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Studies on the Protein of Fish Skeletal Muscle

4. ULTRACENTRIFUGAL ANALYSIS OF CODLING EXTRACTS*

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There is very little information about the ultracentrifugal behaviour of fish-muscle protein and virtually none for marine species. Hamoir (1955*a, b*) has shown the ultracentrifugal diagram of an extract of carp muscle made at low ionic strength and also presented data on certain purified carp myosin and actomyosin fractions and on the crystallized proteins myoglobin (from carp and tuna), tropomyosin and nucleotropomyosin (both from carp). In addition, the sedimentation coefficients of a number of crystalline proteins derived from the myogens of carp (Henrotte, 1952, 1954, 1955; Hamoir, 1957) and of flounder (Henrotte & Dresse, 1955) have been published.

It was therefore of interest to supplement the electrophoretic data previously obtained on codling extracts (Connell, 1953) with ultracentrifuge data. It was also hoped to elucidate some of the problems of the existence in fish extracts of a protein corresponding in its sedimentation behaviour to that of rabbit myosin. Roth (1947) was unable to detect the presence of L-myosin (Schramm & Weber, 1942) in extracts made from carp muscle which normally extract this protein from rabbit muscle, and only components corresponding to S-myosin or actomyosin could be observed. Nevertheless, electrophoretic components corresponding to β -myosin are present in rapidly made extracts of both carp (Hamoir, 1949, 1951) and codling (Connell, 1954). However, with the usual myosin-isolation procedures only heavy or polydisperse preparations were obtained by these authors from such extracts. Some of these results appeared to be due to inattention to a number of factors, e.g. the absolute freshness of the tissue from which the

extract was obtained, the rapidity of working up and examining the extract, the temperature and the pH. Special attention has therefore been paid to such details of procedure. In particular, it is now known that rabbit myosin suffers an apparently irreversible aggregation and may be kept in a monodisperse condition for only a few hours at room temperature or a few days at 0° (Laki & Carrol, 1955; Holtzer, 1956; Holtzer & Lowey, 1956; Rupp & Mommaerts, 1957); cod myosin is even more unstable (J. J. Connell, unpublished work) and its handling poses special problems. Since myosin at or below its isoelectric point is denatured in the presence of salts (Edsall, 1930), acid-extracting solutions as used by Hamoir (1955*a, b*) have been avoided.

The electrophoretic and ultracentrifugal diagrams of extracts of increasing ionic strength were compared; also the amount of protein extracted, the ATP-sensitivity [Portzehl, Schramm & Weber, 1950; see also Table 3 (footnote) of the present paper] and sometimes the actin-combining power of the extracts were measured to indicate when myosin or actomyosin, or both, were being extracted.

EXPERIMENTAL

Materials

Live cod (*Gadus callarias* L.) have been used for all these experiments. The fish were never more than about 40 cm. in length.

Buffers. Buffers of pH 7.5 and 6.5 were made up respectively as follows: 0.00338 M-KH₂PO₄ + 0.0155 M-Na₂HPO₄ (I 0.05) and 0.0217 M-KH₂PO₄ + 0.0095 M-Na₂HPO₄ (I 0.05). Potassium chloride was added to these buffers to bring the total ionic strength up to 0.1, 0.2, 0.3 and 0.5.

* Part 3: Connell, J. J. (1954).

Methods

Electrophoresis. The methods used have been described by Connell (1953).

Viscosity. The methods used have been described by Connell (1954).

Ultracentrifuging. The Spinco Model E was used. The temperature of the runs was kept between 0 and 5° by using a pre-cooled rotor; the temperature was controlled and measured by means of the Spinco Rotor Temperature Indicator and Control Unit. The validity of the large temperature correction was tested on several runs made between 0° and 5° with crystalline bovine plasma albumin (Armour Laboratories, Chicago) when a value of $S_{20,w}$ equal to 4.34 at zero concentration and pH 4.34 was obtained. This figure is in excellent agreement with the published values (for summary see Loeb & Scheraga, 1956). The sedimentation coefficients of the components in extracts were corrected for the variation in the viscosity of water between the temperature of the determination and 20°, but not for the viscosity increment of the solutes or for the buoyancy of the protein. Since the viscosity increment of the mixture of solutes used is small at low ionic strengths and becomes increasingly negative at 1.03 or greater, omission of these corrections will result in an error of at most 5% in the corrected sedimentation coefficients. Sedimentation coefficients are quoted as S_{20} .

