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A Comparative Study of Vitamins in the Trunk Muscles of Fishes

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INTRODUCTION

The ability to move from place to place differs in the animal kingdom. In higher animals, evolution has resulted in increasingly efficient and specialized organs of locomotion, like limbs in mammals and wings in birds, and the body itself often plays a very little, or no role, in movements. In fishes as lower animals, however, the motive force normally is concentrated in the trunk muscles and not in the fins. The importance of the fins is mainly as organs for manoeuvring, but with the exception of the caudal fin, they do not in most fishes promote straight swimming. When fishes swim the movement is due to strokes by the whole body and the tail. This side to side swinging of the body and sweeping motion of the tail are due to alternate contractions of the muscles situated on either side of the trunk of the fish. During the movements the whole trunk musculature is in action. (NORMAN 1931, CURTIS 1949, GRAY 1953).

The speed of swimming and endurance of action varies much from species to species. A consideration of the main factors promoting these qualities inevitably leads to the following conclusions: The speed can be increased by improved shape of the body and by fast and strong muscles. The endurance can be promoted by the staying power of the muscle, which in its turn varies with the structure and its supply of energy for continuous activity.

When a fish swims through the water it meets with a resistance mainly due to the friction between the water and the whole surface of its body. This friction is reduced for objects which are streamlined in shape. Nature has met this demand for several species of fish like tunny, mackerel and herring, which through evolution have developed fusiformed bodies which are excellently shaped for speedy movements through water. The perfection is so good in many species that the body has hollows in which the fins rest during straight, fast swimming, like in many sharks and the tunny. The shape thus being suitable, the limit of the ultimate speed will be decided by the power of the muscles in action.

The trunk muscles are striated muscles, and such muscles have long been recognized as the fastest. It is beyond the scope of this paper to discuss the anatomy and physiology of the muscles, although certain aspects will have to be reviewed. For a long time it has been recognized that the muscles of different animals may differ in colour, structure and physiological properties, and that even within the same animal there are differences in the colour amongst the muscles. Thus GÜNTHER (1880) points out that in bony fishes the development of the skeleton is paralleled by a corresponding development of the muscles. He gives a description of the lateral muscles as divided into a dorsal and a ventral half, "the depression in its middle is filled by an embryonal muscular substance which contains a large quantity of fat and blood vessels, and therefore differs from the ordinary muscle by its softer consistency and by its colour which is reddish or gray". STIRLING (1886) has described the anatomy and histology of the "red and pale muscle in fishes". His work will be referred to in more detail below. CHEVREL (1913) reviews the literature and discusses the morphology and physiology of the lateral muscles in fishes. He supposes that the task of the red muscle is to keep the tail in a bent position for a long time during steering.

The properties and physiology of the red and white muscles have been reviewed by NEEDHAM (1926) and HINES (1927), who both point out that the white muscle is faster than the red muscle, but becomes fatigued more rapidly. PROSSER (1952) in his comparative treatment of animal physiology also points out that in some fishes the striated muscle may be white or red, but that the colour is not so important as other characters. In white muscles the fibrils are close together and abundant, and the muscle cell nuclei are peripheral. In red muscles there is proportionally much more sarcoplasm containing myoglobin, and some of the muscle nuclei are central. DANOIS (1958) describes the red muscle in fish and reviews some of the literature related to the problem. Some references to single papers referred to in these reviews will be given in the discussion of the results of the present study.

Whatever the structure of the muscle, it has to be provided with energy to be able to function. SZENT-GYÖRGYI (1948) has given an account of the chemistry of muscular contraction. He emphasizes that the animal muscle has two sources of energy: fermentation and oxidation. MOMMAERTS (1950) and DUBUISON (1954) have described more in detail the possible processes taking place and the respiratory enzymes which are needed to maintain these metabolic processes. The respiratory enzymes have vitamins as cofactors in the form of coenzymes, thus the vitamins enter the picture. POTTER (1948) has discussed several aspects of the control of metabolism. He emphasizes that most studies show that

the enzymes present in the cells are normally used. In his review of aspects of enzyme environment he also points out that the cofactor concentration may be decisive in determining enzyme activity, and if the cofactor is dissociable, the activity is proportional to the amount of cofactor or vitamin within a limited range. If these assumptions are valid a relation between the occurrence of vitamins in the muscles and their activity may be expected. From the above considerations may further be expected a relation between the occurrence of vitamins in the skeletal muscles and the activity of the fishes.

The present study started out as an attempt to find such a correlation. Accidentally the total muscles regardless of red and white character were sampled, mixed and analysed at the beginning of the investigation. In the course of the study, however, several B-vitamins were determined in the ordinary as well as the red muscle of tunny (*Thunnus thynnus*) (BRÆKKAN 1955). The size of this fish prevented sampling of the total skeletal muscles. The results from analyses of the B-vitamins: niacin, riboflavin, pantothenic acid, vitamin B_{12} and thiamine, revealed an interesting relative distribution. With the exception of niacin, the red muscle contained several times more of the other B-vitamins than the ordinary muscle. These findings were followed up by investigations of other species. Samples from the ordinary as well as from the red lateral muscle (musculus lateralis superficiales = musculus rectus lateralis) of several teleosts were studied. The general findings have been reported briefly (BRÆKKAN 1956). The results focussed the attention on the red muscle in fishes in general. The livers of some species are very fat and have few blood vessels, in contrast to the well developed vascularization in livers of mammals. This prevents a rapid restoration of the energy-giving metabolites from the liver to the bloodstream as well as to the muscles. The possibility of the red muscle in fish being an organ developed to meet the demand for energy thus arises. The present paper gives an account of the present knowledge and discusses the possible function of the red muscle.

While this study was going on, Japanese workers have published several papers dealing with the biochemistry of the red muscle in fish. They also report vitamin investigations with results in general agreement with the present findings. These papers will be referred to and discussed below.

The present study thus presents two related investigations: Firstly, the relation between the activity of different species of fish and the average vitamin contents of the whole muscle has been studied. Secondly, the relation between the vitamin contents of the red and ordinary muscles has been studied in several species, and a proposal has been put forward as to the possible function of the red muscle in fish.

METHODS

Niacin was determined microbiologically with *Lactobacillus arabinosus*. Incubation was carried out for ca. 72 hrs. at 37°C, and the response measured by potentiometric titration of the lactic acid produced.

Pantothenic acid was determined microbiologically with *Lactobacillus arabinosus* as test-organism. The growth was measured turbidimetrically in a Beckman Model B Spectrophotometer at 660 mμ after 20 hrs. incubation at 30°C. The extraction of the vitamin was carried out by digestion of 1 g of the homogenized sample with 1 ml of a suspension of 20 mg takadiastase + 20 mg papain in 8 ml 0.2 N sodium acetate buffer of pH 4.5. The mixture was layered with toluene. The flask plugged with cotton, and incubated ca. 20 hrs. at 37°C. At the end of this time the digest was steamed for 10 min. cooled, neutralized to pH 6.8, made-up to volume, and filtered through a fluted filter. The clear filtrate was used after suitable dilution for the assay response. The enzymes were comparatively free from pantothenic acid, thus a blank could be omitted.

Riboflavin was determined microbiologically by the acidimetric method using *Lactobacillus casei* as test-organism. Extraction as described for pantothenic acid, incubation and response measurements as described for niacin.

The methods used for niacin, pantothenic acid, and riboflavin are essentially as described in "Methods of Vitamin Assay" (1951). The introduction of the incubation temperature of 30°C for pantothenic acid has been found to improve this assay (BRÆKKAN, to be published).

Vitamin B_{12} was determined by its growth promoting activity on *Lactobacillus leichmannii*, using the method described by THOMPSON, DIETRICH & ELVEHJEM (1950). The incubation was carried out for 20—22 hrs. at 37°C, and the response measured turbidimetrically.

The extraction was carried out by autoclaving of 1 g homogenized sample with 50 m. sodium acetate buffer of pH 4.5 + 5 ml 1% KCN-solution for 15 min. at 15 lbs. pressure.

Thiamin was determined microbiologically with *Lactobacillus fermenti 36* as test-organism, using essentially the method described by SARRET & CHELDELIN (1944).

The extraction was carried out as follows: 1 g sample was steamed for 30 min. with 25 ml 0.1 N sulfuric acid. After cooling the pH was adjusted to 4.5 with 2.5 N sodium acetate buffer. 20 mg papain + 20 mg taka-diastase were added and digestion carried out under toluene in a cotton stoppered flask for ca. 20 hrs. at 37°C. The flask was then steamed for 30 min. the sample made up to volume and filtered. Suitable aliquots were used for the determination.

Moisture was determined by drying in an oven at 120°C until constant weight.

Protein was calculated based on Kjeldahl-N multiplied by the factor 6.25.

Fat was determined by ether-extraction of the dried samples in Soxhlet apparatus and calculated in relation to the wet samples.

Ash was determined by ignition of the dried sample, first carefully over a gas burner, then in an oven at 550°C until constant weight was obtained.

THE RELATION BETWEEN THE B-VITAMIN CONTENTS OF THE TRUNK MUSCLES AND THE ACTIVITY OF THE FISHES

Characteristics of the species investigated.

A study of the relation between the B-vitamin contents of the skeletal muscles of fish and their activity, raises as the first problem, the collection of samples from fishes which can be classified according to activity. From marine investigations we know much about the living habits of many of the fishes which can be caught off the coast of Norway, and it should be possible to collect samples of species suitable for the present study. We can, however, not freely pick the species we want, but must limit the investigation to species which are commercially available, as only these fishes can be obtained in number and quality to make representative samples. Fortunately, the commercial fisheries normally comprise a selection of species of such a variety as to make a classification according to activity possible.

In Table 1 is given a list of the species investigated in the present study, with a description of living and feeding habits. Flatfishes have been put up in a separate group as have the freshwater species. The pelagic species are the easiest to classify, as their movements and living habits are rather much studied both with regard to speed and distance. The species tunny, mackerel and herring should therefore be correctly classified, and coalfish as a fairly active pelagic and bottom fish should fit in next. The pollack as a close relative of the coalfish has been put up next, followed by the cod which we know lives as a bottom and pelagic fish. The remaining species in the first section of Table 1 are difficult to classify, as we do not know enough of their living habits. They have, however, been included in the present study as the vitamin concentrations may give further information as to activity and food habits.

The flatfishes are put in a separate group, as the special anatomy and way of life makes it difficult to classify their activity in relation to the above species. With the exception of the halibut which lives on the banks, the classification of the remaining species is casual. Their typical bottom life, partly in muddy water and even partly in fresh water for the flounder,

Table 1. *Living and feeding habits of the species studied.*

Species	Living	Feeding
Tunny (*Thunnus thynnus*)	Pelagic	Herring, mackerel, sprat and other fishes
Mackerel (*Scomber scombrus*).....	—	Plankton, fry and small fishes
Herring (*Clupea harengus*)	—	Plankton, especially Calanus. Krill
Coalfish (*Gadus virens*)	Pelagic (and bottom)	Herring, sprat, fry, Calanus and krill
Pollack (*Gadus pollachius*)	—	Herring, sprat, fry and krill
Cod (*Gadus morrhua*)	Bottom and pelagic	Caplin, bottom invertebrates, small herring, krill and Crustacea
Haddock (*Gadus aeglefinus*)	Mainly bottom	Bottom invertebrates. Roe of fishes. Sand eel
Ling (*Molva molva*)	Bottom (200—500 m)	Little known. Apparently a predacious fish. Probably bottom invertebrates
Torsk (*Brosmius brosme*)	—	—«—
Catfish (*Annarrhichas minor*)	Bottom (100—300 m)	Mussels and snails. Echinoderms and crabs
Redfish (*Sebastes marinus*)	Near bottom (100—300 m)	Krill and prawns. Herring
Halibut (*Hippoglossus vulgaris*) ..	On banks	A predacious fish. All other fishes of reasonable size
Plaice (*Pleuronectes platessa*)	Shallow water. Muddy bottom	Bottom invertebrates. Mainly mussels
Flounder (*Pleuronectes flesus*)	Shallow water. River basin. Lakes	Bottom invertebrates. Mainly mussels. Insect larvae
Lemon sole (*P. microcephalus*) ...	Shallow water. Sandy and stony bottom	Bottom invertebrates. Brittle stars
Salmon (*Salmo salar*)	Pelagic and river	Small fishes, larvae from crustacea, eel and insects
Sea trout (*Salmo trutta*).....	Coast and river	Insects and larvae. Crustacea

may cause other factors also to influence the vitamin levels of their skeletal muscles.

