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Title EFFECTS OF TEMPERATURE ON OSMOTIC REGULATION IN LARVAL AMBYSTOMA GRACILE

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Temperature effects on osmoregulation were studied in larval Ambystoma gracile. There was a pronounced effect on the osmotic uptake of water through the skin. The rates at 5°C, 15°C and 25°C were respectively 0.03 ml/hr., 0.1 ml/hr. and 0.2 ml/hr. for a 10g animal. The Q_{10}'s for these changes in rate are larger than would be expected for simple diffusion, which indicates that an active regulation is causing an increase in the permeability of the skin with a temperature increase.

There was no gross change in the body weight at any temperature studied, indicating that the water loss was closely regulated. A possible mechanism for water regulation is discussed as well as the possibility of hormonal control over this mechanism.

Temperature also has an effect on net potassium loss. The losses at 5°C, 15°C and 25°C were 0.09 µeq/10g hr., 0.23 µeq/10g hr. and 0.48 µeq/10g hr. These changes are comparable to the
change in the rate of uptake of water. This indicates that there is a general change in the permeability of the skin to both salts and water with a change in temperature.

Sodium and chloride were fully regulated at all temperatures studied. Sodium fluxes were obtained using radioactive sodium. There was no difference in the flux values obtained from animals acclimated at 15°C and 25°C, the rates were 1.7 µeq/10g hr. At 5°C the flux values dropped to 0.7 µeq/10g hr.

Reasons are given indicating that a temperature increase causes: (1) a decrease in the activity of the sodium uptake across the skin and gills and (2) an increase in the activity of the sodium uptake across the tubular epithelium. Possible hormonal control of these responses is also discussed.
EFFECTS OF TEMPERATURE ON OSMOTIC REGULATION IN LARVAL AMBystoma gracile

by

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EFFECTS OF TEMPERATURE ON OSMOTIC REGULATION IN LARVAL AMBystoma gracile

INTRODUCTION

Poikilothermic animals are defined as animals whose body temperature corresponds to that of the environment (Prosser and Brown, 1961). At one time it was believed that the rates of physiological processes in these animals were decreased by cool environments and increased by warm environments in a manner that was completely dependent upon the external temperature.

Chemists had already recognized that a rise in temperature generally hastens chemical reactions. When they were able to measure actual rates it was found that, for a 10°C rise in temperature, the rate of a chemical reaction approximately doubles. This relationship is expressed by the van't Hoff equation:

\[ Q_{10} = \frac{k_2}{k_1} \left( \frac{t_2}{t_1} \right)^{10} \]

where \( Q_{10} \) is the temperature coefficient, \( k_1 \) is the rate at temperature \( t_1 \) and \( k_2 \) is the rate at temperature \( t_2 \). A chemical reaction would then have a \( Q_{10} \) of 2 (Giese, 1962).

A more theoretical expression of temperature effects on reaction rates was determined by the Swedish chemist Arrhenius in 1889.
He observed that a straight line relationship is obtained by plotting the logarithm of the rate constant against the reciprocal of the absolute temperature. This may be described by the equation:

$$\ln k = -(E_a) \frac{1}{T} + B$$

where the slope of the line, $E_a$, is called the energy of activation or the critical increment of energy, $k$ is the reaction rate, $T$ is the absolute temperature, $R$ is the gas constant and $B$ is a constant of integration.

These then are the laws that govern the effects of temperature on the rates of molecular reactions. It was thought by early investigators that the same laws could be applied to processes occurring in living systems. On the contrary, it is well known today that poikilotherms are able to escape from the rigidity of these laws by invoking various compensatory homeostatic mechanisms. This was first realized by Krogh in 1916 when he stated,

It would be interesting to compare the respiration in such cases, because it would appear unlikely from a teleological point of view that it should differ so much as would ordinarily be implied from the temperature difference. One would expect that animals living at a very low temperature should show a relatively high standard metabolism at that temperature compared with others living normally at high temperatures (Krogh, 1916 In: Bullock, 1955).

This temperature independence can be seen in many cases where poikilotherms are able to maintain metabolism and other gross characters, measured as rates, at moderately constant
levels, compensating for different temperatures by various homeostatic mechanisms. Thus, any changes at the molecular level will be masked by these gross physiological changes taking place at the tissue or organ level. Krogh seems to have realized this as early as 1914 and indicated this in his statement,

> When animals are studied under standard conditions—-all nervous influence being abolished--the influence of temperature on the metabolism of an animal is regular and constant and can be expressed in a definite curve, which is not a definite straight line and cannot be expressed by Arrhenius' formula or the rule of van't Hoff (Krogh, 1914).

