The total activity of liver glycogen synthetase, assayed in the presence of glucose-6-phosphate, was greatly increased by electrical stimulation of the vagus nerve (Table 1), almost attaining maximum within 5 minutes of the onset of stimulation ($P<.01$). The G6P-i activity of liver glycogen synthetase, assayed without glucose-6-phosphate, changed insignificantly before and after stimulation of the vagus nerve ($P>.3$). Therefore the increase in total activity after vagal stimulation can be attributed to the increase in G6P-d activity of the enzyme, although the exact biochemical meanings of the two types of activity of glycogen synthetase in liver are still unclear.

Steiner *et al.* (*4*) reported that injection of insulin in rats, made diabetic with alloxan, markedly increased both G6P-d and G6P-i activities of liver glycogen synthetase. The result was confirmed in normal rabbits (Fig. 1): when anesthetized rabbits were injected intravenously with insulin (2 units per kilogram) free of glucagon, both types of activity of glycogen synthetase in the liver increased gradually during 20 to 40 minutes after the injection ($P<.01$).

The effect of vagal stimulation on glycogen synthetase activity of the liver is unlikely to be a secondary effect of insulin secreted from the pancreas, since pancreatectomized and intact rabbits gave essentially the same results (Table 1 and Fig. 1).

The response of glycogen synthetase to vagal stimulation is much faster than that to administration of insulin (Fig. 1): the maximum response was delayed only about 5 minutes with the former, versus about 40 minutes with the latter. My observations with liver phosphorylase have been similar: the response of liver phosphorylase to stimulation of the splanchnic nerve is faster than to injection of epinephrine (*1*, *5*). Again, vagal stimulation affected only the G6P-d activity of liver glycogen synthetase, whereas insulin influenced both its G6P-d and its G6P-i activity.

Neither G6P-d nor G6P-i activity of liver glycogen synthetase was affected significantly by electrical stimulation of the splanchnic nerve of rabbits ($P<.3$). Nevertheless, the effect of vagal stimulation on glycogen synthetase was counteracted almost completely by simultaneous stimulation of the splanchnic nerve (Table 1). A similar situation has already been observed with phosphorylase activation, the effect of splanchnic-nerve stimulation on liver phosphorylase being counteracted by simultaneous stimulation of the vagus nerve (*5*).

These results suggest that parasympathetic stimulation results in acceleration of glycogenesis in liver; this acceleration may affect the reported decrease in blood glucose after parasympathetic stimulation (*2*). It is noteworthy that the sympathetic and parasympathetic nerves regulate the activities of separate enzymes implicated in glycogen metabolism in the liver. Stimulation of the sympathetic nerve causes glycogenolysis in liver by stimulating the activation of phosphorylase and glucose-6-phosphatase (*1*), while stimulation of the parasympathetic nerve seems to cause glycogenesis in liver by increasing the activity of glycogen synthetase. These effects of the autonomic nerves on phosphorylase or glycogen synthetase are counteracted by simultaneous action of the antagonistic nerves. It seems that there is a dual regulatory system—hormonal and nervous—in glycogen metabolism in the liver, and that the nervous regulation is much faster than the hormonal.

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Regulation of Intracellular Sodium Concentrations in Rat Diaphragm Muscle

Abstract. *The concept is suggested that the sodium pump mechanism is influenced by the transmembrane electrical potential, and that the pump acts to maintain a constant electrochemical gradient for sodium. Evidence leading to this suggestion was obtained in rat diaphragm muscle by altering systematically the transmembrane chemical gradient for sodium ions and the transmembrane voltage. The voltage changes were produced by varying the extracellular and intracellular potassium ion concentrations. In each case the intracellular sodium concentration changed, presumably by activity of the sodium pump, so that the total electrochemical gradient for sodium was restored.*

At the resting potential of the muscle cell the ratio of internal and external potassium concentrations is close to that predicted by the Nernst equation for a potassium electrode (*1*). Sodium, however, is far from its electrochemical equilibrium potential of about +40 mv. There is a large concentration gradient of sodium from the outside toward the inside, as well as a negative electrical potential in the cell attracting the positively charged sodium ions. The electrochemical potential of the cell is therefore 110 to 120 mv away from the sodium equilibrium, and a driving force of this magnitude is available to promote entry of sodium into the cell. The passive influx-efflux ratio can be calculated from this gradient (*2*) and can be shown to be greatly in favor of influx. Yet in the steady state, where internal sodium concentration is constant, the influx and efflux are necessarily equal. The sodium efflux that is in excess of the predicted passive efflux has been called active transport (*3*) and attributed to a "sodium pump." This part of the sodium efflux has been shown to be dependent on metabolic energy (*4*).

