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THE PROTEINS OF FISH

2. THE PROTEINS OF FISH

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A review of the biochemistry of fish proteins was made in 1948 by Geiger, and here more recent progress in the isolation and characterization of fish proteins will be described. No special consideration will be given to the nutritive value of these compounds or to their possible pharmacological action. It is intended in the present report to give a general picture of the composition of fish muscle and an account of the advances made in the study of the proteins of other fish tissues.

I. PROTEINS FROM SKELETAL MUSCLE

Previous work has shown that fish muscles contain myoalbumin, myogen, globulin X, myosins and stroma. The quantitative estimation of these different fractions was carried out some time ago following Smith's scheme (1937) by Reay (1935) and Reay & Kuchel (1936). At that time, however, the nature and properties of the muscle proteins were not accurately defined; a sufficiently comprehensive study of the conditions of extraction was lacking and the analysis of the protein mixtures was based only upon differences in solubility.

The influence of different factors on the extraction of fish muscle has been re-examined recently by Dyer, French & Snow (1950), and the analysis of extracts by electrophoresis has allowed a better identification of the different components (Hamoir, unpublished). As uncertainty has recently arisen concerning the nomenclature of muscle proteins, precise definitions of these based on our knowledge of rabbit muscle proteins will be given here.

Weber (1934) has given the name myogen to the albumins of the muscle juice. This definition can be generalized to the albumins of muscle extracted at low ionic strength. Recent research has shown that Weber's myogen contains the myoalbumin described by Smith (1937) (known as the component *h* of isoelectric point 4·65 by Jacob (1947, 1948)), together with a group of other proteins of much higher and fairly close isoelectric points (6·00, 6·20, 6·75) which we shall call myogens (Jacob, 1947). All these compounds exist in muscle juice or are extracted by water or dilute salt solution ($\mu \leqslant 0{\cdot}25$), together with Weber's globulin X (1934). This last fraction is removed with denatured myogens by dialysis against distilled water

and is isolated by redissolution in salt solutions. Globulin X represents therefore the muscle globulins extractable at an ionic strength insufficient to bring the myosins into solution. It must not be confused with other globulins such as protein-Y of similar solubility (Dubuisson, 1950) which can only be extracted at much higher ionic strength. The general term 'myosins' includes the 'crystalline' myosin of Szent-Györgyi (1943) and the actomyosin of Banga & Szent-Györgyi (1941–1942) which is a mixture of actomyosins (according to Portzehl, Schramm & Weber, 1950 resulting from the association of actin with the 'crystalline' myosin of Szent-Györgyi. The latter is called L-myosin in the sedimentation diagrams (Portzehl *et al.*, 1950) and β-myosin in the electrophoretic patterns (Dubuisson, 1946 *a*, *b*). Stroma protein can be defined according to Smith (1937) as the residue obtained after repeated extraction by dilute hydrochloric acid or sodium hydroxide.

A. *Qualitative analysis of whole extracts*

The amounts of total nitrogen, non-protein nitrogen, myosins and non-myosins nitrogen in extracts of cod muscles have been determined by Dyer *et al.* (1950) after extraction in the cold for 3–5 minutes in a specially adapted Waring Blendor, while an analysis, using a slightly modified Tiselius electrophoresis apparatus (Dubuisson, Distèche & Debot, 1950), of extracts from carp muscles has been made by me. In my investigations the muscles were cut with a freezing microtome into slices 40 μ thick (Dubuisson, 1947) and extracted in the cold with stirring.

(a) *Extracts at ionic strength* 0·15 *and pH* 7–8. Myosins could not be detected in extracts made under the above conditions by Dyer *et al.* (1950) or by me. Of the total protein content of cod muscle 21% went into solution under these conditions and, according to Smith's analytical scheme (1937), this was made up of 6% myogen, 7% myoalbumin and 8% globulin X. Electrophoretic patterns of one-hour duration of such extracts from carp muscles showed two main components and several others present in small concentrations migrating more rapidly (Fig. 1). Fig. 1 represents a mixture of myoalbumin, myogens and globulin X. As myoalbumin has a very low isoelectric point, it can only be represented by one of the small peaks in front. It is therefore not present in these extracts in a proportion corresponding to the above analytical value of Dyer *et al.* (1950). After dialysis of the extract of Fig. 1 against distilled water, an appreciable precipitate of globulin X and denatured myogens can be removed by centrifugation. The electrophoresis of the clear solution obtained shows a general decrease of all the components except the major one, which becomes very prominent and corresponds to 80–90% of the myogens. Its mobilities in a phosphate-NaCl buffer

of ionic strength 0·15 (0·1 phosphate and 0·05 NaCl) and pH 7·3 are $-3{\cdot}0\times10^{-5}$ cm.^{2}/volt/sec. on the ascending side and -2.5×10^{-5} cm.^{2}/volt/sec. on the descending side.

