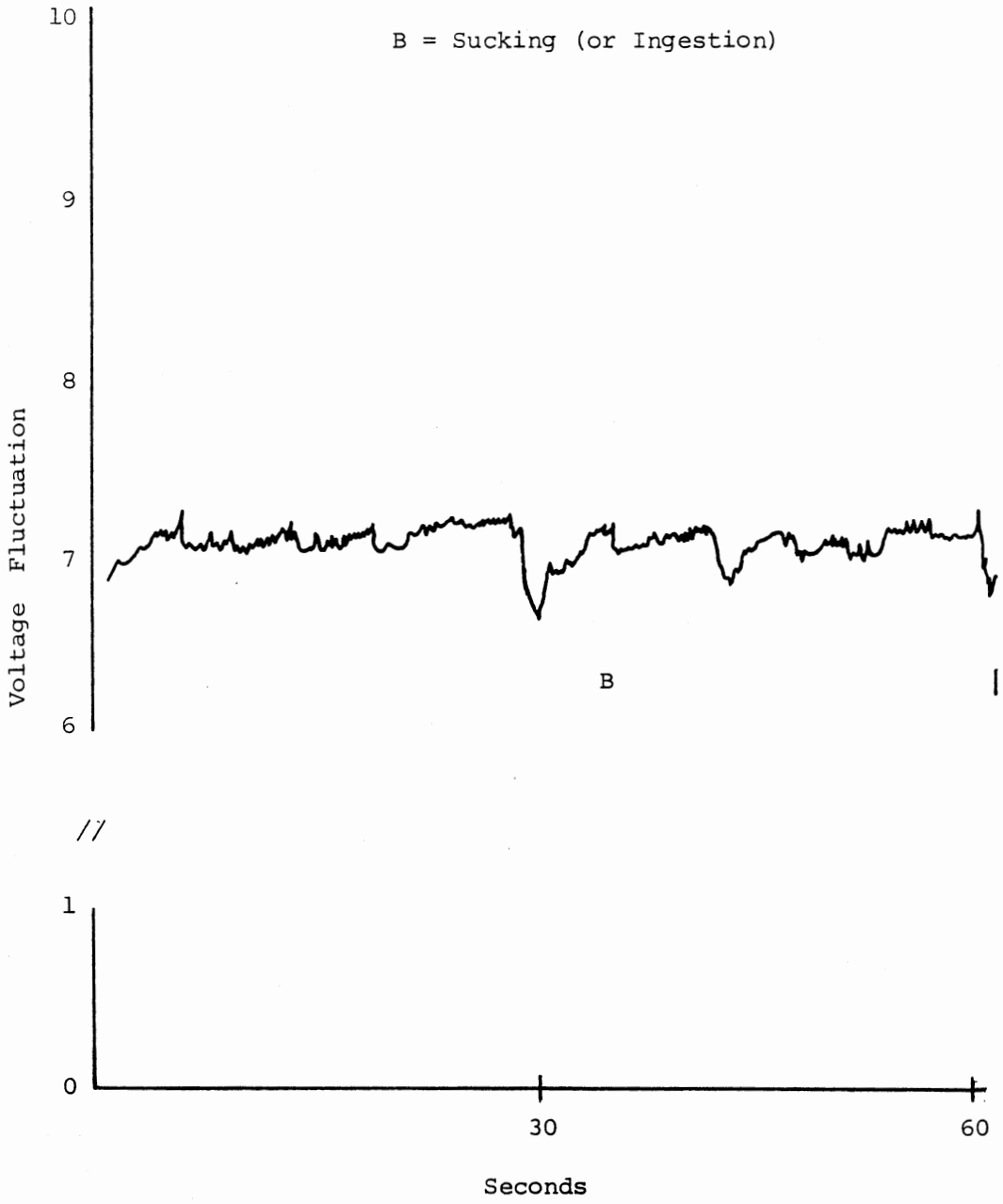

B = Sucking (or Ingestion)
10
9
8
7
6
Voltage Fluctuation
B
//
1
0
30
60
Seconds

Figure 18. This Pattern Recorded for Female Amblyomma americanum (L.) Influenced by Amitraz Shows Erratic Feeding Behavior and was Defined as a Period of Sucking (or Ingestion) for the Length of the Feeding Period Recorded.

