ACTIVE TRANSPORT OF SODIUM ACROSS THE ISOLATED, SHORT - CIRCUITED SKIN OF THE NEWT, NOTOPHTHALMUS VIRIDESCENS

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

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1968

ABSTRACT

ACTIVE TRANSPORT OF SODIUM ACROSS THE ISOLATED, SHORT-CIRCUITED SKIN OF THE NEWT, NOTOPHTHALMUS VIRIDESCENS

By

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The electrical properties of the frog and toad skin are known to result from an active Na^+ transport system which causes a preferrential movement of Na^+ through the skin. In this study the electrical properties of a urodele, Notophthalmus viridescens, are shown to be similar to the frog and toad skin.

The isolated skin was placed in an Ussing-type chamber and the potential difference and the short-circuit current were alternately monitored. After the potentials and currents were equilibrated, either ^{22}Na or ^{36}Cl was added to one side of the chamber. Samples were taken from the "hot" side at one and ten minutes and hourly for at least five hours from the opposite side.

This skin exhibits a transmembrane potential with the inside surface electrically positive to the outside surface. Active transport of Na^+ is demonstrated by the isolated skin under short-circuit, aerobic conditions since the net flux of Na^+ inward equals the recorded short-circuit current and is dependent on metabolic energy.

Under these same conditions Cl^- diffuses passively through the skin; there is no statistically significant net flux of Cl^-.

The short-circuit current is increased by either antidiuretic hormone or adrenalin when applied to the inside surface. It is decreased by potassium iodoacetate, dinitrophenol, malonate or ouabain.

The short-circuit current of the skin is dependent on Na^+, but not K^+, in the outside bathing solution and on K^+, but not Na^+, in the inside bathing solution. This current shows a temperature dependency and seasonal variation. The current and the transmembrane potential are highest in the late fall and winter and lowest in the summer.

Hypophysectomy of the adult reduces the transmembrane potential, suggesting that changes have occurred in the ionic fluxes across the skin. The possibility that one or more hypophyseal hormones may be involved in the maintainance of the electrical properties of the skin is discussed.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

1968

ACKNOWLEDGMENTS

The author expresses his appreciation to both Dr. William L. Frantz, for his guidance and encouragement throughout all phases of this study, and to Dr. Charles S. Thornton, who generously supplied animals for this investigation.

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INTRODUCTION

Because cellular stability and function depend on a dynamic balance between the external and internal environments, the movement of materials across the cell membrane is an important factor in cellular chemistry. The chemical reactions in the cell are dependent on a constant yet controlled supply of substances from the external environment. A membrane completely permeable to all materials would not allow ragulation of substances entering or leaving the cell. The degree of permeability of a substance and the method of transfer across the membrane are of major importance. One such method is active transport; that is, the movement of a substance against an electro-chemical gradient by the expenditure of energy.

In all cells the ions of the inter- and intra-cellular fluids are associated with the existing transmembrane potential. A change in the ion distribution would result in a change in the potential difference and a detectable bio-electric current, so that with suitable electronic instrumentation movement of ions might be detected. Ussing and Zerahn (1951) showed that this was possible when they were able to correlate the net flux of Na^+ with the detectable current in the isolated, short-circuited skin of the frog.

Their experiment proved that the net flux of Na^+ inward was due to an active transport system because there were no chemical or electrical gradients across the skin to influence the particle motion.

The source of the transmembrane potential and the exact mechanism of the movement of Na^+ and Cl^- across frog and toad skin are well studied. Yet little work has been reported on the skin of urodeles. Using an *in vitro* experiment similar to that of Ussing and Zerahn (1951), the aims of this study were to determine:

1. If the movement of Na^+ and Cl^- across the skin of *Notophthalmus viridescens* is due to an active transport system, by determining if the net flux of Na^+ and/or Cl^- is equal to the short-circuit current;
2. If the transmembrane potential is due to the resulting asymmetric distribution of ions.

REVIEW OF THE LITERATURE

In Table 1 the principal contributions of various investigators on active transport of Na^+ in the frog skin are condensed in a temporal order. Not all of the authors cited in this literature review are incorporated in this table.

That the frog skin has electrical properties has been know since 1848, when Dubois-Reymond reported that the frog skin maintains a potential difference across itself. In 1886 Bayliss and Bradford confirmed the observation that the frog skin is an electrically polarized tissue. Reid (1892) demonstrated a net transfer of Na^+ (and water) inward, even when the frog skin was bathed on both sides with identical Ringer's solutions. Using heavy water as a tracer, Hevesy, Hofer, and Krough (1935) showed that the influx and efflux of water across the skin were equal and that there is no reason to assume any active transport of water. An active transport of Na^+ inward across the isolated, surviving frog skin was reported by Huf (1935).

Francis (1933) was the first to show that a current could be drawn from the skin by connecting the two bathing solutions. This short circuiting technique was improved by Lund and Stapp (1947), who used electrodes of low resistance to bring about an almost completely short-circuited skin.

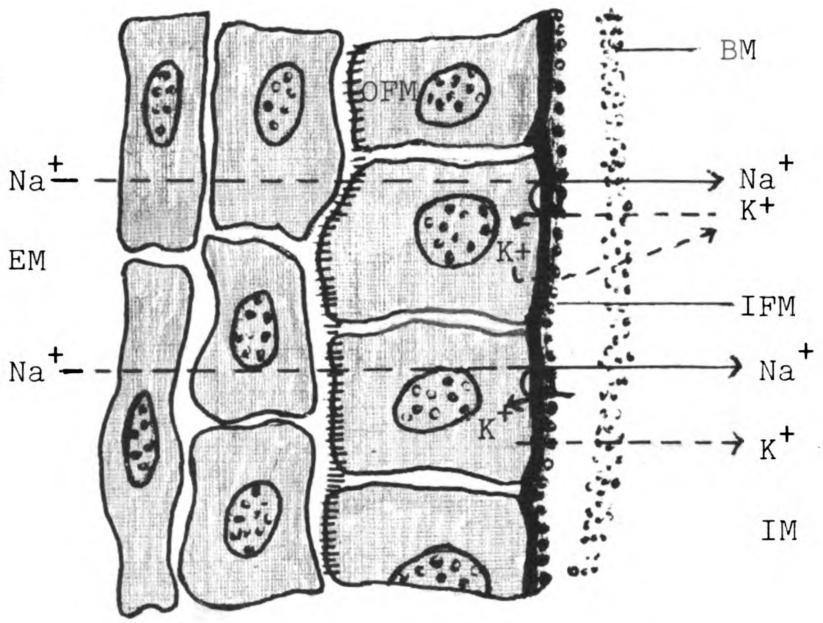
TABLE 1.--A summary of the principal contributions of various investigators' works on active sodium transport in the frog skin. The list is given in temporal order. Additional comments and authors are given in the text.

Investigator	Year	Principal Contribution
Dubois-Reymond	1848	Frog skin has transmembrane potential
Reid	1892	Net transfer of Na^+ and water inward
Galeotti	1904	Na^+ is necessary in external medium to maintain potential Na^+ can be effectively replaced by only Li^+
Hevesy et al.	1935	No active water transport system is present
Huf	1935	Active transport of Na^+ inward
Francis & Gatty	1938	Cyanide abolishes net Na^+ flux, metabolic dependency
Fukuda	1942	K^+ is necessary in internal medium to maintain potential
Ussing & Zerahn	1951	Net flux of Na^+ inward equals recorded short-circuit current, only Na^+ is actively transported across skin
Huf & Wills	1951	K^+ is needed in inside medium to maintain active Na^+ transport
Koefoed-Johnsen et al.	1953	Adrenalin increases Na^+ influx, efflux, and stimulates active transport of Na^+ and causes active Cl^- transport
Kirschner	1953	Cholinesterase inhibitors depress active Na^+ transport
Koefoed-Johnsen and Ussing	1953	Neurohypophyseal hormones stimulate active Na^+ transport
Schoffeniels	1955	Dinitrophenol inhibits net flux of Na^+
Zerahn	1955	Active transport of Li^+ inward, Li^+ competes with Na^+ Na^+ needed in external fluid to maintain active Na^+ transport
Maetz et al.	1958	Aldosterone stimulates active Na^+ transport
Koefoed-Johnsen and Ussing	1958	Proposed a model for active Na^+ transport in frog skin
Curran & Gill	1962	Increasing Ca^{++} in the outside solution inhibits active Na^+ transport
Green & Matty	1963	Thyroxin stimulates active Na^+ transport
Curran & Cereijido	1965	There is no stoichiometric correlation between K^+ influx and active Na^+ transport, no 1:1 coupled Na:K pump
Ussing	1965	Hypotonic conditions stimulate active Na^+ transport Hypertonic conditions inhibit active Na^+ transport
Curran & Cereijido	1965	K^+ influx depends on Na^+ in inside solution
Farquhar & Palade	1966	Pump mechanism is located at all cell membranes facing extracellular spaces

Ussing's (1949) modification of the well known Nernst equation, which can be used to calculate the potential difference generated by asymmetrical distributions in solutions separated by a membrane, enabled him to demonstrate that the movement of Na^+ across the frog skin could not be explained in terms of passive diffusion while the movement of Cl^- and I^- could be explained as a passive diffusion. Ussing and Zerahn (1951) conclusively showed that Na^+ was the only ion actively transported through the isolated, short-circuited skin of the frog and that the net flux of Na^+ equalled the recorded short-circuit current within the accuracy of the measurements.

Models and Microelectrode Studies on the Locus of the Active Transport Mechanism and the Transmembrane Potential

Several theories have been advanced to explain the active Na^+ transport system of frog skin. Koefoed-Johnsen and Ussing (1958) proposed a model for frog skin, which was widely accepted until 1966 (see Figure 1). To explain the observed polarization of the epithelial cells, they postulated two parallel membranes at the apical and basal surfaces of the stratum germinativum. The "outward facing membrane" is highly permeable to Na^+ and Cl^-, but only slightly permeable to K^+ and $SO_4^=$. The "inward facing membrane" is highly permeable to K^+ and Cl^- but impermeable to free or uncoupled N.$^+$ However it is the site of a coupled Na^+ - K^+ active transport pump which maintains a low



"outward facing membrane" - permeable to Na^+
"inward facing membrane" - permeable to K^+
locus of active Na^+ transport system

Figure 1.--The Koefoed-Johnsen-Ussing model of the frog skin.

