THE EFFECTS OF POTASSIUM TRANSPORT ON

VOLTAGE-CURRENT RELATIONSHIPS IN

MANDUCA SEXTA

By

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CHAPTER I

INTRODUCTION

Many diverse types of epithelia have been found to actively
transport ions from one side of the tissue to the other. The charac-
teristics of this mechanism or "pump" are still unclear. However, since
the pump maintains a potential difference across the tissue, electrical
aspects have been applied to this mechanism. Ussing and Zerahn (1951)
stated that the sodium pathway in the frog skin consisted of a driving
force, E_{Na}, in series with a resistor, R_{Na}. The driving force is the
mechanism pumping ions against a concentration gradient and the resistor
is the resistance of the tissue to the transported ion. It is the
driving force, or to put in electrical terms, electromotive force (EMF)
of the pump that this paper is concerned with.

The midgut of the larval stage of Lepidoptera actively transports
potassium from the hemolymph to the lumen. In this system, there are
no specific co-ions or counter-ions required for transport as in mammlian
systems. This type of transport is termed electrogenic transport. The
value of this type of transport is that the effects of the principle
ion can be studied without intervening effects of a counter-ion.
Therefore, the larvae of Lepidoptera, primarily those of Hyalophora
cecropia and Manduca sexta, have become widely used as models for the
study of active transport.

Most of the work done on the EMF of active transport mechanisms has involved either the frog skin or the toad bladder, both of which transport sodium ions. From these studies, several different methods for estimating the EMF have been noted. The method which is utilized in this study is one that was introduced by Civan (1970). When the tissue is voltage clamped at intervals of increasing hyperpolarizations, the resulting voltage-current relationship is linear. However, as the clamped voltage approached the EMF of the pump, the slope of the line, which is equivalent to the resistance of the tissue, changes (See Chapter III). This "breakpoint" is thought to be the EMF of the pump.

This study characterizes the voltage-current (V-I) relationships of the Lepidopteran midgut, a tissue which has a totally different type of transport than those studied previously. The effects of anoxia and concentration changes of potassium on the EMF are also taken into consideration.

CHAPTER II

REVIEW OF THE LITERATURE

The active transport mechanisms in epithelia maintain a potential

difference across the tissue and a current (I_{sc}) is required to keep

the potential at zero when the pump is active. The tissue also has a

resistance to the movement of the transported ions. Therefore,

application of electrical characteristics to the mechanism allows an

easier method of understanding toward the mechanism or "pump".

One of the earliest studies which dealt with the electrical aspects

of the pump was done using the frog skin as a model. The frog skin

transports sodium from the outside to the inside of the tissue. Ussing

and Zerahn (1951) determined that the short circuit current (I_{sc}) was

equal to the net flux of sodium across the tissue. As the clamp voltage

increased, the net flux followed the I_{sc}, but the influx and efflux

began to decrease and increase, respectively. Ussing and Zerahn

determined that the voltage where the influx and efflux were equal was

the EMF, or E_{Na}, of the transport pump. This was approximately 100 mV.

The toad bladder is an epithelia which actively transports sodium

ions from the mucosal to the serosal side of the tissue (Leaf, 1960).

Civan (1970) noted that vasopressin, which enhances sodium conductance

(Biber et al., 1966; Civan and Frazier, 1968) and produced a rectifica-

tion of the sodium flux. He proposed that the true rectifying nature of

the active sodium transport could not be measured by small hyperpolariza-

tions but only when the potential difference was increased beyond the E_{Na} of the sodium pump. At this point, there would be a net driving force on sodium from the serosal to the mucosal side. Civan maintained that the resistance of the tissue would increase above this point due to active transport channels providing a greater resistance to the movement in the opposite direction of normal sodium transport. The point at which this resistance change took place would be the E_{Na} of the pump. By using current pulses of both 50-75 msec and 3-5 sec durations, Civan found the appearance of a transition point beyond which the resistance increased. This point was approximately 172-184 mV. When a inhibitor, such as ouabain was added, the transition point was abolished and the resistance of the tissue was increased. Anoxia and choline-substituted sodium Ringer's solution had the same effect. In each case, the transition point returned to the same point prior to treatment. Vasopressin was found to increase the prominence of the transition point but did not increase the voltage at which it was found. Since anoxia and ouabain are known to depress active sodium transport, the data therefore indicated that the transition point was characteristic of the active sodium transport. Through the use of vasopressin, Civan found that while the resting potential increased, the transition point voltage did not change. However, the hyperpolarizing currnet at the transition point was nearly doubled under the vasopressin influence. Thus, Civan stated, the transition point is voltage-dependent rather than current-dependent. Civan also proposed that while the transition point could be due to the resistance of the active sodium channels, the voltage at the transition point is not necessarily the E_{Na}. Actually the sodium channels could be in one of many possible voltage dependent states and

the transition voltage could reflect any of these states.

In another study using the toad bladder, Yonanth and Civan (1971) demonstrated another technique which produced a reliable and reproducible estimate of E_{Na}. The V-I relationship can be characterized as linear when describing two of the three parameters of the equivalent circuit (See Chapter III). The three parameters are the resistance of the pump, the resistance of non-pump pathways and the I_{sc}. Processes or agents which affect these parameters also effect this linearity. It was determined that factors which changed both parameters, in this case, the tissue conductivity, or resistance, and the I_{sc} would best serve to estimate the E_{Na}. Vasopressin decreases the resistance to sodium entry into the transporting cells or the R_a of the equivalent circuit. This effects both the I_{sc} and R. Therefore, by using vasopressin, the slope of the V-I plot will change. The ratio of the changes in I_{sc} and in R then should equal the E_{Na}. Also, when the V-I relationships of normal tissue and that of vasopressin treated tissue are plotted on the same graph, the intercept of these two lines should be equal to the E_{Na}. In actuality, the mean E_{Na} of this study was in reasonable agreement with previous estimates with the mean being between 170 and 185 mV.

Similar studies involving the frog skin have also been recorded. Helman et al. (1975) used two methods for determining the E_{Na} of the sodium pump in the frog skin. The first method used the bidirectional sodium flux ratio that was utilized by Ussing and Zerahn. The second method took the values of the open circuit voltage, the I_{sc} and the shunt resistance which are estimated when the sodium concentration in the outer solution was zero and calculated the E_{Na}. These values of E_{Na} were then compared to the estimated E_{Na} found using the V-I plots.

ratio of E_{Na} found in the V-I relationships to those found by other
methods was 94.1 ± 4.4 mV for the sodium flux while it was $100 \pm .05$ mV
for the concentrations changes. Thus, Helman et al. concluded that the
values obtained in V-I plots were accurate to those found in other
methods. This then gives a rapid and reliable method for determining
E_{Na}.

Macchia and Helman (1979) studied the V-I relationships of the toad
bladder and colon and the effects of various drugs on the E_{Na} of the
tissues. The E_{Na} of the toad bladder determined by V-I plots was found
to be 124.5 mV which is quite a bit lower than that determined by
Yonanth and Civan. But it was similar to the E_{Na} found in other tissues
such as the frog skin, cortical collecting tubules of the kidney and the
turtle bladder. When subjecting the toad bladder to ADH, it was found
the break at E_{Na} was more pronounced but the voltage at which the change
occurred did not vary significantly. The effect of amiloride on the toad
bladder was to decrease the E_{Na} approximately 9%. Amiloride is thought
to inhibit sodium transport by increasing the resistance to sodium entry
into the transporting cells. The toad colon was found to have an E_{Na} of
96.6 mV.

