# CHEMICAL AND PHYSIOLOGICAL PROPERTIES OF THE NON-PROTEIN NITROGENOUS CONSTITUENTS OF FISH MUSCLE



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by

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The problem of the digestion of foodstuffs in the alimentary tract is of far reaching importance in the maintenance of animal life, and in mammals the question has many aspects which must be approached separately before an understanding of the whole can be obtained. As part of this question it is obvious that a first condition in the utilization of food is the presence of a secretion of digestive juices, and since secretion is produced in part by substances present in foods, it is of interest to find the effects of different foodstuffs on the production of secretion and on the secretory cells. The importance of the so-called non-protein nitrogenous constituents of meats in the production of secretion from the glands of the digestive system has been known for a long time, and was first recognized by Pavlov and his associates. Since that time a very extensive study of the effect of various chemically identified constituents of meat, as well as differently prepared extracts has been made (See Babkin 1928).

In the case of fish muscle, previous work on the nature of the non-protein nitrogenous constituents has been almost entirely chemical. As regards the digestion of fish we have the earlier investigations of Gordeieff (1906) and Boldyreff (1909) on dogs with a Pavlov pouch, showing that the ingestion of fish activated a greater gastric secretion than corresponding preparations of beef meat. According to Hawk, Rehfuss and Bergeim (1926) fish activates in man a secretion of gastric juice with as high an acidity as the secretion from several meat products, e. g. beef and pork, chicken, lamb, turkey and veal. The recent investigations of Komarov (1931) on the study of gastric secretion in a dog with a Pavlov pouch after ingestion of haddock flesh, skin and beef meat has shown that, contrary to the findings of the earlier workers mentioned, in the capacity to activate gastric secretion, beef meat and haddock flesh did not differ considerably. Alley (in press) made a comparative study of the effect of haddock, cod, mackerel and lobster on gastric secretion by a dog with an Armour pouch and gastric fistula. Her experiments showed that cod, haddock and mackerel stimulated the secretion of gastric juice as effectively as meat, and lobster more effectively. Fish was found to remain in the stomach as long as meat, and in the case of mackerel longer, due to its high fat content. A rise in the fluid volume of secretion occurred near the end of the experiment in the case of fish.

The present work was therefore begun with two objects in view, firstly to determine the effect of the non-protein nitrogenous constituents of various fishes on the gastric secretion, with especial regard to the part played by the nitrogenous bases; and secondly, to try to gain some understanding of the nature of the non-protein nitrogenous constituents of fish muscle which cause the secretion.

We have thus attempted to relate the chemical composition of muscle in haddock, cod, salmon and herring with their effect on the gastric secretion of dogs.

At this point it might be appropriate to review very briefly the data in the literature on the chemical nature of fish muscle.

Clifford (1921) using a colour test for the determination of imidazole groups, found that carnosine was absent from the cod or haddock, present in salmon, and present in 0.16% in herring muscle. These results are confirmed in this work and isolation methods failed to reveal the presence of carnosine in cod muscle.

It was observed as early as 1890 by Schroder that the muscles of Selachians were especially rich in ammonia and urea. Suwa isolated betaine in 1908, and trimethylamine oxide in 1909 from Selachian muscle. In 1913 Yoshimura and Kanai isolated creatinine, betaine, methylguanidine, taurine and alanine from dried cod muscle, and Yoshimura (1913) obtained trimethylamine, putrescine and choline from herring roe. Kapeller-Adler and Krael (1930) have presented indirect evidence for the presence of methylguanidine in several species of fish.

Hoppe-Seyler and Schmidt (1927) in an interesting comparative study found that trimethylamine oxide and trimethylamine occurred only in the muscles of sea fishes and that none could be obtained from the muscles of fresh water fish. In a further discussion of the possible causes for this difference, Hoppe-Seyler (1929) states that spawning does not affect it and considers that although the feeding may account for some difference, that this cannot explain it all. Relative to this Kapeller-Adler and Csato (1931) have found considerable amounts of ammonia, methylamine and trimethylamine in sea-weed (_Fucus vesiculosus_ and _F. serratus_).