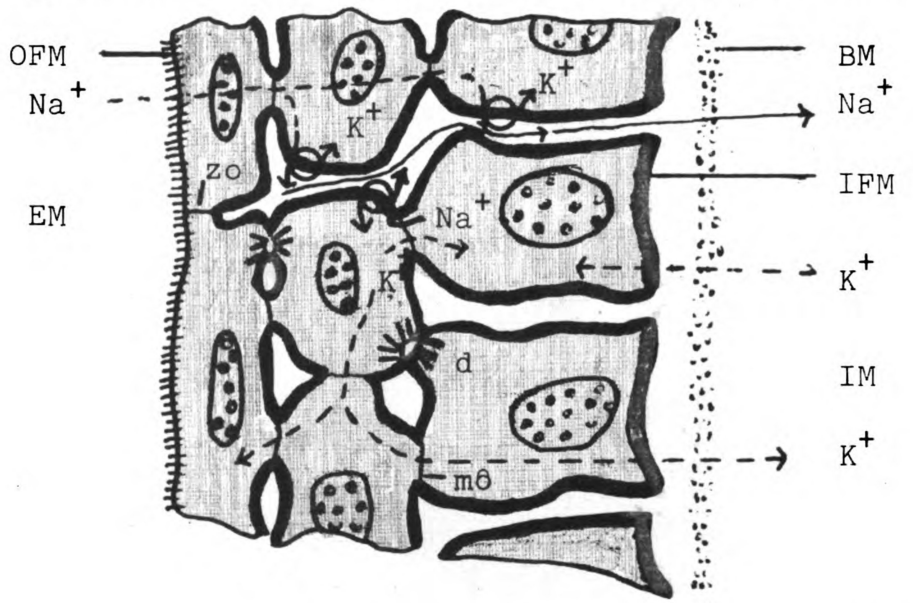


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Figure 2.--The Farquhar-Palade model of amphibian skin.

Figure 1. The Koefoed-Johnsen-Ussing model of the frog skin. The details of the model are explained in the text.

Figure 2. The Farquhar-Palade model of amphibian skin. The details of the model are explained in the text.

Both figures are adopted from Farquhar and Palade (1966). In both figures the three cell layers represent schematically from left to right; the stratum corneum, the s. spinosum, and the s. germinativum. The nuclei are stippled. The intracellular spaces are in gray and the intercellular spaces are in white. The following abbreviations are used in both figures:

BM - basement membrane
EM - external medium
IM - internal medium
IFM - inward facing membrane
OFM - outward facing membrane
d - desmosome
mo - macula occludens
zo - zonula occludens

Na^+ and a high K^+ intracellular concentration. The movement of Na^+ across the apical border, the movement of Cl^- across both borders and the movement of K^+ out of the cell across the inner "membrane" are passive.

After studying the localization of ATPase in the frog skin, Farquhar and Palade (1966) proposed a modified model for frog and toad skin (see Figure 2). This is the most accepted model. They proposed that the Na^+ pump mechanism is located at all cell membranes facing the intercellular spaces. The "outward facing membrane" is the apical border of the stratum corneum. The "inward facing membrane" is the basal border of the stratum germinativum. The permeability and movement of Na^+, K^+, Cl^- and $SO_4^=$ across the inner and outer "membranes" is identical to Koefoed-Johnsen and Ussing's model (1958). Na^+ and K^+ diffuse from cell to cell across the zonula occludens, the macula occludens and the desmosomes.

Several microelectrode studies were done on frog skin to determine the origin or site of the potential difference. Two electrical potential steps were recorded in the studies of Engbaek and Hoshiko (1957), and Scheer and Mumbach (1960). The presence of two steps would be in agreement with the Koefoed-Johnsen-Ussing model (1958). The former group stated that the two steps were at the inner and the outer surfaces of the stratum germinativum while the latter group concluded that the two sites were the stratum germinativum and the tela subcutanea of the epidermis.

One electrical potential step was recorded by Ottosen et al. (1953) and Chowdhury and Snell (1965). The former group interpreted the site of the step as the basement membrane. The latter group found that the potential difference changes did not occur in discrete jumps but occurred in a continuous manner as the microelectrode passed through the cells. They concluded that the pump systems were probably not confined to two cellular "membranes" but were more evenly distributed throughout the epidermis. The technical difficulties of microelectrode studies and the existance of extensive extracellular compartments within the epithelium are reasons for the disagreement of these studies.

Removal of the tela subcutanea reduces the potential difference, increases the Na^+ influx and efflux, and abolishes the net flux of Na^+ inward, suggesting the tela subcutanea as the site of the active transport system (Fleming, 1958). Since cholinesterase inhibitors reduce the active Na^+ transport (Kirschner, 1953), Koblich (1958) suggested the tela subcutanea as a site of the pump, since 90% of the frog skin cholinesterase was localized in the tela. In 1959 Koblich proposed an ion exchange model involving cholinesterase. Franz and VanBruggen (1964) argued that the tela subcutanea was not the locus of the pump process, since the short-circuit current exists after the removal of the tela subcutanea. They also attributed the drop in the transmembrane potential after removal of the tela to the large increase in the Cl^- flux.

Metabolic Involvement

Even though the exact forces involved in the transport of ions is not known, physiochemical considerations point to the fact that in many cases chemical reactions must be involved between the cell constituents and the transported ions. This was clearly expressed by Rosenberg (1948), who gave a theoretical treatment of active transport on a thermodynamic basis. Francis and Gatty (1938) were perhaps the first to suggest that the movement of Na^+ through the frog skin was dependent on metabolism, since cyanide abolished the Na^+ net flux. Ussing (1949) reported that cyanide reduced the Na^+ influx but not the Na^+ efflux. Dinitrophenol was found to reduce the frog skin potential and prevent the net flux of Na^+ by Schoffeniels (1955). Cardiac glycosides were found to inhibit the Na^+ transport by Koefoed-Johnsen (1957). In 1953 Schatzman reported the cardiac glycoside, ouabain, to be a general ATPase inhibitor. Skou (1964) presented evidence that a principal component of the Na^+ pump was a Na^+-K^+-Mg^{++}-activated ATPase and that ATP is the proximate energy donor for active transport. Digitalis was reported to inhibit the "pump" or membrane ATPase almost specifically (Lee and Yu, 1963). Maffly and Edelman (1963) suggested that the energy for the transport is provided by specific metabolic pathways spatially linked to the transport mechanism instead of by a general cellular metabolic pool. It is widely agreed that the metabolic

inhibitors of active transport probably do not act on the transport mechanism directly but interfere with the energy supply.

Effects of Environmental Changes

The electrical properties and the active Na^+ transport system of frog skin undergo changes when certain environmental conditions are altered. Na^+ is necessary in the external bathing solution to maintain the transmembrane potential (Galeotti, 1904) and the active transport of Na^+ (Zerahn, 1955). K^+ is necessary in the internal bathing medium to maintain the potential difference (Fukuda, 1942) and active Na^+ transport (Huf and Wills, 1951). Curran and Cereijido (1965) found that the K^+ influx is dependent on Na^+ in the inside bathing solution, but there is no stoichiometric correlation between K^+ influx and active Na^+ transport, suggesting that there is no 1:1 Na:K coupled pump mechanism in the frog skin.

The effects of pH of the solutions bathing the skin were studied by Ussing (1949) and Schoeffeniels (1955). They found that the active Na^+ transport is more dependent on the pH of the internal solution than the pH of the external one. A pH of about 8 causes a high Na^+ influx, while a pH below 8 in the internal solution (below 6 in the external medium) reduces the active Na^+ transport and increases the passive fluxes of Na^+ and Cl^-.

The effects of temperature on these systems were studied by Snell and Leeman (1957), who found that the Q_{10} for the short-circuit current in the frog skin was about 2 and that the net flux of Na^+ varied 9 to 10% per degree centrigrade (Q_{10} of 2.1 to 2.3).

Increasing the Ca^{++} concentration in the external bathing medium depresses the passive permeability of Na^+ and Cl^-, while increasing it in the internal solution increases the active transport of Na^+ (Curran and Gill, 1962). The Ca^{++} reduces the Na^+ pool size and the short-circuit current (Curran et al., 1963). There is no evidence for a direct effect of Ca^{++} on the active Na^+ transport mechanism (Herrera and Curran, 1963).

Small concentrations of Cu^{++} in the outside medium have little effect on the frog skin Na^+ influx, efflux and short-circuit current; but gradually induce large increases in the potential difference (Ussing and Zerahn, 1941). The Cu^{++} diminishes the permeability of Cl^- (Koefoed-Johnsen and Ussing, 1960).

Osmotic changes profoundly affect the amphibian skin. Ussing (1965) reported that hypotonic solutions in contact with either surface of the skin cause the epidermis to swell and increase active Na^+ transport, whereas hypertonic solutions in contact only with the inside surface cause the epidermis to shrink and inhibit the active Na^+ transport.

Hormonal Influence on the Active Sodium Transport in Amphibian Skin

The following hormones have been reported to stimulate the active Na^+ transport system in amphibian skin: adrenalin (Koefoed-Johnsen et al.; 1952, frog); aldosterone (Maetz et al., 1958, frog), (Alvarado and Kirschner, 1964, larval salamander, Ambystoma); neurohypophyseal hormones (Koefoed-Johnsen and Ussing, 1953, frog), (Bentley and Heller, 1962, terrestrial newts, Notophthalmus); and thyroxin (Green and Matty, 1963, toad). Although the mechanism of adrenalin action is not known, adrenalin is known to increase the active transport of Na^+ inward and to cause an active transport of Cl^- outward in the short-circuited skin (Koefoed-Johnsen et al., 1952). Four explanations of the possible mechanism of aldosterone exist in the literature. Aldosterone may lower the resistance to Na^+ transport so that the energy available for the process is more effectively used (McAfee and Locke, 1961); it may increase the passive diffusion of Na^+ through the "external facing membrane" (Crabbe, 1963); it may stimulate the Na^+ pump independently of the Na^+ permeability increase (Fanestil et al., 1967); or it may stimulate the synthesis of the enzymes involved in the active transport process (Edelman et al., 1963). In support of the last possible mechanism is the fact that puromycin blocks the aldosterone stimulation of active Na^+ transport (Crabbe and DeWeer, 1964).

