

Solute and Water Transport Across Biologic Membranes

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FOREWORD

This is the two hundred and sixth of a series of reports designed to present accounts of progress in saline water conversion with the expectation that the exchange of such data will contribute to the long-range development of economical processes applicable to large-scale, low-cost demineralization of sea or other saline water.

Except for minor editing, the data herein are as contained in the reports submitted by Washington University under Grant No. 14-01-0001-364, covering research carried out through September 1, 1965. The data and conclusions given in this report are essentially those of the contractor and are not necessarily endorsed by the Department of the Interior.

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ABSTRACT

These studies relate to the biology of sodium transport across living membranes. The experimental model employed is the isolated urinary bladder of the fresh water turtle. This structure possesses the capacity to transport sodium ions completely across the cells utilizing endogenous glycogen as substrate. Moreover the transport persists under anaerobic conditions. Thus the opportunity exists to examine the coupling between energy metabolism and a major energy requiring biologic function, sodium transport under conditions where energy is produced via a relatively simple and primitive metabolic pathway. The data document the ability of the bladder to sustain anaerobic sodium transport and a stoichiometric relationship was demonstrated to exist between anaerobic glycolysis and transport. Inhibitors of mitochondrial electron transport failed to inhibit sodium transport markedly whereas glycolytic inhibitors in an anaerobic environment proved to be powerful inhibitors of sodium transport. Tissue ATP concentrations were maintained roughly at the same level under anaerobic as under aerobic conditions and calculations derived from these experiments suggest that approximately 15 sodium ions are transported actively for each molecule of ATP produced anaerobically.

It is believed that continued study of the coupling between anaerobic metabolism and transepithelial sodium transport may greatly clarify the intricacies of salt and water movements across living membranes.

INTRODUCTION

This report describes the results of research performed under the auspices of the Office of Saline Water Grant No. 14-01-001-364 over the two year period ending September 1, 1965. The primary purpose of these studies was to investigate some of the fundamental aspects of ion transport across biologic membranes. One of the most characteristic features of living cells is the ability to maintain a constant volume of water within the limiting cell membrane and simultaneously to preserve the concentrations of sodium and potassium in cell water at levels compatible with life; these levels differ strikingly from those maintained in the fluid surrounding the cells. Thus virtually all living cells, from those of single-celled organisms in a marine environment to the complex cells of the most highly ordered mammalian species maintain a high potassium and low sodium concentration in cell water, while the extracellular fluid characteristically is sodium rich and potassium poor. It is well known that the maintenance of these alkali metal cation concentrations in a steady state condition that is far removed from equilibrium requires the active extrusion of sodium ions from the cell water and in many cell systems, the active transport of potassium into the cell water against their respective electrochemical gradients. It also is known that these ion transport processes are among the most important energy requiring functions of living cells. While the energy requirements for electrolyte transport across cell membranes of the cell types that are concerned

only with maintaining the integrity of their cell water are formidable, the requirements of those highly specialized cell types that are concerned with the movement of ions from one border across the opposite border are far greater. Among these specialized units are the polar epithelial cell systems that transport sodium transcellularly. It would appear that in some of these, at least 50% of the total energy synthesized from metabolism is diverted to ion transport .

Intensive efforts have been made in recent years to examine in depth the relationships between energy production and sodium transport in model systems that lend themselves to a direct experimental approach. Two biologic structures have been employed as the primary experimental models in this type of exploration. One of these is the isolated skin of the frog; the other is the isolated urinary bladder of the toad. Both structures are lined by epithelial cells, and both transport sodium from one border of the epithelia to the other. However, in both, the pathways for energy transformation are primarily oxidative and knowledge of the intimate mechanisms of energy transformation has been slow to evolve for this evolution is dependent upon prior clarification of the molecular basis of mitochondrial energy conservation. We have therefore sought to develop a different model system. The studies which have been performed in our laboratories related to the nature of salt and water movements across membranes of living cells and particularly to the coupling between metabolic energy production and this movement. We have chosen an isolated epithelial cell membrane which transports sodium transcellularly just as do the renal tubule, the frog skin, and the

urinary bladder of the toad. However, in contrast to the latter structures, the present one appears to be capable of synthesizing its energy via anaerobic pathways. It thus permits not only the quantitative measurement of net rates of sodium and water movements from one border of the cells to the other, but it offers a simple schema for the investigation of the energetics of active transport. The model is the urinary bladder of the fresh water turtle. This structure was chosen for two reasons: The first is that the urine obtained from the bladder of living turtles often has a low sodium concentration; this suggests that the epithelial cells lining the bladder might transport sodium out of the urine into the animals. The second is that the intact living turtle is capable of tolerating oxygen deprivation for prolonged periods of time. Thus the possibility was considered that the turtle bladder might provide a model wherein the coupling between metabolism and sodium transport could be investigated in an oxygen free environment in vitro. If this were confirmed, energy production would be derived from anaerobic glycolysis, a pathway more primitive and far less complex than oxidative phosphorylation. It is of interest in this regard that the fresh water turtle appears to have been on earth long before man and it is conceivable that the ability to tolerate oxygen deprivation may account in part for this.