Since then many other authors have questioned the validity of the Arrhenius law or van't Hoff rule in biological systems (Bélehrádek, 1930; Von Brand 1943; McLaren, 1965). As a result, biologists seldom use \( Q_{10} \) in the chemical kinetic sense, but rather to indicate the magnitude of change of a process for a \( 10^\circ\text{C} \) temperature shift. As yet, there is no simple explanation for temperature effects on biological processes.

With the realization that cellular processes in cold blooded animals are not completely dependent upon temperature, attention was focused on determining the extent of temperature independence and the mechanisms by which animals are able to escape from the detrimental effects of temperature changes. These problems have been approached from two points of view: (1) The study of the extent of temperature independence of some poikilotherms, which
has allowed them to extend their range latitudinally into different temperature zones and (2) the study of the extent of the resistance of poikilotherms to seasonal or experimentally induced temperature changes. An extensive review of this work was presented by Bullock in 1955.

Mayer (1914) was the first to observe latitudinal temperature independence of poikilotherms. He noticed that the jellyfish, *Aurelia aurita*, found at Halifax had about the same pulsation rate in the summer where the temperatures were 14°C as *Aurelia aurita* found at Tortugas where the summer temperatures average 29°C. Fox (1936a, 1936b, 1938, 1939) and Fox and Wingfield (1937) worked on a different group of animals and used a different approach. They studied the effect of temperature on scaphognatite beat of crustaceans, pulsation of the dorsal blood vessel and heart beat of polychaetes and crustaceans and rate of cleavage of sea urchin eggs. They showed that, if the temperature at which a particular rate occurred was determined, the species which inhabited the more northern or cooler environment would be able to achieve that particular rate at a lower temperature than the same or similar species from the more southern or warmer environment. Thus, at any given temperature, the rate was greater in the northern species than in the southern species.
The next thorough study was that of Moore (1939, 1942a, 1942b) which showed that *Rana pipiens*, breeding in the northern latitudes develop more rapidly at low temperatures than animals taken from southern latitudes. As the temperature was increased the difference decreased until at the higher temperatures the individuals from the southern latitudes developed faster than those from the northern.

More recently Rao (1953) studied the pumping rate of *Mytilus californianus* collected along the coast from Puget Sound to Los Angeles. He found that the rate was greater in the mussels from higher latitudes, at all temperatures, than in individuals from lower latitudes. In another study Scholander, et. al. (1953) showed that although the metabolic rate of several aquatic animals from Alaska were lower than those obtained for similar animals from Panama, the differences were less than would have been expected from Q₁₀ considerations. Dehnel (1955) has found that larvae of three species of gastropods, *Thais emarginata*, *Crepidula nummaria*, *Lacuna corinata*, from Alaska grow from two to nine times faster, at any given temperature between 10°C and 16°C, than larvae taken from southern California.

The second area, that of resistance or regulation in animals to seasonal or experimentally induced temperature changes, was overlooked for many years because the parameters being observed,
usually metabolism, increased or decreased with an increase or decrease in temperature. And, even though these changes were not as would have been predicted by $Q_{10}$ measurements, reference to temperature dependence of poikilotherms can be cited as late as 1941 (Wimpenny, 1941).

One of the first workers who tried to interpret the effect of experimentally induced temperature changes at the molecular level was Crozier. He and several co-workers analyzed a large volume of experimental data on the temperature effects of various life processes. They plotted the logarithm of the reaction rate against the reciprocal of the temperature. Instead of the straight lines predicted by the Arrhenius equation, simple curves were generally obtained. They postulated that these curves could be represented by a combination of straight lines, usually 2 or 3, each with a different slope, reflecting differences in activation energies. The changing activation energies were then considered to be a type of chemical adaptation. (Crozier, 1924a, 1924b; Crozier and Federighi, 1924a, 1924b, 1924c, 1924d, 1925a, 1925b; Crozier and Libby, 1925; Crozier and Stier, 1925a, 1925b, 1925c, 1925d). Today this interpretation is generally considered an oversimplification; it nevertheless clearly emphasizes the ability of poikilothermic animals to partially escape from the rigidity of the laws governing simple chemical reactions.
Seasonal differences in the physiology of poikilothermic vertebrates had been noticed for many years by several workers. In *Necturus* Riddle (1909) found that, at low temperatures, the digestive rate in December is more than twice that in March. Barcroft (1931) found a difference between the temperature pulse-rate curve of the excised frog heart from summer and winter animals. Carter (1933) and Smith (1951) found that the effect of various hormones was also different on the summer than on the winter curve. Stier (1939) found that the maximum lethal contraction temperature of the excised frog heart was greater in fall and spring than in the winter. Seasonal differences were also found for the temperature at which maximum muscle contraction would take place, being higher in summer than in winter frogs (Hajdu, 1951).