Thus the flatfishes do not in mature state, possess a functional swim bladder as hydrostatic organ and can only keep above the sea bottom by muscular activity.

The fresh water species have finally been put in a separate group. Actually, there are only two species represented, the salmon (*Salmo salar*) and the trout (*Salmo trutta*). But as the latter occurs in very typical differentiated groups with regard to living places and food habits, it has been chosen to report it in three groups: sea trout, brook trout from the lowland, and brook trout from the mountains. These species may be placed high on a general list of classification according to activity. Especially the salmon, may be placed on line with fairly fast pelagic species.

The general data in Table 1 are compiled from several sources and has been confirmed by discussions with marine biologists, familiar with the food habits of the different species, thus detailed discussions and references have been omitted.

Results and discussions.

A comparison on the vitamin contents in the muscles from different species, in order to find a relation to their activity, would be of little value if the vitamin contents showed a marked relation to the size of the fishes within each species. Results from previous investigations do not indicate any such relation (BRÆKKAN, 1955, 1958a), although it may be pointed out that fry have not been included in any of the studies. As a correct assumption on this point is important for the validity of any conclusions to be drawn from the present study, an additional investigation of this relation has been carried out.

Single samples of the total skeletal muscles of 19 cods obtained alive at the fish market, were analysed on all four B-vitamins included in this study as well as their protein contents (N $\times$ 6.25). The fishes were chosen of different size, and their total weight varied over the range from 0.5 to 7.7 kg. The results are reported in Table 2 which gives single values, min.-max., mean and standard error of the mean for the different vitamins. No relation could be observed between the weight of the fish and the protein, niacin, pantothenic acid, riboflavin or vitamin B_{12}-contents of the skeletal muscles. This is in agreement with the findings of YANASE (1956) for vitamin B_6. He investigated two to four samples from different species and found very small individual differences for the vitamin B_6-content among the same species, regardless of the size of the fish. The results of the present study, however, deserve some further comments.

Table 2. *The vitamin contents of the cod muscles in relation to the weight of the fish.*

Fish No.	Total weight	Protein %	Niacin (μg/g)		Pantothenic acid (μg/g)		Riboflavin (μg/g)		Vitamin B_{12} (μg/g)	
			Fresh weight	Protein	Fresh weight	Protein	Fresh weight	Protein	Fresh weight	Protein
19	0.48	18.0	20.0	111	2.0	11.1	1.2	6.7	0.007	0.039
18	0.55	18.6	30.1	161	2.2	11.8	1.2	6.5	0.013	0.070
17	0.56	18.4	27.2	148	1.4	7.6	1.1	6.0	0.012	0.065
15	0.86	17.9	20.1	112	1.7	9.5	1.0	5.6	0.011	0.061
16	0.87	18.2	22.6	124	1.7	9.4	0.9	4.9	—	—
14	0.91	18.3	27.7	150	1.4	7.7	0.6	3.3	0.011	0.060
13	1.20	17.3	17.4	98	2.1	12.1	0.8	4.5	0.009	0.052
1	1.51	18.1	18.8	104	1.9	10.5	1.1	5.5	0.011	0.061
12	1.61	18.2	21.7	119	1.4	7.7	0.8	4.4	0.011	0.060
11	1.72	19.1	29.8	156	1.1	5.8	0.6	3.1	0.011	0.059
10	2.07	18.9	20.6	109	1.6	8.5	0.5	2.6	0.012	0.063
8	2.52	17.9	27.5	153	2.4	13.4	0.7	3.9	0.013	0.073
9	2.78	18.5	21.2	114	1.5	8.2	0.9	4.9	0.013	0.070
7	3.14	19.7	24.3	123	1.6	8.1	0.7	3.6	0.012	0.061
6	3.64	18.2	22.2	122	1.3	7.2	0.6	3.3	0.011	0.060
5	3.86	18.4	21.0	114	2.0	10.8	0.8	4.3	0.014	0.076
4	4.16	18.9	21.9	116	1.7	9.0	0.9	4.8	0.009	0.048
3	6.25	17.5	17.1	98	1.3	7.5	0.5	2.8	0.013	0.074
2	7.69	17.7	21.1	119	2.5	14.1	1.2	6.8	0.011	0.062
Min.-max.		17.3—19.7	17.1—30.1	98—164	1.1—2.5	5.8—14.1	0.5—1.2	2.6—6.8	0.007—0.014	0.039—0.076
Mean ±S		18.3 ±0.132	22.8 ±0.723	124 ±4.54	1.73 ±0.090	9.47 ±0.518	0.85 ±0.054	4.61 ±0.302	0.011 ±0.00038	0.062 ±0.0022

S = Standard error of the mean.

It may be noted that the protein-contents in single fishes in the present study show a very small total variation, between 17.5—19.7%. This result agrees with the finding of BRÆKKAN (1958a), who reported 17.7—19% protein for five representative samples from cod. NOTEVARP (1949) reported variations between 15—19% for cod-fillets from Lofoten. The values for the different vitamins also show very small variations, and the standard error of the mean did not exceed 6.6% when the contents were calculated on the basis of the protein. This variation may be taken as an indication that the vitamins are accumulated in the muscles fairly directly in relation to the metabolic need. The results also give other important information, as they show that it is reasonable to believe that even single analyses of representative samples from different species may give values which can be used in a comparative study as the present one.

Altogether 15 species were studied with regard to their relative contents of the B-vitamins niacin, pantothenic acid, riboflavin and vitamin B_{12}. Each vitamin has been reported separately in Tables 3—6, which give the values for the vitamin content per g fresh weight as well as per g protein. Usuallsy three or more samples were analyzed.

The size of the tunny caught off the coast of Norway varies throughout the season, from an average weight of 170—190 kg in July/August to 70—90 kg in September, when the shoals of smaller fish arrive (BRÆKKAN 1955). For the tunny it would thus be impracticable to homogenize the whole skeletal muscles, as for economical reasons representative samples of this kind could not be obtained. The values for this species are calculated from the results found for the ordinary and red muscle. An approximate proportion of ordinary muscle: red muscle = 8:1 was obtained by averaging the relations in several transverse sections of the trunk.

Niacin is reported in Table 3. This vitamin is discussed first for the following reasons. From the results reported for the relative distribution of the different vitamins between the red and ordinary muscles (BRÆKKAN 1956), it seems that niacin is present in fairly equal concentrations in both types of muscle. Thus any error introduced by insufficient sampling as to the total red and ordinary muscles, as well as insufficient blending of the samples, should influence less on the reliability of the values for niacin than for the other B-factors investigated. Further, niacin plays an important and dominating role in muscle metabolism. As nicotinamide it forms part of the coenzymes DPN (diphospopyridine nucleotide) and TPN (triphosphopyridine nucleotide) (HUNDLEY 1954). These cofactors are phosthetic groups of important dehydrogenases which catalyze the reactions involved in synthesis of high-energy phosphate bound, glycolysis and pyruvate metabolism (SCHLENCK 1951). In the skeletal muscle, niacin is found almost entirely in its bound form as DPN and TPN (HANDLER &

Table 3. *The niacin content of the skeletal muscles from different species of fish.*

Species	µg/g fresh weight		µg/g protein	
	min.-max.	Ave.	min.-max.	Ave.
Tunny (*Thunnus thynnus*)		107		440
Mackerel (*Scomber scombrus*)	62—63	63	340—354	345
Herring (*Clupea harengus*)	28—63	50	159—360	264
Coalfish (*Gadus virens*)	31—44	34	158—215	176
Pollack (*Gadus pollachius*)	16—20	19	86—105	97
Cod (*Gadus morrhua*)	18—19	18	86—136	107
Haddock (*Gadus aeglefinus*)	36—44	40	195—212	202
Ling (*Molva molva*)	21—25	23	113—126	120
Torsk (*Brosmius brosme*)	27—28	28	134—141	138
Catfish (*Anarrhichas lupus*)	18—25	21	100—140	120
Redfish (*Sebastes marinus*)		20		109
Halibut (*Hippoglossus vulgaris*)		44		244
Plaice (*Pleuronectes platessa*)	32—28	35	190—208	201
Flounder (*P. flesus*)		24		140
Lemon sole (*P. microcephalus*)	26—43	34	145—240	195
Salmon (*Salmo salar*)		88		434
Sea-trout (*Salmo trutta*)		56		260
Brook-trout (*S. trutta*)		45		223
Brook-trout (*S. trutta*)*		56		270

* From mountain lakes and rivers.

DAM 1941). Studies with radioactive niacin show that the uptake by various tissues varies. Even if the fixation in the muscle was lower than in most tissues, and the excretion half time was among the longest (8 days), the results show that coenzymes are broken down and renewed at a fairly rapid rate (ROTH, LEIFER, HOGNESS & LANGHAM, 1948). Our present knowledge thus establishes that niacin is usually present in the muscles in an active form directly involved in energy metabolism. The values in the muscle thus should provide an indicator of the activity or more correctly, the possible activity of the muscle. A very active fish with a high metabolic rate should show the highest niacin content in the skeletal muscle.

From the values reported in the first section of Table 3 it can be seen that the niacin contents generally agree with the expected relation. Tunny is possibly the most active and energetic fish of those studied, shows the highest content of niacin, 107 µg per g fresh weight or 440 µg per g protein. Next come the typical pelagic species, mackerel and herring with fairly high values. The gadidae, catfish and redfish show generally

very similar values, which should indicate fairly similar metabolic potentials with regard to reactions where niacin is involved. With the exception of haddock, which unexpectedly show a somewhat higher value than the other gadidae, the observed contents agree with the classification put up in Table 1. Our biological information does not point out the haddock as a very active fish, and the values observed for the other vitamins for this species indicate that an exceptional value for niacin has been found. The possibility that this high value may be caused by special feeding habits in the fishing grounds where these haddocks were caught cannot be ruled out. Thus BAKKEN & BRÆKKAN (1955) found higher values for niacin in herring caught in the fjords during the summer than for niacin in herring caught during the winter and spring, while the remaining B-factors showed only slight differences.

The flatfishes reported in the next section of Table 3 all show relatively high values compared with the gadidae. It is difficult to comment on these results, but it cannot be ruled out that the flat shape of this fish and the somewhat different movements during swimming may demand muscles with a fairly high metabolic potential.

If we finally consider the salmonidae reported in the last section of Table 3, it may be pointed out that the salmon show the second highest value for niacin in the skeletal muscles of all the species studied in the present investigation. 88 μg per g fresh weight or 434 μg per g protein. The salmon is a fairly large fish which inhabits the sea as well as fresh water. In the sea it hunts along the coast and in the ocean, deep-sea as well as pelagic. The three "types" of trout studied also show high values for niacin, indicating high metabolic activities and endurance. The swimming up waterfalls and streams puts extra metabolic demands on the muscles of these species.

Pantothenic acid is reported in Table 4. Pantothenic acid is usually not present in the free form in animal tissues, but mainly or entirely bound as coenzyme A (NOVELLI, KAPLAN & LIPPMANN 1949). Thus the values should bear direct relation to metabolic activities. Coenzyme A is involved in a variety of primary metabolic processes. The reactions as acetyl donor and acetyl acceptor enzyme systems are recognized (CHOU & LIPPMANN 1952). It is also involved in the carbohydrate metabolism, where pyruvate seems to undergo oxidative carboxylation to yield acetyl Co.A, which through reaction with oxalacetate yields citric acid directly (OCHOA 1954). But it is in fat metabolism that coenzyme A seems most heavily engaged. According to present knowledge the fatty-acid chains are broken down to 2-carbon units, which in turn combines with coenzyme A to form acetyl-coenzyme A, which in its turn enter the citric acid cycle. In this way fat is utilized in the energy

Table 4. *The pantothenic acid content of the skeletal muscles from different species of fish.*

Species	µg/g fresh weight		µg/g protein	
	min.-max.	Ave.	min.-max.	Ave.
Tunny (*Thunnus thynnus*)		8.6		35.4
Mackerel (*Scomber scombrus*)	9.7—10.9	10.3	48.5—61.1	54.8
Herring (*Clupea harengus*)	9.3— 9.7	9.5	56.4—66.0	61.2
Coalfish (*Gadus virens*)	3.6— 4.1	3.8	17.6—21.9	19.7
Pollack (*Gadus pollachius*)	2.5— 4.2	3.2	12.6—22.6	16.6
Cod (*Gadus morrhua*)	1.0— 3.1	1.8	5.2—16.9	11.3
Haddock (*Gadus aeglefinus*)	2.0— 2.9	2.5	10.8—15.0	12.9
Ling (*Molva molva*)	2.4— 3.6	3.2	12.9—18.8	16.6
Torsk (*Brosmius brosme*)	3.0— 3.1	3.1	14.3—16.3	15.3
Catfish (*Anarrhichas lupus*)	5.0— 6.4	5.7	27 —36	32.0
Redfish (*Sebastes marinus*)		3.6		19.7
Halibut (*Hippoglossus vulgaris*)		3.6		20.0
Plaice (*Pleuronectes platessa*)	8.8—11.3	10.1	59.0—62.1	59.7
Flounder (*P. flesus*)		11.0		62.7
Lemon sole (*P. microcephalus*)	2.5— 4.2	3.1	14.0—25.4	18.0
Salmon (*Salmo salar*)		20.8		100.7
Sea-trout (*Salmo trutta*)		19.5		90.7
Brook-trout (*S. trutta*)		15.9		79.1
Brook-trout (*S. trutta*)*		23.3		115.9

* From mountain lakes and rivers.

metabolism (BALDWIN 1957). The values for pantothenic acid thus may not always show a direct relation to the activity of the fishes, but may indicate that fat metabolism is involved. In the present study the values found are generally in line with the expected relation.

