CHANGES IN NITROGENOUS EXTRACTIVES IN THE MUSCULAR TISSUE OF THE KING SALMON DURING THE FAST OF SPAWNING MIGRATION.*

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In the preceding paper by Charles W. Greene the general changes in the composition of the muscles of the salmon during the fast of the spawning migration are presented. The question at once arises as to the transformations occurring in this catabolism of the muscle protoplasm.

Since the time of Liebig a bewildering multitude of nitrogenous extractives has been isolated from muscular tissue. The physiological study of the greater number of these compounds, however, has always been hampered by the lack of adequate quantitative methods. Thanks to Folin and to Van Slyke, rapid and accurate methods are now available, especially for the determination of creatine and the amino-acids. These methods have been applied to the study of the changes in mammalian muscle during work and fasting. The enormous tax upon the muscle of the salmon during the spawning migration, however, greatly exceeds any that is usually met with in physiological experimentation, even of the most rigorous character. Consequently this material is most favorable as a basis for the study of the changes in the muscle extractives.

* The experimental data in this paper are taken from the dissertation presented by the author for the degree of Doctor of Philosophy, Yale University, 1917. A brief report was made before the American Society of Biological Chemists, December, 1917, see J. Biol. Chem., 1918, xxxiii, p. xii.

† Currier Fellow in the Graduate School, Yale University, 1915-17.
Recent Observations on Nitrogenous Extractives in Muscular Tissue.

Wilson (1914) reviewed the general literature on the occurrence of the different extractives that have been isolated from muscle. A somewhat similar bibliography is given by von Fürth (1909).

Extractive Nitrogen.

The total non-protein extractive nitrogen in mammalian muscle as determined by von Fürth and Schwarz (1911), Folin and Denis (1912), Buglia and Costantino (1912, a, b), and Catheart (1916), varies between 250 and 390 mg. per 100 gm. of muscle. In the lower vertebrates Buglia and Costantino (1912, c) found values of 470 to 570 mg. per 100 gm., whereas in the invertebrates are found the largest amounts of all, 440 to 1,310 mg. per 100 gm. (Buglia, Wilson). In the elasmobranchs Buglia found the unusually high amount of 1.5 per cent of extractive nitrogen in the fresh tissue. This, however, is associated with the presence of large amounts of urea in the tissues.

Ammonia Nitrogen.

Buglia and Costantino (1912, b) demonstrated that the amino nitrogen in the muscles of mammalian embryos increased from 40 mg. per 100 gm. in a 2 months calf to 58 mg. in a full term fetus. They furthermore found the amino nitrogen in the muscles of the adult to vary between 60 and 90 mg. per 100 gm. Delaunay (1910) estimated from 168 to 252 mg. of amino nitrogen in dog muscle, but admits that his method was designed to give relative and not absolute values. These figures are undoubtedly high, as Van Slyke and Meyer (1913-14, a) proved that dog muscle becomes saturated when it contains approximately 80 mg. per 100 gm.

In common with their content of extractives, the muscles of invertebrates show a much higher content of amino nitrogen than do those of mammals. In Sipunculus, Buglia found 589 mg. per 100 gm. of fresh muscle. This is the highest reported figure for the amino nitrogen in muscle.

Van Slyke and Meyer (1912, 1913-14,a, b) demonstrated that an equilibrium existed between the amino-acids of the tissues and blood. On the injection of digestion mixtures into the blood stream it was possible to raise the amino nitrogen content of the body tissues. The muscles, however, reached a definite "saturation point" above which the amino nitrogen content could not be raised. The liver, on the other hand, very rapidly metabolized the absorbed amino-acids and would take up much greater amounts than the muscles. Catheart (1916) also found that the composition of the muscle was less affected than was that of the liver.

In studies on the effect of different experimental procedures upon the amino-acid content of striated muscles, von Fürth and Schwarz (1911) were unable to affect the amino nitrogen of dog muscle by tetanic stimulation. Van Slyke and Meyer (1913-14, c) found no difference in the amino
nitrogen content of the muscles of dogs on a high or low protein diet. Wishart (1915) likewise found no increase in the residual nitrogen of dog muscle after a heavy meat feeding though there was a slight increase in the urea nitrogen. Mitchell (1918) obtained somewhat similar results in a study of the whole carcass of rats under different dietary conditions. He believes, however, that in young animals a demonstrable increase in the amino nitrogen occurs about 5 hours after feeding. Buglia and Costantino (1913) and Van Slyke and Meyer (1913-14, c) demonstrated that in fasting dogs the muscles contain more amino nitrogen than normally, approaching the saturation limit of Van Slyke. Reference may also be made to Van Slyke's (1915-16) recent summary of the physiology of the amino-acids.

