THE RESPIRATION RATES OF EXCRETORY TISSUES
IN THE CUTTHROAT TROUT
(Salmo clarki clarki)

by

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B.Sc., University of British Columbia, 1958

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ABSTRACT

The oxygen consumptions of gill and kidney tissues of the cutthroat trout (Salmo clarki clarki) were determined by the direct method of Warburg. The respiration rates of tissues from fish ranging from 10 to 100 gm. were examined in relation to body weight. A decline in weight specific oxygen consumption for both tissues was observed. On a log-log plot, the regression coefficient for kidney was -.148 while that for gill was -.139. The decline did not support the .73 rule (Brody, 1945) at the level of tissue respiration.

The oxygen consumptions of kidney and gill tissues were examined during a 168 hour period after transfer of the fish from fresh water to 65% standard sea water. A sharp initial rise in QO₂ of kidney tissue was noted during the first 48 hours after transfer, reaching a maximum at 20 hours. The kidney tissue respiration during the remainder of the experimental period remained significantly higher than the parallel control level. The gill tissue respiration declined rapidly during the first 10 hours after transfer and remained significantly below the control level during the whole experimental period. The results are discussed in relation to recent observations of Holmes, Chester Jones, Phillips, and Sexton, concerning possible hormonal regulation of salt-electrolyte and water metabolism by vasopressin and adrenocortical steroids in euryhaline species of salmonids.
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Department of **Zoology**

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Date **July 9, 1959**
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INTRODUCTION

As a group, the Salmonoidae have remarkable abilities to withstand wide variations in the tonicity of their environment without undergoing commensurate changes in their body fluids. Indeed, the salmon experiences extreme environmental changes during the natural course of its life history. Considerable physiological adaptation with respect to water and electrolyte metabolism must be made for these fish to survive.

The cutthroat trout (*Salmo clarki clarki*) in nature may or may not go to sea. The stocks used in this work probably had some "sea run" propensity, and hence displayed a toleration for a fairly wide salinity range. This study involved the examination of some of the physiological changes occurring when the fish is transferred abruptly from fresh to sea water.

I. Metabolism and Body Size

The size of the fish available for the study of the physiological adaptations associated with the transfer to sea water varied widely during the course of the experiments. Wide variations in the oxygen uptake of gill and kidney tissues from normal fish also occurred. Therefore, a knowledge of any weight specific variation between oxygen consumption and total body weight was necessary before valid interpretations of the experimental data could be made.

There is abundant evidence in the literature to indicate a relationship between body weight and metabolism. Brody (1945)
compared data from mature mammals and found that basal metabolism changed regularly from 8 Calories per Kg. for the elephant to 200 Calories per Kg. for the mouse. Kleiber (1947) carried out a similar comparison of metabolic rates from animals ranging in size from the mouse to the cow. These workers found that the following relationship applied:

\[ \log M = \log b + \alpha \log W \]  
\[ M = bW^\alpha \]

where \( M \) is the metabolism, \( W \), the body weight, the exponent \( \alpha \), the slope of the line on a log-log plot, and \( b \), the intercept indicating the value of \( M \) when \( W \) is 1. Brody (1945) and Kleiber (1947) concluded that the metabolism of mature homoiotherms tended to vary with the \( .73-.75 \) power of the body weight. Benedict (1938) considered metabolism to be directly proportional to the surface area of the body, i.e. \( M = bW^{.66} \). However, the value of \( \alpha \) for Benedict's data was \( .73 \) when certain atypical species were omitted (Brody, 1945).

The generalized formula of Brody, giving an exponent of \( .73 \), was calculated from the regression of log metabolism vs. log body weight for mature homoiothermic species. With the exception of small metazoans, Zeuthen (1953) found the same interspecific generalizations applicable to the poikilotherms. He lists \( \alpha \) values of \( .7 \) for unicellular organisms, \( .95 \) for small metazoans, and \( .75 \) for large metazoans. On a log-log plot, he found the same slope for homoiotherms and poikilotherms. Weymouth's (1944) data showed an \( \alpha \) value for crustaceans of
However, the intercept for the poikilotherms was lower than that for homoiotherms (Zeuthen, 1953).

Zeuthen (1953) also compared the changes in metabolism with ontogenetic increases in the size of an organism. He recognized a similar triphasic curve for the oxygen consumption during the development of several poikilotherms. The larval phases showed $\alpha$ values approaching 1.0 whereas the embryonic and post-larval stages of development showed $\alpha$ values of approximately .7. As in the case of interspecific metabolism, the intraspecific variation in metabolism with increasing body was not so constant among the poikilotherms as the homoiotherms. (Weymouth, 1944).

Both the intraspecific and interspecific log-log plot of metabolism vs. body weight show a positive regression. If the metabolism is expressed in units of metabolism per unit body weight, then a weight specific metabolism is obtained. When equations (1) and (2) are thus treated, the following equations for weight specific metabolism are obtained:

$$\log M - \log W = \log b + \alpha \log W - \log W$$

$$= \log b + (\alpha - 1) \log W \quad \text{..........(3)}$$

or

$$\frac{M}{W} = bW^{(\alpha-1)} \quad \text{.........................(4)}$$

The observed decline in weight specific metabolism with the increasing size of the organism has stimulated several workers to investigate the metabolism of individual tissues from animals of differing sizes. Equation (4) can be used to compare $Q_{O_2}$ values. However, it must be assumed, in relating the tissue metabolism per unit weight of tissue to the total body weight
by equation (4), that the tissue size and the total body size increase proportionately. In the case of weight specific metabolism, exponents of -.27 and -.33 correspond to the .73 and the surface rules respectively. Krebs (1950) studied the oxygen consumption of five different tissues from nine mammalian species, finding the $Q_{02}$ values for homologous tissues lower in the larger animals. He found that the decrease of $Q_{02}$ values for the kidney cortex was much smaller than the decrease of rate of basal heat production. His results gave an interspecific value for $(\alpha-1)$ of -.064. Bertalanffy and Pirozynski (1953) did not find any significant regression of kidney $Q_{02}$ values with body weight for the rat. Krebs (1950) did not find any tissues which conformed to the classical rules, while Bertalanffy and Pirozynski (1953) found only thymus and diaphragm conforming $(\alpha-1) = -.26$. Vernberg (1954) carried out an investigation involving marine teleosts. He examined the weight specific respiration rates of brain, liver, and skeletal muscle for the toadfish (*Opsanus tau*), and found that only liver showed a negative regression $(\alpha-1) = -.145$.