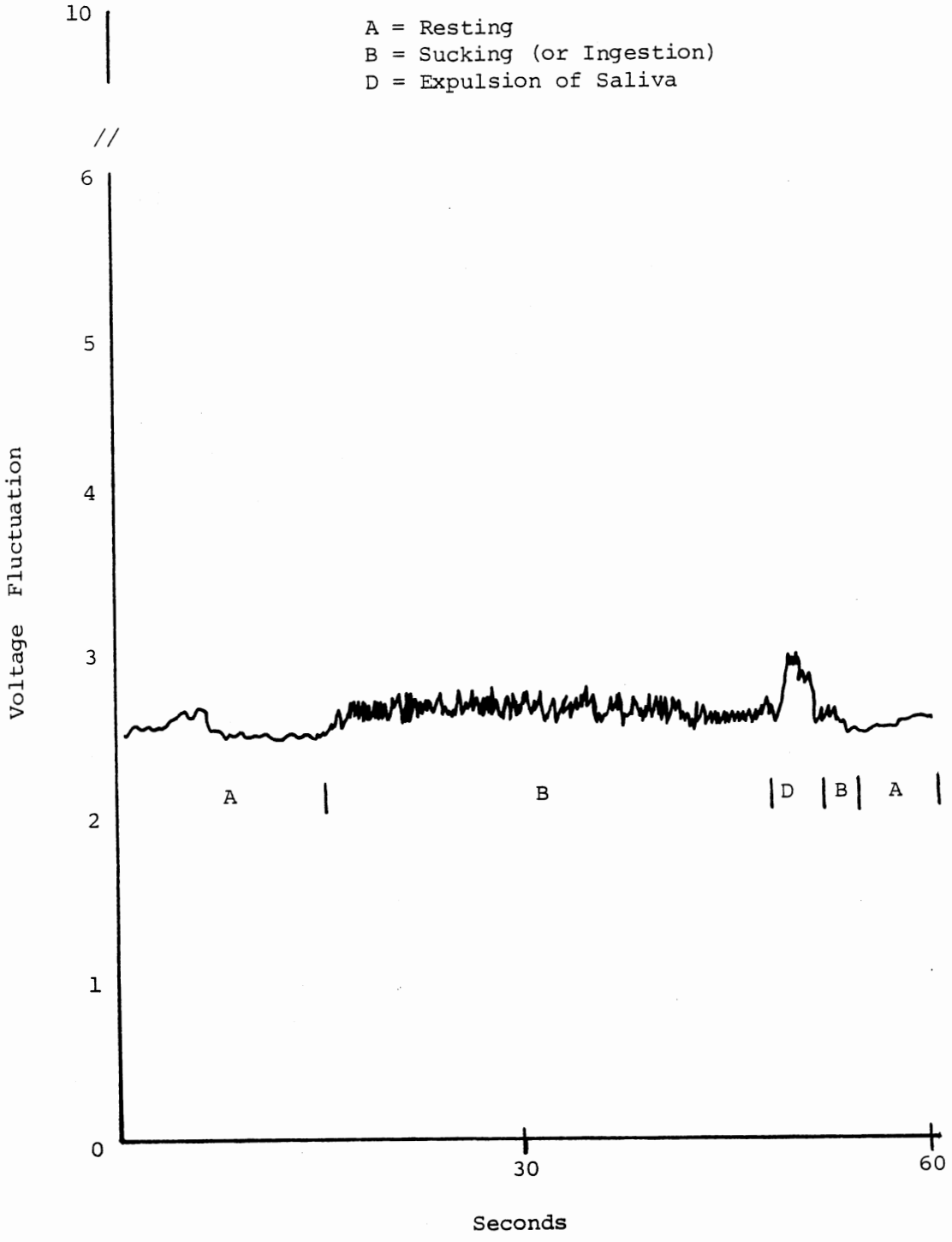

10
A = Resting
B = Sucking (or Ingestion)
D = Expulsion of Saliva
//
6
5
4
3
2
1
0
Voltage Fluctuation
A
B
D
B
A
30
60
Seconds

Figure 19. Pharmacological Alteration of Electronically Recorded Feeding Pattern for Amblyomma americanum (L.) Females, Using Amitraz. The Tracing Reflects Feeding Activities of Resting and Sucking and one Period of Saliva Expulsion.

