MEASUREMENT OF TRANSEPITHELIAL POTENTIALS (*IN VITRO*) FROM THE MIDGUT OF THE GULF COAST TICK *AMBLYOMMA MACULATUM* (KOCH)

By

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MEASUREMENT OF TRANSEPITHELIAL POTENTIALS (IN VITRO) FROM THE MIDGUT OF THE GULF COAST TICK AMBLYOMMA MACULATUM (KOCH)

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PREFACE

This investigation was designed to study aspects of mechanism(s) of ion and water transport across the midgut epithelium of the replete female Gulf Coast tick, Amblyomma maculatum (Koch) employing an in vitro technique. The primary objective was to determine the feasibility of using an in vitro technique which would allow consistent and substantial readings of transepithelial potential differences from the isolated midgut preparations to be established and maintained. Transepithelial potentials were established and subsequent experiments, including the effects of gases (N_2, O_2, CO_2), pH, temperature, chemical inhibitors (2,4 dinitrophenol, ouabain, potassium cyanide) and alterations of the ionic composition of the bathing media were conducted. Hemolymph from feeding and replete females was also introduced to the bathing medium.

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I am especially grateful to my fiancé, Bob Barker, and to my good friend, John Frick for their encouragement and support throughout this study.

A very special "thank you" is extended to Dr. Norman L. Braasch; and to him, this manuscript is dedicated for being such an inspirational entomologist and instructor:

"And now here is my secret, a very simple secret:
It is only with the heart that one can see rightly;
what is essential is invisible to the eye."

** ———Antoine de Saint Exupéry
"The Little Prince"

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CHAPTER I

INTRODUCTION

Because digestion in ticks is specialized toward complete protein assimilation, excess water and various ions in the blood meal must be removed (Balashov, 1972).

Removal of excess ions and water from the hemolymph is accomplished primarily by the salivary glands in ixodid ticks (Kaufman and Phillips, 1973a; Tatchell, 1967). In addition, a large amount of water is moved to and incorporated by the developing eggs in engorged females. The first barrier that must be transversed by the molecules is the midgut epithelium. Mechanisms associated with ion and water transport across midgut epithelial cells in Ixodoidea have not been investigated to date.

This investigation was designed to study various mechanism(s) of electrolyte movement across gut diverticula of the replete female Gulf Coast tick, Amblyomma maculatum (Koch), employing an in vitro technique. Potential differences of the epithelium were established and maintained with subsequent additions and alterations of the external bathing medium (i.e., metabolic inhibitors, gases, pH, temperature, ionic saline substitutions) to see the possible effects on the potential difference. Attempts were made to correlate mechanism(s) to the function of ion and water balance in ixodid ticks.

CHAPTER II

REVIEW OF THE LITERATURE

The Gulf Coast tick, Amblyomma maculatum (Koch) is a highly specialized bloodsucking arthropod and an obligate temporary parasite of birds and certain mammals. This particular ixodid or "hard" tick is primarily distributed in the United States along the Gulf and Atlantic coast states, with its heaviest distribution being in Central and South America (Robinson, 1926).

Amblyomma maculatum is a three-host species with the adults being found primarily on larger mammals (i.e., cattle, horses, sheep, deer) and rather rarely on man. When this tick occurs in large numbers it is a source of great irritation to domestic animals, especially cattle. Other ixodids have been associated with pathogen/toxin transmission (French, 1973; James and Harwood, 1969; McCue et al., 1948; Nutall, 1914; Reik, 1966; Spruance and Bailey, 1973; Sutton and Arthur, 1962); the Gulf Cost tick has also been shown to harbor a rickettsia and it is not known in what animal this organism produces disease in nature (Parker et al., 1939).

Each instar (larva, nymph, adult) requires a bloodmeal for developmental processes. The larvae and nymphs attach to a host for 5 to 7 days and ingest the bloodmeal; when replete, the tick drops to the ground where molting takes place. Adult females may engorge to repletion in less than 14 days. Copulation takes place on the host, and it

appears that some degree of engorgement is necessary for sexual maturation. The adult male remains attached to the host for longer periods of time with a smaller quantity of blood being ingested. The replete female (avg. weight = 1.3 g) can ingest approximately 2 ml of blood (H. Koch, personal communication). This bloodmeal is necessary for oocyte development as well as its nutritional value. The mated female, upon repletion, drops from the host and begins ovipositing as early as three days off the host. Depending upon the environmental conditions, the average life cycle of A. maculatum takes approximately 28 days to complete (Bequaret, 1945; Lancaster, 1973; Robinson, 1926).

Once the food has been ingested, it is moved through the alimentary canal to the stomach where the food materials are broken down by digestive enzymes. Some digestion, but mainly absorption, occurs in the small intestine.

The organs of the digestive system in the tick are the oral apparatus, pre-oral cavity, a pair of salivary glands, pharynx, esophagus, midgut (stomach and diverticulae), small intestine, rectal sac and the anal aperture. The gross anatomy of the gut has been reviewed by Arthur (1962), Balashov (1972), Douglas (1943), and Rhosdy (1961) on several tick species. The midgut, a small saclike stomach and seven pairs of lateral diverticulae, occupies most of the hemocoel dorsally. This basic morphology of the midgut is characteristic of the Ixodidae. Histological changes of the gut epithelium during the process of feeding and digestion have been studied by Chinery (1964), Hughes (1954), Lees (1952) and Till (1961). By direct observation of Dermacentor andersoni (Stiles), Gregson (1960) was able to show the alternating salivary secretory and blood sucking activities during the feeding process.

Chinery (1964) states that the histological appearance of the stomach and diverticulae are similar, being composed primarily of three cell types: digestive (columnar epithelial cells), secretory and undifferentiated cells. The histological and histochemical appearance of the midgut epithelium varies with the age and physiological condition of the tick.

The physiological and biochemical aspects of digestion have also been studied by Araman (1972), Kitaoka (1961) and Tatchell (1962, 1964). Present knowledge on digestion is incomplete as there still remain questions about the functional correlations to be made between extracellular, luminal and intracellular digestive processes; the digestive cell specializations; and the mechanisms of these cells in the midgut epithelium which are responsible for moving molecules across the cytoplasmic membranes.

Osmoregulation (salt and water balance) of the internal body fluids becomes quite critical to bloodsucking arthropods as the host's blood contains a large percentage of water which must be eliminated efficiently by the arthropod to maintain homeostatic functioning of the various systems. The vertebrate blood that is ingested by the arthropod contains 75-85% (avg.) water, 14-16% hemoglobin and other microconstituents (Balashov, 1972). In Rodnius prolixus Stal (Maddrell, 1963; Wigglesworth, 1931), Glossina spp. (Lester and Lloyd, 1928) and other bloodsucking dipterans, excess water is eliminated via the malpighian tubules. Argasids or "soft" ticks remove excess water and ions by the coxal gland (Araman and Said, 1972; Lees, 1946), whereas in the ixodids, the osmoregulatory organ is the salivary gland (Tatchell, 1967; 1969). Although water elimination and solute transport is most intensive on the

attached engorging tick, the same mechanism(s) of transport may also be present and functioning, though not possibly as actively, in the process of moving molecules to the developing eggs in the replete female. Whether it be an engorging or replete tick the midgut may then possess certain mechanisms involved in solute transport which create potential differences.

The intracellular and extracellular media in living cells differ markedly in ionic composition. The cell must then maintain favorable homeostatic conditions to be able to transport and eliminate various ions through the plasmalemma. The cellular ionic differences between the external and internal media are due to a semipermeable membrane. Where a cell membrane separates the intracellular and extracellular fluid, a potential difference is created due to the ionic differences (charges/electrogenic) across that membrane. The potential difference then, is a polarization of the cell membrane as a result of the charge concentrations of the ions in the media. Maintaining the integrity of the cell requires specific cellular mechanisms to regulate the transport of various ions and molecules into and out of the cell (Coster, 1973; Rothfield and Finkelstein, 1968).

The transmural potential difference is the easiest electrical measurement to make in which the potential of the fluid in contact with the serosa is recorded relative to the fluid in contact with the luminal surface of the intestine. Electrical contact is made by KCl bridges linked to calomel half cells and the electrical imbalance is recorded by a voltmeter (Barry and Diamond, 1970). In vitro studies have utilized either open-ended sac preparations or a piece of intestinal tissue clamped between two chambers containing physiological

saline (Caldwell, 1968; Teorell, 1953; Wright et al., 1969). Two potentials are involved in a continuous epithelium; one across the apical cell membrane and the other across the basal membrane. Intracellular recordings show that the inside of the epithelial cell is negative relative to both the serosal and luminal media (Anderson and Ussing, 1960; Whittemburg, 1963; Wood et al., 1969). Koefoed-Johnson and Ussing (1958) have demonstrated that the transporting epithelial cells of the frog skin (Rana temporaria) have a high potassium and a low sodium concentration intracellularly. This Na^+-K^+ concentration is characteristic of living cells and is responsible for maintaining the cell's integrity. Relative to an electrode in the luminal fluid, the potential across the serosal membrane is larger than (and of opposite polarity) that across the luminal membrane. Transepithelial potentials are derived from the sum of these two transmembrane potentials.

The relationships between ion movements and electrical measurements across the frog skin were established by Ussing (1949a, b; 1966). Since ions and water are absorbed across intestinal walls, it might be expected that the electrical characteristics of the gut epithelium would reflect some of the intestine's transfer abilities (Anderson and Ussing, 1957; Ussing and Zerahn, 1951; Ussing and Windhager, 1964).

Salt and water absorption by the intestinal epithelia cells has been the subject for numerous transport studies in vertebrates. Epithelial transport of electrolytes and nonelectrolytes in vertebrates has been reported primarily on toad bladders (Wade et al., 1973), small intestines (Binder et al., 1972a; b; Ellory et al., 1972; Flower et al., 1973; Fromm, 1973) and gall bladders (Diamond, 1962a, b; 1964; 1968). These systems of transcellular absorption appear to depend upon the

operation of processes of transport consisting of pumps and passive leaks located in the cell membranes. Recent studies also suggest that the mechanisms of salt and water transfer by the intestine are closely coupled with those for the transfer of other molecules.

Invertebrate electrolyte transport studies in the intestine are not as extensive as the vertebrate investigations. Harvey et al. (1964, 1968a, b, c; 1971), using isolated midguts of the cecropia silkworm [Hyalophora cecropia (L.)], have discovered that the midgut potential arises from the active transport of potassium from the serosal to the luminal side of the gut. Sodium and lithium are actively transported by the midgut, but the sodium transport system is not sensitive to ouabain. Ouabain is a chemical inhibitor that is specific for the inhibition of the ATP-ase associated with the Na+-K+ pump.