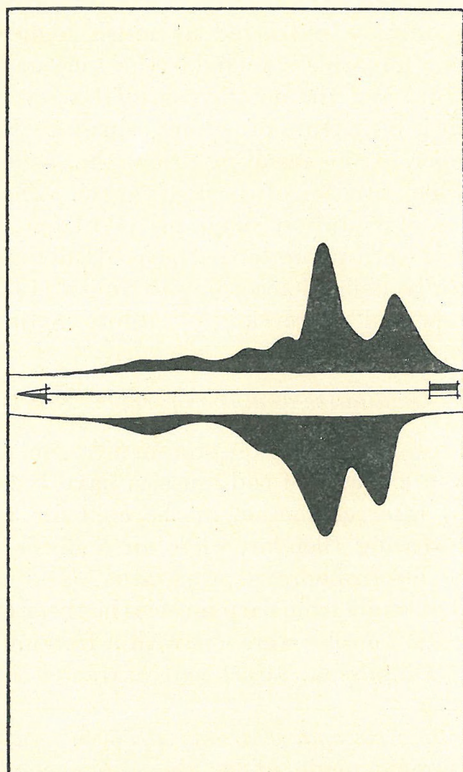

Fig. 1. Electrophoretic pattern of a one-hour extract from carp muscle at $\mu=0{\cdot}15$ and pH 7–8. Ionic strength 0·15, pH 7·3. Migration to the left. Upper part : ascending limb. Lower part : descending limb. Duration of the electrophoresis : 14,100 sec. Electrical field : 3·80 v./cm.

(b) *Extracts at ionic strength* 0·5 *and pH* 7–8. *Conditions for maximum extraction.* When the ionic strength of the extractant is higher than 0·15, structural proteins also go into solution. Extraction of the myosins is already observable at ionic strength of 0·17 in cod muscles (Dyer *et al.*, 1950) ; in carp muscles, no myosins are detectable in extracts made at an ionic strength of 0·35 (Hamoir, 1949), but extraction at $\mu=0{\cdot}5$ proceeds rapidly, giving turbid extracts of high viscosity and high protein content.

Fish muscle differs from mammalian muscle in its very low content of stroma proteins, these being 3% instead of 16% (Reay & Kuchel, 1936 ; Smith, 1937). These determinations were carried out after extraction of the pulp with hydrochloric acid (0·05 to 0·005-N) or sodium hydroxide (0·1 to 0·005-N) (Reay & Kuchel, 1936) ; it is

desirable, however, to use a more gentle method of extraction in order to remove the myosins quantitatively without denaturation.

Some progress has been made in this direction by Dyer *et al.* (1950) by examining the influence of grinding, ionic strength, pH and nature of the extractant, temperature of extraction and cold storage.

It is most important for maximum extraction that the subdivision of the muscle fibrils must be carried as far as possible, and that any denaturation is avoided. Good recovery was obtained by these authors by homogenization of the muscles for 3–5 minutes in a Waring Blendor provided with a plate to prevent the formation of foam. The amount of residue obtained from extractions carried out in these conditions, using a solution 0·85M-NaCl and 0·02M-$NaHCO_3$ to give a pH of 7 to 7·5, varied between 3 and about 15% " depending on the conditions of the fish " (Dyer *et al.*, 1950). Varying the ratio of fish muscle to extractant solution between 1/90 and 1/18 was without influence. The nature of the salt used was of secondary importance (Table 1) and the greater differences previously

Table 1. *Efficacy expressed in per cent. of protein N extracted of normal solutions adjusted to pH* 7–7·5 *of various salts as protein extractants of cod muscle* (after Dyer *et al.*, 1950)

$BaCl_2$	$CaCl_2$	KBr	KCl	KI	K_2HPO_4	K_2SO_4	LiCl
84	87	80	82	91	86	87	86

$MgCl_2$	$MgSO_4$	NaAc	Na_3Citrate	NaCl	$NaHCO_3$	Na_2SO_4
91	83	77	83	91	77	83

observed (Smith, 1937) were probably due to an insufficient subdivision of the muscle fibrils. By the systematic study of these factors, Dyer *et al.* (1950), could separate quantitatively the myosins from the stroma at pH 7–8 by extraction with potassium iodide, magnesium chloride or sodium chloride. It is probable that similar results could be obtained by quickly freezing the fresh muscle solid with a freezing microtome, cutting it immediately in thin slices (Dubuisson, 1947) and grinding the slices with sand in the cold.

Such extracts are unsuitable for electrophoretic analysis because of their high viscosity. They present all the properties of actomyosin solutions, but are from that point of view contaminated by their high content of other proteins. By altering the pH of extraction, extracts of lower viscosity can be obtained giving a general electrophoretic pattern of the different proteins present in fish muscle.

(c) *Extracts at ionic strength* 0·5 *and pH* 5–6. Extractions at high ionic strength and varying pH have been carried out by Dyer *et al.* (1950), on cod muscles. As shown in Fig. 2, the myosins begin to go into solution at pH 4·3, the extractability increasing slowly to pH 5·5 and the process is practically complete at pH 6·0. Maximum extraction is obtained between pH 7 and 9. Similar results (unpublished) were found by me with carp muscles, a shift of the curve to the right being observed with muscles kept for several weeks in frozen state. It is therefore possible, by working at pH 5–6, to get extracts containing only a small proportion of myosins. Such extracts have been analysed by electrophoresis (Hamoir, unpublished).