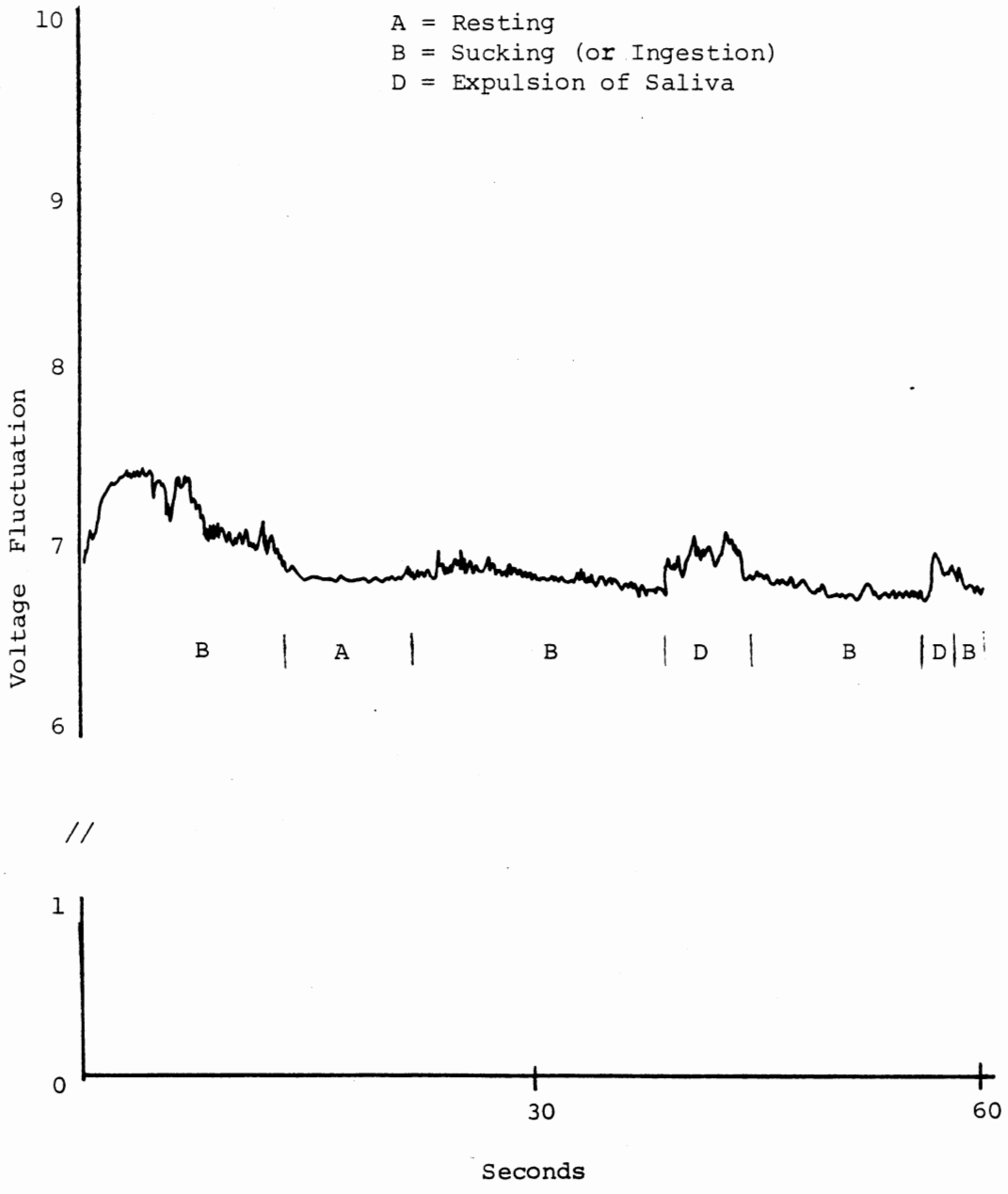

A = Resting
B = Sucking (or Ingestion)
D = Expulsion of Saliva
10
9
8
7
6
Voltage Fluctuation
B
A
B
D
B
D
B
//
1
0
30
60
Seconds

Figure 20. Pattern Recorded for Amblyomma americanum (L.) Females After Topical Application of Amitraz. Feeding Activities Recorded Depict Sucking and Expulsions of Saliva Phases.

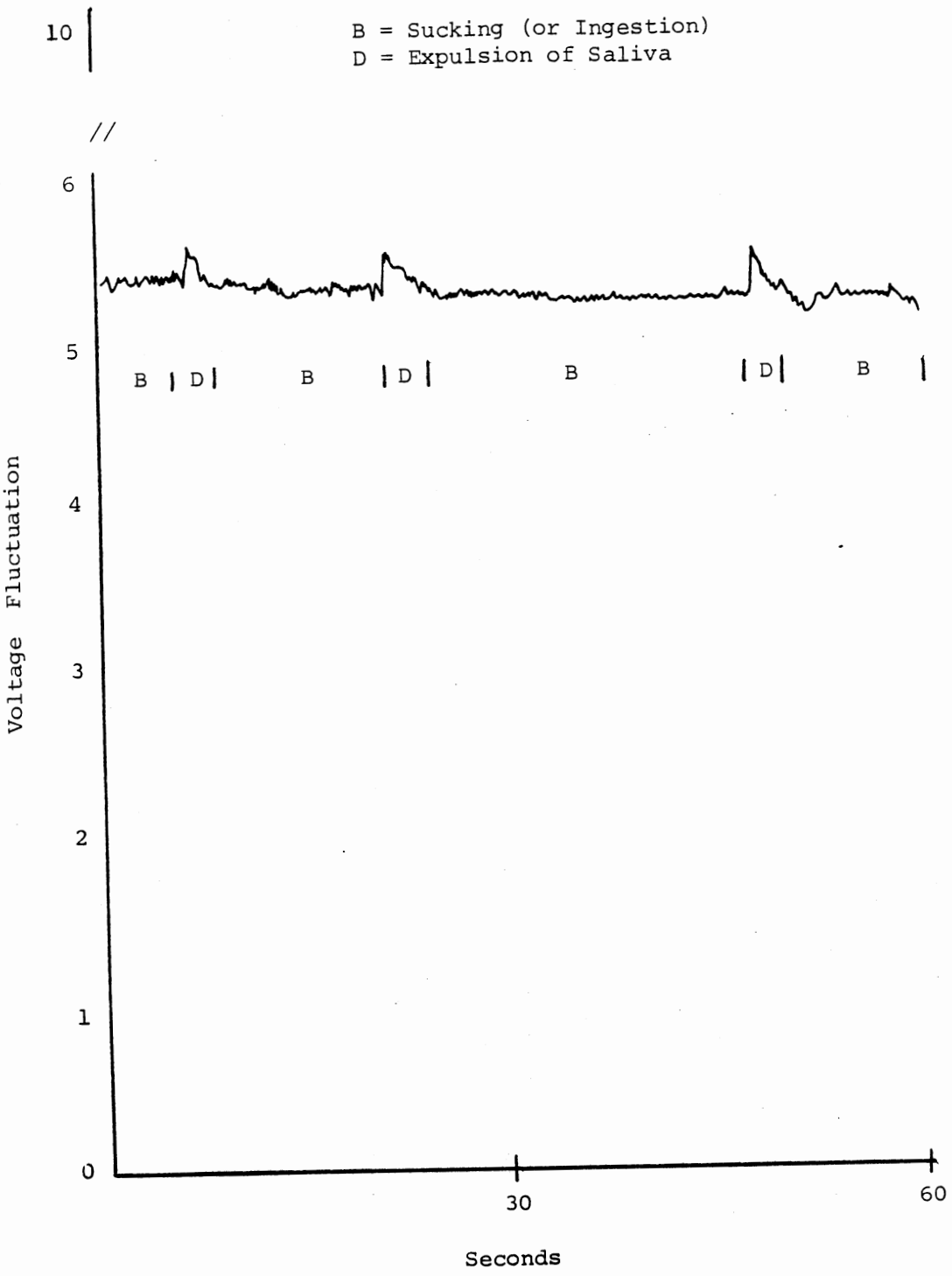

10
B = Sucking (or Ingestion)
D = Expulsion of Saliva
6
5
4
3
2
1
0
B
D
B
D
B
D
B
Voltage Fluctuation
30
60
Seconds

Figure 21. Pattern Recorded by Altering the Normal Feeding Behavior of Amblyomma americanum (L.) Females Using Amitraz. Feeding Activities within the Pattern Included Sucking, Saliva Expulsion, and One Period of Resting.