Wells (1935a, 1935b, 1935c) was the first to clearly demonstrate the temperature adaptation predicted by Krogh in 1916. He observed a higher rate of oxygen uptake, faster opercular movement, and greater drug resistance in cold-adapted killifish (*Fundulus*) than in warm adapted animals, when both were tested at a common intermediate temperature. The greater the difference in adaptation temperature the greater the difference observed in these parameters at the intermediate temperatures. In other studies Freeman (1950) measured oxygen consumption, brain metabolism, and respiratory movements of the goldfish and found that as the temperature
decreased the animals adapted by increasing these parameters. More recently Morris (1965) has shown that *Ictalurus natalis*, the yellow bullhead, can metabolically acclimate to both heat and cold.

Most of the studies on temperature effects have been done on metabolic rate, which is a very gross reflection of physiological activity. It would therefore be of interest to study the more specific adaptive changes that occur in response to temperature changes.

One of the striking features of aquatic animals is their ability to osmoregulate. The level of regulation varies between species, depending upon their external environmental conditions. Most marine animals live in osmotic equilibrium with their environment and have few problems in maintaining a constant internal concentration. They nevertheless have problems in ionoregulation. On the other hand fresh-water organisms maintain the solute concentration of their body fluids well above the concentration of the external medium and regulate the ionic concentration of their body fluids within rigorous limits. For example in amphibia an internal concentration of about 100mM Na, 78mM Cl and 2.6mM K or a total osmotic concentration of 237mOsm/kg water is found while they live in an environment which has about 0.25-2.2mM Na, 0.226-2.54mM Cl and 0.005-1.46mM K (Potts and Parry, 1964).

In aquatic animals osmoregulation can be related to five
factors: (1) permeability of certain epithelia to water, (2) permeability of epithelia to salts, (3) renal loss of salts, (4) renal loss of water, and (5) rate of uptake of salts in their food or across their skin or other epithelia. In order to survive, the aquatic animal must regulate these factors so that the uptake of salts from the environment equals the loss of salts to the environment, similarly the uptake of water must equal the loss of water through the kidneys. Each of the above factors in turn is composed of a multitude of chemical and physical processes operating in an integrated fashion. These must certainly include some temperature sensitive steps. Thus it seems probable that an asymmetric system might exist consisting of a series of steps each with its own temperature coefficient. A temperature change would then affect each step differently and thus disrupt the balance which must exist between the input and output components. The restoration of this balance in response to a temperature change would require the operation of subsystems. Some might be at the local level and others, when regulation between body parts is required would probably be at the organ level. In addition, if these subsystems have different compensation times there would be a period of acclimation before the system could come back into balance.

Temperature effects on osmoregulation were first noticed during the early 1900's. De Haan in 1924 showed that at similar
temperatures the rate of water excretion was 100% greater in summer than winter frogs. Adolph (1925, 1933, 1934) observed that when frogs were transferred to low temperatures they gained weight. Jørgensen (1950) also found that frogs gained weight when transferred from 20° to 8°C. He attributed this to the inability of the kidney to excrete salts taken up through the skin, the resulting hypertonicity being counteracted by retention of the water entering through the skin. Schmidt-Nielsen and Forster in 1954 obtained evidence that a low filtration rate was responsible for the reduced urine flow and thus possibly the inability to eliminate either salts or water.

Wikgren in 1953 reviewed most of the previous work in this field in connection with his work on Potamobius, Petromyzon, and various teleosts. In Petromyzon he showed three effects of acute reduction in temperature on osmoregulation: first there was a reduced permeability of the skin, second a reduction in the rate of urine production, and third a net loss of sodium.

More recently Lockwood (1960) has studied the effects of temperature on the isopod, Asellus aquaticus (L.). He found that the influx, but not the efflux, was affected by temperature. This resulted in an increase in the internal concentration when the temperature was increased. There was, however, a regulation of the
influx, after a slight increase in the internal concentration, which brought the animal back into balance.

Investigations on temperature effects on osmoregulation in amphibia have been limited to adult anurans. Little is known about larval forms of urodeles. Some of these forms live in environments subject to wide seasonal fluctuations in temperature and, to a lesser extent, daily fluctuations. It is the undertaking of this thesis to describe some of the effects of temperature on osmoregulation in larval *Ambystoma gracile*.
MATERIALS AND METHODS

Animals

The experiments were done on larval, *Ambystoma gracile* collected in the vicinity of Corvallis, Oregon. The animals were kept in polyethylene containers in tap water at 5-8°C. The water was changed at least once every 10 days. Animals kept in such a manner have survived for more than a year, however, all animals used in experiments were not kept longer than five months. One week prior to use the animals were transferred to an artificial pond water bath (1.3mM sodium chloride, 0.8mM calcium chloride, 0.1mM potassium chloride and 0.2mM sodium bicarbonate) and equilibrated.

