Energetics of Anaerobic Sodium Transport by the Fresh Water Turtle Bladder

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ABSTRACT Certain of the metabolic events associated with anaerobic sodium transport by the isolated bladder of the fresh water turtle have been investigated. The data suggest that energy for this transport arises from glycolysis and that endogenous glycogen was the major and perhaps the sole source of substrate. The rate of anaerobic glycolysis, as determined by lactate formation, correlates well with the rate as determined by glycogen utilization. Using lactate formation as the index of anaerobic glycolysis, a linear relationship was observed between glycolysis and net anaerobic sodium transport. In the absence of sodium transport, glycolysis decreased by approximately 45 per cent. Tissue ATP concentrations were maintained at about the same level under anaerobic as under aerobic conditions. Finally if it is assumed that in the conversion of glycogen to lactate anaerobically, 3 moles of ATP are generated per mole of glucose residue, an average of over 15 equivalents of sodium were transported for every mole of ATP generated.

The urinary bladder of the fresh water turtle is among the specialized biologic membranes that actively transport sodium transcellularly *in vitro*. However, in contrast to similar structures such as the proximal renal tubule, the frog skin, and the toad bladder, the turtle bladder transports sodium (transepithelially) almost as well in the absence of oxygen as in its presence (1). Presumably, therefore, glycolysis plays a major role in the synthesis of free energy for anaerobic electrolyte transport.

In the present studies, an effort has been made to define the quantitative relationships between anaerobic glycolysis and anaerobic sodium transport in the isolated turtle bladder. Because glycolysis is a more primitive, and certainly less complex, pathway for energy production than mitochondrial-linked oxidative pathways, investigation of the turtle bladder could contribute importantly to the understanding of the coupling between metabolic energy and active transport.

METHODS

The urinary bladders of fresh water turtles (*Pseudemys scripta elegans*) were suspended as diaphragms between the two symmetrical halves of lucite chambers. Twenty-five ml of Ringer's solution were added to each hemichamber in the usual experiment. The composition of this solution is the same as that described previously (1) except that in the present experiments, neither glucose nor any other substrate was added to the media. After the bladders were mounted, they were maintained in a short-circuited state except for the brief intervals required to measure potential difference every 10 to 15 minutes.

Initially the Ringer's solutions were oxygenated, using either room air or 100 per cent oxygen as the gas phase. After steady state conditions were achieved (1), measurements were obtained over a 60 to 90 minute period under aerobic conditions in the majority of the experiments. Thereafter, the gas phase was changed to 100 per cent nitrogen, and unless specifically indicated in the text, the nitrogen was continued throughout the remainder of each experiment. The nitrogen was passed through an oxygen trap (2) before it entered the Ringer's solutions in order to remove trace quantities of oxygen. Fifteen to 20 minutes were allowed for deoxygenation of the Ringer's to occur. Then measurements were made over a 60 to 90 minute period under anaerobic conditions.

Net sodium transport was estimated as the Na^{22} influx (from mucosa to serosa) minus the Na^{24} efflux (from serosa to mucosa). The two flux rates were determined simultaneously as described previously (1). Lactate formation was measured in both serosal and mucosal bathing media during the aerobic control periods and again during the anaerobic periods. Penicillin and streptomycin were added to the bathing solutions in the initial experiments. However, in the absence of substrate in the bathing media, no differences could be detected in the rate of lactate formation with or without the antibiotics. The lactate was measured (3) by the conversion of DPN to DPNH in the presence of LDH in an alkaline medium. Pyruvate formed in the reaction was removed by adding hydrazine. The DPNH was measured using a Farrand fluorometer.

A separate series of experiments was performed for the measurement of ATP and glycogen concentrations. For the ATP measurements individual bladders were cut into 9 to 12 segments approximately 1 cm^2 in area. These were divided into three groups, each of which contained 3 to 4 segments totalling about 100 mg wet weight. One group was processed immediately to obtain control values. The other two groups were transferred into Erlenmeyer flasks containing 10 ml of Ringer's solution. The flasks were incubated at room temperature (approximately 22°C) in a Dubnoff metabolic shaker. For the first hour 100 per cent O_2 was used as the gas phase in all flasks. Thereafter the gas phase was changed to 100 per cent nitrogen in half of the flasks while oxygen was continued in the other half. The second phase of these experiments was continued for 1 to 2 hours.