Extraction. All operations were conducted at 0° and as rapidly as possible. The fish were stunned by a blow on the head, decapitated and then skinned and filleted. The fillets were coarsely minced and the mince slowly stirred with the buffer for periods of 5 and 15 min. and 7 and 24 hr. The ratios of mince to extractant were as follows: 1.0.05 and 0.1, 1:1½; 1.0.2, 1:2; 1.0.3 and 0.5, 1:3. The mixtures were then centrifuged at 10 000 *g* for 10–15 min. and the supernatant solution was decanted. The supernatant solutions from the 5 and 15 min. and 24 hr. extracts were ultracentrifugally examined immediately; the 7 hr. extracts were allowed to stand overnight at 0° before examination. The solutions for electrophoretic examination were dialysed for 2 days at 0° against the same buffer as was used for extraction. In general, each extract was analysed only once.

Analysis of schlieren photographs. The apportioning into separate components of the total area of the schlieren photographs from the electrophoretic and sedimentation experiments was done by Tiselius & Kabat's (1939) method. The mean result of the top and bottom edges of the bar diaphragm tracings of the sedimentation diagrams was used with subtraction of approximate base-lines determined from runs without protein. The areas of the sedimentation diagram were corrected for the dilution occurring in a sector-shaped cell by means of the formula: $A_0 = A(x/x_0)^2$, where A_0 is the area used to assess the proportion of the component, A is the area of the component on the tracing, x is the distance of the maximum ordinate of the component from the centre of rotation and x_0 is the distance of the meniscus from the centre of rotation.

RESULTS

Extracts at an ionic strength of 0.05, pH 7.5. The sedimentation diagrams of extracts taken at 5 and 15 min. and at 7 hr. differed little. They all showed

a very small, rapidly sedimenting component F (Fig. 1*a*) and three relatively slow main components S_1 , S_2 , S_3 (Fig. 1*b*). The fast component remained as a very sharp boundary throughout the period of sedimentation but sedimented with different velocities in different extracts, in contrast with the main components (Table 1). The amounts of all the components showed little variation (Table 2). Sometimes trace components could be detected, especially one moving slightly faster than the fastest of the main groups of components.

The electrophoretic diagram of extracts of several hours' duration has been published before (Connell, 1953) and shows seven clearly distinguishable components (Fig. 2). The numbering of these components is that used by Connell (1953). The fastest electrophoretic components in 5 and 15 min. extracts appear to be present in diminished amount (Table 2); they are absent from extracts of 1 min. duration (Fig. 3). More protein is certainly extracted after 7 hr. stirring than after 5 or 15 min. (Table 3).

Extracts at ionic strengths of 0.1 and 0.2, pH 7.5. These were qualitatively similar and will be dealt with together. The sedimentation diagrams of 5 min. extracts (Fig. 4) showed, in addition to two rapidly sedimenting components (F_1 and F_2) a heavier very polydisperse component H, which contributed as much as 25% of the total area of the diagram of the extract at 1.0.1. The main components were much the same as in extracts at 1.0.05 (Table 1), though there do appear to be increases and decreases in the amounts of the heavier and lighter components respectively as both the ionic strength and duration of extraction increase (Table 2).

It was noticed that the extracts, which were initially quite fluid and only faintly opalescent, became gradually more viscous and cloudy on standing at 0°. The extracts at 1.0.1 eventually gelled, so that the flask containing them could be

Fig. 1. Sedimentation diagrams of a 5 min. extract at I 0.05, pH 7.5 (a) 480, (b) 7812 sec. after reaching full speed of 59 780 rev./min. Temperature 2.3°. F and S_1 , S_2 , S_3 , denote the fast and slow components respectively.