Investigations by Sauer et al. (1969a, b) using the isolated midgut of the American cockroach noted that solute absorption may still take place in the absence of significant net water movement. The midgut mechanism which appears to control water movements across the midgut epithelium is sensitive to the chemical inhibitor dinithrophenol (an uncoupler of oxidative phosphorylation). From these investigations, Mills et al. (1970) and Sauer et al. (1969) identify the midgut as an osmoregulatory organ.

O'Riordan (1969) studied the electrolyte movement in the isolated midgut of Periplaneta americana (L.). The isolated midgut was able to maintain a potential difference of approximately 12mV (lumen negative to hemolymph). The potential could be metabolically inhibited by nitrogen and dinitrophenol. Ouabain was only effective on the serosal side of the tissue. The author suggests that a linked Na+-K+ pump is indirectly

responsible for the potential and directly involved in the Na+ transport into the hemolymph.

Kaufman and Phillips (1973a, b, c) have recently investigated the ion and water balance in the female ixodid tick, Dermacentor andersoni. The authors have shown that the salivary gland is the major route by which excess NaCl and water is eliminated. This gland is an osmoregulatory organ because it controls hemolymph volume by eliminating excess ions and water. The salivery glands could be stimulated to secrete in vitro by adrenaline, noradrenaline, and dopamine. This indicates that salivary secretion is probably under neural rather than hormonal control. The hormonal stimulants were ineffective in vitro. Salivation in D. andersoni also involves passive movements of water coupled to the active transport of a solute or the presence of a chloride pump on the epithelial cells.

CHAPTER III

MATERIALS AND METHODS

Experimental Animals

Ticks used in all experiments were replete female Gulf Coast ticks, Amblyomma maculatum, which were reared at the Oklahoma State University Medical Entomology Laboratory. Stanchioned sheep served as hosts for the unfed adult ticks. The unfed adult ticks were placed in cells which consisted of a piece of orthopedic stocking approximately 6 inches in diameter and 10 inches deep that were glued laterally to the dorsal midline of the thoracic and lumbar regions of the sheep. Formica^{R} brand contact cement was used for cell adhesion to the host (Gladney and Drummond, 1970). The area within the cell was sheared to facilitate tick attachment to the host. The sheep were infested with equal numbers (Range of 38-45 pairs) of male and female A. maculatum and the ticks were allowed to feed until repletion. The replete ticks were collected on a daily basis and transferred to dated glass vials which were kept at laboratory temperatures (23 $\pm$ 2°C).

In Vitro Technique

Midgut tissue was obtained from the dissection of the replete Gulf Coast tick approximately three to seven days off the host. A portion of the midgut diverticulae was carefully removed and placed on one-half of a lucite holder (Figure 1). With a fine probe, the tissue was

incised longitudinally down the length of the diverticula and opened carefully with a fine camel-hair brush. The ingested bloodmeal was scraped free from the midgut epithelium by the camel-hair brush with subsequent mounting of the isolated epithelium over the centrally placed hole (diameter of 0.1 mm) in the lucite holder. Upon proper mounting of the epithelium, the two lucite hemi-tissue chamber holders were brought together and clamped tightly with Swingline^{R} binder clips. The only barrier between the two sides of the holder is the tissue which creates a liquid-junction potential (Bures et al., 1967).

The tissue was bathed on both sides by 300 µℓ of a modified tick culture solution from Rehacek and Brzostowski (1969). The composition of the modified saline solution used in the experiments as the standard saline is shown in Table I. All dissections were done in the standard saline (osmolarity = 0.65-0.69°C; pH 6.8) at room temperature (23 $\pm$ 2°C). Osmolarity determinations of the salines used in the study were measured with a Clifton Technical Physics Nanoliter Osmometer, expressed as the freezing point depression (-Δ°C), being sensitive to the nearest $\pm$ 0.001°C (Frick and Sauer, 1973). The pH of the salines was determined by the Coleman Model 39 Standard pH Meter.

Electrical contact with the bathing solutions is made through moveable saline--3M-KCl agar bridges which also make contact with a 3M-KCl solution containing the two calomel half-cell electrodes. The potential difference across the midgut epithelium was measured on a Keithly 155 Null Detector Microvoltmeter via the electrodes (Figure 1).

An equilibrium period of 15 minutes was allowed for each preparation with potential readings being recorded every five minutes for one hour. The standard saline was removed after 15 minutes and experimental

Figure 1. Diagram of the *in vitro* Apparatus for Transepithelial Potential Difference Measurements.

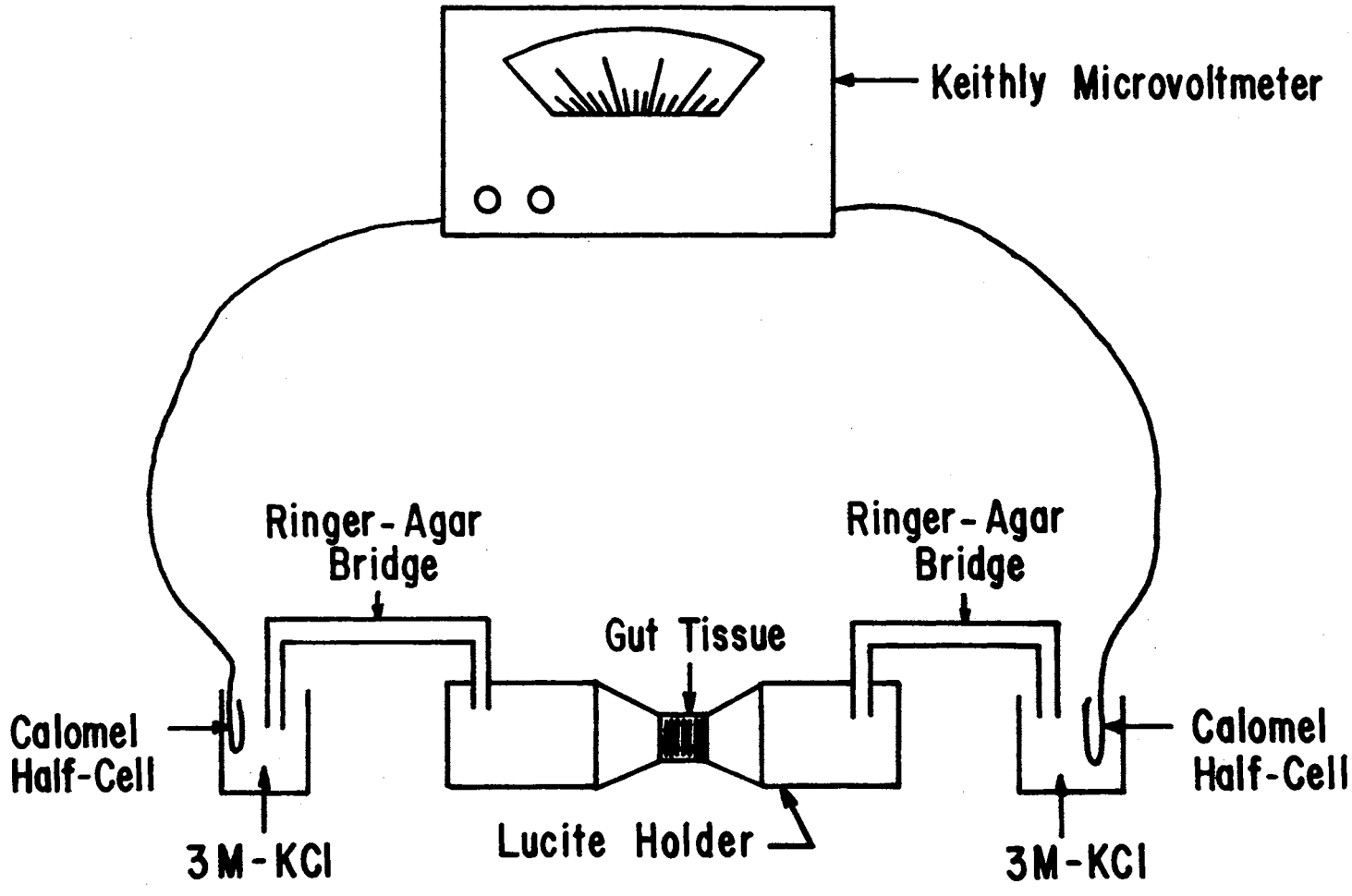

Keithly Microvoltmeter
Ringer-Agar Bridge
Ringer-Agar Bridge
Gut Tissue
Calomel Half-Cell
Calomel Half-Cell
3M-KCl
Lucite Holder
3M-KCl

TABLE I

COMPOSITION OF THE MODIFIED SALINE SOLUTION *+

Salt	mM/l
NaCl	110.00
$Na_2H_2PO_4 \cdot H_2O$	14.45
$NaHPO_4 \cdot 7H_2O$	7.50
$KHCO_3$	16.30
$CaCl_2$	2.80
$MgSO_4$	2.40
Insitol	1.90
Glucose	27.70
Bovine albumin	0.1 g

* pH 6.8

+ $-\Delta^\circ = -0.65$-$0.69^\circ C$

saline (saline of different ionic composition, pH, temp., etc.) was substituted on one or both sides of the gut with the new potential difference being recorded for 30 minutes. The standard saline was reintroduced and the measurements continued. Under every experimental condition, the experiment was repreated on at least 10 preparations.

Resting Membrane Potential

Initial midgut preparations were tried with the replete lone star tick, Amblyomma americanum (L.), but insufficient amounts of tissue made the preparations difficult and potentials were not obtained on a regular basis.

We then chose the replete Gulf Coast tick, since it was twice the size of the lone star tick and sufficient amounts of midgut tissue could be obtained readily. Midgut preparations were also tried on engorging female Gulf Coast ticks that were still attached to the host. Due to the fragility of the tissue during the feeding process, preparations were not feasible. The potentials for all the experiments were obtained from the replete female, A. maculatum, and no potential differences less than 4.8mV were used for an experiment.

The initial preparations were checked for possible tissue damage by adding 10 µℓ of a ^{14}C inulin solution to 290 µℓ of the standard bathing saline on one side of the holder. After a period of 30 minutes, 10 µℓ were withdrawn from the other holder and placed in 15 ml of the liquid scintillant of Wharton et al. (1965). The radioactivity was measured with a Beckman 100 Liquid Scintillation System^{R}.

