Studies on the Proteins of Fish Skeletal Muscle

1. ELECTROPHORETIC ANALYSIS OF CODLING EXTRACTS OF LOW IONIC STRENGTH

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Relatively little is known of the characteristics of fish muscle proteins, though a few preliminary studies have been made. Roth (1947) has demonstrated that the 'classical' muscle protein fractions of some freshwater fish resemble in their general properties the corresponding fractions of rabbit or frog muscle. Using the methods of the Liège Laboratory, Hamoir (1949, 1951*b*) has examined several types of carp muscle extract by electrophoresis and ultracentrifugation, and has compared the results with those from rabbit muscle. Hamoir (1951*a, c*) has also described one of the few well-characterized fish muscle proteins, nucleotropyomyosin; this appears to be a nucleic acid derivative of Bailey's (1948) tropomyosin.

Reay (1935), Reay & Kuchel (1936), and more recently Subba Rao (1948), and Dyer, French & Snow (1950) have quantitatively analysed skeletal muscle of several marine species of fish by methods based on Bate-Smith's (1937) scheme.

Detailed electrophoretic studies of extracts of low ionic strength, such as are described in this paper, have been carried out on rabbit muscle by Jacob (1947, 1948) and Bosch (1951).

EXPERIMENTAL

Electrophoresis was conducted in the Tiselius apparatus at $+0.5^\circ$. The Philpot-Svensson diagonal edge optical system was used throughout. All the results were obtained using the tall-form cell, and the flow-through modification of Kekwick, Lyttleton, Brewer & Dreblow (1951) has been used to facilitate repetition of runs.

Codling (*Gadus callarias* Linn.) were used exclusively. The fish (30–50 cm. in length) were caught from November to May a few miles off Aberdeen, and rested in a shore tank. They were killed by decapitation, and then skinned and filleted in the commercial fashion. The fillet represents the whole of the musculature on both sides of the fish. Occasionally, fish were anaesthetized by urethane before decapitation in order to prevent struggling, but the electrophoretic patterns obtained from such fish were not noticeably different from those of non-anaesthetized fish, nor were the patterns obtained from fish which had been exsanguinated via the tail. The fillets were immediately transferred to a cold room (0°) and finely minced. All subsequent operations were carried out at 0° .

The mince was stirred for 1–3 hr. in phosphate buffer (1–3 ml./g. mince, depending upon ionic strength), and centrifuged at 4000 g for 30 min. Generally, one-half to

two-thirds volume of clear supernatant was obtained in this way. The extract was then dialysed in cellophan tubes for about 48 hr. with frequent changes of outer liquid. Normally the extracts ($I \leq 0.2$) remained quite clear, and only small amounts of protein precipitated on dialysis. Before electrophoresis the extract was always centrifuged at 4000 g for 30 min., and, if necessary, the N content was adjusted by dilution to below 4 mg./ml. The conductivity of the outer dialysis liquid, which was used as supernatant in the electrophoresis cell, was measured at 0° . The values of the extract and supernatant were measured with the glass electrode at 10° ; recorded pH values are the mean of these values.

Fig. 1. Electrophoretic diagram of an extract of codling skeletal muscle at 0.05 *I*, pH 7.46. (a) ascending limb; (b) descending limb. Exp. 43/52: field strength, 7.35 V./cm.; duration of electrophoresis, 4140 sec.

RESULTS

Extracts were made at 0.05, 0.1 and 0.2 *I* in order to find the best conditions for electrophoresis, and to observe any changes in protein composition with changes in ionic strength. These extracts contain the globulin X, myogen and myoalbumin fractions of muscle. The pH was kept close to 7.5 throughout.

Extracts at 0.05 I (Fig. 1). Extractant and diffusate fluid: 0.0156 M- Na_2HPO_4 + 0.0035 M- KH_2PO_4 , pH 7.5 (1 ml./g. mince).