METHODS

Adult turtles (*pseudemys scripta elegans*) of both sexes were used for these experiments. The animals were maintained at room temperature (approximately 22°C) in shallow fresh water. At the time of study, the plastron was removed and the coelomic membrane incised. The exposed urinary bladder, which is bilobed and usually distended with urine, was dissected free, transferred to a dish containing Ringer's solution, opened, rinsed, and then suspended as a diaphragm between two symmetrical halves of a lucite chamber. Both surfaces of the isolated bladder then were bathed with Ringer's solution which usually had the following composition: Na^+ 114 mEq per liter; K^+ 2.4 mEq/L; Ca^{++} 1.4mM; Mg^{++} 2.0mM; Cl^- 120 mEq/L; PO_4^{--} 2.4mM; Osmolality 230 mOs/Kg H_2O ; pH 7.4 ± 1.0 units.

The lucite chambers in which the bladders were suspended provided a membrane area of either 7cm^2 or 2cm^2 . The volume of Ringer solution in each hemi-chamber was 20 to 25 ml with the larger chambers and 5 ml with the smaller chambers. The mixing of the bathing media was accomplished by continuous flow of gas through both compartments. In the studies concerned with the behavior of the isolated bladder under aerobic conditions, the bathing solutions were oxygenated either with 100% oxygen or with room air. Anaerobic conditions were obtained by sustained bubbling of 100% nitrogen through the Ringer's solution. The nitrogen gas was passed through an oxygen trap prior to its entry into the bathing media in order to reduce the oxygen tension to an absolute minimum.

The electrical potential gradient across the bladder and the short-circuit current were measured using the standard techniques of Ussing and Zerahn². Sodium movements across the bladder were obtained using radioactive isotopes. When unidirectional sodium flux measurements were performed, tracer quantities of Na²² were employed. Na²² was added either to the mucosal (i.e. urine side), or serosal (i.e. turtle side), depending upon whether influx or efflux was being studied.

The specific activity of radioactive sodium was determined in the side of addition and the net accumulation of isotope on the opposite side was measured at 30 minute intervals. When the bi-directional movements of Na from mucosa to serosa and vice versa were measured simultaneously, Na²² was added to the mucosal medium and Na²⁴ to the serosal medium. When Na²² alone was used, 200 microliter samples were plated and dried in aluminum planchets and radioactivity was counted using a thin end -window gas flow counter (Tracerlab). When the two isotopes of sodium were used simultaneously, an auto-gamma spectrometer (Packard Model 410A) was used and Na²² and Na²⁴ activities were counted using the appropriate windows on the day of the experiment and again after well over 99% of the Na²⁴ had decayed. Chemical determinations of sodium were performed using a flame photometer.

A second basic protocol was used for a number of these studies. In these experiments, metabolic observations were obtained on bladder tissue segments. In general individual urinary bladders were cut into 9 to 12 segments, each approximately 1 cm² in area. These were divided into

groups and generally each group totalled about 100 mg. wet weight. The segments were incubated in Ringer's solution of the same composition as has been described above under either aerobic or anaerobic conditions. The incubations were performed at 22°C with the tissue immersed in 10 ml of Ringer's solution in Erlenmeyer flasks and agitated gently in a Dubnoff metabolic shaker. During the initial 30 to 60 minutes of study, 100% oxygen was used as the gas phase in all flasks. Thereafter for the anaerobic studies, the gas phase was changed to 100% nitrogen in half of the flasks and oxygen was continued in the control set. The studies were continued for one to two hours thereafter. These experiments were performed to measure the tissue levels of adenosinetriphosphate(ATP), and glycogen and to measure the rate of lactate formation by the tissue slices. Control ATP and glycogen concentrations were measured on tissue segments immediately after removal of the bladders from the turtle. These served as a frame of reference for tissues analyzed after incubation under a variety of experimental conditions. Experiments were performed with and without exogenous substrate (glucose) in the Ringer's solution. Lactate formation was measured in the supernatant Ringer's solution and the total amount of lactate formed could then be correlated with the rate of glycogen utilization. Tissue ATP levels could be correlated with both measurements.

A detailed description of the methods used for ATP, glycogen, and lactate analysis have been included in previous publications from this laboratory^{3,4,5}.