Anesthetic

These animals are sensitive to handling (Alvarado and Kirschner, 1963). Thus for any procedure involving stress, i.e. injections or ligation of the cloaca, the animals were anesthetized by immersion in 0.1% tricane methane sulfonate (pH adjusted to 7.1).

Water Balance

The changes in water content of the animals were monitored
by weighing them at specified intervals on a Mettler balance (precision 0.2%). Each animal was placed in a net and blotted five times on tissue paper before being placed in a tared container of water and weighed.

**Osmotic Uptake of Water**

The animals were anesthetized and the external opening of the cloaca was closed using a purse-string ligature. They were then weighed, placed in separate baths, and weighed every two hours for the next 12 hours. In terminating the experiments each animal was first anesthetized and weighed. The cloaca was then opened to obtain a urine sample. Serum samples were obtained by cutting into the pericardial cavity and severing the blood vessels or heart. A pasteur pipet was then placed in the cavity and the blood drawn up by capillary action. The blood sample was centrifuged in a Beckman/Spinco 152 microfuge and the serum saved for analysis. The serum and urine sample were diluted 100 or 200 times and assayed for sodium by flame photometry (precision 1.0%).

**Sodium Fluxes**

The efflux of an ion is the unidirectional rate of movement of that ion from the animal to the environment and the influx is the
unidirectional rate of movement of the ion into the animal. The difference between the efflux and influx is the net flux. This is summarized in equation (1):

\[
M_n = M_i - M_o
\]

where \(M_i\) is influx, \(M_o\) is efflux and \(M_n\) is the net flux.

The efflux was measured by using Na\(^{22}\). The animals were anesthetized, injected with 0.1 ml. amphibian Ringer's solution containing the isotope (2.1 µc Na\(^{22}\)) and equilibrated for three days before use. Each animal was then rinsed thoroughly in pond water and placed in 250 ml. of Na\(^{22}\)-free pond water. Ten ml. samples were taken from the bath at specific intervals and an equal volume of pond water was replaced. A 2 ml. fraction of each sample was plated on a planchet and evaporated to dryness. The beta radiation was counted with a Nuclear Chicago model 447 counter. A correction for sampling was made by applying the formula:

\[
\sum_{n-1}^{n} = C_{n-1} \left( \frac{10}{250} \right)
\]

where \(n\) is the number of the sample, \(C_{n-1}\) is the concentration of Na\(^{22}\)(cts/ml.) in the \(n-1\) sample, and \(\sum_{n-1}^{n}\) is then the correction for the \(n^{th}\) sample. At the conclusion of the experiment the animals were anesthetized and killed. A serum and when possible a urine sample were taken. The carcass of each animal was then
dried (48 hours at 100°C) and a dry weight obtained. The carcass was digested in 30 ml. of concentrated HNO₃ and diluted to one liter. A 2 ml. sample was plated and counted and another fraction was assayed for sodium by flame photometry. The specific activity was then determined and from this the efflux was calculated by applying equation (3):

\[ M_o = \frac{(dNa^{22})}{dt} \frac{X}{(S_a)} \]  (3)

where \( \frac{dNa^{22}}{dt} \) is the rate of appearance of Na²² in the bath, \( S_a \) is the specific activity of the animal and \( X \) is the weight of the animal in grams. \( M_o \) is then the efflux in \( \mu \text{eq/10gr.-hour} \). All experiments were of short enough duration that the radioactivity in the bath remained low, relative to the activity in the animal. This eliminated the necessity of correcting for back flux of the isotope.

The net flux was obtained from changes in the bath sodium which was monitored by flame photometry. The influx was then calculated from equation (1).

Sodium fluxes were measured on both acclimated and acutely stressed animals. All animals were kept one week at the temperature of interest before being used. The animals were then run at the same temperature and the fluxes obtained were termed "acclimated fluxes". After 24 hours the animals were changed to a temperature 10°C higher or lower and the fluxes obtained were
termed "acute fluxes". The temperature change was made by moving the container with the animal from one temperature bath to another. This minimized the disturbance of the animal. The container required one hour to come into equilibrium with the new bath.

**Statistical Treatment**

Results are represented as the mean ± one standard error. The number of samples is indicated in parenthesis. The Student's T-test was used for determining the significance of the difference between sample means (Simpson, 1960). The 95% level of significance was used in all cases.
RESULTS

Ion Balance

When animals are isolated in separate containers in a bath of limited volume, changes in the quantity of a given ion inside the animal are reflected by changes in the bath concentration. Accordingly the bath concentrations of sodium, potassium and chloride were followed for a period of time to determine the ability of larval *Ambystoma gracile* to maintain themselves in a steady state with respect to these ions at various temperatures.