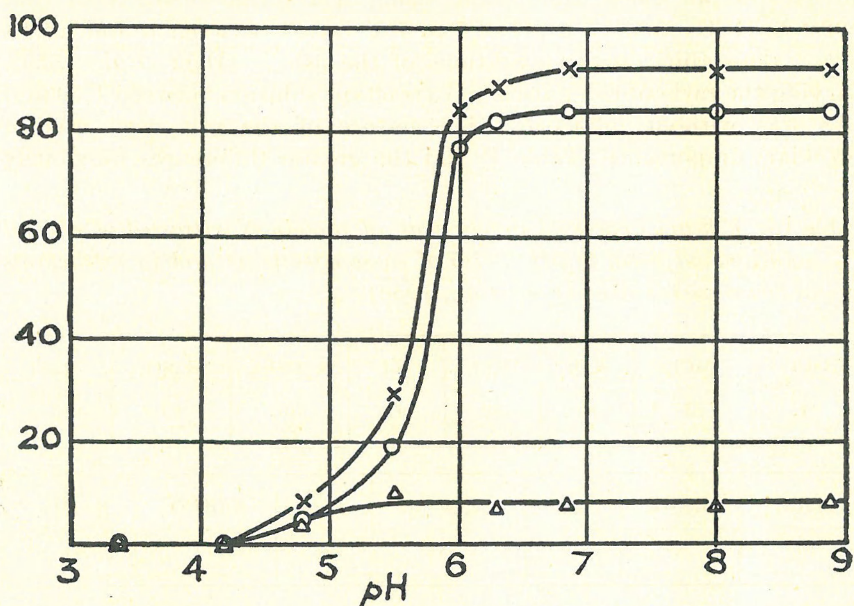

Fig. 2. Influence of the pH on the extractability of the muscle proteins of cod at ionic strength 0·85 (after Dyer *et al.*, 1950). Ordinate : per cent. of the total protein N extracted ; ×——×, as soluble protein N ; ○——○, as myosin N ; △——△ as non-myosin N. Abscissa : pH.

Carp muscles were extracted for ten minutes with phosphate-KCl buffers of ionic strength 0·5 (μ=0·3 KCl and 0·2 phosphate) and of pH 5·8 or 5·1. After removal of the muscles debris by centrifugation, the extracts were dialysed against phosphate-NaCl buffers of ionic strength 0·35 and pH 7·1 (μ=0·1 phosphate and 0·25 NaCl) or of ionic strength 0·15 and pH 7·3 (μ=0·1 phosphate and 0·05 NaCl).

The electrophoretic patterns obtained are given in Figs. 3 and 4. At μ=0·35 and pH 7·1, carp myosins are perfectly soluble, although they cannot be extracted at this ionic strength and pH (Fig. 3). On the ascending side, the myosins form two peaks migrating very closely together in front of the myogens, the rapid one being very sharp and

the slower one showing a more symmetrical shape ; in front of these two peaks a new much more rapid component, representing only few per cent. of the protein content of the extract, can be observed. An important asymmetry exists between the ascending and the descending limbs due to the presence of the myosins : the components do not separate well on the descending side and the two peaks of the myosins are not visible. The mobilities of those different peaks have been determined ; mean values of several electrophoresis are given in Table 2.

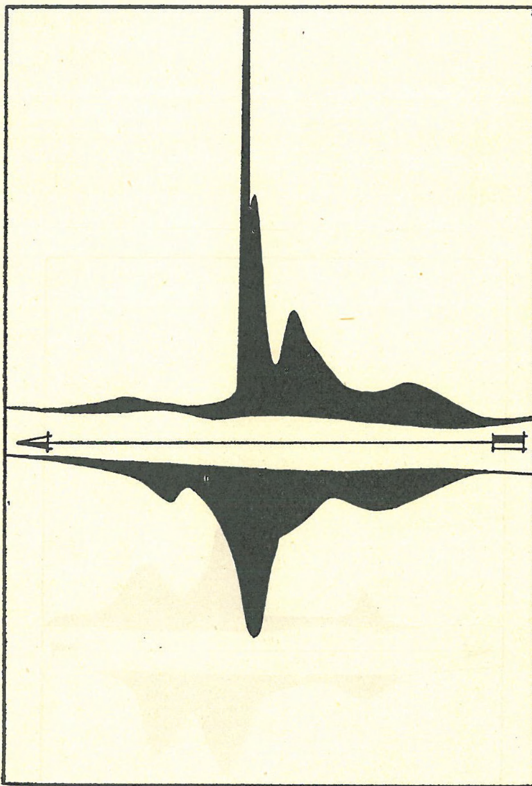

Fig. 3. Electrophoretic pattern of a ten-minute extract from carp muscle at $\mu=0{\cdot}5$ and pH 5·8. Ionic strength 0·35, pH 7·1. Migration to the left. Upper part : ascending limb. Lower part : descending limb. Duration of the electrophoresis : 79,100 sec. Electrical field : 2·0 v./cm.

By dialysis of such an extract against a solution of $\mu=0{\cdot}15$ and pH 7·3, a precipitate of myosins forms which can easily be removed by centrifugation. The composition of the supernatant is given in Fig. 4.

This electrophoretic pattern differs from the previous one obtained at low ionic strength (Fig. 1) by the presence of the peak of high mobility already observed in Fig. 3. This component behaves as a

structural component : it seems not to be extractable in appreciable amount at low ionic strength, but once extracted, it is soluble at much lower salt concentrations. Its mobilities at $\mu=0{\cdot}15$ and pH 7·3 are $-6{\cdot}45\times10^{-5}$ cm.^{2}/volt/sec. on the ascending side and $-6{\cdot}0\times10^{-5}$ cm.^{2}/volt/sec. on the descending one.