Since the action of the pump is to extrude sodium from the cell, it would be expected that the internal sodium concentration itself might be a factor in the regulation of pump activity. This relationship has been shown by several workers, and there also appears to be some dependence on external sodium concentration (*5*). In almost all studies of the ionic regulation of sodium efflux the assumption has been made that the transmembrane electrical potential gradient does not influence sodium "pumping." However, Conway and his co-workers (*6*, *7*) have suggested that the regulating factor is the total chemical and electrical gradient. According to their suggestion the cell maintains a constant electrochemical gradient for sodium (the "critical energy

Table 1. Effect of varying extracellular potassium ion concentration on intracellular sodium ion concentration $(Na)_i$ and transmembrane potential (E_m). The sodium electrochemical gradient in millivolts is calculated from the equation in the text. We used a minimum of six diaphragms for each group.

$(Na)_o$ (mM)	$(K)_o$ (mM)	$(Na)_i$ (mM)	E_m (mv)	Sodium electrochemical gradient (mv)	Inulin space (ml/100 g)
115	4.7	23.1 ± 0.8	−70 ± 3.3	113.5	15.5 ± 0.6
115	7.2	22.8 ± 0.9	−67 ± 3.4	111.5	12.8 ± 0.6
115	9.7	21.8 ± 1.1	−64 ± 3.3	109	13.6 ± 0.2
115	12.2	19.7 ± 1.6	−58 ± 5.1	105.5	12.1 ± 0.4
115	14.7	18.9 ± 1.2	−52 ± 5.0	101	11.1 ± 0.4
115	19.7	14.9 ± 0.8	−45 ± 3.2	100	9.9 ± 0.4
115	34.7	9.6 ± 1.1	−36 ± 4.0	102	7.8 ± 0.6

barrier") by pumping sodium out when the gradient falls below the "barrier" and ceasing to pump when enough sodium is removed to restore the total gradient to the level of the "barrier." The pump would then be expected to adjust the internal sodium concentration, and thereby the sodium chemical gradient, so as to restore the total electrochemical gradient. According to this explanation of the control of the sodium pump, it should be sensitive not only to internal and external sodium concentrations but also to the membrane potential. A simple way to alter the membrane potential in the range where the cell approximates a potassium electrode is to change the internal-external potassium concentration ratio. A certain dependence of the pump on external potassium has been known since the work of Steinbach (*8*). Horowicz and Gerber (*9*) have recently studied further the effects of external potassium on the sodium efflux rate and found evidence supporting the suggestion that the efflux is voltage dependent. The experiments reported here were made in order to examine the electrochemical gradients for sodium at a new steady state after alterations were made in the chemical gradient of sodium, the electrical gradient, or both.

Intact rat diaphragms (*10*) were used in these studies. The standard incubation solution was hypotonic: Na, 115 mM; K, 4.7 mM; Ca, 2.03 mM; Cl, 100 mM; PO_4, 0.95 mM; SO_4, 0.95 mM; and HCO_3, 20 mM. This solution was saturated by a 95 percent O_2 and 5 percent CO_2 mixture, with a pH of 7.2 to 7.4. In experiments where the sodium concentration was lowered, Na was replaced by choline. Potassium concentration was increased by addition of solid KCl to the standard solution. Hyperosmotic solutions were made by addition of sucrose to a concentration of 220 mM in the standard solutions containing 19.7, 14.7, 9.7, and 4.7 mM potassium. Resting membrane potentials were measured with glass micropipettes filled with 3M KCl and selected for resistances of 10 to 20 Mohm and low tip potentials. The average value from impalement of 15 to 20 cells was accepted as the membrane resting potential. The intracellular sodium was measured by the quantity of Na^{22} in the intracellular phase at isotopic equilibrium (*11*). For these determinations extracellular space was measured by isotopic inulin distribution. The electrical and chemical determinations were done on separate diaphragms treated identically, and each value reported is the average of at least three diaphragms. The experimental procedure for the chemical measurements was an incubation of the tissue for 45 minutes with Na^{22} in the standard solution. The tissue was then exposed to the test solution, and analysis was made after 30 minutes. Earlier studies had shown that this was sufficient time for isotopic equilibrium and for a new steady-state internal sodium concentration to be achieved. The electrical measurements were also made after a 30-minute exposure to the test solution. The transmembrane electrochemical gradient for sodium was calculated by the formula

$$\left[\frac{RT}{F}\ln\frac{(Na)_o}{(Na)_i}\right] - E_m$$

where R, T, and F have their usual meanings, $(Na)_o$ and $(Na)_i$ are external and internal sodium concentrations, respectively, and E_m is the intracellular potential (inside negative with respect to the bathing solution).