Table 1. *Sedimentation coefficient of components of codling-muscle extracts, pH 7.5*

Ionic strength of extract	Duration of extraction	Concn. of total protein in extract (%)	S_{20} of component* (s)				
			Fast components		S_1	S_2	S_3
0.05	5 min.	2.5	93	—	6.4	4.4	1.3
0.05	15 min.	2.5	—	33	7.5	4.3	1.0
0.05	7 hr.	3.2	59	—	6.2	4.3	1.2
0.10	5 min.	2.9	64, 53	30	6.8	4.7	1.3
0.10	15 min.	3.0	59	—	†	4.5	1.4
0.10	7 hr.	3.1	57	—	6.8	4.4	1.2
0.20	5 min.	2.0	80, 50	30	7.0	4.9	1.5
0.20	7 hr.	2.5	66	—	†	4.8	†
Arithmetical mean	—	—	—	—	6.6	4.5	1.3
S.D.	—	—	—	—	0.49	0.25	0.17

* For designation of components see Figs. 1 (a) and 1 (b).

† The component had not been resolved sufficiently for an accurate determination.

Table 2. *Composition of codling-muscle extracts assessed by sedimentation and electrophoretic analysis, pH 7.5*

		Component* (percentage of total area)														
		Sedimentation†				Electrophoresis										
Ionic strength of extract	Duration of extraction	Fast components	S ₁	S ₂	S ₃	1		sp	2		3	4		5	6	7
0.05	5 min.	3	17	56	24	4	4		11			42			39	
0.05	15 min.	3	17	55	25	3.5	3.5		10			35			48	
0.05	7 hr.	7	16	57	20	4	6		8	7		9	25		30	11
0.10	5 min.	5	19	60	16	8			10			22			60	
0.10	15 min.	4	24	60	14											
0.10	7 hr.	8	22	54	16	1	10		12			77				
0.20	5 min.	15	21	50	14	7	1		30			62				
0.20	7 hr.	16	20	50	14	11			10			22		57		

* For designation of components see Figs 1 (a), 1 (b) and 2.

† Corrected for dilution in a sector-shaped cell as described in the text.

Fig. 2. Ascending-limb electrophoresis diagram of a 7 hr. extract at I 0.05, pH 7.5; 7.35 v/cm.; 4140 sec. δ denotes the ascending-limb anomaly, and the designation of the components is that used by Connell (1953). Fig. 3. Ascending-limb electrophoresis diagram of a 1 min. extract at I 0.05, pH 7.5; 6.34 v/cm.; 3870 sec. For designation of components see Fig. 2.

tilted through 45° before the gel broke. On shaking the gel it became fluid, but although becoming lumpy on standing never set completely to a gel again: it was not truly thixotropic. It was also noticed that on the addition of adenosine triphosphate (ATP) to the initial fluid extract a copious precipitate appeared which could be dissolved in neutral 0.5M-KCl. When the extracts were made up to 0.5M-KCl before addition of ATP a large reduction in viscosity occurred (Table 3). These reactions towards ATP are characteristic of the superprecipitation and dissociation of actomyosin in the gel and sol states respectively (Szent-Györgyi, 1947), and it must be concluded that actomyosin is in fact being extracted in quantity from the fish muscle at ionic strengths as low as 0.1. The actomyosin becomes eventually insoluble, as is shown by the absence of the heavy polydisperse component from 5 and 15 min. extracts which had

stood until they had gelled. Although 7 hr. extracts had a high ATP-sensitivity (Table 3) no very heavy polydisperse component could be observed in their sedimentation diagrams; this indicates that the actomyosin has become polydisperse during extraction or standing so that it no longer forms a discrete peak, although not aggregated enough to be sedimented at 10 000 g. The extracts at *I* 0.1 took about 2–3 hr. to gel, and those at 0.2 about 5–6 hr. or overnight, which indicates that the incipient solubilization process is greater at the higher ionic strength.

The extraction of actomyosin at such a low ionic strength appears to be dependent on the absolute freshness of the fish muscle, since no indications of the presence of actomyosin were found in extracts of *post rigor* muscle at *I* 0.2 either in sedimentation diagrams or from ATP-sensitivity measurements. That the presence of ATP is necessary for the extraction of actomyosin under these conditions was indicated in an experiment in which portions of the same lot of *post rigor* muscle were extracted for the same length of time with solvent *I* 0.2, both in the presence and absence of 3 mM-ATP (25 mg. of ATP + 7.5 ml. of buffer and 5 g. of muscle). The presence of small amounts of the heavy polydisperse component was noted in the solution containing the ATP, but not in the other.

