been applied to a urinary extract to which 17 α :20 β :21-trihydroxypregn-4-en-3-one (III) was added: reasonable recoveries of formaldehyde and 17-ketosteroid formed from III were obtained.

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Studies on the Proteins of Fish Skeletal Muscle

2. ELECTROPHORETIC ANALYSIS OF LOW IONIC STRENGTH EXTRACTS OF SEVERAL SPECIES OF FISH

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The comparative biochemistry of skeletal-muscle proteins has so far attracted only occasional and incidental attention. From the published work it is possible to make a few comparisons which, however, largely relate either to the fibrillar proteins or particular enzymes of a few types of animal muscle, e.g. rabbit, frog, carp, snail, rat, mouse and insect muscle. In particular, the electrophoretic properties of the fibrillar proteins from different

animals are rather similar (Weber & Portzehl, 1952; Hamoir, 1952). Of the proteins which are extractable with dilute salt solutions, only those from frog (Jacob, 1945; Dubuisson & Jacob, 1945), rabbit (Jacob, 1947; Bosch, 1951), carp (Hamoir, 1951) and human (Haan, 1952) muscle have been studied electrophoretically. The derived diagrams show some marked differences between different animals, but the results have been obtained under

such diverse conditions as to make detailed comparisons difficult. In a previous paper (Connell, 1953) suitable conditions for the electrophoretic analysis of fish-muscle protein extracts of low ionic strength have been described, and it was thought worth while to compare extracts from several species of fish under the same conditions.

EXPERIMENTAL

Methods. The conditions of electrophoresis, extraction of the muscle and dialysis have been described previously (Connell, 1953). The extractant was 0.05 *I* buffer (0.0156 M-Na₂HPO₄+0.0035 M-KH₂PO₄), pH 7.5 (1 ml./g. mince), and the same buffer was used for equilibrium dialysis and electrophoresis. The recorded pH values are the means of the values of diffusate fluid and protein solution measured at 10°. In the main, the mean pH values varied between 7.5 and 7.6, but in three experiments (68/52, 74/52 and 75/52) they were unaccountably higher than expected.

In some cases (whiting, hake, herring, plaice, lemon sole, turbot and carp) the extracts were quite turbid, and were given a final centrifugation at about 20000 *g* for 15 min before electrophoresis; in others (coalfish, herring, carp) the extract retained a pink colour due to blood or muscle pigments. Analysis of the electrophoretic diagrams by projection, tracing and dividing by perpendicular extrapolation was normally carried out only once.

In some diagrams protein components overlap or completely obscure the δ and ϵ boundaries, rendering accurate evaluation impossible, but an approximate result has been arrived at by the following procedure. It is assumed that the ϵ boundary constitutes 5% of the total area on the descending side, that it remains stationary during the course of electrophoresis, and that it 'spreads' to an extent determined from the average behaviour in experiments without overlapping. All these assumptions are very nearly true as judged from experiments without overlapping. It is then possible to obtain the 'true' areas of the components overlapping the ϵ boundary by subtraction, and thus to evaluate the whole of the descending side. If it is further assumed that the contributions of the components which overlap the stationary boundaries are the same on the ascending as on the descending side, then the area of the δ boundary may be calculated, and consequently the rest of the ascending diagram evaluated. This procedure was adopted with the diagrams of herring, grey skate, thornback skate and cuckoo-ray extracts, and the values for these species in Table 2 must be viewed with this in mind.

Mobilities on the ascending side have been corrected as described previously (Connell, 1953).

Materials. The following twenty species, representing several quite different biological types, have been examined:

Cod (and codling)	<i>Gadus callarias</i> L.
Haddock	<i>G. aeglefinus</i> L.
Whiting	<i>G. merlangus</i> L.
Coalfish	<i>G. virens</i> L.
Ling	<i>Molva molva</i> L.
Hake	<i>Merluccius merluccius</i> L.
Herring	<i>Clupea harengus</i> L.
Angler	<i>Lophius piscatorius</i> L.