It is widely agreed on that antidiuretic hormone (ADH) increases the permeability of Na^+ of the external facing membrane such that more Na^+ is available for the pump mechanism. Several explanations have been given for this effect. Schwartz et al. (1960) suggest that the action of ADH is mediated via disulfide interchange reactions between hormonal disulfide and a membrane receptor sulfhydryl which alter the permeability of the outer membrane. Indeed sulfhydryl blocking agents interfere with the stimulatory action of ADH (Rasmussen et al., 1960). Whittembury (1962) found that ADH widens the pore radii of the outside surface of toad skin from 4.5 to 6.5 Å, suggesting that the permeability of the outer barrier is increased. Curran et al. (1963) found that ADH increases the Na^+ permeability at the outward facing membrane and thus affects the Na^+ pool size but does not affect the active transport system directly. Orloff and Handler (1962) proposed that, in the toad bladder at least, cyclic 3'-5' adenosine monophosphate (AMP) is the agent which alters the permeability of the outer barrier and that the action of ADH is mediated via increased production of the cyclic AMP. Evidence in favor of this was given by Schultz and Zalunsky (1963), who found that ADH increased the activity of phosphorylase and stimulated the production of the cyclic AMP.

Contrary to Green and Matty (1963), Taylor and Barker (1967) were unable to show a stimulatory effect in

vitro with thyroxin. Yet they suggested that thyroxin may be responsible in part for establishing the active transport mechanism since in tadpoles the active Na^+ transport process becomes demonstrable only immediately before metamorphosis from the aquatic to the terrestrial habitat; a process which involves thyroxin (Etkins, 1964). The thyroid hormone may be of evolutionary significance in amphibians, since it possibly conditioned the assumption of terrestrial modes of life.

Seasonal Differences in the Transmembrane Potentials of Frog Skins

The time of the year is a factor in the transmembrane potential and the ionic fluxes in frog skin. Franz and VanBruggen (1964) reported that summer frogs have lower skin potentials and higher passive ionic fluxes than in the other seasons. Ussing and Zerahn (1951) noticed that autumn forgs showed much lower outfluxes than summer fluxes. Myers, Bishop, and Scheer (1961) observed higher ionic fluxes in both directions across the skin and lower transmembrane potentials in summer frogs than in winter or spring frogs.

Adenohypophysectomy Effects on the Frog Skin

Bishop, Mumbach, and Scheer (1961) have shown that removal of the adenohypophysis of the frog is followed by both a decrease in the resting membrane potential and the

short-circuit current and a long lasting increase in the outflux of Na^+ through the skin. These changes are opposed by the administration of mammalian ACTH or aldosterone. The frog seems to survive satisfactorily without the adenohypophysis even without the administration of ACTH or aldosterone. The increased permeability of the skin to Na^+ appears to be associated with a decrease in the amount of a mucopolysaccharide in the dermis (Bishop et al., 1961).

Research on the Salamander Skin

Few reports have appeared in the literature on the active transport systems in urodeles. Koefoed-Johnsen and Ussing (1949) reported that ACTH increased the net Na^+ uptake in intact axolotls, Ambystoma. Alvarado and Kirschner (1964) showed that aldosterone increased the Na^+ influx in larval Ambystoma but had no effect on the Na^+ efflux. The pressor fraction of the posterior pituitary induces a net Na^+ uptake in larval Ambystoma (Jorgensen, 1946). The neurohypophyseal hormones decrease the net loss of Na^+ and promote Na^+ uptake in terrestrial newts, Notophthalmus, when they are placed in water (Bently and Heller, 1962). They also reported that the short-circuit current in the isolated skin is stimulated by these hormones. Although they did not do flux experiments with radioisotopes to determine the source of the short-circuit current, they assumed that the short-circuit current was a measure of the active transport of Na^+, citing Ussing and

Zerahn's work (1951) as the reason for their assumption. In 1964 they reported values of 22±5.4 mV and 25 to 45 μamps/cm^2 for N. cristata. These are mostly terrestrial forms. The transmembrane potential in the aquatic forms is much smaller than that in the terrestrial forms. This difference even extends to larval and metamorphosed axolotls. The neurohypophyseal hormones do not influence the short-circuit current of the axolotl (Bentley and Heller, 1964).

MATERIALS AND METHODS

Experimental Animals

Adult, aquatic spotted salamanders, Notophthalmus viridescens, were obtained from a commercial supplier in Petersham, Massachusetts. They were kept in five gallon aquaria, which contained aged, aerated, recirculated, and filtered tap water (pH, 7.0-7.2; temperature, 21-23°C). The degree of hardness of the water was not measured or controlled. Continuous light was supplied from an overhead source, although the aquaria were shaded with floating duckweed, Spirodela polyrhiza, and ditch moss, Anacharis canadensis. No gravel was used in the aquaria. The animals were fed daily either rinsed, newly hatched brine shrimp, Artema salina, or frozen calf liver scrapings. Uneaten portions were removed to avoid contamination of the water with bacteria or decomposed matter. The animals were kept for at least two weeks before experimental use.

The animals were killed by decapitation. With the aid of a dissecting microscope, a slit was made along the length of the spinal cord into the body cavity. The internal organs were removed and the skin was placed in a glass dish containing an amphibian Ringer's solution for the cleaning procedure. The connective tissues and the

musculature were gently peeled from the inner surface with care being taken to avoid touching the area to be bathed in the chamber. The skin (area of bathed section, 0.785 cm^2) was placed in an interlocking ring system, which was placed between the halves of a lucite bathing chamber (see Figure 1) for electrical and ionic flux measurements. Four ml. of amphibian Ringer's (pH, 7.4±0.1; temperature, 22±2°C) solution were placed in each side of the chamber. The block system was placed in a box fitted with grounded copper wire screening. The entire operation was performed in ten to fifteen minutes.

Solutions

The composition of the amphibian Ringer's solution used in these studies was:

Na^+	123.6 meq./l	$SO_4^=$	1.7 meq./l
K^+	3.8	$H_2CO_3^-$	2.4
Ca^{++}	1.6	Cl^-	116.6
Mg^{++}	1.7	$H_2PO_4^-$	10.0

The Ringer's low in potassium contained the ions listed above except that sodium was substituted for potassium. The Ringer's low in sodium contained the following ions:

Na^+	12.4 meq./l	$SO_4^=$	1.7 meq./l
K^+	3.8	$H_2CO_3^-$	2.4
Ca^{++}	1.6	Cl^-	116.6

Mg^{++}	1.7	$H_2PO_4^-$	10.0
Choline	111.2		

All solutions also contained 11.1 meq./l glucose, were buffered with 12.4 meq./l tris (hydroxymethyl aminomethane) at a pH of 7.4±0.1 and had an osmolarity of 241-246 milliosmoles as measured on an Osmometer (Perfection Instrument Co., Newton Heights, Massachusetts). The levels of Na^+ and K^+ were measured on a Beckman DU flame photometer (Beckman Instruments Inc.; Fullerton, California). The level of Cl^- was measured on a Chloriometer (Buchler Instruments; Fort Lee, New Jersey). The levels of all other ions were calculated.

Apparatus

A lucite chamber similar in principle to that used by Ussing and Zerahn (1951) was used in this study (Figure 3). Pressurized air was passed through a filter-flow equalizer (Koby Corporation; Melrose, Massachusetts) and three distilled water filled wash bottles to saturate it with water vapor before it entered the bathing chambers. In the chambers the air served as an air lift siphon for mixing the solutions and as a source of oxygen. The mixing also aided in the removal of carbon dioxide. Lucite caps were used to prevent splashing and excessive evaporation of fluid.