Kapeller-Adler and Krael (1930) and (1931) found that there were certain pronounced differences between carp, cod, shark, and skate muscle with respect to their preformed and altered volatile bases. By the methods used it is claimed that determinations of ammonia, methylamine, trimethylamine, trimethylamine oxide, methylguanidine and betaine can be made. Much more trimethylamine was found in Selachian muscle than had been found by Hoppe-Seyler and Schmidt, the difference being attributed to bacterial action which might have occurred during transit, leading to a conversion of trimethylamine oxide to trimethylamine.

The work of Hunter (1929) on the creatine content of fish muscle is discussed later, as is the work of Johnston (1932) on the nitrogenous extractives of fish muscle.

Komarov (1933) found by silver-barytra fractionation of haddock muscle extracts that they differed greatly from ox, dog or horse muscle extracts, being characterized especially by a high proportion of nitrogen in the lysine fraction, and a low proportion in the histidine-arginine fraction. Komarov ($1933_2$) also found interesting differences in the distribution of nitrogen after autolysis in haddock muscle. The nitrogen in the lysine and histidine-arginine fractions remained practically the same, the changes occurring in the other fractions.

EXPERIMENTAL

For the purposes of this investigation, a large number of extracts were made from the muscle of cod, haddock, salmon and herring at the Fisheries Experimental Station (Atlantic) at Halifax during the summer months (1932). The cod and haddock were caught by line trawl set in the evening, and were taken for extraction the next morning, so that the fish were in a relatively fresh condition. The herring were caught by netting and were obtained for extraction about 18 hours later. The salmon were either single fish caught locally or had been preserved in a frozen condition at the Station for some months.

The muscle "fillets" on either side of the fish were removed, care being taken to avoid any contamination with debris or the slime and scales of the skin. Two lots of muscle, A and B, were always made, the two fillets of one fish being put in separate lots A and B. The part A was used for immediate extraction and B for autolysis. Both A and B were minced.

To weighed quantities of A in large beakers were added 2 volumes of distilled water, and with stirring, sufficient 0.5 N HCl to bring to a pH of 4.9. This pH had been found by test experiments to be the point where maximum precipitation of the muscle proteins occurred. (10 cc. of a brei of haddock muscle, similar to the above, were placed in several test tubes, and 0.5 N HCl and water added to each to make a total of 2 cc. fluid and give a pH range between 3 and 7. The test tubes were placed in a boiling water bath for 20 minutes, filtered, and to 5 cc. aliquots of the filtrate trichloracetic acid was added to 5% concentration. The consequent turbidity was estimated by eye and was seen to be least at pH 4.9 ).

To 500 grams muscle about 65 cc. of 0.5 N HCl were added with stirring. The beakers were then placed in boiling water baths and stirred. A temperature of 80°C. was reached in about 10 minutes and the material was heated for 20 minutes longer. It was then filtered through gauze on the Buchner funnel, giving a clear solution and was washed twice with boiling water. In the second extraction, 1.5 volumes of water were used with 4 cc. of 0.5 N HCl per 500 grams muscle and the material heated for 30 minutes before filtration and washing. A third extraction was made with 1 volume of water and 1 cc. acid per 500 grams muscle. The combined extracts were evaporated "in vacuo" in the presence of thymol at a temperature of the water bath not exceeding 60°C. When a small volume was reached the material was transferred quantitatively to a bottle. A little thymol was then added and an equal volume of 95% alcohol.

The B portion, after mincing was placed in a wide mouthed bottle and an equal volume of distilled water was added. As antiseptics, toluol, thymol and chloroform were used. The bottles were well stoppered and the material was shaken each day. After 15 days one more volume of water was added and the material treated as in A.