The bathing salines were initially aerated before being introduced into the holder; because there was no effect upon the potential, this

step of the technique was excluded.

Chemical Inhibitors

The chemical inhibitors used in this study were potassium cyanide (KCN), 2,4 dinitrophenol (DNP) and ouabain with each inhibitor being used at the following concentrations: 3×10^{-3}, 3×10^{-4}, 3×10^{-5}M. Ten µℓ of the inhibitor were added to one or both sides of the gut after a minimum equilibration period of 15 minutes. Potentials were recorded for an hour with no restoration of the original standard saline.

Bathing Media Alterations

The ionic composition of the modified saline (Table I) was altered to see the effects of various ions on the potential. Sulfate ($SO_4^=$ ions substituting for Cl^- ions) and choline (choline ions replacing Na+ ions) salines were used. The composition of the altered salines is shown in Table II. The sulfate and choline salines had a pH of 6.05 and 6.9, respectively. Electrolytes were balanced in both salines, thereby creating no change in the osmolarity of the solutions. Three hundred µℓ of the altered salines were introduced to one or both sides of the gut and after a 30 minute period the standard saline was reintroduced for 15 minutes.

Temperature Variations

The temperature range of the experimental salines that was introduced on both sides of the tissue after 10 minutes was 5°, 15°, 25° and 37°C. The standard saline (23°C) was placed in a cold water bath or on a temperature controlled hot plate to obtain the necessary experimental

TABLE II

COMPOSITION OF THE ALTERED SALINE SOLUTIONS

Sulfate Saline (pH 6.85)	
Salt	mM/l
Na_2SO_4	55.00
$Na_2H_2PO_4 \cdot H_2O$	14.45
$NaHPO_4 \cdot 7H_2O$	7.50
$KHCO_3$	16.30
$CaCl_2$	2.80
$MgSO_4$	2.40
Insitol	1.90
Glucose	78.40
Bovine albumin	0.1 g
Choline Saline (pH 6.9)	
Salt	mM/l
ChCl	111.28
$Na_2H_2PO_4 \cdot H_2O$	14.45
$NaHPO_4 \cdot 7H_2O$	7.50
$KHCO_3$	16.30
$CaCl_2$	2.80
$MgSO_4$	2.40
Insitol	1.90
Glucose	27.70
Bovine albumin	0.1 g

temperature. All experimental salines were placed on both sides of the tissue for 40 minutes.

pH Alteration

Since the standard saline had a neutral pH, 6.8, we decided to use a range of $\pm$ 1.0 from the standard to obtain the acidic and alkaline salines. The composition of the acidic and alkaline salines is shown on Table III. All the experimental pH salines were introduced on both sides of the tissue.

Gases

One hundred percent carbon dioxide, oxygen, and nitrogen purchased locally in compressed form were the experimental gases employed in this study. The standard saline was continually gased for 15 minutes with the respective gas before introduction on both sides of the tissue.

Hemolymph

Feeding adult female ticks having a weight range of 0.3 to 1.4g, were removed from the host daily. Hemolymph was obtained by inserting a probe through various portions of the abdominal cuticle into the hemocoel while gently pressing the cuticle so that hemolymph would flow through the cuticular puncture. Ten µl of hemolymph were collected directly by calibrated capillary tubes and introduced to 290 µl of standard saline on one or both sides of the gut.

The same method of collection and introduction of hemolymph as that described for feeding ticks was employed also with replete adult female ticks (weight range of 0.9 to 1.4g).

TABLE III

COMPOSITION OF THE PH SALINE SOLUTIONS

Acidic Saline (pH 5.85)	
Salt	mM/l
NaCl	110.00
$Na_2H_2PO_4 \cdot H_2O$	57.70
$NaHPO_4 \cdot 7H_2O$	0.00
$KHCO_3$	16.30
$CaCl_2$	2.80
$MgSO_4$	2.40
Insitol	1.90
Glucose	27.70
Bovine albumin	0.1 g

Alkaline Saline (pH 7.85)	
Salt	mM/l
NaCl	110.00
$Na_2H_2PO_4 \cdot H_2O$	7.20
$NaHPO_4 \cdot 7H_2O$	7.50
$KHCO_3$	16.30
$CaCl_2$	2.80
$MgSO_4$	2.40
Insitol	1.90
Glucose	27.70
Bovine albumin	0.1 g

CHAPTER IV

RESULTS

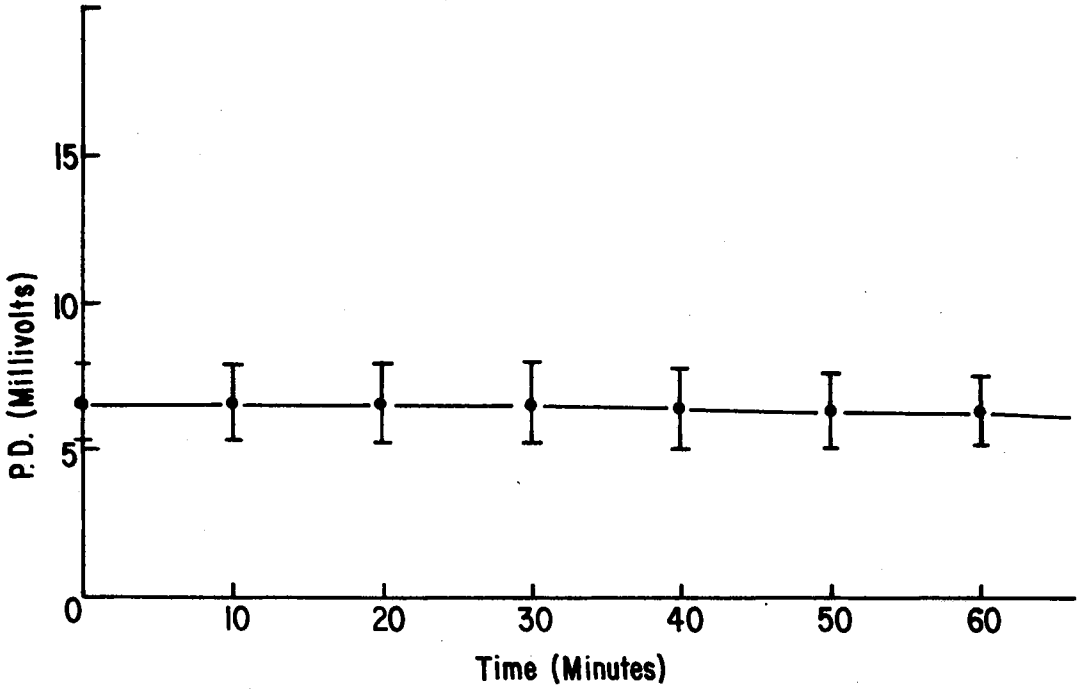
Establishment of Transepithelial Potentials

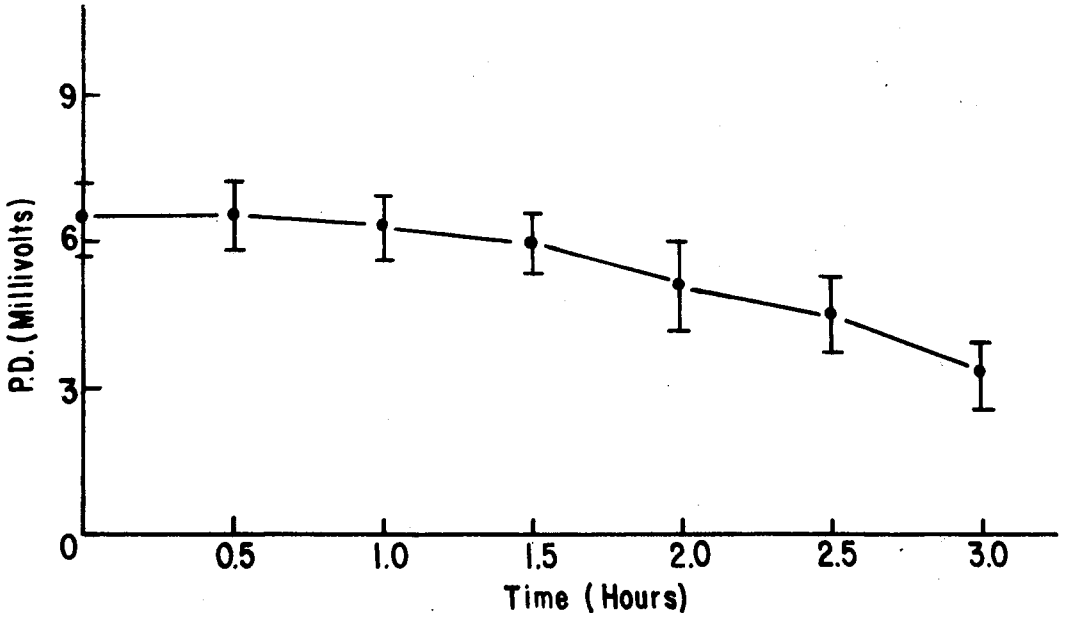
The relationship between the transepithelial potential and time is shown in Figure 2. A minimum of 20 experiments were done to obtain the average transepithelial potential of 6.8 $\pm$ 1.2mV ($\pm$ means S.D.). The lumen was negative to the serosal (hemolymph(side in all experiments. Preparations could be maintained for up to three hours but were not used for more than one hour as the potential steadily declined at a rate of 1.0-1.5 mV/hr. Low potential differences were observed on replete ticks recently fallen from the host and those beginning to oviposit. The most stable and highest potentials were obtained from replete ticks approximately 3 to 7 days off the host. The decline in potential when correlated with the number of days off the host may be due to the natural degeneration of the tissue and/or the physiological state (i.e., oviposition).

Effects of Chemical Inhibitors

The first chemical inhibitor of transport used was potassium cyanide (KCN). Cyanide combines with cytochrome oxidase to prevent its transfer of electrons in the respiratory chain thus preventing high energy phosphate formation (ATP). The effects of adding 10 $\mu\ell$ of KCN to the standard saline on one or both sides of the tissue at three

Figure 2. The Relationship Between the Transepithelial Potential and Time. Each point (●) represents the mean of 10 measurements ± S.D.


15
10
5
0
P.D. (Millivolts)
10
20
30
40
50
60
Time (Minutes)


9
6
3
0
P.D. (Millivolts)
0.5
1.0
1.5
2.0
2.5
3.0
Time (Hours)

concentrations are shown in Figure 3. Slight inhibition was induced by 10^{-3}M potassium cyanide when present in either or both sides of the bathing saline solutions. As the concentration of potassium cyanide decreased, less inhibition was observed; but neither side responded to 10^{-5}M of this inhibitor.