In the first group the tunny shows a fairly high value, 8.6 µg pantothenic acid per g fresh weight or 35.4 µg per g protein. But contrary to the finding for niacin, mackerel and herring show higher values for pantothenic acid than the tunny, ca. 10 µg per g fresh weight or ca. 60 µg per g protein. The values indicate that fat is the dominating source of energy in these pelagic species, an assumption which is further supported by their usually rather high contents of depot fat. The rest of this group show the expected relation between the pantothenic acid content and activity, with the exception of the catfish. The results for the flatfishes show that plaice and flounder contain more pantothenic acid in the skeletal muscles than halibut and lemon sole, respectively ca. 10 µg per g fresh weight or ca. 60 µg per g protein and ca. 3.5 µg per g fresh weight

Table 5. *The riboflavin content of the skeletal muscles from different species of fish.*

Species	μg/g fresh weight		μg/g protein	
	min.-max.	Ave.	min.-max.	Ave.
Tunny (*Thunnus thynnus*)		2.5		10.2
Mackerel (*Scomber scombrus*)	3.5—3.6	3.6	18.0—18.2	18.1
Herring (*Clupea harengus*)	2.7—3.4	3.1	15.5—23.1	17.4
Coalfish (*Gadus virens*)	1.4—3.0	2.0	6.9—16.8	10.6
Pollack (*Gadus pollachius*)	0.9—1.3	1.0	4.5— 7.0	5.4
Cod (*Gadus morrhua*)	0.7—1.3	1.1	3.7— 7.7	5.5
Haddock (*Gadus aeglefinus*)	0.8—1.6	1.1	3.9— 8.0	5.8
Ling (*Molva molva*)	0.6—1.0	0.8	3.2— 5.2	4.2
Torsk (*Brosmius brosme*)	1.0—2.0	1.5	5.2— 9.6	7.4
Catfish (*Anarrhichas lupus*)	0.7—0.9	0.8	4.0— 4.9	4.5
Redfish (*Sebastes marinus*)		1.1		5.5
Halibut (*Hippoglossus vulgaris*)		1.0		6.8
Plaice (*Pleuronectes platessa*)	0.9—1.1	1.0	5.0— 6.6	5.8
Flounder (*P. flesus*)		1.1		6.4
Lemon sole (P. *microcephalus*)	0.7—0.9	0.8	4.5— 5.0	4.8
Salmon (*Salmo salar*)		2.2		13.7
Sea-trout (*Salmo trutta*)		1.9		8.8
Brook-trout (*Salmo trutta*)		1.7		8.4
Brook-trout (*Salmo trutta*)*		2.7		13.1

* From mountain lakes and rivers.

or ca. 20 μg per g protein. This relation is rather interesting as of the four species, the former two are living on more muddy bottom, and the latter on banks and sandy and stony bottom.

The salmonidae reported in the last section of Table 4, show comparatively very high values for pantothenic acid, on the average ca. 20 μg per g fresh weight or ca. 100 μg per g protein. These results show that fat apparently plays a dominating role in their energy metabolism, and generally the values support the expected high activity of these species which was expressed in the results for niacin.

The results for riboflavin are reported in Table 5. Riboflavin is active as part of the coenzymes flavin mononucleotide (riboflavin- 5-phosphate) and flavin adenine dinucleotide, which are the prostetic groups in a series of flavoproteins. Either of the coenzymes are bound to specific proteins (apoenzymes), and this combination is at least for riboflavin-5-phosphate reversible in a stoechiometric manner (Theorell 1934, 1935), and thus the level of the vitamin may be expected in relation to the enzymatic activity (Potter 1948). Riboflavin-5-phosphate as part of the enzyme

Table 6. *The vitamin B_{12} content of the skeletal muscles from different species of fish.*

Species	µg/g fresh weight		µg/g protein	
	min.-max.	Ave.	min.-max.	Ave.
Tunny (*Thunnus thynnus*) ...		0.100		0.410
Mackerel (*Scomber scombrus*) .	0.100—0.130	0.120	0.500—0.679	0.589
Herring (*Clupea harengus*) ...	0.120—0.150	0.140	0.815—0.915	0.715
Coalfish (*Gadus virens*)	0.022—0.050	0.035	0.112—0.247	0.175
Pollack (*Gadus pollachius*) ...	0.008—0.022	0.011	0.040—0.118	0.077
Cod (*Gadus morrhua*)	0.004—0.011	0.008	0.021—0.060	0.046
Haddock (*Gadus aeglefinus*) ..	0.014—0.022	0.018	0.070—0.108	0.095
Ling (*Molva molva*)	0.004—0.008	0.006	0.020—0.040	0.029
Torsk (*Brosmius brosme*).....	0.011—0.012	0.012	0.057—0.058	0.058
Catfish (*Anarrhichas lupus*) ...	0.018—0.020	0.023	0.099—0.164	0.131
Redfish (*Sebastes marinus*) ...		0.010		0.054
Halibut (*Hippoglossus vulgaris*)		0.009		0.050
Plaice (*Pleuronectes platessa*)...	0.009—0.011	0.010	0.059—0.061	0.060
Flounder (*P. flesus*)		0.010		0.059
Lemon sole (*P. microcephalus*).	0.007—0.010	0.008	0.034—0.060	0.046
Salmon (*Salmo salar*).....		0.040		0.186
Sea-trout (*Salmo trutta*)		0.040		0.186
Brook-trout (*Salmo trutta*) ...		0.044		0.213
Brook-trout (*Salmo trutta*)* ..		0.060		0.290

* From mountain lakes and rivers.

cytochrome c reductase and flavin adenin nucleotide as flavoprotein dehydrogenase are involved in oxidation-reduction processes in carbohydrate metabolism, but maybe of greater interest, is the part the latter plays in the protein synthesis as different amino acid oxidases (BALDWIN 1957).

The values reported for riboflavin are very much in agreement with the expected relation between the different species. The pelagic species tunny, mackerel and herring show the highest contents, then follows coalfish, while the remaining species in the first group show fairly constant but lower values.

The flatfishes show relatively low values for all the investigated species, while the salmonidae, as expected, give values of the same order as for herring and coalfish.

Vitamin B_{12} is reported in Table 6. We know that this vitamin is essential for survival, especially of the very young, and for the normal growth. It seems involved in metabolic reactions connected with methyl group synthesis, reduction of disulphide to sulphydrol groups, and protein

synthesis in animals of various species (ARNSTEIN 1958). A speculation as to the effect of these functions of vitamin B_{12} in the skeletal muscle, and thus a relation to the activity of the fishes will not be presented. Vitamin B_{12} seems, however, very much connected with marine ecology (DROOP & DAISLEY 1957, ADAIR & VISHNIAC 1958, BRÆKKAN 1958b) and it was therefore considered of interest to include this vitamin in the present study.

The results show that the vitamin B_{12} content in the skeletal muscles of the different species investigated is generally related to the activity of the fishes. Tunny, mackerel and herring show the highest contents in the first group, with coalfish next and the remaining species showing generally lower contents with the exception of the catfish. This species also showed a deviating value for pantothenic acid, and for both vitamins the contents were somewhat higher than for the rest of the bottom fishes. The flatfishes show fairly consistent values, on an average 0.05 μg per g protein. This result seen in relation to the vitamin B_{12}-values for muscles of cod of different weight (Table 2) deserves some further comments. The liver of small cod living in the sea-weed area along the coast, as well as from some flatfishes, often contain extra higher contents of vitamin B_{12} (BRÆKKAN 1958a 1958b). This is probably a result of relatively high vitamin B_{12} supply from the food as well as possibly from the sea water in these waters. The present results indicate that the vitamin B_{12} levels of the muscle of fish are relatively independent of the supply through the food, and for each species probably related to the metabolic need of the muscle cells. If we finally consider the salmonidae, the muscle of these species show fairly high values in agreement with their general activity.

In general, there seem to be a significant relation between the vitamin contents of the skeletal muscle of the fishes investigated and their activity. The results, however, indicate that this relation is mainly pronounced and correctly expressed when species of the same types with regard to external anatomy are compared. Factors besides the activity of swimming may be of importance, thus basal metabolism may influence the vitamin levels. As to the energy required during swimming, this will naturally vary considerably with the speed, and shoals and individuals of the same species may under circumstances show differences in their vitamin contents from this habitual reason. SUNDNES (1956) has calculated the relation between the energy consumption and the speed of a cod from results of model experiments. Over a distance of 50 km a cod weighing 5.5 kg should consume ca. 1 k.cal. when swimming with a speed of 1 m per sec., and ca. 20 k.cal. over the same distance when the speed is 5 m. per sec. We do know that some of the migratory pelagic species do swim for often many days with rather high speed, and thus consume a considerable lot of energy. Most fishes, however, only now and then swim fast

for a few seconds hunting or chased, but mostly the speed is moderate. The present results from the vitamin contents in the skeletal muscles of cod of different size indicate that the individual variation in activity is more evened out than may be expected. It shall, however, not be ruled out that this uniformity results from some days' stay in well-boats and storage nets.

As to the vitamin supply in fishes, this is apparently mostly or entirely derived from the food consumed. Contrary to mammals and birds, the fishes do not seem to have a regular intestinal microflora which supply additional vitamins (KLINGLER 1958). Thus in haddock, MARGOLIS (1953) found that the microflora had entirely disappeared from the digestive tract during fasting. It may also be pointed out that antibiotics added to the food of young fishes do not promote the growth, contrary to the finding in birds and mammals. Thus supplemental feeding of auromycin to guppies (*Lebistes reticulatus*) even caused a marked inhibition of somatic development and growth (BJERKE, SILVER & KUPPERMAN 1953). There is apparently no intestinal flora which can be "regulated" and give an improved digestion. YANASE (1955), however, found a higher vitamin B_{12} content in the intestinal content of some fishes than in their gastric content, and suggested that synthesizing bacteria are present in the intestinal tract of these species. For other species investigated such relations could not be observed. Comparative investigations at this laboratory indicate a possible synthesis of vitamins in the polyric cæca, but further studies are needed before any conclusion can be drawn.

It may further be noted that the migratory pelagic species which show the highest vitamin values generally are fat or semifat fishes, and it seems that fat is their most important source for energy. YANASE (1956) found that in general, meat of pelagic and migratory fishes contained far greater amounts of vitamin B_6 than bottom fishes. The present study shows that this is generally true also for the vitamins niacin, pantothenic acid, riboflavin and vitamin B_{12}.

THE RELATION BETWEEN THE B-VITAMIN CONTENTS OF THE RED AND ORDINARY MUSCLE IN FISH. THE POSSIBLE FUNCTION OF THE RED MUSCLE

Description of the skeletal muscles of the species investigated.

The present study of vitamins in the red and ordinary muscles of fish comprise an investigation of the species tunny (*Thunnus thynnus*), mackerel (*Scomber scombrus*), herring (*Clupea harengus*), coalfish (*Gadus virens*), salmon (*Salmo salar*), halibut (*Hippoglossus vulgaris*) and the sharks porbeagle (*Lamna cornubica*) and mako (*Isurus Oxyrhynchus*). The living habits and food of the teleosts are described in Table 1. Our knowledge of the diet of the porbeagle is fairly good, but very little is known of the diet of the mako. Borh species pray on shoals of scombroids, clupeids and other small fishes, which they catch in great quantities. But also larger fishes are caught by these predacious sharks, thus coalfish is frequently found in the porbeagle.

