Bioenergetics, Exercise, and Fatty Acids of Fish

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SYNOPSIS. The bioenergetic aspects of pentachlorophenol poisoning and exercise in fish are discussed. When cichlids are exposed to 0.2 ppm of pentachlorophenol, the intake of food was increased and energy losses were also increased. Growth was decreased. The cost of specific dynamic action was higher and the cost of exercise was increased above the cost of similar exercise in nonpoisoned controls. In salmon swimming to exhaustion at 52 cm/sec fatty acids 18:1, 16:0, and 16:1, and at 59 cm/sec fatty acids 22:6, 18:2, and 20:4 suffered the greatest depletion. At 52 and 59 cm/sec, respectively, average exhaustion times were 1141 and 598 minutes; the equivalents of distance traveled were 26.0 and 12.7 miles; the loss in lipids, 54 and 10 mg; and the average weight losses, 830 and 480 mg per salmon. Total caloric losses calculated from the data on lipid and weight losses were approximately 1118 and 566 calories. Calculated from the data of Brett (1964) on O2-consumption, caloric losses were estimated at only 344 and 188 calories. The difference between observed values and values calculated from the data of Brett may lie in the duration and severity of the exercise. Brett collected his data on O2-consumption on the basis of at most two hours at high velocity. Possibly when maximum effort is involved each succeeding mile and each succeeding hour is more difficult and more costly to the salmon.

Nutrition is concerned with food-intake through all its ramifications and implications. The science of nutrition deals primarily with the interactions of the animal body and its food in order to define quantitatively the fully adequate food supply for any combination of animal functions and for any type of internal and external stress (Griffith, 1967). Food-intake must supply the calories that are dissipated as heat or excreted, and the calories that are stored as carbohydrate, fat, and protein. Food-intake should also supply needed vitamins, amino acids, and salts.

Tepperman (1962) indicates that the balance or imbalance of caloric intake in food with caloric outgo, among other components, depends on the interrelations of (1) psychological factors such as hunger and appetite; (2) the integrative activity of the central nervous system; (3) the delivery of signals from the mouth, pharynx, and gastro-intestinal tract (that may allow an approximate metering of food and whose cumulative effect when integrated by the central nervous system with other information over a period of time leads to satiety, hunger, appetite, or in special cases, anorexia); (4) the storage of energy in adipose tissue; (5) the utilization of carbohydrates, lipids, and proteins in growth and repair of tissues; (6) basal metabolism and specific dynamic action; and (7) the activity pattern or muscle work. About 50% of body weight of many animals is skeletal muscle. The activity and the energy requirement of muscle can be varied by a factor of 20 or more.

It is the purpose of this manuscript to present an equation of energy balance which shows some of the bioenergetic relationships in nutrition (Equation 1), to examine the components of the bioenergetic equation on exposure of cichlid fish to potassium pentachlorophenate (KPCP), and then to provide data on the material and bioenergetic aspects of lipid and fatty acid metabolism of coho salmon in severe and very severe muscular activity, and to suggest the relationship of the events in severe...
and very severe exercise to nutritional requirements. Bioenergetic relationships, the methods available for study, their evaluation, and their interpretation are extensively discussed by Warren and Davis (1967).

THE EQUATION FOR ENERGY BALANCE

Some important bioenergetic relationships in animal nutrition are given in equation (1) based on the law of conservation of energy. E is used where chemical energy from oxidation is still available and H where the chemical energy has been degraded into heat.

Basically any animal at a specified zero time has an energy content, \(E_o\), equivalent to its heat of combustion. Subsequently over a period of time \(t\) the energy content of the food taken will be \(E_f\) and at time \(t\) the remaining energy content of the animal will be \(E_t\). Energy losses will have developed due to basal metabolism and to the metabolic requirements of exercise and of specific dynamic action (SDA) of the food substances. Energy will also have been lost as compounds with measurable heats of combustion in the urine and feces. Similar losses may have to be considered from the lungs, gills, and skin. Milk, ova, and sperm are another category of energy displacement rather than energy lost; many experiments on bioenergetics are so conducted that milk, ova, and sperm displacement are not involved and do not appear in equation (1). When involved, another term, perhaps \(E_{secretion}\), should appear on the right hand side of the equation.