For the determination of ATP, the tissue segments in each group were transferred into 10 ml of distilled water and placed in a boiling water bath for 10 minutes. The volume then was reconstituted to 10 ml and ATP concentrations were measured

using the firefly technique (4). Values were expressed as micrograms per milligram of wet weight.[1]

A similar system was used for glycogen determinations, except that larger segments of bladder (2 to 4 cm^2) were used. After incubation, the tissues were homogenized and the glycogen was hydrolyzed to glucose in 2 N H_2SO_4 (5). Glucose then was determined by a glucose oxidase method.[2] In a number of glycogen experiments, lactate was measured in the Ringer's solutions in order to compare glycogen utilization with lactate formation.

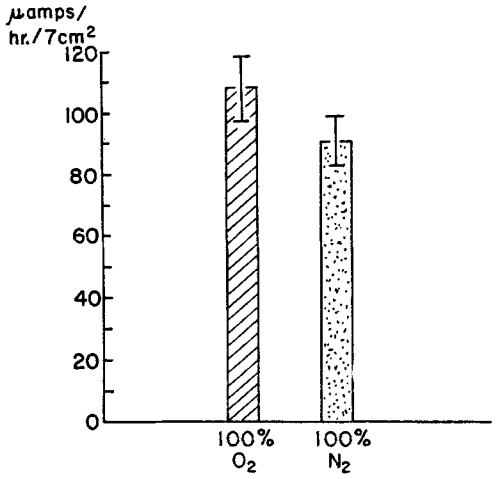

FIGURE 1. In each experiment, 2 to 3 30-minute periods were obtained, with oxygen as the gas phase. Then a similar number of observations was obtained with nitrogen as the gas phase. Net transport represents the Na^{22} influx (from mucosa to serosa) minus Na^{24} efflux (from serosa to mucosa). The two flux rates were determined simultaneously. Each value represents the mean ±1 standard error.

RESULTS

Fig. 1 depicts the results of 18 bidirectional sodium flux studies.[3] The net flux (*i.e.* influx minus efflux) was only 16 per cent less under anaerobic than under aerobic conditions.

Lactate formation was measured in the serosal and mucosal bathing solutions in 25 experiments and the data are shown in Fig. 2. In 24 of the 25 studies, lactate formation increased after the gas phase was changed from 100 per cent O_2 to 100 per cent N_2. The mean value was 162×10^{-3} μmoles (per 7 cm^2 of bladder per hour) in O_2 compared to 347×10^{-3} μmoles in N_2. The increase was 114 per cent and is highly significant ($p < 0.01$).

In 8 experiments, oxygen was reintroduced after the anaerobic measurements were completed. In each experiment O_2 suppressed lactate formation. The results of these studies are summarized in Table I. In 2 experiments a second period of anaerobiosis was established and in both, lactate formation increased again.

[1] The recovery of ATP, added to the tissue before boiling, averaged 104 per cent (±1.6 SE).
[2] Glucostat kits, Worthington Biochemical Company, Freehold, New Jersey.
[3] Five of these 18 studies were included in the previous paper (1).

In order to investigate the relationship between sodium transport and glycolysis, measurements were made of the rate of lactate formation in an anaerobic system before and after removal of sodium ions from the bathing Ringer's solutions. Control measurements were made with conventional sodium Ringer's solution in both hemichambers. Thereafter the bladders were

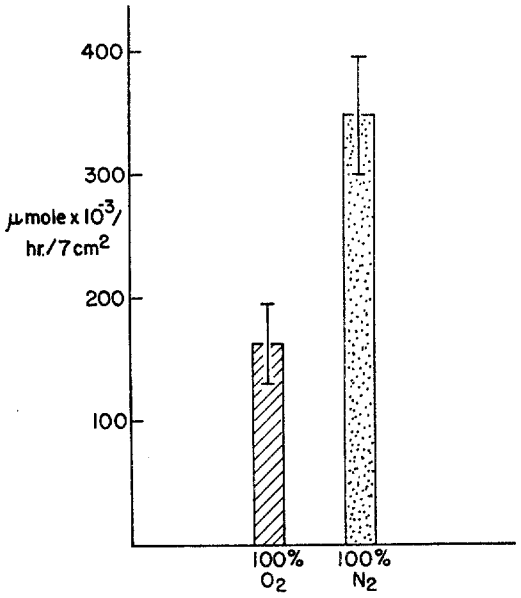

FIGURE 2. Lactate formation was measured for 1 hour under aerobic conditions and for 1 hour under anaerobic conditions. Studies were initiated after the membranes had reached steady state conditions in oxygenated Ringer's (1). Each value represents the mean ± 1 standard error.