As the terms "red and ordinary" muscles have been used in this publication, and not the terms "red and white" more commonly used in literature on muscles, the following reason is given in explanation. The different types of muscles in fish have been termed "red and pale". (RANVIER 1874 STIRLING 1886). The terms most frequently applied in descriptions of muscles in general are "red and white", NEEDHAM 1926 and HINES 1927). These papers and reviews, however, mainly deal with mammalian and avian muscles, where the different muscle fibres normally are mixed and not confined to separate muscles of special function. Most of the work with the different fish muscles has been done by Japanese workers. They have applied somewhat different terms as: "red and white muscle" (SAITO 1954), "red and white meat" (FUKUDA and HIGUCHI 1954), and "red and ordinary muscle" (MATSUURA and HASHIMOTO 1954). In later papers the term "ordinary" gains further application, although still different descriptions are given as: "bloody and ordinary muscles" (ENDO and SEMIDU, 1955) and "dark meat and ordinary meat" (FUKUDA 1958). The colours of the muscles of the different species of fish actually vary considerably. Thus, in tunny the muscles are dark red and red, in salmon brownish red and orange-pink and in most species reddish

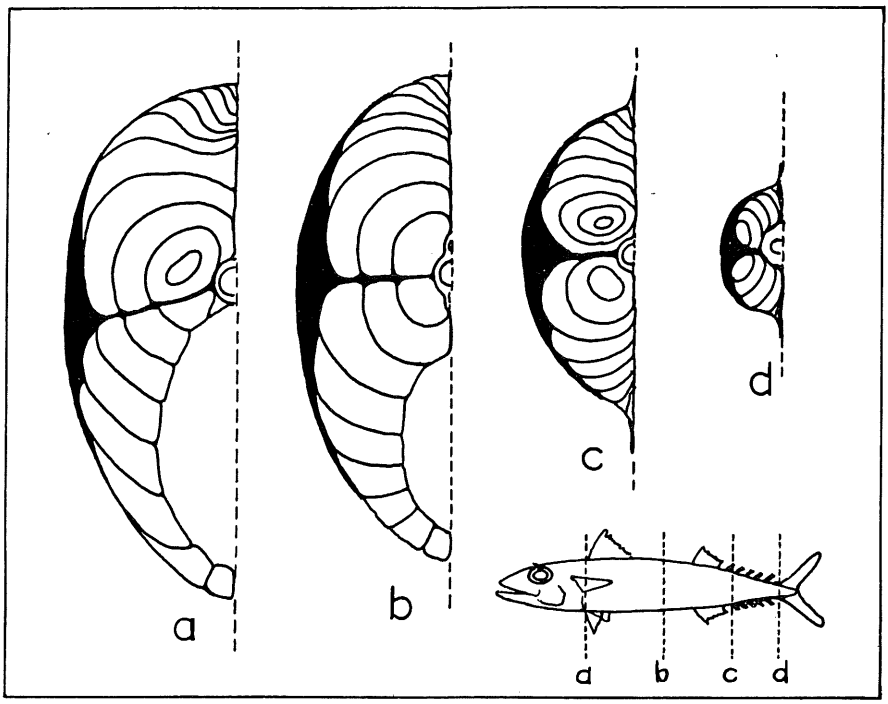

Fig. 1. Mackerel (*Scomber scombrus*). Transversal sections through the trunk showing the layout and relative distribution of the red and ordinary muscles.

or brown-greyish and pale white. As the different types of muscles in fish are occurring clearly in separate tissues, with the striated paler muscle most common, "red and ordinary muscles" is in the authors opinion, the best term in work dealing with their biochemistry.

The anatomy of the trunk musculature in fishes been has generally described by ROMER (1950). It forms much of the bulk of the body, dorsally extending from the vertebrae on either side upward and outward to the skin, ventrally an enveloping sheath around the body cavity. The trunk musculature is of direct myotomic origin, and shows a segmental arrangement. Successive vertical segments — the myomeres — run along each flank, corresponding in number to the vertebrae. The muscle fibres are oriented in an anteroposterior position in each myomere. The myomeres are separated by septa of connective tissue, the myocomata, into which the muscle is bound. The myocomata reach from the skin to the vertebrae. When the fish is boiled the connective tissue will dissolve and the myomeres can be easily separated one from another. They will usually be found to pursue in a complicated zig-zag pattern. This folding seems to promote muscular efficiency, and is especially prominent in

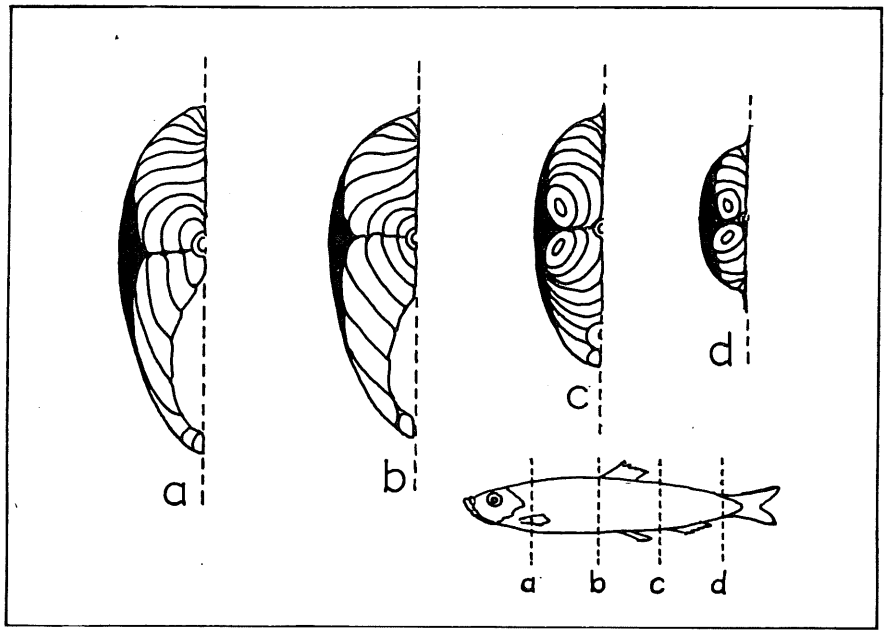

Fig. 2. Herring (*Clupea harengus*). Transversal sections through the trunk showing the layout and relative distribution of the red and ordinary muscles.

sharks. Seen on the surface, each myomere is usually in the form of a W with the upper edge turned forward. In fishes above the cyclostome level there develops a horizontal septum of connective dividing every myomere into dorsal and ventral portions just below the tip of the anteriorpointing V. The trunk muscles of these species thus are divided into two major groups, the dorsal or apaxial musculature and the ventral or hypaxial musculature of the flanks and belly.

The occurrence of the red muscle in fish has been described by several workers (STIRLING 1886, CHEVREL 1913, IHLE, VAN KAMPEN, NIERSTRASZ & VERSLÜYS 1927 and DANOIS 1958). Normally the red muscle is situated under the skin along the horizontal septum, and it is divided in segments corresponding with the myomeres in the ordinary muscle. The horizontal septum also divides the red muscle into two. In some fishes deep-seated red muscles have developed. As the occurrence of the red muscle in the species investigated is an important factor in the discussion of the present findings, it has been found convenient to give a description of the relative distribution in the different species in the following paragraphs.

Mackerel. When a mackerel is cut in transversal sections, the strongly marked red muscle can clearly be seen. It has a deep colour and is highly developed. In Fig. 1 is shown four transversal sections, to illustrate the

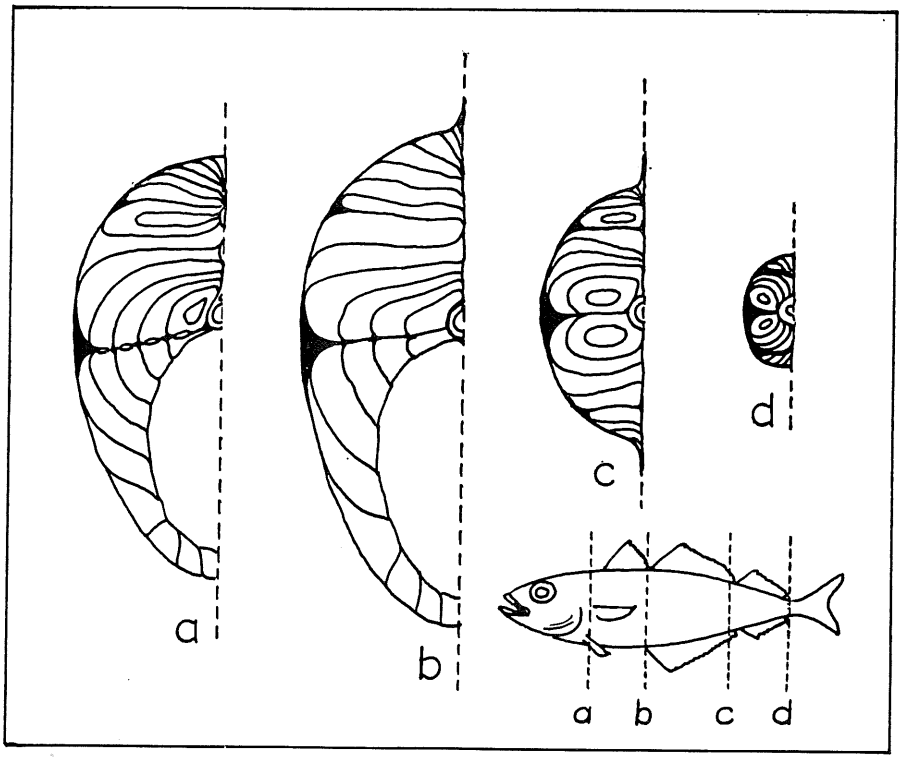

Fig. 3. Coalfish (*Gadus virens*). Transversal sections through the trunk showing the layout and relative distribution of the red and ordinary muscles.

extension of the red muscle along the body of the fish. It can be seen how it is thickest in the interval between the dorsal and ventral lateral muscles and tapers away dorsally and ventrally. It may also be observed that a horizontal prolongation of the red muscular substance is attached to the vertebral column.

Herring. In Fig. 2 is shown four transversal sections of a herring. The red muscle in the herring is, as in mackerel, covering continuously most of both sides. It has a reddish-brown colour and shows in general the same extension as in mackerel. A thin slip, which cannot be clearly drawn on the figures without spoiling the proportions, passes inward horizontally to be attached to the vertebral column.

Coalfish. In Fig. 3 is shown four transversal sections of a coalfish. A similar arrangement of the red muscle as in coalfish exists in most gadidae and several other closely related fishes. In the transversal sections can be noted how the red muscular tissue is thickest under the position of the lateral line and apparently between separate muscle bundles. In a

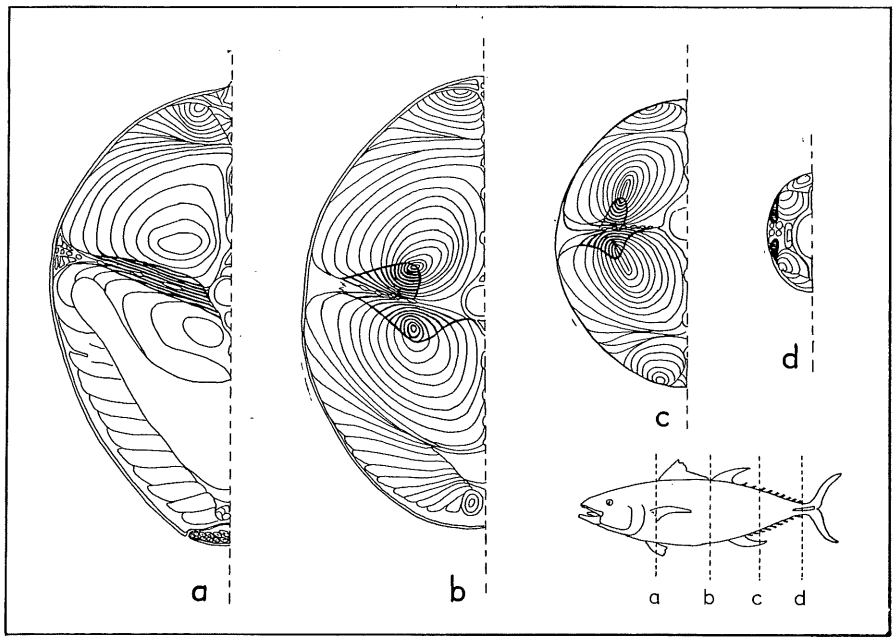

Fig. 4. Tunny (*Thunnus thynnus*). Transversal sections through the trunk showing the layout and relative distribution of the red and ordinary muscles.

way it coats the ordinary muscles. Another very important aspect of the appearance of the red muscle in this species is that is does not form quite so continuous a layer as in the herring and mackerel. It is thickest in the centre of each myotome and gets much thinner opposite the septa. If the fish is boiled the typical appearance of zig-zag stripes can be observed along each myotome or muscle segment.