Table 2. *Mobilities of the chief components of fish muscles at* $\mu=0{\cdot}35$ *and pH* 7·1 ($\mu=0{\cdot}1$ *phosphate and* 0·25 *NaCl*)

Nature of component	Mobility in 10^{-5} cm.^{2}/volt/sec.	
	Ascending value	Descending value
Quick peak	−4·1	−2·9
Myosin α	−2·88	−2·3 (Myosin α, Myosin β, Myogen)
Myosin β	−2·78	
Myogen (*major component*)	−2·3	

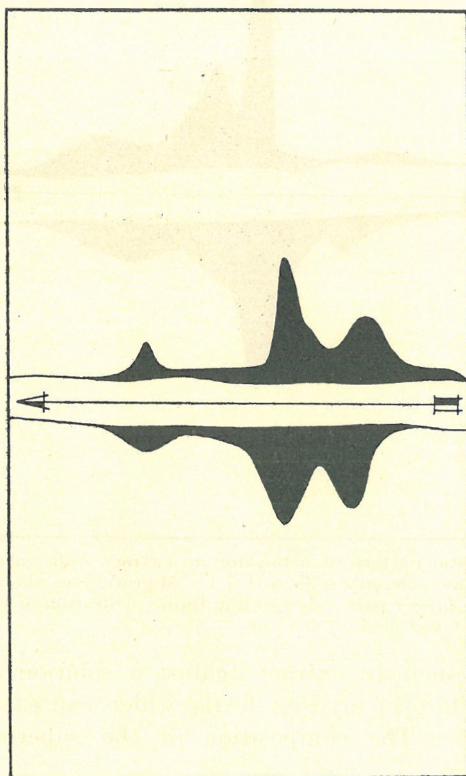

Fig. 4. Electrophoretic pattern of a ten-minute extract from carp muscle at $\mu=0{\cdot}5$ and pH 5·1. Ionic strength 0·15, pH 7·3. Migration to the left. Upper part : ascending limb. Lower part : descending limb. Duration of the electrophoresis : 16,500 sec. Electrical field : 3·70 v./cm.

The identification and characterization of the different peaks observed makes dubious the validity of the scheme of fractionation devised by Smith in 1934 and still in use. A better knowledge of the muscle components can only be achieved by their isolation.

B. *Isolation and properties of the main protein components of fish muscle*

Many of the properties of fish proteins have been described by Roth (1947).

Myoalbumin. Roth (1947) has expressed the opinion that the existence of myoalbumin is dubious. The absence in the electrophoretic patterns (Fig. 1) of a well-defined component migrating at the high mobility characteristic of myoalbumin proves that, if it exists in the extracts, its concentration must be low. It seems quite probable that the data obtained, following Smith's scheme for cod (Dyer *et al.*, 1950), are erroneous.

Myoglobin. This heme protein has been isolated in crystalline form from whale muscles by Keilin & Schmid (1948). Schmid (1949 *a*, *b*) has made a detailed study of its properties. He has found by electrophoresis that whale myoglobin like human myoglobin (Theorell & de Duve 1947) is not homogeneous, and has carried out a complete quantitative analysis of its amino-acid content (Schmid, 1949 *b*, *c*).

Myogens. Fish myogens differ markedly from mammalian myogens. The mobilities of fish and rabbit myogens are very different. Under the same conditions, the quickest main component *l* of the rabbit myogens (Jacob, 1947) migrates two to three times more slowly than the major component of the fish myogens. The latter also have a higher electrophoretic homogeneity. The difference in mobility appears not to be due to a different buffer action.

An electrotitration-curve of a fraction of haddock myogens has been made by Subba Rao (1948). The preparation used was obtained by dialysis of the muscle juice against water to remove globulin X and unstable myogens which precipitate. After centrifugation, the solution was saturated with ammonium sulphate and brought to pH 5·4. Only 50–60% of the myogens could be precipitated under these conditions. This fraction, dialysed free of salts, was used for the determinations. The curve obtained does not show any difference in buffer action when compared with rabbit myogens in the pH range 5–9 (Weber, 1927).

It is quite possible that the isoelectric points of fish and rabbit myogens are much more different than previously stated (Roth, 1947).

Globulin X. The globulin X fraction of cod muscle amounts to 8% of the total protein content (Dyer *et al.*, 1950). An analysis of haddock muscle juice (Subba Rao, 1948) shows a globulin X content of 11% of the total nitrogen of the solution and, since the remaining 89% is albumin-nitrogen, this value corresponds (see Table 4) to a

globulin X content of about 2% of the total protein. Such variations are unlikely.

According to Roth (1947), the isolation of globulin X from whole fish extracts can easily be carried out by two successive dialyses, the first one against a salt solution of pH 7 and ionic strength 0·2 to remove the myosins and the second one against water to isolate globulin X. But, according to Snow (1950), the precipitation of the myosins is incomplete in such conditions, 12% remaining in solution. We have been unable to confirm Snow's results; no myosins were observed in the electrophoretic pattern of extracts dialysed against a buffer of μ=0·15 and pH 7·3.

New determinations based upon Roth's method of isolation are necessary to know the content in globulin X of fish muscle. Since the investigations of Jacob (1947) on rabbit extracts have shown that globulin X is a very complex mixture of globulins, the values obtained depend on the method used, and an accurate description of the conditions of isolation seems particularly important.

Tropomyosin and nucleotropomyosin. Tropomyosin has been isolated from mammalian and fish muscles previously dehydrated by organic solvents (Bailey, 1948). More recently, two tropomyosins have been isolated from fish muscle by salt extraction; one is practically identical with Bailey's tropomyosin, while the other one is an association of the same compound with nucleic acid (Hamoir, 1950). Both compounds have been found homogeneous by electrophoresis and ultracentrifugation. They crystallize in different forms. The electrophoretic mobilities are the same for both compounds and are very close to the values of the small, quick peak observed in Fig. 4. Their ultracentrifugal behaviour is very different, as shown by the sedimentation-concentration curves (Fig. 5). Their ultraviolet absorption spectra show the typical difference already observed between a protein and the corresponding nucleoprotein. The yields of the preparations of nucleotropomyosin and tropomyosin do not amount to more than 0·07 and 0·03% respectively.