2,4 dinitrophenol (DNP), the second chemical inhibitor employed in this study is known to effect transport mechanisms by uncoupling the cellular respiration process of oxidative phosphorylation (Hochster and Quaster, 1963). A substantial fall in the potential difference was induced by 10^{-3}M dinitrophenol when placed only on the serosal side of the tissue (Figure 4). This serosal inhibition was also effective, though more slowly, at 10^{-4}M and relatively ineffective to this inhibitor at 10^{-5}M. Somewhat surprisingly only slight changes in the potential difference were observed when DNP was placed on both sides of the tissue at all concentrations (Figure 4).

Ouabain (strophanthin-G), a cardiac glycoside, which is thought to be a specific inhibitor of sodium transport, is assumed to exert its chief action on the ATP-ase system associated with a transport mechanism. The effects of the addition of ouabain to the bathing saline solutions are shown in Figure 5. Ouabain was ineffective when placed on the lumen side bathing medium, but a significant potential decline was induced on the serosal and both side(s) of the tissue by 10^{-3} and 10^{-4}M of this inhibitor. Ouabain was also somewhat effective on the serosal side, though more slowly, at 10^{-5}M. The results suggest that the potential difference is dependent upon the activity of a ouabain-sensitive "pump ATP-ase" on the serosal side of the gut epithelium.

Figure 3. The Effect of Potassium Cyanide (KCN) on the Potential Difference in the Standard Saline. Each point represents the mean of 10 measurements + S.D. KCN placed on lumen (●), serosal (o) and both (Δ) side(s) of the gut epithelium.

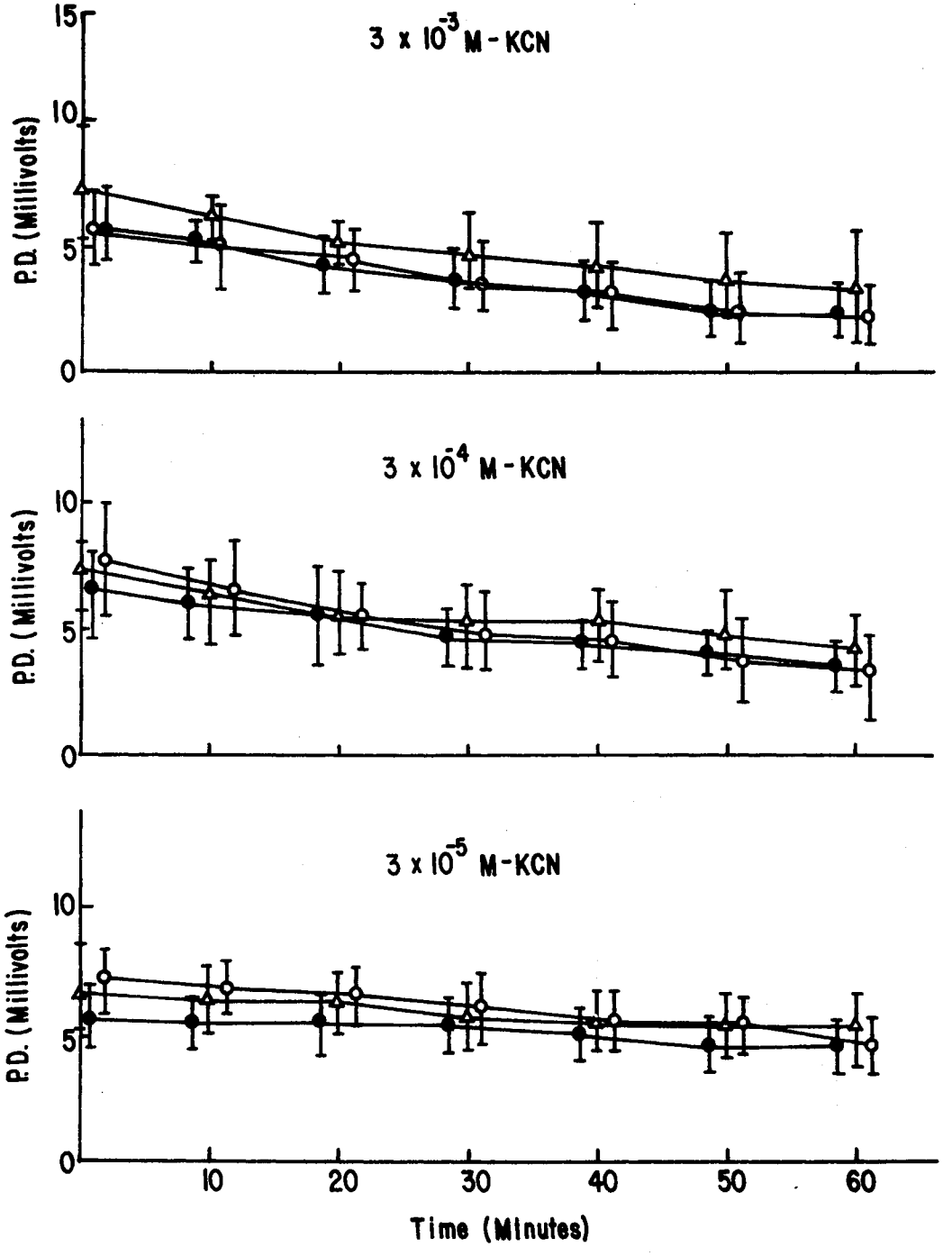

3 x 10⁻³ M - KCN
3 x 10⁻⁴ M - KCN
3 x 10⁻⁵ M - KCN
P.D. (Millivolts)
15
10
5
0
10
20
30
40
50
60
Time (Minutes)

Figure 4. The Effect of 2,4 Dinitrophenol on the Potential Difference in the Standard Saline. Each point represents the mean of 10 measurements ± S.D. DNP placed on lumen (●), serosal (o) and both (Δ) side(s) of the gut epithelium.

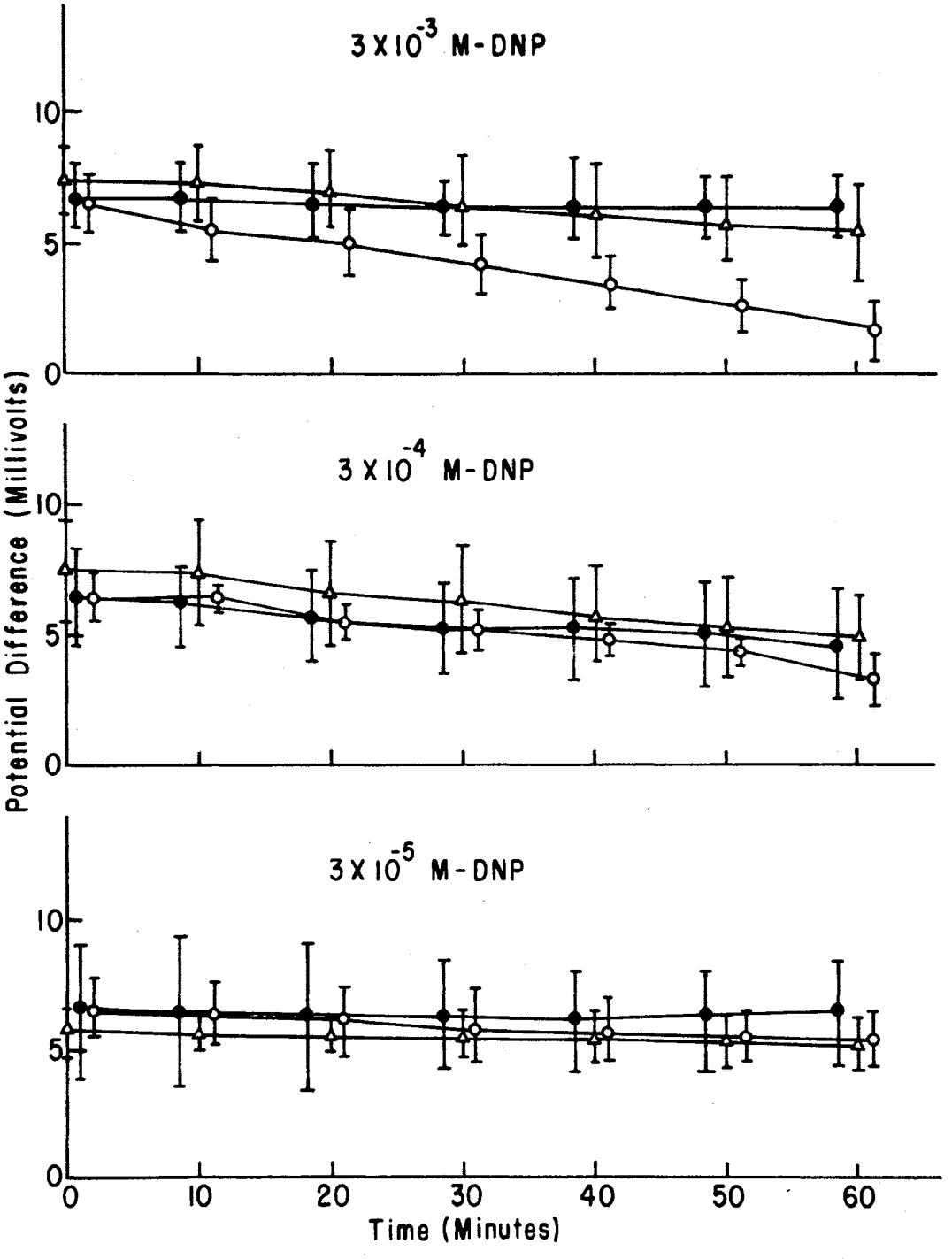
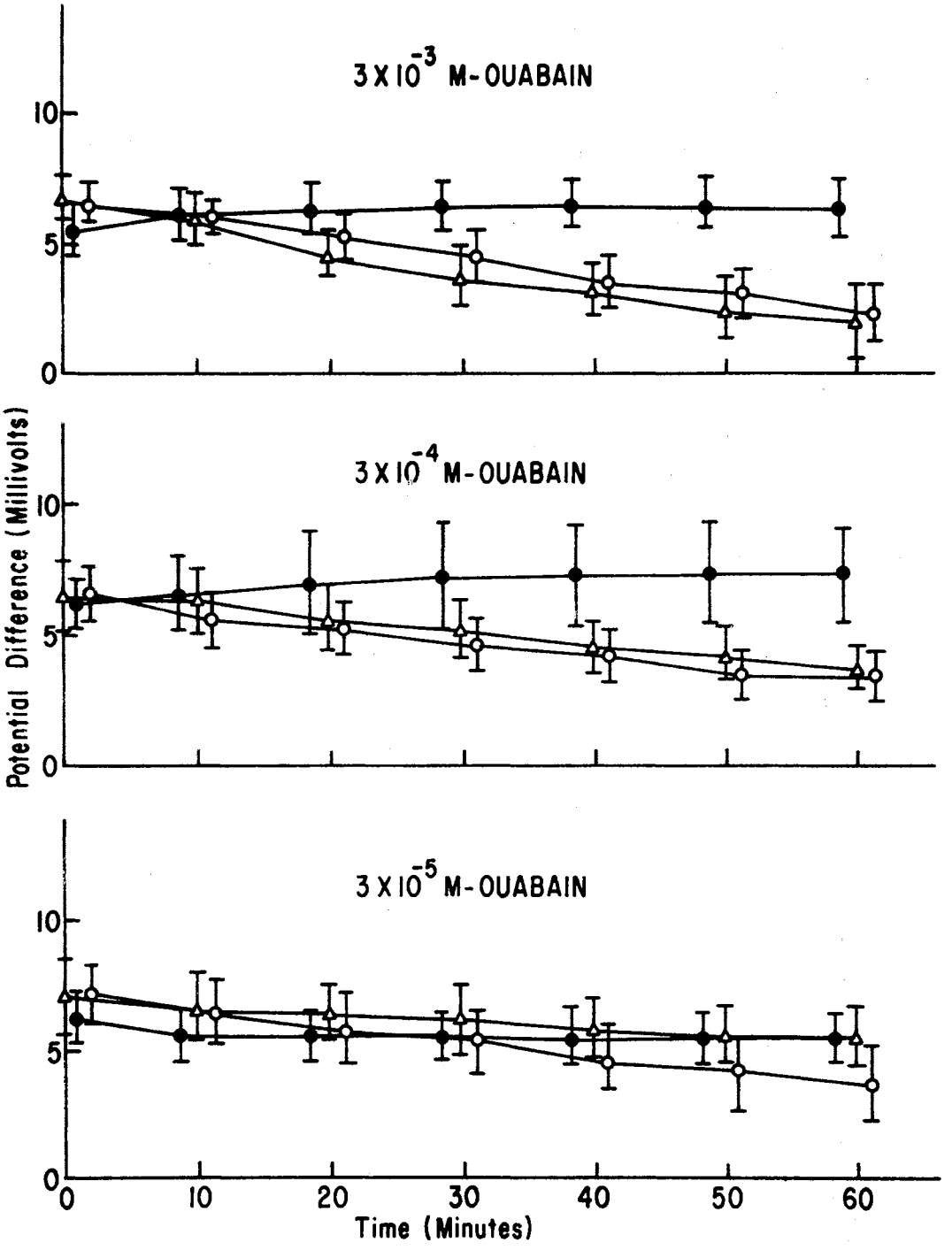