The more obvious components have been numbered arbitrarily from 1 to 7 in decreasing order of mobility. There was no simple correlation between the diagrams of codling and rabbit, therefore neither the nomenclature of Jacob (1947) nor that of Bosch (1951) has been used. It is possible to divide the codling diagram into the same general mobility groups of I–III as for the rabbit and frog diagrams (Jacob, 1945, 1947). Group III, with the highest mobility, separates very quickly from the initial boundary, and comprises in the present case only one small and usually diffuse peak, 1. Group II, of average mobility, subdivides into three peaks, 2, 3 and 4. Between groups III and II is situated an ill-defined region of refracting material, the top of which lies almost parallel to the base-line. Following Jacob (1947) this region was designated *sp* (space), contributing up to about 7 % of the total area of the

contributions of the components to the total area of the diagram for several analyses are shown in Table 1.

All the components in both limbs moved anodically at pH 7.5, consequently there was no difficulty in accounting for the stationary δ or ϵ boundaries. The results of the present experiments bear no relation to the protein concentration, to small variations in pH or to the duration of electrophoresis. The variations between individual values of the amount of the same component were fairly large and probably outside the experimental error, but were least for experiments with the same extract or with the same fish, which indicates that the variations may be physiological in nature. Variations in the electrophoretic composition of rabbit muscle extracts have been explained on the same basis (Jacob, 1947; Bosch, 1951).

Fig. 2. Tracing of ascending limb diagram of Exp. 43/52 showing construction of approximate error curves.

diagram. In most cases, there was a suggestion of a small component in *sp* somewhat faster than 2, but it was too ill-defined to warrant separate designation. Group I subdivides into peaks 5–7, and contributes about 65 % of the total area. A small part of the turbidity of the extracts migrated with 1 in both limbs; in the ascending limb, most of the turbidity migrated with 2, the apex of 2 sharply marking the edge of the turbidity; in the descending limb, the turbidity migrated with 2 and 3, increasing from 2 to 3. Especially on prolonged electrophoresis, further separation of peaks between 5 and 6 occasionally occurred, and the gradual tailing off of the slowest part of the diagram indicated the presence of small components slower than 7. That the division into these seven components did not fully represent the complexity of the diagram is illustrated by Fig. 2 in which the ascending side of Exp. 43/52 has been analysed by constructing approximate error curves (Svedberg & Pedersen, 1940; Wiedemann, 1947). There is a large degree of arbitrariness in the selection of appropriate error curves for the complex diagrams met with here, and the disposition and number of the overlapping curves varies from one diagram to another. The simpler perpendicular extrapolation method (Tiselius & Kabat, 1939) has therefore been used for estimating the proportions of the components. The

The pronounced variations in the amount of 2 may be due to another factor which becomes evident when aged or partially heat-denatured extracts are examined. Extracts which have been kept at 0° for longer periods than about 7 days, or at room temperature for about 18 hr. became more opaque, but very little more protein could be removed from them by centrifugation. The diagrams of such modified extracts appear normal except for a pronounced increase in the proportion of 2, and small and variable decreases in the proportions of all the other components (Table 2). Most of the turbidity of aged extracts migrated precisely with 2. Jacob (1948) also noted an increase in the turbidity of group II, whilst Bosch (1951) found that the only change brought about by ageing was a decrease in the slowest component of group I. It is possible that denaturation in phosphate buffers is associated with the formation of a complex whose mobility coincides with that of 2; the large variations of 2 in 'normal' extracts might then be accounted for by incipient denaturation.

Extracts made at ionic strengths sufficient to bring the actin-myosin group into solution (0.3–0.5 *I*) and then dialysed to 0.05 *I* (Exp. 36/52) gave diagrams similar to those of extracts at 0.05 *I*, though the low protein content made precise analysis difficult.

Table 1. *Composition of codling extracts, 0.05 I*
(A, ascending diagram; D, descending diagram.)