II. The Sites of Osmotic Regulation

A. Osmoregulation and Environment

In fish, osmotic regulation varies according to the environment. Stenohaline forms are able to tolerate only narrow ranges of salt concentration in the water. In fresh water fish, the osmotic concentration of the body fluids (depression of
freezing point, $\Delta = 0.57^\circ C$) is higher than that of their environment ($\Delta = 0$) (Black, 1957). Water tends to diffuse into the tissues and salts diffuse from the body to the external environment. The fresh water fish must, therefore, alleviate the tendency towards hydration of body tissues and conserve electrolytes. In marine fish the opposite problems arise. Since the concentration of the body fluids ($\Delta = 0.78$) is lower than that of sea water ($\Delta = 2.0$) (Black, 1957), the fish tend to lose water across the gills and other body surfaces. They gain water by the ingestion of food and by swallowing sea water, but much of this is lost extra-renally. Smith (1930) found that 81% of the water swallowed by the eel was absorbed through the gut and 59% was lost extra-renally, while in the sculpin 63% was absorbed and 31% lost.

Euryhaline fishes are able to tolerate a wider salinity range. On transfer to fresh water, marine forms tend to gain water and lose salts. They adapt by increased urine flow and increased reabsorption of electrolytes. The anadromous and catadromous fishes, such as the salmon and the eel, may utilize hormonal activity to produce physiological changes enabling them to survive in their new environments.

B. Sites of Osmotic Exchange

The main sites of osmotic exchange in the fishes are skin, the kidney, and the gills.

1) The Skin

Injury to the skin or removal of the mucus covering impairs
the resistance of the fish to the osmotic pressures of their environment. In marine species, injury leads to a water diuresis and an increase in body chloride concentration (Grafflin, 1931). Raffy (1949) found that \textit{Blennius pholis L.} became stenohaline if the mucus was washed off, and the removal of mucus from the elvers of \textit{Anguilla anguilla} led to a rise in the osmotic pressure of the blood and to death (Firly, 1932). Krogh (1937) also found that damage to the skin accelerated the loss of salt by eels held in fresh water. Pickford (1957) states that the results of Grafflin, Raffy, and Firly indicate that the intact skin of teleosts is impermeable to the external medium and that the impermeability depends on the secretion of the mucus glands. The skin of the fresh water teleost has a very low permeability to water. This is emphasized by the finding that 1 ml. of water takes 158 days to pass across 1 sq. cm. of goldfish skin, and 5 years to pass across the same area of eel skin (Krogh, 1939, and Keys, 1933). Holmes (1959) also found that the skin of the rainbow trout was only a minor site of sodium transfer.

2) \textbf{The Kidney}

Fresh water fish have a well developed glomerular kidney, which enables them to excrete water as a hypotonic urine ($\Delta = 0.7-0.9$) (Black, 1957). The urine flow is copious, that for the rainbow trout ranging 60-106 ml. per kg. body weight per day (Krogh, 1937). Marshall (1934) found that the catfish produced 300 ml. of urine per kg. per day, and Smith (1932)
indicated urine flows of 200-400 ml. per kg. per day for the carp, the goldfish and the eel.

In marine fish, glomeruli may be present or absent, but the distal convoluted tubule is normally absent (Edwards, 1935). The proximal convolution is thus the only indispensible part of the kidney. Marshall and Grafflin (1932) found that water reabsorption takes place in the kidney tubule in glomerular teleosts. Clarke (1934) demonstrated a 78.6% reabsorption of the glomerular filtrate by the kidney of the sculpin. Only a small amount of urine is excreted by marine teleosts, about 3 ml. per Kg. per day (Clarke, 1934). Grafflin (1931) recorded urine flows of 2.5 ml. per kg. per day in the aglomerular toadfish and less than 4 ml. per kg. per day in the sculpin possessing glomerular kidneys. Although the urine is hypotonic ($\Delta = 0.62-0.79$), the concentration is very close to that of the blood ($\Delta = 0.72-0.82$) (Smith, 1932 and Forster, 1953). Therefore, most of the water must be reabsorbed although Smith attributes the low urine flow in the sculpin to a greatly reduced glomerular filtration rate. Studies of the structure and function of the aglomerular kidney of marine fishes have indicated that the agglomerular kidney corresponds to the second segment of the proximal convoluted tubule in glomerular forms (Edwards and Condorelli, 1928, Edwards, 1929, Marshall, 1930 and Grafflin, 1937). The substances to be excreted from the blood are secreted into the kidney tubule accompanied by small amounts of water (Marshall and Grafflin, 1932). Studies involving the
isolated kidney of the flounder showed that phosphate bond energy, oxygen, cytochrome oxidase, and dehydrogenases were necessary for phenol red transport (Forster, 1948, Taggart and Forster, 1950, and Forster and Taggart, 1950). Calcium, magnesium, sulfate, and phosphate ions, absorbed through the gut, are excreted actively by the kidney (Smith, 1930, 1932, Forster, 1948, Forster and Berglund, 1956 and Berglund and Forster, 1958).

In euryhaline fish entering fresh water, increased glomerular filtration rate and diuresis are necessary to enable the fish to excrete excess water crossing the integument. Clarke (1934-) noticed that the diuresis due to handling of fish was caused by increased glomerular filtration rate with a constant reabsorption of water. Reabsorption of salts by the kidney tubule is important in a hypotonic environment. When euryhaline fish enter sea water, the increased concentration of body fluids due to water loss automatically reduces the urine loss.

3) The Gills

Keys (1931a, b), by means of a heart-gill perfusion apparatus, studied osmoregulation by the gill of the eel, Anguilla vulgaris. He found a dilution of the perfusion medium when the external medium was fresh water, due to the inward diffusion of water through the gill surface. Krogh (1937) found that fresh water fishes actively absorbed chloride ions from very dilute solutions when the salt content of the body fluids was depleted. These ions were usually accompanied by cations but
could be exchanged against bicarbonate. Krogh states that the gills of the goldfish have independent mechanisms for absorbing anions and cations. The anion mechanism absorbs chloride and bromide. The cation mechanism takes up sodium ions, but not potassium. Sexton and Meyer (1955) found that goldfish gills could absorb lithium as well as sodium, but not potassium or cesium. Meyer (1952) and Sexton and Russell (1955) have suggested an analogy between the function of gill and kidney epithelial tissues. These workers found that mercurial diuretics inhibited the active sodium uptake and increased the sodium loss from the goldfish gill.

Marine fish, which swallow sea water to prevent dehydration must excrete the excess salts to maintain a low body fluid concentration. Smith (1930) noted that osmotic work was necessary for extra-renal excretion of water and electrolytes in a marine environment. He suggested that the gills were the site of this extra-renal regulation. Perfusion experiments by Keys (1931b) involving a hypertonic environment showed that chloride was secreted from the perfusion medium against a large concentration gradient. He pointed out a similarity between the activity of the gill of the sea water eel and the normal mammalian kidney. Keys and Wilmer (1932) found secretory cells in the gills of the eel, and indicated a possible correlation between them and the chloride secreting activity of the gills.