Creatine Nitrogen.

Myers and Fine (1913, a) have recently reviewed the chemistry of creatine in the muscle in great detail and reference for the extensive literature on the subject may be made to their papers.

Though creatine is present only in vertebrates and the amounts are distinctive for the species, there is no marked phylogenetic relationship in the amounts present in the muscles. Rabbit muscle contains more creatine (0.52 per cent) than that of man (0.39 per cent). Grindley and Woods (1906-07) and Mellanby (1907-08) found that the muscles of the lower vertebrates, frogs and fishes, contain relatively small amounts of creatine, 0.28 to 0.35 per cent. In a number of teleosts Okuda (1912) determined creatine in quantities of from 0.42 to 0.75 per cent. In salmon muscle he found 0.55 per cent. These results of Okuda are probably high; certainly they are not in agreement with the analyses of Mellanby, or the experiments of Suzuki and Joshimura (1909) on the direct isolation of creatine from fish muscle.

Since the time of Liebig much attention has been paid to the variations in the creatine content of muscle under different experimental conditions. The earlier studies were made by the Salkowski-Neubauer method and are consequently not reliable. Since the introduction of the Folin method for the determination of creatinine several papers have appeared upon the changes in the muscle creatine. Thompson (1917-19) has recently summarized the present views as to the effect of work on the amount of creatine in the muscle. His own experiments made on decerebrated cats demonstrated that there was no change in muscles with an intact circulation after intermittent stimulation for over 2 hours. On the other hand, when the circulation was interfered with by arterial ligation the stimulation resulted in a loss of over 6 per cent of the muscle creatine. Similar or even greater losses occurred in the total nitrogen and solids of the affected muscle. Accordingly Thompson believes that with an intact blood supply there is a complete restitution of the substances used up by the muscular contraction, but that if the blood supply is restricted there is a reduction in the energy-yielding material of the muscle. This loss involves not only the non-nitrogenous substances but the total nitrogen and creatine of the muscle.
460 Nitrogenous Extractives of King Salmon

muscle as well. In contrast to these results, Scott and Spohn (1916-17) report that fatigue of the isolated muscle has no effect upon the total creatinine, but that fatigue of the living cat results in a loss of from 18 to 30 per cent of the creatine in the muscles. The views of Pekelharing (1911) as to the dependence of the creatine content of a muscle upon the condition of tonus rather than upon the direct muscular activity are well known.

There has been even greater confusion concerning the behavior of the muscle creatine during starvation than during muscular work. Myers and Fine (1913, b) put forward the following view.1

"The experiments . . . indicate that the creatine concentration of the muscle in the rabbit is first increased during starvation, then subsequently decreased. The increase is apparently due to the removal of the non-creatine portion, e.g., glycogen, fat, etc., of muscle more rapidly than that containing creatine. The elimination of creatine in the urine increases with the length of fast and in the days preceding death relatively large amounts are eliminated. This results in a depletion of the creatine supply of the body and in a decreased content of muscle creatine. . . . The loss of creatine in the urine is still, however, the most important factor in causing a depletion in the creatine content of the muscle."

Benedict and Osterberg (1914) working on fasting dogs secured results agreeing with those of Myers and Fine.

Howe and his coworkers (1911, 1912) emphasized most strongly the possibility of washing out the creatine from the muscle without affecting the other constituents but their conclusions have been strongly questioned by Mendel and Rose (1911-12) and by Cathcart and Orr (1914). The belief of Urano (1907) and of Folin and Denis (1914) that creatine is an integral part of the muscle protoplasm is also opposed to this view of Howe and Hawk.

Materials Investigated.

The samples of salmon muscle used were duplicates kindly supplied for this study of nitrogenuous extractive distribution by Professor Charles W. Greene from his research collection taken on the Columbia River during the summer of 1918 and reported in this Journal, page 435. The analytical samples were selected, weighed, and preserved in alcohol in glass-stoppered bottles and sealed by paraffin as described in his paper. The samples are a composite from the middle section of the great lateral muscle described by Greene (1912), and Greene and Greene (1913).