The animals used in these experiments were acclimated to $15^\circ C$ for one week prior to the experiment. At zero time 5 animals were transferred to $5^\circ C$, another 5 to $25^\circ C$ and the remaining 5 were left at $15^\circ C$. At $25^\circ C$ three of the five animals underwent metamorphosis during the experiment. Since this greatly upset their ion balance only data from the two animals which did not transform were used.

Figure 1 shows that sodium was lost to the bath for the first 48 hours by all groups. This was probably due to handling, rather than to temperature shock, since the animals at $15^\circ C$ responded in the same manner. For the remainder of the experiment the animals were able to maintain a steady state, with respect to sodium, at all temperatures.
Figure 1. The effect of temperature on sodium balance in larval *Ambystoma gracile*. The bath was 250 mls. of artificial pond water with an initial sodium concentration of 1.25 meq/l. At time zero the animals were transferred from 15°C to the temperature indicated in the graph. Each point represents a mean; the number of observations is given in parenthesis. The maximum standard error observed was ±5.0 µeq/10g for 5°C and 15°C and ±13 µeq/10g for 25°C. The final weight of the animals was 9.28±0.38g (12).
Chloride changes are shown in Figure 2. The animals maintained a steady state with respect to this ion for the entire experiment.

The results for potassium were obtained from a different group of animals which had been treated in the same manner described above. The animals lost potassium at all temperatures (Figure 3). Similar results were reported by Alvarado and Kirschner (1963). The net fluxes of potassium at 5°C, 15°C and 25°C were respectively: 0.09 μeq/10g hr., 0.23 μeq/10g hr., and 0.48 μeq/10g hr. giving a Q10 of 3 between the 5°C and 15°C group and a Q10 of 2 between the 15°C and 25°C groups. There was no apparent acute effect of temperature on the loss of potassium.

**Sodium Flux Measurements**

Figures 4, 5 and 6 show the accumulative loss and uptake of sodium as a function of time for animals at 5°C, 15°C and 25°C. In each case the slope of the curve indicates the flux.

Figure 4 shows the results from animals which had been previously acclimated for at least one week at 5°C. At this temperature the sodium influx equals the efflux \( M_i = 0.7 \pm 0.1 \) (30) μeq/10g hr., \( M_o = 0.8 \pm 0.1 \) (10) μeq/10g hr.) except for the first hour when the efflux exceeded the influx resulting in a slight net loss of sodium. This is a characteristic response seen after handling (Alvarado and Kirschner, 1963).
Figure 2. The effect of temperature on the chloride balance in larval Ambystoma gracile. The maximum standard error observed was ±6.0 μeq/10g for 5°C and 15°C and ±35 μeq/10g for 25°C. The initial chloride concentration was 2.19 meq/l. See Figure 1 for details.
Figure 3. The effect of temperature on potassium balance in larval Ambystoma gracile. The maximum standard error observed was $\pm 6.1 \, \mu\text{eq}/10\text{g}$. The final weight of the animals was $11.02\pm0.94\text{g}$ (14). See Figure 1 for details.
Figure 4. Accumulative sodium gained (---) or lost (---) by larval *Ambystoma gracile* acclimated at 5°C and acutely stressed at 15°C. The arrow indicates when the animals were transferred from 5°C to 15°C. The animals were in a 250 ml. artificial pond water bath with an original sodium concentration of 1.20 meq/l. The final weight was 12.69±0.88g (5).
The animals were then transferred to 15°C and the acute fluxes measured. The influx increased to 2.1±0.4 (20) µeq/10g hr. or about 3 times ($Q_{10} = 3$) the acclimated influx, while the efflux increased only about 1.5 times ($Q_{10} = 1.5$) to a value of 1.3±0.1 (30) µeq/10g hr. These disproportionate shifts in rates resulted in a positive net flux of 0.6 µeq/10g hr. After 12 hours this positive net flux was checked by a decrease in the influx to 0.8±0.2 (10) µeq/10g hr. without a change in the efflux. This resulted in a negative net flux of 0.41 µeq/10g hr. which presumably allowed the animals to lose some of the excess sodium gained after the transfer. Eventually the animals must come into a steady state, but the time course has not been followed.

Figure 5 shows the results from animals which were acclimated for one week at 15°C. The sodium fluxes were then measured for 24 hours, and the animals were immediately transferred to 5°C and the fluxes again measured. At 15°C there was a negative net flux of 1.2 µeq/10g hr. in the first 4 hours due to handling. This was partially corrected in the next 4 hours when the influx increased, without a change in the efflux, resulting in a positive net flux of 0.7 µeq/10g hr. For the next 16 hours the influx and efflux were 1.4±0.3 (9) and 1.9±0.2 (30) µeq/10g hr. respectively, the difference is not significant ($P>0.05$). When the animals were transferred to 5°C both the influx and efflux were reduced by a factor of about
Figure 5. Accumulative sodium gained (——) or lost (---) by larval Ambystoma gracile acclimated at 15°C and acutely stressed at 5°C. The arrow indicates when the animals were transferred from 15°C to 5°C. The animals were in a 250 ml. artificial pond water bath with an original concentration of 1.30 meq/l. The final weight was 9.52±2.46g (3).
2 (Q₁₀ = 2) the influx was 0.7±0.2 (18) µeq/10g hr. and the efflux was 0.7±0.1 (18) µeq/10g hr. They were thus able to maintain a steady state at all times when the temperature was lowered from 15°C to 5°C.

