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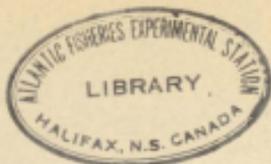
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No. 135

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by

J. Campbell



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The nitrogenous constituents of skate and lobster muscle.

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FINAL REPORT TO THE ATLANTIC BIOLOGICAL STATION  
ST. ANDREWS, NEW BRUNSWICK, CANADA.

THE NITROGENOUS CONSTITUENTS OF SKATE AND LOBSTER MUSCLE

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McGill University, Montreal, September, 1933.

The work undertaken during the Summer of 1933 at the Atlantic Biological Station, St. Andrews, N. B. was a continuation of the work begun the previous summer at the Fisheries Experimental Station, Halifax, N. S. and carried on throughout the year at McGill University in the Department of Physiology under Dr. B.P. Babkin and Dr. S.A. Komarov.

As will be found in the Report of June 1933 to the Halifax Station, the partition and nature of the nitrogenous constituents of fish muscle has been investigated and their effects on gastric secretion has also been studied. The muscles which have been studied include cod, haddock, salmon and herring; of which cod and herring muscle extracts were fractionated and the partition of nitrogen in them established.

While the chemical composition of the muscles differed importantly in many respects we shall only remark in this place that cod muscle extracts showed a preponderance of the lysine fraction over the histidine-arginine fraction; and showed a striking similarity to haddock muscle, fractionated by Komarov (1933).

In these respects herring muscle appeared to be intermediate between cod and ox muscle, the latter also having been studied by Komarov (1933).

The physiological effects of the extracts were also striking. Extracts of cod and haddock muscle appeared to produce gastric juice of stronger peptic power and lower acidity than herring muscle, and herring muscle, in turn, than meat.

Juice of great peptic power and low acidity occurred with the lysine fraction of cod, while the histidine-arginine fraction produced a juice high in acid and low in pepsin.

The character of the juice secreted by the stomach therefore seems to depend on the nature and partition of the nitrogenous constituents of the tissue. Cod muscle being high in the lysine fraction and low in the histidine-arginine fraction gives a choline-like response; high in pepsin and low in acid as compared to ox muscle. Herring muscle is mid-way between these.

During this summer Lobster (*Homarus americanus*) muscle and Skate (*Raja stabiiformis*) muscle were investigated. Lobster muscle is of interest since Alley (1933) found great secretagogue effect from its ingestion, giving a juice very high in pepsin and of moderate acidity. The reason for investigating the nitrogenous constituents chemically and physiologically is obvious as they have been said to regulate gastric secretion. Skate muscle is also of interest through its evolutionary position, its importance as an object of physiological investigation by Dr. Bekkin and others, and the known peculiar nature of its composition by the large amounts of urea and ammonia present. Time permitted the fractionation of lobster muscle alone but the nature of some of the constituents of both muscles was investigated.

While a study of physiological effects of the extracts and the fraction thereof was also planned and was attempted, this part of the work was unsuccessful. The dog used, having an Armour pouch and gastric fistula, was extremely variable and was later found to have a chronic gastric ulcer near the fistula border.

The surprising nature of lobster muscle composition and of the less extensively studied skate muscle is shown below.

## METHODS

The methods used are similar to those used in the fractionation of the cod and herring extracts described in the Report to the Halifax Experimental Station, Campbell (1933); with the exception, that in the fractionation of the lobster extracts, the histidine-arginine, purine and lysine fractions were liberated from the silver salt formed by hydrochloric acid instead of hydrogen sulphide.

Particular care was paid to the extraction of lobster muscle because of the rapidity with which it has been found to autolyse (Reed, Rice and Sinclair 1933). The lobster muscle was excised immediately after killing the animal by splitting with a cleaver, and after weighing and mincing was put into water at 90°C. The muscle was therefore only kept fresh for about 30 minutes after death. Muscle from the thorax and lesser claws was used as well as that from the tail and large claws. After extraction in two volumes of hot water for 20 minutes at 85°C. and pH 5.0 the suspension was filtered and the clear fluid evaporated. The muscle cake was washed and extracted again twice as described in the previous communication.

In the case of skate the "wing" muscles were excised from a fresh specimen and extracted in the same way.

The weight of lobster muscle used was 3280 g. (obtained from 12 lobsters) and the weight of skate muscle was 1000 g.

## RESULTS

The results of the analyses and of the fractionation appear in table 1. In this table the amount of the various constituents and partitions of the extracts are expressed as mg. of nitrogen per 100 g. of fresh muscle (mg.%), and also as percent of the total non-protein nitrogen (%).