Exp. no.	pH	N content (mg./ml.)		Component, % total area							
				1	<i>sp</i>	2	3	4	5	6	7
23/52*	7.49	3.58	A	2.9	7.3	8.3	6.6	8.6	24.2	30.8	11.3
			D	2.1	4.8	6.0	6.9	12.3	26.2	30.4	11.3
24/52*	7.49	3.58	A	—	—	7.1	6.8	7.8	25.9	32.7	11.7
			D	4.0	3.7	7.9	7.2	11.4	25.5	29.4	10.9
36/52†	7.54	0.89	D	5.9	6.0	11.4	12.0		22.2	42.5	
41/52	7.40	4.10	A	—	7.0	7.6	5.2	8.4	25.7	32.8	10.3
			D	3.4	4.3	7.9	6.0	8.9	27.4	31.5	10.6
43/52	7.46	3.30	A	3.5	6.6	10.4	7.6	7.9	22.4	26.5	15.1
			D	4.5	7.0	12.5	7.0	10.7	24.9	22.2	11.2
57/52‡	7.55	3.10	A	2.5	6.6	4.7	6.8	9.6	26.0	31.2	12.6
58/52	7.58	3.31	A	2.6	5.4	5.0	6.6	9.1	26.1	33.6	11.6
			D	3.2	6.1	7.9	8.7	10.3	25.7	27.8	10.3
59/52‡	7.59	3.61	A	2.4	7.2	6.3	7.0	8.3	27.7	30.9	10.2
			D	3.0	5.2	5.4	7.2	10.8	25.8	31.3	11.2
60/52	7.59	3.04	D	4.1	7.1	6.1	6.6	8.7	24.9	31.6	10.9
Arith. mean				3.5	5.9	7.6	6.9	9.5	25.4	30.2	11.4
s.d.§				1.0	1.2	2.3	0.8	1.4	1.3	2.9	1.3

* Same extract.
† Same fish.
‡ Obtained by dialysis of an extract at 0.3 I.
§ Standard deviation = $\sqrt{[\Sigma d^2/(n-1)]}$.

Table 2. *Composition of partially denatured extracts 0.05 I*
(A, ascending diagram; D, descending diagram.)

Exp. no.	pH	N content (mg./ml.)		Component, % total area							
				1	sp	2	3	4	5	6	7
42/52*	7.43	3.90	A	0.9	7.4	13.8	5.9	6.8	25.0	29.8	10.4
			D	1.6	5.3	13.4	6.2	10.9	25.6	26.9	10.3
44/52†	7.42	3.60	A	2.4	7.7	17.5	8.6		21.1	42.7	

* 7 days at 0°.
† 18 hr. at room temperature.

Fig. 3. Electrophoretic diagram of an extract at 0.1 I, pH 7.45. (a) ascending limb; (b) descending limb. Exp. 2/52: field strength, 4.47 V./cm.; duration of electrophoresis, 12060 sec. The lines X denote the position on the cell faces of engravings which are used as markers.

Extracts at 0.1 I (Fig. 3). Extractant and diffusate fluid: 0.0312 M- Na_2HPO_4 + 0.007 M- KH_2PO_4 , pH 7.5 (1 ml./g. mince).
The diagrams as a whole were less clearly defined than those at 0.05 I. Group III was very diffuse, and in most cases could only be estimated immediately after separation from the initial boundary.

In group II, 3 did not separate clearly and appeared to be partly associated with both 2 and 4, whilst in group I, 7 did not separate at all on the descending side.
Evaluation of the diagrams (Table 3) shows that the leading components (1-4) contribute less and the slower components (5-7) more to the total area than

Table 3. *Composition of codling extracts, 0.1 I*

(A, ascending diagram; D, descending diagram.)