3 X 10⁻³ M-DNP
3 X 10⁻⁴ M-DNP
3 X 10⁻⁵ M-DNP
Potential Difference (Millivolts)
0
5
10
0
10
20
30
40
50
60
Time (Minutes)

Figure 5. The Effect of Ouabain on the Potential Difference in the Standard Saline. Each point represents the mean of 10 measurements ± S.D. Ouabain placed on lumen (●), serosal (o) and both (Δ) side(s) of the gut epithelium.


3 X 10⁻³ M-OUABAIN
3 X 10⁻⁴ M-OUABAIN
3 X 10⁻⁵ M-OUABAIN
Potential Difference (Millivolts)
10
5
0
0
10
20
30
40
50
60
Time (Minutes)

Effects of Altered Ionic Composition of the Bathing Media

Figure 6 shows the effects of a sulfate (usually an impermeable anion) and choline (usually an impermeable cation) bathing salines upon the potential difference. Each experimental saline was substituted in one or both sides of the gut tissue after a minimum equilibration period of 15 minutes with subsequent restoration of the standard saline.

The substitution of sulfate ions for chloride ions in the standard saline led to a slight increase in the potential when placed on the lumen and both sides of the gut epithelium. This increase was maintained until the standard saline was restored. The sulfate saline on the serosal side of the tissue caused a rapid, pronounced decrease and reversal of the potential followed by an almost complete restoration upon reintroduction of the standard saline.

A choline saline, in which all but 21.95 mM/l Na^+ was placed with choline, caused a decrease in the potential when placed on the luminal side. This decrease was maintained but the normal potential was restored with the replacement of the standard saline. The choline saline on the serosa induced a slight increase. Choline saline on both sides of the tissue caused a slight decrease in the potential.

Effects of Various Gases, pH, and Temperatures

The effects of various gases (N_2, CO_2, O_2) on the potential are shown in Figure 7. The experimental gased salines were introduced on both sides of the tissue for 30 minutes with the standard saline reintroduced at the end of the experimental recording period. The presence of nitrogen in the bathing saline led to a decrease which was maintained

Figure 6. The Potential Difference in Sulfate and Choline Bathing Saline Solutions. Each point represents the mean of 10 measurements $\pm$ S.D. Experimental salines placed on lumen (●), serosal (o) and both (Δ) side(s) of the gut epithelium.

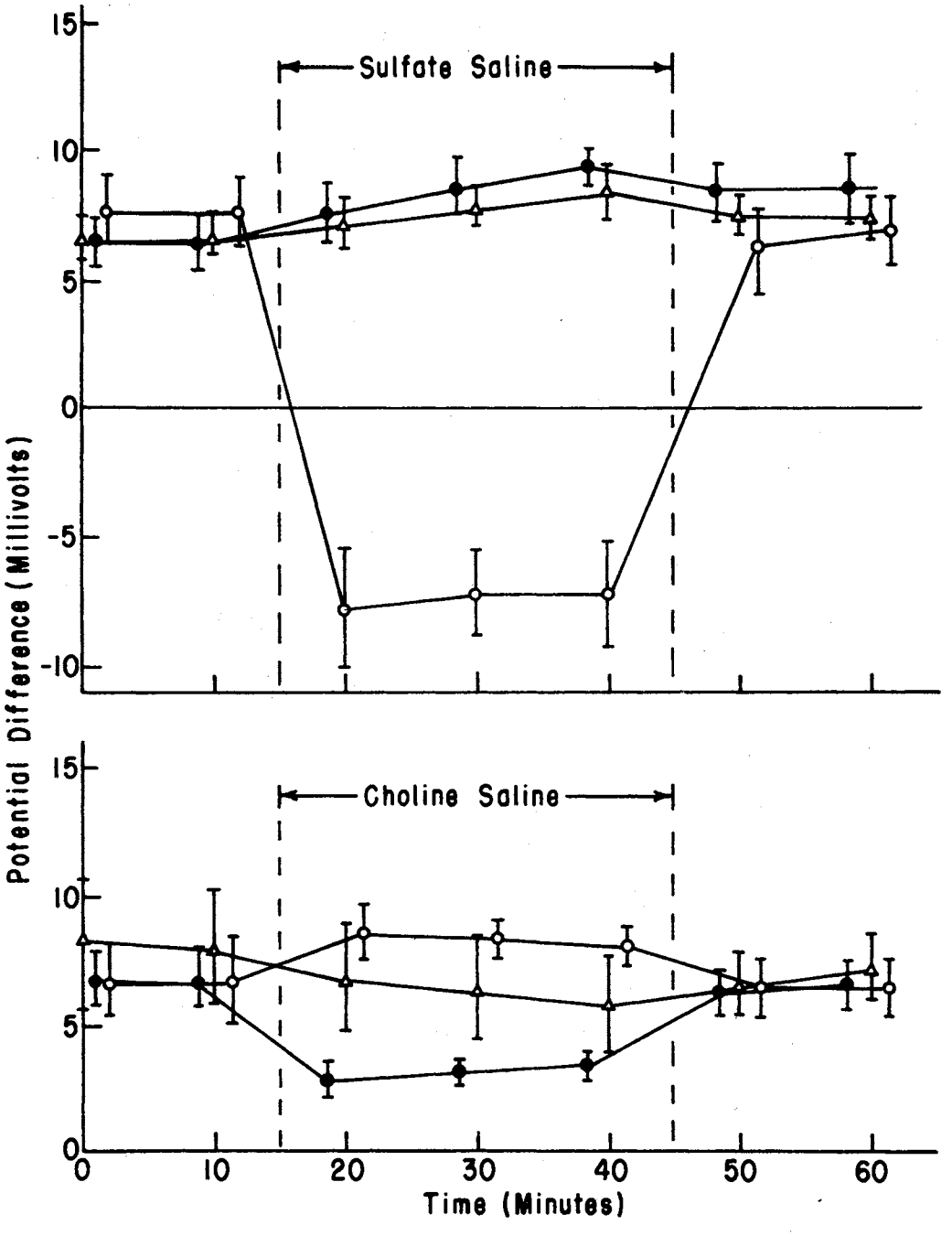
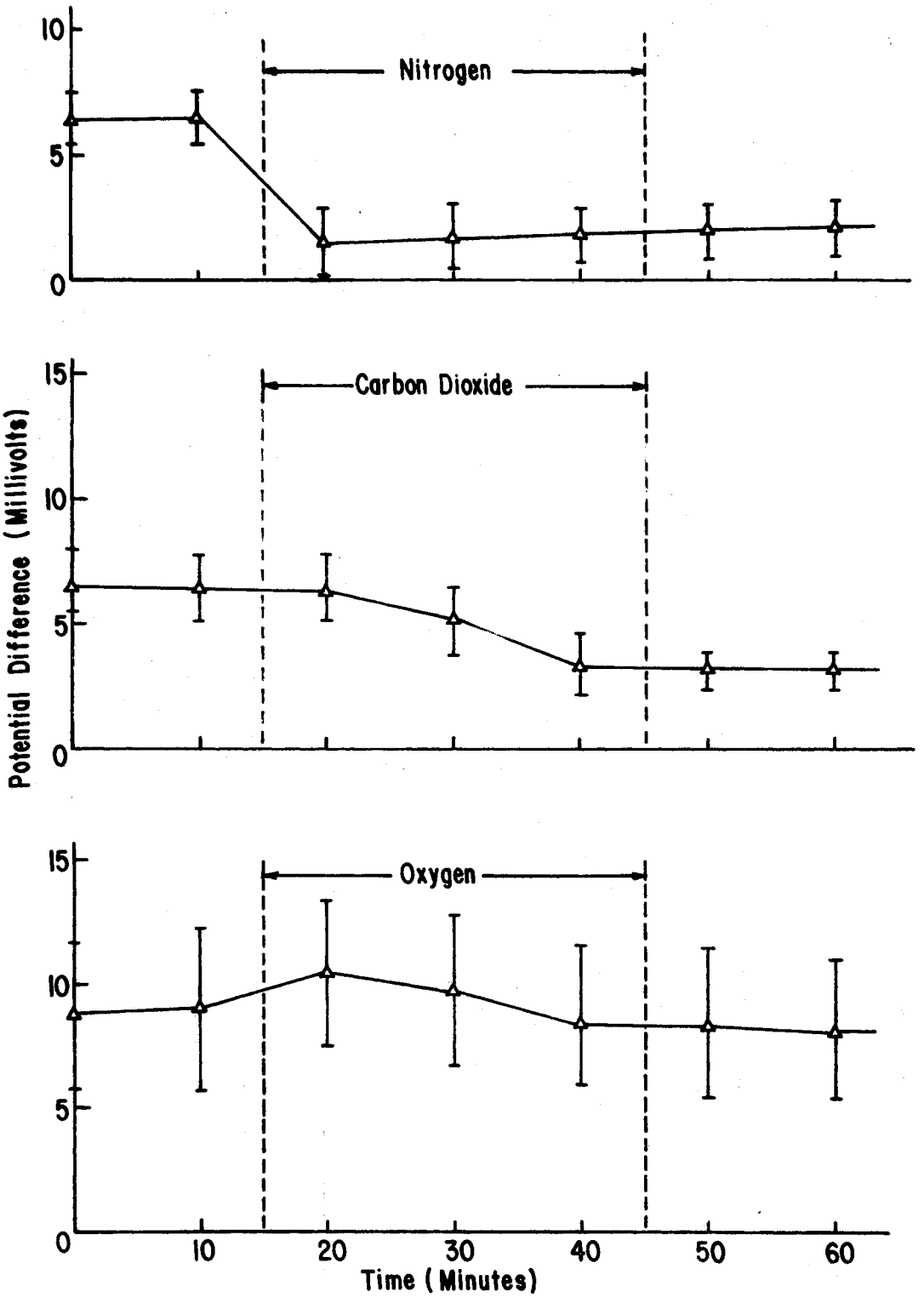