The resolution of components during the electrophoresis of fish-muscle extracts at *I* 0.1 and 0.2 is poor, and it is hardly possible to decide whether changes in the protein composition of the solution are occurring as extraction proceeds or whether altering the ionic strength has resulted in a different

protein composition. This problem has been briefly discussed by Connell (1953). The analyses which can be made (Table 2) show that no gross changes are occurring. Only slightly greater amounts of protein can be extracted if the ionic strength is increased to 0.2 (Table 3).

Extracts at an ionic strength of 0.3, pH 6.5 and 7.5. Only 5 min. and 24 hr. extracts have been examined. Neither of these extracts or others at a higher ionic strength showed any tendency to gel. The sedimentation diagram of the 5 min. (pH 7.5) extract showed only traces of rapidly sedimenting material, and that of the 24 hr. (pH 7.5) extract rather more; the sedimentation coefficients of

Fig. 4. Sedimentation diagram of a 5 min. extract at *I* 0.10, pH 7.5; 480 sec. after reaching full speed of 59 780 rev./min. Temperature 3.3°. H denotes the polydisperse and *F*₁, *F*₂ the fast components respectively.

Table 3. Amount of protein extracted and ATP-sensitivities of various extracts of codling muscle

Ionic strength of extract	Duration of extract	pH	Percentage of total protein extracted	ATP-sensitivity* of extract after making up to 0.5 M-KCl
0.05	5 min.	7.5	21	Not determined
0.05	15 min.	7.5	21	Not determined
0.05	7 hr.	7.5	27	Not determined
0.1	5 min.	7.5	20	68
0.1	15 min.	7.5	21	42
0.1	7 hr.	7.5	22	119
0.2	5 min.	7.5	25	21
0.2	7 hr.	7.5	31	36
0.3	5 min.	7.5	26	10
0.3	24 hr.	7.5	42	21
0.3	5 min.	6.5	16	11
0.3	27 hr.	6.5	32	12
0.5	5 min.	7.5	26	11
0.5	24 hr.	7.5	53	81

* ATP-sensitivity is defined by Portzehl *et al.* (1950) as follows:

$$\frac{\log \eta_{\text{rel.}} - \log \eta_{\text{rel., ATP}}}{\log \eta_{\text{rel., ATP}}} \times 100,$$

where $\eta_{\text{rel.}}$ and $\eta_{\text{rel., ATP}}$ are the relative viscosities of the solution before and after addition respectively of ATP.

these heavy components were in the region of 25–30s. The main portion of the diagram differed markedly from those of extracts at lower ionic strength in showing a remarkably sharp boundary M (Fig. 5a), which in the 5 min. extract retained its extreme sharpness during the whole of the run (143 min.). This alteration in diagram was shown not to be solely a consequence of the increase in ionic strength on sedimentation behaviour, by increasing the ionic strength of an extract at 0.2 up to 0.3; the sedimentation diagram of this solution did not differ from that of the extract of *I* 0.2. On diluting the 5 min. (*I* 0.3) extract with buffer of the same ionic strength, component M was resolved into

Fig. 5. Sedimentation diagrams of a 5 min. extract at *I* 0.30, pH 7.5 (a) 8580 sec., (b) 4080 sec. after reaching full speed of 59 780 rev./min. (b) is the extract diluted with an approximately equal volume of extracting solution. Temperatures: (a) 2.1°, (b) 2.1°. M denotes myosin.

a single sharp peak (Fig. 5b). It could be precipitated from the solution by reducing the ionic strength to about 0.05; the sedimentation diagram of the main group proteins remaining in solution was very like that of extracts at *I* 0.05–0.2. The precipitated protein had the properties of a true myosin, i.e. it had a very small or zero ATP-sensitivity and combined with actin (Table 4); the original extract also showed actin-combining power (Table 4).