Exp. no.	pH	N content (mg./ml.)	Component, % total area								
			1	<i>sp</i>	2	3	4	5	6	7	
3/51	7.5	—	A	—	—	3.7	4.3	7.8	27.9	35.6	12.0
			D	3.4	5.3	5.3	9.8	29.1	47.1		
4/51	7.5	—	A	—	—	8.4		8.4	28.4	36.1	12.7
			D	—	2.9	4.8	10.5	27.0	51.8		
6/51	7.5	—	A	—	—	7.4		9.7	26.5	37.8	9.7
			D	—	5.9	5.3	6.4	27.4	52.0		
2/52	7.45	5.10	A	—	—	2.9	4.3	7.4	27.0	39.4	10.4
			D	2.4	6.1	6.1	8.7	26.9	49.8		
Arith. mean				2.9	5.0	15.2			27.5	37.2	11.2

in the case of 0.05 I extracts. This is probably a consequence of the boundary anomalies rather than an actual change in composition of the extract, since extracts at 0.1 I when dialysed to 0.05 I lose little protein and have similar composition to 0.05 I extracts. The moving boundary theories of Svensson (1946) and Dole (1945) predict that the amounts of leading and slowest ions, as judged from their refractive index increments, will be diminished and enhanced respectively on passing from lower to higher ionic strength at equivalent protein concentration. The same tendency is shown at 0.2 I though the interpretation of the diagrams at this ionic strength is somewhat ambiguous.

Extracts at 0.2 I (Fig. 4). Extractant and diffusate fluid: 0.1 M - KCl + 0.0312 M - Na_2HPO_4 + 0.007 M - KH_2PO_4 , pH 7.5 (2 ml./g. mince).

An extract at 0.05 I dialysed to 0.2 I (Exp. 49/52, Table 4) gave diagrams which were very similar in appearance to those obtained with 0.2 I extracts and therefore it was concluded that the gross differences between extracts at 0.2 I and at 0.05 and 0.1 I were due to the influence of increased ionic strength on electrophoretic separation rather than to differences in the protein composition of the extracts. However, solutions at 0.2 I do extract more protein than solutions at 0.05 I, and on dialysis of an 0.2 I extract to 0.05 I about one-quarter of the protein is precipitated. It has not been possible to apportion the extra protein extracted at 0.2 I among the components with great exactitude. Both the proportions of group II and of 6 and 7 appear to increase in relation to the total at 0.2 I. It is possible that group II may represent in

Fig. 4. Electrophoretic diagram of an extract at 0.2 I, pH 7.41. (a) ascending limb; (b) descending limb. Exp. 4/52: field strength, 3.94 V./cm.; duration of electrophoresis, 13320 sec.

The diagrams showed much less detail than those at 0.05 or 0.1 I, even after prolonged electrophoresis. Group III was again diffuse and group II consisted of only one distinct peak, with which the turbidity of the extract moved precisely. In group I, 5 and 6 could be distinguished but 7 separated on only one occasion. The evaluation of these extracts is shown in Table 4.

part the myosin group of proteins since an electrophoretic component with the properties of myosin, and which travels at the rate of group II, becomes evident at 0.3 I (Connell, unpublished). Even at 0.2 I a small amount of protein is dissolved from a washed actomyosin gel by phosphate buffer and, although difficult to observe in the electrophoresis apparatus, it travels with a mean mobility between

Table 4. *Composition of codling extracts, 0.2 I*
(A, ascending diagram; D, descending diagram.)

Exp. no.	pH	N content (mg./ml.)		Component, % total area							
				1	<i>sp</i>	2?	3?	4?	5	6	7
4/52*	7.41	4.20	A	3.5	6.4	8.6			27.3	54.2	
			D	3.1	18.6			21.8	56.5		
33/52	7.44	2.20	A	5.1		16.0			23.5	55.4	
			D	5.1		17.0			23.2	54.7	
47/52	7.32	2.30	A	5.0	4.0	9.9			27.1	54.0	
			D	5.0	5.1	11.6			28.4	49.9	
49/52†	7.44	3.30	A	4.8	6.0	7.2			28.5	53.5	
			Arith. mean‡			4.6	16.2			25.2	54.1

* 1 ml. extractant per g. muscle. † Extract at 0.05 I dialysed to 0.2 I. ‡ Excluding Exp. 49/52.