Isolation of tropomyosin from muscle without previous treatment with organic solvent confirms its existence as a natural component of the fibril.

Myosin of Szent-Györgyi. Data from the literature are somewhat contradictory about the existence of this protein in fish muscle extracts. Roth (1947) could not detect its presence. The electrophoretic pattern given in Fig. 3 shows, however, the presence of two peaks corresponding to the myosins. Comparison with patterns of rabbit myosins (Dubuisson, 1946 *a*) suggests that the slower peak corresponds to the β-myosin or myosin of Szent-Györgyi. Extractions of carp muscles with solutions of μ=0·5 and pH 5 allowed us to isolate by fractionation a compound having the properties of this protein. Its solution in

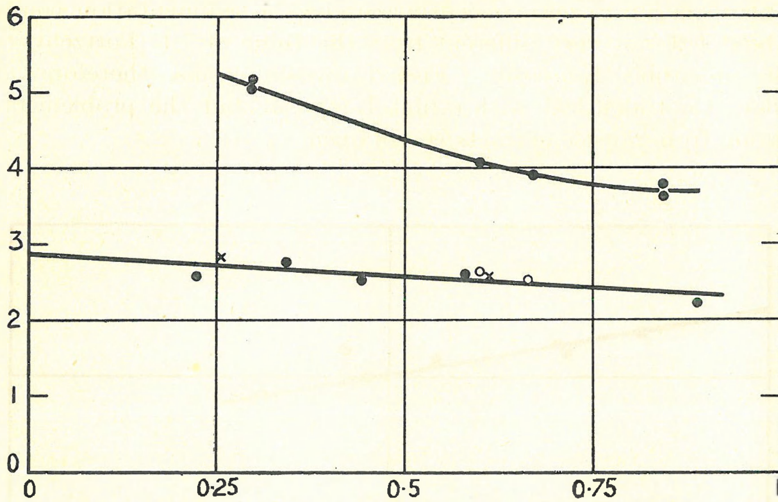

Fig. 5. Variation of S_{20} for carp tropomyosin and carp nucleotropomyosin with the concentration of the solution. Lower curve: tropomyosin. Upper curve: nucleotropomyosin.
O : values taken from Bailey *et al.* (1948) (rabbit).
X : values obtained by splitting of nucleotropomyosin at low pH.

saline was water-clear, did not show a decrease of viscosity by addition of adenosine triphosphate and was homogeneous by electrophoresis. The mobility corresponded to the value found for rabbit β-myosin (Hamoir, 1949). These results were, however, not confirmed by a further ultracentrifugal analysis. No compound sedimenting at the expected rate could be observed on a great number of extracts made in very different conditions. Addition of adenosinetriphosphate during the extraction, or shortening of the time of extraction, did not improve the results. More work is clearly necessary in this direction.

A sedimentation-concentration-curve of fish L-myosin can, however, be determined by ultracentrifugation of actomyosins in the presence of adenosinetriphosphate (Fig. 6). The solutions of twice-precipitated actomyosins were dialysed against the usual NaCl-phosphate buffer of μ=0·35 and pH 7·1; quantities of calcium ATP and magnesium chloride were added just before the ultracentrifugation so as to have a concentration of 0·5% calcium ATP and 0·01M-magnesium. The solutions were examined in the Svedberg oil-turbine ultracentrifuge, using the Philpot optical system (Philpot, 1938) at 60,000 r.p.m. The concentration of the proteins was determined refractometrically, before the addition of ATP, assuming a specific refractive increment of 0·00180. The values of the sedimentation constants have been corrected to 20° and to a water basis, using the partial specific volume given by Svedberg & Pedersen (1940) and the viscosity and density correction for ATP determined by myself.

Extrapolation to zero concentration gives a sedimentation constant of about 6·9, not very different from the value of 7·1 (Portzehl *et al.*, 1950) of rabbit L-myosin. Fish L-myosin seems, therefore, very similar, if not identical, with rabbit L-myosin, but the problem of its isolation from muscle extracts is still open.

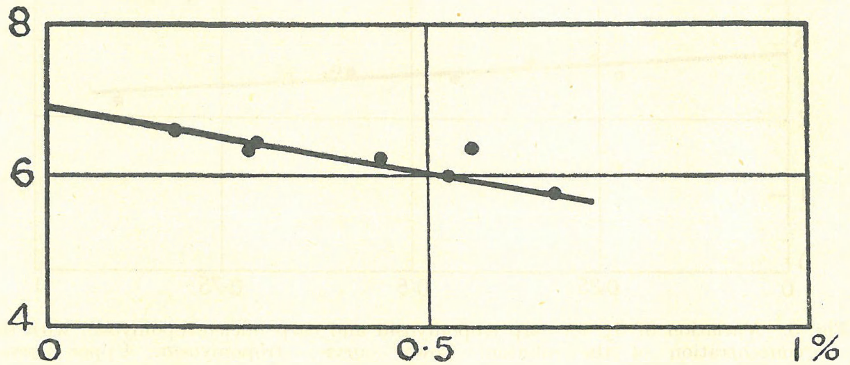

Fig. 6. Variation of S_{20} for carp L-myosin with the concentration of the solution.