1 Myers, V. C., and Fine, M. S., J. Biol. Chem., 1913, xv, 301.
The chemical examination was completed in the Sheffield Laboratory of the Yale University, during the winter of 1916-17. The majority of the samples used were of the larger series and varied between 28 and 37 gm. The total volume of the alcohol and sample was from 90 to 100 cc., so that the preserving concentration of the alcohol, taking into account the dry matter of the samples, varied between 70 and 80 per cent. Wells and Caldwell (1914) have given evidence to show that to prevent completely autolysis in blocks of liver tissue requires an alcohol concentration of at least 90 per cent. With alcohol strengths of 80 per cent or over, he found only a slight degree of autolysis, but below that concentration the autolytic action was distinctly measurable. In the light of their experiments it would seem that slight autolysis might be expected in these muscle samples. However, the alcohol concentrations approached the limiting concentrations advised by Wells and Caldwell and the tissues were finely ground, quickly and intimately mixed, and heated somewhat during the sealing, all of which favor the inhibition of autolysis. Furthermore, Hoagland, McBryde, and Powick (1917) have recently reported the changes during the aseptic autolysis of beef muscle during cold storage, the most characteristic being that in the amino-acids. The amino nitrogen increased from 78 to 766 mg. per 100 gm. of fresh sample, a total increase of 840 per cent. In view of these results they suggest the study of the amino nitrogen as a sensitive indicator to the presence of autolysis in animal tissues. In the present investigation, the marked constancy of the amino nitrogen content of a series of diverse samples speaks against the presence of any such disturbing factor as autolysis. The close agreement of the total protein content with the data presented by Charles W. Greene (1919) from earlier analyses, made in 1909, is proof of the adequate preservation of the samples.

Methods of Analysis.

The analysis of the muscle was carried out according to the improved method of Janney. The sample was placed in a casserole and boiled

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2 The writer is under deepest obligation to Prof. Lafayette B. Mendel for friendly advice and direction during the progress of the study.

three or four times with 50 cc. portions of alcohol. This completed the coagulation of the muscle proteins and also removed some of the fat from the tissue. The samples highest in fat received a preliminary extraction with ether, since the removal of the excess fat greatly facilitates the subsequent extraction with water.

After the coagulation with alcohol, the muscle was repeatedly extracted with boiling water, in 50 to 100 cc. portions. This water extraction was repeated seven or eight times, the total volume of the extract being between 800 and 900 cc. The protein residue was then washed onto a tared filter, the filter folded around it, and the whole extracted with 95 per cent alcohol in a Soxhlet for 3 to 6 hours. This was followed by extraction with absolute alcohol for 15 to 20 hours. Although Janney considered an ether extraction of the protein residue unnecessary, yet on account of the high fat content of these samples, a 12 hour extraction with ether was resorted to as a measure of precaution. After completion of the extraction the filter and residue were transferred to a glass-stoppered weighing bottle, dried to constant weight at 105°, and the weight of the filter was deducted.

The alcohol and water extracts were combined in a large Claisen flask and evaporated to dryness under diminished pressure. The extracts were transferred to a small bottle with ether and water and extracted in a Soxhlet with ether for 15 hours, as suggested by Saiki. This separation of the ether- and water-soluble extractives was not entirely satisfactory. It seemed, however, to be preferable to the acid-chloroform-water method of Koch. The greatest difficulty occurred in the recovery of the water-soluble material carried over into the ether. Recovery was attempted by shaking out the ether extract, with water, in a separatory funnel. Owing to extensive emulsion formation, this was not a success. The procedure finally adopted was to evaporate the ether extract and redissolve in a minimum of anhydrous ether. The solution was filtered, the ether distilled off, and the semipurified ether extract dried to constant weight at 100°, as provided by the official methods of analysis.

That portion of the crude ether extract insoluble in anhydrous ether was dissolved in hot water and returned to the water extract. When the original alcohol-water extract was concentrated, a small amount of insoluble material separated. This was filtered off, dried, and weighed. The weight of this recovered material was then added to that of the original protein residue, to secure the total.

The filtered solution of the water-soluble extractives was transferred to a volumetric flask and made up to a definite volume, usually 300 cc. To avoid the possibility of change during drying, this water extract was divided into aliquots. One of these, of 100 cc., was evaporated on a water bath and dried to constant weight at 105°, an aluminum capsule with

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5 Koch, W., *J. Biol. Chem.*, 1907, iii, 159.
closely fitting lid being used as a container. The weight of the total solids in the water extract was then calculated.

The weight of the total solids in the muscle was taken to be the sum of the weights of the insoluble residue, the ether-soluble, and the water-soluble materials. In their first paper, Janney and Csonka\textsuperscript{7} mentioned that the results obtained in this way were reasonably accurate. They recovered between 99.7 and 100.6 per cent of the amounts found by direct determination. The water was taken as the difference between the weights of the fresh sample and the total solids. Owing, therefore, to losses in the manipulation of the other materials, the water determinations should show the greatest error.