At 25°C only an acclimated rate was obtained and is shown in Figure 6. These animals showed a constant influx and efflux throughout the entire 24 hours of the experiment which were 1.7±0.2 (30) and 2.0±0.2 (30) µeq/10g hr. respectively, the difference is not significant (P>0.05).

When the acclimated fluxes at the three temperatures were compared it was found that there was a significant difference (P<0.05) between the influx at 15°C and 5°C, with a Q₁₀ of 2.1 between these temperatures. There was also a significant difference (P<0.05) between the effluxes at these temperatures (Q₁₀ = 1.9). There was however no significant difference (P>0.05) between the influxes at 25°C and 15°C and likewise there was no significant difference (P>0.05) between the effluxes at these two temperatures.

It should also be noted that when the animals that had been acclimated to 15°C were acutely stressed at 5°C their flux values immediately dropped to the flux values obtained for animals that had been acclimated to 5°C. There was no significant difference between these values (P>0.05).
Figure 6. Accumulative sodium gained (---) or lost (---) by larval Ambystoma gracile acclimated at 25°C. The animals were in a 250 ml. artificial pond water bath with an original concentration of 1.41 meq/1. The final weight was 9.52±0.52 (5).
Water Balance

The ability of animals to maintain a constant body weight was impaired at 25°C and 15°C. This was shown by a 10% and 13% decrease in body weight, for 15°C and 25°C respectively, over a period of eight days (Figure 7). Animals at 5°C held a constant weight. Since these animals were not being fed it is possible that the loss in weight experienced by the animals at 15°C and 25°C was due to starvation. If we assume that fat and carbohydrates are being metabolized the loss of weight would amount to about 1% of the body weight in 8 days.

The rate of osmotic uptake of water, which is a measure of the permeability of the exposed epithelia to water (these animals do not drink water, Alvarado and Kirschner, 1963), was changed markedly with a change in temperature (Figure 8). The extent of the change varied with temperature, the Q₁₀ between 5°C and 15°C was 3.3 while the Q₁₀ between 15°C and 25°C was 1.8.

Serum Sodium

A summary of the serum samples taken at the end of the experiment shows that the serum sodium concentration remained constant in all experiments (104±0.5 (21) µeq/ml.), except in the
Figure 7. Effect of temperature on body weight in larval *Ambystoma gracile*. The animals were acclimated at 15°C in artificial pond water. At zero time the animals were transferred to the temperature indicated. The initial weight was 7.61±0.32g (15). The maximum standard error was ±3.23%.
Figure 8. Rate of water uptake, in larval *Ambystoma gracile*, as measured by the increase in body weight after cloaca ligation. Each animal was immersed in 250 mls. of artificial pond water. The initial weight was 7.61±0.32g (15). Vertical lines represent ±1 SE.
permeability studies where the cloaca was blocked. The serum in these cases became diluted (5°C, 96±4.1 (3) μeq/ml.; 15°C, 86±2.9
(4) μeq/ml.; 25°C, 84±2.4 (3) μeq/ml.).
DISCUSSION

Water Balance

Larval *Ambystoma gracile* at 5°C maintain a constant body weight. This was true for both acclimated and acutely stressed animals. The increase in body weight observed by Jørgensen (1950) in adult anurans, acutely stressed at 5°C, was not seen in the larval urodeles. Animals at 15°C and 25°C suffered a slight loss of weight. This loss could partially be the result of potassium loss, since the maintenance of a constant internal osmotic concentration would require a concomitant loss of water. A slight loss may also have resulted from metabolic needs. Both of these effects would be greater on the 15°C and 25°C groups than on the 5°C group. On the whole, however, there were no gross changes in body weight of acclimated or acutely stressed animals at any of the temperatures studied.

Acclimated animals, with their cloaca blocked, take up water at a rate of 27% of their body weight per day at 15°C. At 25°C the rate approximately doubles (*Q_{10} = 1.8*), and at 5°C the rate is reduced to 1/3 its original value (*Q_{10} = 3*). Since both *Q_{10}* changes are greater than 1.5, which is the *Q_{10}* for simple diffusion (Giese, 1962), they indicate a change in the permeability of the epithelia to water.
A change in temperature, therefore, causes a change in the rate of water uptake by the animal. Since there were no gross changes in body weight, this change must be detected, possibly by volume or osmotic receptors, and the rate of water excretion by the kidneys adjusted accordingly. This response is probably mediated by hormones which change the rate of loss of water by changing the glomerular filtration rate and/or tubular reabsorption. Arginine vasotocin has been shown to reduce the glomerular filtration in *Ambystoma tigrinum* and *A. gracile* (Alvarado and Johnson, 1965). An increase in temperature might cause a reduction in the production of this hormone, thus increasing glomerular filtration and presumably water loss through the kidney.