Table 5. *Correction of ascending mobilities*

(A, ascending mobility; C, corrected ascending mobility; D, descending mobility; Δ_a and Δ_d are the total traced areas on the ascending and descending sides, respectively.)

Exp. no.	Δ_a	Δ_d	δ	ϵ	Mobility ($10^5 \text{ cm.}^2 \text{ V.}^{-1} \text{ sec.}^{-1}$)							
	Arbitrary units				1	2	3	4	5	6	7	
41/52 (0.05 <i>I</i>)	488	447	160	31	A	13.9	8.2	7.0	5.7	4.9	3.6	2.7
					C	10.9	6.6	5.4	4.5	3.5	2.7	1.8
					D	10.8	6.7	5.5	4.5	3.2	2.1	1.5
6/51 (0.1 <i>I</i>)	415	401	57	27	A	9.5	5.4		3.7		2.7	
					C	9.3	4.8		3.5		2.5	
					D	9.4	4.2		3.2		2.1	
49/52 (0.2 <i>I</i>)	493	454	93	24	A	7.6		4.7	3.6		2.8	
					C	7.1		4.2	3.3		2.5	
					D	7.4		3.9	3.0		2.3	

that of groups I and II. On the basis of isoelectric points Jacob (1947) has suggested that group II may represent myosin.

Mobility determinations. In all these analyses, but especially at 0.05 and 0.1 I, the ascending and descending diagrams showed a pronounced non-enantiography, and the stationary boundaries contribute up to 30 % of the total area of the tracings on the ascending side and 5–7 % on the descending side. Mobilities on the ascending side were consequently appreciably higher than on the descending side, the difference being particularly large with 6 and 7. The boundaries on the ascending side were always sharp, but on the descending side considerable spreading and overlapping of adjacent boundaries occurred, so that in some cases accurate mobility determinations could not be made. In order to use the ascending boundaries for mobility determinations the correction derived by Longworth & MacInnes (1940) was applied where the traced areas of the ascending and descending diagrams were similar enough to justify it. The

observed mobility of any component, *j*, on the ascending side is corrected by the factor

$$\frac{A_{ja} + A_{\epsilon}(V_{jd}/V_{ja})}{A_{jd} + A_{\epsilon}}$$

where A_{ja} , A_{jd} and A_{ϵ} are the traced areas of the ascending *j* boundary, descending *j* boundary and ϵ boundary, respectively. V_{ja} and V_{jd} are the volumes swept through by the ascending and descending *j* boundaries, respectively. This correction is approximate only since it takes no account of pH, conductivity and viscosity changes at each boundary, but the general agreement between the descending and corrected ascending mobilities suggests that it is reliable. Table 5 gives three examples of the application of the correction.

In general, the mobilities of the slowest components tend to be undercorrected. The results of several experiments at 0.05, 0.1 and 0.2 I are given in Table 6. In these experiments there appears to be no correlation between mobility and electrical field strength.

Table 6. *Mobilities of codling muscle components at pH 7.5*(M, Arithmetical mean of corrected ascending and descending mobilities. S.D., standard deviation = $\sqrt{[\Sigma d^2/(n-1)]}$.)

<i>I</i>	Electrical field (V./cm.)	No. of experiments		Mobility ($10^5 \text{ cm.}^2 \text{ V.}^{-1} \text{ sec.}^{-1}$)						
				1	2	3	4	5	6	7
0.05	4.0-7.5	10	M	10.7	6.7	5.6	4.4	3.6	2.6	2.0
			S.D.	0.45	0.36	0.37	0.16	0.34	0.41	0.35
0.1	3.2-9.0	4	M	8.9	4.5	3.5		3.0	2.1	
			S.D.	0.46	0.63	0.59		0.45	0.41	
0.2	3.3-4.7	5	M	7.1	3.8			3.1	2.3	
			S.D.	0.51	0.40			0.26	0.22	