Copeland (1948, 1950) studied the histology of the chloride secreting cells of the euryhaline fish, Fundulus heteroclitus.
He ascribed the function of chloride exchange to certain columnar acidophilic cells rich in mitochondria. The cells have a reversible polarity, secreting chloride in sea water, and absorbing it from a hypotonic environment. Since an "excretory vesicle" containing chloride was present in sea water and in saline injected fish, Copeland (1948) concluded that the activity of the cells was determined by the internal environment. The activity of the cells in fresh water was correlated with a high concentration of alkaline phosphatase (Copeland and Pettengill, 1948). Getman (1950) noticed similar changes in the chloride secreting cells of *Anguilla rostrata*. Bevelander (1935, 1936) assigned the function of chloride secretion to the whole respiratory epithelium rather than to single cells, although he noticed that the mucus cells, of various stages of secretory activity, could be localized or scattered. Copeland (1950) agrees that the chloride cell may be a modified mucus cell.

III. Changes Occurring on Transfer to Sea Water

A. Physiological Changes

Busnel (1942) found that exposure of stenohaline and euryhaline fishes to varying salinities caused changes in the concentration, the hematocrit, and the pH of the blood. Portier and Duval (1922a, b) and Fontaine, Dallatre, and Callamand (1945) showed that transfer of fresh water fishes to moderate salinities caused an initial rise in the blood con-
centration and the plasma-cell ratio. Busnel (1942) indicated that there is a gradual return to normal. Parry (1958) found that salmonid fishes able to withstand transfer into sea water showed an initial rise in blood concentration with a subsequent fall to normal levels. In rainbow trout, Busnel, Drilhon, and Raffy (1946) found that adult fish could withstand a gradual transfer to high salinities ($\Delta = 1.90$), but the concentration of their internal milieu increased (from $\Delta = .50$ to $\Delta = 1.03$). These workers suggested that rainbow trout were intermediate between the stenohaline (carp) and euryhaline (eel) teleosts in their ability to regulate osmotically.

B. Hormonal Changes

In the mammal, hormonal principles from the neurohypophysis and adrenal cortex are active in water and salt-electrolyte metabolism. Polypeptides have been isolated from the neurohypophysis which have vasopressor, antidiuretic, and oxytocic effects. Vasopressin, or antidiuretic hormone, causes increased water reabsorption in the distal convoluted tubule of the kidney. The amphibian water balance principle from the neurohypophysis also causes water retention in the Anura. From the adrenal cortex, the mineralocorticoids, especially aldosterone, effect the retention of sodium by modifying the active reabsorption from the kidney tubule. These hormones may also govern osmoregulation in fishes.

The hormones of the neurohypophysis have been isolated in
fishes. Heller (1941, 1945) demonstrated the presence of the antidiuretic hormone in the cod pituitary. Fontaine (1956) found strong antidiuretic activity, according to mammalian bioassay techniques, in the pituitary of eels and salmon in fresh water, and in the pike, a fresh water teleost. Many workers found that fish do not respond to the antidiuretic action of either mammalian or fish vasopressin by a weight increase (Burgess, Harvey and Marshall, 1933, Boyd and Dingwall, 1939, Fontaine and Raffy, 1950, Callamand, Fontaine, Olivereau, and Raffy, 1951, and Fontaine, 1956). Sexton (1955), however, found that vasopressin caused an increased flow of urine in the goldfish, accompanied by an increased water uptake by the gill. These observations may explain the failure of other workers to record a weight increase in fishes injected with vasopressin.

Several adrenocortical steroids have been characterized in fish blood. Hatey (1954) studied the corticosteroids in the blood of the salmon and found the concentration to be highest at the smolt stage. Phillips, Holmes, and Bondy (1959) characterized cortisol, cortisone, corticosterone, and aldosterone in the plasma of spawning male salmon. Chester Jones (1956) has outlined the features common to the teleost interrenal organ and the mammalian adrenal cortex. In Astyanax mexicanus, the interrenal body responded to mammalian corticotropin (ACTH) as well as to fish pituitary extracts, and hypophysectomy of Anguilla anguilla, caused a decrease in the
weight of the interrenal which could be restored by ACTH (Rasquin, 1951, Rasquin and Atz, 1952, Fontaine and Hatey, 1953). He also found that hormones affecting salt-electrolyte metabolism in eutherians (desoxycorticosterone acetate and cortisone) influence the sodium and potassium levels in the blood and muscle in fish (Chester Jones, 1956).

IV. Scope of the Thesis

The purpose of this thesis is to investigate the changes in the metabolic activity of the gill and kidney tissues when cutthroat trout are transferred abruptly from fresh water to a hypertonic medium. The preceding sections show that there is ample evidence for the osmoregulatory function of the gill and kidney tissues. From the proved experimental differences in the active excretion of water and electrolytes in fresh water and marine fishes, one might expect variations in the metabolism of these tissues in the different environments. Oxygen consumption, since tissue oxidation and energy-producing phosphorylation systems are normally linked, is used as an index of the energy expended by the gills and kidney during osmoregulation.

The effects of vasopressin, the posterior pituitary hormone active in mammalian water balance, on oxygen consumption was investigated. Holmes (1959) found that vasopressin almost completely blocked renal sodium excretion in saline loaded fish. Since vasopressin appears to be active in fishes, an attempt was made to correlate a possible action of vasopressin
in the osmoregulation of fishes, by comparing the metabolic activity of tissues from sea water and vasopressin treated fish.
MATERIALS AND METHODS

I. Respirometry

Cutthroat trout, \textit{(Salmo clarki clarki)} which had been reared in the British Columbia Game Department Hatchery at Cultus Lake, B.C., were used throughout these experiments. No attempt was made to sex the animals. Prior to use the fish were held in running dechlorinated tap water (temperature about 8°C) in large cement troughs.

The oxygen consumption of gill and kidney tissue was determined by the direct method of Warburg as outlined by Umbreit, Burris, and Stauffer (1958). The centre well of each flask contained 0.2 ml. of 20\% KOH solution and a wick of Whatman #1 filter paper. The main compartment contained 2.7 ml. of Krebs' saline solution (1950) modified for trout tissues.