Plaice	<i>Pleuronectes platessa</i> L.
Lemon sole	<i>P. microcephalus</i> Day
Witch	<i>P. cynoglossus</i> Day
Megrim	<i>Arnoglossus megastoma</i> Day
Turbot	<i>Rhombus maximus</i> Day
Common skate	<i>Raja batis</i> L.
Thornback skate	<i>R. clavata</i> L.
Cuckoo ray	<i>R. circularis</i> Day.
Lesser-spotted dogfish	<i>Scyliorhinus caniculus</i> L.
Pike	<i>Esox lucius</i> L.
Perch	<i>Perca fluviatilis</i> L.
Common carp, mirror carp	<i>Cyprinus carpio</i> L.

The names are taken from Jenkins (1936).

The marine species were caught throughout the years 1951-2, a few miles off Aberdeen; the pike and perch were caught in Loch Luichart, Ross and Cromarty, during April 1952; the common and mirror carps were commercial samples of unknown origin. Most of the fish were obtained alive, and in the other cases they were never more than a few hours dead. Unless stated otherwise, the fish were filleted and boned in the commercial fashion and the musculature from both sides used for extraction. In the case of the angler, only the flesh from both sides of the 'tail' was used, whilst only the flesh from the 'wings' of the three skates was used, the tails being omitted. No attempt was made to distinguish the sex or age of the individuals, but none was in any way abnormal in size and appearance.

RESULTS

The results of mobility determinations and of percentage composition are given in Tables 1 and 2, respectively. Occasionally, components failed to resolve in the descending limb and are therefore assessed together, e.g. coalfish, hake, ling, angler. Considerations related to the percentage composition of the diagrams are based on the assumption that the extracts represent essentially similar protein fractions from one species to another. In the case of codling, cod, haddock, dogfish, grey skate, angler, herring, turbot, perch and common carp, more than one individual of each species was examined, and the diagrams from individuals of the same species were found to be substantially the same. In all other cases only one individual was examined. Except with codling and haddock, detailed analysis by division into components was carried out on only one diagram from each species. However, variations between the individuals of one species, as exemplified by the results on codling (Connell, 1953), are small, and it can reasonably be assumed that the variations which occur with other species are also small. The electrophoretic diagrams are depicted in Fig. 1; only the ascending limb is shown. The diagram of both the carp extracts could not be reproduced or accurately traced because of the lack of clarity in the photographic plates, but an approximate tracing of the descending limb of the mirror-carp extract is shown in Fig. 2. Evaluation of the carp diagrams was not justifiable.

Table 1. *Mobilities of fish-muscle components*
(0.05 I phosphate buffer, 0.5°)