Usually \(\Delta E\), equal to \((E_t - E_o)\), is positive and indicates growth or an increase in protein, carbohydrate, or fat. If \(\Delta E\) is negative, food deprivations or conditions of starvation prevail. \(E_o\) and \(E_t\) can be evaluated for a population by running heats of combustion on individuals selected by proper sampling techniques; \(E_f\) may be similarly evaluated from aliquots of food.

Basal metabolic rates, minimal metabolism, the specific increments of heat production with food-intake, and the heat production of muscular activity can be evaluated in several ways. Among others, heat production can be measured in calories; oxygen consumption can be measured in ml and related to heat production; a variety of experiments can be developed to allow estimation of the right hand factors from data on heat of combustion; and changes in energy can be estimated from material data such as protein, carbohydrate, and lipid composition.

Each of the four methods for measuring chemical energy degraded into heat (the measurement of the heat produced, the measurement of the oxygen consumed, the estimation from heat of combustion data, and the estimation from analyses of protein, carbohydrate, and lipid) has advantages in that different types of information are provided, and each may have disadvantages from the point of view of time required, the accuracy and the adequacy of the data for specific purposes, and the experimental designs that may be allowable or that may have to be excluded. The production of heat and consumption of oxygen are very variable from hour to hour, but their measurement allows a detailed insight into the physiological history of an organism. Because of the high variability, it is difficult to design collection-procedures that allow evaluation of net heat losses over periods such as a week or a month.

The production of heat is generally esti-
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Estimated from O₂-consumption on the basis that 1 ml of oxygen equals 4.8 calories or 1 mg of oxygen equals 3.36 calories at a respiratory quotient of 0.8 (Brody, 1945). For mammals and birds where lipids, proteins, and carbohydrates are being converted to carbon dioxide, water, urea, and uric acid, the conversion error in this procedure lies between 0.0 and 4.0%, and is usually of the magnitude of 1%. The validity of this procedure for fish has yet to be established because of inadequate information on the heat of combustion of fish protein. Considerable error can occur in evaluating heat production indirectly from O₂-consumption if partially oxidized metabolic fragments are accumulating or are being excreted. Wiegert emphasizes this point (1968).

Heitz (1967) has considered the problem of the heat of combustion of fish protein. Since the caloric equivalent of mammalian protein when burned in a calorimeter is 5.65 cal/g (Brody, 1945), and since protein is 16% nitrogen, protein calories can be derived from mg of nitrogen × 6.25 × 5.65, or nitrogen × 35.3. In the experiments of Heitz with cichlid fish, the protein calories determined by nitrogen × 35.3 were greater than the protein plus carbohydrate calories determined from combustion data. Thus, N × 35.3 considerably overestimates the protein calories in cichlids. Further by dividing heat of combustion of the dry mass of cichlids, decreased by the heat of combustion of the lipids, by the corresponding mg of nitrogen, one obtains a series of estimates for the heat of combustion per mg of nitrogen which are too high for protein (because the heat of combustion of carbohydrate should be considered), but where 19 out of 24 values were below 35.3, four were 35.3 and one was 39.2. The heat of combustion of cichlid protein probably is significantly below 5.65 cal/g.

The basal rate of heat production is usually described as the minimum recordable metabolism, compatible with normal existence of the animal. It is very difficult to approximate zero activity in fish. Basal rates of metabolism for fish are usually predicted from data on O₂-consumption by extrapolation back to zero activity along metabolism/activity curves, obtained 48 hr or more after the last intake of food (Fry, 1957; Brett, 1965). The use of drugs to attain minimum activity has not been sufficiently investigated. The data provided by Brett serve as standards for Oncorhynchus nerka and are probably representative for closely related species, but give only the order of magnitude for more distant species in different environments. However, for many problems under different experimental conditions than those supplied by Brett, ad hoc estimates of basal or standard energy degradation are needed. Frequently a compromise value can be derived by replacing basal metabolism of Equation (1) with post-digestive starvation-maintenance costs. In the absence of food the excess of catabolic over anabolic metabolism includes basal metabolism, a variable but usually small component from exercise, and a component from excretion.