TABLE I

LACTATE FORMATION DURING SEQUENTIAL CHANGES IN THE GAS PHASE MICROMOLES × 10^{-3}/HR./7 CM^2

No. of experiments	100 per cent oxygen	100 per cent nitrogen	100 per cent oxygen
8	225 ±56.2	421 ±71.6	184 ±21.9

Studies were initiated after the membranes had equilibrated in O_2 (1). Each of the gas phases was maintained for 60 minutes.
Values represent the means and standard errors of means.

rinsed from 2 to 3 times with deoxygenated choline chloride Ringer's and fresh deoxygenated choline Ringer's was used as the bathing solution in contact with the two surfaces of the bladder. 100 per cent N_2 was maintained as the gas phase. The potential differences (PD) and the short-circuit current (S.C.C.) fell precipitously to zero after the choline chloride Ringer's was added. Values for lactate formation are shown in Fig. 3. In the presence of

sodium transport the mean value was 380 × 10^{-3} μmoles (per 7 cm^2 per hour) and in the absence of sodium transport the value fell to 208 × 10^{-3} μmoles. The net decrease is 45.4 per cent. In 9 studies, the experimental sequence was reversed and measurements were made first with choline chloride Ringer's then with sodium Ringer's. Lactate formation increased in 8 of the 9 experiments by an average of 57 per cent. A decrease was noted in one experiment and for the group the increase averaged 36 per cent.

Lactate accumulated on the opposite sides of the turtle bladders in an asymmetric fashion regardless of the experimental conditions. The values for

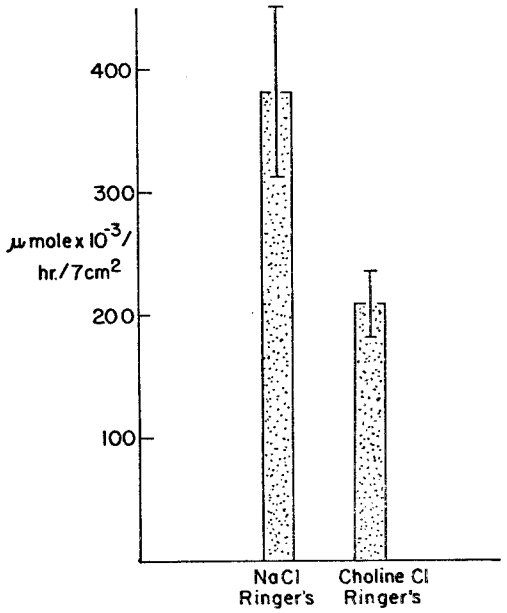

FIGURE 3. Sixteen experiments were performed to measure lactate formation and the details are described in the text. Each bladder was exposed to 1 hour of incubation in NaCl Ringer's (after steady state conditions were achieved) and 1 hour of incubation in choline Ringer's. Each value represents the mean ±1 standard error.

serosal *versus* mucosal lactate accumulation are shown in Fig. 4. In sodium Ringer's solution, the serosal accumulation markedly exceeded the mucosal accumulation both in oxygen and in nitrogen. The same phenomenon was observed with choline chloride Ringer's solution.[4]

The values for ATP concentrations of bladder segments incubated under aerobic and anaerobic conditions are shown in Table II. The mean value for the control tissues (*i.e.* those processed immediately after removal from the animals) was 0.25 μg per mg wet weight. The mean value for tissues from the same animals incubated in oxygenated Ringer's was 0.20 μg per mg wet weight, while the value for the tissues incubated under anaerobic conditions

[4] A similar pattern has been reported by Leaf for the urinary bladder of the toad (6).

was 0.18. The difference between the aerobic and anaerobic values is not statistically significant ($p < 0.2$).

Fig. 5 depicts the glycogen concentrations of bladder segments. Aerobic incubation in substrate-free media was associated with a decrease in glycogen