Expt. no.	Species	No. of fish	Mobility ($10^5 \text{ cm}^2 \text{ v}^{-1} \text{ sec}^{-1}$)							
			1	1a	2	3	4	5	6	7
7	Codling*	7	10.7±0.45	—	6.7±0.36	5.6±0.37	4.4±0.16	3.6±0.34	2.6±0.41	2.0±0.35
80/52, 80a/52	Cod	2† +	10.4 (10.3-10.4) 10.6 (10.5-10.6)	—	6.8 (6.7-6.8) 6.9 (6.8-6.9)	5.6 (5.5-5.7) 5.7 (5.5-5.8)	4.5 (4.3-4.6) 4.5 (4.4-4.5)	3.8 (3.7-3.8) 3.4 (3.3-3.5)	3.2 (3.0-3.3) 2.5 (2.4-2.6)	2.2 (2.0-2.3) 2.0 (1.9-2.0)
15/52, 16/52, 22/52	Haddock	3	9.2 (8.1-9.8) 8.6 (7.7-9.5)	—	6.7 (6.1-7.4) 6.2 (5.7-7.0)	5.2 (4.9-5.6) 5.1 (4.4-5.9)	4.2 (4.0-4.4) 3.8 (3.5-4.0)	3.4 (3.3-3.5) 3.2 (2.4-4.3)	2.6 (2.4-2.8) 2.0 (1.8-2.2)	1.6 (1.3-1.7) 1.3 (1.1-1.5)
65/52, 65a/52	Whiting	2	10.5 (10.3-10.8) 11.1 (11.0-11.2)	—	6.6 (6.4-6.8) 6.8 (6.7-6.9)	5.1 (5.1-5.1) 5.4 (5.3-5.4)	3.7 (3.6-3.8) 3.8 (3.7-3.9)	3.1 (3.0-3.2) 2.9 (2.8-2.9)	2.2 (2.0-2.3) 1.8 (1.7-1.8)	1.4 (1.3-1.5)
73/52	Coalfish	1	10.4 10.6	—	6.5 6.4	— —	4.2 4.1	3.6 3.1	3.0 2.7	2.6 1.9
66/52	Ling	1	8.4 8.8	6.4 6.5	5.1 5.1	4.3 4.1	3.3 3.2	3.1 2.5	2.3 1.6	1.9 1.6
67/52	Hake	1	10.3 10.3	—	6.8 6.4	5.4 4.8	4.0 3.3	3.1 2.3	2.5 1.3	1.7 1.3
64/52, 64a/52	Herring	2	10.5 (10.0-11.0) 11.1	—	5.5 (5.4-5.6) 5.3 (5.2-5.3)	4.4 (4.3-4.5) 4.1 (4.0-4.2)	3.6 (3.6-3.6) 3.2 (3.1-3.3)	2.5 (2.4-2.5) 2.3 (2.2-2.4)	1.4 (1.3-1.5) 1.5 (1.3-1.6)	0.0 (0.0-0.0) 0.0 (0.0-0.0)
68/52, 68a/52	Angler	2	— 11.0 (10.7-11.3)	7.5 (7.1-7.9) —	5.8 (5.7-5.9) —	4.8 (4.7-4.9) 5.1 (5.0-5.2)	4.0 (3.9-4.1) 3.9 (3.8-3.9)	3.1 (3.1-3.1) 3.0 (2.9-3.0)	2.0 (2.0-2.0) 2.0 (1.9-2.1)	— — —
70/52	Plaice	1	9.3 9.6	6.6 6.5	5.5 5.3	4.3 4.3	3.1 3.0	2.4 2.3	1.6 1.6	— —