An estimate of some of the energy loss in urine and feces can be obtained by iodometric estimation of the ml oxygen required for wet combustion of the excreta and multiplication by 4.8 cal (Warren and Davis, 1967). This procedure provides reasonable values for unoxidized or partially oxidized carbon and hydrogen atoms but the nitrogen probably remains in a reduced form such as ammonia, and a correction for reduced nitrogen in urine must be added to the energy lost in urine as determined from data on wet combustion.

The heat of combustion of urea is 2,528 small calories per gram. The formation of urea from two molecules of ammonia and one of carbonic acid can be considered as due to a withdrawal of two molecules of H₂O. The increase in free energy in the combination of glucose and fructose to produce sucrose with the elimination of one molecule of water is 5500 cal per mole (Lehninger, 1965). Thus a reasonable evaluation of the change in free energy from carbonic acid and ammonia would be 11,000 cal/mole of urea, or 183 cal per gram of urea. The heat of combustion of
NH₃ would thus be (2528—183) or 2345 cal for the ammonia obtainable from one gram of urea (34/60 g) or 4137 cal/g of ammonia or 5024 cal/g of nitrogen.

On the combustion of 1 g of protein, the ammonia nitrogen possible would be 160 mg and the heat of combustion in a bomb calorimeter of the ammonia equivalent would be 894 cal. Thus, if ammonia is the end product of protein combustion the energy loss would be 894 cal for every 5650 heat-of-combustion cal in the protein, or 14.2%.

If the nitrogen is excreted as urea, the 16% nitrogen in protein will yield 343 mg of urea with a heat of combustion of 0.343 X 2528 or 882 cal, and the loss would be 15.6% of the heat-of-combustion cal in protein.

If the nitrogen is excreted as uric acid, each gram of protein would yield (168/56) X 160 or 480 mg of uric acid and the energy loss in the urine per gram of protein metabolized would be 1315 calories and the percentage loss would be 23.3%. The non-nitrogenous fraction of the loss of uric acid would be picked up by wet combustion methods. (The heat of combustion of uric acid is 2740 cal/g.)

Thus, the urinary loss of energy from protein would have to be 14% or so at a minimum for nitrogen with additional losses from uric acid, amino acids, and other excreted metabolites. Tentatively, an estimate of 14% may be taken for the nitrogen calorie losses, and the carbon and hydrogen caloric losses can be estimated from wet combustion data.

Specific dynamic action (SDA) can be evaluated by a combination of starvation and feeding experiments. If the calories fed a fish are reduced by the calories impounded as caloric growth, by the calories excreted, and by the metabolic costs as estimated in a starvation experiment, a reasonable figure of SDA is obtained provided there is no unusual muscular activity. Specific dynamic action can also be evaluated by subtracting starvation metabolism plus excreted calories from the caloric intake required for constancy of body weight and of caloric content. It should be mentioned that SDA will vary with food-intake.

Post-digestive starvation-metabolism is not a neat mental concept, because both basal metabolism and a variable exercise-metabolism are involved. While precise definitions can be given to basal and exercise-metabolism, they are not always precisely determinable experimentally. However, the metabolic cost of starvation, although including basal, exercise, repair, and other components, is easily definable and determinable under laboratory conditions from data on heat of combustion. The metabolic costs of starvation form an important physiological and ecological unit.

Under laboratory conditions fish may be made to swim at constant velocities for long periods of time. For any given velocity the heat losses for starvation and for starvation exercise can be determined from E₀ and Eₜ values. The difference between starvation and starvation exercise loss estimates the overall cost of the exercise in calories.

Data on heat of combustion are fairly easy and inexpensive to obtain and provide an avenue for the solution of many simple laboratory problems. For many problems, data on heat of combustion alone will be inadequate. The use of heat of combustion and other methods is more extensively discussed by Warren and Davis (1967).

PENTACHLOROPHENOL AND THE ENERGY ACCOUNT OF CICHLID FISH

One of the areas where simple heat-of-combustion data can supply valuable information is in the analysis of the costs of exposure to toxic substances. As an example we shall present some data on the toxic effects of the exposure of Cichlasoma bimaculatum to the non-lethal level of 0.2 ppm of potassium pentachlorophenate at 25°C. Pentachlorophenol interferes with the production of adenosine triphosphate and is an example of the class of toxic substances known as general protoplasmic poisons. The basic data (Tables 1 and 2) portraying the effects of KPCP are derived from the thesis of Chapman (1966).