The effect of changes in extracellular potassium concentration was similar to that found by Hodgkin and Horowicz (*1*) (Fig. 1). With the high concentrations of extracellular potassium the potential remained more negative than the prediction of the simple Nernst equation, probably because under the conditions of these experiments the concentration of intracellular potassium rose somewhat. The results of increasing the extracellular potassium on the internal sodium concentration are shown in Table 1. In each case the sodium concentration within the cell fell to a new steady-state value. Calculation of

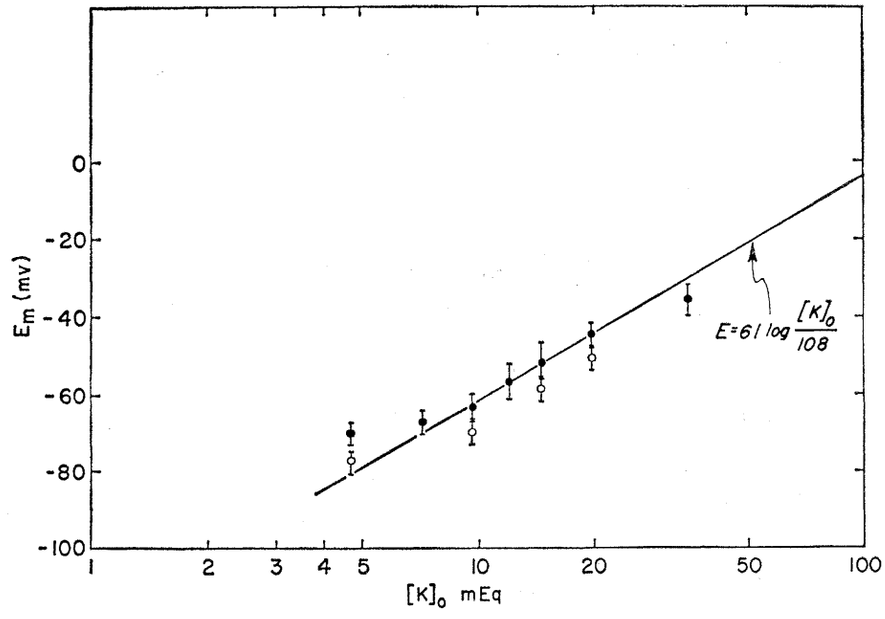

Fig. 1. Relation between membrane potential (E_m) and log $[K]_o$. The closed circles indicate average values of membrane potential after 30-minute equilibration in hypotonic solution with different potassium ion concentrations. Open circles indicate values of membrane potential after increase in osmolality by addition of 220 mM sucrose. The vertical bars indicate 2 standard deviations. The line is the predicted behavior of a potassium electrode with internal potassium concentration assumed to be 108 mM.

the electrochemical gradient for sodium, by use of the new internal sodium concentration and the new transmembrane voltage value, showed that the gradient had changed very little from its original value of about 110 mv (2600 cal). No influence of these varying potassium concentrations on the rate of Na^{22} uptake of the tissues was found, which suggests that influx was not significantly changed.

The chemical gradient of sodium was decreased by substitution of choline for sodium in the bathing solution. There was no significant change in the resting membrane potential. The internal sodium concentration fell under these conditions until the chemical and the electrochemical gradients were restored approximately to their previous values (Table 2). When the muscles were exposed to solutions made hyperosmotic by addition of sucrose, they may be presumed to have lost water and to have experienced an increase in both sodium and potassium concentrations. That the internal potassium concentration was raised is supported by the finding that the cells became hyperpolarized (Fig. 1). It is interesting to note that with the external potassium concentration of 4.7 mM, where the cell does not appear to be a good potassium electrode, the cell hyperpolarizes in response to an increased intracellular potassium concentration. Calculation of the expected electrochemical gradient for sodium indicated that no change should have occurred if sucrose caused a loss of only water. Measurement of the sodium concentrations confirmed this prediction, in that the internal sodium concentrations were increased by the amount expected from the water loss so that the electrochemical gradient was not altered (Table 3). The slight differences in internal sodium concentrations shown in Table 1 and Table 3 exist because these experiments were performed on separate groups of diaphragms. Mullins and Awad (*12*) have reported that doubling the osmotic pressure of their bathing solution with sucrose did not alter the isotopic sodium efflux rate in frog sartorius muscle. Keynes (*13*) has reported some change in efflux when he increased the osmotic pressure with dextrose.