The total extractive nitrogen was determined in a aliquot of the water extract. The distillation method of Bock and Benedict\textsuperscript{8} was used and the ammonia in the distillate not Nesslerized but titrated with 0.5 N acid, as suggested by Folin.\textsuperscript{9} The amino-acid nitrogen in the water extract was determined in a 150 cc. portion by the micro apparatus of Van Slyke.\textsuperscript{10} In the removal of the urea and residual traces of protein, urease and trichloracetic acid were used, according to the method chosen by Bock,\textsuperscript{11} for blood. The creatine in the water extract was determined by the procedure of Janney and Blatherwick.\textsuperscript{12} Because of its greater convenience the Myers autoclave method of conversion was employed in place of heating on the water bath. Janney and Blatherwick found this method accurate when used on muscle extracts, though it gave erroneous results with other tissue extracts. In order to reduce to a minimum the errors in the colorimetric reading, varying amounts of extract were taken, so that the reading of the unknown and the standard were always approximately equal.\textsuperscript{13}

A general table (Table I) is presented giving the grand totals of the separations. This table is valuable chiefly as a background for the special study of nitrogenous extractives. However, attention is called to the very close agreement with the earlier analyses of Charles W. Greene on this series of fishes. His analyses are based primarily on Koch's methods, mine on Janney's—both modified in adaptation to the salmon muscle tissue.

\begin{itemize}
  \item Bock, J. C., and Benedict, S. R., \textit{J. Biol. Chem.}, 1915, xx, 47.
  \item Folin, O., \textit{J. Biol. Chem.}, 1915, xxi, 185.
  \item Van Slyke, D. D., \textit{J. Biol. Chem.}, 1913-14, xvi, 121.
  \item Bock, J. C., \textit{J. Biol. Chem.}, 1916, xxviii, 357.
  \item Janney, N. W., and Blatherwick, N. R., \textit{J. Biol. Chem.}, 1915, xxi, 567.
  \item The picric acid was purified as suggested in Folin, O., and Doisy, E. A., \textit{J. Biol. Chem.}, 1916-17, xxviii, 349.
\end{itemize}
### TABLE I.

**Changes in Composition of Muscle of Salmon during Migration.**

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* The original analysis was lost. The results of Charles W. Greene are given here.
Chemical Changes in Extractives of Muscle.

Hatai (1917) found that in the white rat the extractives in the muscle varied with the amount of protein, and were, he considered, an index to the activity of the tissue. The same seems to be true of the salmon muscle (Table II). The average figure for the extractives in the fat-free tissue for the samples from Ilwaco is undoubtedly too high, owing to suspected error in the analysis of one of the samples (No. 1279, Table I). The figure for the corrected average is probably not much over 4.5 per cent. There is a slight increase in the muscle extractives of the fishes obtained through the successive stations as far as Ontario and a very slight decrease to the amount present at Cazadero. The variation in

| Table II. Changes in Composition of Muscle of Salmon During Migration.* |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Station         | Location        | Protein residue | Water extract  | Extractive nitrogen | Amino nitrogen  | Total creatine nitrogen |
|                 |                 | per cent        | per cent       | per cent           | per cent        | per cent           |
| Ilwaco          | Tide-water      | 20.3            | 4.79           | 0.482              | 0.061           | 0.081              |
| Warrendale      | 130 miles       | 19.9            | 4.65           | 0.524              | 0.076           | 0.094              |
| Seufert         | 210 "           | 19.7            | 4.70           | 0.521              | 0.078           | 0.078              |
| Ontario         | 700 "           | 18.3            | 4.75           | 0.553              | 0.074           | 0.089              |
| Cazadero        | Spawning grounds| 13.8            | 4.34           | 0.534              | 0.094           | 0.089              |

* Averages in percentages as calculated on the fat-free basis.

the amount of extractives present in the muscle, therefore, parallels the activity of the catabolic processes in the salmon in somewhat the same manner in which it parallels the anabolic processes in the growing rat.

Total Extractive Nitrogen.

The total extractive nitrogen shows variations comparable with those in the total extractives. Charles W. Greene (1919) found that the ash content of the muscle was relatively constant, or else slightly decreased during the migration. With this in mind, it becomes evident that the extractive nitrogen remains approximately constant in its relation to the extractives of the muscle.
Phylogenetically it is of interest to note that the percentage of extractive nitrogen in the salmon muscle (Table III) is very much higher than in mammalian tissue. The figures are practically identical with those given by Buglia for other teleosts, and by Wilson for some invertebrates. It should be noted that among the invertebrates Buglia found much more extractive nitrogen than did Wilson. They analyzed different species, however, so that much emphasis cannot be placed upon this difference.