Considerable difficulty was encountered in the treatment of salmon by this method. A suspension formed on heating the tissue with water, which was extremely difficult to filter and almost invariably gave cloudy filtrates. The cake on the Buchner funnel could not always be sucked dry so that a quantitative extraction of the tissue was hindered. Treatment of the tissue with alcohol, acetone or gasoline before aqueous extraction was not entirely successful or led to practical difficulties. Finally, in the case of the fresh fish, the aqueous extraction was carried out as described above, leaving the precipitates in the turbid filtrates for later removal. In the case of the frozen salmon, the tissue was heated for half an hour in beakers in boiling water baths before aqueous extraction. This treatment produced a surprising change, the filtrates being as clear and the filtration as rapid as in the case of the other fishes.

In all, the muscle of 18 Kg. cod, 17 Kg. haddock, 6 Kg. herring and 7 Kg. salmon were treated in this way.

The extracted muscle residues were dried in an oven at approximately 70°C. and kept.

At the Department of Physiology, McGill University, under the direction of Dr. B. P. Babkin and Dr. S. A. Komarov the investigation of the chemical and physiological nature of the extracts was begun. The work may be arranged for convenience under the following headings:

Chemical Investigation of the extracts

Fractionation of Cod Muscle

Effect of Fish Extracts on Gastric Secretion

Effect of the Cod muscle Fractions on Gastric Secretion

Properties of Cod Muscle Fractions.

CHEMICAL INVESTIGATION OF FISH MUSCLE EXTRACTS

The extracts were prepared for analysis by removing the alcohol by evaporation "in vacuo". (In all this work, unless when otherwise stated, it is understood that evaporation was carried out "in vacuo"). The material was then diluted till the ratio of grams tissue to cubic centimeter volume was about 1.5 to 1. This was than placed on the water bath for about half an hour and filtered from the precipitate, which was well washed, and the volume then made up to a ratio of about 1 gram tissue to 1 cc. fluid. The salmon and herring extracts were first extracted four times with toluol by shaking in a separatory funnel, the layers being separated by centrifuging. The combined toluol extracts were then extracted four times with water and the aqueous layers combined with the previous aqueous layers.

The methods used in the analysis are as follows:
Total nitrogen: This was determined in the micro-Kjeldahl apparatus of the Parnas-Wagner type, or by the micro-Kjeldahl method. The latter method obviated dilution of the solution.
Non-protein nitrogen: The extracts contained less than 1% of nitrogen precipitable in 5% trichloracetic acid. To determine the non-protein nitrogen, a sample of the extract was diluted in the ratio 3 to 4 with 20% trichloracetic acid (e.g. 3 cc. extract plus 1 cc. 20% trichloracetic acid) and filtered after 1 hour. The nitrogen in the filtrate was determined.
Volatile base nitrogen: This was determined in the apparatus of Van Slyke and Cullen (1916). 2 cc. of the extract and 10 cc. of saturated sodium carbonate being used in the reaction tube. The bases were aerated into 5 cc. of N/50 HCl.
Urea nitrogen: The urea was determined by the urease method, the ammonia formed being aerated into N/50 HCl as described above. The increase in the titration figure of the urea nitrogen determination over that of the volatile base represents the ammonia due to urea. Blank determinations were always made for volatile bases and urea.
Ammonia nitrogen: The ammonia nitrogen was determined by aeration of a sample similar to that used for the volatile base determination into N/50 HCl and Nesslerising, the unknown being compared in a Dubosc colorimeter with a standard. It was found necessary to remove the caprylic alcohol used to reduce frothing, "in vacuo" before Nesslerising as its presence seemed to cause turbidity.

<u>Amino nitrogen:</u> The amino nitrogen was determined by formol titration under carefully controlled conditions. The material was first titrated to a standard pH with phenolphthalein as indicator, and to the same pH after the addition of formalin at this pH. In detail we proceeded as follows: titrations were made in 50 cc. centrifuge cups. Immediately before using, to 10 cc. of commercial 40% formaldehyde 3 drops of 1.0 N NaOH were added. The first standard A contained 2 cc. of distilled water, 1 drop of 0.1% phenolphthalein and sufficient N/10 NaOH to cause a complete change of colour. A standard B contained 2 cc. distilled water, 1 drop of 0.1% phenolphthalein, 1 cc. of the formaldehyde solution and sufficient N/10 NaOH to cause no further change of colour. To 2 cc. of the extract was now added 3 drops of 0.5% phenolphthalein and this was titrated to the standard A with N/20 NaOH. 1 cc. of the neutralized formol was then added and the material further titrated to the colour of the standard B with the standardized N/20 NaOH. The standards A and B are made equal in volume to the solution titrated just before the end-point.