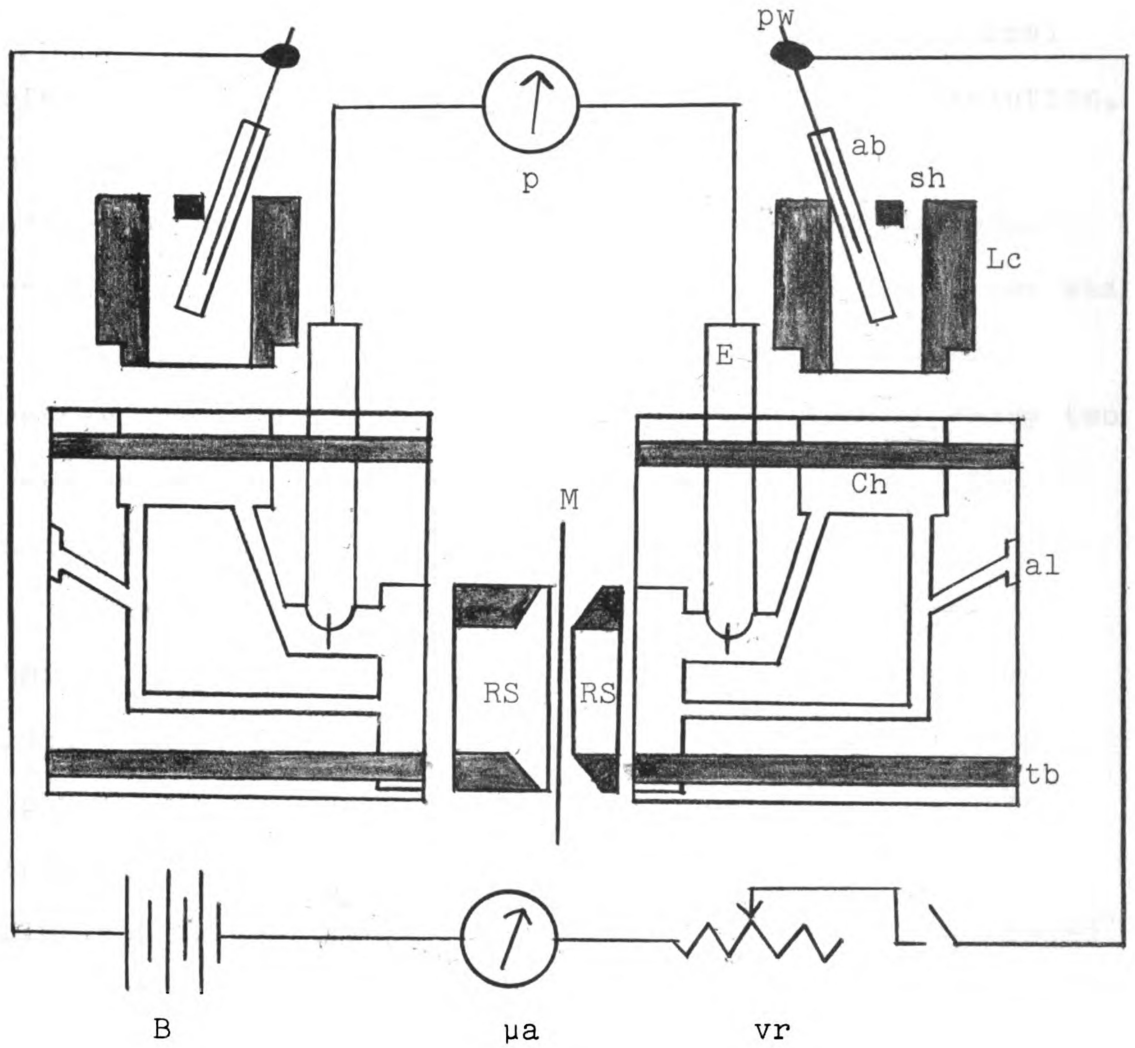

Figure 3.--Diagram of the perfusion blocks and the electrical circuits

ab	-	agar bridge	μa	-	microammeter (0 - 100 μamps)
al	-	air line	p	-	potentiometer (0 - 100 mV)
B	-	battery (4.5 v)	pw	-	platinum wire
Ch	-	chamber	RS	-	ring system
E	-	electrode	sh	-	sampling hole
Lc	-	lucite cap	tb	-	tube for bolt
M	-	membrane	vr	-	variable resistor (0 - 11 x 10^6 ohms)

Transmembrane potentials were recorded via calomel reference electrodes, filled with a saturated KCl solution, on a potentiometer (Model AW, Esterline-Angus Co.; Indianapolis, Indiana). Pairs of electrodes were equilabrated for several days before use. An electrode set was not used if the potential difference between them was greater than 1.0 mV. The electrodes were checked every two weeks in a NaI crystal gamma scintillation counter for radioactive contamination of the KCl solution.

The skin was short-circuited with an instrument similar in principle to that used by Ussing and Zerahn (1951) (see Figure 3). The skin was short-circuited by applying a counter emf from three 1.5 volt batteries in series through a variable resistor. Contact with the solutions in the chambers was made through platinum coated copper wires inserted in a Ringer-agar bridge (5 g. agar/ 100 ml. Ringer's). By varying the external resistor (VR, Figure 3), the skin potential was nulled; that is, both sides of the skin were at the same potential. The current flowing in the external circuit when the transmembrane potential was nulled was read from a microammeter (Model 420; Triplet Electrical Instruments Co.; Bluffton, Ohio) and was called the short-circuit current. Manual adjustment of the external resistor was made whenever necessary to keep the transmembrane potential nulled. The skin was constantly short-circuited, except when the potential difference was checked for 1--20 seconds every fifteen minutes.

Sampling

The skin was considered equilibrated if there were no changes greater than 10% within 30 minutes in the potential or the current. Using micro-pipettes (Drummond Scientific Co.; Broomall, Pennsylvania) 100 µl of radioactive solution containing either 0.166 µc of 0.02 N ^{22}NaCl (Abbott Laboratories; Chicago, Illinois) or 0.185 µc of 0.44 N H^{36}Cl (Nuclear Science Engineering Corp.; Pittsburgh, Pennsylvania) were added to one side of the chamber; each side contained 4 ml. of Ringer's. A twenty µl sample was taken from that same side at one and ten minutes. Since these samples counted identically, the distribution was considered complete and rapid. Twenty µl samples were taken hourly from the opposite side for at least five hours and were placed in glass counting vials (Packard Instruments Co., Inc.; Downers Grove, Illinois) containing a scintillation solution for determination of the radioactive content.

Each counting vial contained 15 ml. of a scintillation solution, which contained a primary scintillator (5 g. PPO or 2.5 - Diphenyloxazole), a secondary scintillator (50 mg. 2- (-1- naphthyl) -5- phenyloxazole), 80g. naphthalene and enough dioxane to make one liter of solution. The samples were assayed in a Nuclear Chicago Mark I Liquid Scintillation Spectrometer (Model 6860). The counting efficiency was 72% for ^{22}Na and 87% for ^{36}Cl. The counting error was not greater than 5%. Each sample was corrected

for background activity and quenching by using a ^{133}Ba external standard.

Statistical Analyses

The results were reported as mean ± standard deviation. The number in parentheses after the standard deviation is the number of experimental animals. The agreement between the short-circuit current and the net flux of an ion was analyzed with a linear regression analysis of variance and a correlation coefficient for the data was determined. Analyses of differences between groups were made with an appropriate t-test, F-test, or an unweighted means analysis of variance.

RESULTS

Electrical Properties of the Newt Skin

The isolated skin of the adult salamander, Notophthalmus viridescens, generates a transmembrane potential, when it separates identical amphibian Ringer's solutions, such that the inner surface of the skin is electrically positive with respect to the outer surface. When the transmembrane potential is nulled, a short-circuit current (J_{sc}) can be recorded. In Table 2 average values for this skin are listed. An unweighted means analysis of variance was done to test the possibility that either the potential difference or the short-circuit current varies with the seasons and/or between sexes. The analyses of variance are given in Tables 3 and 4. Both the potential difference (p.d.) and the J_{sc} were found to vary significantly ($p = 0.99$) with the seasons but not between sexes. Higher values occurred in the late fall and the winter. There are no significant seasonal-sexual interactions.

Evidence for Active Transport of Sodium

Evidence for an active transport of an ion is best obtained from an experimental model using the principles of Ussing and Zerahn's experiments (1951). Such a set of experiments on the isolated skin of the adult newt was

TABLE 2.--Average transmembrane potentials and short-circuit currents of the salamander skin. The results are reported as mean ± standard deviations.

Season	Transmembrane Potential (mV)	Short-Circuit Current ($\mu amps/cm^2$)
Late fall (Nov.) Winter (Dec.-Feb.)	16.3 ± 7.4 (19)	17.4 ± 3.9 (8)
Spring (Mar.-May)	5.6 ± 2.8 (31)	10.1 ± 3.5 (31)
Summer (June-Aug.)	5.3 ± 2.7 (39)	8.5 ± 3.4 (39)

TABLE 3.--An unweighted means analysis of variance on seasonal and sexual differences in the potential differences.

Source	Degrees of Freedom	Sums of Squares	Mean Square	F-Ratio	Critical Value $F(0.01, df_n, 83)$
A season	2	945.4	472.7	21.6**	4.93
B sex	1	7.1	7.1	0.32	6.99
AB interaction	2	131.0	65.5	2.96	4.93
error	83	1815.2	21.8		

performed during the summer months. The aim of these studies was to equate the net movement of Na^+ and/or Cl^- with the J_{sc} measured under aerobic, short-circuited conditions. Since under these conditions there are no concentration, osmotic, or electrical gradients between the two solutions, any net fluxes of ions would be due to an

TABLE 4.--An unweighted means analysis of variance on seasonal and sexual differences in short-circuit currents.

Source	Degrees of Freedom	Sums of Squares	Mean Square	F-Ratio	Critical Value $F(0.01, df_n, 74)$
A season	2	265.75	132.87	8.32**	4.86
B sex	1	7.44	7.44	0.47	7.02
AB interaction	2	2.56	1.28	0.05	4.86
error	74	1181.59	15.97		

active transport process. The amount of current (the J_{sc}) used to maintain the transmembrane potential equal to zero can be calculated from the Faraday law of electrolysis:

$$\mu\text{eq. of electrons} = \frac{\text{current } (\mu\text{amps}) \text{ X time (sec.)}}{\text{Farad } (\mu\text{amps sec. / } \mu\text{eq. of } e^-)}$$

$$= \frac{15\ \mu\text{amps X } 3600 \text{ sec.}}{96500\ \mu\text{amps sex. / } \mu\text{eq of } e^-} \qquad (1)$$

$$= 0.56\ \mu\text{eq. of } e^-$$

The numbers used are not actual data. For the same amount of time the amount of an ion crossing the skin would be calculated as:

15,000 cpm of ion initially in solution A (not actual
0 cpm of ion initially in solution B data)
398 µeq of ion in both solution A and B
21 cpm of ion in solution B at time t

$$\text{µeq. of ion crossing skin} = \frac{\text{µeq of ion initially in solution A}}{\text{cpm ion initially in solution A}} X \text{ cpm ion in solution B at time t} \quad (2)$$

$$= \frac{398 \text{ µeq}}{15{,}000 \text{ cpm}} X \text{ 21 cpm}$$

$$= 0.56 \text{ µeq ion}$$

Three assumptions are made in these calculations:

1. The bathing media are homogeneously and instantaneously mixed after isotope dilution.
2. The addition of the isotopic solution does not significantly alter existing chemical, electrical, or osmotic activities.
3. The tissue reacts similarly to the radioactive ion as to the non-radioactive one.

The average values of the fluxes of Na^+ and Cl^- from nine sets of skins are given in Table 5. The Na^+ data skins had an average transmembrane potential of 4.8±1.3 mV and an average J_{sc} of 9.0±2.2 µamps; the Cl^- data skins had values of 4.8±0.8 mV and 8.8±1.2 µamps. Inspection of the table shows that the net fluxes of Na^+ are nearly equal to the J_{sc} whereas the net fluxes of Cl^- are not. These data are plotted against time in Figure 4. It is apparent that with time there is a high degree of correlation between the accumulated fluxes of Na^+ and the accumulated J_{sc}, but little correlation between the accumulated fluxes of Cl^- and the accumulated J_{sc}.

TABLE 5.--The accumulated short-circuit currents and accumulated fluxes of sodium and chloride. The values are averages of nine sets of skins under short-circuit conditions. For the Na^+ data skins the potentials were 4.8±1.3 mV and the currents were 9.0±2.2 μamps, for the Cl^- skins they were 4.8±0.8 mV and 8.8±1.2 μamps.