Krebs' saline solution was specifically formulated for mammalian tissues. The total osmolar concentration of this solution was varied until the depression of freezing point and pH were equal to those of cutthroat trout blood (\(\Delta = 0.58^\circ\text{C}, \quad \text{pH} = 7.2\)). The composition of this modified Krebs' solution was as follows:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (%)</th>
<th>Parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>0.935</td>
<td>103</td>
</tr>
<tr>
<td>KCl</td>
<td>1.19</td>
<td>4</td>
</tr>
<tr>
<td>(\text{KH}_2\text{PO}_4)</td>
<td>2.19</td>
<td>1</td>
</tr>
<tr>
<td>(\text{MgSO}_4\cdot 7\text{H}_2\text{O})</td>
<td>3.97</td>
<td>1</td>
</tr>
<tr>
<td>Substance</td>
<td>Concentration</td>
<td>Parts</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------</td>
<td>-------</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>1.35</td>
<td>3</td>
</tr>
<tr>
<td>PO₄ Buffer</td>
<td>---</td>
<td>18</td>
</tr>
<tr>
<td>PO₄ Buffer:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1.47</td>
<td>4</td>
</tr>
<tr>
<td>NaH₂PO₄·H₂O</td>
<td>1.43</td>
<td>1</td>
</tr>
</tbody>
</table>

No substrates were used in this medium since previous results had been more variable in the presence of the recommended substrates.

The fish were killed by a blow on the head and immediately weighed. After the digestive tract and swim bladder were removed, the dorsal side of the kidney was gently scraped free with curved forceps and the organ removed. The kidney was then blotted to remove blood and laterally sliced freehand with a razor blade. The gills were removed whole and rinsed in ice cold Krebs' solution. The filaments were then cut free of the cartilaginous gill bars. Approximately 100 mg. wet weight of tissue were placed in each flask.

All flasks were gassed with therapeutic oxygen (B.P. and U.S.P. specifications) for 12 minutes and were allowed to equilibrate in the constant temperature bath at 30°C for 15 minutes. The flasks were agitated at the maximum rate and amplitude of shaking. Manometer readings were taken at 10 minute intervals and the total oxygen consumption for the 60 minute period was calculated from a graph of reading vs. time. After the final
reading, the tissues were dried for 48 hours at 101°C to determine the dry weight values. The oxygen consumptions of the tissues were expressed in microlitres (mm.\(^3\)) per mg. dry weight of tissue per hour (\(Q_{O_2}\)).

**II. Regression Lines**

Log \(Q_{O_2}\) for each tissue was plotted against log body weight (in gm.). The ALWAC III-E digital computer was used to calculate regression coefficients by the method of least squares. The programme for this statistical analysis is available at the computing centre, U.B.C., Vancouver, B.C. Correlation coefficients (r) were calculated according to the method outlined by Snedecor (1946). The 95% confidence limits on mean values of \(Q_{O_2}\) appropriate to various values of body weight were calculated by the method outlined by Snedecor (1956). The observed regression coefficients were compared to a theoretical regression of \(-.27\) using the procedure of Snedecor (1946).

**III. Transfer to Sea Water**

To determine the changes occurring on transfer of the fish to a hypertonic medium, the fish were pretreated by holding them in sea water. The sea water was prepared by mixing 3 parts of ocean sea water to 1 part of dechlorinated tap water. This had a salinity of 62-65% of standard sea water (NaCl = 31.88%), when tested by a conductivity meter, and caused a depression of freezing point, \(\Delta = 1.29^\circ\text{C}\).

During the experiment, the fish were transferred from the
storage tanks to battery jars containing 4 litres of sea water for a specified period of time. Two fish were placed in each jar, and duplicate determinations were made. Parallel controls were run in jars containing dechlorinated tap water. The experiment was repeated varying the length of time the fish were left in the jars. The water temperature was held constant between 7-8°C by keeping the jars in a trough of running water, and the water in the jars was aerated by individual bubbling stones connected to a compressed air source.
RESULTS AND DISCUSSION

I. Tissue Respiration and Body Weight

A. Results

1) Kidney (Table I and figure 1)

The weight specific equation (4) was applied to the $Q_{O_2}$ values obtained for cutthroat trout tissues. The kidney values plotted in figure 1 were obtained from 83 estimations involving fish varying in body weight from 10 to 100 gm. These values were found to have a regression of -.148 with a mean body weight of 23.2 gm. and a mean $Q_{O_2}$ of 6.77. The equation for this line was:

$$\log Q_{O_2} = 1.0327 - .148 \log W$$

or

$$Q_{O_2} = 10.78 W^{-0.148}$$

The correlation coefficient (r) of .42 was rather low, but with 81 degrees of freedom, the fit of the line was highly significant (p < .001) (Table I). The weight specific values were used since it was impossible to remove the entire kidney from the fish.

2) Gill (Table I and figure 2)

The equation (4) was again applied to the $Q_{O_2}$ values for gill tissue of the cutthroat trout. Estimations were made on 143 fish varying in weight from 10 to 100 gm. The regression of the values was -.139 with a mean body weight of 26.1 gm. and a mean $Q_{O_2}$ of 5.79. The equation for the line was:
\[
\log Q_02 = 0.9597 - 0.1391 \log W \\
\text{or } Q_02 = 9.114 W - 0.139
\]

The wide variation in values gave an even lower correlation coefficient than the kidney tissue (\( r = 0.32 \)), but with 141 degrees of freedom, the fit of the slope was significant (\( p < 0.001 \)). (Table I).

The dotted lines on figures 1 and 2 represent the intervals within which 95\% of the mean \( Q_02 \) values would fall for the corresponding body weight. Table I shows the results of the regression lines for gill and kidney.
### TABLE I. REGRESSION OF KIDNEY AND GILL Q\textsubscript{O2} WITH BODY WEIGHT

<table>
<thead>
<tr>
<th>Tissue</th>
<th>N</th>
<th>Mean Body Weight in gm.</th>
<th>Mean (Q_{O2})</th>
<th>(b^1)</th>
<th>((\alpha - 1)^2)</th>
<th>(S(\log y, \log x)^3)</th>
<th>(r^4)</th>
<th>P value 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>83</td>
<td>23.2</td>
<td>6.77</td>
<td>10.780</td>
<td>-.148</td>
<td>.0112</td>
<td>.42</td>
<td>&lt;.005 &gt; .001</td>
</tr>
<tr>
<td>Gill</td>
<td>143</td>
<td>26.1</td>
<td>5.79</td>
<td>9.114</td>
<td>-.139</td>
<td>.0100</td>
<td>.32</td>
<td>&lt;.005 &gt; .001</td>
</tr>
</tbody>
</table>

1 \(Q_{O2}\) when body weight = 1 gm.

2 Gradient of line on a log-log plot

3 \(S(\log y, \log x)\) = standard deviation of the regression coefficient

4 Correlation coefficient

5 P value when \((\alpha - 1)\) compared to theoretical regression of -.27
FIGURE 1. Regression of kidney $Q_{O2}$ with body weight of the cutthroat trout.
log \( y = 1.0327 - 0.148 \log x \)

**FIGURE 1**
FIGURE 2. Regression of gill $Q_02$ with body weight of the cutthroat trout.
log y = 0.9597 - 0.139 log x
B. Discussion

Neither the exponent for the kidney nor that for the gills agreed with the surface rule or Brody's (1945) .73 rule relating body weight and metabolism. The regression coefficients of the $Q_{O_2}$ values for kidney and gill (-.148 and -.139) were statistically compared to a theoretical value of .27 corresponding to the .73 rule. Since the comparisons showed a significant difference from the .73 rule for both tissues (kidney, $p < .005 > .001$, and gill, $p < .005 > .001$), the observations do not support the .73 rule at the level of tissue respiration.