The number of experiments is sufficiently large to show graphically the dependence of mobility upon ionic strength (Fig. 5). For the faster components 1 and 2, and possibly 3 and 4, the dependence resembles that found by Tiselius & Svensson (1940) for ovalbumin and can be satisfactorily explained on the basis of the Debye-Hückel-Henry theory. The mobilities of 5 and 6 are, however, greater at 0.2 *I* than at 0.05 or 0.1 *I*. This deviation may be due to the change from a purely phosphate buffer at 0.05 and 0.1 *I* to a potassium chloride-phosphate buffer at 0.2 *I*; Davis & Cohn (1939) observed that pronounced effects on electrophoretic mobility were brought about by changes in ionic environment.

Fig. 5. Influence of ionic strength on electrophoretic mobility of codling skeletal muscle constituents.

The myogen and globulin X fractions. On dialysis for 2-3 days of a 0.05 *I* extract to a conductivity of $3 \cdot 10^{-5} \Omega^{-1} \text{ cm.}^{-1}$, about one-third of the protein precipitated and could be easily separated by centrifugation. This insoluble fraction corresponds to the globulin X of Weber & Meyer (1933). After washing with distilled water, dispersion in half the original volume of 0.05 *I* phosphate buffer, pH 7.5, and dialysis to equilibrium against the same buffer, only about one-third of the globulin X could be brought into solution again. The insoluble portion was removed completely only by centrifuging at

20 000 *g*. A little more of the insoluble portion was soluble in 0.3 or 0.5 *I* potassium chloride solution, but it undoubtedly consisted mainly of irreversibly denatured protein. The portion which was soluble in

Fig. 6. Myogen fraction of codling skeletal muscle. (a) ascending limb; (b) descending limb. Exp. 56/52: 0.05 *I*; pH 7.62; field strength, 6.23 V./cm.; duration of electrophoresis, 4080 sec. Fig. 7. Globulin X fraction of codling skeletal muscle. Ascending limb. Exp. 54/52: 0.05 *I*; pH 7.61; field strength, 6.34 V./cm.; duration of electrophoresis, 3600 sec.

0.05 *I*, and the completely water-soluble fraction (myogen) after equilibrium dialysis against 0.05 *I* phosphate buffer, pH 7.5, gave the electrophoresis diagrams shown in Figs. 6 and 7.

Table 7. *Composition and mobilities of the myogen fraction* 0.05 I phosphate, pH 7.5*

	1	<i>sp</i>	3	4	5	6	7
% of total area†	1.4	3.4	2.4	9.6	37.6	33.1	12.4
Mobility $10^5 \text{ cm}^2 \text{ V}^{-1} \text{ sec}^{-1}$ †	7.2-10.3	—	6.0	4.5	3.6	2.6	2.2

* Average N content: 2.00 mg./ml.

† Average of four experiments.

The myogen diagram is similar to that of the original extract with the exception that 2 is undetectable. The components can be identified almost exactly by their mobilities, apart from 1, whose mobility varied from 7.2 to $10.3 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ sec}^{-1}$ in four experiments (Table 7).

An examination of the average composition of the myogen fraction (Table 7) and of the average protein contents before and after complete removal of salts by dialysis reveals that the absolute amounts of all the components, except perhaps 5, have decreased. Jacob (1947) observed a similar effect with rabbit-muscle extracts dialysed against distilled water, and noted the parallel with the euglobulins of blood serum (Raffel, Pait & Terry, 1940; Svensson, 1941).

The globulin X diagrams show three diffuse but quite distinct components *A*, *B* and *C* whose mobilities (Table 8) correspond to 1, 3 and 7, respectively. However, the proportionate losses of all the components on dialysis and the absolute amounts of *A*, *B* and *C* (Table 8) indicate that *B* and *C* partially represent 2 and 4, and 6, respectively. Also, the losses of all the components, except perhaps 7, cannot be accounted for by the absolute amounts calculated from the globulin X diagram, and therefore nearly all the components must suffer some denaturation. The duration of dialysis cannot wholly explain this denaturation, since only small amounts of protein are precipitated from normal 0.05 I extracts on prolonged dialysis against 0.05 I phosphate buffer.