**TABLE III.**

*Total Extractive Nitrogen.*

<table>
<thead>
<tr>
<th>Station</th>
<th>Location</th>
<th>Fat-free sample</th>
<th>Water extract</th>
<th>Protein residue</th>
<th>Water in muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>Ilwaco</td>
<td>Tide-water</td>
<td>0.482</td>
<td>10.1</td>
<td>2.37</td>
<td>0.708</td>
</tr>
<tr>
<td>Warrendale</td>
<td>130 miles</td>
<td>0.524</td>
<td>11.2</td>
<td>2.63</td>
<td>0.694</td>
</tr>
<tr>
<td>Seufert</td>
<td>210 “</td>
<td>0.521</td>
<td>10.8</td>
<td>2.67</td>
<td>0.689</td>
</tr>
<tr>
<td>Ontario</td>
<td>700 “</td>
<td>0.553</td>
<td>11.7</td>
<td>3.04</td>
<td>0.700</td>
</tr>
<tr>
<td>Cazadero</td>
<td>Spawning grounds</td>
<td>0.534</td>
<td>12.6</td>
<td>3.82</td>
<td>0.652</td>
</tr>
</tbody>
</table>

**Amino Nitrogen.**

The salmon muscle is unique in the extent and character of the protein utilization during the spawning migration. Great interest, therefore, attaches to the amino-acids at known stages in the process of protein decomposition. The amino nitrogen in the muscle shows a striking increase from 61 to 84 mg. per 100 gm. of fat-free tissue, or 39 per cent (Table IV). This increase is not confined to the muscle alone, but also includes the

**TABLE IV.**

*Non-Protein Amino Nitrogen.*

<table>
<thead>
<tr>
<th>Station</th>
<th>Location</th>
<th>Fat-free sample</th>
<th>Water extract</th>
<th>Protein residue</th>
<th>Water in muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>Ilwaco</td>
<td>Tide-water</td>
<td>0.061</td>
<td>1.280</td>
<td>0.300</td>
<td>0.082</td>
</tr>
<tr>
<td>Warrendale</td>
<td>130 miles</td>
<td>0.076</td>
<td>1.640</td>
<td>0.380</td>
<td>0.101</td>
</tr>
<tr>
<td>Seufert</td>
<td>210 “</td>
<td>0.078</td>
<td>1.660</td>
<td>0.400</td>
<td>0.098</td>
</tr>
<tr>
<td>Ontario</td>
<td>700 “</td>
<td>0.074</td>
<td>1.520</td>
<td>0.410</td>
<td>0.096</td>
</tr>
<tr>
<td>Cazadero</td>
<td>Spawning grounds</td>
<td>0.084</td>
<td>1.860</td>
<td>0.590</td>
<td>0.099</td>
</tr>
</tbody>
</table>
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amino nitrogen in proportion to the rest of the extractives, indicating that the amino-acids are, perhaps, more active during the migration than are the extractives as a whole.

Particularly striking is the enormous increase in the amount of amino-acids relative to the protein from which they must originate. The amino nitrogen in the fat-free muscle increases by over 35 per cent. At the same time the protein content of the muscle decreases by nearly the same amount (Greene, 1919). In relation to the protein the increase in the amino nitrogen is, in round numbers, 100 per cent. Evidently the amino-acids are liberated at the expense of an ever increasing wastage of the muscle substance.

The extractive nitrogen shows an increase of 33 per cent in the amount present relative to the protein content of the muscle. Though this increase is not so extreme as that of the amino nitrogen, it alone is sufficiently striking to emphasize the great exaggeration of the protein catabolism occurring in the salmon during the migration fast.

While emphasis is laid on the fact of the increase in the amino-acids at the expense of the muscle protein, even more importance is to be attached to the effect of the influx of water into the tissue. The increase in the water content of the muscles during migration has already been pointed out by Greene (1915), also previously in this paper. The amino nitrogen relative to the water present in the tissues increases from 80 to 100 mg. per 100 gm. of water at the very beginning of the migration and remains unaffected by subsequent changes in the muscles. With the stimulated protein catabolism during the migration fast, the muscles and particularly the fluid in the muscles become saturated with amino nitrogen early in the migration period. This saturation then continues to the end (Table IV). The limiting factor in the behavior of the amino-acids would seem to be the volume of water present in the tissue, and not the protein content.