The exponents of the body weight for kidney and gill tissues did not differ significantly (-.148 vs. -.139), suggesting that the rate of decline in weight specific oxygen consumption was the same for gill and kidney tissue.

Bertalanffy and Pirozynski (1953) recalculated Krebs' (1950) data for nine mammalian species and obtained an interspecific ($\alpha - 1$) value of -.064 for kidney. They did not find any significant regression of kidney $Q_{O_2}$ values in their own data. Examination of the integration constants ($b$) showed that the $Q_{O_2}$ values of the kidney and gill tissues were on the same level (Table I, $b = 10.78$ vs. 9.11). This fact provides further evidence to support the idea that the osmoregulatory work is equally distributed between the gills and kidneys in teleosts. Holmes (1959) showed that, in the sodium loaded rainbow trout, the excretion of sodium was equally divided
between the gills and the kidney.

Vernberg (1954) studied the weight specific respiration rates of brain, liver and skeletal muscle from the marine teleost, *Opsanus tau*. Both brain and muscle showed positive regressions with \((\alpha-1)\) values of .202 and .118 respectively. Liver showed a negative regression of approximately similar value to those obtained for gill and kidney of the cutthroat trout \((\alpha-1) = -.145\). Since Vernberg expressed the \(Q_{o2}\) values in microlitres of oxygen per gm. wet weight of tissue per minute, it was necessary to evaluate his results in terms of mg. dry weight of tissue per hour to compare the two sets of data. The dry weight values for Vernberg's data were calculated from the mean percentage water content given in his results. This conversion gave integration constants \((b)\), i.e. \(Q_{o2}\) when \(W\) is 1, of .734 for brain, 2.29 for liver, and .110 for muscle. All these values were lower than those for excretory tissues in the fresh water cutthroat trout.

As a result of their studies of the oxygen consumptions by the major tissues of the mammalian body, Krebs (1950) and Bertalanffy and Pirozynski (1953) abandoned the theory that the weight specific decline in the total body metabolism was due to an integrated decline in the metabolic rates of the tissues. From the data presented in this thesis and those of Vernberg, there appears to be a parallel decline in the metabolically more active tissues, liver, kidney, and gill, in teleosts. The less active tissues, the brain and skeletal
muscle, with lower integration constants, show positive regressions. Thus the evidence indicates that the weight specific decline in overall metabolism in teleosts is not due to an integrated decline, and supports the work of Krebs (1950) and Bertalanffy and Pirozynski (1953) for mammals.

The correlation coefficients (r) for kidney (.42) and for gill (.32) are very much lower than those found by Krebs (1950) and calculated by Bertalanffy and Pirozynski (1953) (r = .846-.969). This inconsistency may have been because the animals were not from highly inbred stock. It could also have been a manifestation in part of the poikilothermic nature of the teleosts. Weymouth, Crimson, Hall, Belding, and Field (1944) reported low correlation coefficients when weight specific oxygen consumption was related to body weight in crustaceans.
II. Effect of Transfer to Sea Water

A. Results

The mortality rate after transfer of cutthroat trout from fresh water to sea water was dependent on the concentration of the sea water. After transfer to 100% standard sea water, from a sample of 10 fish, 5 were dead within 22 hours and all were dead by 30 hours. The fish survived for 96 hours in 75% standard sea water but were in poor condition at the end of this period. All the fish survived and were in good condition 168 hours after transfer into 65% standard sea water. For this reason, 65% standard sea water was used in all these experiments.

The body weight of the fish used in this experiment was within a 15 gm. range. Since the regression of $Q_0$ and body weight on a log-log plot was slight, and the scatter of the $Q_0$ values for a single weight was large, the uncorrected values for $Q_0$ were used in plotting the graphs.

1) Kidney (Tables II and IV, and figure 3)

Control fish kept in 4 litres of fresh water during the experimental period showed a slight initial rise in kidney $Q_0$ value followed by an apparent fall, between 48 and 168 hours, below the zero hour control level. This variation did not differ significantly from the zero hour oxygen consumption ($p > .1$).

The experimental animals, transferred into 65% standard
sea water, showed an initial rise in kidney $QO_2$ values during the first 48 hours. This rise was maximal at 20 hours and highly significant when compared to the parallel controls ($p < .001$). From 48 hours onwards, there was a progressive decline in the $QO_2$ values which levelled off slightly higher than the parallel controls. The combined values for the 48-168 hour samples showed that the mean $QO_2$ value for the experimental fish was significantly higher than the mean value for the controls ($p < .001$).

2) Gills (Tables III and IV, and figure 4)

During the experimental period of 168 hours there was no significant change in the oxygen consumption of gill tissue from control fish kept in 4 litres of fresh water. Gill tissue from animals exposed to sea water showed a significant decline during the first 10 hours. The combined values for the 48-168 hour samples showed that the mean $QO_2$ value for the experimental fish was significantly lower than the mean value for the controls ($p < .001$).

All curves on figures 3 and 4 were drawn freehand to the best fit of the points. The first point on each figure corresponds to the zero hour control fish.
TABLE II. Changes in the oxygen consumption of kidney tissues after transfer of fish to sea water.

| Time after Transfer (Hr.) | **SEA WATER** | | **FRESH WATER** | | **P Value** |
|---------------------------|---------------|-----------------|-----------------|-----------------|
|                           | Mean Body Weight (Gm.) | No. of Fish | $Q_O_2$ | Mean Body Weight (Gm.) | No. of Fish | $Q_O_2$ | |
| 0                         | 14.02 ± 1.54 | 4 | 7.211 ± 0.288 | 14.02 ± 1.54 | 4 | 7.211 ± 0.288 | — |
| 5                         | 14.02 ± 0.69 | 4 | 7.988 ± 0.739 | 14.78 ± 0.37 | 4 | 9.242 ± 0.500 | <.2 > .1 |
| 10                        | 22.00 ± 0.95 | 4 | 10.356 ± 0.283 | --- | - | --- | --- |
| 15                        | 21.95 ± 0.64 | 4 | 10.996 ± 0.580 | --- | - | --- | --- |
| 20                        | 21.37 ± 3.55 | 3 | 11.840 ± 0.453 | --- | - | --- | --- |
| 24                        | 13.88 ± 1.57 | 4 | 10.086 ± 0.500 | 14.05 ± 0.70 | 4 | 6.922 ± 0.620 | <.01 > .005 |
| 30                        | 12.92 ± 0.88 | 4 | 7.411 ± 0.760 | --- | - | --- | --- |
| 48                        | 13.84 ± 0.64 | 8 | 8.193 ± 0.600 | 14.50 ± 0.87 | 4 | 5.422 ± 0.303 | <.005 > .001 |
| 72                        | 13.20 ± 1.62 | 4 | 8.007 ± 0.231 | 15.12 ± 0.69 | 4 | 7.165 ± 0.803 | <.4 > .2 |
| 96                        | 17.90 ± 1.68 | 4 | 7.860 ± 0.289 | 16.22 ± 2.36 | 4 | 7.586 ± 0.445 | >.5 |
| 120                       | 19.02 ± 5.57 | 2 | 8.579 ± 1.556 | 13.45 ± 2.10 | 4 | 5.963 ± 0.504 | <.2 > .1 |
| 144                       | 10.95 ± 1.02 | 4 | 6.143 ± 0.283 | 12.50 ± 1.08 | 4 | 6.713 ± 0.590 | <.5 > .4 |
| 168                       | 15.50 ± 1.76 | 4 | 7.902 ± 0.737 | 12.35 ± 1.32 | 4 | 6.715 ± 0.600 | <.2 > .4 |