71/52	Lemon sole	1	9.5	—	5.9	5.3	4.2	3.9	3.2	2.2	1.3	—
			10.0	—	5.9	5.1	4.2	3.1		2.3	1.5	—
72/52	Witch	1	11.3 10.9	—	7.0 6.8	6.3 5.5	4.8 4.2	3.8 2.9	3.0 2.3	—	—	—
74/52	Megrim	1	—	—	5.6	5.2	3.7	2.7	2.6	2.1	1.9	—
			—	—	5.2	4.4	2.7	2.1	1.7	1.1		—
69/52, 69a/52	Turbot	2	9.4 (9.1-9.6) 10.6 (10.0-11.2)	—	6.3 (6.1-6.5) 6.2 (6.1-6.2)	5.2 (5.0-5.4) 4.5 (4.3-4.6)	3.7 (3.6-3.8) 3.4 (3.4-3.4)	2.9 (2.9-2.9) 2.5 (2.4-2.5)	1.9 (1.8-2.0) 1.6 (1.5-1.6)	—	—	—
75/52, 75a/52	Common skate	2	10.1 8.9 (9.7-8.7- 10.5) 9.2)	6.0 (5.7-6.3)	5.1 (5.0-5.2)	4.5 (4.5-4.5)	3.7 (3.6-3.8)	2.8 (2.6-3.0)	2.3 (2.1-2.5)	0.6 (0.5-0.6)	+0.7† (+0.6- +0.7)	+1.7 (+1.6- +1.7)
			9.0 (8.4-9.7)	5.9 (5.7-6.0)	4.7 (4.6-4.8)	2.8 (2.6-3.0)	1.8 (1.6-2.0)	0.6 (0.5-0.6)	+0.7 (+0.6- +0.7)	+1.7 (+1.7- +1.8)	—	—
76/52	Thornback skate	1	10.6	6.8	6.2 5.3	4.6 4.3	4.1 3.6	1.9 1.6	0.8 0.8	+0.4 +0.4	—	—
77/52	Cuckoo ray	1	11.0 11.3	6.1 6.1	5.6 5.3	3.9	3.3	1.9 1.5	0.7 0.5	0.2 0.2	+0.5 +0.5	—
61/52, 61a/52, 62/52	Dogfish	3	— 9.7	6.4 (5.9-7.2) 7.7 (7.0-8.1)	5.5 (5.3-5.7) 5.8 (5.3-6.1)	4.3 (4.4-4.6) 4.7 (4.4-4.9)	3.8 (3.7-3.8) 3.4 (3.3-3.5)	2.7 (2.7-2.9) 2.2 (2.1-2.3)	1.5 (1.4-1.7) 1.4 (1.3-1.5)	1.0 (0.9-1.1) 1.0 (0.9-1.1)	0.5 (0.4-0.5) 0.4 (0.4-0.5)	—
39/52	Pike	1	9.6	—	6.8 6.2	5.3 5.3	4.6 4.4	3.4 2.9	2.9 2.2	1.5 1.3	—	—
40/52, 40a/52	Perch	2	11.1 (10.6-11.7) 10.4 (10.4-10.4)	—	6.6 (6.3-6.8)	5.5 (5.3-5.7)	3.9 (3.8-3.9)	2.6 (2.6-2.6)	1.7 (1.6-1.7)	1.2 (1.1-1.2)	0.6 (0.5-0.6)	—
			—	—	6.2 (6.1-6.2)	4.8 (4.7-4.8)	3.4 (3.3-3.4)	1.9 (1.8-1.9)	0.9 (0.7-1.0)	—	—	—

* Values taken from Connell (1953); arithmetical mean of 'corrected ascending' and descending mobilities $\pm$ s.d.

† The first and second lines for each species (except codling) denote the 'corrected ascending' and descending mobilities, respectively. Arithmetical mean; range in brackets.

‡ A plus sign denotes movement towards the cathode.