Time After Isotope Dilution (hrs.)	1	2	3	4	5
Na^+ influx (μeq Na^+/0.785 cm^2)	1.56	3.15	4.50	6.34	7.94
Na^+ efflux (μeq Na^+/0.785 cm^2)	1.23	2.39	2.47	5.02	6.30
Na^+ net flux (μeq Na^+/0.785 cm^2)	0.33	0.76	1.03	1.32	1.64
J_{sc} (μeq e^-/0.785 cm^2)	0.34	0.68	1.02	1.36	1.70
Cl^- influx (μeq Cl^-/0.785 cm^2)	3.60	6.91	10.41	14.11	17.85
Cl^- efflux (μeq Cl^-/0.785 cm^2)	3.58	6.87	10.31	13.98	17.76
Cl^- net flux(μeq Cl^-/0.785 cm^2)	0.02	0.04	0.10	0.13	0.08
J_{sc} (μeq e^-/0.785 cm^2)	0.33	0.66	0.99	1.32	1.65

A simple linear analysis of variance was done on these data. In Figure 5 the net fluxes of Na^+ and Cl^- are plotted against the J_{sc}. The solid line is the theoretical plot of 1.0 μeq. of ion moving across the skin for each 1.0 μeq. of electron flowing in the external circuit. The dotted lines are the best fit lines predicted from simple linear regression statistics. The regression line for Na^+ is: $y = 0.92 \pm 0.18\ x + 0.08 \pm 0.15$. The origin is not statistically significant from zero and the slope is not significant from one ($p = 0.99$). The correlation between

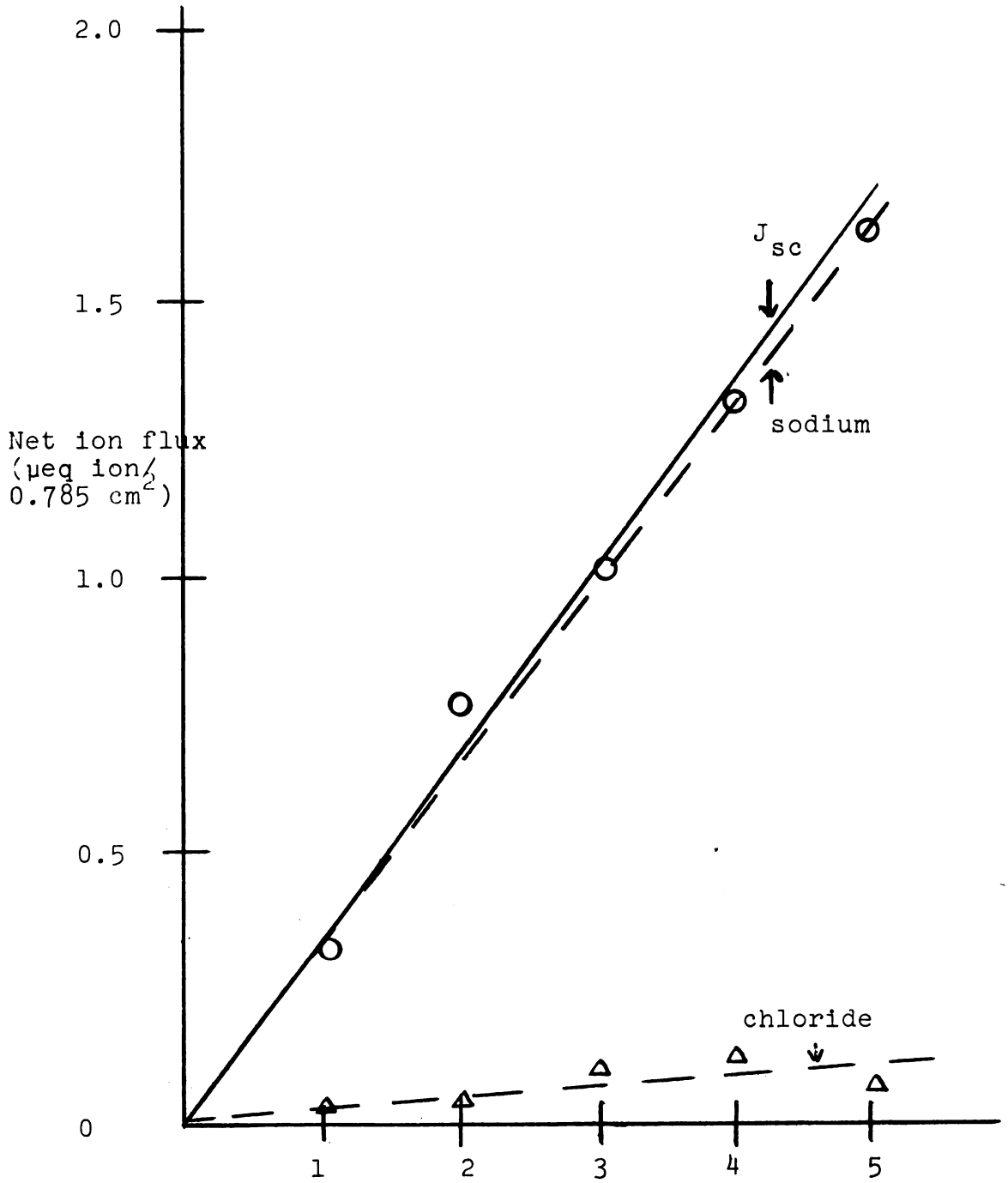

Fig. 4.--The accumulated short-circuit current and net fluxes of sodium and chloride against time. Each point is the average of nine membranes. The standard deviation for any point is not greater than ±0.21. Note the high degree of correlation between the net flux of Na^+ and the accumulated J_{sc}.

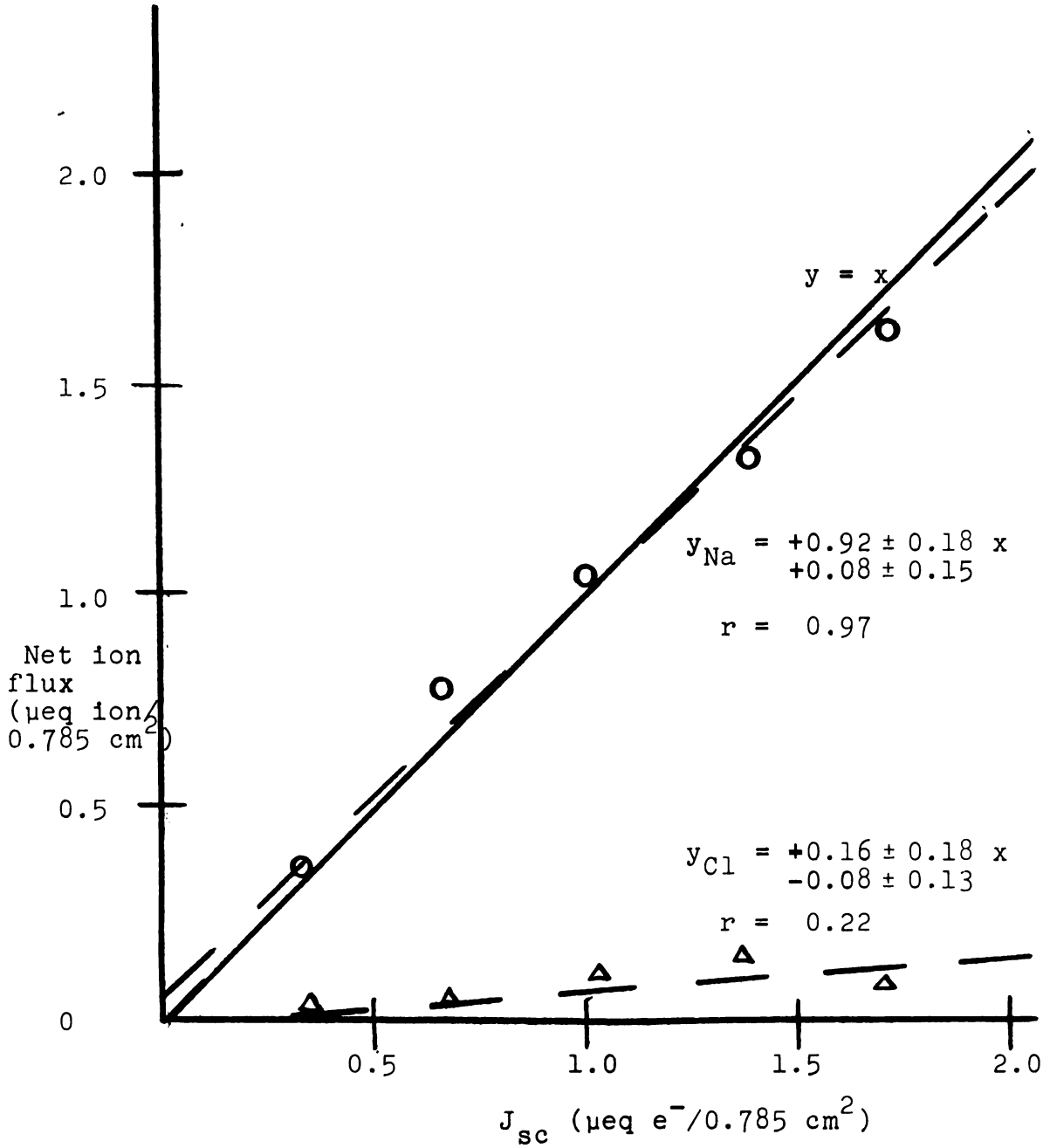

Fig. 5.--The net flux of sodium and chloride versus the short-circuit current. The solid line is the theoretical plot of 1.0 μeq of ion moving across the membrane for each 1.0 μeq of electron flowing in the external circuit. The dotted lines are the best fit lines predicted by simple linear regression statistics. The statistical significance of the slopes and the origins of the lines is discussed in the text. The standard deviation of any point is not greater than ±0.21. The r value is the degree of correlation between the net flux of an ion and the short-circuit current measured simultaneously.

the net flux of Na^+ and the J_{sc} is 0.97. The regression line for Cl^- is: $y = 0.16 \pm 0.18\ x\ -0.08 \pm 0.13$. Although the origin is not statistically significant from zero, the slope is significantly different from one ($p = 0.99$). The slope is not significantly different from zero. The correlation between the net flux of Cl^- and the J_{sc} is 0.22. Since the Na^+ net flux equals the J_{sc}, Na^+ is the only ion actively transported across the short-circuited skin.