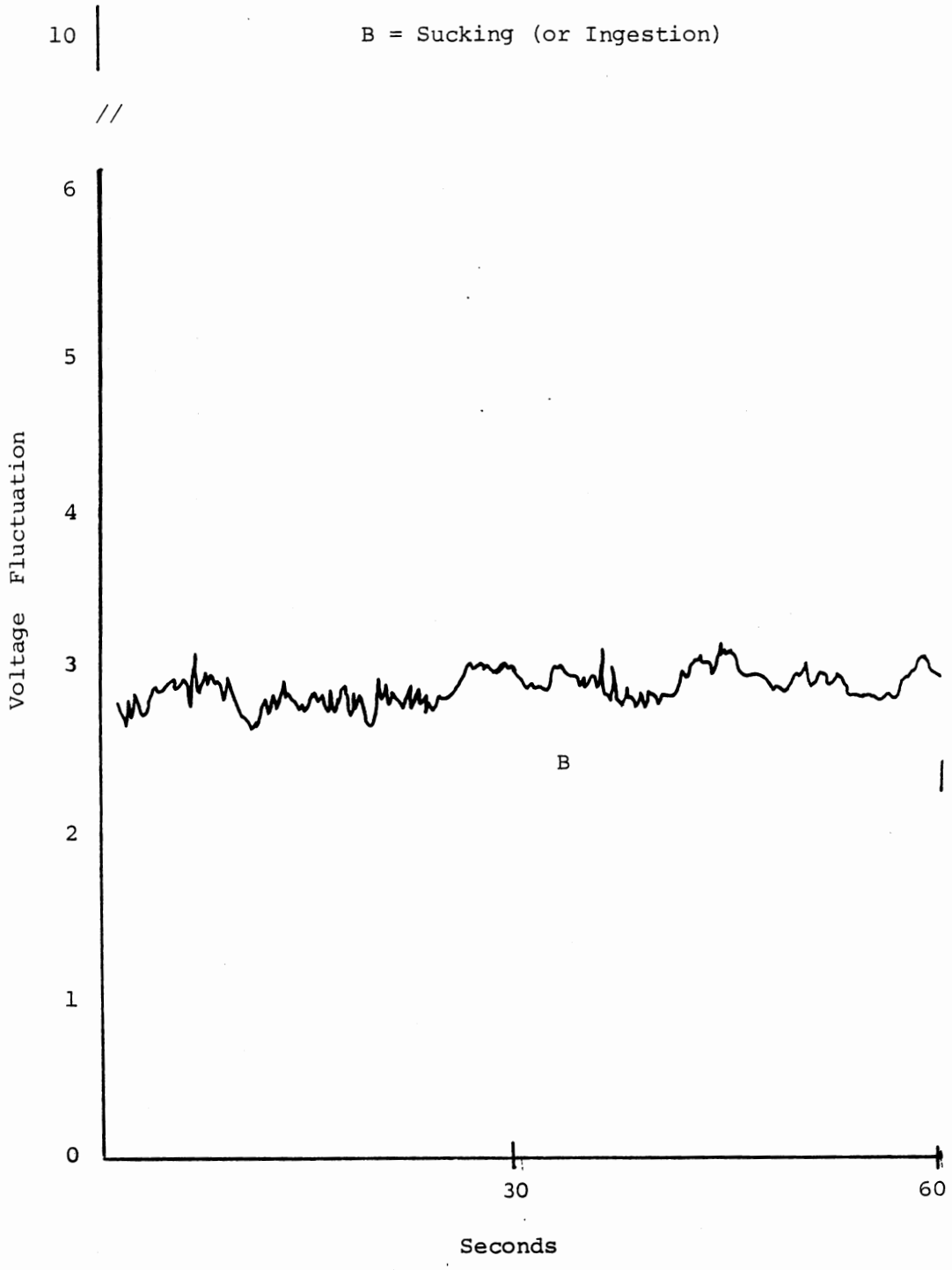

10
B = Sucking (or Ingestion)
//
6
5
4
3
2
1
0
Voltage Fluctuation
B
30
60
Seconds

Figure 22. Pilocarpine-Influenced Amblyomma americanum (L.) Females Recorded a Feeding Activity of Continuous Salivation. This Pattern was Similar to the Feeding Behavior of Paired Females Prior to Engorgement.