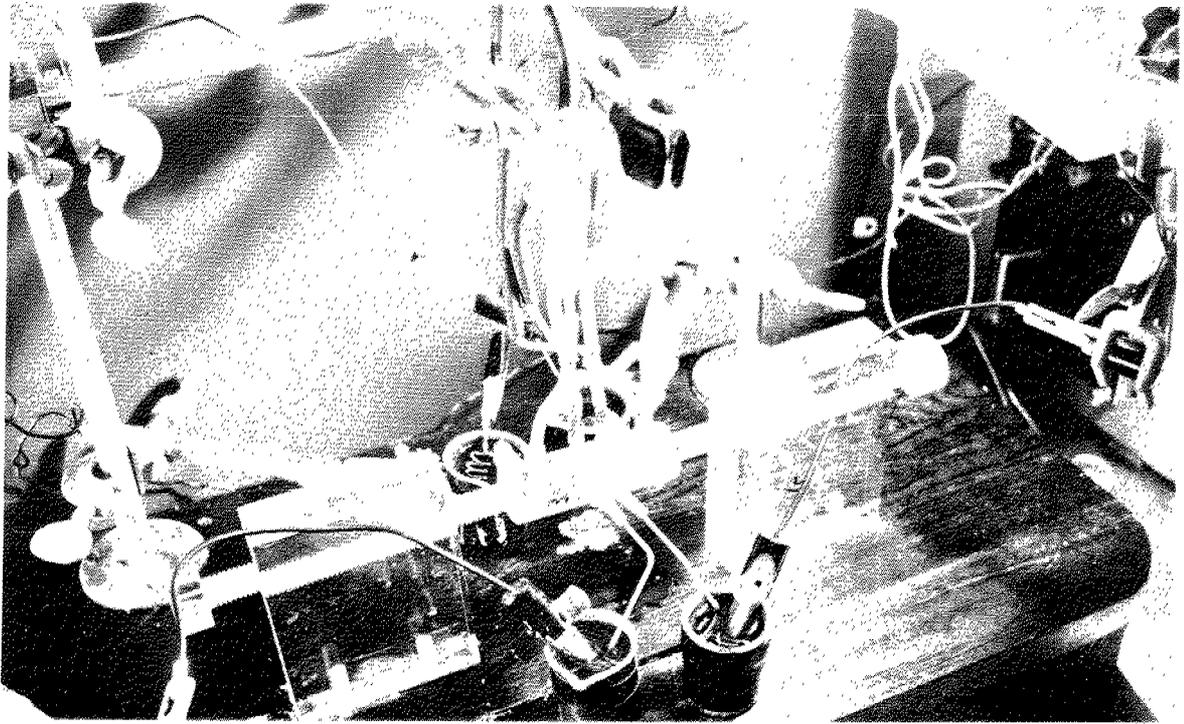


Figure #1

RESULTS

A photograph of the lucite chamber with bladder mounted between the two hemi-chambers is shown in Figure 1. The electrodes closest to the bladder are employed for the measurement of transbladder potential difference; the electrodes entering at the two poles of the chambers are used for the external (i.e. "bucking") current which provides a measure of the short-circuit current as defined by Ussing and Zerahn².

The histologic appearance of the isolated bladder is shown in Figure 2.



Figure #2

The membrane shown was fixed and sectioned after it had been in the transport chamber for three hours. The mucosal surface consists of 1 to 2 cell layers of epithelial cells which are the sodium transporting structures. The remainder of the bladder wall includes a supporting layer containing muscle fibers and connective tissue, an open space which presumably may be a lymphatic channel, and a serosal layer of mesothelial cells. When examined by electron microscopy, the epithelial cells include "dark cells" which contain a dense network of endoplasmic reticulum and have many mitochondria, clear cells which have a more loosely arranged endoplasmic reticulum and few mitochondria, and a group of cells which fall somewhere between the two main cell types. The mucosal surface of the epithelial cells contains microvilli that extend into the bladder lumen and the serosal or basal surface of the epithelia is characterized by multiple invaginations of the plasma membrane into the cell interior³.

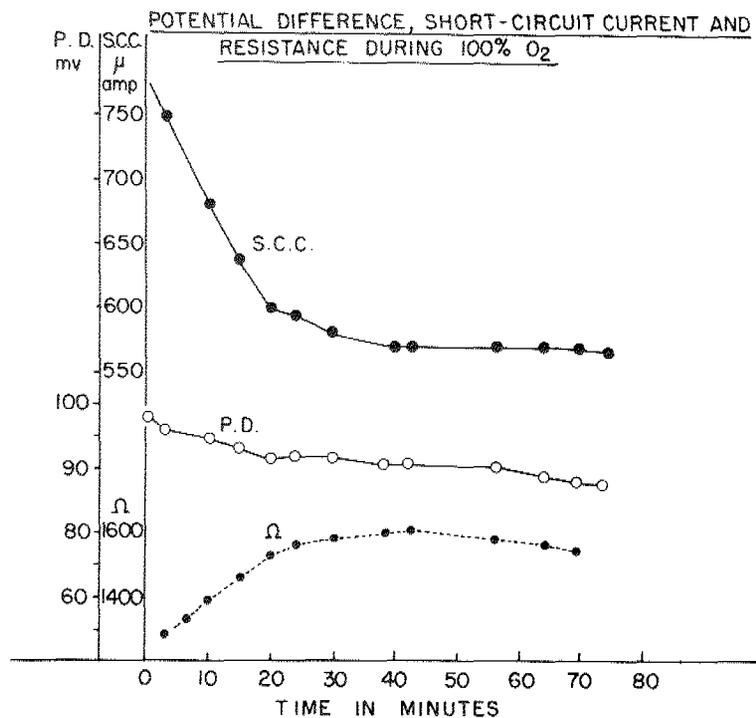


Figure #3

Potential Gradient and Short-Circuit Current:

The intact turtle bladder exhibits electrical asymmetry characterized by a trans-bladder potential gradient(P.D.). The serosal surface is electrically positive to the mucosal surface. The magnitude of this trans-bladder P.D. varies from 10 to over 100 millivolts in aerobic studies and in the majority of instances, the steady state values range from 30 to 60 millivolts. Figure 3 depicts the values for P.D. in a representative bladder studied under aerobic conditions. The drop in the P.D. that is evident during the first 30 minutes is quite characteristic and so, also, is the recovery. In the majority of experiments, the decrease occurred over approximately a 30 to 60 minute interval, and recovery was complete within 90 minutes. Once steady state

conditions were achieved, P.D. remained relatively constant for periods up to several hours in duration.

The short-circuit current (S.C.C.) is an expression for the amount of electric current necessary to "buck-out" the spontaneous P.D. The external current is adjusted so as to maintain the P.D. at 0 mV. and in the frogskin and toad bladder, the value for S.C.C. has been found to equal the value for net transport of sodium.* The values for S.C.C. also fell immediately after the bladder was suspended in the plastic chamber, but recovery then occurred and values ultimately stabilized at levels ranging from 40 to over 400 microamps per 7 cm². Once stability was achieved, the values for S.C.C. tended to remain constant for an extended interval.

The patterns observed under anaerobic conditions differed from those observed under aerobic conditions. In Figure 4, a representative experiment performed under anaerobiosis is shown.