The effects of toxicants on the utilization
TABLE 1. Metabolic alterations in starving Cichlasoma bimaculatum with potassium pentachlorophenate (KPCP). The data given are in calories per gram of cichlid per day for cichlids weighing around 3 g.

<table>
<thead>
<tr>
<th>Calories lost</th>
<th>Non-poisoned</th>
<th>KPCP-poisoned</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starvation loss</td>
<td>8.7</td>
<td>19.6</td>
</tr>
<tr>
<td>Starvation + exercise loss</td>
<td>27.6</td>
<td>35.1</td>
</tr>
<tr>
<td>Exercise loss</td>
<td>15.5</td>
<td>15.5</td>
</tr>
<tr>
<td>Starvation urinary loss</td>
<td>0.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Basal metabolism</td>
<td>8.0</td>
<td>18.6</td>
</tr>
</tbody>
</table>

of body energy reserves during starvation were determined for groups of cichlids for periods of 10 days. About 200 fish were fed in troughs for over one week, and 48 hr before the start of the experiment, all food was removed and feeding discontinued. At the beginning of the experiment, the fish were weighed on a Mettler top-pan balance to the nearest 0.01 g, and were then placed into groups of eight fish each. Some fish were sampled on day zero and others on day 10. Data were obtained on wet weights, dry weights, fat, ash, protein + carbohydrate, and water content, and heats of combustion of control and poisoned cichlids. In the exercise experiments the cichlids were forced to swim continuously for 10 days at a velocity of 13 cm/sec.

Pentachlorophenol increased the caloric loss during starvation by 125%. However, exercise was equally costly in control and poisoned cichlids.

It is interesting to note the similarity of the basal loss of 8 cal per gram of cichlid per day at 25°C with the same caloric loss for a goldfish weighing 32 g at 24°C as calculated from the graph of Spoor's data presented by Fry (1957). At zero activity the graph gives 0.04 ml of oxygen consumed by the 32 gram goldfish per min. This reduces to 1.84 ml/g/day. At a caloric equivalent of 4.8 cal/ml O₂ the heat loss becomes 8.8 cal.

The effect of pentachlorophenol on the growth of cichlids was studied over a period of 11 days. Control and toxicant-exposed cichlids were held in the stainless steel troughs at 25°C. The daily food consumption, the initial and final weights, and the fat, water, and ash contents of the cichlids were determined for poisoned fish and for controls. Throughout the growth studies, the cichlids were fed tubificid worms. The worms left from the previous day's feeding were quantitatively recovered to make possible a determination of the food consumption.

Table 2 discloses some of the effects of 0.2 ppm of KPCP when cichlids are fed. Food intake calories, growth calories and total calories lost were derived from heat of combustion of cichlids on day zero and day 10, and of tubificids. Basal metabolism was derived from the starvation data of Table 1. Swimming activity was not noticeably different in the feeding experiments and in the starvation experiment and, therefore, was omitted. In the starvation experiment it is unlikely that muscular activity contributed much to the low value of 8 cal/g/day.

Loss of urinary reduced-nitrogen calories was derived by multiplying calories from metabolized protein by 0.14. Other urinary and fecal losses were obtained by multiplying calories in food intake by 0.17. To derive this figure, cichlids were starved for 48 hr, and then fed known amounts of tubificid worms. The cichlids were each placed in 45 ml water for 36 hr. Fecal contents were then pressed out of the exposed intestinal tract and wet combustible oxygen equivalents were obtained on the urine and feces. The oxygen equivalents in ml were multiplied by 4.8 cal/ml to yield estimates.
of the wet combustible energy lost in urine and feces.

SDA was estimated by subtracting the sum of basal, urinary, and fecal losses from the total calories lost.

In the presence of 0.2 ppm of KPCP, the intake of food was slightly greater, and growth slightly less, than in controls. The total calories lost were significantly higher with KPCP. Total calories lost increased 49% with KPCP while SDA calories increased 65%.