Blank titrations of 2 cc. distilled water plus 3 drops of 0.5% phenolphthalein are titrated to the colour of A. On the addition of the formol solution (1 cc.) the colour exactly matched that of the standard B. A difference in colour, due to a difference in the pH of the formol solution, necessitates a correction. The titration figure from A to B in terms of $-NH_2$ groups, minus the ammonia nitrogen gives the value for amino nitrogen.

Creatine and Creatinine: Benedict's boiling down method (1914) was used for the conversion of creatine to creatinine, and Folin's (1914) method for the determination of creatinine. The standard was prepared from a solution of pure creatinine zinc chloride.

Imidazole groups: The procedure of Kossel and Hanke (1919) was first used but the procedure of Jorpes (1932) was later adopted as it was found to be more satisfactory.

Organic solids: The organic solids were determined by drying 5 cc. of the extract to constant weight in an oven at 120°C., then ashing.

The results of these experiments are shown in Table I.

TABLE I

Non-Protein Nitrogen of Fish Muscle Extracts

mg. per 100 gram muscle

| Nitrogen | Haddock mg.% | Cod mg.% | Salmon mg.% | Herring mg.% |
|---|---|---|---|---|
| Non-protein | 417.0 | 400.0 | 466.0 | 425.0 |
| Volatile base | 7.6 | 12.5 | 12.5 | 14.0 |
| Ammonia | -- | 9.5 | 11.9 | 9.3 |
| Urea | 2.0 | 2.3 | 1.3 | 1.7 |
| Amino- | 28.3 | 34.2 | 39.1 | 45.4 |
| Creatine & Creatinine | 205.0 | 169.0 | 193.0 | 185.0 |
| Imidazole | (0.87) | (0.87) | 2.36 | 28.1 |
| Organic solids | 2.52g.% | 2.16g.% | 2.87g.% | -- |

In these four species of fish there is seen to be a considerable variation in the content of non-protein nitrogen in the extracts, and it must be noted that a certain amount of this variation may be due to proteoses or higher peptides formed by the process of extraction. In cod, salmon and herring the values for ammonia do not vary significantly. In the case of herring the volatile bases other than ammonia are notably higher than in the other fishes. The urea nitrogen figures do not vary significantly. They are, however, much lower than the value of 14.5 mg.% for haddock, found by Komarov (1933). The cause of this discrepancy is not known. Johnston (1932) found a value of 1.7 mg.% urea in the case of haddock. The amino nitrogen values which become increasingly greater in haddock, cod, sâlmon and herring, seem to be significantly higher in the last two cases, especially in herring.

The values for creatine and creatinine, expressed in mg. creatinine per 100 grams muscle, are - haddock 552, cod 454, salmon 521, and herring 498. Hunter (1929) found a value of 740 mg.% for Pacific coast herring (Clupea pallasii), and 636 mg.% for Coho salmon (Oncorhynchus kisutch). It may be noted that these figures apply to muscle from the tail region which is richer in creatine than the muscle anterior to this. The figures given here are for the whole muscle.

Johnston (1932) in a study of the non-protein nitrogenous constituents of cod, haddock, hake, mackerel and herring muscle finds figures of approximately the same order for ammonia, urea and creatinine. It is doubtful whether the differences may be considered as constant due to variations within a species, but a comparison of the latter worker's figures with our own is as follows. By Johnston's figures, the order in decreasing magnitude for ammonia is herring, cod, haddock; for urea cod, herring, haddock; for creatine herring, haddock, cod. In the results in this report the order for volatile bases is herring, cod, haddock; for urea cod, haddock, herring; for creatine haddock, herring, cod.