The values for the sedimentation coefficient of this 'myosin' in extracts (Table 5) also definitely

Fig. 6. Sedimentation diagram of the precipitate obtained by decreasing to 0.05 the ionic strength of a 5 min. extract at *I* 0.3, pH 7.5. The precipitate was redissolved in KCl-phosphate buffer, *I* 0.4, pH 7.5; 5160 sec. after reaching full speed of 59 780 rev./min. Temperature 1.0°. A, T, D and M denote actomyosin, trimeric, dimeric and monomeric myosin respectively.

Table 4. *ATP-sensitivity and actin-combining power of a codling-muscle extract and of a precipitated protein*

Duration of extract, 5 min. (*I* 0.3, pH 7.5); the protein was precipitated from the extract when *I* was decreased to 0.05. Conditions for ATP-sensitivity: 0.45M-KCl + phosphate buffer (*I* 0.05, pH 7.5); myosin:actin ratio, 3:1.

	$\eta_{rel.}$				ATP-sensitivity	
	Alone	With ATP	With actin*	With actin and then ATP	Without actin	With actin
Extract	1.67	1.57	2.13	1.69	14	44
Precipitated protein	2.06	2.00	2.76	2.61	4	35

* Prepared from cod according to Connell (1954).

Table 5. *Sedimentation coefficients of myosin components in codling-muscle extracts*

Duration of extract	Ionic strength	pH	Concn. of total protein in extract (%)	S_{20} of component (s)		
				Myosin	Dimer	Trimer
5 min.	0.3	6.5	1.0	4.5	—	—
5 min.	0.3	7.5	1.5	4.5	—	—
24 hr.	0.3	6.5	1.8	4.2	—	—
24 hr.	0.3	7.5	1.4	4.9	5.4	—
24 hr.	0.3	7.5	0.7	6.0	7.4	8.2
5 min.	0.5	6.5	0.8	5.5	6.4	—
5 min.	0.5	7.5	1.3	3.5	—	—
24 hr.	0.5	7.5	0.4	6.2	7.1	—

establishes it as an L-myosin, since the values for this protein vary from about 4 to 7s, whereas those for S-myosin vary from about 10 to 90s (Portzehl *et al.* 1950). On isolation, however, it appears to undergo the same type of spontaneous aggregation shown to occur with rabbit myosin by Holtzer & Lowey (1956), and what are presumably the dimer and trimer are always present in fish myosin prepared by precipitation (Fig. 6), even in preparations analysed only 3–4 hr. *post mortem*. The presence of a heavy polydisperse component ($S_{20}=14$), presumably actomyosin (A in Fig. 6), is often detected in preparations having an ATP-sensitivity. This component and the ATP-sensitivity can be reduced in amount by precipitating it after adjusting the ionic strength to about 0.27. The aggregation of the myosin is very rapid and occurs overnight even in a 5 min. ($I\ 0.3$) extract undisturbed at 0° , and the presence of myosin aggregates is also evident in the 24 hr. extract at $I\ 0.3$.

No component corresponding to myosin could be found in extracts of *post rigor* muscle, but it was

Fig. 7. Ascending-limb electrophoresis diagram of a 5 min. extract at $I\ 0.3$, pH 7.5; 2.07 v/cm.; 19 980 sec. S and M denote the sarcoplasmic group of proteins and myosin respectively.

Fig. 8. Sedimentation diagram of a 24 hr. extract at $I\ 0.5$, pH 7.5; 3780 sec. after reaching full speed of 52 640 rev./min. Temperature 0.9° . A denotes actomyosin; M is possibly myosin and its aggregates.

present in extracts of muscle from fish 3–4 hr. dead, i.e. before the first signs of rigor mortis were apparent. On the other hand it has not so far been detected in extracts of cod muscle rapidly frozen *pre rigor* and then extracted during the thawing process. This contrasts with Crepax & Hérion's (1950) finding on rabbit muscle.

The myosin in 5 min. extracts at $I\ 0.3$ appeared as a typical β -myosin peak in the electrophoretic diagram (Fig. 7).

There is a considerable increase in the amount of protein extracted at $I\ 0.3$ between 5 min. and 24 hr. (Table 3), but there is only a moderate increase in ATP-sensitivity, so that most of the protein further extracted is probably myosin. As judged from the sedimentation diagrams the amount of myosin in the extracts does increase up to as much as one-half of the total protein content.