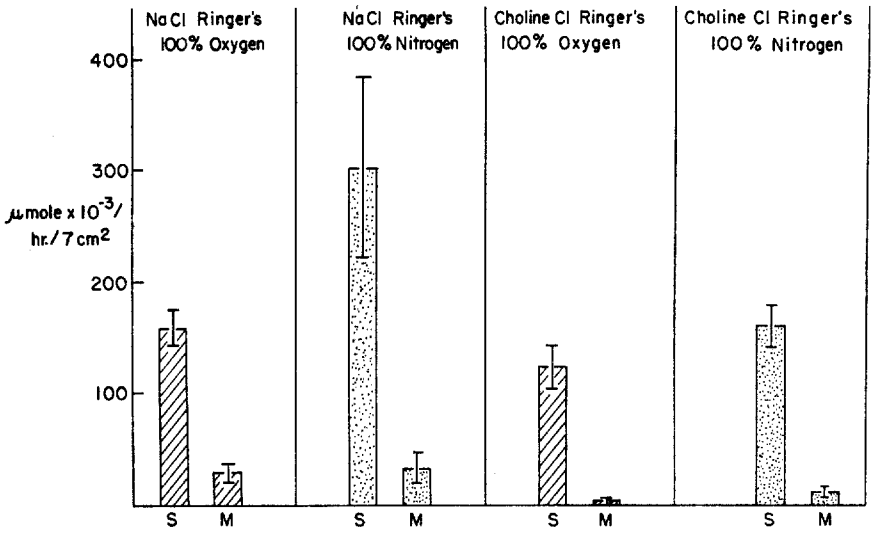

FIGURE 4. The number of experiments in each group varied from 8 to 12. Measurements of lactate were made for 1 hour periods. Each value represents the mean ±1 standard error.

TABLE II

ATP CONCENTRATION OF ISOLATED TURTLE BLADDER MICROGRAMS PER MILLIGRAM WET WEIGHT

No. of experiments	Control	100 per cent O_2	100 per cent N_2
8	0.25 ±0.03	0.20 ±0.02	0.18 ±0.02

Control values for ATP concentrations were measured immediately after removal of the bladder from the animal. The other values represent ATP concentrations after 2 hours of incubation. (See text for details.)
Values represent the means and standard errors of means.

concentration (in comparison to control values). Furthermore glycogen utilization was greater in an anaerobic than in an aerobic environment; the difference between these two groups is statistically significant ($p < 0.01$).

The correlation between glycogen utilization and lactate formation in bladder segments is shown in Table III for 19 experiments. The glycogen values are expressed as micromoles of glucose residue and theoretically 1 mole

of glucose should give rise to 2 moles of lactate if all of the lactate arises from glycogen and if none of it is further metabolized. The data show quite good agreement between the two expressions of anaerobic glycolysis. Thus the mean value for glycogen utilization was 10.4 μmoles per gm (1.88 μg/mg) while the mean value for lactate recovery was 21.9 μmoles/gm (1.97 μg/mg).

The relationship between net sodium transport and anaerobic glycolysis, as measured by lactate formation, is shown in Fig. 6. The two parameters were measured simultaneously under anaerobic conditions in 13 experiments. The

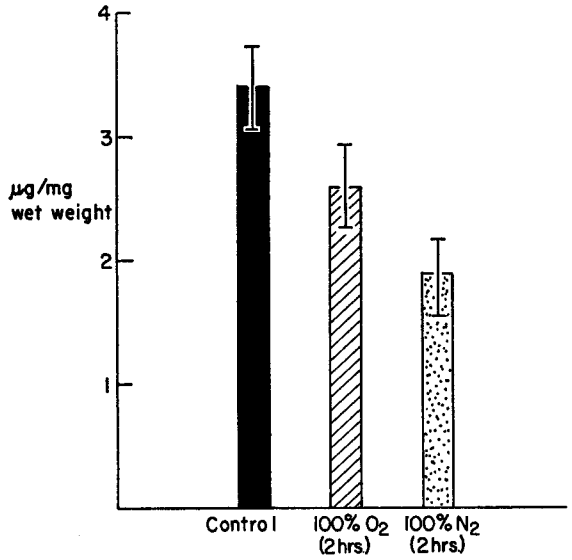

FIGURE 5. Eight experiments were performed. The control tissues were analyzed for glycoger immediately after removal from the turtles. The system of incubation is described in the text. Each value represents the mean ±1 standard error.

rate of lactate formation is plotted on the ordinate against the rate of net sodium transport on the abscissa. Each value for sodium transport represents the mean of at least two 30 minute periods. The linear regression line is derived by the method of least squares and the correlation coefficient is 0.81. The function is statistically significant ($p < 0.02$).

DISCUSSION

The foregoing data indicate that glycolysis comprises the major, and perhaps the sole, source of energy for anaerobic sodium transport across the isolated turtle bladder. Furthermore 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 a direct coupling between anaerobic glycolysis and anaerobic transepithelial sodium transport is suggested by two observations.[5] First, when sodium was removed from the bathing media (*i.e.* when the bladders were suspended in choline chloride Ringer's solutions), anaerobic glycolysis was diminished by approximately 45 per cent. 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 the lactate values reflect total rates of glycolysis and not just that portion of anaerobic metabolism linked to sodium transport.