Salmon. Salmon shows generally the same anatomical picture as coal-fish, but the red muscle is brownish red and ordinary muscle is orange-pink in colour.

Halibut and other flat fishes have very developed red muscles. They are generally paler in colour, but can be clearly seen when the fish is boiled and the skin removed. Also in these species it follows the zig-zag pattern of the myotomes. In the fin rays can also be observed a red muscle lying along the white one.

In all the species described the red muscles pass into the rays of the tail fin.

Tunny. In the tunny the red muscles have an arrangement which differ from the species described above. In Fig. 4 is shown four transversal sections through a tunny. These slices of the fish were cut out at the indicated transverse sections *a* — *d*, and the surfaces of these slices were

planed with a sharp gutting-knife. By means of transparent paper the layout of the muscle segments were copied minutely, thus detailed pictures of the muscular arrangement were obtained. It may be noted that the red muscles in section *a* just behind the gills at the side fins form horizontal layers which reach inwards to the vertebral column. It may further be noted in section *b* and *c* that the red muscles are of such extension as to border on the apieces of the myotomes of the main muscles. Finally, the red muscles diminish towards the tail, and run fairly superficially into the tail fins. When the frozen slices were thawed, the red muscles loosened from the ordinary muscle and could be picked out as if they constituted an organ, separate from the main muscular tissue.

Porbeagle. Also the shark porbeagle has a deepseated red muscle, and as in tunny, it can be fairly easily removed when frozen slices are thawed. By chance, a closely related shark the *mako* (*Isurus oxyrhynchus*) could be investigated. In the porbeagle the red muscles are situated close to the vertebra and the body-cavity and only extended partly along the trunk (Fig. 5), while in the mako the red muscles are extended all along the trunk and do not lie directly up to vertebra (Fig. 6). BRÆKKAN (1959) has proposed the usefulness of this character for indentification purposes in fishery research. From section *c* and *d* in Fig. 6 can further be noted that in the mako the myotomes are twice as many in the red as in the ordinary muscle along the main part of the skeletal muscles and show in these sections the similarity with the tunny, that the red muscles border on the apices of the "total" myotomes.

The histology of the red and ordinary muscles in fishes has not been studied comparatively to any great extent. STIRLING (1886) has given a general description of the histological structure of whiting, haddock and mackerel, and discussed some details of the differences between the cells in the red and ordinary muscles. As very few reports regarding these problems are found in the literature (DANOIS 1958), it was considered of value to carry out some additional investigations of the species in the present study. It may be pointed out that the aim of these studies has not been to give a detailed histological description of the muscles in fishes, but merely a general comparative investigation as related to the present problem. Unfortunately, fresh samples of tunny and porbeagle could not be obtained at the time of this investigation, thus the anatomical structure of deepseated red muscles could not be studied. Coalfish and cod from the lean fishes, and herring and mackerel from the fat fishes were chosen as representative samples of the other fishes. The sections were stained in hematoxylin eosin (H + E) for the general studies, with Sudan for localization of fat and with Schiff's reagent for the study of the distribution of mucopolysaccharides and glycogen. Transverse and longitudinal sec-

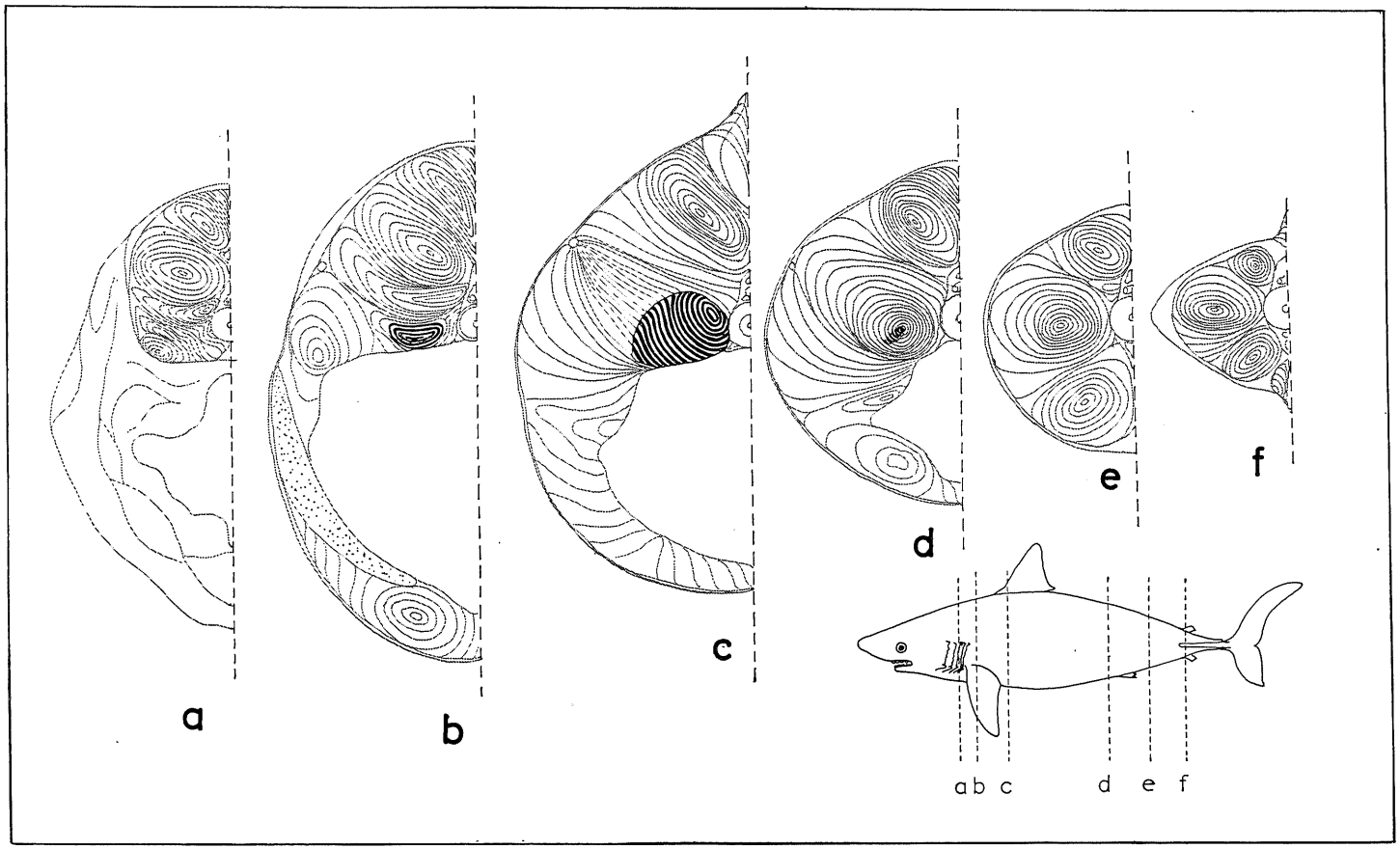

Fig. 5. Porbeagle (*Lamna cornubica*). Transversal sections through the trunk showing the layout and relative distribution of red and ordinary muscles.

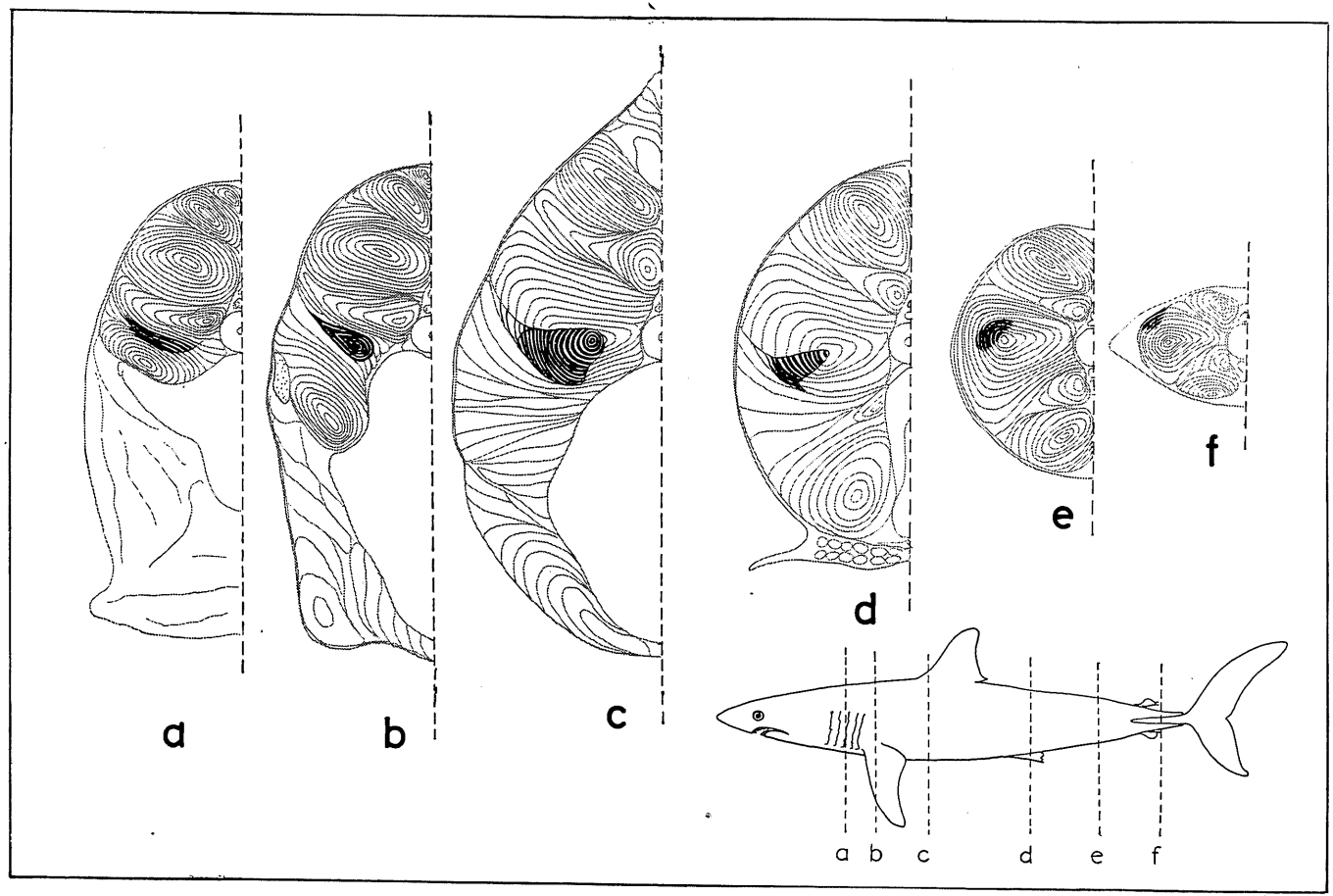

Fig. 6. Mako (*Isurus oxyrhynchus*). Transversal sections through the trunk showing the layout and relative distribution of the red and ordinary muscles.

tions were prepared. Although the details may show some variations, the following general pictures can be described for the lean fishes (coalfish and cod) and the fat fishes (herring and mackerel) respectively.

In coalfish (Pl. I, Fig. 1 and 2) and cod the borderline between the red and the ordinary muscles is clearly marked, but some "red" fibres can be observed in the ordinary muscle and a few "ordinary" fibres in the red muscle. In the ordinary muscle the striation is dense and the histological structure shows fibres usual for such musculature, while in the red muscle the striation is not so dense and as a whole looks more compact and granulated. With regard to fat, the ordinary muscle showed no sudanophilic reaction, while in the red muscle droplets of fat could be observed within the muscle cells between the fibres. The test for glycogen, however, was altogether negative.