Very little work has been done on the driving force of the potassium
pump found in the midgut of Lepidopteran larvae. The midgut transports
potassium when isolated and placed on a flat Ussing chamber from
hemolymph to lumen when both sides of the tissue are bathed in identical
solutions (Harvey and Nedergaard, 1964). The potential difference
illicited usually ranges from 130-180 mV and gradually decreases over
a six to eight hour period. Potassium is the only ion transported and
Wood (1972) determined that this transport was responsible for 99% of

the I_{sc} in <u>A</u>. <u>pernyii</u>.

Blankemeyer (1978) showed that the tissue in the midgut of <u>H</u>. <u>cecropia</u> and <u>M</u>. <u>sexta</u> consisted of basically two separate types of cells. By using microelectrodes and anoxic resistance measurements of each cell type, Blankemeyer demonstrated that the cell type with a low potential difference or LPD cell had a large resistance change while the cell with a high potential difference or HPD cell had little resistance change. From this he concluded that the active transport site was in the apical region of the LPD cell. By analyzing the ratio of columnar cells to goblet cells in electron micrographs by Anderson and Harvey (1966) and the ratio of HPD to LPD impalements, Blankemeyer tentatively identified the LPD cell as the goblet cell, the site of active potassium transport. He also estivated the EMF of the potassium pump of the midgut to be 180 mV using the bidirectional flux ratio calculations used by Ussing and Zerahn.

CHAPTER III

THEORETICAL CONSIDERATIONS

The active transport mechanism in the midgut can be shown in the
same electrical consideration as Ussing and Zerahn demonstrated with the
frog skin using the Thevenin equivalent (Figure 1). The potassium ions
are thought to be transported through certain pathways by the potassium
pump which has a driving force of E_K and a resistance to this movement
termed R_a. Potassium can also move in passive pathways which are
independent of the pump and have a resistance termed R_1.

By using Ohm's Law, the change of voltage from one side of the tissue
to the other can be described as:

$$V = \frac{(E_K/R_a) + I}{1/R_a + 1/R_1} \tag{1}$$

The voltage can be divided into the potential difference caused by the
pump and that caused by the passive pathway. E_K/R_a is equivalent to the
current through the pump mechanism while I is the current outside the
pump mechanism. Since the resistance of the tissue is in parallel, the
total resistance, R_t, can be termed:

$$R_t = \frac{1}{1/R_a + 1/R_1} \tag{2}$$

When the tissue is short circuited, (ie. the voltage is clamped at zero),
the passive movement decreases to insignificance and the short circuit

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Figure 1. The Thevenin Equivalent Circuit of the Midgut of <u>Manduca</u>
<u>sexta</u>. The midgut can be broken down into two components,
the active transport mechanism and its associative resis-
tance and the resistance of the tissue to passive movement
of potassium. These have been labelled with R_a being the
resistance of the mechanism or "pump" and R_l being the
resistance to passive movement. E_k refers to the driving
force of the pump or the electromotive force of the pump.
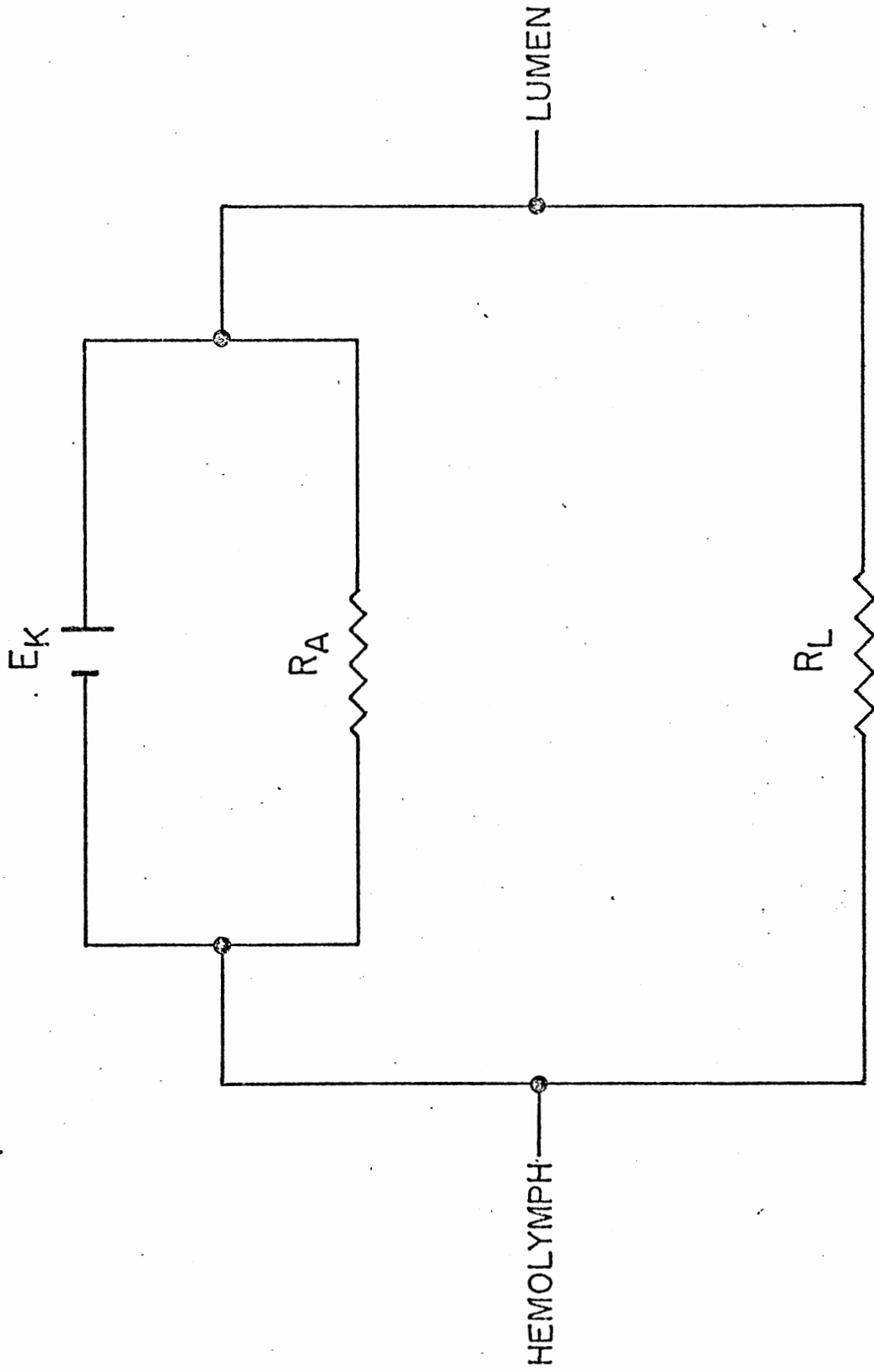
current, I_{sc}, is a product of the pump alone.

$$I_{sc} = E_k/R_a \tag{3}$$

If the voltage is clamped at some other value besides zero, V_c, then the current, I_c, is equivalent to:

$$I_c = \frac{(E_k - V_c)}{R_a} + \frac{V_c}{R_1} \tag{4}$$

Again, the movement of ions can be divided into passive and active constituents. The clamp voltage reduces the voltage being produced by the E_k of the pump. This should change the resistance in a linear fashion that can be demonstrated by combining equation (2) with equation(4):

$$R_t = \frac{(E_k - V_c)}{I_c + V_c/R_1} + \frac{1}{1/R_1} \tag{5}$$

As V_c approaches E_k, the resistance changes radically so that the breakpoint of the linear V-I plot can be seen as a change in resistance which alters the linearity.