TABLE III

CORRELATION BETWEEN GLYCOGEN UTILIZATION AND LACTATE FORMATION

Experiment	Glycogen utilized	Lactate evolved
	μmole/gm	*μmole/gm*
1	9.5	22.6
2	11.7	22.7
3	15.8	41.0
4	13.4	29.4
5	12.9	14.9
6	4.1	9.4
7	6.7	15.6
8	4.1	8.9
9	9.3	19.0
10	11.7	23.9
11	6.0	20.2
12	13.3	24.4
13	17.6	42.3
14	21.7	42.1
15	3.2	3.0
16	14.0	42.4
17	7.2	2.7
18	14.6	28.8
19	1.4	2.6
Mean	10.4 (±1.2)	21.9 (±3.1)

Measurements of ATP demonstrate the ability of the isolated bladder to maintain tissue ATP concentrations at the same level under anaerobic conditions as under aerobic conditions; although active sodium transport, a major

[5] A close coupling between sodium transport and oxygen consumption has been established previously in several epithelial cell systems and a discussion of these data together with pertinent references is contained in a recent paper by Whittam (7).

source of energy utilization remains roughly the same in the anaerobic system. Hence, although measurements of ATP turnover are not available, this provides indirect evidence that ATP synthesis continued in the absence of molecular oxygen.

It is possible on the basis of the present data to examine the relationship

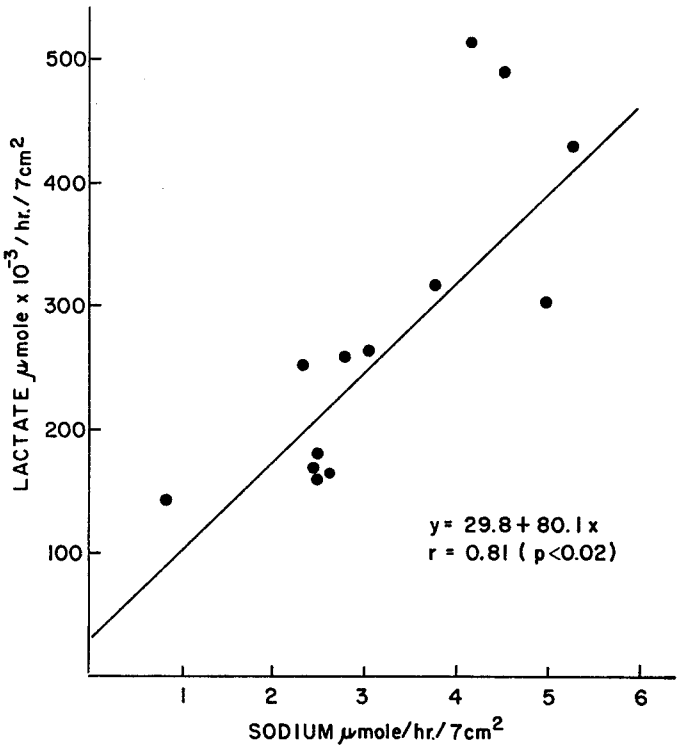

FIGURE 6. Values for sodium transport and lactate represent the mean of 2 to 3 consecutive 30 minute periods of measurement. The transport term represents net flux (*i.e.* influx minus efflux).

between ATP and anaerobic sodium transport. In the 13 experiments in which bidirectional sodium flux rates and lactate accumulation rates were measured simultaneously (Fig. 6), the average value for net sodium transport was 3.16 μmoles/hr./7 cm². This corresponded to a mean value of 0.28 μmole of lactate for the same bladders during the same period of time. In the experiments using choline chloride Ringer's it was found that slightly less than half of the total lactate formation was linked to sodium transport. Hence for purposes of the present calculations it will be assumed that out of the total of 0.28 μmole of lactate, 0.13 μmole was linked to sodium transport. Endogenous glycogen served as the source of the lactate; therefore, 3 moles of ATP

were generated per mole of glucose residue metabolized, or 1.5 moles of ATP were formed for every mole of lactate generated. It would appear, therefore, that 3.16 moles of Na were transported for 0.195 mole of ATP. Hence over 15 equivalents of sodium would be actively transported per mole of ATP generated from anaerobic glycolysis. This must be considered as a rough approximation, since the utilization of preexisting cellular stores of ATP is not taken into account and, on the other hand, intracellular lactate was not measured. Nevertheless, it would appear that the cost of transporting sodium in this system may be extremely small[6]; it also is evident that none of the existing models of active sodium transport will allow for this high a ratio between sodium ions and ATP (8).

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[6] This conclusion would obtain even if more than 50 per cent of the lactate produced were coupled to sodium transport (as is suggested by the intercept in Fig. 6).