As explained in the previous Report, the non-protein nitrogen is the true non-protein nitrogen value, the proteoses and peptones are not included in the purine fraction nitrogen. The nitrogen of the purine fraction was determined when the bases were in the form of nitrates and is therefore somewhat higher than the true value for this fraction.

The results are interesting in many respects and one is struck at the outset by the profound differences shown between the composition of skate and lobster muscle and the muscle of cod, haddock, salmon and herring.

A full description comparative with the last mentioned group which have been described in the previous report would be too tedious here so that the main differences will be touched on.

#### NON-PROTEIN NITROGEN

In the group cod, haddock, salmon and herring the non-protein nitrogen, while showing certain characteristics in cases, is in the neighbourhood of 400. mg.% (390 to 460 mg.%). In lobster the value is 682.8 mg.% and in skate 1423. mg.%; or an increase of 50% and 350% respectively over the average value for the first group.

#### VOLATILE BASE NITROGEN OTHER THAN AMMONIA

The value of the volatile bases nitrogen other than ammonia (probably mostly due to trimethylamine) is great in skate muscle being 152. mg.% or 10.7% of the total non-protein nitrogen, while in lobster it is very low being 0.6 mg.% or 0.09% of the total. In the former group herring has the highest value of 4.7 mg.% or 1.1% of the total non-protein nitrogen.

#### AMMONIA NITROGEN

The ammonia nitrogen of skate, being 35.4 mg.% is also very high, but due to the high non-protein nitrogen value the percent of the total, 2.69%, is not high comparatively. The amount in lobster, 19.9 mg.% is fairly high as is the % of the total, which is 2.9%.

### UREA NITROGEN

The urea nitrogen of skate muscle (about 800 mg.%), is extremely high. This figure is unfortunately not known exactly, hence the brackets in the table. It represents more than half the total non-protein nitrogen (56%). In lobster muscle the urea nitrogen is the lowest of all the cases including the former group.

### AMINO GROUP NITROGEN

The value determined by the formal titration is here termed amino group nitrogen and is calculated as such, the ammonia nitrogen being, of course, subtracted.

The values of the amino group nitrogen are interesting. Skate muscle contains 52.9 mg.%; a high value, the next highest in the former group being that of herring amounting to 48.8 mg.%. In skate muscle however the percent of the total is low being 6.2% while in herring it is 11.2%. In lobster muscle the amino nitrogen is extremely high being 211. mg.% or 30.9% of the total non-protein nitrogen.

### CREATINE AND CREATININE NITROGEN

The creatine plus creatinine nitrogen of skate is 107. mg.%; lower than in the former cases of which the value of cod muscle, 163. mg.%, is lowest. Creatine and creatinine are practically absent in lobster muscle.

To sum up this first portion of the work we note that the enormous value of the non-protein nitrogen of skate muscle is caused mainly by the amount of volatile base, ammonia and urea nitrogen. The amino nitrogen is high in absolute amount but not as percent of the total.

Lobster muscle while having a high non-protein nitrogen value is low in the combined volatile base, ammonia and urea nitrogen. Creatine and creatinine are practically absent and the amino nitrogen is extremely high. This condition in lobster muscle is further investigated by the fractionation of the extracts whereby the partition of nitrogen is established, and which will now be discussed.

## FRACTIONATION OF LOBSTER MUSCLE

### NITROGENOUS BASES FRACTION

The nitrogen of the nitrogenous bases fraction of lobster muscle is 382.6 mg.%. This is a very high value as the values in cod and herring were 257.6 mg.% and 269.9 mg.% respectively. As percent of the total non-protein nitrogen the figure is low (56.0%) and compares to ox muscle (53.6%), Komarov (1933) rather than to fish muscle.

### PURINE FRACTION

The purine fraction nitrogen (10.7 mg.%) is not markedly different from the other cases (24.5 mg.% in cod and 8.21 mg.% in herring muscle).

### HISTIDINE-ARGININE FRACTION

The nitrogen of the histidine-arginine fraction (191. mg.%) is very high both in absolute amount and as percent of the total non-protein nitrogen (26.0%). Herring muscle contains 72.7 mg.% and cod muscle 32.7 mg.%. The nitrogen of this fraction of lobster muscle is also twice as great as that found in ox muscle (89.8 mg.%) by Komarov (1933).

### LYSINE FRACTION

The nitrogen of the lysine fraction of lobster muscle (150.6 mg.%) is high but not nearly so much so as the histidine-arginine fraction. Cod muscle contains 157.9 mg.% and herring muscle 145.6 mg.%. The percent of the total non-protein nitrogen is much lower in lobster muscle 22.1% than in cod or herring muscle (37.7% and 33.3% respectively).