The changes here observed in the amino nitrogen content of the salmon lend a new significance to, and afford an illuminating extension of, the studies of Van Slyke and Meyer. It is to them that we are at present largely indebted for our knowledge of the behavior of the amino-acids in the body after absorption from the intestine. Van Slyke and Meyer (1912) demonstrated
that in dogs the amino-acids are absorbed into the blood stream from which they rapidly disappear as the blood circulates through the tissues. Their experiments have been confirmed by Delaunay (1913) and others. Van Slyke and Meyer (1913–14) have further shown that this disappearance from the blood stream is due to the absorption of the amino-acids by the muscles.\textsuperscript{14} They write as follows:

"The disappearance of the intravenously injected amino-acids from the circulation is the result of neither their destruction, synthesis, nor chemical incorporation into the cell proteins. The acids are merely absorbed from the blood by the tissues, without undergoing any immediate chemical change. In the case of the muscles at least, a fairly definite saturation point exists, which sets the limit to the amount of amino-acids that can be absorbed. We have never been able to force the amino-nitrogen figure of the striated muscles above 75–80 mg. per 100 grams. The capacity of the internal organs is more elastic; we have raised the amino figure of the liver to 125–150 mg.

"The absorption of amino-acids from the circulation by the tissues, although extremely rapid, is never complete; the blood contains 3–8 mg. of amino-acid nitrogen per 100 cc. even after a fast of several days' duration. The amino-acids of the blood appear, therefore, to be in equilibrium with those of the tissues, a condition which accounts for all the observed phenomena, and would also account for any transfer of amino-acids which may occur from organ to organ, or from maternal organs to fetus.

"The process by which the amino-acids are taken up and held by the tissues cannot be wholly osmotic, because the normal concentration of the amino nitrogen in the tissues is five to ten times that in the blood; and even when the latter is suddenly loaded with injected amino-acids, they quickly gather in not equal, but greater, concentration in the tissues. The most probable explanations of the process are, that it is either; (1) a mechanical absorption, or (2) the formation of loose molecular compounds between the amino-acids and the tissue proteins, such as Pfeiffer has recently shown can be formed by the amino-acids themselves with inorganic salts."

Van Slyke and Meyer found a varying behavior on the part of the amino-acids after absorption by the tissues. They disappeared rapidly from the liver, with a simultaneous increase in the urea content of the blood indicating that the liver was able to metabolize the absorbed amino-acids. The muscles on the contrary showed but little such power, and the absorbed amino-acids were lost but slowly.

Van Slyke and Meyer (1913–14, c) in continuation of their investigations found that the amino-acid content of the muscles of dogs was not affected by heavy protein feeding, but did increase upon fasting. These writers summarize their views in the following manner:\footnote{Van Slyke, D. D., and Meyer, G. M., \textit{J. Biol. Chem.}, 1913–14, xvi, 232.}

"The amino-acids appear, therefore, to be intermediate steps, not only in the synthesis, but in the breaking down of body proteins. Otherwise, in order to explain their maintenance in the tissue during starvation, one would be forced, contrary to the conclusions of all experimental work on the subject, to assume that they are inert substances, lying unchanged for long periods, even when most urgently needed to build tissue or supply energy. The maintenance of the amino-acid supply by synthesis, from ammonia and the products of fats or carbohydrates, seems excluded. The supply of raw material in the form of fat and carbohydrates nearly disappears during starvation, and the ammonia could originate only from broken-down protein, as the normal store of ammonia nitrogen is only a fraction of that of the free amino-acids. These considerations, and the self-evident wasting of starved tissues, point strongly to autolysis as the main source of the free amino-acids in the fasting body."

The changes in the muscles of the salmon during the fast of the spawning migration furnish clear-cut and uncomplicated evidence that the muscle tissue is the source of the free amino-acids in the fasting body. This is independent evidence confirmatory of that quoted above and derived by Van Slyke and Meyer from studies on fasting dogs. Furthermore, we emphasize the fact that in the salmon a mechanism is present capable of maintaining the amino nitrogen at a constant level of saturation in the water present in the tissues, even though this water increases in amount during the fast. This saturation is kept up regardless of the great loss in muscle protein until, in the end, the exhaustion of the organism proceeds to a degree culminating in death. It is evident that this previously undescribed water-volume effect is a strong regulating factor and must be added to those suggested by Van Slyke as probably controlling the amino-acid content of the tissues.

\textit{Creatine Nitrogen.}

The figures obtained for the total creatine nitrogen show a very great amount of variability (Table V). That the variations
cannot entirely be charged to manipulation of samples is shown by the fact that the amino nitrogen determinations underwent nearly the same manipulation and yet they gave consistent results. Some other undiscovered factor was involved. No effort was made to determine the creatine and creatinine separately.

The creatine nitrogen in percentage of the fat-free muscle revealed no constant variation in relation to the fast. The variability of the analyses was sufficient to mask any slight degree of change. Certainly, there was no evidence of any such enormous lowering of the creatine content of the muscle as that described by Howe and Hawk (1911) in fasting dogs. Instead of decreasing, the creatine in the salmon muscle increased in proportion to the protein residue. This increase is definite and above the influence of possible variations in the muscle samples.