* Net transport is defined as influx from mucosa to serosa (the direction of active transport) minus efflux from serosa to mucosa (the passive movement).

POTENTIAL DIFFERENCE, SHORT-CIRCUIT CURRENT AND RESISTANCE
DURING ANAEROBIOSIS

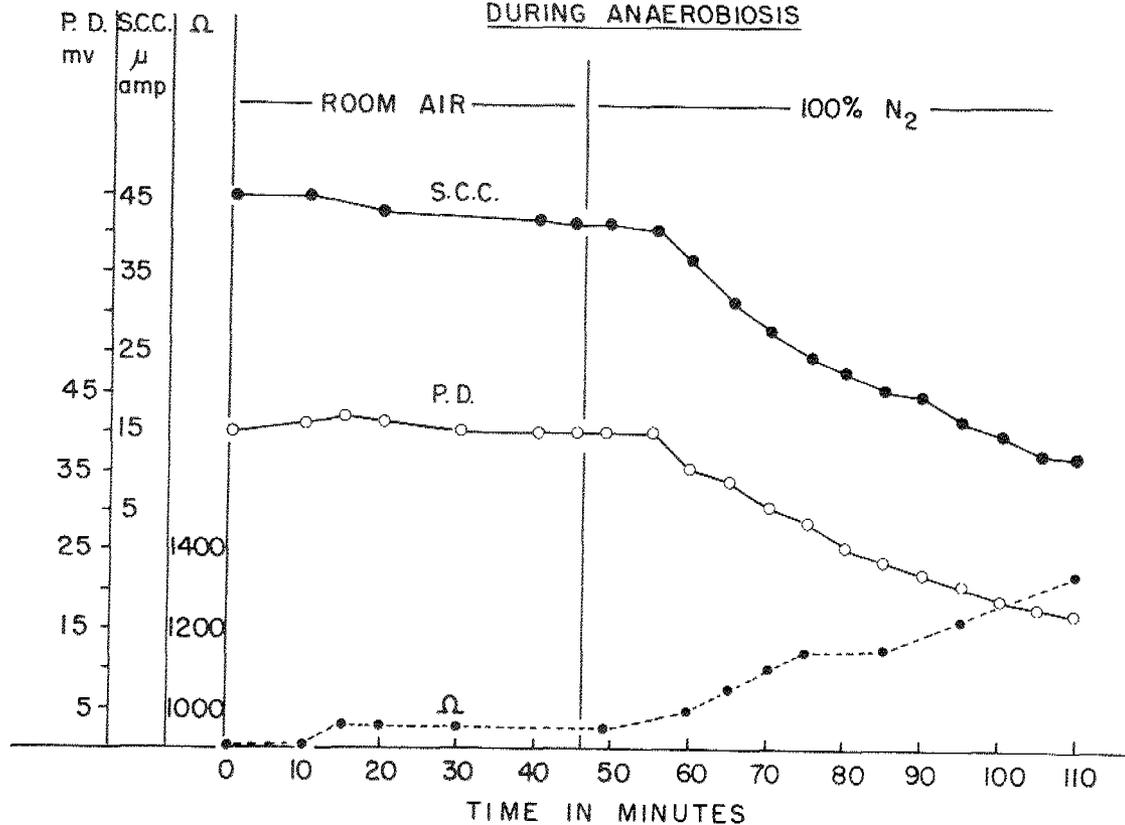


Figure #4

Both the P.D. and the short-circuit current fell slowly and progressively after the gaseous environment was changed from oxygen to 100% nitrogen. However, positive values persisted throughout the duration of observation which, in many experiments, exceeded 3 hours. The marked increase in electrical resistance shown in the figure was a regular occurrence. In approximately 5% of the experiments, the values for P.D. and S.C.C. remained constant in nitrogen. When S.C.C. and P.D. fell, this did not represent death of the membrane for recovery of both parameters typically could be induced by re-introduction of oxygen into the Ringer's solution.

Experiments also were performed on a group of turtles which were totally submerged under water for periods of over 12 hours. Robin et. al.¹ have shown that during this type of sustained diving, the fresh water turtle is unable to extract dissolved oxygen from the water and oxygen tensions of the blood fall to zero. In the present experiments, animals were taken from the water after diving with their tracheae occluded, and their bladders were rapidly removed, transferred to deoxygenated Ringer's solution, opened, and suspended in lucite chambers with deoxygenated Ringer's solution bathing both surfaces. Positive values were observed for both P.D. and short-circuit current. Although these values fell during a 60 minute period of observation, introduction of oxygen into the system was associated with an increase in values which was progressive over a period of approximately four hours.

The Short-Circuit Current Versus Sodium Transport:

As already indicated, there is a close correspondence between net sodium transport and the short-circuit current in the frog skin and urinary bladder of the toad. Accordingly, one might anticipate on a priori grounds that a similar correspondence would be observed in the urinary bladder of the turtle. This was not the case. Moreover, the discrepancy between net sodium transport and S.C.C. was different under aerobic and anaerobic conditions.

Aerobic Conditions:

The relationship between net sodium flux and short-circuit current in oxygenated Ringer's solutions is shown in Table I. Net flux was equated with the Na^{22} influx minus the simultaneously determined Na^{24} efflux. (Influx is defined as transport from mucosal to serosal solutions and efflux transport in the opposite direction). The net sodium flux averaged 82.4% of the short-circuit current in the 5 experiments shown. The difference between the two values is statistically significant ($p < 0.01$).