In experiments where the extracellular potassium was increased, the electrochemical gradient of sodium was somewhat lower than that found in tissue incubated with more normal potassium concentrations. It was first thought possible that a new steady state had not been achieved with these conditions after 30 minutes. This was investigated by measurement at longer times (up to 90 minutes) with no further fall in internal sodium concentration. The technique of measuring internal sodium employed in these experiments depends on an accurate measurement of the extracellular space and a subtraction of the sodium in this space from the total present in the tissue. Because of this, quite small errors in the extracellular space measurement produce large errors in the calculated internal sodium concentration, especially where the internal sodium concentration is low. A systematic error yielding a consistently higher internal sodium concentration than is actually present would lead to results as we have reported them, even if the sodium electrochemical gradient has returned to normal. Routine chemical measurements of sodium by flame photometry were not done in these experiments. A separate experiment was done that suggested that the chemical measure might be slightly higher than the one determined by isotopic dilution (*14*). Because of the difficulty of being certain what is extracellular space, we made no further test of this possibility. It remains possible that the small reduction in the electrochemical gradient for sodium in high-potassium solutions is a real phenomenon, either because the potassium injures the cells in some way or because the pump is unable to compensate for the considerable reduction in membrane potential.

The implication of the pattern of changes in intracellular sodium concentration in this work is that the cell establishes an electrochemical gradient for sodium across the membrane. Such a mechanism would operate to achieve the asymmetrical distribution of sodium and potassium as well as the transmembrane potential. According to this interpretation, the rate of sodium extrusion would depend not only on the sodium intracellular-extracellular ratio (the chemical potential gradient for sodium) but also on the transmembrane potential. A precedent for such an effect can be found in the sodium pump in frog skin. Ussing and Zerahn (*15*) studied the effect of trans-skin potential on active sodium movement when the chemical gradient was abolished. They found that the pumping was inversely related to the magnitude of the potential, and appeared to cease at a potential of 110 mv. The flux measurements of Horowicz and Gerber (*9*) with frog semitendinosis muscle lend strong support to the concept of voltage dependence. They found that increasing extracellular potassium from 2.5 mM to 7.5 mM exerted only minor effects on Na efflux. Above 7.5 mM potassium a marked increase in the sodium efflux rate was seen. This corresponded to the point where the cell began to be depolarized by the increased extracellular potassium. They observed no effect of increased extracellular potassium (15 mM) on Na in-

Table 2. Effect of varying extracellular sodium ion concentration on intracellular sodium ion concentration $(Na)_i$, transmembrane potential (E_m), and the calculated sodium electrochemical gradient. Three diaphragms are included in each group.

$(Na)_o$ (mM)	$(K)_o$ (mM)	$(Na)_i$ (mM)	E_m (mv)	Sodium electrochemical gradient (mv)	Inulin space (ml/100 g)
140	4.7	30 ± 3.4	−73.6 ± 1.8	106	15.5 ± 0.6
115	4.7	23 ± 2.2	−72 ± 2.1	116	15.5 ± 0.6
70	4.7	17.3 ± 1.4	−72 ± 2.1	110	15.5 ± 0.6
40	4.7	11.8 ± 1.1	−72 ± 2.1	106	15.5 ± 0.6
25	4.7	7.5 ± 0.5	−72 ± 2.1	107	15.5 ± 0.6
10	4.7	3.2 ± 0.2	−72 ± 2.1	106	15.5 ± 0.6

Table 3. Effect of hyperosmotic solutions on intracellular sodium concentration $(Na)_i$, transmembrane potential (E_m), and the sodium electrochemical gradient. Six diaphragms were included in each group.

$(Na)_o$ (mM)	Sucrose (mM)	$(K)_o$ (mM)	$(Na)_i$ (mM)	E_m (mv)	Sodium electrochemical gradient (mv)	Inulin space (ml/100 g)
115		4.7	24.9 ± 2.4	−70 ± 3.3	111	15.5 ± 0.6
115	220	4.7	31.7 ± 2.9	−77 ± 3.8	112	14.9 ± 1.0
115		14.7	19.3 ± 1.6	−52 ± 5.0	102.5	11.1 ± 0.4
115	200	14.7	25.9 ± 3.0	−59 ± 1.5	99	11.8 ± 0.7

flux. In *Sepia* axons, however, Hodgkin and Keynes (*16*) were not able to influence sodium efflux by passage of hyperpolarizing currents.