### TABLE V.

**Total Creatine Nitrogen.**

<table>
<thead>
<tr>
<th>Station</th>
<th>Location</th>
<th>Fat-free sample per cent</th>
<th>Water extract per cent</th>
<th>Protein residue per cent</th>
<th>Water in muscle per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ilwaco</td>
<td>Tide-water.</td>
<td>0.081</td>
<td>1.720</td>
<td>0.400</td>
<td>0.108</td>
</tr>
<tr>
<td>Warrendale</td>
<td>130 miles.</td>
<td>0.094</td>
<td>2.030</td>
<td>0.470</td>
<td>0.125</td>
</tr>
<tr>
<td>Seufert</td>
<td>210 &quot;</td>
<td>0.078</td>
<td>1.690</td>
<td>0.400</td>
<td>0.103</td>
</tr>
<tr>
<td>Ontario</td>
<td>700 &quot;</td>
<td>0.089</td>
<td>1.580</td>
<td>0.510</td>
<td>0.115</td>
</tr>
<tr>
<td>Cazadero</td>
<td>Spawning grounds</td>
<td>0.089</td>
<td>1.930</td>
<td>0.610</td>
<td>0.106</td>
</tr>
</tbody>
</table>

Unfortunately no metabolism studies in fishes, involving complete examination of the excreta, are available as a means of throwing light upon the behavior of the creatine during the breaking up of the muscle. The further study of creatine metabolism in the salmon should be of marked interest in the light of the present views of Thompson (1917, a, b) regarding the probable metabolic relationship of arginine and creatine, and of the heightened arginine metabolism in the salmon incident to the development of the reproductive organs. The material available in the present instance was not sufficient to permit of such investigation.

The present studies are inadequate to decide whether the relative constancy of the muscle content of creatine nitrogen
Nitrogenous Extractives of King Salmon

is due to the liberation of increased amounts proportionate with the disintegration of the muscle protein, or to the maintenance of a constant saturation of the muscle fluids, as is true of the amino-acids.

General Significance of the Changes in the Muscle of the Salmon during the Spawning Migration.

The mechanism concerned in the transference of material in the salmon is still unsettled. Miescher (1897) interpreted the changes he observed as being a fatty degeneration associated with “liquefaction” of the muscle. The cause of this “liquefaction” Miescher associated with the increased vascularity of the gonads and a reduced flow of blood to the muscles. The resulting insufficient tissue respiration he believed to be the primary cause of the breakdown of the muscles. It is not necessary to discuss the details of the method of protein transportation hypothesized by Miescher. They were based upon Voit’s idea of organized and circulating protein, which is now abandoned in view of the present day conception of the rôle of the amino-acids in protein metabolism.

In his critique of Miescher’s theory, Paton says: ¹⁶

¹⁶ Paton, D. N., Report Fishery Board of Scotland, 1898, iv, 175.
nection it must be remembered that throughout the whole period the muscles remain active, and not only excrete material to the ovaries and testes but also set free energy of the proteids and fats stored within them, a state of matters irreconcilable with the idea of the existence of a degenerative process."

In this connection, reference should also be made to the histological studies of Charles W. Greene (1913) upon the behavior of the fats in the muscle of the Columbia River salmon. He points out and emphasizes that not only the intermuscular but also the intramuscular fat is storage fat and that the muscles are the great fat storage organs of the salmon. Regarding the smaller amounts of intramuscular fat present, he says:\(^\text{17}\)

"The salmon muscle fat is a filtration fat, not a fatty degeneration. It may be stated here that the studies on the king salmon tend to disprove Miescher's theory that the intracellular fat of the salmon muscle, of whatever type the muscle, is a fatty degeneration, a 'Fettentartung,' and support the observations of Mahalanobis that the process is an 'infiltration.' In short, the observations made on the king salmon have tended to confirm the view . . . . that the intracellular fat of the king salmon is an expression of the nutritive state of the muscle. It is a loading of fat by a process of infiltration . . . . and is not a degeneration of the muscle substance."

Under the topic "The mechanism of fat transference in the salmon body" Greene further advocates the view that fat transformations and transferences are under enzymatic regulation, that the whole process is in this case a function of lipase reactions, in short, is governed by chemical factors.

To extend this view to the field presented here would lead us to advocate that chemical factors govern the regulation of the protein changes during the migration, as well as the fat changes. Undoubtedly the breakdown of the muscle is autolytic in nature.