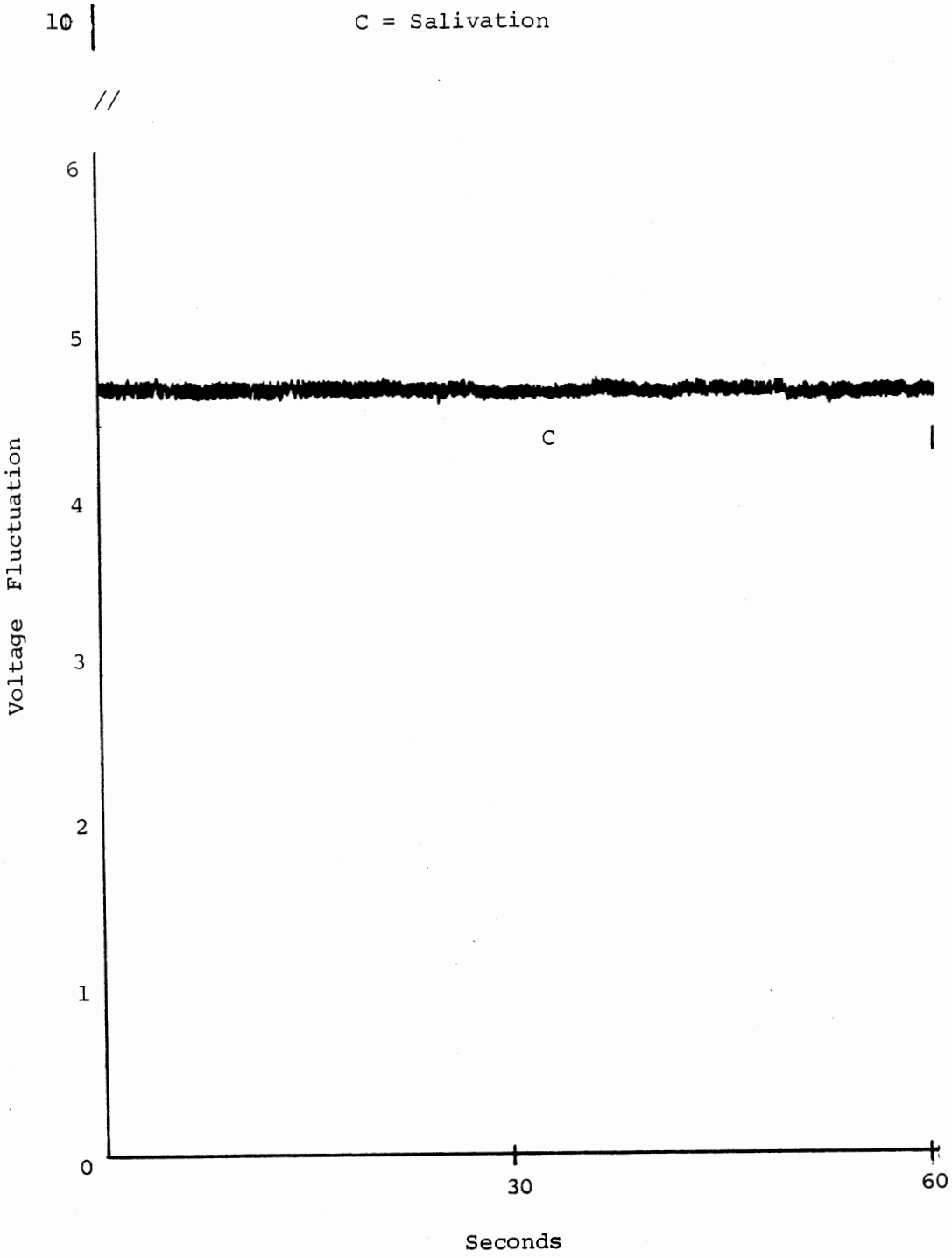

10
C = Salivation
6
5
4
3
2
1
0
C
Voltage Fluctuation
30
60
Seconds

Figure 23. Pattern Type Electronically Recorded for Amblyomma americanum (L.) Females by the Injection of Ivermectin. A Period of Constant Salivation with Fluctuations Noted in the Voltage was the Feeding Response Recorded.