While considerably more calories were lost by the poisoned cichlids, it is interesting that most of the loss appeared as decreased storage of fat. Of the 116 cal stored by the control cichlids per gram per day (as additional carbohydrate, fat, and protein), 62 were stored as fat and 54 as carbohydrate + protein. In the presence of KPCP, 44 cal were stored as fat and 55 as protein. The pentachlorophenol did not destroy or damage the growth machinery; it merely made it more costly to operate.

It seems probable that the great deposits of lipid materials in cichlids fed tubificids ad libitum are due to an inadequate supply in tubificids of amino acids essential for cichlids. In the presence of KPCP, the cichlid tissues scavenge in the tubificid amino acid aggregate as well as do non-poisoned tissues. So little interference in the integrated mechanism is there that food-intake is increased beyond that of non-poisoned fish. Appetite and food-intake are greater in the poisoned fish than in the control-non-poisoned cichlids, and the 17% increase in food-intake compensates for some of the losses due to pentachlorophenol and delays the development of permanent damage to the tissues.

**FATTY ACIDS USED BY COHO SALMON IN SWIMMING**

Bioenergetics, the application of thermodynamic principles to biological phenomena, provides an important integrating point of view. But living matter is highly organized and the simple thermodynamic scheme outlined in Equation (1) gives no assay of the value of this organization. We shall now consider some of the simpler components of this organization and their alteration with severe and very severe muscular activity.

To investigate metabolic changes during muscular activity, coho salmon were forced to swim against sustained water velocities. Fish were selected with lengths between 75 mm and 85 mm. Initially the velocity of the water was adjusted to 8 cm/sec and the salmon were allowed to remain in the test chamber for 20 hr. After this initial 20-hr period, the velocity was increased to 15 cm/sec for 1 hr; immediately following, the velocity was increased to 52 cm/sec or 59 cm/sec. Fish were allowed to swim against the 52 or 59 cm/sec velocities for a maximum of 24 hr or until they ceased to swim. When a salmon ceased to swim, (or after 24 hr), it was removed from the swimming chamber and its swimming time, length, and wet weight recorded and the fatty acid composition determined. Some fish were selected as non-swimming controls at the time others were forced to begin swimming and these were immediately subjected to analysis. Data from salmon which were forced to swim are given in Table 3. Table 4 gives the mean amounts per salmon of individual fatty acid methyl esters in controls. Twenty-seven fatty acids were found in the lipids of control and exercised coho salmon (Saddler, 1967).

The salmon selected to swim at each velocity had an average length of 7.9 cm. The difference in length from controls was probably a sampling difference, but there remains the possibility that the severe bout of exercise may have produced some attrition in length. The salmon that swam less than 24 hr had lengths of 7.79 and 7.82 cm and the 24-hr swimmers had lengths of 7.98 and 8.25 cm at 52 and 59 cm/sec, respectively. At each velocity the forced exercise divided the salmon into a group that was able to swim at a given velocity for shorter periods of time and a group that was able to swim for longer periods of time. The shorter and lighter salmon were less competent at a given velocity than were the longer and heavier salmon. The
TABLE 3. Average values for length, weight, weight of lipid, and weight of fatty acid methyl esters per Coho salmon used for experiments on swimming performance.

<table>
<thead>
<tr>
<th>Velocity (cm/sec)</th>
<th>Salmon Used (no.)</th>
<th>Length (cm)</th>
<th>Weight (g)</th>
<th>Weight of Lipid (mg)</th>
<th>Weight of Esters (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32</td>
<td>8.06</td>
<td>5.22</td>
<td>233</td>
<td>189.2</td>
</tr>
<tr>
<td>52</td>
<td>8</td>
<td>7.79</td>
<td>4.16</td>
<td>177</td>
<td>145.0</td>
</tr>
<tr>
<td>59</td>
<td>30</td>
<td>7.82</td>
<td>4.48</td>
<td>203</td>
<td>101.8</td>
</tr>
<tr>
<td>52</td>
<td>20</td>
<td>7.98</td>
<td>4.44</td>
<td>180</td>
<td>150.3</td>
</tr>
<tr>
<td>59</td>
<td>8</td>
<td>8.25</td>
<td>5.14</td>
<td>299</td>
<td>237.1</td>
</tr>
<tr>
<td>Total salmon swimming</td>
<td>28</td>
<td>7.91</td>
<td>4.39</td>
<td>179</td>
<td>148.8</td>
</tr>
<tr>
<td>59</td>
<td>33</td>
<td>7.90</td>
<td>4.74</td>
<td>223</td>
<td>177.7</td>
</tr>
</tbody>
</table>

The salmon swimming at 52 cm/sec, whether for 24 hr or less, had less lipid than the controls that had not been forced to swim. In the salmon swimming at 59 cm/sec, those swimming for less than 24 hr had less lipid (180 vs. 233 mg per salmon) than controls. There was no increase in lipid during 24 hr from the severe exercise, but the forced exercise was only possible for 24 hr at the 59 cm/sec velocity in salmon that had above-average masses of lipid before the exercise began.