In mackerel (Pl. II, Fig. 1 and 2) and herring (Pl. III, Fig. 1 and 2) the distinction between the red and the ordinary muscles is very clear, and they are separated by a connective tissue septum. The ordinary muscle shows a normal structure, but the size of the fibres vary considerably. The red muscle, however, shows a very compact structure with fibres of very uniform size arranged in bundles. The sarcoplasma is crowded with vacuoles. With regard to fat, both species show layers of depot fat just under the skin. In the ordinary muscle diffuse fat is observed between the muscle fibres, while distinct droplets can be observed within the fibres of the red muscle. The Sudan reaction is so intense as to make detailed observations difficult. The findings agree with the observations of GREENE (1913) in king salmon. Also in herring and mackerel the reaction for glycogen was negative.

Results and discussion.

The present study was instigated by the observation of the relation between the B-vitamins in the red and ordinary muscle in the tunny. These first results indicated a relative distribution and concentrations of the vitamins in the red muscle very similar to the values found for the liver. As this relation might give a clue as to the importance of the distribution between the two types of muscles and to the functional importance of the red muscle, the liver was included in the subsequent investigations of other species. Care has been taken to secure that the samples of red muscle, ordinary muscle and liver came from the same fishes. For the porbeagle and the halibut this could not be arranged, and the livers from these fishes are sampled separately. Analyses of the contents of water, protein, fat and ash were included as they might reveal relations of interest in a comparative study as the present. The number of different analyses which thus had to be carried out made it necessary to secure sufficient samples.

Table 7. *Water, protein, fat and ash in liver, red muscle and ordinary muscle from species of fish.*

Species	Sample of	Water %	Protein Nx 6,25	Fat %	Ash %
Tunny (*Thunnus thynnus*)	Liver	63.4	17.0	17.5	1.09
	Red muscle	66.4	22.9	6.7	1.16
	Ordinary muscle	68.5	25.1	4.6	1.34
Mackerel (*Scomber scombrus*)	Liver	69.8	19.8	7.7	1.90
	Red muscle	54.2	14.9	29.7	1.18
	Ordinary muscle	65.5	21.2	13.1	1.24
Herring (*Clupea harengus*)	Liver	78.0	18.1	1.8	1.60
	Red muscle	57.8	15.5	28.2	1.30
	Ordinary muscle	74.0	22.0	13.0	1.40
Coalfish (*Gadus virens*)	Liver	41.8	13.3	42.2	1.30
	Red muscle	77.8	18.6	2.5	1.13
	Ordinary muscle	78.4	19.9	0.5	1.27
Salmon (*Salmo salar*)	Liver	75.8	17.2	7.4	1.87
	Red muscle	66.3	16.2	15.1	1.66
	Ordinary muscle	73.7	22.6	2.0	1.72
Halibut (*Hippoglossus vulgaris*)	Liver	49.6	10.8	38.2	1.15
	Red muscle	62.0	11.3	27.3	0.82
	Ordinary muscle	77.7	14.5	7.0	1.10
Porbeagle (*Lamna cornubica*)	Liver	14.1	2.8	84.2	0.44
	Red muscle	75.6	23.7	1.2	1.58
	Ordinary muscle	76.4	23.9	0.7	1.50

Species like tunny, mackerel, herring and porbeagle did not cause any problems as the red muscle is plentiful and can be readily removed in these fishes. In the gadidae, however, the red muscle occurs more sparsely, and it can neither be readily separated from the ordinary muscle nor from the skin. As the aim of the study was to supply general information, it was decided to include only the coalfish. This fish has a fairly developed and distinct red muscle (Fig. 3) which can be sampled exactly and relatively easy.

The results of the chemical analyses are reported in Table 7. The fishes are as in previous tables put up in order of assumed activity. Instead of discussing the results from species to species it is more convenient to discuss them in relation to the general findings. The results show that the content of dry matter generally is higher in the red than in the ordinary

muscles. This is a result of a higher storage of fat in the red muscle, as the protein content generally is higher and often much higher in the ordinary than in the red muscle. That definite chemical differences exist between the red and ordinary muscle was already pointed out by NAMIKI (1933), who found a higher content of dry matter and more cholesterol in the red than in the ordinary muscle. In mackerel and herring both types of muscles are fat, the red muscles containing almost 30%. The histological investigations revealed that a rather well-defined layer of fat could be observed between the skin and the red muscle, thus erroneous high values may be found as it is difficult to obtain a "clean" red muscle. One may assume that muscular tissue with such high fat content is unfit for strong muscular activity. The red muscle in fish seems to be used as a fat storage organ. This function has been emphasized for the red muscle of Pacific salmon by GREENE (1913), and VANSTONE (1957) reports that the said stores undergo depletion during the migration fast to the spawning grounds. The analyses of the liver from mackerel and herring show that they are lean, and histological studies reveal the ordinary trabeculated arrangements of the liver cells, containing small intracellular vacuoles. (Pl. IV, Fig. 1). In the halibut the red muscle shows a similar chemical picture as in herring and mackerel, but the liver is rather fat, containing 38% in the present sample. Salmon also has a fat red muscle, but a medium fat liver. In coalfish the red muscle is comparatively lean, but fatter than the corresponding ordinary muscle. The liver from coalfish, however, is very fat and the structure consists almost only of large fat-containing cells. (Pl. IV, Fig. 2). This is usually the case in gadidae, where livers with up to 70% fat are encountered (BRÆKKAN 1958a). If we consider the species with deepseated red muscles, the tunny and the porbeagle, both types of muscles show the same general composition, the red containing slightly more fat. In the tunny the liver is semi-fat with a relatively rich vascularization. The liver of the porbeagle is large and extremely fat, 84% in the present sample. Actually, the fat is present in such amount as to make sampling difficult without bursting the lobes. Functionally such a liver may be considered less fit, with sparse distribution of active cells and limited vascularization. It may be noted that the protein content is only 2.8%. A certain compensation for the relatively sparse content of "active" cells is usually obtained by the larger size of liver in these fish.

The results from the vitamin determinations are reported in Table 8, which gives values calculated per g fresh weight as well as per g protein.

Niacin shows relatively moderate differences between the three organs when calculated per g fresh weight. An exception is the porbeagle, where the muscles contain ca. 70 μg, while the liver only contains 8 μg per g

Table 8. *Niacin, pantothenic acid, riboflavin, vitamin B_{12} and thiamine in liver, red muscle and ordinary muscle from some species of fish.*

Species	Sample of	Niacin μg/g		Pantothenic acid μg/g		Riboflavin μg/g		Vitamin B_{12} μg/g		Thiamine μg/g	
		Fresh weight	Protein	Fresh weight	Protein	Fresh weight	Protein	Fresh weight	Protein	Fresh weight	Protein
Tunny (*Thunnus thynnus*)	Liver	87	510	26.0	156	46.0	270	3.10	18.2	3.7	21.6
	Red muscle ...	100	435	32.8	146	7.9	35	0.38	1.66	4.3	18.8
	Ordinary muscle	122	485	5.0	20	1.5	6	0.047	0.19	2.5	9.9
Mackerel (*Scomber scombrus*)	Liver	79	398	33.3	168	11.3	57	0.52	2.62	—	—
	Red muscle ...	68	455	30.0	200	13.7	92	0.47	3.15	3.7	25.0
	Ordinary muscle	112	530	3.8	18	1.4	7	0.018	0.12	0.2	0.9
Herring (*Clupea harengus*)	Liver	65	365	21.0	116	5.9	32	0.34	1.86	1.6	8.9
	Red muscle ...	69	441	30.0	193	12.7	82	0.54	3.48	1.1	7.1
	Ordinary muscle	60	274	2.8	13	1.1	5	0.07	0.32	0.03	0.13
Coalfish (*Gadus virens*)	Liver	35	264	8.0	60	6.3	47	0.25	1.88	3.0	22.6
	Red muscle ...	58	312	13.9	73	7.0	38	0.20	1.07	8.0	43.0
	Ordinary muscle	28	143	2.0	10	0.9	5	0.029	0.15	0.5	2.3
Salmon (*Salmo salar*)	Liver	46	267	17.3	100	8.0	46	0.45	2.62	4.0	37.0
	Red muscle ...	64	393	28.0	173	8.8	54	0.22	1.35	4.3	26.6
	Ordinary muscle	94	415	10.0	45	0.9	4	0.036	0.16	1.3	5.8
Halibut (*Hippoglossus vulgaris*)	Liver	30	280	10.8	100	7.3	68	1.00	9.25	2.5	23.2
	Red muscle ...	31	275	5.2	47	2.5	22	0.05	0.45	0.4	3.1
	Ordinary muscle	44	304	3.6	28	0.6	4	0.009	0.06	0.4	2.8
Porbeagle (*Lamna cornubica*)	Liver	8	278	5.6	200	2.5	89	0.04	1.45	0.9	32.2
	Red musle	70	295	33.0	138	6.4	27	0.37	1.58	6.0	25.3
	Ordinary muscle	67	280	15.0	64	1.0	4	0.026	0.11	0.8	3.4

fresh weight. When niacin is calculated per g protein, the total picture shows a typical trend in the relation between the values. They are still of the same order of magnitude for all organs within each species, but now the liver and the red muscle generally show fairly equal niacin contents while the values for the ordinary muscle deviates moderately.

All the other B-vitamins in the species investigated show comparatively the same picture with regard to the relative distribution. The contents are usually several times higher in the red than in the ordinary muscle, but the differences seem to depend on the species, and are most pronounced in fishes with highly developed and dark red muscles. The feature and the magnitude of the values in the red muscle and the liver are generally very similar. The liver from the tunny, however, shows extra high contents of riboflavin and vitamin B_{12} compared with the relations prevailing in the other species. The values for thiamine are given with reservation for any possible effect of thiaminases, which can cause erroneous results if present (HARRIS 1951). During the present study some Japanese papers reported results in general agreement with the present findings. HASHIMOTO, YAMADA & MORI (1953) found about ten times higher values for vitamin B_{12} in the red muscle than in the ordinary muscle of some Pacific scombroids. MORI, HASHIMOTO & KOMATA (1956) investigated the contents of thiamine, riboflavin, niacin and vitamin B_{12} in the red and ordinary muscles of several species and found the same general distribution as reported in this investigation. Although they did not present analytical data from the livers, they pointed out the similarity between the feature of the accumulation in the red muscle and in the liver, and the possibility that the red muscle may carry out physiological functions performed by the liver. This aspect has also been put forward in a preliminary communication from the present study (BRÆKKAN 1956). MORI, KONOSU and MIYAGAWA (1957) investigated the choline content of the muscles of aquatic animals and found two to three times higher values in the red than in the ordinary muscle.

When the fish consume food it is digested in the alimentary canal, where it is broken down to substances which pass the wall of the canal and into the blood. Through the vascular system they are carried to the storage organs or the cells in need of metabolites. The vascular system may vary somewhat in different groups of fishes, but in general it is much less developed than in mammals and covers mainly the internal organs. The livers in some species are very fat and have few blood-vessels compared with the highly developed blood-system in mammalian livers. In fishes the amount of blood present is much less than in the higher vertebrates, and it flows through the vascular system in a sluggish manner. The temperature of the blood is but little higher than the surrounding

water. There are some exceptions, like the tunny, in which the blood is abundant, and where it attains comparatively warm temperatures during muscular activity, (NORMAN 1931). In general, however, a rapid restoration of energy-giving metabolites from the liver as well as a rich supply to the muscle by the bloodstream are not possible. On the other hand some of the fishes are very active, with a performance of muscular work which results in high energy consumption. This demand for energy has caused the organism to develop means of meeting this need, and in the author's opinion the red muscle is Nature's answer.

The red muscle normally has an anatomical situation which prevents it from taking part in the main muscular work. The chemical analyses reveal that the red muscle usually has a much higher fat content than ordinary muscle, thus the present samples from mackerel, herring and halibut contain 27—29%. Muscles with such high fat contents are less suitable for strained and continued activities. The high fat contents indicate that the red muscle acts as a storage organ for this nutrient.

The histology of the fibres in the red muscles show that they have a less dense striation than in the ordinary muscle. They are more or less granulated and have intracellular fat. The red muscles, because of their situation must have a contractile system, even if they should have other main functions. It has clearly been established that white muscles are stronger than red muscles, although the latter for metabolic reasons may show greater endurance. (NEEDHAM 1926, HINES 1927, PROSSER 1952). The situation of the red muscle in relation to the ordinary muscle actually provides for a relatively short transport of metabolites from the former to the latter through the lymphatic system, which is a fine network of tubules distributed in the connective tissue of different parts of the body.