Bradley and Taylor (1916) have reemphasized the fact that autolytic processes are very sensitive to the reaction, being markedly accelerated by slight increases in the hydrogen ion concentration of the medium. On this basis they explain the postpartum involution of the uterus as due to autolysis. This autolysis is favored by vascular changes in the organ which may

\(^\text{17}\) Greene, Chas. W., *U. S. Bureau of Fisheries Bull.*, 1913, xxxiii, 123-
result in the development of a local acidosis. A similar set of
differences can scarcely determine the autolytic processes in the
salmon muscle. Greene (1904) found that in the Pacific salmon
the blood pressure was maintained at its original high level even
at the end of the migration. The muscles are also exceedingly
active through the whole migration period, and the oxidations
therein must be correspondingly intense. Certainly there is no
evidence of "Zenker's degeneration" such as Wells (1914) pro-
duced by the action of acids or by overstimulation of the muscle.

The true factor regulating the catabolism of the muscle protein
is probably the energy requirement of the salmon. Fat is the
chief and primary source of energy during the spawning migration.
As migration proceeds, however, more and more of the protein
as well is catabolized. Protein is also consumed in the develop-
ment of the growing genitalia. According to the present day
theories of metabolism this protein cannot be catabolized directly,
but only after breaking down into the so called "building stones."
The muscle shows a remarkable constancy in the relative amounts
of extractives present during the migration. Particularly con-
stant is the concentration of the extractives in the water contained
in the protoplasm. Van Slyke and Meyer (1913–14) have shown
that an equilibrium exists between the amino-acids of the muscles
and blood, and it is possible that similar equilibria exist for others
of the muscle extractives. With the removal of amino-acids from
the muscles or blood either by synthesis into testicular or ovarian
tissue, or by oxidation for the production of energy, these partic-
ular equilibria are disturbed. This is the state in which more of
the tissue protein is broken down to bring the amino-acid content
of the muscles back to the normal level.

Physiologically the changes in the muscles of the salmon during
the fast of the spawning migration are most interesting. Ordinarily,
in mammals especially, fats and carbohydrates form the greater
part of the energy-producing substances in the body.
In the salmon, on the other hand, fats and proteins are the sole
energy-producing substances. Charles W. Greene (1913) has found
that carbohydrate does not enter into the physiological economy
of these fish, and has followed the course of the fats, showing
their great importance as a source of energy during the migration.
He also has laid stress on the significance of the great decrease
in the amount of protein arguing that this is stored protein necessary for the maintenance of muscular and other activity in the fasting salmon.

In the present paper, evidence has been presented for the presence in the fasting salmon of a regulatory mechanism that maintains the concentration of amino nitrogen in the muscle fluids at an approximately constant level. The significance of such a mechanism for the control of protein metabolism in general is obvious.

SUMMARY

The results of this investigation that call for especial emphasis may be stated as follows.

1. A table giving the percentage composition of salmon muscle from the different stations is presented which confirms by a different method the determinations of Charles W. Greene and extends the analyses to the separation of the non-protein nitrogen fractions.

2. The total extractives in the fat-free tissue increase slightly during the greater part of the migration. The amount of extractives in muscles seems, therefore, to be an index of the metabolic activity of these tissues. This is true both for the tissues of the growing rat as studied by Hatai, and for those of the fasting salmon studied in the present instance.

3. The percentage of nitrogen in the organic extractives remains approximately constant during the migration period. The changes in the extractive nitrogen are, therefore, the same as those in the extractives as a whole.

4. The non-protein amino nitrogen increases in percentage in the fat-free muscle during the migration. The increase in the amount of amino nitrogen relative to the protein from which it must be formed is especially marked, being nearly 100 per cent.

5. The concentration of amino nitrogen relative to the water in the muscles increases from 82 to 100 mg. per 100 gm. of water coincidently with the augmentation of catabolism at the beginning of the migration. The amino nitrogen then shows no further change during the migration. The amount of amino-acids in the tissue is dependent, therefore, upon the volume of water present. This water-volume effect must be added to those factors suggested by Van Slyke as controlling the amino-acid content of the tissues.
6. The creatine nitrogen determinations were rather variable. In general it may be stated that the total creatine content of the fat-free muscle is not essentially changed during the migration fast. Relative to the protein in the muscle the creatine nitrogen increases sharply.

7. The presence in the body of a mechanism regulating the concentration of amino nitrogen in the fluids of the tissues is indicated. The importance of this observation and its significance relative to the theories of protein metabolism are discussed.

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CHANGES IN NITROGENOUS EXTRACTIVES IN THE MUSCULAR TISSUE OF THE KING SALMON DURING THE FAST OF SPAWNING MIGRATION
Carl H. Greene


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