Creatine, creatinine, urea or uric acid have not been shown to be active in producing secretion from the digestive glands, although many experiments have been made in this connection.

The imidazole group nitrogen varies very greatly in these fishes. The figures for cod and haddock are given in brackets to indicate that they are not reliable as the colour developed was so weak as to make a determination almost impossible. The colour developed by salmon is also not so constant as that developed by herring. If the value ascribed to cod and haddock be accepted for comparison the ratio for cod: haddock: salmon: herring is about 1: 1: 5: 30. Such values were found by Clifford (1921) who made an extensive investigation of animal tissues, using this test. This imidazole group is of great physiological importance.

The values for organic solids parallel the non-protein nitrogen values.

FRACTIONATION OF COD MUSCLE EXTRACTS

The extractives of cod muscle were next studied more fully with respect to their chemical nature and physiological properties.

An analysis similar to that previously described was carried out on the extract which was then fractionated by the silver-barytra method. Throughout the fractionation portions were kept for a determination of the ability to produce gastric secretion. Finally an investigation of the fractions for the isolation of crystalline substances was made.

Experimental

The extract from 4295 grams of cod muscle was concentrated in a volume of 1000 cc. 75 cc. were removed for analysis and physiological assay and the remainder was fractionated. The silver-barytra method was used in this investigation, precisely as by Komarov (1933) in the study of the extractives of haddock muscle, but with some slight variations. When the filtrate of free nitrogenous bases was being concentrated "in vacuo" the distillate was lead into standard N/5 $H_2SO_4$ containing methyl red indicator. The volatile bases were thus collected and determined by titration of the excess acid with standard N/10 NaOH. A part of the monoamino acid fraction corresponding to 1000 grams muscle was neutralized with hot barytra and concentrated to a small volume. To precipitate the nitrogenous bases 23.4 grams phosphotungstic acid per 100 grams muscle were used.

It was seen that the purine fraction contained proteoses or higher peptides by the formation of slight flocculent precipitates. A portion of this fraction was therefore precipitated by tannic acid, following the procedure of Wasteneys and Borsook (1924), and it was found that while the purine fraction contained 73.1 mg. of nitrogen per 100 grams muscle (i.e. 73 mg.%) the proteoses and higher peptides constituted 48.8 mg.% of this, and the remainder, purines, 24.3 mg.%. This nitrogen, precipitable by tannic acid, was subtracted from the non-protein nitrogen, the nitrogenous bases, and from the purine fraction. Proteoses were found in the extracts of meat by Fürth (1911), and are regarded as artificial products formed as a result of hydrolysis occurring during the extraction with hot water.

The results are shown in Table II. The distribution of amino nitrogen is shown in Table III.

## TABLE II

### Partition of Non-Protein Nitrogen in Cod Muscle

The results are expressed both as mg. per cent. of wet fresh tissue and as per cent. of total non-protein nitrogen.

| Nitrogen in the form of | mg. % | % of total |
|---|---|---|
| Non-protein | 419.2 | 100.0 |
| Volatile bases and ammonia | 9.84 | 2.35 |
| Ammonia | 7.74 | 1.85 |
| Urea | 1.86 | 0.44 |
| Amino- | 39.16 | 9.34 |
| Creatine and creatinine | 163.2 | 38.9 |
| Imidazole group | Trace | -- |
| Nitrogenous bases | 253.6 | 60.49 |
| *Purine bases | 24.5 | 5.84 |
| Histidine-arginine fraction | 32.7 | 7.80 |
| Lysine fraction | 157.9 | 37.67 |
| Monoamino acid fraction | 95.7 | 22.8 |

*The purines present as nitrates

TABLE III

Partition of Amino Nitrogen in Cod Muscle Extract

The results are expressed both as mg. per cent. of wet fresh tissue and as per cent. of total amino nitrogen.

| Amino nitrogen in | mg.% | % of total |
|---|---|---|
| Whole extract | 46.9 | 100.0 |
| Nitrogenous bases | 11.8 | 25.1 |
| Purine bases | 0.0 | 0.0 |
| Histidine-arginine fraction | 0.997 | 2.13 |
| Lysine fraction | --- | --- |
| Monoamino acid fraction | 22.2 | 47.4 |