Dependence of the Short-Circuit Current on Sodium and Potassium

When a Ringer's solution containing a low concentration of Na^+ is placed in the inside chamber, the J_{sc} increases slightly but not significantly (Figure 6). This slight increase in the J_{sc} could be due to the increased concentration gradient for Na^+. Under these conditions the efflux of Na^+ would be lowered. When a Ringer's solution with a low Na^+ concentration is placed on the outside of the skin, the J_{sc} falls significantly towards zero (Figure 6). The J_{sc} does not fall entirely to zero because a small amount of Na^+ still is present and the choline is capable of supporting a small J_{sc}. When normal Ringer's replaces the low Na^+ Ringer's, the J_{sc} returns to near normal.

In Figure 7 the effects of low K^+ Ringer's are shown. A low K^+ Ringer's solution on the inside of the skin causes a reduction in the J_{sc} with time. When this solution is replaced by normal Ringer's, the J_{sc} returns to the

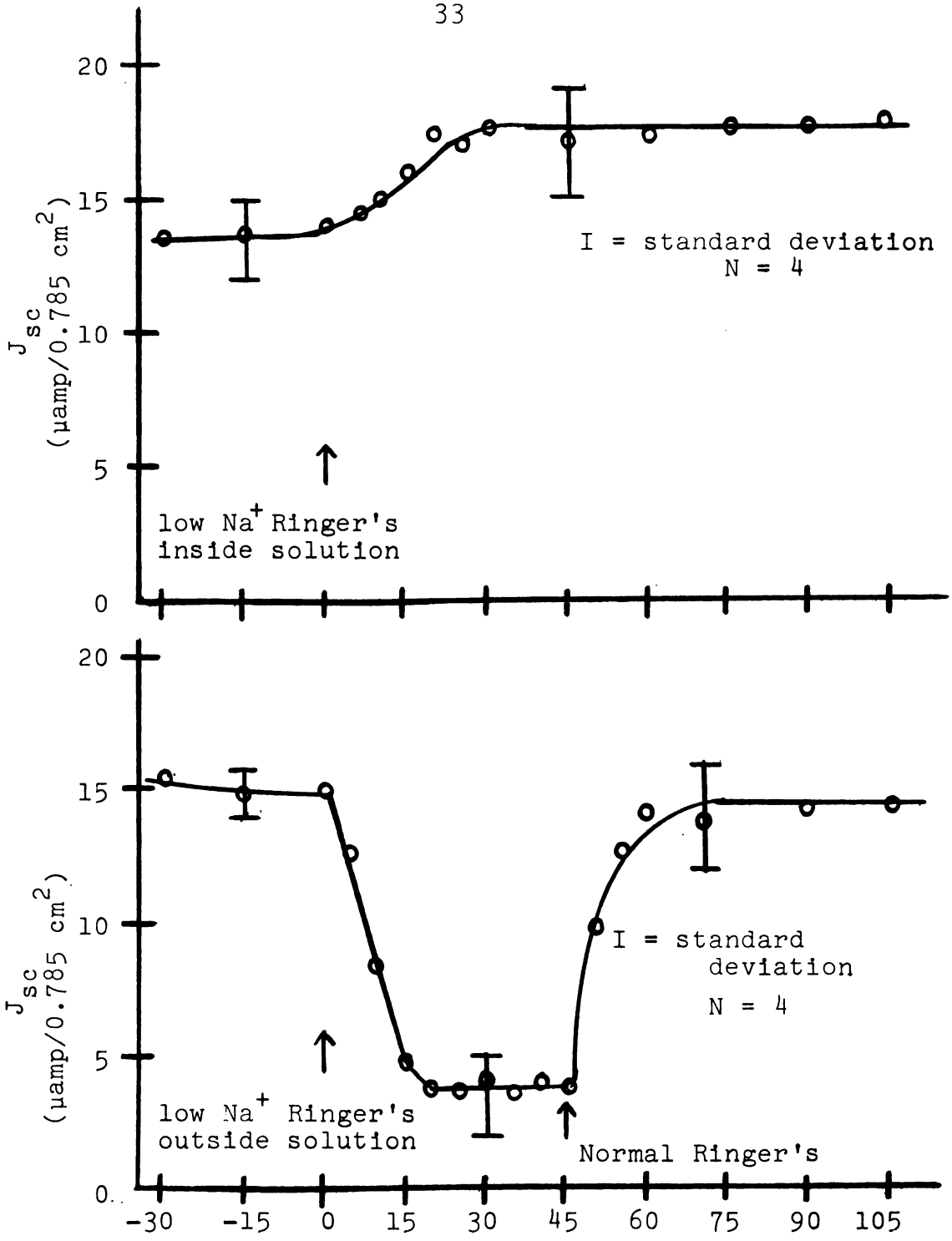

Fig. 6.--The effects of low sodium Ringer's solutions on the short-circuit current of the salamander skin. Choline chloride was used to replace the sodium.

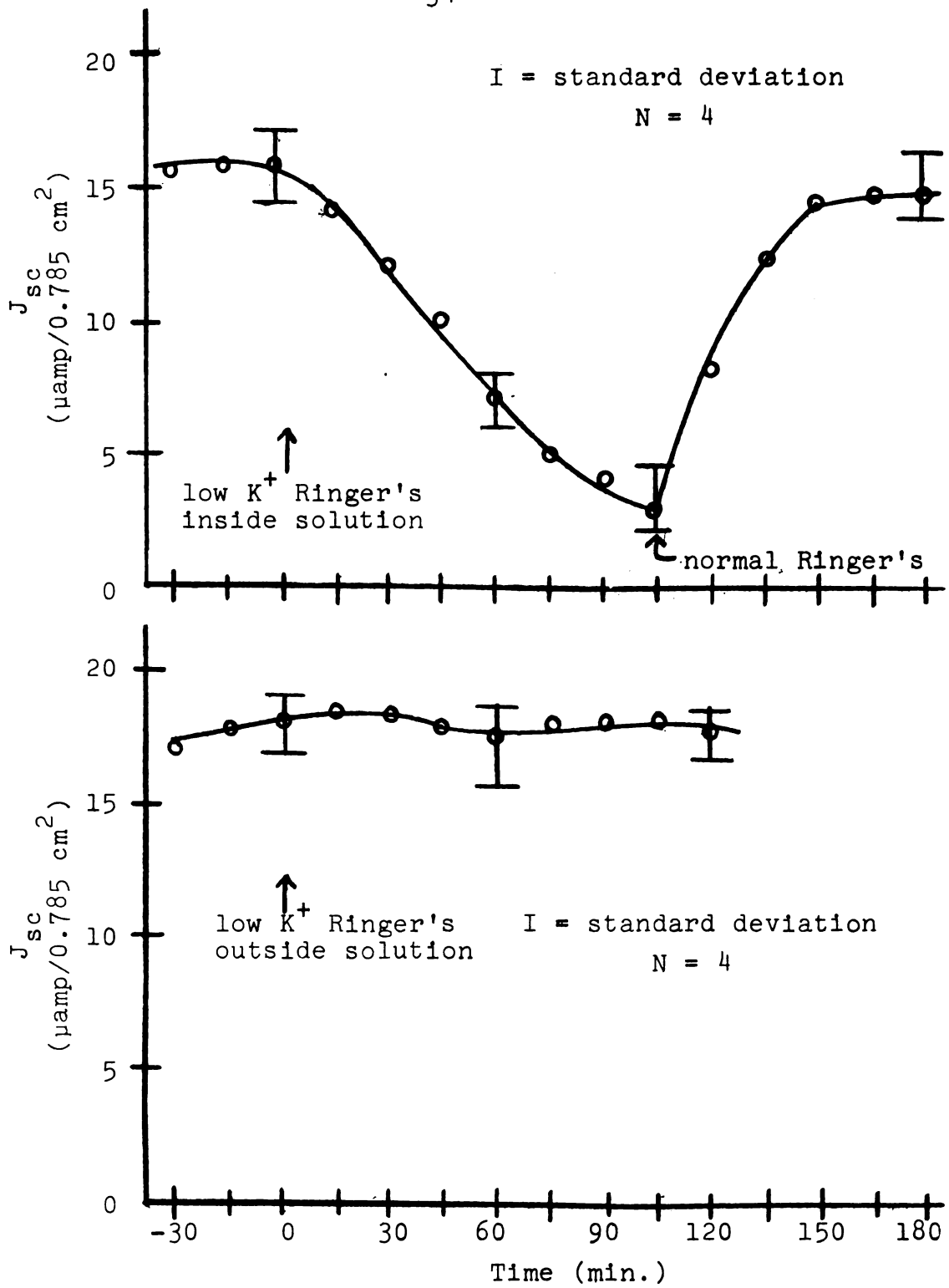

Fig. 7.--The effects of a low potassium Ringer's solution on the short-circuit current. The potassium was replaced by Na^+.

pre-experimental value. If a low K^+ Ringer's is placed on the outside of the skin, the J_{sc} remains constant. Thus for the maintenance of the J_{sc} Na^+, but not K^+, must be present in the outside solution, whereas K^+, but not Na^+, must be in the inside solution.

Dependence of the Short-Circuit Current on Metabolic Energy

Four inhibitors were used *in vitro*: 7.0 mM malonate, 0.5 mM potassium monoiodoacetate, 0.1 mM 2,4-dinitrophenol, and 0.1 mM ouabain (g-strophauthin. In Table 6 it can be seen that each inhibitor significantly ($p = 0.95$) reduced the Na^+ pump activity as measured by the J_{sc}, suggesting that these parameters have a dependence on metabolic energy. Since the J_{sc} was reduced by these agents, the net flux of the actively transported Na^+ must have been reduced also.

TABLE 6.--The effects of four inhibitors and two stimulants on the short-circuit current of the newt skin. The results are given as mean ± standard deviation. Each agent was placed in the inside bathing solution. These studies were done in the spring.