Table 8. *Composition and mobilities of the globulin X fraction* 0.05 I phosphate, pH 7.5*

	<i>A</i>	<i>sp</i> ?	<i>B</i>	<i>C</i>
% of total area†	5.7	6.4	31.0	56.9
Mobility. $10^5 \text{ cm}^2 \text{ V}^{-1} \text{ sec}^{-1}$ †	10.6	—	6.0	2.1

* Average of two experiments.

† Average N content: 0.90 mg./ml. ($\approx 0.45 \text{ mg./ml.}$ in Tables 1 and 7).

DISCUSSION

Whereas rabbit muscle proteins can be analysed satisfactorily by electrophoresis at 0.15 I, the present work has shown that lower ionic strengths must be used in order to resolve the components of codling muscle extracts satisfactorily. Extracts of codling muscle at 0.15 I were intermediate in character between those at 0.1 and 0.2 I, and showed almost as little resolution as extracts at 0.2 I. Good resolution was obtained at 0.05 I, and

this ionic strength is being used in routine work on fish muscle proteins, although such low ionic strengths are disadvantageous for quantitative electrophoretic analysis because of boundary anomalies, especially at relatively high protein concentrations. Because of this, the analyses recorded here are not necessarily absolute, although useful for comparison. Even at 0.1 I, the boundary anomalies and asymmetries were almost as great as at 0.05 I.

The electrophoretic diagrams of extracts of codling muscle at low ionic strengths, although of equal complexity, show the same general characteristics as those of rabbit or frog muscle. The appearance of the faster components and components of intermediate mobility of codling suggests that they may be identified with Jacob's components *h*, *j*, *k*₁ and *k*₂, and Bosch's components 2, 3, 4 and 5, although the mobilities of the fish proteins are higher. The value and disposition of the mobilities of the slowest group of codling components, however, are sufficiently different to establish their non-identity with the members of the corresponding group of rabbit components. It would be interesting to work out this difference in terms of the muscle enzymes of rabbit and codling.

Jacob (1947) has attempted to correlate the electrophoretic components of rabbit muscle with the classical muscle proteins, and in general his conclusions apply to codling muscle. It is probable that group III represents myoalbumin; quantitatively this means that it is present as less than 1 % of the total codling muscle proteins (cf. Hamoir, 1951b). Dyer *et al.* (1950) found about 7 % of myoalbumin in cod muscle using Bate-Smith's (1937) method, but attempts to estimate this fraction (Connell, unpublished) by the same method failed to reveal significant amounts of acid-stable protein in codling. The globulin X of codling muscle is electrophoretically almost as complex as the total non-myosin proteins, and it is evident that the characterization of this type of fraction by simple loss of solubility on removal of salts is far from adequate.

SUMMARY

1. Extracts of codling skeletal muscle at 0.05, 0.1 and 0.2 I, pH 7.5, have been examined in the Tiselius electrophoresis apparatus.

2. Extracts at 0.05 I, pH 7.5, proved to be the most informative, and revealed the presence of at

least seven components which may be divided into Jacob's groups I, II and III.

3. Whereas groups II and III of codling bear similarities to the corresponding groups of rabbit, group I is quite different.

4. Group II appears to be particularly significant in relation to the denaturation of these protein mixtures.

5. The mobilities of codling muscle proteins are appreciably greater than those of rabbit.

6. The influence of ionic strength on the mobilities of these proteins has been examined.

7. On dialysis of a 0.05 *I* extract against distilled water, component 2 precipitates completely, and all the components, except perhaps 5, suffer a partial loss of solubility. The precipitate of globulin X is only partially re-soluble; the soluble portion shows three main components which on the basis of mobility can be related to components in the original extract. However, estimates of composition reveal that the relationship is complex.