1 Standard error of mean value

2 P value compared to corresponding freshwater controls
<table>
<thead>
<tr>
<th>Time after Transfer (Hr.)</th>
<th>Mean Body Weight (Gm.)</th>
<th>No. of Fish</th>
<th>$Q_{O_2}$</th>
<th>Mean Body Weight (Gm.)</th>
<th>No. of Fish</th>
<th>$Q_{O_2}$</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>SEA WATER</strong></td>
<td></td>
<td></td>
<td><strong>FRESH WATER</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14.02 ± 1.54</td>
<td>4</td>
<td>6.410 ± 0.144</td>
<td>14.02 ± 1.54</td>
<td>4</td>
<td>6.410 ± 0.144</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>14.02 ± 0.69</td>
<td>4</td>
<td>5.414 ± 0.300</td>
<td>14.78 ± 0.37</td>
<td>4</td>
<td>6.776 ± 0.236</td>
<td>&lt;.025 &gt;.010</td>
</tr>
<tr>
<td>10</td>
<td>22.00 ± 0.95</td>
<td>4</td>
<td>4.846 ± 0.100</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>21.95 ± 0.64</td>
<td>4</td>
<td>5.582 ± 0.462</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>21.37 ± 3.55</td>
<td>3</td>
<td>5.790 ± 0.095</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>13.88 ± 1.57</td>
<td>4</td>
<td>7.552 ± 0.215</td>
<td>14.05 ± 0.70</td>
<td>4</td>
<td>6.060 ± 0.276</td>
<td>&lt;.010 &gt;.005</td>
</tr>
<tr>
<td>30</td>
<td>12.92 ± 0.88</td>
<td>4</td>
<td>4.487 ± 0.202</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>12.76 ± 0.99</td>
<td>3</td>
<td>5.177 ± 0.537</td>
<td>14.50 ± 0.87</td>
<td>4</td>
<td>5.314 ± 0.266</td>
<td>&gt;.5</td>
</tr>
<tr>
<td>72</td>
<td>13.20 ± 1.62</td>
<td>4</td>
<td>5.457 ± 0.253</td>
<td>15.12 ± 0.69</td>
<td>4</td>
<td>6.396 ± 0.305</td>
<td>&lt;.1 &gt;.05</td>
</tr>
<tr>
<td>96</td>
<td>17.90 ± 1.68</td>
<td>4</td>
<td>5.001 ± 0.366</td>
<td>15.53 ± 1.05</td>
<td>3</td>
<td>6.159 ± 0.217</td>
<td>&lt;.050 &gt;.025</td>
</tr>
<tr>
<td>120</td>
<td>19.02 ± 5.57</td>
<td>2</td>
<td>4.320 ± 0.344</td>
<td>13.45 ± 2.10</td>
<td>4</td>
<td>6.183 ± 6.340</td>
<td>&lt;.01 &gt;.005</td>
</tr>
<tr>
<td>144</td>
<td>10.95 ± 1.02</td>
<td>4</td>
<td>5.294 ± 0.231</td>
<td>12.50 ± 1.08</td>
<td>4</td>
<td>7.609 ± 0.556</td>
<td>&lt;.01 &gt;.005</td>
</tr>
<tr>
<td>168</td>
<td>15.50 ± 1.76</td>
<td>4</td>
<td>5.687 ± 0.331</td>
<td>12.35 ± 1.32</td>
<td>4</td>
<td>5.826 ± 0.359</td>
<td>&gt;.40</td>
</tr>
</tbody>
</table>

1 Standard error on mean value

2 P value compared to corresponding freshwater controls
TABLE IV

Comparison of combined values (48-168 hr.) for gill and kidney tissue respiration of fish in sea water and fresh water.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>SEAWATER</th>
<th>FRESHWATER</th>
<th>P Value2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean $Q_{O_2}$</td>
<td>N</td>
<td>Mean $Q_{O_2}$</td>
</tr>
<tr>
<td>Gill</td>
<td>$5.237 \pm 0.425$</td>
<td>21</td>
<td>$6.254 \pm 0.510$</td>
</tr>
<tr>
<td>Kidney</td>
<td>$7.856 \pm 0.825$</td>
<td>26</td>
<td>$6.593 \pm 0.707$</td>
</tr>
</tbody>
</table>

1 Standard error of mean value

2 P value of combined values compared corresponding combined control values over the same period
FIGURE 3. Effect of transfer of cutthroat trout to seawater on the kidney $Q_{O_2}$ values.
Figure 3

Oxygen Consumption (mm$^3$/mg dry weight/hr)

- **SEA WATER**
- **FRESH WATER**

Time after transfer (hr.)

---

**FIGURE 3**
(facing figure 4)

FIGURE 4. Effect of transfer of cutthroat trout to seawater on the gill $\dot{Q}_{O_2}$ values.
B. Discussion

There are three possible sites of osmotic regulation in fishes: the kidney tubule, the gill epithelium, and the skin.

The well developed glomerular kidney possessed by fresh water teleosts enables them to excrete a copious dilute urine. Urine flows vary from 60-400 ml. per kg. body weight per day (Smith, 1932, Marshall, 1934, and Krogh, 1937). In contrast to these high rates of diuresis, the marine teleosts have a relatively scant urine flow of less than 4 ml. per kg. per day (Grafflin, 1931). Although Smith (1955) attributes the low urine flow in the sculpin to a greatly reduced glomerular filtration rate, Clarke (1934) reported a 78.6% tubular reabsorption of glomerular filtrate in the sculpin. There is no evidence at present to indicate that the anadromous species, such as the salmon, or euryhaline species, such as the trout, undergo such a drastic reduction of glomerular filtration rate when they are transferred to a hypertonic medium.