These results show a striking similarity in the distribution of non-protein nitrogen in the cod as compared with that of the haddock investigated by Komarov (1933). We find that, in contrast to meat, cod has a higher proportion of nitrogen in the nitrogenous bases. The nitrogen of the histidine-arginine fraction of cod is even lower than that of haddock, and that of the lysine fraction of cod is about the same as in haddock. The monoamino acid fractions are of the same order in cod and haddock, and the purine bases are somewhat higher in cod. Thus there is a marked parallel in the nitrogenous constituents of cod and haddock as contrasted with meats, and in view of the wide variation between the two phyla in which these forms occur, the resemblance is striking.

The fractionation of herring extracts is in progress.

SECRETAGOGUE ACTION OF EXTRACTS FROM FISH MUSCLE AND OX MUSCLE

The ability of the extracts to produce gastric secretion was determined by means of a dog with an Armour pouch (see Armour, 1930) and gastric fistula. The extract was introduced into the stomach through the fistula and the secretion from the Armour pouch was used to determine the effect. There were thus no psychic influences present and we can attribute any effect on secretion to the influence of the fluid given. Experiments were done when the dog was in a fasting condition, i. e. about 18 to 20 hours after the last meal. Control experiments with distilled water, tap water and physiological saline showed that the greatest secretion occurred with distilled water, less with tap water and a negligible secretion with saline. To avoid such disturbances due to variations in the osmotic pressure of the extracts, their osmotic pressure was determined by the lowering of the freezing point, and before giving to the experimental animal they were diluted so as to be of the same osmotic pressure as physiological saline. They were also warmed to 37°C. before introducing into the stomach. The amount of extract given was always equivalent to that from 200 grams muscle. The herring and salmon extracts were first extracted with toluol as described previously, and in the second series of experiments the salmon was also extracted with ether.

The first series of experiments was made some months before the second series, and in that time a change occurred in the composition of the pouch juice.

The results are shown in Tables IV and V.

TABLE IV

Secretagogue Action of Fish and Meat Extracts

Dog - "Monday"

Extracts were introduced into the stomach by a fistula, and the secretion obtained from the Armour pouch. The extracts each equivalent to 200 grams muscle.

| Extract from Muscle of | Volume of Secretion In 15 minute periods | | | | | Total | Free HCl | Total Acidity | Pepsin (Mett' units) |
|---|---|---|---|---|---|---|---|---|---|
| | 0-15 | 15-30 | 30-45 | 45-60 | 60-75 | | | | |
| **First Series** | | | | | | | | | |
| Cod | 0.4 | 2.6 | 0.9 | 0.2 | -- | 4.1 | 56.7 | 64.9 | 13.0 |
| Haddock | 0.43 | 2.45 | 1.00 | 0.30 | -- | 4.18 | 57.6 | 65.7 | 21.1 |
| Salmon | 1.5 | 3.4 | 0.45 | 0.2 | -- | 5.55 | 60.6 | 68.5 | 29.1 |
| Herring | 0.60 | 4.2 | 1.45 | 0.40 | -- | 6.65 | 60.0 | 68.1 | 27.0 |
| **Second Series** | | | | | | | | | |
| Distilled water | 0.60<br>0.70 | 2.00<br>0.68 | 0.30<br>0.43 | 0.20<br>0.32 | 0.05<br>0.18 | 3.10<br>2.13 | 105.0<br>65.0 | 122.0<br>83.0 | --<br>77.4 |
| Tap water | 0.46 | 0.83 | 0.30 | 0.18 | -- | 1.85 | 76.0 | 91.0 | 100.0 |
| 0.9% NaCl | 0.06 | 0.05 | 0.15 | 0.05 | -- | 0.31 | -- | -- | --- |
| Cod | 0.25 | 4.15 | 1.80 | 0.39 | 0.70 | 6.59 | 100.0 | 116.0 | 135.0 |
| Haddock | 0.18 | 3.10 | 2.10 | 0.90 | 0.60 | 6.28 | 98.0 | 113.0 | 117.0 |
| Salmon | 0.50<br>1.10 | 2.40<br>3.55 | 1.90<br>0.73 | 0.45<br>0.22 | --<br>0.19 | 5.25<br>5.60 | 95.0<br>111.0 | 111.0<br>125.0 | 125.0<br>125.0 |