Extracts at pH 6.5 were also examined in the hope that they would contain smaller amounts of actomyosin, but, although the total protein extracted was considerably less, the ATP-sensitivity of even a 5 min. extract was 11 (Table 3). The ATP-sensitivity of myosins prepared from 5 min. extracts with a pyrophosphate-containing solution of pH 6.5 and $I\ 0.35$ had been found to be much less than this (Connell, 1954), but it has been subsequently discovered that the presence of pyrophosphate bound to the myosin preparation interferes with the ATP-sensitivity test and results in anomalously low results. The properties of extracts at pH 6.5 were otherwise like those at pH 7.5.

Extracts at an ionic strength of 0.5, pH 7.5. The 5 min. extracts at $I\ 0.5$ were very much like the corresponding extract at $I\ 0.3$ in respect of amount of protein extracted (Table 3), ATP-sensitivity (Table 3), sedimentation diagram and electrophoretic diagram, though the last diagram suggested that more myosin is extracted at the higher ionic strength. The 24 hr. extract had the typical high opacity, viscosity and ATP-sensitivity of a solution rich in actomyosin. The actomyosin was revealed in the ultracentrifuge as an extremely sharp boundary sedimenting at about 11.5s (Fig. 8). The presence of myosin and its aggregates is also suggested in this diagram.

DISCUSSION

The electrophoretic and sedimentation data on the extracts of low ionic strength suggest that there is a differential extractability of the protein of the muscle. The amounts of the components of greater electrophoretic mobility or of greater sedimentation velocity appear to increase as the time of extraction or ionic strength is increased. This perhaps reflects some differing accessibility in the muscle structure, though the extraction equilibrium must be established quite quickly.

It appears that in some circumstances fish actomyosin can be appreciably soluble at low ionic strength. For example, the initial extracts of fresh muscle made at low ionic strengths are quite clear; also the actomyosin in such extracts sediments at a rate (about 60s) which suggests that its particle weight, though large, is well within the range generally attributed to dissolved colloidal substances. However, the protein soon aggregates and becomes insoluble. No account of similar experiments with extractants of low ionic strength and different types of muscle appears to have been published; so it is not known whether this behaviour is a peculiarity of fish muscle. Hamoir (1955*a*) has shown that the precipitation of carp actomyosin is complete at a lower ionic strength (0.25) than that of rabbit actomyosin (0.30), and Roth (1947) gives the salting-in of carp actomyosin as beginning at *I* 0.24; so that fish actomyosins prepared by precipitation from long extracts at *I* 0.5 are more soluble than is the rabbit protein, but certainly insoluble at *I* 0.2 or less.

The amount of codling actomyosin appearing in rapidly made extracts diminishes as the ionic strength is increased from 0.1 to 0.3, although actomyosin may be said to be truly extractable only at *I* 0.5 or greater. The particle weight of the actomyosin soluble at low ionic strength is greater than that soluble at *I* 0.5, since their rates of sedimentation at approximately the same concentration are about 60 and 10–20s respectively. The reason why the actomyosin of larger particle weight dissolved from very fresh muscle should be soluble at an ionic strength at which actomyosin of smaller particle weight is insoluble is not known.

Another feature of the extracts which may be bound up with the unusual solubility of actomyosin is the appearance of myosin only when an *I* 0.3 is reached and the simultaneous virtual disappearance of the rapidly sedimenting components after their amount had been increasing as the ionic strength was raised from 0.05 to 0.2.

The nature of the other rapidly sedimenting components in low-ionic-strength extracts is unknown. Their sedimentation coefficients appear to be grouped approximately around three values of about 30, 50–65 and 80–90s, though the number of experiments is not large enough to be certain that these values can be associated with three distinct components. The component sedimenting with a sharp boundary at about 50–65s occurs most often; it is possible that this may be identified with F-actin since it is said to sediment at about 50–65s (Portzehl *et al.* 1950; Johnson & Landolt, 1950; Mommaerts, 1951).