A second water regulatory mechanism might operate through the skin, since the change in permeability of the skin is itself a sign of water regulation. For example, if volume regulators detected an increase in volume, resulting from a decrease in water loss, they could initiate a response which would decrease the permeability of the skin and thus bring the animal into balance. Probably the animals make use of some combination of these two mechanisms, so that they can regulate both the inflow and the outflow of water.

Adult frogs acutely stressed at 5°C cannot cope with the change in water movement unless their body fluids first become diluted (Jørgensen, 1950). This may be caused by their inability
to reduce the permeability of their skin. Thus the kidneys may not be able to eliminate all the incoming water. It has been shown that the skin of adult Ambystoma tigrinum is more permeable to water than that of larvae (Alvarado and Kirschner, 1963). Therefore adults may have more difficulty in decreasing the permeability of their skin; this may explain why larval urodeles can maintain a body weight at 5°C while adult frogs cannot.

**Ion Balance**

The net potassium loss was affected the same by temperature as the uptake and loss of water, exhibiting a decrease to 1/3 of the original value when the temperature was decreased from 15°C to 5°C. The major routes through which this loss could occur are the skin and kidney. If the loss through both these routes were reduced to 1/3 of the original value, then the total loss would also be reduced to 1/3 of the original value. If this were the case, the Q10 for the loss of potassium across the skin would be 3 which indicates a general change in the permeability of the skin to both water and salts. In nature the potassium loss may be replaced through the food.

The animals maintained a steady state with respect to chloride at all temperatures. As only net values were obtained there is no information as to how the regulation of this ion is brought about.
Under acclimated conditions larval Ambystoma gracile are able to maintain a steady state with respect to sodium in their normal environmental temperature range (5°C to 25°C). The net loss of sodium at any temperature studied was negligible. Accordingly they must be able to compensate for any changes which a temperature shift might cause in the numerous components involved in maintaining sodium balance.

At higher temperatures, between 15°C and 25°C there seems to be a range where the animals can completely regulate the influx and efflux of sodium and thus maintain the flux values constant even with a temperature change. The influx of sodium results primarily from active transport which is believed to be a chemically mediated process involving several reactions (Skou, 1965). A temperature increase would be expected to increase the rate of these reactions. Yet the animals are able to exert a control over the active uptake of sodium which overrides the effect of temperature. This control over temperature may be mediated through the endocrine system. The interrenal hormone, aldosterone (Alvarado and Kirschner, 1964), and the neurohypophyseal hormone, arginine vasotocin (Alvarado and Johnson, 1965), have been shown to stimulate the uptake of sodium across the skin of urodeles. Thus an increase in temperature might cause a reduction in these hormones which would reduce the influx of sodium.
With a reduction in temperature from 15°C to 5°C there is a reduction in the influx of acclimated animals to 1/2 the original value \((Q_{10} = 2)\). It is impossible to predict how much of the reduction is due to the direct action of temperature on the transport systems and how much is due to some regulatory system. However, the presence of some regulatory mechanism was revealed when the sodium fluxes were followed during an acute temperature increase from 5°C to 15°C. The system was thrown out of balance, the influx increasing above the efflux, causing a positive net flux for 12 hours. This overshoot of the influx was then corrected by a reduction in the influx which resulted in a negative net flux, allowing the animals to lose the excess sodium gained during the first 12 hours after transfer. Presumably the influx and efflux eventually approached some intermediate value commensurate with the values obtained for animals acclimated to 15°C. There is thus some oscillation in the magnitude of the fluxes when the temperature is acutely raised from 5°C to 15°C. This is characteristic of control systems. Perhaps the increase in temperature from 5°C to 15°C is compensated for in the same manner as a rise from 15°C to 25°C -- by a decrease in the concentration of a hormone in the blood, since in both cases a decrease in influx is required with a temperature rise. There could be a lag between the shut down of hormone release into the blood and the fall in the titer of the blood. This could also
account for the lack of an effect when the temperature was decreased from 15°C to 5°C, since this would require the release of hormone which can occur rapidly.