Anaerobic Conditions:

Values for net sodium flux and the simultaneous values for short-circuit current are included in Table I. During exposure to 100% nitrogen, net flux was greater than short-circuit current in 11 of 12 measurements. The data recorded in Table I also allow a comparison between aerobic and anaerobic net sodium transport in the same bladders. In three experiments, values fell after changing from oxygenated to deoxygenated Ringer's solution.

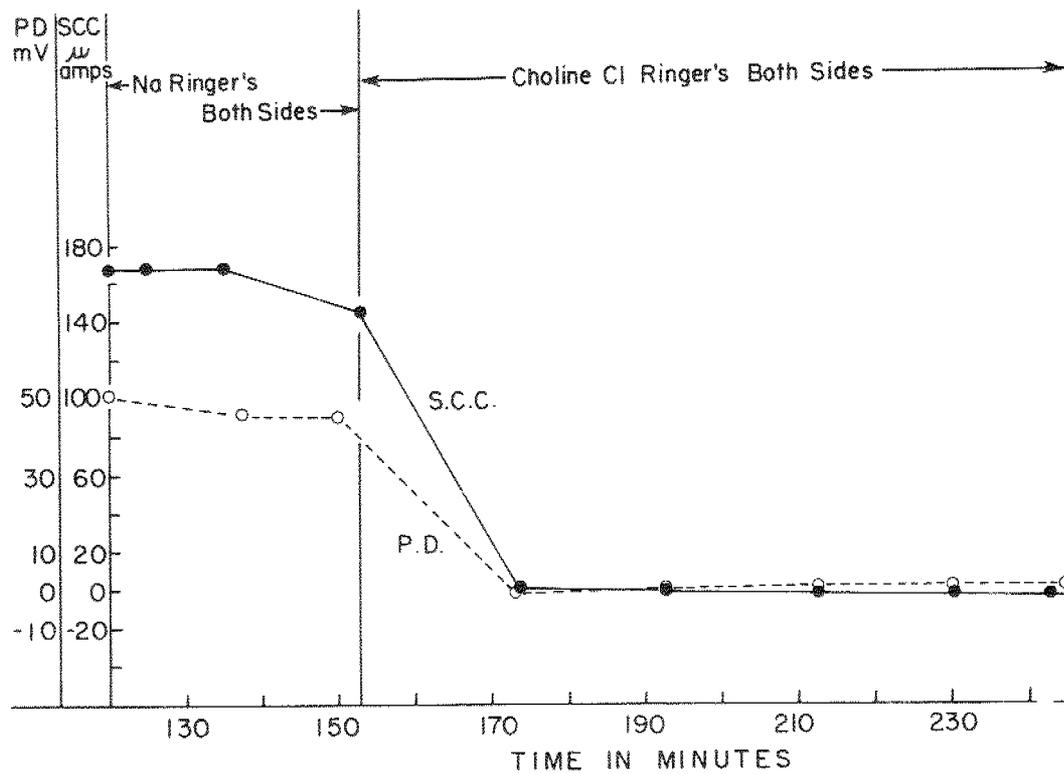
Energy Metabolism Versus Sodium Transport:

In view of the demonstrated ability of the turtle bladder to transport sodium anaerobically, studies were undertaken to define the quantitative relationships between anaerobic glycolysis and anaerobic sodium transport. Glycolysis as noted is a more primitive and less complex pathway for energy production than mitochondrial-linked oxidative pathways; hence investigation of the turtle bladder could contribute importantly to the understanding of the coupling between metabolic energy and active transport.

Lactate Formation Versus Sodium Transport:

In order to investigate the relationship between sodium transport and anaerobic glycolysis, measurements were made of lactate formation in an anaerobic environment before and after removal of sodium from the bathing Ringer's solutions. The control studies were performed with conventional sodium Ringer's solution in both hemi-chambers. Thereafter the bladders were rinsed from two to three times with de-oxygenated choline chloride Ringer's solution and fresh de-oxygenated choline chloride Ringer's was used as the bathing solution in contact with the two surfaces of the bladder. 100% nitrogen was maintained as the gas phase. The potential difference and short-circuit current fell precipitously to 0 after removal of sodium ions (See Figure 5). Lactate formation averaged $380 \times 10^{-3} \mu\text{m}$ per 7cm^2 of bladder per hour in the presence of sodium whereas in the absence of sodium the value fell to 208. This represents a net decrease of 45.4%. In 9 studies, the experimental sequence was reversed and measurements were made first with choline chloride Ringer's and then with sodium Ringer's. Lactate formation

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 IN THE ABSENCE OF SODIUM TRANSPORT



increased in 8 of the 9 experiments by an average of 57%. In one experiment the value fell and for the group the increase averaged 36%.

The rate of lactate formation also was studied with and without oxygen in the Ringer's in 25 experiments. In 24 of these lactate formation increased after the gas phase was changed from 100% oxygen to 100% nitrogen. The mean value was $162 \times 10^3 \mu\text{m}$ per 7 cm^2 per hour in oxygen compared to 347 in nitrogen. The increase was 114% and the difference is highly significant ($p < 0.01$).

Glycogen Utilization Versus Sodium Transport:

The glycogen concentrations of bladder segments were measured under control conditions (i.e. immediately after removal from the turtles), after two hours of incubation in oxygenated Ringer's solutions, and after 2 hours of incubation in anaerobic Ringer's solutions. No glucose was used in these studies. Glycogen concentrations were significantly decreased after aerobic incubation in relation to control and glycogen utilization was greater in anaerobic than in aerobic environment. This difference also was statistically significant ($p < 0.01$).