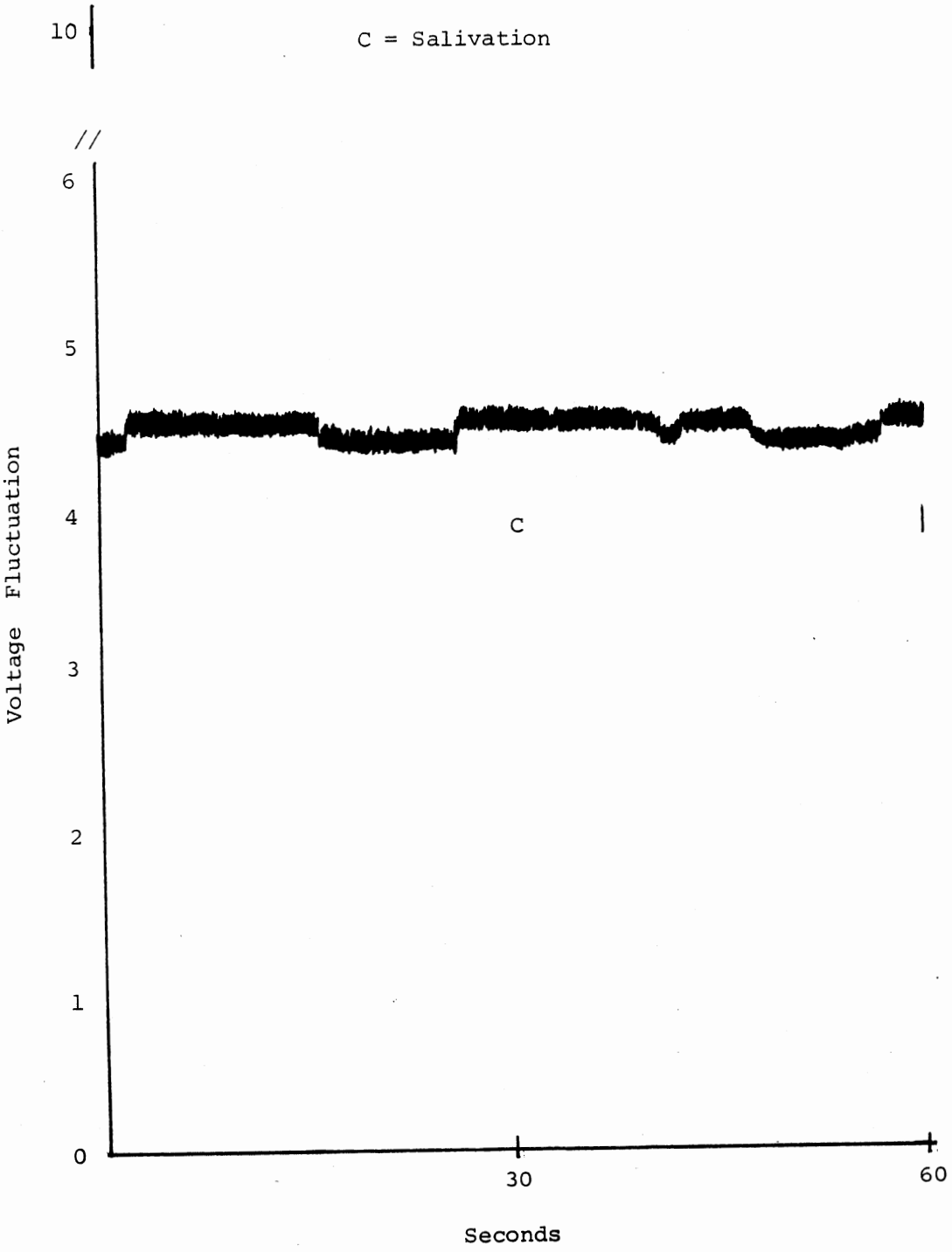

10
C = Salivation
//
6
5
4
3
2
1
0
Voltage Fluctuation
C
30
60
Seconds

Figure 24. Pharmacological Alteration of Electronically Recorded Feeding Pattern for Amblyomma americanum (L.) Females, Using Ivermectin. Characterizations of Feeding Activities within the Feeding Pattern were Limited to Alternating Periods of Resting and Sucking.

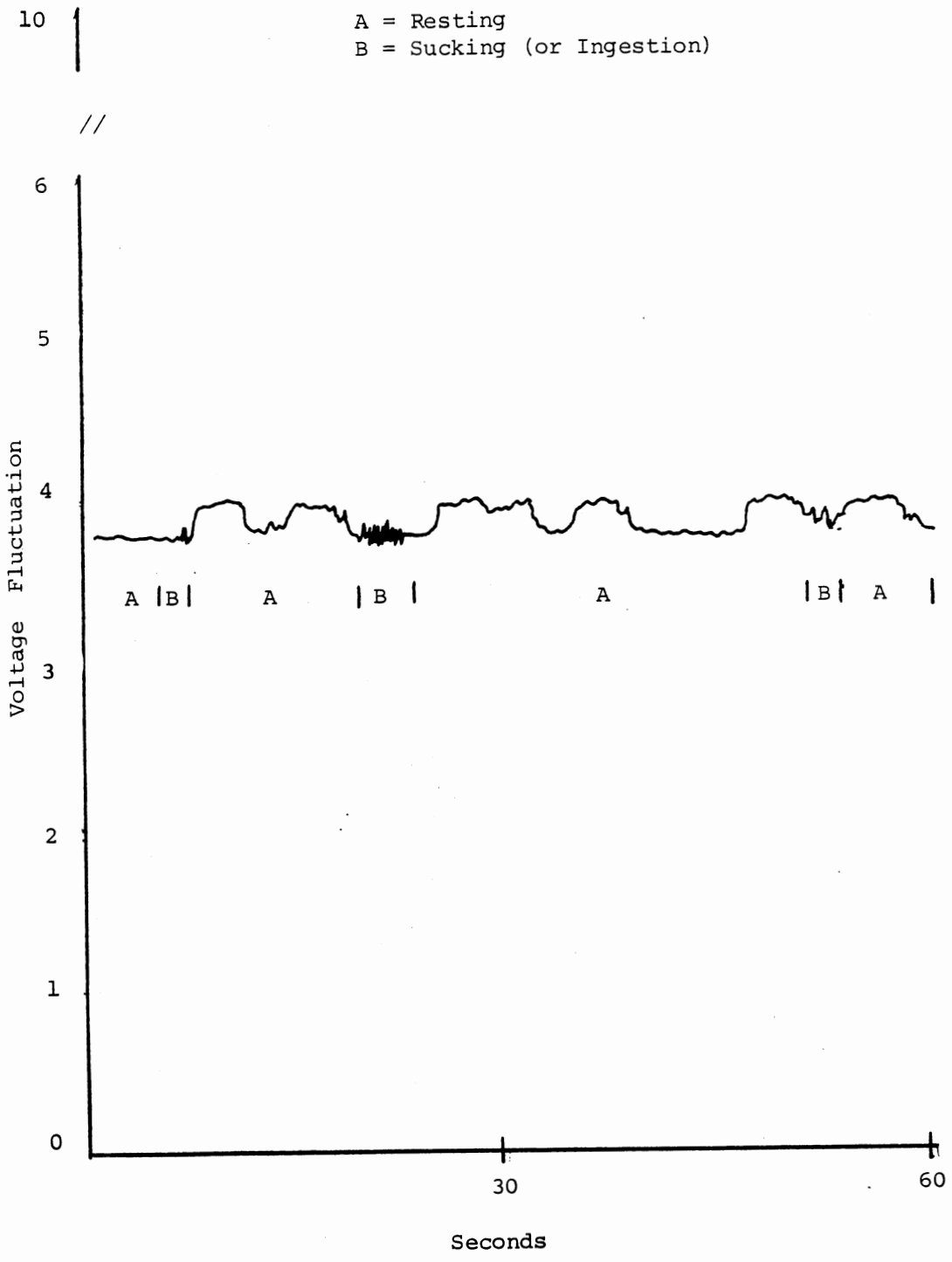

10
A = Resting
B = Sucking (or Ingestion)
//
6
5
4
3
2
1
0
Voltage Fluctuation
A
B
A
B
A
B
A
30
60
Seconds

Figure 25. Ivermectin-Influenced Amblyomma americanum (L.) Females Recorded 80% Sucking (or Ingestion) Activity and the Remaining 20% was Either Saliva Expulsion or Resting Phases.