Table 2. *Composition of fish-muscle extracts*

Expt. no.	Species	No. of fish	% total area									
			1	2	3	4	5	6	7	8	9	10
7	Codling*	3.5±1.0	5.9±1.2	—	7.6±2.3	6.9±0.8	9.5±1.4	25.4±1.3	30.2±2.9	11.4±1.3	—	—
80/52, 80a/52	Cod	2† 1.3 (1.1-1.6)	1.9 (1.6-2.2)	—	3.8 (3.7-3.9)	5.5 (5.3-5.7)	11.0 (11.0-11.1)	21.6 (20.9-22.2)	34.9 (34.7-35.0)	20.0 (19.6-20.4)	—	—
		2.9 (2.6-3.3)	3.9 (3.8-3.9)	—	4.3 (3.7-4.8)	6.7 (6.7-6.8)	13.1 (12.9-13.3)	20.8 (20.5-21.0)	32.2 (32.0-32.4)	16.2 (15.1-17.2)	—	—
15/52, 16/52, 22/52	Haddock	3	0.5 (0.3-0.7)	1.7 (2.6-4.8)	—	3.7 (5.1-9.3)	7.2 (7.0-8.7)	33.3 (33.1-33.5)	8.4 (6.7-10.0)	3.9 (3.3-4.4)	—	—
		2.7 (2.5-2.8)	2.1	—	—	7.3 (4.6-5.3)	12.6 (10.6-14.6)	28.9 (27.4-30.3)	8.0 (7.8-8.2)	31.3 (28.6-34.0)	—	—
65/52, 65a/52	Whiting	2	2.1 (2.1-2.2)	6.3 (6.0-6.6)	—	8.1 (7.8-8.3)	19.0 (18.8-19.2)	30.8 (30.0-31.5)	16.4 (16.2-16.7)	10.2 (10.0-10.3)	—	—
		3.9 (3.7-4.0)	5.7 (4.9-6.4)	—	—	8.3 (8.3-8.3)	20.3 (20.0-20.6)	30.7 (30.5-31.0)	22.3 (20.8-23.7)	—	—	—
73/52	Coalfish	1	1.3	5.1	—	5.8	3.7	9.0	6.7	44.0	5.3	14.1
		1.9	6.0	—	7.0	—	12.3	19.7	37.6	15.5	—	—
66/52	Ling	1	3.2	2.3	4.9	6.8	13.2	33.3	13.8	19.3	—	—
		2.8	2.0	3.4	9.4	5.8	15.2	32.7	28.7	—	—	—
67/52	Hake	1	2.4	4.2	—	10.1	9.8	31.8	6.8	20.7	—	—
		3.9	5.9	—	11.3	14.7	16.3	31.3	16.6	—	—	—
64/52, 64a/52	Herring	2	0.8 (0.5-1.0)	3.3 (3.1-3.5)	—	8.1 (7.9-8.2)	10.7 (10.2-11.1)	26.5 (25.6-27.3)	30.8 (30.4-31.2)	12.0 (11.8-12.3)	—	—
		0.7	6.2 (5.7-6.6)	—	—	8.5 (8.3-8.7)	13.0 (12.7-13.2)	24.4 (23.0-25.9)	28.0 (27.8-28.2)	10.7 (6.0-12.3)	—	—
68/52, 68a/52	Angler	2	—	1.9 (1.5-2.3)	4.9 (4.7-5.0)	7.5 (6.2-8.7)	6.7 (6.5-6.8)	37.0 (36.2-37.8)	25.5 (24.0-27.0)	—	—	—
		1.5 (0.9-2.0)	—	20.4 (19.8-21.0)	—	18.2 (17.9-18.4)	8.7 (8.7-8.7)	31.0 (30.7-31.2)	20.4 (20.3-20.4)	—	—	—
70/52	Plaice	1	4.5 7.2	3.0 6.1	3.6 6.9	10.8 12.2	32.8 32.4	20.3 16.6	19.3 10.8	—	—	—