Sulfate Saline
Choline Saline
Potential Difference (Millivolts)
15
10
5
0
-5
-10
0
10
20
30
40
50
60
Time (Minutes)

Figure 7. The Effects of Various Gases (N_2, CO_2, O_2) on the Potential Difference When Placed on Both Sides of the Tissue. Each point (Δ) represents the mean of 10 measurements $\pm$ S.D.


Nitrogen
Carbon Dioxide
Oxygen
Potential Difference (Millivolts)
Time (Minutes)
0
5
10
15
20
30
40
50
60

but the normal potential value could not upon reintroduction of the standard saline be restored. Carbon dioxide induced a gradual decline in the potential which was irreversible. The substitution of oxygen for air resulted in a 3.0mV rise which gradually declined to the normal potential value in 20 minutes. The experimental gas results indicate that the transporting mechanisms do require aerobic conditions for optimum functioning.

An acidic saline (pH 5.85) was introduced after a minimum equilibration period of 15 minutes to both sides of the tissue and caused a decrease of 3.3 mV after 60 minutes (Table IV). The alkaline saline (pH 7.8) when substituted for the standard saline induced a 1.0mV rise in the potential followed by a slight decline (Table V). From this data it appears that the mechanisms present do function at both alkaline and acidic pH. However, the mechanisms appear to be most stable and consistent at a neutral pH, 6.8 but the potential can also be maintained though not as well in the alkaline and acidic media. The highest and most stable potential is best achieved at a neutral pH 6.8.

The effects of temperature on the potential difference are shown in Figure 8. All of the experimental salines were introduced to both sides of the tissue after the minimum equilibration period. A chilled saline (5°C) produced a prompt, irreversible fall in the potential. A slight decrease was observed upon the addition of a 15°C and 25°C saline respectively. The 37°C saline induced a sharp, rapid increase in the potential with a gradual decline as the saline cooled to room temperature. Temperature does appear to effect the energy-requiring mechanisms associated with potential maintenance.

TABLE IV

THE EFFECT OF AN ACIDIC SALINE SOLUTION*
ON THE POTENTIAL DIFFERENCE **

Time (min.)	Potential Difference ± S.D. *** (mV)
00	5.7 ± 1.0
10	4.5 ± 1.5
20	3.9 ± 1.3
30	3.4 ± 1.5
40	3.0 ± 1.3
50	2.5 ± 1.0
60	2.4 ± 0.8

* Saline pH, 5.85

** Each potential reading was obtained by placing the acidic saline on both sides of the tissue.

*** Each potential difference represents the mean of 10 measurements ± S.D.

TABLE V

THE EFFECT OF AN ALKALINE SALINE SOLUTION*
ON THE POTENTIAL DIFFERENCE **

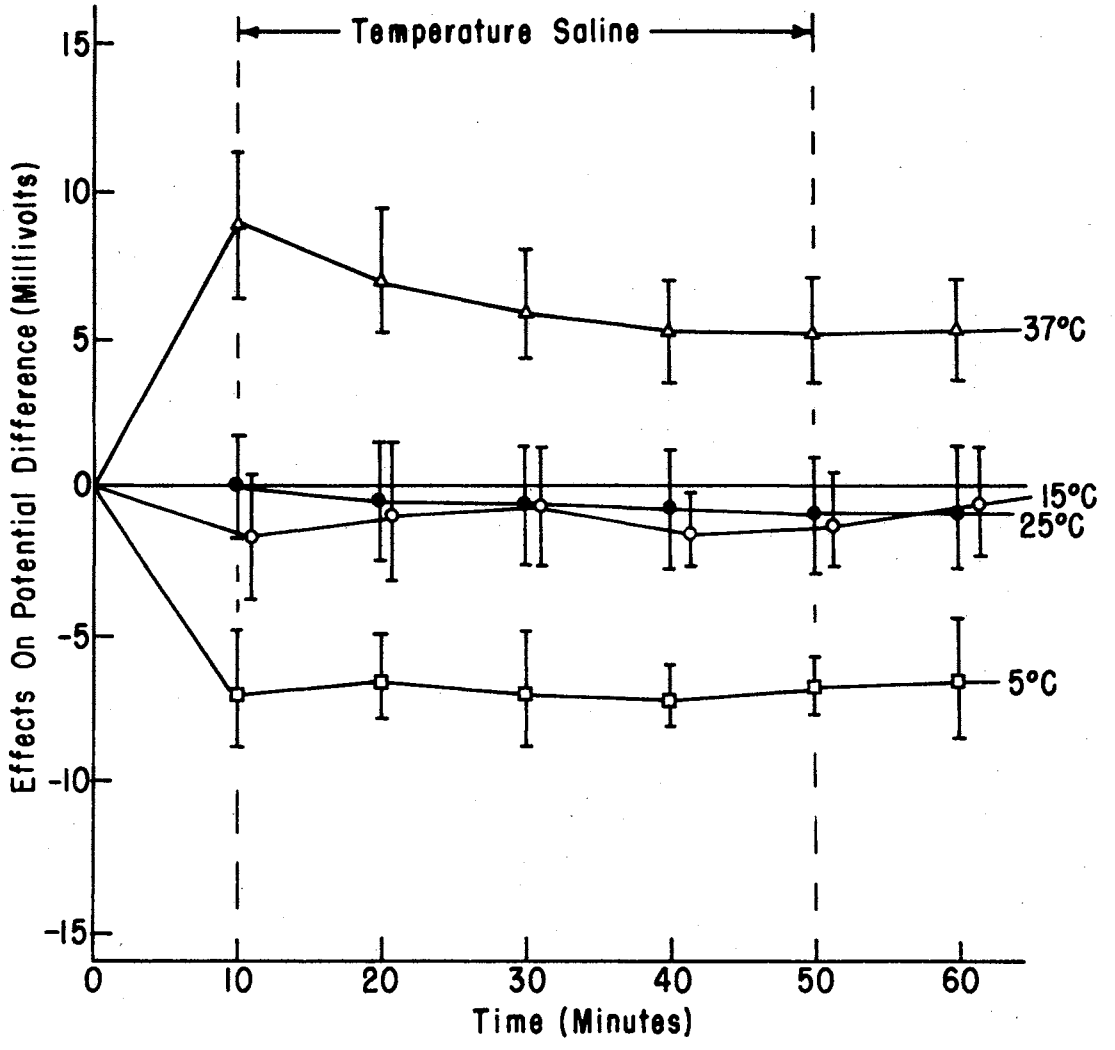
Time (min.)	Potential Difference ± S.D.*** (mV)
00	6.8 ± 1.7
10	6.9 ± 1.8
20	6.4 ± 1.7
30	6.0 ± 1.8
40	5.8 ± 1.7
50	5.5 ± 1.7
60	5.4 ± 1.6

* Saline pH 7.80

** Each potential reading was obtained by placing the alkaline saline on both sides of the tissue.

*** Each potential difference represents the man of 10 measurements ± S.D.

Figure 8. The Effects of Various Temperatures on the Potential Difference. Each point represents the mean of 10 measurements $\pm$ S.D. The experimental temperature salines were placed on both sides of the tissue (Δ).


Temperature Saline
15
10
5
0
-5
-10
-15
Effects On Potential Difference (Millivolts)
0
10
20
30
40
50
60
Time (Minutes)
37°C
15°C
25°C
5°C

Effects of Hemolymph

Preliminary studies were initiated to observe the effects of hemolymph from feeding and nonfeeding adult female A. maculatum on the potential difference. The initial data does indicate that there is a factor present in the feeding tick's hemolymph which induces a slight increase of approximately 2.0-3.0 mV in the potential difference. No change in the potential was observed when the hemolymph was placed on the luminal side of the tissue. Hemolymph from nonfeeding ticks did not appear to effect the midgut potential significantly. At this time, no conclusions may be drawn from this preliminary data as further experiments need to be conducted to investigate the "hemolymph factor" to verify these observations.