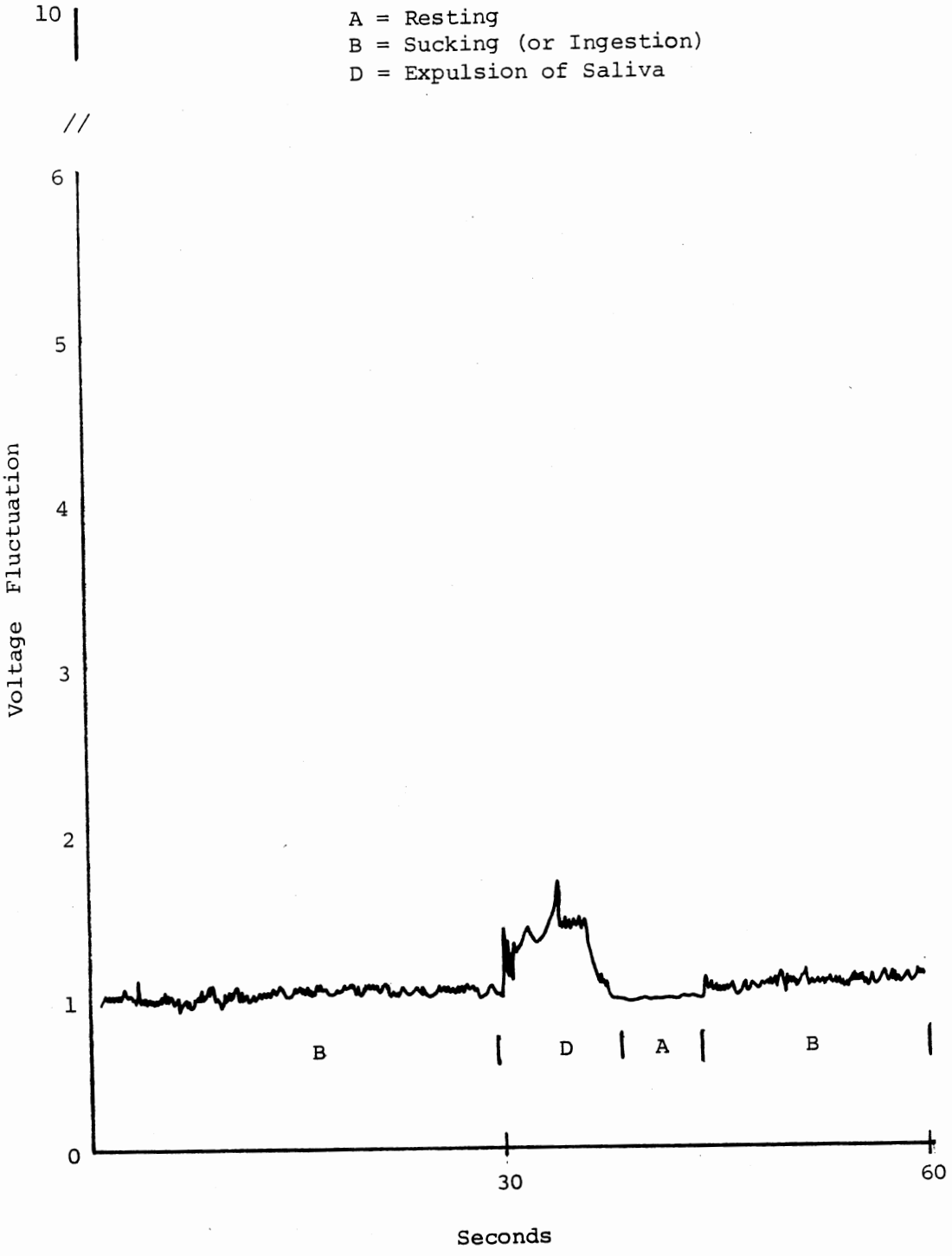

A = Resting
B = Sucking (or Ingestion)
D = Expulsion of Saliva
10
//
6
5
4
3
2
1
0
Voltage Fluctuation
B
D
A
B
30
60
Seconds

Figure 26. Mean Weights (mg) of Unpaired and Paired Feeding Female Amblyomma americanum (L.) by Day.