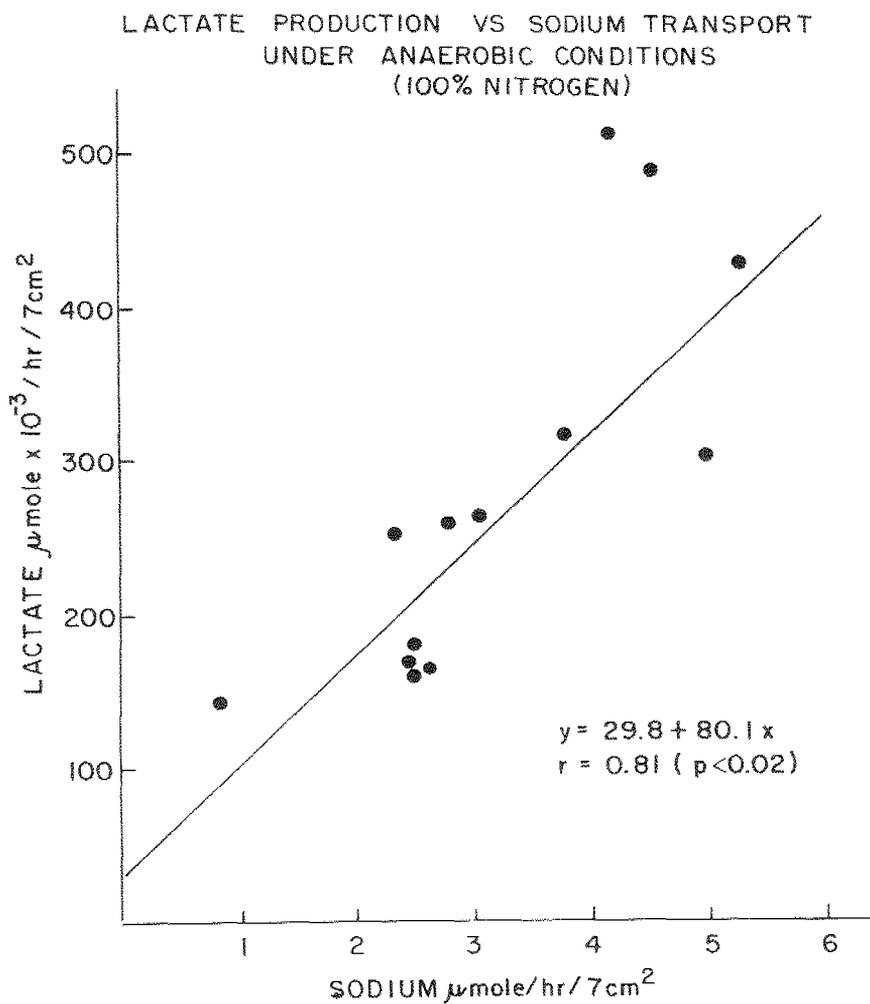
Lactate Formation Versus Glycogen Utilization:

Lactate formation and glycogen utilization were measured simultaneously in 19 experiments. Glycogen was measured as glucose residue; hence if all the glycogen utilized were converted to lactate, 2 moles of lactate should be evolved for every mole of glucose utilized. The results in the 19 experiments were as follows. Glycogen utilization averaged $10.4 \mu\text{m}$ per gram of tissue per hour (standard deviation ± 1.2); lactate evolution averaged $21.9 \mu\text{m}$ per gram of tissue per hour (standard deviation ± 3.1).

ATP Concentrations:

The values for ATP concentrations of bladder segments incubated under aerobic and anaerobic conditions were measured in 8 experiments. The mean value for the control tissues processed immediately after removal from the animals incubated in oxygenated Ringer's was 0.20 μg per mg wet weight. Finally the value for the tissues incubated under anaerobic conditions was 0.18 μg per mg of wet weight. The difference between the aerobic and anaerobic values was not statistically significant ($p < 0.2$)

Figure #6



The Relationship Between Net Sodium Transport and Anaerobic Glycolysis:

In Figure 6 anaerobic glycolysis, as reflected by lactate formation, is plotted against net sodium transport determined with bidirectional flux techniques. These measurements were made simultaneously in 13 experiments performed using de-oxygenated Ringer's solutions. Each value for sodium transport shown in the plot represents the mean of at least two 30 minute periods. The linear regression line was drawn using the method of least squares and the correlation coefficient is 0.81. The function is statistically significant ($p < 0.02$).

The Effects of Inhibitors of Electron Transport on the Behavior of the Turtle Bladder Under Aerobic Conditions:

The capacity of the turtle bladder to function when the gas phase in the bathing Ringer's solutions is 100% nitrogen would support the view that this structure is capable of sustaining sodium transport via anaerobic metabolism. The preservation of ATP levels under anaerobic conditions would provide additional support for this thesis and finally the relationships between sodium transport and anaerobic glycolysis depicted in Figure 6 would seem to document this phenomenon. Nevertheless it is possible that trace quantities of oxygen are present in the Ringer's solutions and that the metabolism is at least in part oxidative. To examine this thesis experimentally, studies were performed on turtle bladders suspended in oxygenated Ringer's and inhibitors of mitochondrial electron transport were employed. These should render this system operationally anaerobic.