If we assume the red muscle is less active than the ordinary muscle, it should be expected that under the anaerobic conditions which prevail during death struggle, the breakdown of glycogen and the formation of lactic acid should be less in the red than in the neighbouring ordinary muscle. A check on this point by analyses of the red and ordinary muscle in fresh frozen tunny gave the following values: 1 per cent and traces respectively of glycogen, 0.8 per cent and 1.6 per cent respectively of lactic acid. (BRÆKKAN 1956). NAMIKI (1934) studied the creatine, creatinine, and fat contents of fish muscles and found the following ratios, respectively in ordinary and red muscle: 2 : 1, 1 : 1, and 1 : 7. For Fe he found respectively 0.66 — 1.04 and 4.44 — 7.19 mg %. The latter results are of interest in relation to the investigations of myoglobin reported below. The higher creatine content in the ordinary muscle indicates higher muscular activity with increased breakdown of phosphagen to creatine and free inorganic phosphate. AMANO, BITO & KAWABATA (1953)

investigated the differences in glycolysis in the frigate mackerel killed by various methods. Unfortunately, their analysis of glycogen and lactic acid were only carried out on the ordinary muscle. Results, however, from their observation of pH in different parts of the fish show, that while the values were fairly equal in both types of muscle when the fish was killed by decapitation, a clearly lowered pH value could be observed in the ordinary but not in the red muscle after death struggle.

At this point attention was drawn to the report of the finding of myoglobin in the lateral muscle of the carp (HAMOIR 1953). Investigations of samples of neighbouring muscles from tunny at this Institute gave as results: The red muscle contained an average of 0.14 and the ordinary 0.04 standard Clegg & King units per g wet tissue. The same values expressed as hemin being respectively 0.70 and 0.19 μg hemin per g wet tissue (JEBSEN 1954). MATSUURA & HASHIMOTO (1954) investigated the distribution of hemoglobin, myoglobin and cytochrome c in several fishes, mainly scombroids, and found the red muscle to be richer in these pigments than the ordinary muscle. Particularly the deepseated red muscle in tunny and bonito were rich in myoglobin. Their results were in agreement with relation reported above. MATSUURA & HASHIMOTO (1955) also prepared crystalline myoglobin from the red muscle of fish and compared it with horse heart myoglobin. They found different absorption spectra for oxymyoglobin obtained from different sources, but other myoglobin derivates showed nearly identical absorption curves. The same authors found myoglobin from fish to be fairly species specific with regard to solubility (MATSUURA & HASHIMOTO 1956). MATSUURA, YAMADA & HASHIMOTO (1955) prepared cytochrome c from the red muscle of the tunny, and found it to be identical with horse muscle cytochrome c.

If the red muscle performs metabolic activities of an organ more than the function of a muscle, the enzyme contents should differ in the red and ordinary muscles. If further, the former carries out physiological functions normally performed by the liver, a similar quantitative and qualitative distribution of the enzymes in the red muscle and the liver should be observed. The literature was found to report several comparative studies of enzymes in fish, and during the progress of the present study several papers dealing comparatively with the red and ordinary muscle in fish appeared. MATSUURA, BABA & MORI (1953) compared the arginase activity of the ordinary and red muscle of various kinds of fish. They found no arginase, or only traces, in the ordinary muscle, while activity could be shown in the red muscle. This result is interesting in relation to the finding of ENDO & SIMIDU (1955), who studied the distribution of extractive nitrogen in the red and ordinary muscle and found four to

six times higher arginine-histidine fraction in the ordinary than in the red muscle of some scombroid fishes. FUKUDA 1954) and FUKUDA & HIGUCHI (1954) studied the catalase in different organs from several species of fish. They found the liver normally to have the highest content, but the red muscle was also much richer than the ordinary muscle with respect to this enzyme. In large pelagic fishes they found the liver to be comparatively poor but the red muscle richer in catalase, and they claimed that this finding indicates "that the red meat of pelagic fishes is not only a muscle but also an organ quite distinct from it". Thus they reached a similar conclusion for the function of the red muscle in pelagic fishes, as has been proposed generally in fishes from a different approach in the present study. FUKUDA (1958) also studied the succinic dehydrogenase activity in different organs from various species. He found the highest activity in the red muscle, 1—2 times as high as in the liver and 5—10 times the activity in the ordinary muscle from the same fish. He also found higher activity in the pelagic species than in the non-pelagic and fresh water species. The results are in agreement with the findings of UMEMURA (1951), who reported 8 times as high succinic dehydrogenase activity in a red muscle homogenate as in an ordinary muscle homogenate from a silver carp. SAITO & SAMESHIMA (1958) studied the proteolytic activity of fish muscle extract as measured by tyrosin liberation and found highest activity in extracts from the red muscle.

The red muscle in fish thus contains relatively high concentrations of the B-vitamins compared with the neighbouring ordinary muscle, and enzyme studies report high contents of myoglobin, cytochrome c, arginase, catalase and succinic acid dehydrogenase. All these findings indicate a higher metabolic activity than would be expected if only muscular activity should be maintained, and suggest that the main function of the red muscle is that of a specialized organ. If the relative distribution of vitamins and enzymes is compared with the conditions in the liver, a general similarity is observed. This indicates that the red muscle is able to carry out similar metabolic processes as those typical for the liver. In most fishes the liver is very fat, in cases even extremely fat as in the porbeagle and the coalfish (Pl. IV Fig. 2), and the vascularization is poorly developed compared with the mammalian liver. But even in fishes with lean livers, as for instance in the herring (Pl. IV Fig. 1), the structure differs from the "normal" mammalian liver. No speculation as to the reason for, and processes of, these evolutionary differences shall be advanced. One aspect, however, shall be commented on. The liver usually functions as a storage organ for nutrients which are broken down and brought to the muscles by way of the vascular system. This system is, particularly in fishes with fat livers, so insufficiently present that develop-

ment of accessory means to keep up this function would be expected. The red muscle answers to this demand in metabolic ability and anatomically suitable localization. If we finally consider the presence of the red muscle in relation to the known activities of the species, there exists a relation which supports this view. Tunny as a very active and fast swimming fish has the highest proportion of red muscle. This species has even further improved its metabolic endurance by having a more complete vascular system and relatively much more blood than most other fishes. Mackerel and herring, which also are fast swimming migratory pelagic species have a relatively high proportion of fairly strongly pigmented red muscles. Halibut, however, as a flatfish has less and a paler red muscle. Further considerations along this line give the same picture of a relation between activity and extension and pigmentation of the red muscle. The sharks, porbeagle and mako, are examples of this relation. The mako lives in warmer waters where the higher temperature will result in a higher metabolic rate. Compared with the porbeagle it has red muscles which have a considerable greater extension (Fig. 5—6).

Further studies chemically, anatomically and histologically, are necessary to establish the proposed function of the red muscle. The present paper reports some vitamin studies and discusses some enzyme studies. How recently the attention in general has been drawn to the problem of the red muscle in fish, is illustrated by the fact that even recent handbooks on the physiology of fishes have not discussed the topic (BROWN 1957).

GENERAL SUMMARY

A comparative study of vitamins in the trunk muscles of fishes is reported. The investigation comprises two separate studies. Firstly, the relation between the activity of different species and the average vitamin contents of the whole muscle was studied. Secondly, the relation between the vitamin contents of the red and ordinary muscles were studied in several species, and a proposal is put forward as to the possible function of the red muscle in fish.

All B-vitamins investigated were determined by microbiological methods, and the results are reported per g fresh weight as well as per g protein. In addition analyses of moisture, protein, fat and ash are reported in the second part of the study.

Single samples of the total trunk muscles from 19 cods weighing from 0.5 to 7.7 kg were analysed on niacin, pantotheic acid, riboflavin and vitamin B_{12}. No relation could be observed between the weight of the fish and the vitamin contents of the muscles.

Altogether 15 species were studied with regard to their relative contents of niacin, pantothenic acid, riboflavin and vitamin B_{12} in the trunk muscles. In general, there seem to be a significant relation between these vitamin contents and the activity of the fishes. The relation was mainly pronounced for species of the same type with regard to external anatomy. Flatfishes were therefore reported as a separate group.

In seven species the relations between the contents of niacin, pantothenic acid, riboflavin, vitamin B_{12} and thiamine in the red and ordinary muscles from the same fishes were reported. The anatomical arrangement and the general histology of the different muscles were briefly described. In addition, analyses of livers from the same species were carried out.

The results show that, with the exception of niacin, all other vitamins investigated are present in much higher concentrations in the red than in the ordinary muscle. The general feature of the distribution was very similar to the conditions prevailing in the liver. In most fishes the red

muscle had a much higher fat content than the neighbouring ordinary muscle.

The results are discussed in relation to other findings reported in the literature with regard to the relative distribution of vitamins and enzymes in the red and ordinary muscle of fish.

It is proposed that the main function of the red muscle is not muscular activity, but the function of an organ able to carry out several metabolic processes normally taking place in the liver.

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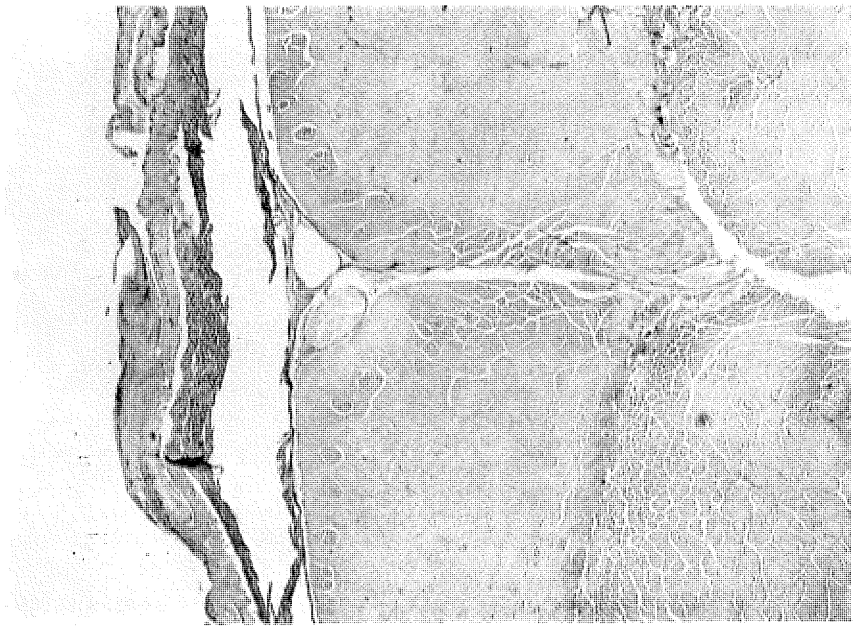

Fig. 1. Transversal section through the red and ordinary muscles of coalfish (*Gadus virens*). (Schiff × 13).

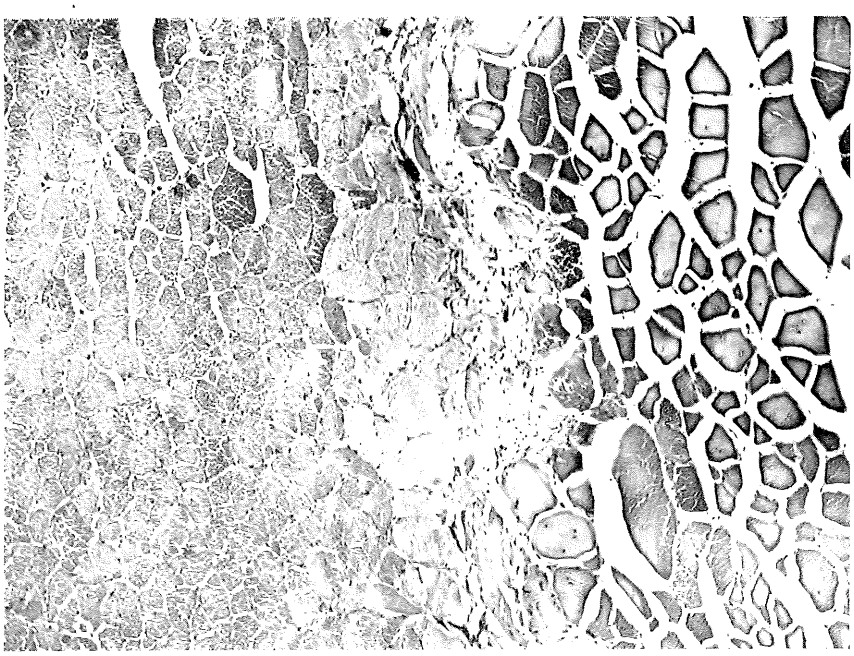

Fig. 2. Transversal section through the red and ordinary muscles of coalfish (*Gadus virens*) showing details of the structure on the borderline. The red muscle has a compact structure with granulated cells. The ordinary muscle has a homogeneous cell structure. (H + E. × 130).