The molecular mechanism by which the sodium extrusion system could be influenced by transmembrane voltage is not readily apparent. Conway (*6*) has proposed an oxidation-reduction reaction, as in the cytochrome system, and Kernan (*17*) has found evidence that the cytochrome chain is indeed active during pumping. This suggestion has been criticized on the basis of its energetic inefficiency. It is not difficult to imagine that charged elements in the membrane might be sensitive to electrical potential or that the electrical field in the membrane might influence binding sites for sodium, but our understanding of the sodium pump is inadequate at this time to warrant speculation about its mechanism.

An interesting part of this interpretation that the cell acts to maintain constant its electrochemical gradient for sodium is its voltage dependence. As a result, depolarization alone will activate the pump. An action potential, while produced by a current of sodium ions into the cell, might itself activate the pump. The prolonged action potential of cardiac muscle (250 to 500 msec) should be a good stimulus, and as suggested by Page (*18*) an electrogenic pump might even contribute to the repolarization of the action potential. Little information is available to indicate how rapidly the pump may be stimulated or depressed. Adrian and Slayman (*19*) have found that pump activity appeared to follow temperature changes with a lag of less than 2 seconds, but further discrimination was not possible in their experiments because of the slowness with which the cells were warmed.

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Regulation of Body Temperature in the Blue-Tongued Lizard

Abstract. *Lizards* (Tiliqua scincoides) *regulated their internal body temperature by moving back and forth between 15° and 45°C environments to maintain colonic and brain temperatures between 30° and 37°C. A pair of thermodes were implanted across the preoptic region of the brain stem, and a reentrant tube for a thermocouple was implanted in the brain stem. Heating the brain stem to 41°C activated the exit response from the hot environment at a colonic temperature 1° to 2°C lower than normal, whereas cooling the brain stem to 25°C delayed the exit from the hot environment until the colonic temperature was 1° to 2°C higher than normal. The behavioral thermoregulatory responses of this ectotherm appear to be activated by a combination of hypothalamic and other body temperatures.*

Vertebrates regulate internal body temperature by activation of two classes of responses in varying proportions. One class, termed physiological, comprises the secondary functions of organ systems that can modify rates of heat transfer from the core to body surface or from surface to environment, or modify the level of heat generation; shivering, panting, and vasomotor responses are obvious examples. An older class of thermoregulatory responses has been termed behavioral; they are the coordinated activity of the whole animal, selecting or creating a microenvironment in which the optimal internal temperature may be achieved passively. The animal may also modify by behavior one or more of the physical factors effecting heat exchange, such as body shape or orientation, when choice of environment is not possible.

Ectotherms such as lizards (*1*) and fish (*2*) regulate their body temperatures when not constrained from doing so. When a range of thermal environments is available to the animal, it apparently selects one in which a preferred body temperature can be achieved. Although the major thermoregulatory responses in ectotherms are behavioral, certain physiological responses also have been described (*3*). The way in which these responses are activated has not been explored.

We now report that the blue-tongued lizard, *Tiliqua scincoides* (Shaw), also can regulate its body temperature primarily by behavioral responses. Our results suggest that the regulatory responses are activated by a combination of brain and other body temperatures.

A pair of thermodes (1.0 mm in outside diameter, thin-wall, stainless-steel tubes spaced 3 mm apart) were implanted astraddle the brain stem of each of ten lizards. A stainless-steel reentrant tube (0.5-mm outside diameter) was implanted 0.5 mm from the midline and 1.0 mm rostral to the thermodes to accommodate a thermocouple. The temperature of the thermodes was controlled by water from a circulator (*4*) mounted on the lizard's head; water was pumped to and from the circulator through small plastic tubes at 70 ml/min from a constant-temperature bath (45° or 25°C). Three or more weeks after the thermodes were implanted, the lizard was placed in a chamber with a choice of two environments: a dimly lit courtyard at 15°C was surrounded by six aluminum boxes (10 by 20 by 30 cm) heated to 45°C by electrical ribbon heaters which, with thermal insulation, were wrapped around them. The door closed when the lizard entered a heated box, but could be readily opened by the lizard when it was ready to return to the cold courtyard. While the lizard oscillated between the two environments, its colonic, dorsal skin surface, and brain temperatures were continuously recorded by 36-guage, nylon-coated thermocouples (Fig. 1 and Table 1).

When lizard No. 8 entered a hot box, it remained there until its colonic temperature increased to 37.1° ± 1.2°C, when it returned to the cold courtyard until its colonic temperature fell