CHAPTER IV

MATERIALS AND METHODS

Larvae of the tobacco hornworm, Manduca sexta, were obtained from
Carolina Biological Co.. The larvae were maintained on both artificial
diet (Yamamoto, 1968) and leaves of the thornapple plant, Datura
metalloides, until the fifth instar stage. All experiments were carried
out in vitro using 32 K/16 Cs-S-TRIS as a standard bathing solution.
One series of experiments included several changes in the potassium con-
centration of the bathing solution. The osmotic difference due to these
changes was made up for by adding cesium chloride to the solution.
Cesium is a halide which is not transported by the pump mechanism of the
midgut (Zerahn, 1973). The constituents of the bathing solutions of
each concentration change is shown in Table I.

The midgut was isolated and placed on a flat-sheet, Ussing-type
chamber described by Wood (1972). Stirring and oxygenation of the bath
was by a gas lift pump using 100% oxygen with a complete stirring time
of approximately 20 seconds. This allowed rapid solution changes. The
midgut was stretched across the opening of the chamber which had an area
of 0.396 cm^2. The area of the opening could be changed by inserting a
plexiglass disk inside the original opening. The plexiglass disk had
an opening with an area of 0.107 cm^2. Since resistance is a function
of area, the resistance drops so that with a fixed applied voltage, the
I_{sc} drops into a range that is in the optimum range of the clamp meter.

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TABLE I

BATHING SOLUTIONS OF VARYING POTASSIUM CONVENTRATIONS*

	KCl	CsCl	$MgCl_2$	$CaCl_2$	TRIS	Sucrose
48 K/0 Cs	48	0	1	1	5	166
32 K/16 Cs	32	16	1	1	5	166
24 K/24 Cs	24	24	1	1	5	166
16 K/32 Cs	16	32	1	1	5	166
8 K/40 Cs	8	40	1	1	5	166

*Concentrations in millimoles

The clamp used in this study was a Biologic VC-3 voltage-current clamp which was interfaced with a Southwest Technical Products Model 6800 microcomputer. The computer was programmed to control the clamp, record the voltage pulsed through the tissue and the resulting current, and to calculate the resistance of the tissue using Ohm's Law. The experimenter could vary the pulse duration, the time between pulses, and the voltages at which the tissue would be clamped. The standard V-I run was between -100 mV and 300 mV at 20 mV intervals. The pulse duration was at 500 and 800 msecs. The time interval between each pulse was 20 seconds. The tissue was under open circuit conditions between each V-I run and between the pulses. The data was recorded on both a Heath single pen recorder and on computer memory. The data on memory was printed out along with resistance calculations at the end of each run.

Nitrogen was substituted for oxygen as the stirring gas in one series of experiments to study the effects of increasing resistance of the tissue on the breakpoint. Both gases were hydrated in Fisher Gas Wash bottles. The nitrogen was only used as a stirring gas until the potential reached zero and a V-I run could be made. If the tissue was left in nitrogen longer than this, irreparable damage occurred. The effect of the inhibitor, sodium azide, was also studied. The azide was administered to the tissue by dissolving it directly into the bathing solution. The potential was allowed to decrease until it reached a steady level at which a V-I run was made. The azide could be washed out by washing the tissue with standard bathing solution.

CHAPTER V

RESULTS

A typical voltage-current plot using diet reared larvae in 32 K/ 16 Cs is shown in figure 2. The voltage was clamped over a range of -100 mV to 300 mV at 20 mV intervals. It was noted that when the tissue was clamped at more than 60 mV above the breakpoint of the tissue, damage seemed to occur to the tissue so that consistant values were lost and the remainder of the experiment was questionable. The Y-axis intercept is equivalent to the potential difference (PD) of the tissue under open circuit. The slope of the line is the resistance of the tissue to the movement of potassium.

The resistance of the leaf reared larvae when compared to diet reared larvae was considerabley higher (Table II). In 32 K/16 Cs, the resistance of the tissue before breakpoint in diet reared animals averaged 147 ohms, whereas in leaf reared larvae, the resistance was 256 ohms. However, the voltage of the breakpoint, V_b, did not significantly differ between the two types of larvae. In leaf reared larvae, V_b was 252 mV while the V_b of diet reared larvae was 242 mV. The resistance of the tissue did increase in a prolonged experiment. Over the course of 1 1/2 hours, the resistance increased at least 100 ohms. V_b did not change in this experiment, though.

Resistances of the tissue as well as the breakpoint voltage, V_b, changed when potassium concentration was varied. As con be seen from

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Figure 2. A V-I Plot of the Active Transport Pump in 32 K/16 Cs-S-TRIS Bathing Solution. The system was clamped over a range of -100 to 300 mV at 20 mV intervals. The slope of the line is equal to the resistance of the tissue. The resistance before the breakpoint, R_b, is 151 ohms. The resistance after breakpoint, R_k, is 94 ohms. The breakpoint of the tissue, V_b is 245 mV. The Y-axis intercept is the voltage of the tissue under open circuit.
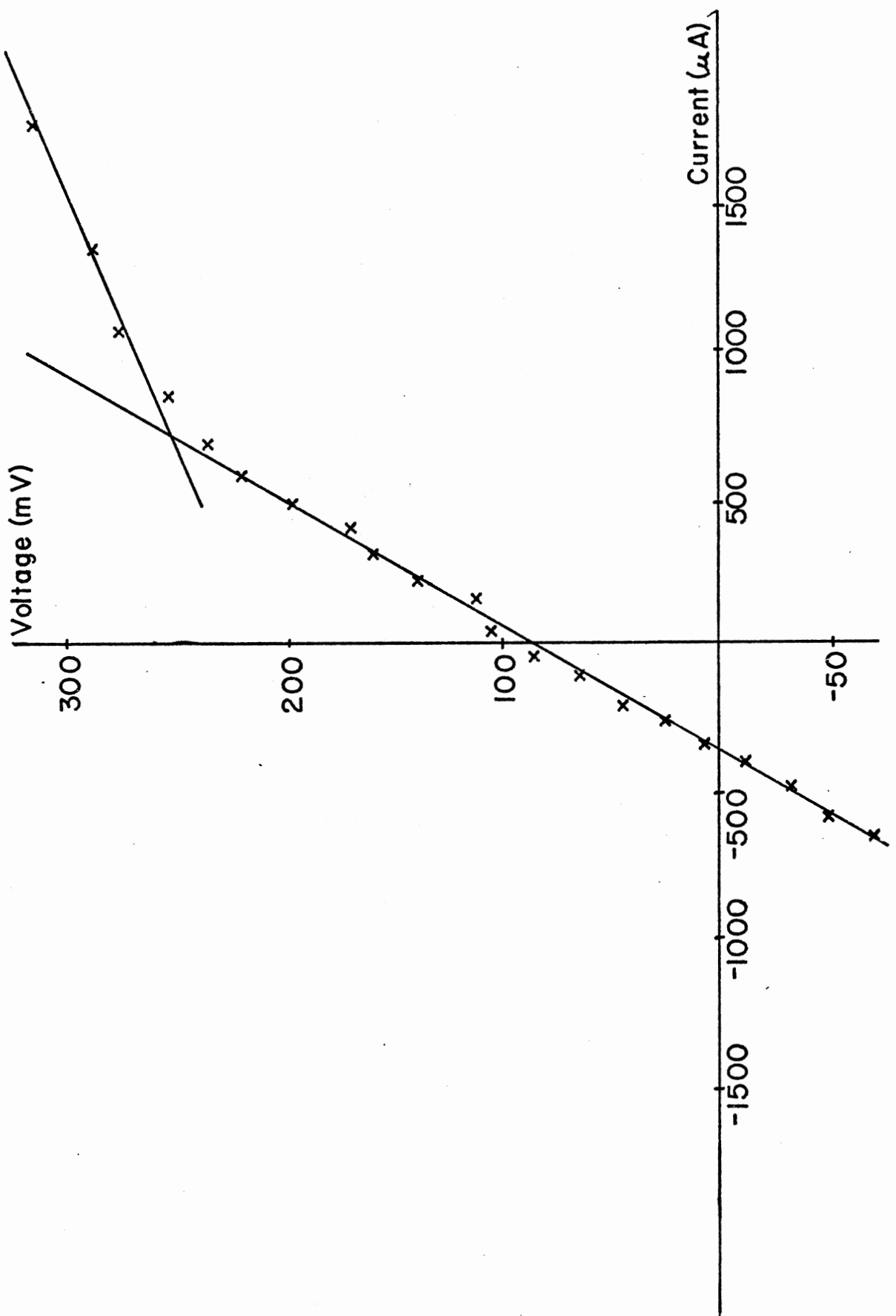
TABLE II