Twelve experiments were performed to examine the behavior of sodium flux and short-circuit current and the relationship between these two

parameters in an aerobic system following the addition of KCN in a concentration known to inhibit mitochondrial respiration in a wide variety of cell types. KCN was added to the serosal solution in a final concentration of 10^{-3} M. In each experiment, net sodium transport increased while the short-circuit current remained unchanged. A comparable relationship was observed in 8 unidirectional flux studies (4 influx and 4 efflux). The response to cyanide therefore resembles that observed in de-oxygenated Ringer's solutions in that the ratio of net sodium flux to short-circuit current increased in every experiment. It is of considerable interest that sodium transport persisted in the presence of this inhibitor of electron transport. In studies performed with antimycin, net sodium transport diminished but once again persisted at levels in excess of 50% of control aerobic values. Short-circuit current diminished to a degree greater than the decline in net sodium transport and transport current ratios increased. Thus with antimycin as well as KCN the turtle bladder retained the ability to sustain trans-cellular sodium transport. Under the influence of both inhibitors, lactate formation increased markedly.

Inhibitors of Anaerobic Glycolysis:

Sodium fluoride and iodoacetic acid are both known inhibitors of anaerobic glycolysis. Fluoride inhibits the enolase reaction involved in the conversion of 2-phosphoglyceric acid to phosphoenolpyruvate while iodoacetate inhibits the conversion of glyceraldehyde-3-phosphate to 1,3-disphosphoglyceric acid presumably by inhibiting glyceraldehydephosphate dehydrogenase activity. Both of these inhibitors were studied in experiments on the isolated turtle bladder suspended in de-oxygenated Ringer's

solutions. If the source of energy for anaerobic transport is anaerobic glycolysis inhibition of glycolysis should interfere both with lactate formation and with sodium transport. These were the results that were observed. Iodoacetate produced a fall in lactate formation towards 0, a decrease in net sodium transport towards 0, and a fall in tissue ATP concentrations of over 99%. Sodium fluoride produced similar but less striking effects.

Isolated Epithelial Cell Sheets:

One of the major virtues of the isolated urinary bladder of the turtle is that it is a simplified model which permits the detailed study of the coupling between energy metabolism and sodium transport. Despite its relative anatomic simplicity however, the turtle bladder still consists of several different layers as noted earlier in this discussion. In addition to the epithelial cell layer, there is smooth muscle, connective tissue, and mesothelial cells. In an effort to obtain a more discrete experimental model, we developed a technique for isolating the epithelial cells in intact sheets suitable for transport and metabolic studies. The urinary bladders were opened and rinsed as described above and then positioned mucosal side down in a dish containing fresh Ringer's solution. The dissection procedure was performed manually. Using small blunt forceps, the serosal and muscular layers were retracted gently away from the mucosa. The index finger was used to support the mucosa as the dissection proceeded. When connective tissue adhesions appeared between mucosa and muscular layers, these were cut with the dissecting scissors. The dissection was continued until an area in excess of 2 cm² was freed or in metabolic studies until enough tissue was obtained to provide approximately 200 mg of material.

The dissections usually were completed in less than 5 minutes. For transport studies, it was essential that the epithelial cell sheets be intact and that the dissected material be examined carefully in the dish before it was removed and again after it was placed as a diaphragm over the plastic transport hemi-chamber having an exposed area of 2 cm². This inspection was performed under bright illumination. For the transport studies short-circuit current and transpeithelial cell potential gradients were measured as previously described. Sodium influx and sodium efflux measurements were performed with Na²² when unidirectional fluxes were measured and both Na²² and Na²⁴ were used in the mucosal and serosal solutions respectively for bidirectional flux studies. For metabolic studies the dissected sheets of epithelium were cut into segments weighing approximately 50 mg each and these were transferred to Erlenmeyer flasks containing 10 ml of Ringer's solutions. These studies were performed at room temperature and the tissues were agitated in a Dubnoff metabolic shaker.

Potential differences with the serosal surface positive to the mucosal surface were observed in these preparations. The values were somewhat lower than those for the intact bladder and they persisted for shorter periods of time. Nevertheless it was possible to perform satisfactory studies with the preparation. S.C.C. values also were lower than in the intact bladder, but values remained positive under anaerobic conditions although the rate of decline was more rapid than for the whole bladder. With reintroduction of oxygen S.C.C. values increased.

Bidirectional flux studies showed a closer correspondence between net sodium transport and S.C.C. under both aerobic and anaerobic conditions than was observed with the whole bladder. Tissue ATP concentrations were preserved at approximately 75% of the aerobic value in tissues incubated in oxygen-free media. Lactate formation increased after transferring the tissues from aerobic to anaerobic systems.

DISCUSSION

The major area of scientific interest in these studies is the biology of ion transport. The isolated urinary bladder of the fresh water turtle was chosen as the experimental model after it was found to be capable of sustained and vigorous sodium transport in vitro under anaerobic conditions. We have confirmed the fact that the turtle bladder will transport sodium anaerobically at virtually the same rate as it transports sodium aerobically. Moreover sodium transport continues when KCN and antimycin A, both inhibitors of electron transport by mitochondria, are added in inhibitory concentrations. The turtle bladder was found to engender an electrical potential gradient with the serosal surface positive to the mucosal surface. This could be maintained for several hours in oxygen but values declined progressively in an anaerobic system. As the short-circuit current fell, however, the net sodium transport values persisted; thus a progressively greater discrepancy emerged between transport and current. This property of the bladder is unusual and sets it aside from aerobic models which transport sodium such as the isolated urinary bladder of the toad and the isolated skin of the frog. In the latter two structures net sodium transport is equal to short-circuit current under most conditions.