In order to evaluate the changes during exercise, the failing salmon and salmon swimming for 24 hr were combined to form a single population, and information is presented for total salmon swimming at each velocity (Table 3). When failing salmon and salmon that were able to swim for 24 hr were combined, differences in lengths between controls and swimming salmon at the water velocities of 52 and 59 cm/sec were not significant at the 1% level. Therefore, combination of the data eliminated some of the selection problem induced by swimming and allowed the salmon to be compared on the basis of a sample population of controls and sample populations that were forced to swim but with no consideration of the swimming ability of individuals within a sample.

The average weight of control salmon (5.22 g) was greater than the average weight of salmon swimming at 52 cm/sec (4.39 g) and of salmon swimming at 59 cm/sec (4.74 g).

The average amount of lipid for control salmon (233 mg) was much greater than the 179 mg found in salmon swimming at 52 cm/sec, but only slightly greater than the 223 mg from salmon swimming at 59 cm/sec.

Table 5 gives the statistically significant differences obtained on subtracting amounts of fatty acid methyl esters of all salmon swimming at a velocity of 52 cm/sec from comparable amounts for control salmon. The total loss of fatty acid methyl esters for salmon swimming at 52 cm/sec was 40.4 mg. The total loss in saturated fatty acids is negligible.
TABLE 5. Significant differences obtained by subtracting amounts of fatty acid methyl esters of all salmon swimming in water-velocity of 52 cm/sec from comparable amounts from control salmon.

<table>
<thead>
<tr>
<th>Number of Carbons</th>
<th>Double Bonds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>1.07</td>
</tr>
<tr>
<td>14</td>
<td>7.53</td>
</tr>
<tr>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>1.76</td>
</tr>
<tr>
<td>20</td>
<td>2.12</td>
</tr>
<tr>
<td>22</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 5 gives the significant differences obtained by subtracting amounts of fatty acid methyl esters of all salmon swimming at 52 cm/sec from comparable amounts from control salmon. Significant losses are indicated by differences greater than 0.5 mg.

The greatest losses of fatty acids occurred with 18:1, 16:0, and 16:1. The greatest percentage decreases (33, 28, 28%) occurred with 20:5, 16:1, and 20:1. The identity of the fatty acids lost would suggest that fish exercising at 52 cm/sec are burning triglyceride. These fatty acids, particularly the one double-bonded acids, are incorporated preferentially in triglyceride.

Table 6 gives the significant differences obtained by subtracting amounts of fatty acid methyl esters of all salmon swimming at 59 cm/sec from comparable amounts from control salmon. Significant losses are indicated by differences greater than 0.5 mg.

The average distances traveled for salmon swimming at 52 and 59 cm/sec were 26.0 and 12.7 miles, respectively. The average swimming times for salmon swimming at 52 and 59 cm/sec were 1141 and 398 min, respectively.

The average distances traveled for salmon swimming at 52 cm/sec could be expected to exceed the total metabolic cost of salmon swimming at 59 cm/sec, because the average distance traveled by salmon swimming at 52 cm/sec was more than twice the average distance traveled by salmon swimming at 59 cm/sec. The loss of 22:6, 18:2, and 20:4 in fish swimming at the higher velocity is rather unusual as it suggests that phospholipid is being destroyed. Loss of 22:6 is especially surprising, since in other situations this acid appears to be conserved. In an extreme does the fish lose its ability to control what lipid is utilized? (Elimination of differences not statistically or technically significant contribute to rounding out errors, so that the sum of the fatty acid differences was only 9.15 mg while the total difference on direct subtraction was 11.5 mg).