CHAPTER V

DISCUSSION

From the results it is evident that a bioelectric potential does exist across the tick midgut epithelium *in vitro*. The potential does not appear to arise from passive physical forces because the potential can be maintained up to three hours in the absence of any chemical gradients between the lumen and serosal side of the tissue; and the potential does decline in the presence of various metabolic inhibitors.

The chemical inhibitions were relatively slow and sometimes less with the inhibitor on both sides of the tissue which may be due to a diffusion factor within the lucite holder or the difficulty of penetration by these molecules into the tissue. Irvine and Phillips (1971) report very ambiguous results upon the addition of dinitrophenol and potassium cyanide to *in vitro* preparations of the desert locust rectum. O'Riordan (1969), using an *in vitro* cockroach ventriculus preparation, observed no inhibition of the potential with cyanide and iodacetic acid which apparently failed to reach the appropriate tissue sites.

Dinitrophenol, an uncoupler of oxidative phosphorylation, was an effective inhibitor especially on the serosal side of the tissue. This potential decrease indicates that there are probably energy-requiring processess associated with the potential that are dependent upon aerobic metabolism. Additional evidence for oxidative metabolism by the midgut cells is the effect of carbon dioxide and nitrogen on the potential.

Both gases deprived the tissue of a ready source of energy (i.e., oxygen) which caused a potential decline that could not be restored upon the addition of aerated salines. The gases may have effected the intracellular pH thereby deactivating the metabolic enzymes associated with transport mechanisms.

On the serosal side, ouabain induced a significant decrease in the potential difference. Ouabain is believed to be a specific inhibitor of membrane ATP-ases and has been associated with Na^+-K^+ pumps in vertebrate and invertebrate tissues (Coplon and Maffly, 1972; Glynn, 1964; Treherne, 1966). Several investigators (Cooperstein and Brockman, 1958; O'Riordan, 1969) report that ouabain is ineffective when placed on the lumen side of the epithelium as is the case in the tick midgut. On the basis of this evidence, one could suggest that the tick midgut serosal pump is a linked Na^+-K^+ pump. Other ixodid ticks, Amblyomma americanum and Dermacentor andersoni, have a high hemolymph sodium concentration (Kaufman and Phillips, 1973a; Shih et al., 1973). An active transport mechanism, (i.e., Na^+-K^+ pump) is probably responsible by creating gradients for the high sodium concentration in the hemolymph. Care should be taken when interpreting these results as passive forces may alter as well as complicate or change the true normal potential value.

The involvement of various ions in the transepithelial potential is confirmed by the experiments in which concentrations of these ions in the standard saline solution were altered. The experiment using the sulfate saline, non-penetrating sulfate ions substituting for chloride ions, discounts a possible serosal chloride pump. The sulfate saline induced an increase when placed on the luminal side but a pronounced decrease resulted on the serosal side. The potential

difference then is not dependent upon a chloride pump and probably some chloride ions leaked through the midgut epithelium in the standard saline, thereby partly disguising the true size of the potential by short-circuiting the system. The rapid and dramatic reversal in tissue polarity when sulfate was placed on the hemolymph side only but not when on both sides or in the lumen only indicates an ability of anions (probably chloride) to diffuse easily from the lumen to hemolymph in the presence of a concentration gradient.

The luminal border of the midgut was sensitive to the choline saline, choline ions substituting for sodium ions, indicating that sodium does move across this border rather easily and after a period of time is transported across the serosal membrane into the hemolymph. This serosal potential increase verifies the cellular sodium movement.

It seems reasonably certain from the experimental results of this study that a $Na^+ - K^+$ pump is situated in the tick midgut epithelium. Sodium ions diffusing into the epithelial cells from the midgut lumen would mostly be pumped out again on the serosal side and into the hemolymph. This sodium pump may indirectly be responsible for the midgut potential. This net sodium transport which creates a solute gradient (probably chloride ions passively involved also) might induce an osmotic movement of water in the same direction. The pump would then be entirely responsible for maintaining a high potassium concentration and a low sodium concentration inside the cell, thus indirectly regulating the hemolymph volume of the replete tick. Active sodium transport accounts for most of the net ionic movements observed across many epithelia. Across the frog skin and toad bladder, the net transcellular ionic movements consists almost entirely of sodium accompanied by

chloride.

Kaufman and Phillips (1973a; b; c) and Tatchell (1969) cite the salivary glands of two ixodid ticks, [D. andersoni; B. microplus (Canestrini)] as osmoregulatory organs in feeding ticks because of their ability to regulate hemolymph volume via the salivary secretion. These authors suppose that it is the act of salivation which ultimately regulates the volume of hemolymph. From this study on A. maculatum, a midgut pump may also be indirectly related to hemolymph volume as it may create the necessary gradients to eliminate excess ions and water via the salivary glands. The effect of the addition of hemolymph on the potential suggests that a hemolymph factor (i.e., hormone(s)) may be present which alters the permeability of the membrane thereby contorlling ionic movements across the midgut epithelium.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This study describes the effects of metabolic (chemical) inhibitors, gases, pH, temperature and ionic saline substitutions on the midgut transepithelial potential difference of the replete female Gulf Coast tick, Amblyomma maculatum (Koch) using an in vitro technique.

The isolated tick midgut epithelium maintains an average potential difference of 6.8 ± 1.2mV (± mean S.D.) (Lumen negative to serosa) in the standard saline solution.

The size of the potential could be reduced by various metabolic (chemical) inhibitors, i.e., potassium cyanide, ouabain and dinitrophenol. Ouabain significantly inhibited the potential on the serosal side but was ineffective when placed on the lumen side of the tissue.

The sulfate saline enhanced the potential on the luminal border but caused a rapid decrease which was reversible upon the reintroduction of the standard saline on the serosal side. The choline saline induced a potential increase on the serosal side and a decrease on the luminal side.

Nitrogen and carbon dioxide when present in the bathing media produced a potential decline which could not be restored to its normal value. Oxygenated saline solutions produced a slight increase in the potential. The potential appears to be oxygen dependent.

The potential is best maintained at a neutral pH 6.8, but can also

be maintained though not as stable and consistently in an alkaline or acidic media.

The effects of temperature on the potential indicates that temperature does appear to effect the energy-requiring mechanisms associated with potential maintenance.

Hemolymph obtained from feeding ticks does appear to have a "factor" which causes an increase in potential differences when placed on the serosa of the tissue.

The experimental results appear to indicate a Na^+ - K^+ pump situated in the tick midgut epithelium. This sodium pump may be indirectly responsible for the midgut potential but is primarily involved in the transport of sodium into the hemolymph.

SELECTED BIBLIOGRAPHY

Andersen, B. and H. H. Ussing. 1957. Solvent drag on non-electrolytes during osmotic flow through the isolated toad skin and its response to antidiuretic hormone. Acta. Physiol. Scand. 39: 228.

Andersen, B. and H. H. Ussing. 1960. Active Transport. In Comparative Biochemistry. II. M. Florkin and H. S. Mason (ed.'s). Academic Press, New York. pp. 371-402.

Araman, S. F. 1972. Biochemical and physiological studies of certain ticks (Ixodoidea). The ionic composition of Hyalomma (Hyalomma) dromedarii Koch and H. (H.) anatolicum excarvatum Koch (Ixodidae). J. Parasitol. 58: 354-357.

Araman, S. F. and A. Said. 1972. Biochemical and physiological studies of certain ticks (Ixodoidea). The ionic regulatory role of the coxal organs of Argas (Persicargus) persicus (Oken) and A. (P.) arboreus Kaiser, Hoogstraal, and Kohls (Argasidae). J. Parasitol. 58: 348-353.

Arthur, D. R. 1962. Ticks and Disease. Pergamon Press, Oxford. 445 p.

Balashov, Y. S. 1972. A translation of bloodsucking ticks (Ixodoidea) --vectors of diseases of man and animals. Entomol. Soc. Amer. Misc. Pub. 8: 166-376.

Barry, P. H. and J. M. Diamond. 1970. Junction potentials, electrode standard potentials, and other problems in interpreting electrical properties of membranes. J. Membr. Biol. 3: 93-122.

Bequaret, J. C. 1945. The ticks or Ixodidae of the northeastern U.S. and eastern Canada. Entomol. Amer. 25: 73-232.

Binder, H. J., D. W. Powell, and P. F. Curran. 1972a. Effect of hexoses on ion transport in guinea pig ileum. Amer. J. Physiol. 223: 538-544.

Binder, H. J., D. W. Powell and P. F. Curran. 1972b. Electrolyte secretion by the guinea pig ileum in vitro. Amer. J. Physiol. 223: 531-537.

Bures, J., M. Petran and J. Zachar. 1967. Electrophysiological Methods in Biological Research. Academic Press, New York. pp. 17-31.

Caldwell, P. C. 1968. Liquid junction potentials and their effect on potential measurements in biological systems. Int. Rev. Cytol. 24: 345.

Chinery, W. A. 1964. The midgut epithelium of the tick Haemaphysalis spinigera Neuman 1897. J. Med. Entomol. 1: 206-212.

Cooperstein, I. L. and S. K. Brockman. 1958. The electrical potential developed by the large intestine: its relation to electrolyte and water transport. Clin. Res. 6: 277.

Coplon, N. S. and R. H. Maffly. 1972. The effect of ouabain on sodium transport and metabolism of the toad bladder. Biochem. Biophys. Acta. 282: 250-254.

Coster, H. G. L. 1973. The double fixed charge membrane solution-membrane ion partition effects and membrane potentials. Biophys. J. 13: 133-142.

Diamond, J. M. 1962a. The reabsorptive function of the gallbladder. J. Physiol. 161: 442.

Diamond, J. M. 1962b. The mechanism of solute transport by the gallbladder. J. Physiol. 161: 474.

Diamond, J. M. 1964. Transport of salt and water in rabbit and guinea pig gallbladder. J. Gen. Physiol. 48: 1-16.

Diamond, J. M. 1968. Transport mechanisms in the gallbladder. In Handbook of Physiology. V. Williams and Wilkins, Baltimore. pp. 2451-2482.

Douglas, J. R. 1943. The internal anatomy of Dermacentor andersoni Stiles. Univ. Calif. Publ. Entomol. 7: 207-272.

Ellory, J. C., M. H. Evans, J. W. Hsall and M. W. Smith. 1972. Intracellular and transepithelial recording from the goldfish intestine. J. Physiol. 226: 29-30.

Flower, R. J., R. J. Pollitt, P. A. Sanford and D. H. Smyth. 1972. Metabolism and transfer of choline in hamster small intestine. J. Physiol. 226: 473-489.