THE DIFFERENCE IN E_k AND RESISTANCE VALUES

BETWEEN LEAF REARED AND DIET REARED LARVAE

	V_b (mV)	R_b (ohms)	R_k (ohms)
Diet reared	242 ± 10.8	147 ± 18.9	91 ± 4.3
Leaf reared	252 ± 7.3	256 ± 60.3	70 ± 7.5

TABLE III

VALUES OF E_k AND RESISTANCES BEFORE AND AFTER BREAKPOINT

DURING K CONCENTRATION CHANGES AND INHIBITORS

	V_b (mV)	R_b (ohms)	R_k (ohms)
48 K/0 Cs	253 + 17.7	133 + 23.9	93 + 5.2
32 K/16 Cs	242 + 10.8	147 + 18.9	91 + 4.3
24 K/24 Cs	139 + 20.3	163 + 13.6	87 + 3.7
16 K/32 Cs	57 + 11.3	171 + 7.4	89 + 4.7
8 K/40 Cs	NB*	208 + 23.2	--
Nitrogen	NB	514 + 73.9	--
Sodium Azide	NB	100 + 18.3	--

*No Breakpoint

Figure 3. A V-I Plot of the Pump in 48 K/0 Cs-S-TRIS. The resistance, R_b is 134 ohms and R_k equals 95 ohms. V_b is 248 mV and the tissue was clamped over a range of -100 to 300 mV.
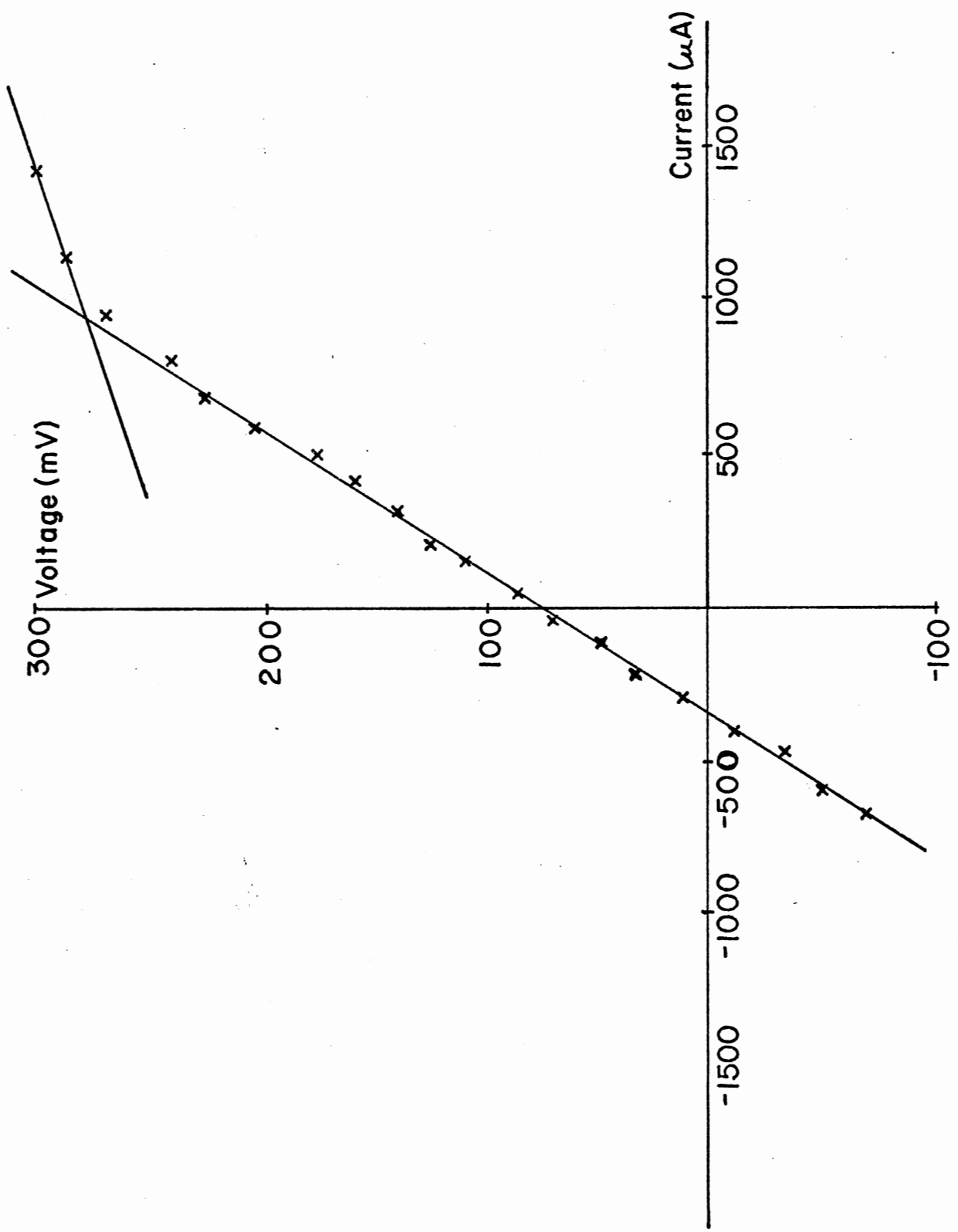
Voltage (mV)

Current (μA)