Several possibilities could explain this dissociation between current and transport. 1) Sodium ions could enter the serosal bathing medium together with an anion (i.e. as a salt) or there could be independent transport of an anion from mucosa to serosa. 2) Another cation could move in a direction opposite to that of sodium. 3) The transfer of isotopic sodium from cell water to serosal medium could reflect exchange diffusion rather than net transport. However this would probably require the occurrence of exchange diffusion across the serosal border but not across the mucosal border inasmuch as efflux rates of sodium measured isotopically were not modified by introduction of anaerobiosis.

The metabolic studies indicate that glycolysis comprises the major and perhaps the sole source of energy for anaerobic sodium transport across the isolated turtle bladder. In the absence of exogenous substrate, endogenous glycogen, which is located within the epithelial cells lining the bladder, serves as substrate for the anaerobic glycolysis.

That there is direct coupling between anaerobic glycolysis and anaerobic transepithelial sodium transport is suggested by two observations. First when sodium was removed from the bathing media, anaerobic glycolysis was diminished by approximately 45%. Second, when the net rate of sodium transport was compared with lactate accumulation in the bathing media, a linear relationship was observed. Thus in group data a stoichiometric relationship exists between anaerobic glycolysis and net sodium transport despite the fact that lactate formation reflects the total rate of glycolysis, including that involved in cell functions other than sodium transport.

The ATP studies document the ability of the isolated bladder to maintain tissue ATP concentrations at approximately the same level under anaerobic as under aerobic conditions. This occurs despite the fact that ATP utilization from sodium transport remains relatively unchanged. Thus glycolytic energy production must be extremely efficient. It is possible on the basis of the data obtained to examine the relationship between ATP production and anaerobic sodium transport. In 13 experiments in which bidirectional sodium flux studies and lactate accumulation measurements were made simultaneously (Figure 6) the average value for net sodium transport was $3.16 \mu\text{m}$ per hour per 7 cm^2 of bladder. The mean value for lactate formation was $0.28 \mu\text{m}$ for the same bladders during the same period of study. Using the results of the choline chloride Ringer's study to deduce the fraction of lactate related to sodium transport it was calculated that $0.13 \mu\text{m}$ of the total lactate formed were linked to sodium transport. Endogenous glycogen served as a source of the lactate; therefore 3 moles of ATP were generated per mole of glucose residue metabolized or $1 \frac{1}{2}$ moles of ATP were formed for every mole of lactate generated. Thus, 3.16 moles of sodium were transported for 0.195 moles of ATP. Consequently over 15 Eq of sodium are actively transported per mole of ATP generated from anaerobic glycolysis. This is an extremely high value and suggests that there may be an extraordinarily efficient system for anaerobic ATP synthesis in the turtle bladder.

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TABLE I

Simultaneous measurements of Na^{22} influx, Na^{24} efflux, and short-circuit current (S.C.C.) in oxygenated and deoxygenated Ringer's solutions

Exp.	Period	100% O_2					100% N_2					
		Influx, μamps	Efflux, μamps	Net, μamps	S.C.C., μamps	% Net flux S.C.C.	Period	Influx, μamps	Efflux, μamps	Net, μamps	S.C.C., μamps	% Net flux S.C.C.
1	1	210.1	11.5	198.6	270.4	73.4	4	320.3	45.8	274.5	105.5	260.2
	2	213.9	44.5	169.4	265.7	63.8	5	168.0	23.9	144.1	22.8	632.0
	3	249.5	27.9	221.6	257.7	86.0	6	71.0	18.5	52.5	7.6	690.8
Mean				196.5	264.6	74.4						
2	1	163.7	45.0	118.7	152.0	77.6	4	112.3	27.3	85.0	83.9	101.3
	2	142.0	33.5	108.5	141.0	77.0	5	112.3	37.3	75.0	47.3	158.6
	3	165.6	27.9	137.7	129.5	106.3						
Mean				121.6	141.1	87.0						
3	1	178.2	17.7	160.5	129.5	123.9	4	120.7	26.8	102.9	104.3	98.7
	2	150.9	47.2	103.7	130.7	79.3	5	119.5	21.7	97.8	52.6	185.9
	3	117.4	7.8	109.6	140.8	77.8	6	71.8	7.8	64	35.1	182.3
Mean				124.6	133.7	93.7						
4	1	131.1	84.2	46.9	69.5	67.5	4	105.1	7.8	97.3	47.0	207.0
	2	120.9	53.9	67.0	69.5	96.4	5	84.2	15.8	68.4	41.7	164.0
	3	37.5	10.7	26.8	68.5	29.1						
Mean				46.9	69.2	67.7						
5	1	109.3	37.3	72.0	78.4	91.8	4	169.7	42.6	147.1	68.0	216.3
	2	108.3	40.2	68.1	83.8	81.3	5	115.8	22.8	93.0	40.1	231.9
	3	112.8	33.8	79.0	84.0	94.9						
Mean				73.0	82.1	86.0						

S.C.C. - net flux: Mean value - 25.59
(SE - 8.0)
 $P < 0.01$

Net flux - S.C.C.: Mean value - 53.81
(SE - 14.20)
 $P < 0.01$

Measurements in oxygenated Ringer's solution were initiated after steady-state conditions were achieved and 30 min after addition of the Na^{22} to the mucosal solution and Na^{24} to the serosal solution. Three consecutive 30-min periods were obtained in oxygenated Ringer's solution. Thereafter the gas was changed to 100% N_2 , and a 20-min interval was allowed for deoxygenation before measurements were continued. Values are expressed in $\mu\text{amps}/7 \text{ cm}^2$ of bladder.