French, E. L. 1973. A review of arthropod-borne virus infections affecting man and animals in Australia. Aust. J. of Exp. Biol. and Med. Sci. 51: 131-158.

Frick, J. H. and J. R. Sauer. 1973. Examination of a biological cryostat/nanoliter osmometer for use in determining the freezing point of insect hemolymph. Ann. Entomol. Soc. Amer. 66: 781-783.

Frizzell, R. A., H. N. Nellans, R. C. Rose, L. Markscheid-Kaspi and S. G. Schultz. 1973. Intracellular Cl concentrations and influxes across the brush border of rabbit ileum. Amer. J. Physiol. 224: 328-337.

Fromm, D. 1973. Na and Cl transport across isolated proximal small intestine of the rabbit. Amer. J. Physiol. 224: 110-116.

Gladney, W. J. and R. O. Drummond. 1970. Mating behavior and reproduction of the Lone Star Tick, Amblyomma americanum. Ann. Entomol. Soc. Amer. 63: 1036-1039.

Glynn, I. M. 1964. The action of cardiac glycosides on ion movements. Pharm. Rev. 16: 381-407.

Gregson, J. 1960. Morphology and functioning of the mouth parts in Dermacentor andersoni Stiles. Acta. Trop. (Basel) 17: 48-79.

Harvey, W. R., J. A. Haskell and R. D. Clemons. 1964. Active transport by the cecropia midgut. I. Inhibitors, stimulants, and potassium transport. J. Cell. and Comp. Physiol. 65: 45-56.

Harvey, W. R., J. A. Haskell and S. Nedergaard. 1968a. Active transport by the cecropia midgut. III. Midgut potential generated directly by active K^+ transport. J. Exp. Biol. 48: 1-12.

Harvey, W. R. and S. Nedergaard. 1968b. Active transport by the cecropia midgut. IV. Specificity of the transport mechanism for potassium. J. Exp. Biol. 48: 13-24.

Harvey, W. R., J. A. Haskell and R. M. Clark. 1968c. Active transport by the cecropia midgut. V. Loss of potassium transport during larval-pupal transformation. J. Exp. Biol. 48: 25-37.

Harvey, W. R. and K. Zerahn. 1971. Active transport of sodium by the isolated midgut of Hyalophora cecropia. J. Exp. Biol. 54: 269-274.

Hochster, R. M. and J. H. Quastel. 1963. Metabolic Inhibitors: A Comprehensive Treatise. II. Academic Press, New York. pp. 503-505.

Hughes, T. E. 1954. Some histological changes which occur in the gut epithelium of Ixodes ricinus females during gorging and up to oviposition. Ann. Trop. Med. Parasitol. 48: 397-404.

Irvine, H. B. and J. E. Phillips. 1971. Effects of respiratory inhibitors and ouabain on water transport by isolated locust rectum. J. Insect Physiol. 17: 381-383.

James, M. T. and R. F. Harwood. 1969. Herm's Medical Entomology. MacMillan Co., New York. pp. 320-361.

Kaufman, W. R. and J. E. Phillips. 1973a. Ion and water balance in the Ixodid tick Dermacentor andersoni. I. Routes of ion and water excretion. J. Exp. Biol. 58: 523-536.

Kaufman, W. R. and J. E. Phillips. 1973b. Ion and water balance in the Ixodid tick Dermacentor andersoni. II. Mechanism and control of salivary secretion. J. Exp. Biol. 58: 537-547.

Kaufman, W. R. and J. E. Phillips. 1973c. Ion and water balance in the Ixodid tick Dermacentor andersoni. III. Influence of monovalent ions and osmotic pressure on salivary secretion. J. Exp. Biol. 58: 549-564.

Kitaoka, S. 1961. Physiological and ecological studies on some ticks. VI. Date of digestion of blood meal and nitrogen, iron and sterol economy during the ovipositing process in the tick. Nat. Inst. Anim. Hlth. Quat. 1: 105-112.

Koefoed-Johnsen, V. and H. H. Ussing. 1958. The nature of the frog skin potential. Acta. Physiol. Scand. 42: 298-308.

Lancaster, J. L. Jr. 1973. A guide to the ticks of Arkansas, Univ. Ark. Agric. Exp. Stat. Bull. 779: 3-32.

Lees, A. D. 1946. Chloride regulation and the function of the coxal gland in ticks. Parasitology 37: 172-184.

Lees, A. D. 1952. The role of cuticle growth in the feeding process of ticks. Proc. Zool. Soc. Lond. 121: 759-772.

Lester, H. M. O. and L. Lloyd. 1928. Notes on the process of digestion in tsetse-flies. Bull. Entomol. Res. 19: 39-60.

Maddrell, S. H. P. 1963. Excretion in the bloodsucking bug, Rhodnius prolixus Stol. I. The control of diuresis. J. Exp. Biol. 40: 247-256.

McCue, C. M., J. B. Stone and L. E. Sutton Jr. 1948. Three cases of tick (Dermacentor variablis, Say) paralysis in Virginia. Pediatrics 1: 174.

Mills, R. R., J. R. Sauer and R. D. Wright. 1970. Midgut epithelium of the American cockroach: probable mechanisms of water secretion. J. Insect Physiol. 16: 417-427.

Nutall, G. H. F. 1914. Tick paralysis in man and animals. Parasitology 7: 95-104.

O'Riordan, A. M. 1969. Electrolyte movement in the isolated midgut of the cockroach (Periplaneta americana L.) J. Exp. Biol. 51: 699-714.

Parker, R. R., G. M. Kohls, G. W. Cox and G. E. Davis. 1939. Observations on an infectious agent from Amblyomma maculatum. U. S. Pub. Hlth. Repts. 54: 1482-1484.

Rehacek, J. and H. W. Brzostowski. 1969. A tick culture medium based on analysis of tick hemolymph. J. Insect Physiol. 15: 1431-1436.

Reik, R. F. 1966. The development of Babesia spp. and Theileria spp. in ticks with special reference to those occurring in cattle. In biology of Parasites. E. J. L. Soulsby (ed.) Academic Press, New York. pp. 15-30.

Robinson, L. E. 1926. The Genus Amblyomma. Cambridge University Press London. pp. 40-44; 285-286.

Roshdy, M. A. 1961. Comparative internal morphology of subgenera of Argas ticks (Ixodoidea; Argasidae). I. Subgenus Carios: Argas vespertilionus (Latreille, 1802). J. Parasitol. 47: 987-994.

Rothfield, L. and A. Finkelstein. 1968. Membrane Biochemistry. J. Biol. Chem. 243: 463-496.

Sauer, J. R. and R. R. Mills. 1969a. Movement of potassium and sodium across the midgut epithelium of the American cockroach. J. Insect Physiol. 15: 1489-1498.

Sauer, J. R., R. Schlenz-True and R. R. Mills. 1969b. Salt and water transport by the in vitro cockroach midgut. J. Insect Physiol. 15: 483-493.

Shih, C. Y., J. R. Sauer, P. Eikenbary, J. A. Hair and J. H. Frick. 1973. The effects of desiccation and rehydration on the Lone Star Tick. J. Insect Physiol. 19: 505-514.

Spruance, S. L. and A. Bailey. 1973. Colorado tick fever: a review of 115 laboratory confirmed cases. Arch. Intern. Med. 131: 286-294.

Sutton, E. and D. R. Arthur. 1962. Aspects of disease transmission by ticks. Symp. Zool. Soc. Lond. 6: 223-252.

Tatchell, R. J. 1962. The digestion of blood proteins by the tick Argas persicus. Parasitology 52: 13-14.

Tatchell, R. J. 1964. Digestion in the tick Argas persicus Oken. Parasitology 54: 423-440.

Tatchell, R. J. 1967. Salivary secretion in the cattle tick as a means of water elimination. Nature (London) 213: 940-941.

Tatchell, R. J. 1969. The ionic regulatory role of the salivary secretion of the cattle tick, Boophilus microplus. J. Insect Physiol. 15: 1421-1430.

Teorell, T. 1953. Transport processes and electrical phenomena in ionic membranes. Prog. Biophys. Chem. 3: 305.

Till, V. M. 1961. A contribution of the anatomy and histology of the brown ear tick Rhiphicephalus appendiculatus Neumann. Mem. Entomol. Soc. S. Afr. 6: 1-124.

Treherne, J. E. 1966. The effect of ouabain on the efflux of sodium ions in the nerve cords of two insect species (Periplaneta americana and Carausius morosus). J. Exp. Biol. 44: 355-362.

Ussing, H. H. 1949a. Active ion transport through the isolated frog skin in the light of tracer studies. Acta. Physiol. Scand. 17: 1-37.

Ussing, H. H. 1949b. The distinction by means of tracers between active transport and diffusion. The transfer of iodide across the isolated frog skin. Acta. Physiol. Scand. 19: 43-56.

Ussing, H. H. and K. Zerahn. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta. Physiol. Scand. 23: 110-127.

Ussing, H. H. and E. E. Windhager. 1964. Nature of shunt path and active sodium transport path through frog skin epithelium. Acta. Physiol. Scand. 61: 484.

Ussing, H. H. 1966. Anomalous transport of electrolytes and sucrose through the isolated frog skin induced by hypertonicity of the outside bathing solution. Ann. N. Y. Acad. Sci. 37: 543.

Wade, J. B., J. P. Revel and V. A. DiScala. 1973. Effect of osmotic gradients on intercellular junctions of the toad bladder. Amer. J. Physiol. 224: 407-415.

Wharton, D. R. A., M. L. Wharton and J. Lola. 1965. Blood volume and water content of the American cockroach, Periplaneta americana L. Methods and influence of age and starvation. J. Insect Physiol. 11: 391-404.

Whittemburg, G. 1963. Site of potential difference measurements in single renal proximal tubules of Necturus. Amer. J. Physiol. 204: 401-403.

Wigglesworth, V. B. 1931. The physiology of excretion in a bloodsucking insect, Rhodnius prolixus (Hemiptera: Reduviidae). III. The mechanisms of uric acid excretion. J. Exp. Biol. 8: 443-451.

Wood, J. L., P. S. Farrand and W. R. Harvey. 1969. Active transport of potassium by the cecropia midgut. VI. Microelectrode potential profile. J. Exp. Biol. 50: 169-178.

Wright, E. M., P. H. Barry and J. M. Diamond. 1969. An electrical method of measuring nonelectrolyte permeability. Proc. Roy. Soc. (London) 172: 203.

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