Smith (1932) states that marine teleosts drink large amounts of sea water to offset the water lost by diffusion through the gills. It is assumed that a similar phenomenon occurs on transfer of euryhaline species to sea water. The absorption of both salts and water from the gut and the loss of water by diffusion through the gills would cause a rise in the tissue fluid concentration reflected by a rise in the blood concentration (Portier and Duval, 1922 a,b, Busnel, 1942, and Fontaine, Dellatre, and Callamand, 1945). In mammals, the
kidney controls the loss of water through the antidiuretic effect of increased active tubular reabsorption of water. The rapid rise in trout kidney $Q_{O_2}$ upon transfer to sea water indicates an increased energy turnover which may be associated with increased tubular reabsorption of water and/or electrolytes. Immediately upon transfer, the fish tends to dehydrate. Parry (1958) found that salmonid fishes transferred to sea water showed an initial rise in blood concentration with a subsequent fall to normal levels. The fish must, therefore, conserve body water. The initial rise in $Q_{O_2}$ could thus be associated with some antidiuretic process.

Physiological doses of vasopressin administered intraperitoneally to the cutthroat trout are capable of increasing the $Q_{O_2}$ values of kidney tissue by an increment equal to that observed upon transfer to sea water (Holmes, unpublished, and Appendix). Adrenocortical steroids are capable of increasing the oxygen consumption also, but not to the extent of vasopressin (Holmes, unpublished). Previous work on this subject (Holmes, 1959, Chester Jones, Phillips and Holmes, 1959) indicated that adrenocortical steroids were effective in enhancing the reabsorption of sodium by the kidney of the rainbow trout. One would not expect this to occur in a fish situated in a hypertonic environment. However, the increased reabsorption of water may be accompanied by increased reabsorption of salts. The demonstration of appreciable amounts of cortisol, cortisone, corticosterone, and aldosterone in
Sockeye salmon blood (Phillips, Holmes, and Bondy, 1959) suggests that the synthetic pathways of adrenocortical steroids are at least present among the Salmonoidea.

The shape of the experimental curve in figure 3 may possibly be explained as follows: the initial rise and peak values for the $Q_{O_2}$ values may be due to regulatory effects of vasopressin and adrenocortical steroids, while the maintained higher level may be due to a persistent effect of adrenocortical steroids. In mammals, vasopressin has been shown to have a short term action, while the adrenocortical steroids may have more lasting effects, as in the case of stress. The later stages of the adaptation of euryhaline fishes to sea water may include anatomical, as well as functional changes in the kidney. Ford (1958) has shown that salmon raised in sea water have fewer glomeruli than those raised in fresh water. Black (1957) has also pointed out a gradation in the functional activity of glomeruli in marine teleosts.

Holmes (1959) found that saline loaded rainbow trout exhibit an enhanced net output of sodium in the presence of adrenocortical steroids. Although these steroids caused the classical effect, increased reabsorption of salts, in the kidney, they increased the extra-renal output. The extra-renal increment was greater than the increased tubular reabsorption. Inhibition of the extra-renal re-uptake of sodium from the environment seemed to be a major factor in enhancing the net output. Sexton (1955) reported a similar inhibition
of sodium re-uptake in the presence of desoxycorticosterone acetate in the goldfish. The observed decline in gill $\dot{Q}_{O_2}$ values is consistent with an inhibitory mechanism.

The shape of the experimental curve in figure 4 may be explained by an inhibited re-uptake of salts by the gill epithelium, which thus expends less energy in sea water than in fresh water. The inhibition may be controlled by adreno-cortical steroids. Anatomical or histological changes may also follow the hormonally induced functional changes in the gills, the number of chloride secreting cells in Fundulus heteroclitus varying in fresh water and sea water (Copeland, 1948, 1950).
CONCLUSIONS

(1) The weight specific oxygen consumption of gill and kidney tissue for the cutthroat trout (*Salmo clarki clarki*) decreased with increasing body weight. The following relationships were found:

Kidney:

\[ Q_{02} = 10.78 \ W^{-0.148} \]

Gill:

\[ Q_{02} = 9.114 \ W^{-0.139} \]

The decline of the oxygen consumption did not support Brody's .73 rule or the surface rule at the level of tissue respiration.

(2) On transfer of the cutthroat trout from fresh water to sea water, changes occurred in the oxygen consumption of the excretory tissues. A sharp initial rise in the \( Q_{02} \) of kidney tissue was noted during the first 48 hours after transfer, reaching a maximum at 20 hours. The tissue respiration during the remainder of the experimental period remained significantly higher than the parallel control level. The gill tissue respiration declined rapidly during the first 10 hours after transfer and remained significantly below the control level during the whole experimental period. Hormonal regulation of salt-electrolyte and water metabolism by vasopressin and adrenocortical steroids may be correlated with these changes in energy output in euryhaline species of salmonids.
APPENDIX - EFFECT OF VASOPRESSIN ON OXYGEN CONSUMPTION IN THE KIDNEY

In an attempt to correlate antidiuretic processes and the transfer of the cutthroat trout to sea water, the effects of vasopressin, administered in vitro and in vivo, on the kidney oxygen consumption values was investigated.

I. Materials and Methods

A. Effects of Vasopressin in Vitro

To test the effects of vasopressin in vitro, 5 milliunits (mU) of vasopressin (Parke-Davis pitressin) were added to the experimental flasks. This was accomplished by making up a solution of modified Krebs' medium containing 2.0 mU of vasopressin per ml. The experimental flasks contained 2.5 ml. of the vasopressin fortified medium, while the control flasks contained 2.5 ml. of Krebs' medium in the main compartment. Substrates, 0.2 ml. of .1 M disodium succinate, .1 M disodium fumarate, or .1 M trisodium citrate, were added to the main compartment. Substrates containing .1 M disodium succinate plus .1 M disodium malonate, and .1 M disodium fumarate plus .1 M disodium malonate were used to test the effects of malonate inhibition. The respirometry was carried out as before.

B. Effects of Vasopressin in Vivo

To determine the effects of vasopressin in vivo, the fish were taken from the storage tanks and injected intraperitoneal-
ly with a dose of 10 mU of vasopressin. This was administered as 0.1 ml. of vasopressin solution containing 100 mU per ml. The fish were held in dechlorinated tap water for 5 hours until sacrificed.