Continued on Page 20

TABLE IV (Continued)

| Extract from Muscle of | Volume of Secretion In 15 minute periods 0-15 | 15-30 | 30-45 | 45-60 | 60-75 | Total | Free HCl | Total Acidity | Pepsin (Mett' units) |
|---|---|---|---|---|---|---|---|---|---|
| Second Series | | | | | | | | | |
| Herring | 0.08 | 3.90 | 2.60 | 0.60 | 0.50 | 7.18 | 105.0 | 123.0 | 85.0 |
| Meat | 0.43 | 2.40 | 1.30 | 0.30 | 0.70 | 5.13 | 120.0 | 131.0 | 36.0 |
| | 0.18 | 1.75 | 0.70 | 0.60 | 0.30 | -- | -- | -- | -- |
| | -- | -- | -- | -- | 1.40 | 5.43 | 104.0 | 117.0 | 58.0 |
| | 1.0 | 4.15 | 0.63 | 0.50 | 0.15 | 6.43 | 109.0 | 122.0 | 71.0 |

TABLE V

Secretagogue Action of Cod Muscle Fractions

Dog - "Monday"

Extracts were introduced into the stomach by a fistula and the secretion obtained from the Armour pouch. The extracts were each equivalen to 200 grams muscle.

| Fraction of Cod Muscle | % of Total Nitrogen | Volume of Secretion In 15 minute periods 0-15 | 15-30 | 30-45 | 45-60 | Total | Free HCl | Total Acidity | Pepsin (Mett' Units). |
|---|---|---|---|---|---|---|---|---|---|
| Whole Extract | 100.0 | 0.25 | 4.15 | 1.80 | 0.39 | 6.59 | 100.0 | 116.0 | 135.0 |
| | --- | -- | -- | -- | 0.70 | -- | -- | -- | -- |
| Nitrogenous Bases | 60.0 | 0.75 | 4.00 | 1.25 | 0.55 | 6.55 | 105.0 | 120.0 | 85.0 |
| Histidine-Arginine Fraction | 8.0 | 1.90 | 1.10 | 0.20 | 0.25 | 3.25 | 90.0 | 107.0 | 52.0 |
| Lysine Fraction | 38.0 | 0.25 | 1.05 | 0.12 | 0.11 | 1.53 | -- | -- | 219.0 |

The results of these experiments, of which the second series is more complete, show that aqueous extracts from herring give the largest volume of secretion, cod and haddock follow, and salmon gives the least volume. In the first series the order is herring, salmon, cod and haddock equal, but on the basis of the more complete later experiments salmon should apparently be last. Meat tended to give a secretion over a more prolonged period than fish and the secretion is apparently equal to that of cod or haddock. Meat gives a more acid juice than cod or haddock, and one with lower pepsin values.

SECRETAGOGUE ACTION OF FRACTIONS OF COD MUSCLE EXTRACT

The determination of the ability of the fractions of cod muscle extract to produce gastric secretion was carried out in the same way as for the crude extracts, with the exception that the adjustments of osmotic pressure were not made. The results are shown in Table VI.

It is seen that on the basis of volume of secretion produced the nitrogenous bases have 99% of the activity of the extract but 60% of the nitrogen, the histidine-arginine fraction has 47% of the activity but 8% of the nitrogen, and the lysine fraction 19% of the activity but 38% of the nitrogen.

A loss of about 30% of the activity occurs on treatment of the extract with silver. (This has also been found lately by Dr. Komarov in the fractionation of gastric juice.)