Actomyosins. All the fish myosins described so far in the literature are in fact actomyosins. Some of the properties of fish actomyosins have been investigated recently. Snow (1950) has studied their denaturation by freezing. Insolubilization proceeds very quickly in the gel and slowly in the solution. Rapid freezing causes much less denaturation than slow freezing. Above the temperature of the cryohydric point of the salt used to dissolve the actomyosins, no denaturation occurs, whereas below that point denaturation is rapid. A comparison of the denaturation by freezing of actomyosin gels *in vitro* and of muscle *in situ* has been made by Subba Rao (1948). The influence of the speed of freezing and the duration of cold storage is the same in both cases, but denaturation occurs more slowly in muscle. The myogens are not influenced by freezing (Subba Rao, 1948). The value of such investigations to the preservation of fish, the elucidation of the physical state of the myosins in muscle and the curious contracture observed during thawing (Crepax & Herion, 1950) is obvious.

Subba Rao (1948) has studied the solubility and the water-retaining capacity of haddock actomyosin gels at varying pH values. A minimum is observed at pH 5·4 and a rapid increase at lower and higher pHs. Electrotitration curves of actomyosins in presence of 0·5 M-KCl from haddock, cod, lemon sole, skate and dogfish were also carried out. The maximum acid- and base-binding capacities found are given and compared with the same values for rabbit myosins (Dubuisson, 1941) in the following table (Table 3).

Table 3. *Maximum binding capacities of fish actomyosins and of rabbit myosins dissolved in* 0·5M-KCl

Species	Maximum binding capacity in 10^{-5} eq./g. protein		Authors
	Acid range	Alkaline range	
Haddock	163	165	Subba Rao (1948)
Cod	162	165	Subba Rao (1948)
Lemon sole	160	165	Subba Rao (1948)
Skate	172	177	Subba Rao (1948)
Dogfish	169	180	Subba Rao (1948)
Rabbit	156	180	Dubuisson (1941)

Some data have also been obtained by the study of the actomyosins by electrophoresis and ultracentrifugation (Hamoir, unpublished). The mobilities of twice-precipitated fish actomyosins are not influenced by the conditions of extraction (long or short duration, pH 5 or 8, fresh muscles or kept for weeks in frozen state). The mean values of many experiments are $-2{\cdot}83$ ($\alpha = -2{\cdot}88$; $\beta = -2{\cdot}78$) $\times 10^{-5}$ cm.^{2}/volt/sec. on the ascending side and $-2{\cdot}59 \times 10^{-5}$ cm.^{2}/volt/sec. on the descending one in the phosphate-NaCl buffer of pH 7·1 and $\mu = 0{\cdot}35$ previously described. If the charges of the particles are independent of the conditions of extraction, their dimensions seem to be very influenced. Extraction at $\mu = 0{\cdot}5$ and different pHs shows that the sedimentation constants of the particles decrease when the pH of extraction decreases. Several peaks can be observed in preparations made at low pH, while extractions of 10 or 60 minutes with 0·5M-KCl and 0·03M-$NaHCO_3$ (pH of extraction 7–8) always give single peak preparations. Some extractions have been made under these conditions from muscles which have been kept frozen for different periods of time; the corresponding sedimentation-concentration-curves are given in Fig. 7.

No difference is observed between actomyosin extracted for ten minutes from fresh muscles and actomyosin extracted for sixty minutes from muscles kept frozen for different periods of time; but a definitely lower rate of sedimentation can be observed with an actomyosin extracted for ten minutes from carp muscles kept for three weeks in frozen state. If the reciprocal value of S is taken as ordinate instead of S, linear relations are obtained for each preparation corresponding to the equation $1/S = 1/S_0 + kc$. Extrapolation to $c = 0$ gives values of $1/S$ very near to zero or even slightly negative. Values of S_0 seem,

therefore, to be very high and cannot be determined at present. Fish actomyosins appear to be very similar to the rabbit actomyosins (Dubuisson, 1946 *a*, *b*) from the point of view of their electrochemical behaviour but notably different in their size or shape.

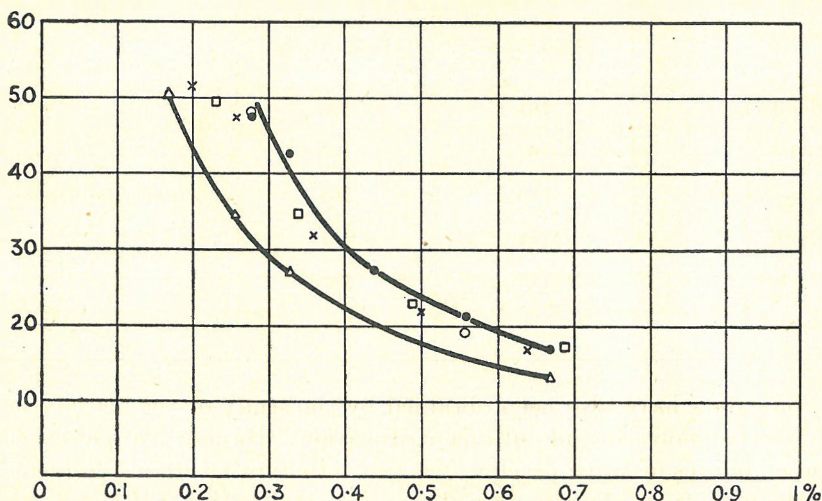

Fig. 7. Variation of S_{20} for carp actomyosins prepared by (*a*) ten-minute, or (*b*) one-hour, extraction of muscles kept frozen for different periods of time : (*a*) 2 days □ ; 21 days △ ; (*b*) 5 days × ; 24 days ○ ; 38 days ●.