Figure 4. A V-I Plot of the Pump in 24 K/24 Cs-S-TRIS. R_b equals 165.
ohms and R_k is 91 ohms. V_b is 148 mV. The range over which
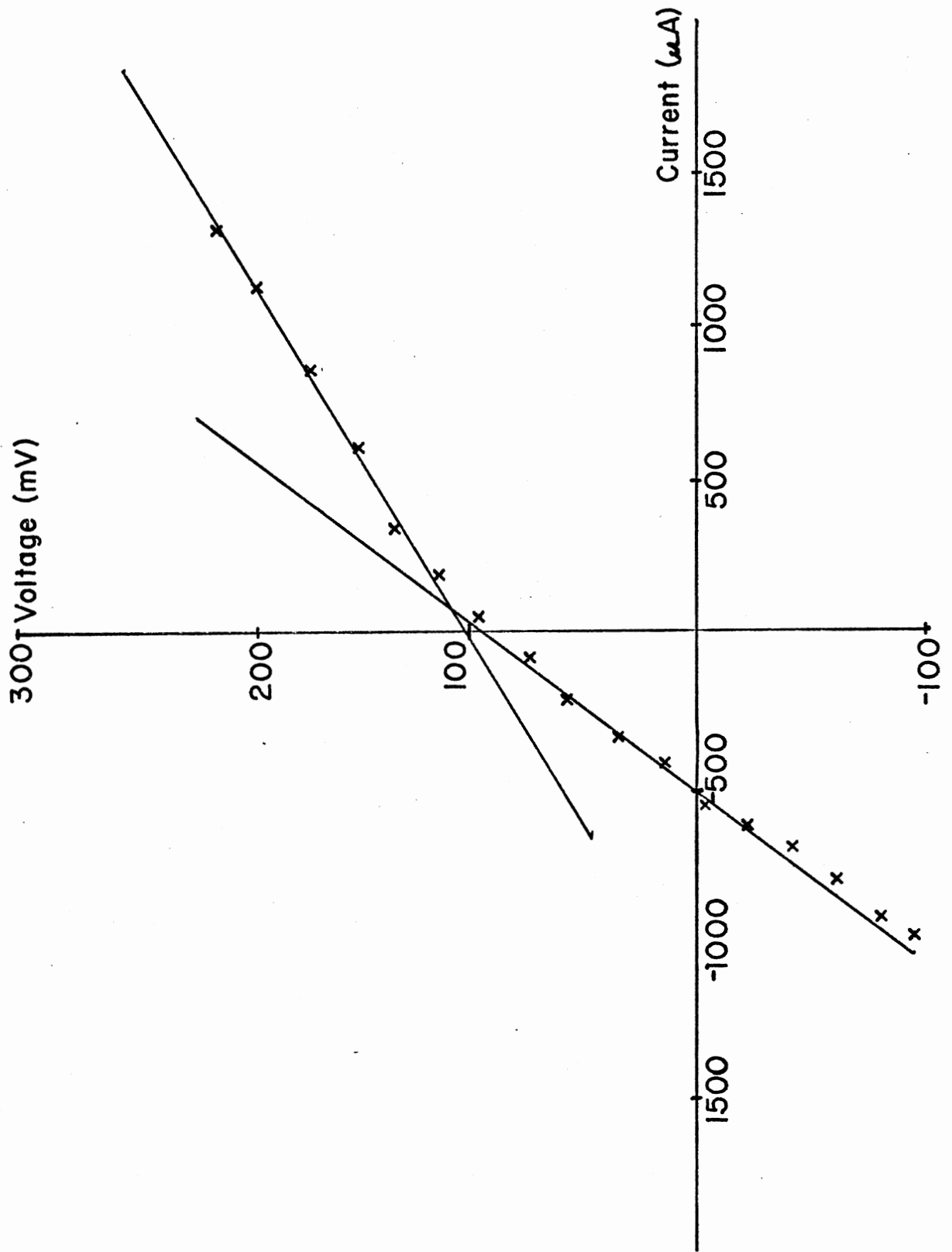the tissue was clamped was -100 to 260 mV.

Table III and figures 3,4,5 and 6, V_b was directly proportional to the potassium concentration while the resistance of the tissue before reaching breakpoint, R_b, was inversely proportional to concentration. There was little difference between 32 K and 48 K solutions shown in either V_b or R_b. The change in these two factors was much greater when the potassium concentration decreased to 24 K or 16 K. The largest drop was in 16 K where the V_b dropped to 57 mV and R_b increased to 171 ohms in diet reared animals. This concentration and below had an adverse effect on the tissue. The effect of 16 K solution on the tissue is demonstrated in figure 7. The line using squares shows the normal V-I plot of a tissue in 32 K before low potassium. The line using triangles shows the same tissue after exposure to low potassium and returned to 32 K bathing solution. Both V_b and R_b are altered. 8 K bathing solution was used in an experiment and was found to cause a disappearance of V_b and a reduction of R_b (figure 6). This also damaged the tissue so that a return to 32 K did not return the breakpoint or the R_b of normal bathing solution.

The resistance of the tissue after breakpoint, R_k, was similar in each of the changes using diet reared larvae. R_k for 16 K, 32 K, and 48 K were 89.4, 90.9 and 93.0 ohms, respectively. The leaf reared larvae were somewhat different with the R_k of each bathing solution being inversely related to the potassium concentration. These values were not significantly different from those of the diet fed animals though.

Nitrogen has been shown to increase the resistance of the tissue to potassium. In this study, when nitrogen replaced oxygen, the resistance of a diet reared tissue increased to 514 ohms. The breakpoint disappeared as the PD reached zero in nitrogen but as the PD decreased,