THE METABOLIC COST OF FORCED EXERCISE IN SALMON

All salmon used in this study were forced to swim for 1200 min at 8 cm/sec and 60 min at 15 cm/sec; then, the salmon were forced to swim against water velocities of 52 and 59 cm/sec for a maximum of 24 hr. At each velocity tested, some salmon failed to swim for the maximum 24 hr and some salmon were able to swim 24 hr. The average swimming times in minutes for salmon swimming at velocities of 52 and 59 cm/sec were 1141 and 398 min, respectively.

The average distances traveled for salmon swimming at 52 and 59 cm/sec were 26.0 and 12.7 miles, respectively. These distances include approximately 8.6 miles at 8 cm/sec and 0.3 miles at 15 cm/sec. Thus, the total metabolic cost for salmon swimming at 52 cm/sec could be expected to exceed the total metabolic cost of salmon swimming at 59 cm/sec, because the average distance traveled by salmon swimming at 52 cm/sec was more than twice the average distance traveled by salmon swimming at 59 cm/sec.
An increase in velocity from 52 to 59 cm/sec reduced the average distance traveled by 52%, because average swimming time was reduced from 1141 to 398 min.

The average loss in weight for all salmon swimming at 52 cm/sec was 830 mg and the average loss for all salmon swimming at 59 cm/sec was 480 mg (loss in weight was obtained by subtracting the weight of swimming salmon from the weight of control salmon). Hence, the loss in weight for salmon swimming at 52 cm/sec was approximately twice the loss for swimming at 59 cm/sec.

The total loss of fatty acids for salmon swimming at 52 and 59 cm/sec (total amount of fatty acids for swimming salmon subtracted from total amounts of fatty acids for control salmon) was 40 mg and 11 mg, respectively. Thus, the loss in fatty acids for salmon swimming at 52 cm/sec was nearly four times the loss for salmon swimming at 59 cm/sec. (Compare this with the two times loss in weight and average distance traveled, mentioned in the preceding paragraphs, for salmon swimming against the lesser velocity of 52 cm/sec).

Saturated fatty acids accounted for 27.5% of the total loss in fatty acids for salmon swimming at 52 cm/sec while the saturated fatty acids lost from salmon swimming at 59 cm/sec accounted for only 18% of the total loss. The salmon swimming for shorter distances at high velocities preferentially metabolize a higher percentage of unsaturated fatty acids.

Brett (1965) has provided information relating swimming velocity and O\textsubscript{2}-consumption for sockeye salmon. From his data it is possible to calculate the expected production of heat and obtain estimates of material losses with reasonable reliability for coho salmon forced to swim at high velocities. From Figure 2 of the paper by Brett the estimated O\textsubscript{2}-consumption for a 5.2 g salmon swimming against a water velocity of 8 cm/sec for 20 hr would be 18.2 mg and for 1 hr at 15 cm/sec would be 1.0 mg. For 1141 min at 52 cm/sec the O\textsubscript{2}-requirement would be 79.1 mg and for 398 min at 59 cm/sec, 34.5 mg. Thus, the average O\textsubscript{2}-consumptions for the fish swimming at 52 cm/sec should equal 98.4 mg (18.2 \(+\) 1.0 \(+\) 79.1) and at 59 cm/sec, 53.7 mg (18.2 \(+\) 1.0 \(+\) 34.5). Since 1 mg of oxygen will burn 0.376 mg (3.5/9.3) of lipid, the lipid equivalents of the total estimated oxidative costs for exercise are 37.0 mg at 52 cm/sec and 20 mg at 59 cm/sec. The observed losses in lipid were 54 mg at 52 cm/sec and 10 mg at 59 cm/sec; the loss at 52 cm/sec is considerably higher than the calculated value even when all oxidative losses are attributed to lipids.

Of the total loss in weight for swimming salmon (830 mg at 52 cm/sec and 480 mg at 59 cm/sec), lipids represented 54 and 10 mg, respectively. Losses other than lipids were 776 mg and 470 mg, and possibly involved modifications in water, protein, carbohydrate, and salt. In general, the water content of juvenile salmonid fish is approximately 80% (Phillips, Livingston and Dumas, 1960). Thus, of the total losses (830 mg and 480 mg), approximately 80%, or 664 and 384 mg, would have been water. On subtracting losses of both water and lipid, the protein plus carbohydrate would be approximately 112 mg and 86 mg, respectively. The losses of lipid represent 502 and 98 cal, the protein plus carbohydrate losses approximately 616 and 473 cal, and the total caloric losses 1118 and 566 cal at 52 and 59 cm/sec, respectively. Similar losses calculated from the O\textsubscript{2}-consumption data of Brett on Oncorhynchus nerka give only 344 and 188 cal, respectively.