Stroma proteins. The very low content in stroma proteins of fish muscle suggests that this residue is formed from connective tissue. As no investigation has been carried out recently on the stroma proteins, readers are referred to the section on proteins from connective tissue.

C. *Quantitative estimation of fish muscle proteins*

The two preceding sections have shown that much of the data relating to the quantitative estimation of fish proteins are dubious. Smith's fractionation scheme is out of date and some of the results obtained by this method by Dyer *et al.* (1950) or by Subba Rao (1948) are incorrect. Only the most reliable values will be given here, together with the description of the methods used. Values for mammalian muscle, obtained under similar conditions, have been added for comparison (Table 4).

All the determinations of stroma proteins have been carried out by exhaustive extractions with dilute hydrochloric acid (Smith, 1937). The albumin content of cod muscle (Dyer *et al.*, 1950) was determined by dialysis against distilled water of the extract previously diluted tenfold to remove myosins. Total protein was obtained by Subba Rao (1948) by trichloracetic precipitation of the finely minced muscle (Latapie mincer, 1 mm. holes). Different methods have been used

by him for the determination of the albumins after exhaustive extraction of the muscle at pH 7 with 7% LiCl : (*a*) The extract was dialysed free of salt ; the precipitate separated by centrifugation was redissolved in 10% KCl at pH 7. The residue was taken to be denatured albumin and added to the protein content of the dialysed extract. (*b*) The extract was diluted twenty times with cold water. The precipitate was redissolved in neutral 10% KCl and the residue taken to be denatured albumin, while the solution was dialysed free of salt. The protein content of the dialysed solution and the denatured albumin gave the albumin content. Good agreement was found between these two methods. The nitrogen values were expressed in protein by multiplying by 6·025.

Table 4. *Content and partition of nitrogen in fish muscle*

Species	Total N (in per cent. of the wet weight)	Total coagulable N (in per cent. of the wet weight)	Stroma proteins (in per cent. of the total coagulable N)	Albumins (in per cent. of the total coagulable N)	Authors
Haddock	2·91	2·54	3	15	Subba Rao (1948)
Cod	3·14	2·50	3	15	Subba Rao (1948)
Cod	2·90	2·50	3	13	Dyer *et al.* (1950)
Lemon Sole	3·03	2·75	4	16	Subba Rao (1948)
Skate	4·25	2·73	8	—	Subba Rao (1948)
Dogfish	4·94	2·71	10	17	Subba Rao (1948)
Rabbit (white muscle)	2·9	2·54	17	22	Weber & Meyer (1933)
Rabbit (white muscle)	—	—	16	9	Smith (1937)

Fish muscles differ from mammalian muscles in their very low content of connective tissue and perhaps in their content of albumins. The difference in stroma proteins presented by fishes of the elasmobranch class and the teleosts is remarkable. Much could probably be learned from other analytical determinations. As present knowledge of fish proteins is now on a safer basis for further analytical work, it is hoped that progress will soon be made in this direction. The systematic use of an improved fractionation scheme is, however, not to be recommended in view of the possibility of variation in solubility or extractability of the proteins in different species. A preliminary study of the best conditions of isolation in the case considered will always be a necessary step for accurate analytical work.

D. *Nutritive value of fish muscle proteins*

Although it is not intended to discuss this problem here, it seems worth while to point out some recent work in this direction. A book on the nutritional value of fish and the different ways in which this food can be prepared has been written by Rudolph (1946 ; see also Rudolf, 1947). An analysis of British Columbia fish-and-meat meals have been carried out by March & Biely (1948). The influence of canning on the amino-acid content of fish food has been investigated by Dunn *et al.* (1949), and by Neilands *et al.* (1949). This treatment apparently does not alter the amino-acids present.

II. FISH ENZYMES

The enzyme from fish tissues which has received the most attention during recent years is thiaminase, which destroys thiamine by cleaving the pyrimidylmethylene group from the thiazole portion. A short review on thiaminase has been written by Abderhalden (1947). Its distribution has been examined by Jacobson & de Avezedo (1958 *a*, *b*) and particularly by Neilands (1947). The last author has determined the thiaminase activity of muscle and viscera of a large number of aquatic animals from Nova Scotia, showing that the enzyme when present exists in both tissues. It has been found in many fresh-water fishes, one marine teleost, the herring and some invertebrates. The activation and the inhibition of the enzyme by several amines and the splitting of an isoster of thiamine, called neopyrithiamine, have been described by Sealock & Davis (1949) and Sealock & White (1949). Important data could be gained in this way concerning the mechanism of action of the ferment.

A study of carbohydrate metabolism in different dolphin tissues has been made by Dubois, Geiling, McBride & Thomson (1947). The enzymic reactions examined were quantitatively the same in dolphin as in rat tissues. Adaptation of these mammals to an aquatic environment does not noticeably influence their carbohydrate metabolism. Among the enzymes of glycogenolysis, special attention has been given by different workers to the adenosinetriphosphatases. The apyrase activity of homogenized muscle tissue of different vertebrates were determined at different temperatures by Steinbach (1948, 1949). No relationship could be observed between normal environmental temperatures of the animals and the temperature coefficients found ; fishes of cold and warm water showed a definitely lower coefficient than the other animals independent of their ecological milieu. The adenosinetriphosphatase activity of myosins from marine animals has been investigated by Humphrey (1949), while Roche *et al.* (1949) have isolated from carp muscles the soluble adenosinetriphosphatase recently described by Kielley & Meyerhof (1948) and have

described the influence of some metallic ions on its activity and of the activity of the adenosinetriphosphatase associated with the myosins.