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REFERENCES

- Bailey, K. (1948). *Biochem. J.* **43**, 271.
 Bate-Smith, E. C. (1937). *Proc. Roy. Soc. B*, **124**, 136.
 Bosch, M. W. (1951). *Biochim. biophys. Acta*, **7**, 61.
 Davis, B. D. & Cohn, E. J. (1939). *J. Amer. chem. Soc.* **61**, 2092.
 Dole, V. P. (1945). *J. Amer. chem. Soc.* **67**, 1119.
 Dyer, W. J., French, H. V. & Snow, J. M. (1950). *J. Fish. Res. Bd Can.* **7**, 585.
 Hamoir, G. (1949). *Bull. Soc. Chim. biol., Paris*, **31**, 118.
 Hamoir, G. (1951a). *Biochem. J.* **48**, 146.
 Hamoir, G. (1951b). *Biochem. Soc. Symp.* no. 6, p. 8.
 Hamoir, G. (1951c). *Biochem. J.* **50**, 140.
 Jacob, J. J. C. (1945). *Bull. Soc. Sci. Liege*, no. 4, 231.
 Jacob, J. J. C. (1947). *Biochem. J.* **41**, 83.
 Jacob, J. J. C. (1948). *Biochem. J.* **42**, 71.
 Kekwick, R. A., Lyttleton, J. W., Brewer, E. & Dreblow, E. S. (1951). *Biochem. J.* **49**, 253.
 Longworth, L. G. & MacInnes, D. M. (1940). *J. Amer. chem. Soc.* **62**, 705.
 Raffel, S., Pait, C. F. & Terry, M. C. (1940). *J. Immunol.* **39**, 317.
 Reay, G. A. (1935). *Rep. Fd Invest. Bd, Lond.*, no. 65.
 Reay, G. A. & Kuchel, C. C. (1936). *Rep. Fd Invest. Bd, Lond.*, no. 93.
 Roth, E. (1947). *Biochem. Z.* **318**, 74.
 Subba Rao, G. N. (1948). Physico-chemical investigations on the muscle proteins of some commercial species of fish caught in N.E. Scottish waters. Ph.D. Thesis, University of Aberdeen.
 Svedberg, T. & Pedersen, K. O. (1940). *The Ultracentrifuge*, p. 296. London: Oxford University Press.
 Svensson, H. (1941). *J. biol. Chem.* **139**, 805.
 Svensson, H. (1946). *Ark. Kemi Min. Geol.* **22A**, no. 10, p. 1.
 Tiselius, A. & Kabat, E. K. (1939). *J. exp. Med.* **69**, 119.
 Tiselius, A. & Svensson, H. (1940). *Trans. Faraday Soc.* **36**, 16.
 Weber, H. H. & Meyer, K. (1933). *Biochem. Z.* **266**, 137.
 Wiedemann, E. (1947). *Helv. chim. Acta*, **30**, 892.

The Lipids of Fish

1. CONTENT AND CONDITION OF LIPIDS IN THE FLESH OF THE HADDOCK (*GADUS AEGLEFINUS*)

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The series of papers which it is hoped to present under the general title 'The Lipids of Fish' will describe studies preparatory to investigations of the lipoprotein constituents of fish tissues. From the outset, therefore, considerable emphasis has been placed on the relative ease with which any particular lipid may be extracted from the tissue, since much of the evidence for the existence of lipid-protein complexes is based on the occurrence of so-called 'bound' lipids, i.e. lipids which cannot be extracted from the fresh tissue by solvents in which they are normally readily soluble. At the same time, segregation of constituents according to ease of

extraction may well assist in the separation of the extremely complex mixture of lipids present in the tissue.

The flesh of the haddock has been chosen as the most suitable raw material for the present series of studies, since it is quite free from adipose tissue, it can be obtained absolutely fresh in virtually unlimited quantity from this laboratory's own fishing vessel, and, the haddock being a small fish, it offers the advantage that lipid samples are usually representative of a very large number of individuals.

The present paper reports the results of successive extractions of haddock flesh with a range of solvents.