Agent	(N)	Final Concentration	J_{sc} Before (μamps/0.785 cm^2)	J_{sc} After (μamps/0.785 cm^2)
DNP	(7)	0.1 mM	9.2 ± 2.9	3.1 ± 0.7
Ouabain	(7)	0.1 mM	10.3 ± 3.1	2.3 ± 0.8
K iodoacetate	(6)	0.5 mM	13.8 ± 2.4	2.8 ± 1.2
Malonate	(6)	7.0 mM	12.3 ± 3.5	3.3 ± 1.7
ADH	(7)	25 mU/ml	10.4 ± 2.5	19.3 ± 3.4
Adrenalin	(6)	2.5 mg%	9.8 ± 2.6	17.8 ± 2.8

Stimulation of the Short-Circuit Current

The salamander skin's active transport system as measured by the J_{sc} is stimulated by ADH (25 mU/ml) and by adrenalin (2.5 mg%) when these agents are placed in the internal bathing solution (Table 6). ADH is not effective when placed on the outside surface of the skin. Since the J_{sc} increased, the net fluxes of actively transported ions must have increased.

Dependency of the Short-Circuit Current on Temperature

Because temperature affects metabolic processes, it would be expected to influence the rate of active transport of a substance, since the rate of energy liberation would likely be a limiting factor. It would be reasonable to expect a temperature coefficient for active transport which is characteristic of thermochemical reactions; that is, the rate of transport should double or triple with a 10°C rise in temperature. A representative response of a single newt skin is shown in Figure 8. The rate of change was one °C every two minutes. The J_{sc} shows a Q_{10} of about 2.0 and falls in the expected range. The J_{sc} reaches a peak at 36°C. It should be remembered that a high temperature coefficient would not of itself prove that there is an active transport process.

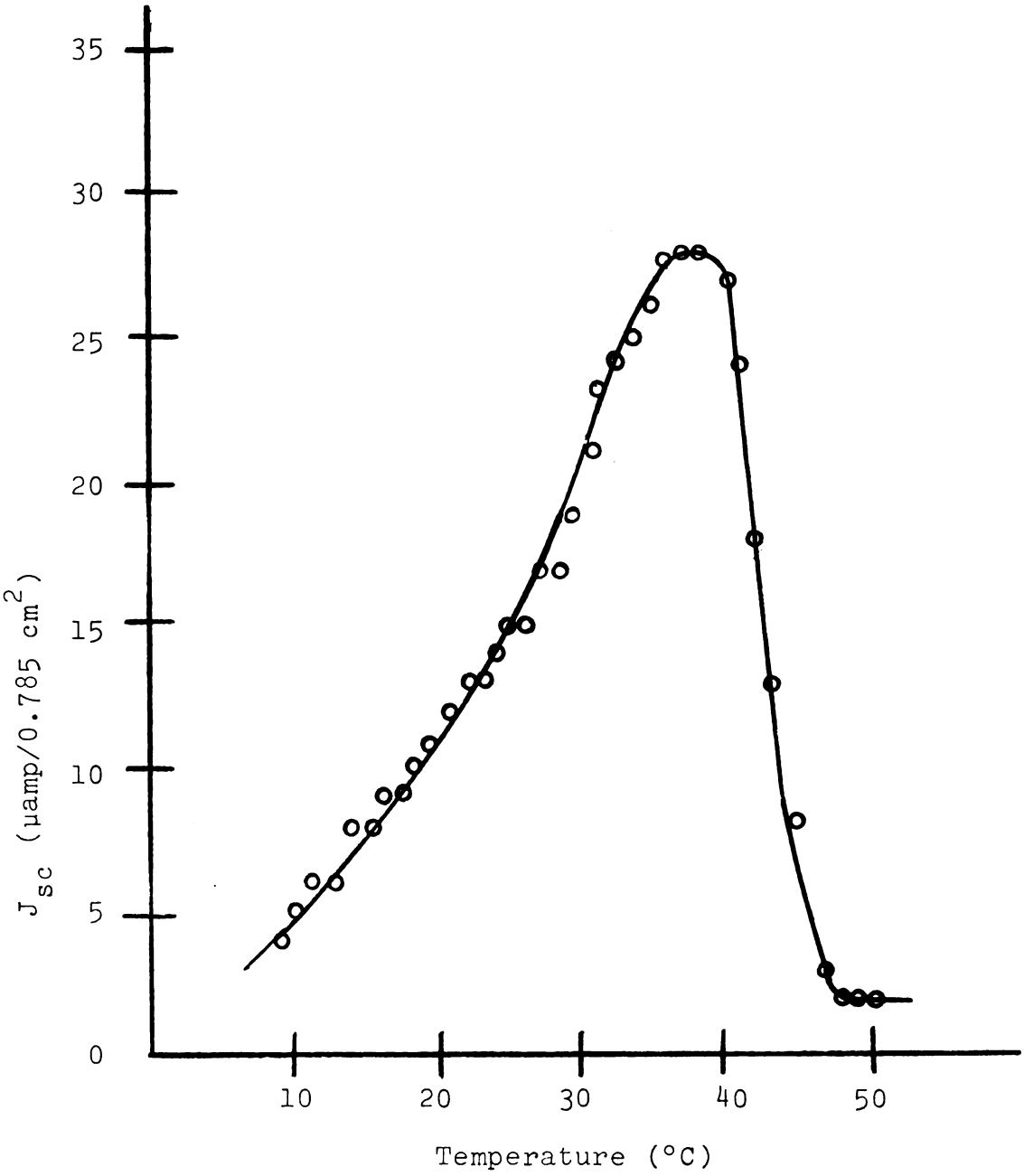

Fig. 8.--Dependency of the short-circuit current on temperature. A representative curve from a single skin is shown. The rate of change of temperature was one degree centigrade every two minutes. The Q_{10} of the short-circuit current is about two.

Minimal Total Sodium Pool Size

An estimate of the Na^+ pool size of the salamander skin was made according to the method of Anderson and Zerahn (1963). The method is to plot the accumulated Na^+ influx against time. A second line is drawn parallel to the straight slope of the first line such that the second line passes through the zero point. The difference between the two lines is the amount of labeled Na^+ used to bring the skin to a steady labeled state. The method assumes that the back diffusion of Na^+ is negligible and gives a minimal total pool size which includes the radiosodium not in the transport pool. The average influxes of Na^+ across 14 skins are plotted in Figure 9. A minimal Na^+ pool size of 0.063 ± 0.027 μeq Na^+/0.785 cm^2 was obtained for the newt skin.

The Effect of Hypophysectomy on the Transmembrane Potential of the Newt

During the winter a comparison between the transmembrane potentials of normal and hypophysectomized newts was made. The newts were hypophysectomized for seven days before the potentials were measured. The transmembrane potentials of hypophysectomized animals were significantly ($p = 0.99$) lower than those of the normal animals. Hypophysectomy caused a reduction in the potential difference from 16.3 ± 7.4 mV (19) to 3.5 ± 2.7 mV (25).

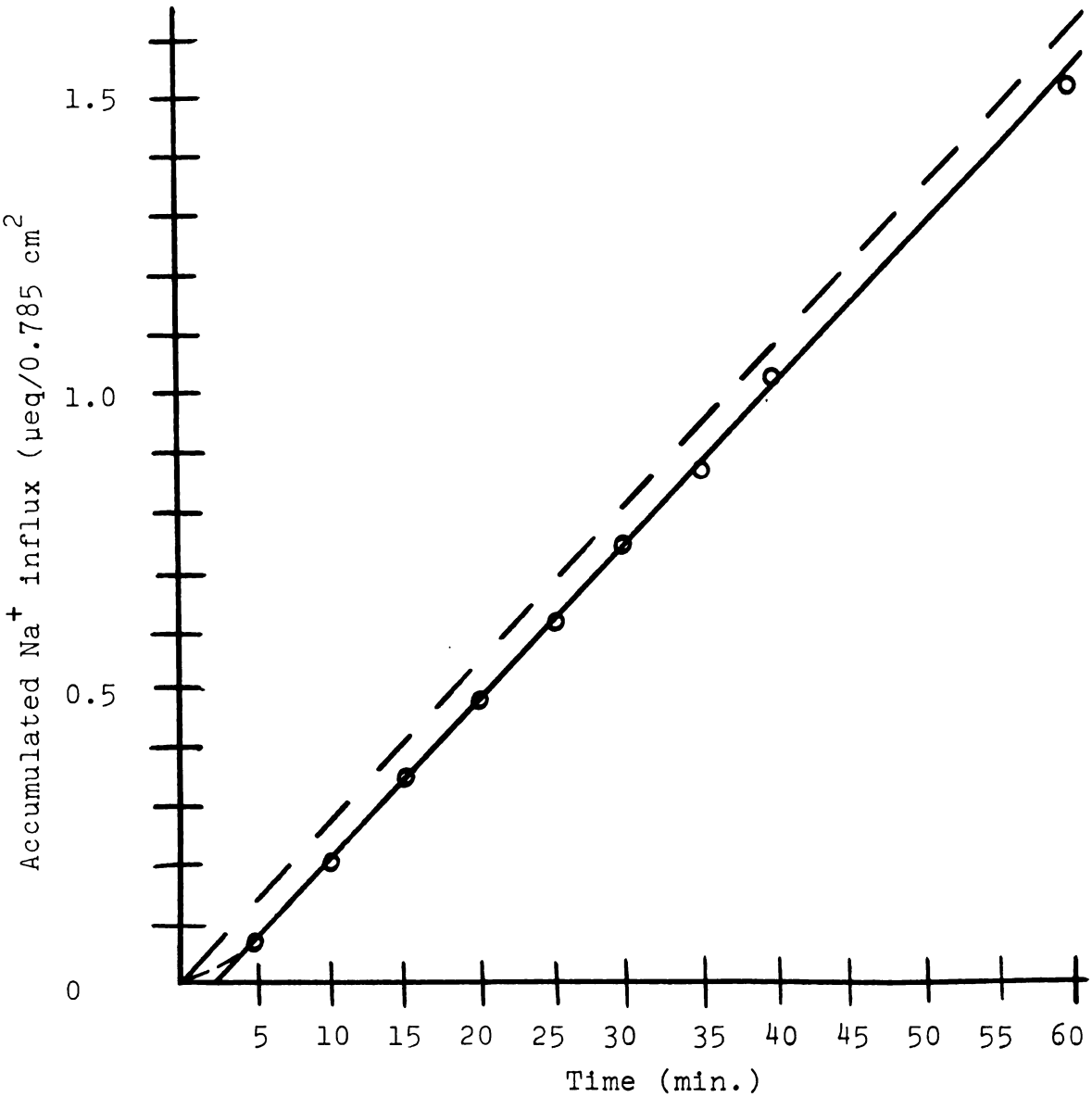

Fig. 9.--Minimal total sodium pool size. The average accumulated influxes of Na^+ from 14 skins are plotted against time. The difference between the dotted line and the solid line is an estimate of the total Na^+ pool size. An estimate of 0.063 ± 0.027 μeq. Na^+/0.785 cm^2 was obtained for the newt skin after the method of Anderson and Zerhan (1963). The standard deviation of any point is not greater than ±0.034.