Figure 5.   A V-I Plot of the Pump in 16 K/32 Cs-S-TRIS bathing solution.
V_b is equal to 58 mV and R_b is 174 ohms. R_k is 87 ohms.
the tissue could not be clamped at high potentials due to
tissue damage so the run only extended to 200 mV.
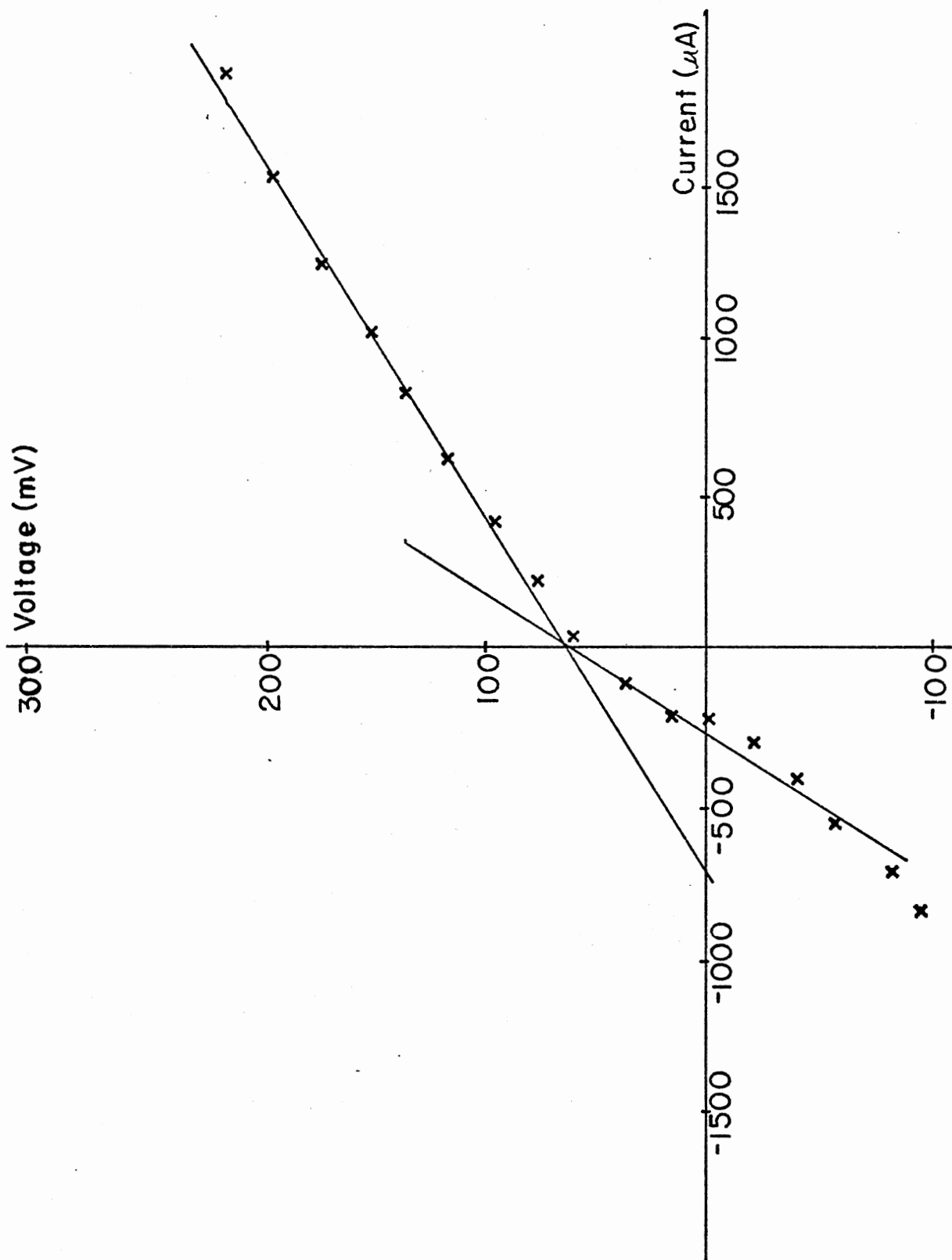
Figure 6.   The V-I Plot of the Pump in 8 K/40 Cs-S-TRIS solution.   There
            is no significant breakpoint and the resistance of the
            tissue remains the same throughout the run.   This resistance
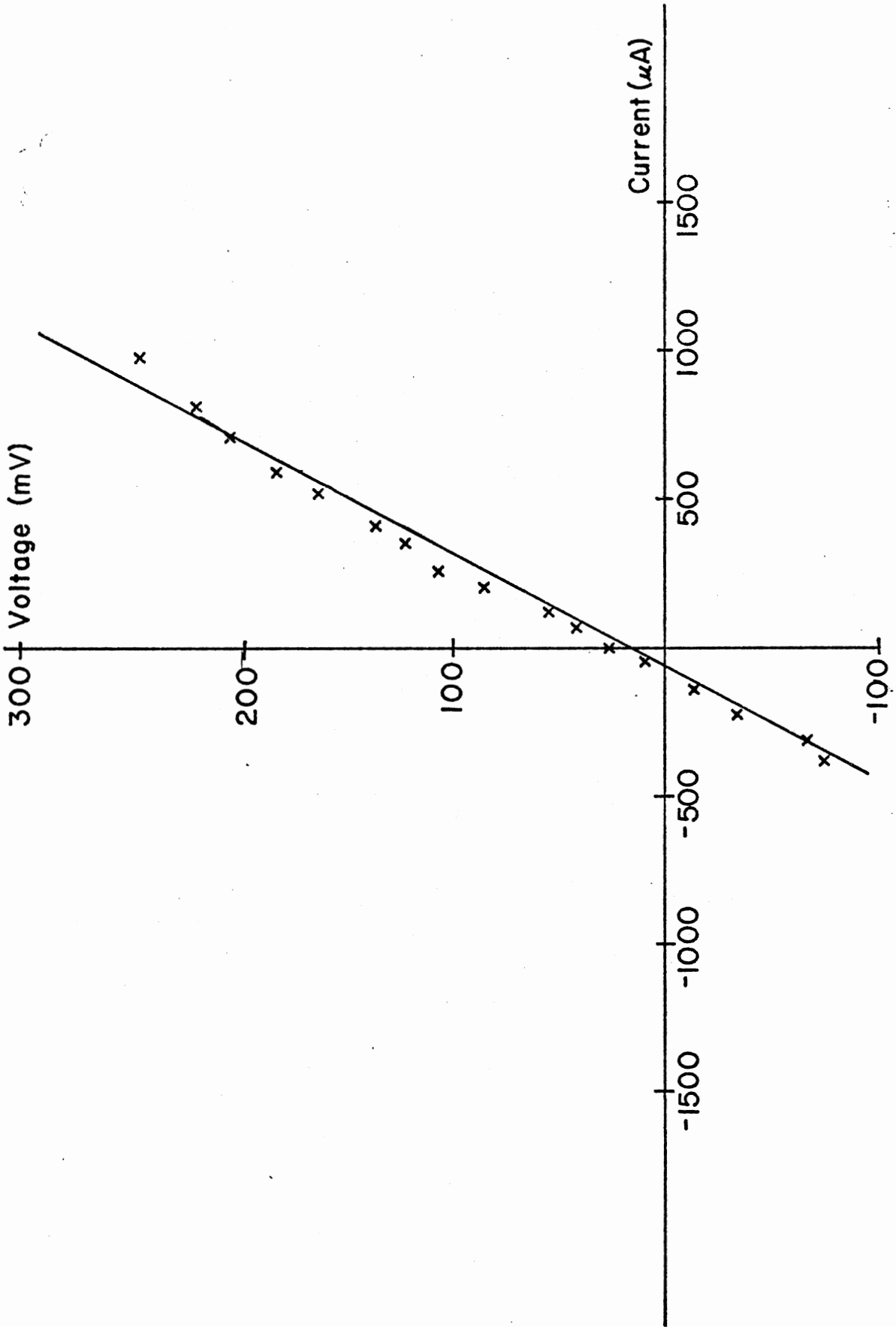   equals 208 ohms.

Figure 7. A V-I Plot Demonstrating Changes in the Tissue After Being
Exposed to Low Potassium. The line with squares represen-
ting data points is a plot of a tissue in normal 32 K
solution before exposure to low potassium. The line with
triangles is a tissue in 32 K after being exposed to low
potassium. In the former tissue, V_b is 239 mV and R_b is
149 ohms. In the later tissue, V_b is difficult to determine
and the resistance drops to 88 ohms. The tissue never
recovers from this treatment.
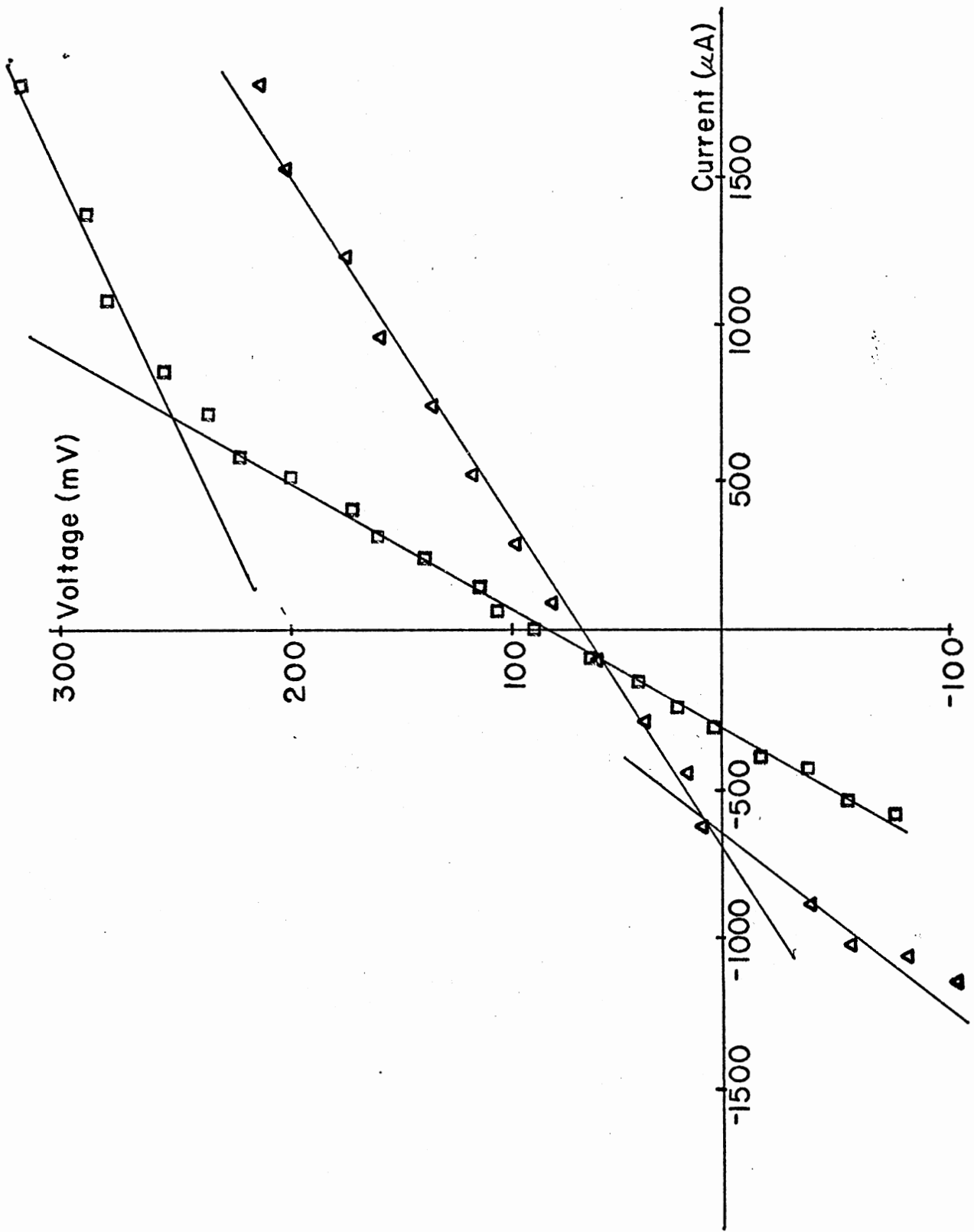
V_b would still be present at approximately the same value as in oxygen (figure 8 and Table III). Sodium azide is a compound which inhibits the electron transport chain at cytochrome a/a_3. When azide was placed in the bathing solution, the resistance of the tissue decreased to the same levels as R_k and V_b would disappear after the tissue had been under the influence of azide for one minute (figure 9). The concentration of sodium azide used was approximately 0.01 mM.