### MONOAMINO ACID FRACTION

Lobster muscle is also characterized by having a high proportion of the total non-protein nitrogen in the monoamino acid fraction. The value is 32.44% while in cod and herring muscle the values are 22.8% and 22.4% respectively. Since the value of the total non-protein nitrogen is high in lobster muscle the amount of the monoamino acid fraction

nitrogen (221.5 mg.%) far exceeds that present in cod and herring muscle (95.7 mg.% and 97.9 mg.% respectively).

#### THE HUMINE NITROGEN

The humine nitrogen of lobster muscle is low as compared to cod and herring muscle. In the humine nitrogen l. or nitrogen unrecovered in the first phosphotungstic acid precipitation it forms 11.5% of the total non-protein nitrogen as contrasted with 16.7% for cod and herring muscle.

The total humine nitrogen of lobster muscle (16%) is even lower as compared to cod and herring muscle (25%).

#### SUMMARY

The investigation of the nitrogenous constituents of skate and lobster muscle has shown extreme contrasts between them and also between the muscle of cod, haddock and herring which have been previously studied.

The total non-protein nitrogen of lobster is 632.8 mg.% or 50% greater than that found in the latter cases where fairly uniform values occur. This value in skate muscle is 1423. mg.% or 3.5 times as much as in the latter cases.

In skate muscle this increase is due to urea, ammonia and volatile bases other than ammonia. The urea of skate muscle is especially high comprising over half of the total non-protein nitrogen.

In lobster muscle the urea and volatile bases other than ammonia are low while the ammonia nitrogen is fairly high.

Creatine and creatinine nitrogen are practically absent in lobster muscle but present to the extent of 108. mg.% in skate muscle.

The amino nitrogen of lobster muscle is extremely high both in absolute amount and as percent of the total non-protein nitrogen (211. mg.% and 30.9% respectively). In skate muscle the absolute

amount (52.9 mg.%) is high but the proportion of the total non-protein nitrogen is low (6.20%).

The results of the fractionation procedures show that the nitrogenous bases of lobster muscle form a lower percent of the total non-protein nitrogen than cod or herring muscle (56.04% in lobster muscle and 61.44% and 61.77% in cod and herring muscle respectively). The absolute amount present however is much greater in the former cases.

The Purine bases of lobster muscle are quite low.

The histidine-arginine fraction in lobster muscle is however very high, the nitrogen of this fraction being 6 times that of cod muscle and 2½ times that of herring muscle. The percent of the total non-protein nitrogen is also greater.

The lysine fraction of lobster muscle is high, but the nitrogen of this fraction in lobster muscle is slightly less than that present in cod muscle.

The nitrogen of the monamino acid fraction is very high both in amount and as percent of the total non-protein nitrogen.

It is important to note that the increase in the total non-protein nitrogen in lobster muscle over the average value for cod, haddock, salmon and herring muscle is due to the increase in the nitrogen of the monamino acid and histidine-arginine fractions.

The writer's greatest thanks are due to Dr. B. P. Babkin and Dr. S. A. Komerov who directed this work. The Director and Staff of the Atlantic Biological Station, St. Andrews, N. B. gave facilities and great help in it.

DISTRIBUTION OF NON-PROTEIN NITROGEN IN MUSCLE

Results are expressed as mg. nitrogen per 100.g. wet weight of tissue, and as per cent of the total non-protein nitrogen.

| NITROGEN IN THE FORM OF             | LOBSTER |       | SKATE  |       |
|-------------------------------------|---------|-------|--------|-------|
| Total Non-protein Nitrogen          | 682.8   | 100.  | 1423.  | 100.  |
| Volatile bases (other than ammonia) | 0.60    | 0.09  | 152.1  | 10.7  |
| Ammonia                             | 19.9    | 2.92  | 35.4   | 2.69  |
| Urea                                | 0.07    | 0.010 | (800.) | (56.) |
| Amino-nitrogen                      | 211.    | 30.9  | 52.9   | 3.51  |
| Creatine and Creatinine             | 4.67    | 0.68  | 107.6  | 7.56  |
| <hr/>                               |         |       |        |       |
| Nitrogenous Bases                   | 362.6   | 56.04 |        |       |
| *Purine Bases                       | 10.7    | 1.57  |        |       |
| Histidine-Arginine fraction         | 191.    | 28.0  |        |       |
| Lysine fraction                     | 150.6   | 22.1  |        |       |
| Monosmino acid fraction             | 221.5   | 32.4  |        |       |
| Humine fraction 1.                  | 78.1    | 11.6  |        |       |
| Total Humine nitrogen               | 108.4   | 15.93 |        |       |

\*In the form of nitrates

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