Two sets of flasks were prepared: one as outlined before with 2.7 ml. of modified Krebs' medium in the main compartment, and the other differing by containing only 2.5 ml. of modified Krebs' medium and 0.2 ml. of substrate. The substrates were the same as those used before, and the respirometry was carried out as explained earlier.
### TABLE V. THE EFFECT OF SUBSTRATE ON THE UTILIZATION BY ISOLATED KIDNEY TISSUES OF VASOPRESSIN IN VITRO AND IN VIVO

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Treatment</th>
<th>N</th>
<th>Mean $Q_O^2$</th>
<th>Corrected mean $Q_O^2$</th>
<th>% increase over controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Controls</td>
<td>30</td>
<td>9.236</td>
<td>9.236</td>
<td>0</td>
</tr>
<tr>
<td>Vasopressin</td>
<td>VP.A ³</td>
<td>4</td>
<td>10.126</td>
<td>9.255</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>(Controls) 4</td>
<td></td>
<td>10.104</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VP.B ⁵</td>
<td>26</td>
<td>9.832</td>
<td>9.832</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>(Controls)</td>
<td></td>
<td>9.236</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td>Succinate</td>
<td>12</td>
<td>11.666</td>
<td>11.856</td>
<td>28.4 *</td>
</tr>
<tr>
<td></td>
<td>(Controls) 11</td>
<td></td>
<td>9.087</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Succinate + VP.A</td>
<td>8</td>
<td>15.190</td>
<td>11.917</td>
<td>29.0</td>
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<td></td>
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<td>15.112</td>
<td></td>
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<tr>
<td></td>
<td>Succinate + VP.B</td>
<td>12</td>
<td>12.400</td>
<td>11.965</td>
<td>29.5 *</td>
</tr>
<tr>
<td></td>
<td>VP.B 11</td>
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<td>10.188</td>
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<td></td>
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<tr>
<td>Fumarate</td>
<td>Fumarate 4</td>
<td>8</td>
<td>8.888</td>
<td>12.152</td>
<td>20.6</td>
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<td></td>
<td>(Controls) 4</td>
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<td>7.377</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fumarate + VP.A</td>
<td>4</td>
<td>10.525</td>
<td>15.169</td>
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<tr>
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<td>(Fumarate) 4</td>
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<td>7.726</td>
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</tr>
<tr>
<td></td>
<td>Fumarate + VP.B</td>
<td>4</td>
<td>11.953</td>
<td>12.170</td>
<td>31.7</td>
</tr>
<tr>
<td></td>
<td>(VP.B) 4</td>
<td></td>
<td>9.655</td>
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<tr>
<td>Citrate</td>
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<td>4</td>
<td>10.659</td>
<td>9.820</td>
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<td></td>
<td>(Controls) 4</td>
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<td>10.024</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Citrate + VP.A</td>
<td>3</td>
<td>13.348</td>
<td>9.149</td>
<td>-0.9</td>
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<td>11.369</td>
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<td></td>
<td>F + M + VP.B</td>
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<td>(VP.B) 4</td>
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<td>9.568</td>
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</table>

1 Controls for the various samples corrected to value for combined controls
2 Controls = 100%
3 VP.A - Vasopressin in vitro, added to flasks
4 Bracketed values act as parallel controls for preceding samples
5 VP.B - Vasopressin in vivo, injected into fish
* P value comparing value and bracketed control significant at the 5% level
II. Results and Discussion

Most of the results shown in Table V were not significantly different from the controls. This may have been due to the small sample size. The experiments could not be repeated because of lack of fish, since a disease of the trout at the Cultus Lake Hatchery necessitated destroying the stock of cutthroat trout. Thus, inferences only could be made concerning the results.

These inferences were:

1) Both succinate and fumarate enhanced the oxygen consumption of control tissue.
   Citrate was not active as a substrate.
2) Fumarate, only, was active as a substrate in the presence of vasopressin in vitro, succinate acting at the same levels in the absence and presence of vasopressin.
3) Both succinate and fumarate were inhibited by malonate to the same degree in the control experiments.
4) In the presence of vasopressin in vitro, fumarate was inhibited to a greater degree than succinate by the addition of malonate.

The oxygen consumption levels for the vasopressin in vivo experiments were not markedly different from the control values, indicating that either the dosage of vasopressin was too low to cause an effect or the time between injection and sacrifice of the fish was not optimum. Other experiments (Holmes, unpublished) indicated that injection of vasopressin
significantly raised the level of kidney $QO_2$.

Substrates from the tricarboxylic acid cycle in catalytic amounts have been found to stimulate cellular metabolism (Krebs and Johnson, 1937), although work on bacterial whole cell preparations indicates that the ability of the cell to utilize the substrates depends on the permeability of the cell wall to the substrate. As a rule, certain forms of bacteria cannot utilize citrate as a substrate until they have manufactured permease enzymes which enable the substrate to enter the cell (Barrett, Larsen, and Kallio, 1953). This fact seemed to hold true also in the cutthroat kidney slices where fumarate and succinate were active and the larger citrate molecule was not. Sexton and Russell (1955) noted an increased uptake of oxygen by the filaments of the goldfish gill in a succinate fortified medium, citing this as proof of the presence of succinic dehydrogenase in the gills of the goldfish. The results in this thesis showed that succinate (28.4%) and fumarate (20.6%) increased the oxygen consumption of kidney tissues, and both were inhibited by malonate to the same degree (succinate depressed to 20.8% and fumarate to 10.9%). This might indicate succinic dehydrogenase activity.

Vasopressin, added to excretory tissues in vitro, has been found to enhance their activity. Bentley (1958) found that vasopressin, in the presence of a substrate, increased water transport across the wall of the isolated urinary bladder
of the toad. After testing the effects of several metabolic inhibitors, he concluded that oxidative metabolism utilizing phosphate bond energy acted in water transfer. Vasopressin, added in vitro to trout kidney slices did not enhance the $Q_{O_2}$ significantly without substrate (0.2%). It caused a much greater enhancement in the presence of fumarate (64.3%) than in the presence of succinate (29.0%) as a substrate. Also, malonate caused a much greater depression of the $Q_{O_2}$ of the kidney slices with vasopressin in vitro, when fumarate was a substrate (10.6%) than when succinate was the substrate (26.4%).

Thus it might appear that vasopressin acts at an enzymatic level, activating fumarate as a substrate. The succinic dehydrogenase enzyme may be involved since malonate is active in inhibiting the fumarate as a substrate. However, if succinic dehydrogenase were involved and fumarate was inhibited to a greater degree than succinate, one would suspect that the tricarboxylic acid cycle was reversed by vasopressin. This is not so since energy is released only by the forward reactions of the cycle. The relatively small malonate inhibition of succinate as compared to fumarate in the presence of vasopressin in vitro might suggest the involvement of the actual fumarate molecule as the target for vasopressin. The results in this thesis do, however, agree with Bentley's (1958) finding that vasopressin can act in vitro in the presence of a substrate.
To summarize: vasopressin is active in vitro in the presence of a substrate with trout kidney slices. The nature of the substrate may indicate the action of vasopressin at an enzymatic level.

The statistical analyses in Table V were done on the raw data, not the corrected QO2 values.
BIBLIOGRAPHY