Figure 8. V-I Plots Showing the Differences Between an Oxygenated
Tissue and a Nitrogenated One. The nitrogen run, designated
by circles, shows an absence of V_b and a resistance through
the run of 319 ohms after the PD reaches zero. The oxygen
run, designated by squares, exhibits a V_b of 247 mV and an
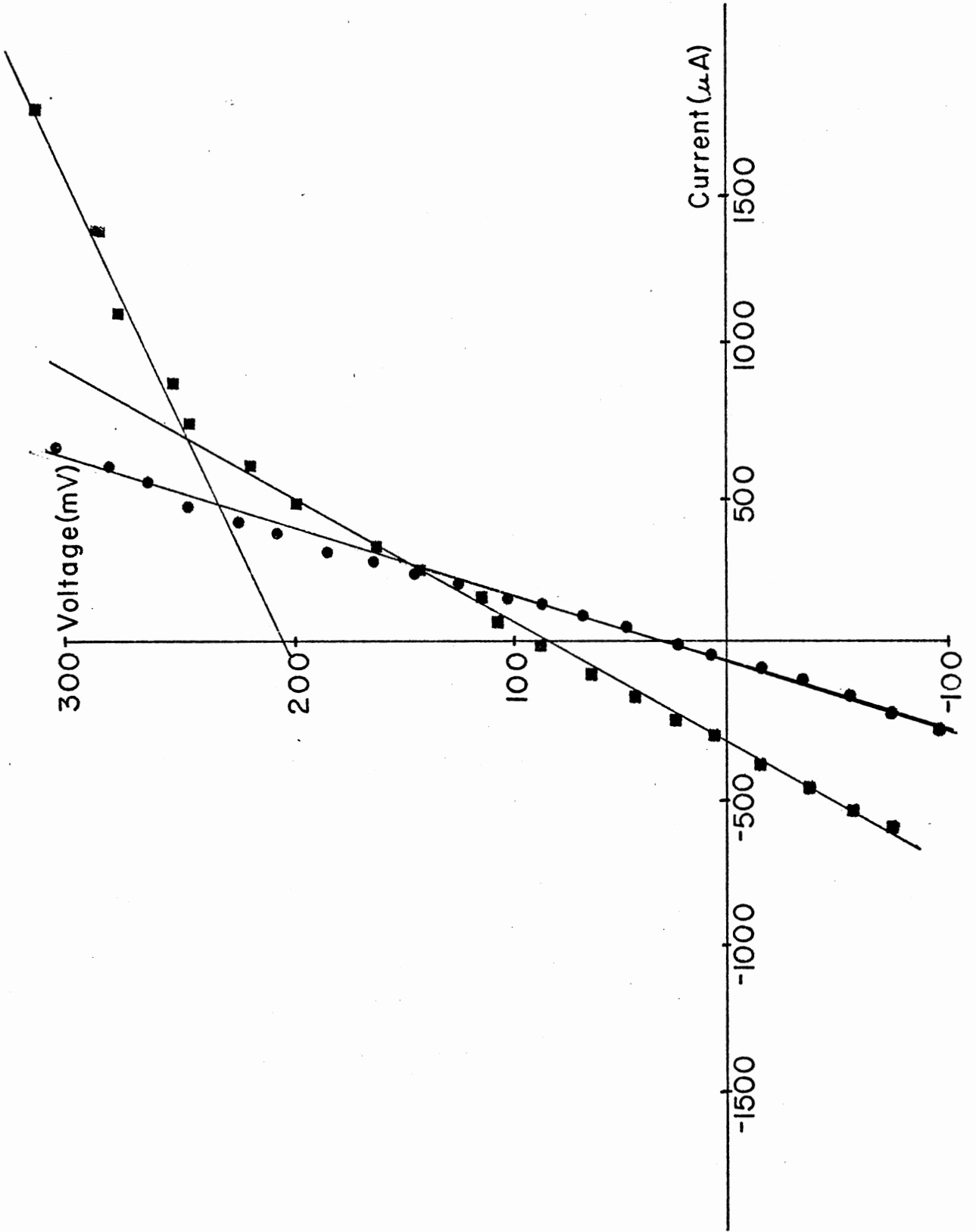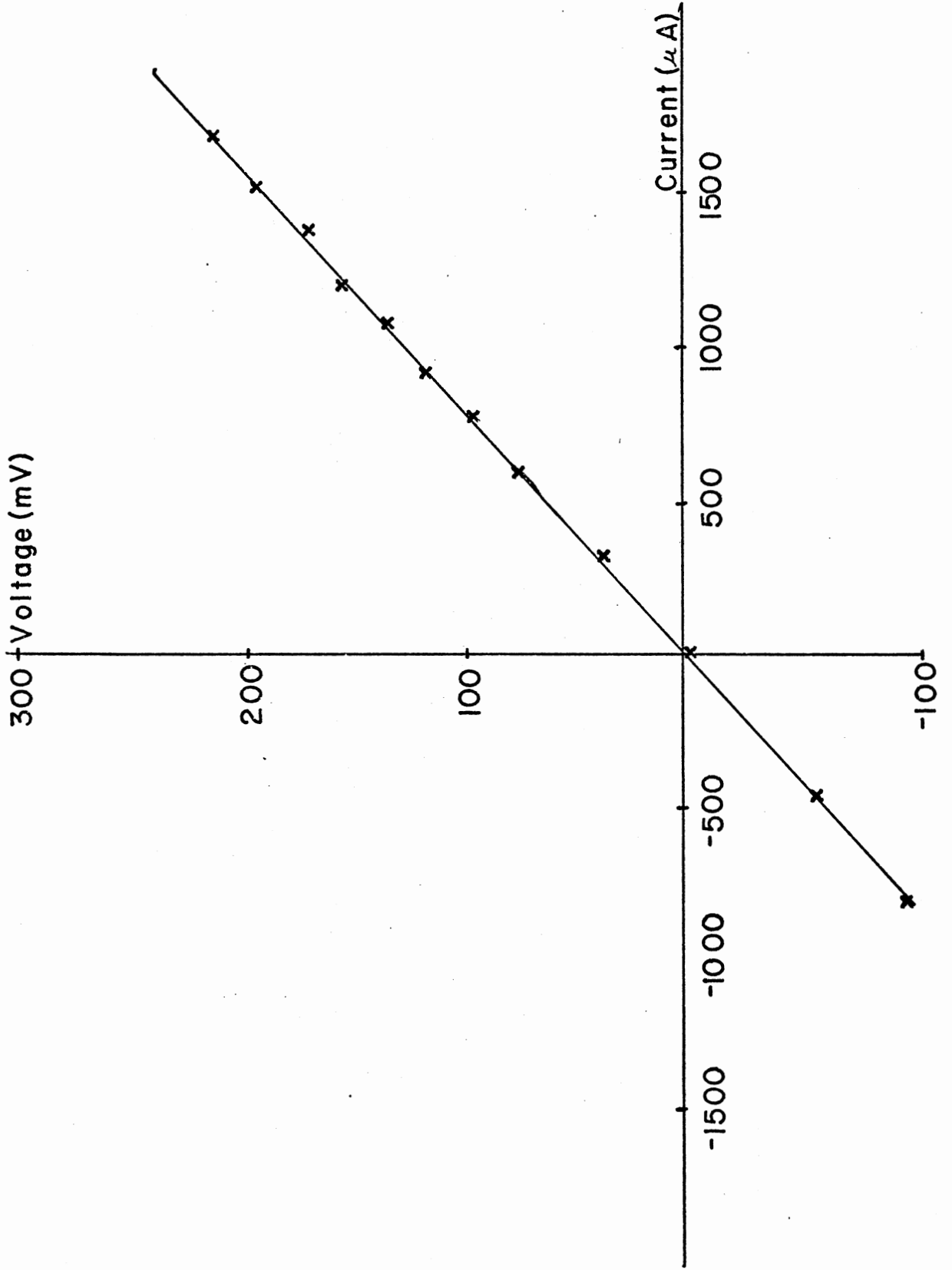R_b of 150 ohms. Both runs are in normal 32 K/16 Cs bathing
solution.