The pepsin is seen to be four times as great in the lysine fraction as in the histidine-arginine fraction, indicating a "choline-like" effect in the lysine fraction in contrast to the "histamine-like" effect in the histidine-arginine fraction. (Choline injections produce a juice very high in pepsin, while histamine injections produce a large volume of very acid juice, low in pepsin).

Experiments by Krimberg and Komarov (1928) have shown that there is very little activity in the lysine fraction from meat, and that the activity is present preponderantly in the histidine-arginine fraction. It was seen above that in the fractionation of cod muscle extracts, the value for nitrogen in the lysine fraction was very high, and that of the histidine-arginine fraction small in comparison to meat.

It is therefore believed that the high pepsin secretion produced by cod or haddock is due to the presence of the large lysine fraction, giving a choline-like response, while the preponderance of the effect due to the histidine-arginine fraction in meat produces a more histamine-like response, low in pepsin.

<u>CHEMICAL INVESTIGATION OF THE FRACTIONS OF COD MUSCLE</u>

A considerable amount of time was spent in separating crystalline substances from the lysine and histidine-arginine fractions of cod muscle. In meats the histidine-arginine fraction contains a large amount of carnosine. It is calculated by colorimetric determinations that carnosine constitutes 0.3% of the wet tissue. By isolation carnosine is present in meats in 0.15% to 0.2%. Histidine has been found by Engeland but this is strongly disputed. Arginine has been found but in very small amounts, e. g. 5.4 mg. from 2.5 kg. of meat, by Komarov (1929). Carnitine was first isolated from the lysine fraction of meats by Gulewitsch and Krimberg. Choline was found by Kutscher and later by Somordinzew. Many other substances belonging to the quarternary ammonium bases have been said to be present but are not definitely proved (e. g. Novain, oblitin, carnomuscarin, neurin, neosin and myokinin.)

In this work the procedure which usually leads surely and quickly to the isolation of carnitine from meat extract did not give any definite result in the case of cod. (The method for the isolation of carnitine in the form of the mercuric salt was elaborated by Krimberg, and was used in the present investigation). In this tissue, carnitine may be present in such small quantities as to make the usual means of isolation ineffective.

The method of Gulewitsch and his co-workers (see Hoppe-Seyler's Handbuch) for the isolation of carnosine from the histidine-arginine fraction of meats by fractional crystallization from dilute alcohol, gave no result in the case of cod. Carnosine must be practically absent from this tissue. This confirms the observations of Clifford (1921) and the determinations of imidazole group concentration in this paper (Table I).

At least three crystalline substances have been obtained from the lysine fraction of cod in the form of the mercuric salts, but the determination of the nature of these substances has been discontinued for lack of time.

In cod, therefore, we find substances which are present in high concentrations in meat, are present in low concentrations or are absent, and also we find that other substances are present in larger quantities in cod than in meat.

These experiments indicate once more that the composition of fish muscle is quite different from meat - different not only with regard to the partition of nitrogen but even with regard to the chemical nature of the substances present. A more extensive chemical and physiological study seems to be quite desirable.

SUMMARY

A chemical investigation of some of the non-protein nitrogenous constituents of fish muscle (haddock, cod, salmon and herring) has been made.

By silver-barytra fractionation of cod muscle extracts it is seen that in this respect, cod is very similar to haddock and different in many respects from meat muscle.

The fractions of cod muscle have been studied chemically and are seen to be decidly different from those of meat.

The power of the non-protein nitrogenous constituents of fish to produce gastric secretion varies, the order in decreasing magnitude being - herring, haddock and cod equal, and salmon. The secretion with meat extracts equals that of cod

and haddock. The secretion with fish is higher in pepsin and slightly lower in acidity than with meat extracts.

The secretagogue action of cod muscle has been related to the chemical composition of the muscle by fractionation. In this study the nitrogenous bases had 99% of the activity of the extract but 60% of the nitrogen, the histidine-arginine fraction had 47% of the activity but 8% of the nitrogen and the lysine fraction 19% of the activity but 38% of the nitrogen. The pepsin value through the lysine fraction was four times that produced by the histidine-arginine fraction.

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