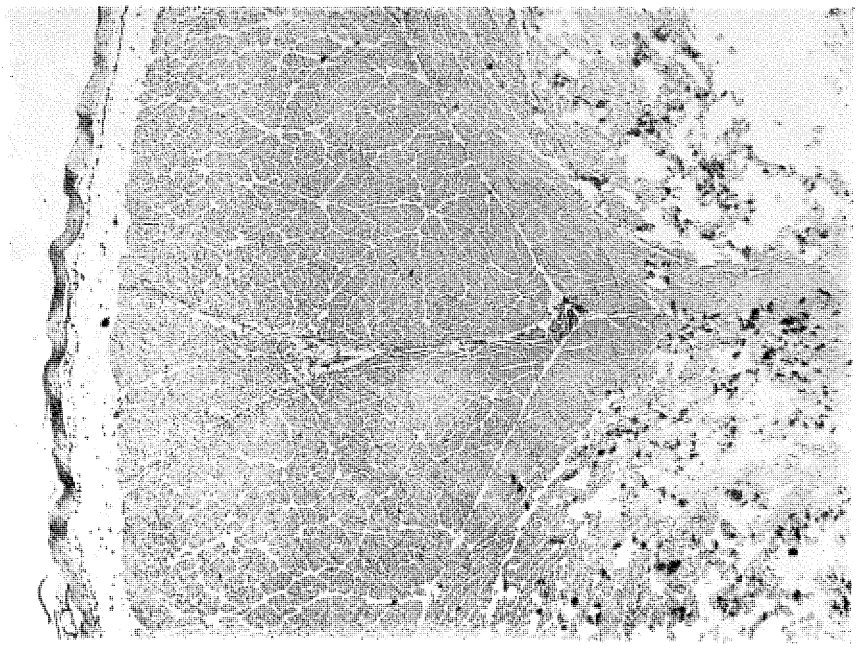

Fig. 1. Transversal section through the red and ordinary muscles of mackerel (*Scomber scombrus*). (Schiff × 13).

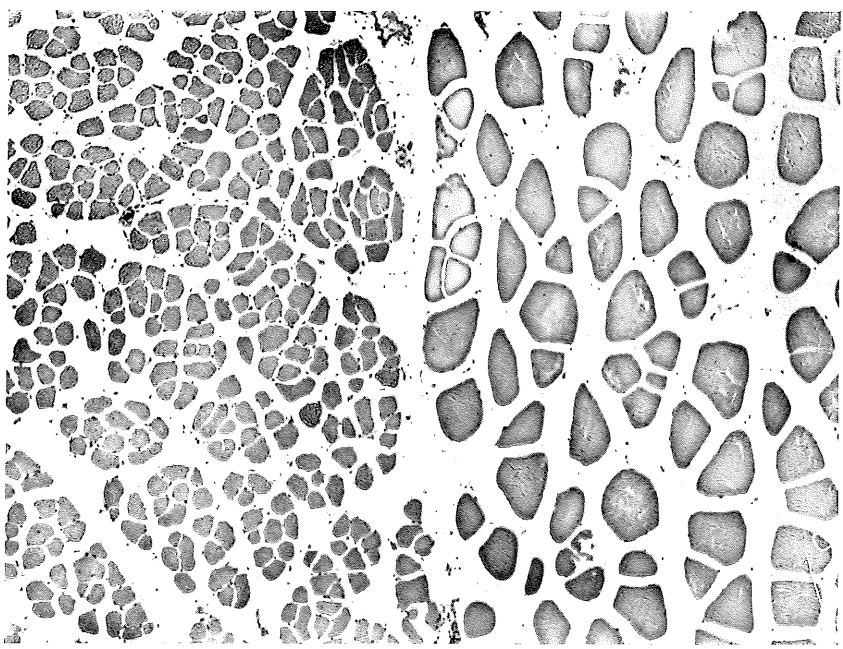

Fig. 2. Transversal section through the red and ordinary muscles of mackerel (*Scomberscombrus*) showing detail of the borderline. (Schiff × 130).

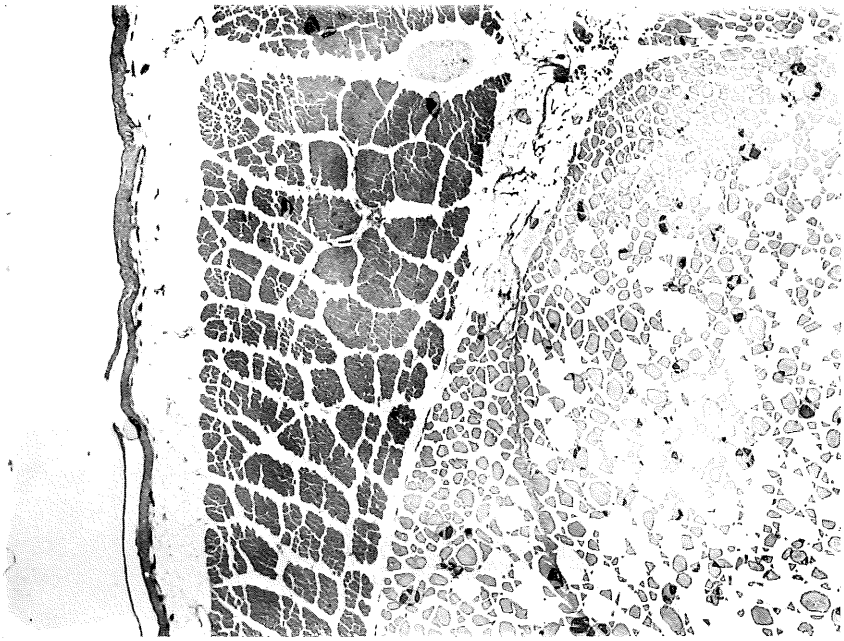

Fig. 1. Transversal section through the red and ordinary muscles of herring (*Clupea harengus*). The septum on the borderline can be clearly seen. (Schiff × 35).

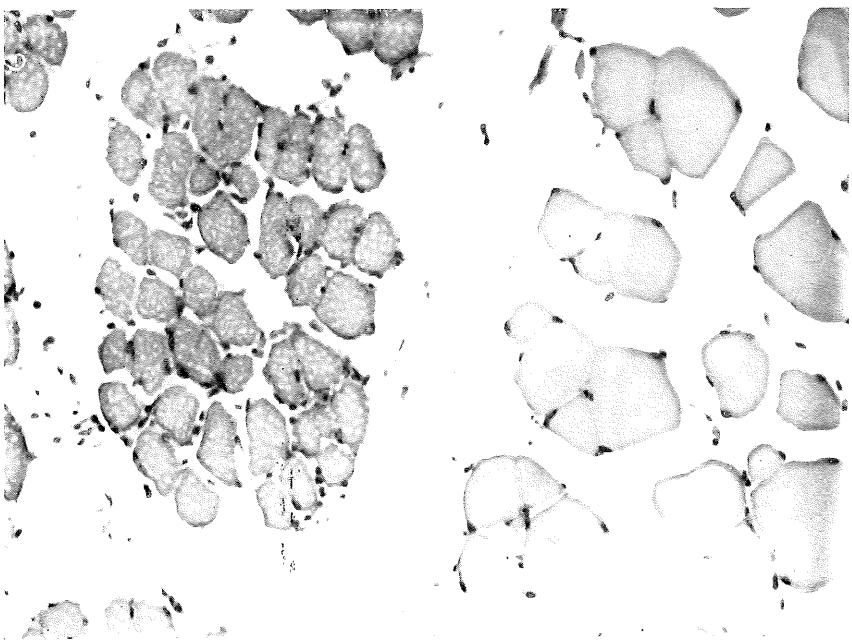

Fig. 2. Detail fig. 1. 10x. Transversal section through the borderline of the red and ordinary muscles of herring (*Clupea harengus*). In the red muscle the fat is seen intracellular while in the ordinary muscle no intracellular vacuoles are visible. (Schiff × 350).

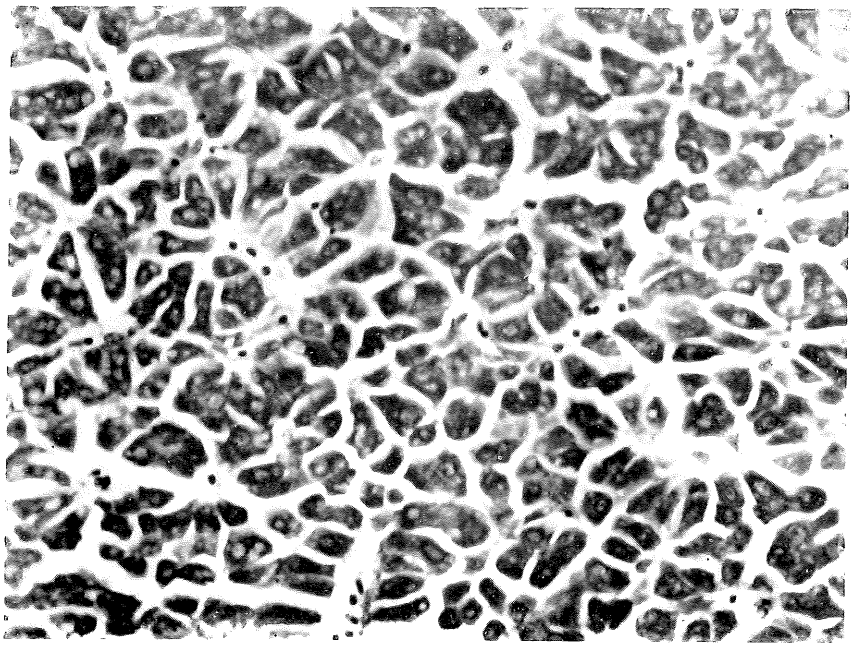

Fig. 1. Section of the liver from herring (*Clupea harengus*). Trabeculated arrangement of parenchyma with small intracellular vacuoles.
(Schiff × 500).

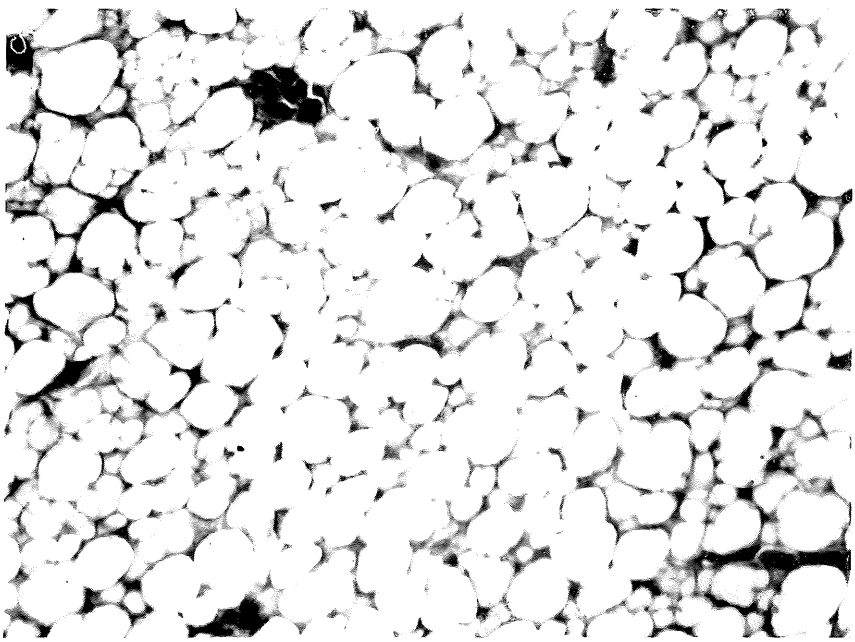

Fig. 2. Section of the liver from coalfish (*Gadus virens*). No "parenchymatic" cells are visible. The structure shows only large, fat-containing vacuoles.
(H + E. × 500).