The caloric costs as estimated from material losses are three times the losses calculated from the O\textsubscript{2}-consumption data of Brett (1964). Differences between species and possible errors in materials, since losses are not measured within animals but between controls and exercised salmon, account for some of the differences. There is also the possibility of greater water losses and some salt loss. Some additional material losses may be attributed to excretion of metabolites via the gills and kidneys. However, even gross errors cannot account for all of the difference.
In Brett's experiments the salmon swam a maximum distance of 5.2 miles, about half of the distance at velocities below 45 cm/sec and the other half divided approximately equally between velocities of 52 and 60 cm/sec. In our study, the average swimming distance was 26 miles for salmon selected to swim in water at a velocity of 52 cm/sec, and 12.7 miles at 59 cm/sec, with three miles of the distance for both at low velocities. The caloric data calculated from Brett's graph for O₂-consumption and from material losses have taken time, distance, and velocity factors into consideration. However, Brett's data on O₂-consumption were collected during one, and at the most, two hours at high velocity. It is still a moot question whether O₂-consumption during the third, fifth, tenth, or even the twenty-fourth hour of swimming at high velocities would be the same as during the first hour. However, when a maximum effort is involved, it seems reasonable that each succeeding mile and each succeeding hour without a recovery period would be more difficult and costly to the salmon.

In general, Brett found a ratio for maximum active metabolism to standard metabolism of 8:1 for a fish of 5 grams. If the data in this study on material balance are supported by bioenergetic data, the ratio of active to standard metabolism with prolonged exercise may even reach a value of 24:1 if computed on the basis of calories lost. There may be significant destruction of tissue components apart from oxidative destruction.

The data suggest that at the velocity of 52 cm/sec about 55% of the caloric losses came from protein and 45% from lipids. But at the very high velocity of 59 cm/sec protein contributed 84% of the loss and lipid only 16%. The obvious implication is that activity with a high intensity factor will require a source of protein (either tissue or diet) but lower intensities can be supplied by either fat or protein. (The data on material balance must be augmented by determinations of the direct heat of combustion and the content of protein in exercised salmon in order to evaluate the validity of this deduction.)

It is now worthwhile to return to the equation for bioenergetic balance. If an intake of 30 cal food per kcal of fish (Fig. 7, Warren and Davis, 1967) represents an approximation of the maximum intake at 15°C; and if there is an obligatory loss of 17% of the calories in feces and urine, and another 5% for protein-nitrogen losses, only 24 cal per kcal would be available for repair. On the basis of 80% water and 2.5% ash (Phillips, Livingston, and Dumas, 1960) and 4.58% lipid, the fat calories per gram of wet salmon would be 409 and the protein calories would approximate 609 (residual mass × 5.65, before correction for carbohydrate). This amounts to roughly 1 kcal/g.

Under the rigorous conditions of fingerling salmon swimming against a stream velocity of 52 cm/sec for extended periods, the loss was 1118 cal in 1141 min for a salmon of 5.22 g, or 270 cal/g/day on the average. This is eleven times the likely food-intake. In order to allow and provide for maintenance only, such a stress could be endured for only 128 min per day.

At 59 cm/sec the loss was 392 cal over an average of 398 min for a 5.2 g salmon, or 228 cal/g/day. This stress could be endured for 88 min in one day under similar conditions.

Swimming problems of the magnitude placed before experimental salmon in these experiments are highly improbable in nature. But the experiments do give a measure of the potentialities the salmon have for swimming and of the cost to the salmon. They indicate that the potentialities of living structures are tremendous; but when events require maximum utilization of the potentialities, the problems of recovery become tremendous.

REFERENCES

Brett, J. R. 1965. The relation of size to rate of oxygen consumption and sustained swimming...
speed of sockeye salmon (Oncorhynchus nerka).