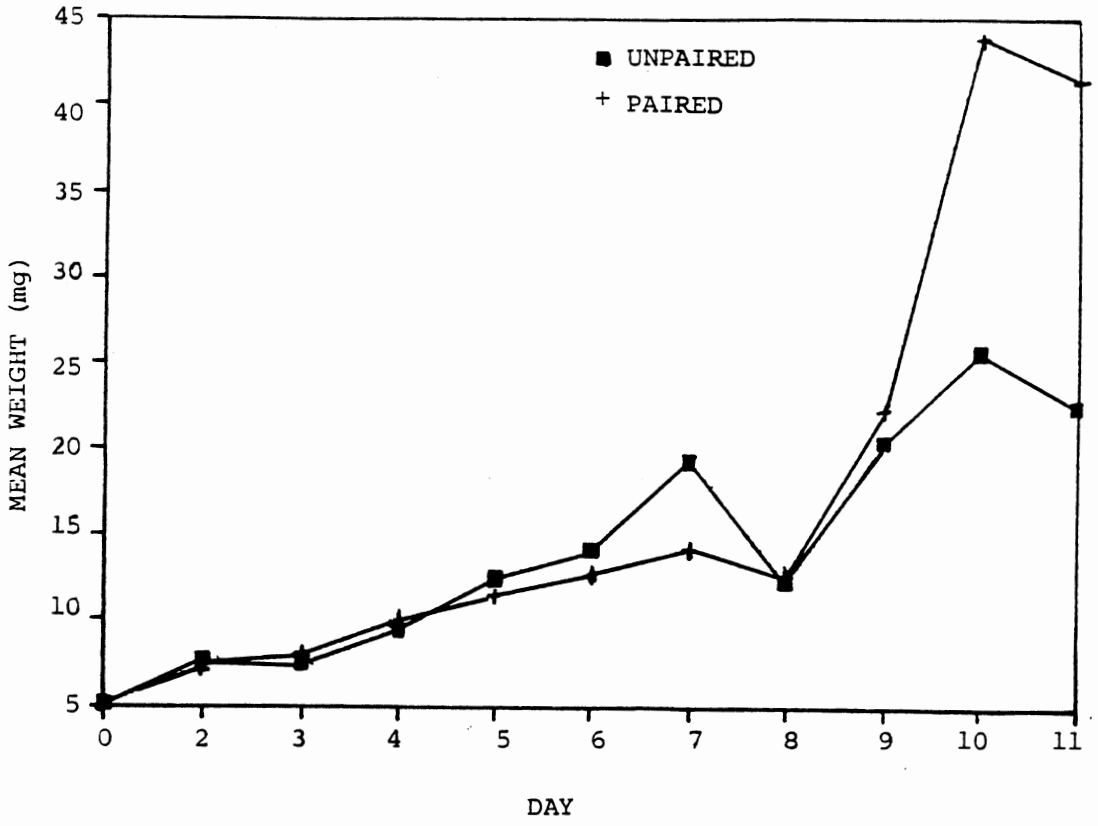
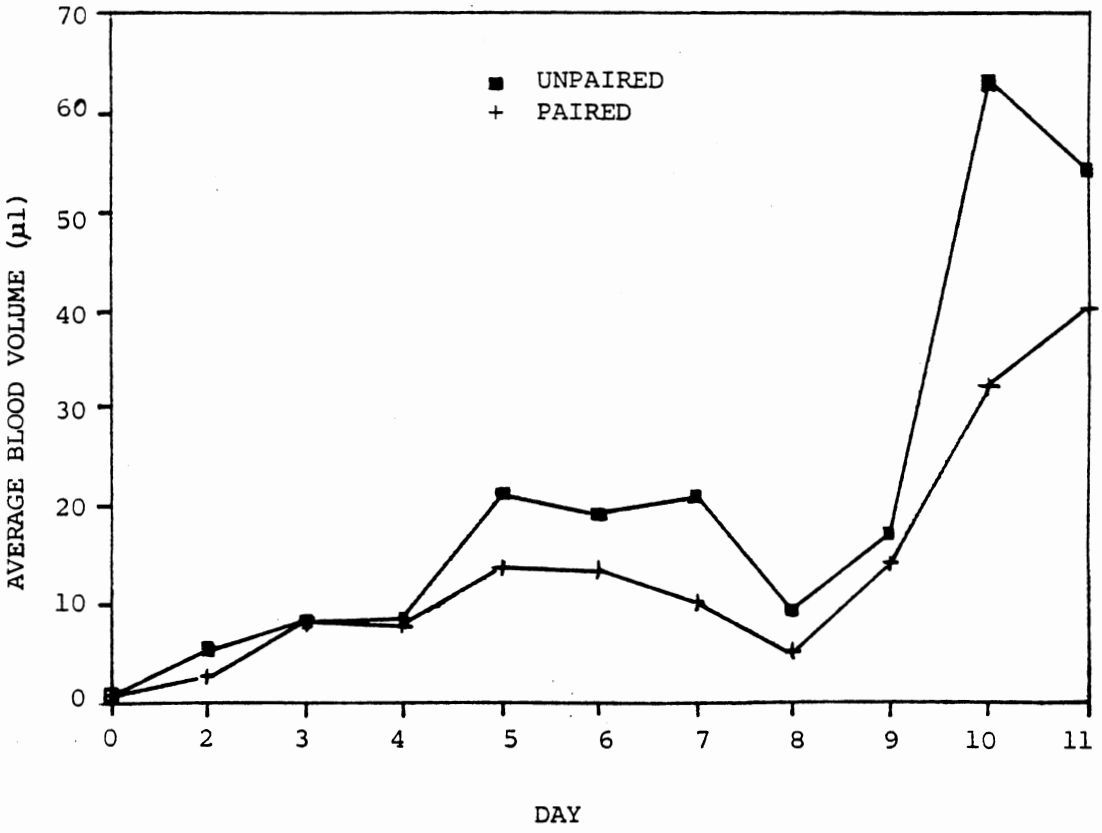

45
40
35
30
25
20
15
10
5
MEAN WEIGHT (mg)
UNPAIRED
PAIRED
0
2
3
4
5
6
7
8
9
10
11
DAY

Figure 27. Average Blood Volumes (μl) of Feeding Unpaired and Paired Female Amblyomma americanum (L.) by Day.


70
60
50
40
30
20
10
0
AVERAGE BLOOD VOLUME (µl)
UNPAIRED
PAIRED
0
2
3
4
5
6
7
8
9
10
11
DAY

VITA[1]

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Candidate for the Degree of

Master of Science

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