The optimum temperature of cholinesterase activity prepared from Torpedo's electrical tissue has been determined by Ghiretti (1948). But a much more important contribution to the cholinesterase and other esterase activity in different tissues of bony fishes, sharks and even myxine has been made by Augustinsson (1948) in an extensive study in comparative enzymology.

Since the pyloric caeca of fish possess proteolytic activity, Norman, Cooke & Chowdhury (1948) have suggested their use as substitutes for commercial bait.

III. FISH BLOOD PROTEINS

Since no general investigation appears to have been carried out recently on these proteins, only limited aspects will be considered.

The effect of temperature upon antibody production in cold-blooded vertebrates has been investigated by Bisset (1948). No lyzozyme could be found by Caselli & Melluci (1947) in fish serum. The cholinesterase and other esterase activity of the plasma and erythrocytes of different species of fish have been determined by Augustinsson (1948) in his extensive study in comparative enzymology. Papers have also been published by Fontaine and collaborators on the antigonadotropic action of the serum of the eel (Fontaine & Callamand, 1947), the iodine content of the salmon and the lamprey during migration (Fontaine & Leloup, 1950 *a*, *b*, *c* ; Leloup, 1949 *a*, *b*) and the lipase content of the salmon during migration and of different species of fish (Fontaine & Callamand, 1949 *a*, *b*).

IV. PROTEINS FROM REPRODUCTIVE ORGANS

The action of proteolytic enzymes and protaminase on salmine has been carefully reinvestigated by Portis & Altman (1947), while Block, Bolling, Gershon & Sober (1949) have described a simplified procedure for the preparation of salmine and clupein ; the purity of the preparations obtained was checked and their amino-acid content determined quantitatively. The amino-acid composition of commercial salmine has also been determined by Hamer & Woodhouse (1949).

The molecular weight of sturin prepared from the sperm of sturgeon has been determined by Plaskeev, Yaroveirko & Passynski (1948) and a value of 2,700 was found. Nuclear proteins in selachians have been investigated by Cardoso & Piro (1947).

The pharmacological action of these proteins will not be considered here.

V. PROTEINS FROM CONNECTIVE TISSUE

Very little is known about the proteins which form the connective tissue. However, some progress has been made on fish collagen, for Salo (1948) has determined its arginine and histidine content, while Loofbourow, Gould & Sizer (1949) have measured its absorption spectrum. No absorption characteristic of phenylalanine, tyrosine and tryptophan could be detected. Collagen from the skin of the whale has been isolated by Ichimaro (1944) and some analyses have been carried out on this protein. A method of preparation and crystallization of procollagen from different sources mainly from the skins of different animals has been given by Orekhovich & Plotnikova (1948) and Orekhovich, Tustanovsky & Plotnikova (1948). The highest yields of procollagen are obtained from fish skins, but its isolation from the swim-bladder has also been reported. The isolation of gelatin and a collagen-like protein from herring scales has been described by Block, Horwith & Bolling (1949).

Comparative Biochemistry of Fish Proteins

Many of the comparative studies which have been made on fish proteins concern mainly the enzymes, and as they involve very special problems and are more closely related to comparative metabolism than to the comparative biochemistry of fish proteins, they will not be considered here. Very little seems to have been done so far on the comparative study of tissue proteins. Apart of the work of Labhart, Süllman & Viollier (1947) on lens proteins, comparative studies in this field have only been carried out on muscle (Jacob, 1945 ; Cigada, Citherio, Ranzi & Tosi, 1948 ; Subba Rao, 1948 ; Hamoir, 1949). A first contribution to the comparative biochemistry of tissue proteins can now be attempted by comparison with the muscle proteins of different classes of vertebrates.

The scientific importance of the study of fish proteins becomes still more evident when considered from this angle. As fish represent the lowest classes of vertebrates and mammals the highest, a comparison of fish and mammalian proteins should allow one to draw very general conclusions on the proteins of the vertebrates. Such a comparison will also contribute to bridge the gap existing between our knowledge of muscle chemismus and physiology based upon studies on frogs and of muscle protein chemistry which has developed mainly from studies on rabbits.

In comparing different animals which have been more or less completely studied (fish, amphibia, mammalia), it is important to make the distinction between qualitative and quantitative variation. One qualitative difference only has so far been observed and this concerns the myogens ; this has already been described in the case of carp and

rabbit muscle. Data concerning frog myogens (Jacob, 1945 ; Roth, 1947) are still very imcomplete, but they show that this group of proteins is much more related to the fish myogens than to the mammalian myogens. An important distinction can therefore be made between cold-blooded and warm-blooded vertebrates.

Other differences of minor importance are observed between fish and frogs or cold-blooded and warm-blooded vertebrates. The formation of actomyosins during the extraction is much quicker in the case of fish muscle than in the case of any other animal (Guba, 1943). The proportion of stroma proteins in muscle, the content of the myosin of Szent-Györgyi in the extracts, and the differences in sedimentation constants of the actomyosins allow us also to characterize the muscle proteins. But striking similarities are also observed. Fish myosins and the rabbit myosins have the same electrophoretic mobilities. Fish tropomyosin has been found to be practically identical with rabbit tropomyosin by the different methods used (Bailey, 1948 ; Hamoir, 1950). The first lineaments of a common protein pattern underlying muscular contraction is beginning to appear through the wealth of data already acquired.

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