Figure 9. A V-I Plot Using the Inhibitor, Sodium Azide. The bathing
solution is normal 32 K/16 Cs with the sodium azide
dissolved in it. The V_b disappeared within 30 seconds of
addition. Diring this run the PD was zero.

CHAPTER VI

DISCUSSION

The purpose of this study was to test the concept of voltage-current
relationships on a tissue which does not pump sodium, as in all other
mechanisms studied thus far, but potassium. To study this relationship,
the method introduced by Civan (1970) was used. By clamping the voltage
at intervals of increasing positive voltage and recording the subsequent
current produced, the resistance of the tissue to movement of potassium
could be calculated and any changes noted. According to theory, the
point at which the resistance changes dynamically should be the breakpoint
or the EMF of the active transport mechanism.

From the data presented, the voltage-current relationship reached
the breakpoint voltage, V_b, at a potential difference of 242 mV, beyond
which the resistance of the tissue decreased in normal bathing solution.
This differed from previous studies of voltage-current relationships
both in V_b and the R_k of the tissue. The V_b is higher than the values
of those found in either the frog skin or the toad bladder. However,
the active transport mechanism in the midgut produces a higher initial
potential difference and sustains a higher PD than these other two types
of epithelia. The R_k did not increase as the breakpoint was passed
as in the other models. Instead, the resistance after the breakpoint
decreased which suggests that the active channels for this transport
open and that the resistance in both directions decreases.

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The differences between the leaf reared and diet reared larvae was definite. While the V_b was essentially the same in both types, the resistance of the tissue varied greatly. The diet reared larvae had a stable resistance of the tissue before breakpoint, R_b, (ie. the resistance of each clamp interval were approximately equal). In leaf reared larvae, R_b was irregular and ranged between 200 and 500 ohms. V_b was located in these experiments by noting when the resistance dropped to a low value and remained steady. This value, which the resistance decreased to was arbitrarily set at 120 ohms. The resistance after the breakpoint, R_k, averaged 70 ohms in all experiments conducted on leaf reared animals which numbered 20 experiments. R_b was much higher than that in diet reared larvae. The leaf reared larvae had a much helathier appearance than the diet reared larvae. The animal was a much darker green color and was much larger. The midgut, when removed from the animal was also much larger and, when looked at under a light microscope, had a much tighter cell structure than did the diet reared. The leaf reared produced higher potentials which were sustained longer. V_b was not significantly different from that of the diet reared larvae. This suggests that the differences noted between diet reared and leaf reared larvae was not due to the E_k of the pump but to the resistances R_l and R_a, increasing the efflux of potassium. The irregularity of R_b can then be explained as the differences in the two resistances of the equivalent circuit.

V_b was observed to be a function of concentration of potassium in the bathing solution. There was little difference between the breakpoint of 32 K and 48 K bathing solutions. This suggests that these concentrations are nearing the maximum concentration of potassium that the system can pump. Below 32 K, the V_b dropped in relation to the potassium

concentration until the potassium concentration reached 8 mM. At this point, the V_b of the system disappeared and R_b was equivalent to R_k throughout the V-I run. It was noted that the tissue never fully recovered from this concentration change or that in 16 K. The tissue is thought to become irreversibly damaged when placed in a bathing solution containing no potassium (Blankemeyer, personal communication). Potassium concentration therefore is essential for maintenance of the pump and is assumed to effect the E_k of the mechanism.

Inhibitors of the active transport mechanism also effected the pump. When nitrogen was used as a stirring gas to replace oxygen, the PD decreased to zero. If a V-I run was made before the PD reached zero, it was observed that the resistance increased but the V_b did not change. When the PD reached zero and a V-I run made, the breakpoint disappeared. With sodium azide, the breakpoint disappeared as soon as the azide had a chance to take effect. These two inhibitors would then seem to have two different methods of action. Nitrogen would effect only the resistances, R_l and R_a, increasing these until the PD dropped to zero. When the potential was negated, the E_k of the pump faltered. Sodium azide works on the electron transport chain on cytochrome a/a_3 so that formation of ATP is decreased. The active transport mechanism in the midgut is an ATP dependent process. In this way, sodium azide effects the E_k immediately.

CHAPTER VII

CONCLUSIONS

The midgut of the Lepidopteran larvae, Manduca sexta, demonstrated

an EMF of the active potassium transport mechanism the same way as shown

in the toad bladder frog skin and other sodium transporting epithelia.

In normal bathing solution, the EMF or E_k of the transport was equal to

242 mV in diet reared larvae. The resistance of the tissue before the

breakpoint, R_b, was 147 ohms while after breakpoint R_k was equal to 90

ohms. V_b of the midgut tissue is higher than that of the sodium trans-

porting tissues studied but the initial potential difference of the

midgut and the sustained potential is higher than the sodium transporting

tissues also.

The V_b of the pump was essentially the same for leaf reared larvae

and diet reared larvae. However the R_b was much higher in leaf reared

larvae. This was attributed to the healthier aspects of the leaf reared

animals. Since R_b is effected by the diet, it is likely that R_1 and R_a

of the equivalent circuit and not the E_k of the pump is effected.

Concentration of potassium was found to have an effect on V_b. As

the concentration decreased, so did the breakpoint voltage. 16 K

bathing solution produced a V_b of 57 mV. However, an increase in potas-

sium concentration did not significantly change V_b, suggesting that 32 K

is slightly lower than the maximum concentration which would effect the

pump. If the potassium concentration was 16 K or below, the pump was

impaired so that full recovery was never achieved. R_b was also concentration dependent with the resistance increasing with lower potassium concentrations. R_k was not dependent on potassium concentration, reaching a value of approximately 90 ohms at each concentration change.

Nitrogen and sodium azide were two inhibitors used on the tissue to note their effects on V_b and R_b. Nitrogen was found to cause V_b to disappear only when the PD reached zero. When a V-I run was made prior to zero, V_b did not change. However, when the PD reached zero and the resistance of the tissue was at its greatest, V_b disappeared. Sodium azide caused the disappearance of V_b immediately after addition. Sodium azide is presumed to have a direct effect on the E_k of the active transport mechanism by decreasing the amount of ATP available for use by the pump.

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