DISCUSSION

It is well known that the isolated skins of frogs and toads (anurans) possess an active Na^+ transport system. Other amphibian skins would likely have similar properties. The skin of the adult newt, N. viridescens, exhibits a transmembrane potential with the inside surface electrically positive to the outside. This potential is due mainly to the active transport of Na^+ inward. Under short-circuit conditions a net flux of Na^+ inward occurs while the movement of Cl^- is entirely passive. Under these conditions the net flux of Na^+ is equal to the current drawn from the skin (the J_{sc}). The net flux of Na^+ inward is due to an active transport for the following reasons:

1. In this in vitro preparation there are no chemical, electrical or osmotic gradients to influence particle motion, yet a net flux of Na^+ exists;
2. The dermal surface is electrically positive to the epidermal surface, hence Na^+ ions increase in electrochemical energy as they pass through the skin inward;
3. Metabolic poisons depress the J_{sc} and the transmembrane potential (and hence the source of these). Energy must be supplied by metabolism.

Chloride ions must move through the skin passively for the following reasons:

1. In this *in vitro* preparation there is no statistically significant net flux of Cl^-;
2. Replacement of the Cl^- by the highly impermeable $SO_4^=$ ion does not reduce the J_{sc}.

Active Na^+ transport and passive Cl^- diffusion have been shown in the frog skin, *Rana pipiens* (Ussing and Zerahn, 1951) and in the toad skin, *Bufo bufo*, (Green and Matty, 1963). However, the skin of the frog, *Leptodactylus ocellatus*, possesses an active transport of both Na^+ and Cl^- (Zadunaisky and DeFisch, 1964). Hence differences in amphibian skins occur. The skin of the salamander, *N. viridescens*, has properties similar to *R. pipiens*. However this salamander's active Na^+ transport (J_{sc}, 10-20 μamps/cm^2) is not as strong as that of *Rana* (J_{sc}, 30-60 μamps/cm^2), the newt Na^+ transport is more on the order of the toad (J_{sc}, 15-35 μamps/cm^2) (Taylor and Barker, 1967).

Several metabolic inhibitors depress the J_{sc} of the newt skin. Ouabain is a general Mg^{++}, K^+ dependent ATPase inhibitor (Schatzman, 1953) and also affects the frog skin (Koefoed-Johnsen, 1957). DNP uncouples oxidative phosphorylation; iodoacetate inhibits phosphoglyceraldehyde dehydrogenase; and malonate competes with succinate in the TCA cycle, thus inhibiting the cycle (Krebs, 1954). DNP also affects the frog skin (Schoffeniels, 1955). Malonate has

not been reported to affect the frog skin. Operation of the Kreb's cycle (TCA) is essential for the operation of the Na^+ transport mechanism, since malonate decreases the J_{sc}.

The results on the dependence of the J_{sc} on Na^+ in the outside bathing medium and on K^+ in the inner medium are similar to those found in the frog (Zerahn, 1955). When the concentration of Na^+ in the outside solution is decreased, less Na^+ is available for diffusion into the cells and hence less Na^+ is available to the pump mechanism. K^+ may be necessary in the inside solution to stimulate the Na^+-K^+ dependent ATPase in the cells or it may be involved in a Na^+-K^+ couple pump. When a low K^+ Ringer's solution is present in the inside chamber, K^+ probably diffuses from the cells with time so that the cellular K^+ becomes depleted.

Inspection of the data shows that the unidirectional fluxes of Na^+ and Cl^- are quite high. Although the unidirectional fluxes of Cl^- are two to three times higher than those of Na^+, the net fluxes of Cl^- are much lower than the net fluxes of Na^+. In the frog skin the influx of Cl^- is usually smaller than the influx of Na^+ (Ussing, 1949). Two explanations for the high unidirectional fluxes in the newt are possible. These flux experiments were performed in the summer. Since the transmembrane potential and the J_{sc} were lowest in the summer, it would be reasonable to expect higher passive fluxes of ions in the summer. In the

frog high passive ion fluxes exist in the summer (Myers et al., 1961). Another possible reason may be the small surface area of skin used (0.785 cm^2). Dobson and Kidder (1968) showed that when frog skin is mounted in a conventional chamber, part of the exposed tissue is damaged by the clamping process. In the damaged areas the intercellular spaces are enlarged and the passive ion conductivity is increased, accounting for the drop in the transmural potentials observed in small chambers, even though the potential difference should be theoretically independent of the surface area. The J_{sc} (in $\mu amps/cm^2$) was not altered by changing the total area of skin exposed.

ADH in the internal bathing medium increases the J_{sc} of the newt skin. ADH has a similar response in the skins of N. alpestris and N. cristata (Bentley and Heller, 1962, 1964). These are however mostly terrestrial forms. Although they did no flux experiments with radioisotopes to determine the origin of the J_{sc}, they assumed that the J_{sc} was a measure of the active transport of Na^+, citing Ussing and Zerahn's work (1951) as evidence for the validity of this assumption. The transmembrane potential (16.3 ± 7.4 mV) and the J_{sc} (17.4 ± 3.9 $\mu amps/cm^2$) of N. viridescens are smaller than those reported for N. alpestris (22 ± 5.4 mV and 25 - 45 $\mu amps/cm^2$) and for N. cristata (39 ± 2.8 mV and 25 - 40 $\mu amps/cm^2$) (Bentley and Heller, 1964). This may be due to the fact that the

transmembrane potential is lower in the aquatic forms than in the terrestrial forms (Bentley and Heller, 1964). The adult newt, N. viridescens is completely aquatic.

The transmembrane potential is lower in hypophysectomized, adult newts. This suggests that changes in the fluxes of ions have occurred. Removal of the adenohypophysis in the frog is followed by a decrease in both the resting potential and the J_{sc} and by an increase in the efflux of Na^+ across the skin. These changes are opposed by treatment with mammalian ACTH or aldosterone (Bishop et al., 1961). However the frog appears to survive satisfactorily without its adenohypophysis for several months. But the hypophysectomized, adult newt dies within four weeks (Dent, 1967), unless prolactin (ovine) is administered (Connelly et al., 1968). The latter group also reported that an injection of prolactin and thyroxin together was more effective in promoting survival than an injection of prolactin alone. Thyroxin alone is ineffective. It would be interesting to see what effects hypophysectomy has on the fluxes of ions through the skin and the J_{sc}, and what hormones, if any, oppose these changes. If prolactin is effective, the studies might be of value in studying the mechanism of prolactin action. Perhaps the amphibian equivalent of prolactin has a role in maintaining normal osmotic and electrolyte balance in the newt.

A similar phenomenon occurs in euryhaline fish. Hypophysectomized Fundulus heteroclitus fail to survive in fresh water without prolactin administration (Burden, 1965). Prolactin administration to hypophysectomized fish allows them to maintain a positive Na^+ balance when transferred to fresh water (Maetz et al., 1967). Hypophysectomy has the same effect on certain other fish, for example, Salmo gairdnerii (Donaldson and McBride, 1967).

The newt, N. viridescens, has three stages in its life cycle and undergoes two metamorphoses. The larval, aquatic form has external gills and a smooth skin and undergoes a metamorphosis to a terrestrial form which has a granular, cornified skin and no gills. Thyroxin is known to be involved in the metamorphosis of amphibians (Etkins, 1964). In 2-3 years the terrestrial eft undergoes a second metamorphosis to the aquatic, adult form, which has a smooth skin but no gills. Prolactin is known to cause this second change (Chadwick, 1940). Grant and Cooper (1964) reproted that thyroxin partially reverses the second metamorphosis by producing a migration to land and a return of the skin to the eft condition of a loose, convoluted, and cornified epidermis. They also found that prolactin inhibits primary metamorphosis in larvae. It would be interesting to investigate the effects of these hormones on the permeability and active transport systems of these different forms, since profound changes must occur

in water and electrolyte metabolism to accommodate these shifts from aquatic to terrestrial habitats. This research has led to several questions:

1. How does hypophysectomy affect the Na^+ pump and the fluxes of other ions through the skin?
2. Does prolactin or other hormones reverse these changes?
3. What changes in the Na^+ pump and fluxes of ions through the skin might prolactin cause in the terrestrial eft?
4. What changes in these parameters might thyroxin cause in the larval and adult forms?

SUMMARY

The following results were obtained in this study:

1. The skin of Notophthalmus viridescens exhibits a transmembrane potential of 16.3 ± 7.4 mV, with the inner surface electrically positive to the outer surface, and a short-circuit current of 17.4 ± 3.9 μamp/cm^2.

2. Sodium is actively transported across the skin while chloride diffuses across the skin passively.

3. Under aerobic short-circuit conditions the net flux of sodium inward is equal to the recorded short-circuit current.

4. Malonate (7.0 mM), potassium monoiodoacetate (0.5 mM), 2,4- di-nitrophenol (0.1 mM) or ouabain (0.1 mM) decreases the short-circuit current.

5. Antidiuretic hormone (25 mU/ml) or adrenalin (2.5 mg%) increases the short-circuit current.

6. The short-circuit current depends on sodium, but not potassium, in the outside bathing medium and on potassium, but not sodium, in the inside medium.

7. The temperature coefficient or Q_{10} of the active transport system is about 2.0.

8. The transmembrane potential and the short-circuit current vary with the season. They are highest in the winter (Nov.-Feb.) and lowest in the summer (June-Aug.).

9. The minimal total sodium pool size is 0.063 ± 0.027 μeq. $Na^+/0.785\ cm^2$).

10. Hypophysectomy of the adult newt significantly reduces the transmembrane potential to 3.5 ± 2.7 mV after seven days.

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