The results also indicate a regulation of the efflux. This can be partitioned into renal and extra-renal components. It is known that, at 15°C the efflux of sodium is 1.7 µeq/10g hr. and of that 60% is renal loss (Alvarado and Kirschner, 1963). Since the rate of sodium loss through the kidney is about 1.0 µeq/10g hr. and since the rate of urine production is 0.1 ml/hr. for a 10g animal the urine concentration at 15°C must be 10 µeq/ml. At 25°C no urine samples were obtained and the efflux was not partitioned. However it can be reasoned that if the urine concentration was not changed (if it was 10 µeq/ml), since the urine production at 25°C is 0.2 ml/10g hr. the renal efflux would be 2.0 µeq/hr. The efflux at 25°C was 1.9 µeq/hr. and it seems unlikely that the extra-renal component would drop to zero. Thus at the higher temperature the loss of sodium through the kidney must be reduced. Since there is an increase in urine production at the higher temperature, a more diluted urine must be produced at 25°C than at 15°C. This reflects a more efficient tubular reabsorption of sodium.

A reduction in temperature from 15°C to 5°C reduces the loss of water to 0.03 ml/10g hr. If the urine concentration remained the same (10 µeq/ml), the urine sodium loss would be 0.3 µeq/10g hr.
With an efflux of 0.8 µeq/10g hr. this would mean an extra-renal loss of 0.5 µeq/10g hr. Since the extra-renal loss at 15°C was 0.7 µeq/10g hr. this would give a Q₁₀ of 1.4 for the extra-renal sodium loss. The loss of potassium and the uptake of water both decreased by a factor of 3 (Q₁₀ = 3). It seems unlikely that the skin would change its permeability to potassium and water differently than to sodium. Therefore at 5°C the extra-renal loss is probably less and the renal loss is greater than at 15°C. This would mean a reduction in the tubular reabsorption of sodium at 5°C. Schmidt-Nielsen and Forster (1954) working on the frog found a decrease in the tubular reabsorption of water when frogs were exposed to 5°C. This observation was based on U/P creatinine ratios and could reflect a decrease in the tubular reabsorption of sodium, which is usually associated with water absorption. There is thus a good indication that when the temperature is increased two effects are seen: (1) the active uptake of sodium across the skin and gills is decreased and (2) the active uptake of sodium across the tubular epithelium is increased.

The changes in Q₁₀ that were seen for the rate of water uptake, rate of potassium loss and flux values, cannot fully be explained. However, they are not peculiar to studies on osmotic and ionic regulation. Bullock (1955) reviewed a large array of data which demonstrated that an increase in temperature would result
in an increase in $Q_{10}$. Similar effects were seen in a number of poikilotherms by Rao and Bullock (1954) and in several fish by Morris (1962). However in the present studies a decrease in $Q_{10}$ was observed with an increase in acclimation temperature for water uptake, potassium loss and efflux of sodium. There are only a few other experiments showing this effect. Shurmann presented data showing that the $Q_{10}$ of metabolic rate of the carp, measured at the upper temperature range, decreased with an increase in the acclimation temperature (Shurmann, 1955 In: Morris, 1965). Similar results were also obtained on the catfish, Ictaturus natalis, by Morris (1965).

It should be noted that the $Q_{10}$'s discussed by Bullock (1955) were acute $Q_{10}$'s. The animals were first acclimated to one temperature and then acutely stressed at some other temperature. These values were then used to compute the $Q_{10}$'s. In the present experiments the $Q_{10}$ values for the potassium loss and water uptake were acclimated $Q_{10}$'s, that is the values used to obtain the $Q_{10}$ were both from acclimated animals. The only $Q_{10}$ values directly comparable to Bullock's discussion would be the values for sodium influx and efflux. These were obtained when the value of an acclimated and acutely stressed animal were used. When this is done the $Q_{10}$ for the influx for animals acclimated to 5°C and acutely stressed at 15°C was 3.0. Conversely the $Q_{10}$ for the influx for
animals acclimated to 15°C and stressed at 5°C was 2. This is the same change found by Bullock. On the other hand the corresponding values for the efflux are 1.6 and 2.4, again the opposite effect.

Changes in $Q_{10}$ may indicate that either the regulatory mechanism is itself being affected by the temperature shift or that one of the components of the rate process has reached its limit of regulation (it can no longer be increased or decreased by the regulatory mechanism). Therefore for an animal to come into a steady state, the only choice is to increase or decrease the other components in the system until they are all in balance with the errant component. Also at the new steady state the effect of temperature might be different. High $Q_{10}$'s may thus indicate that an animal is poorly adapted to a change in temperature in this temperature range. In general, most of the animals discussed by Bullock (mainly aquatic animals) were better adapted to lower temperatures. Both of the exceptions cited above, the carp and the catfish, are warm water animals and might be expected to be better adapted to a warmer environment. The sodium transport system is then better adapted to warmer environments.

The questions which have been raised in discussing the present experiments can be more specifically answered when further experiments are performed which will elucidate the role played by the kidney during temperature changes.
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