The three main ultracentrifugal components of extracts of low ionic strength compare quantitatively

with these described for carp by Hamoir (1955*a*), who quotes values from three extracts at *I* 0.15 of 6.86–7.26, 4.75–5.11 and 1.44–1.70s for the rates of sedimentation and 7–9, 36–51 and 40–57% for the proportions of the components. Although the rate of sedimentation of these three molecular-weight groups seems to be similar for carp and cod, their amounts in the two species differ considerably. Hamoir (1955*a, b*) has remarked on the large amounts in carp of the components sedimenting at about 1.5s, which indicates an unusually low average molecular weight. Similar low-molecular-weight proteins are evidently present in cod, as they seem to be in frog but not in rabbit (Deuticke, 1934; Amberson, Erdős, Chinn & Ludes, 1949).

The ultracentrifuge experiments at *I* 0.3 and 0.5 establish that myosin substantially free of its oligomers is present in extracts of fish muscle. Hamoir (1955*a, b*) detected β -myosin in a number of different kinds of extract of carp; but on isolating this component he always found that it gave rise to multiple peaks in the ultracentrifuge, although it was apparently pure by electrophoresis. It seems certain from the present results that these preparations consisted of mixtures of myosin and its aggregates, which are apparently not separable by the techniques of electrophoresis used. The heavy polydisperse component observed in the cod myosin prepared with a pyrophosphate extractant (Connell, 1954) was probably also an aggregated or denatured protein, since several days had elapsed before it could be analysed.

The properties of the structural proteins of fish muscle thus fall a little more closely in line with those of rabbit. There are nevertheless important differences, particularly in stability. It appears that fish myosin cannot be precipitated in the ordinary way without aggregating, neither can it be kept longer than about 12 hr.; rabbit myosin is stable for at least three days (Holtzer & Lowey, 1956) and is normally prepared by precipitation.

The minimum ionic strength for extraction of cod myosin has been confirmed at 0.3, or perhaps slightly lower. This figure is much lower than that quoted for carp by Hamoir, namely 0.45. The reason for this difference is not apparent.

SUMMARY

1. Extracts of codling muscle with solvents of pH 6.5 and 7.5 and of ionic strengths from 0.05 to 0.50 have been analysed by electrophoresis and ultracentrifuging and the results compared with previous data.

2. There are indications of a differential extractability of the proteins soluble at low ionic strength (0.2 or less).

3. Actomyosin is extractable from very fresh codling muscle at ionic strengths as low as 0.1 and pH 7.5, but quickly becomes insoluble. It is not extractable from fish under the same conditions after rigor mortis.

4. Monodisperse myosin is present in extracts of codling muscle made at an ionic strength of 0.3 or higher, pH 6.5 and 7.5.

5. Codling myosin is extremely labile and rapidly aggregates either on standing in the cold or during precipitation.

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The Charged Groups at the Interface of Some Blood Cells

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It has been implied that, apart from their quantitative differences in charge density, the ionized groups comprising the electrophoretic surface of foreign particles or cells suspended in plasma are similar and are probably derived from adsorbed protein. This conception is unsatisfactory in that it fails to explain the quite different colloidal properties of the formed elements of blood suspended in their common protein pool at a common bulk pH. Abramson, Moyer & Gorin (1942) emphasize the unusual fact that erythrocytes do not appear to adsorb protein, a property which, from the biological point of view, may help to explain many of their unusual properties. Thus erythrocytes do not ordinarily stick to one another, they do not adhere like leucocytes to glass, they survive for many weeks in a circulation constantly and efficiently

being filtered of most other particles by reticulo-endothelial cells, and although they represent a huge surface area they do not, apparently, accelerate the clotting reaction in shed blood. With these simple biological properties in mind, it was thought worth while to investigate the nature of the ionized groups responsible for the surface charge of the various blood cells and particles added to blood, and perhaps to correlate any differences or similarities with their known biological properties. It was thought that lymphocytes, for instance, since they are not phagocytic, do not aggregate with polymorphonuclear leucocytes (Jago, 1956) and do not stick to glass, might have ionized groups similar to those found on an erythrocyte. On the other hand, it was thought probable that polymorphonuclear leucocytes, platelets and glass