71/52	Lemon sole	1	3.1	3.5	—	6.5	4.6	6.8	21.8	12.7	2.2	38.8	—
			4.1	6.4	—	6.0	6.9	14.3	28.0		4.0	30.3	—
72/52	Witch	1	4.8	4.8	—	7.9	23.4	8.3	27.4	23.4	—	—	—
			2.7	7.3	—	8.9	24.3	10.8	26.7	19.4	—	—	—
74/52	Megrim	1	—	1.8	—	5.9	20.3	19.3	4.9	26.5	6.1	16.2	—
			—	6.0	—	6.8	16.4	20.2	6.7	23.1	20.8		—
69/52, 69a/52	Turbot	2	4.9	2.7	—	4.6	20.8	3.9	15.5	45.6		—	—
			(4.7-5.1)	(2.1-3.2)	—	(4.2-5.0)	(20.6-21.1)	(3.3-4.5)	(14.7-16.3)	(44.5-46.7)		—	—
			5.6	4.3	—	8.5	20.5	4.5	17.7	39.0		—	—
			(5.6-5.6)	(4.0-4.6)	—	(7.8-9.1)	(20.0-20.9)	(4.4-4.5)	(17.0-18.4)	(38.4-39.6)		—	—
75/52, 75a/52	Common skate	2	1.3	2.9	3.6	9.9	3.0	34.0		4.9	8.9	20.0	0.8
			(0.7-1.9)	(2.2-3.7)	(3.2-3.9)	(9.2-10.6)	(2.7-3.2)	(32.4-35.6)	(4.7-5.1)	(8.5-9.3)	(19.0-21.0)	(0.7-0.9)	(6.3-6.7)
			4.0	3.9	4.5	8.6	39.8		11.9		20.0	0.8	6.5
			(2.7-5.4)	(3.6-4.2)	(4.5-4.5)	(8.1-9.1)	(38.6-40.9)		(11.6-12.2)		(19.0-21.0)	(0.7-0.9)	(6.3-6.7)
76/52	Thornback skate	1	2.0	2.8	3.0	10.8	3.0	16.6	31.5	20.8	9.5	—	—
			7.3			9.5	2.5	13.8	36.6	20.8	9.5	—	—
77/52	Cuckoo ray	1	4.9	6.0	7.9	9.8	21.9	13.3	11.9	24.3		—	—
			4.4	8.5	7.4	6.7	37.0		11.7	11.0	5.5	7.8	—
61/52, 61a/52, 62/52	Dogfish	3	—	0.8	1.3	5.2	6.4	21.3	28.1	9.1	2.1	25.6	—
			—	(0.6-1.1)	(0.7-2.0)	(3.5-6.9)	(5.9-6.6)	(20.0-23.1)	(27.9-28.4)	(8.3-10.1)	(1.3-3.0)	(24.2-27.0)	—
			0.3?	1.5	3.9	6.9	7.0	18.7	24.6	8.5	3.6	25.6	—
			—	(0.9-2.1)	(3.6-4.2)	(5.9-7.8)	(6.9-7.2)	(18.4-19.2)	(23.0-26.0)	(7.0-9.6)	(3.1-4.0)	(24.2-27.0)	—
39/52	Pike	1	1.7	3.9	—	8.2	4.1	10.3	22.3	43.3	5.5	—	—
			—	6.2	—	6.2	3.7	11.2	32.7	35.0	5.0	—	—
40/52, 40a/52	Perch	2	1.5	4.6	—	3.9	4.2	23.7	28.2	3.3	12.8	18.1	—
			(1.3-1.6)	(3.9-5.2)	—	(3.6-4.2)	(3.9-4.4)	(22.9-24.5)	(28.0-28.5)	(2.9-3.6)	(12.6-13.0)	(17.7-18.6)	—
			1.8	3.7	—	5.0	6.6	24.9	31.0	27.1		—	—
			(1.5-2.0)	(3.5-3.9)	—	(4.8-5.2)	(5.8-7.3)	(23.1-26.8)	(30.0-31.0)	(25.6-28.5)		—	—

* Values taken from Connell (1953); arithmetical mean of ascending and descending values $\pm$ s.d.

† The first and second lines for each species (except codling) denote ascending and descending values, respectively. Arithmetical means; range in brackets.

Fig. 1 a-j. For legend see p. 385.

Fig. 1 k-s.

Fig. 1. Electrophoretic diagrams of fish-skeletal-muscle extracts at 0.05I, pH 7.5. Ascending-limb diagrams only are shown. The direction of migration is from left to right, except in the case of (n) and (r) where it is from right to left. The diagrams of plaice and pike show the course of electrophoresis at an intermediate stage, because the final photographs were accidentally destroyed. The dark rectangular-shaped patch in the top left-hand corner of some of the diagrams is an aberration in the cell.

The N contents and pH's of the extracts, and the duration and the electrical-field strengths of the experiments are shown in Table 3.

Fig. 2. Approximate tracing of the descending electrophoretic diagram of a muscle extract of mirror carp, 0.05*I*, pH 7.5.

The division of the diagrams into components is somewhat arbitrary and only serves to indicate one possible solution. In some cases it probably over- or under-represents the 'true' picture. This must necessarily lead to errors in both the evaluation of

1953), the component with the largest anodic velocity has been designated 1 and the other components numbered consecutively to that with the smallest anodic velocity or largest cathodic velocity. In addition, the number of the component with which the opacity moved has been retained as 2, since it appeared to occupy a special position in all the diagrams studied. This has occasionally resulted in a subdivision of numbering into 1, 1*a* and 1*b* for the faster components.

It is emphasized that components having the same designation in two different species are not necessarily identical, and that Tables 1 and 2 should be read in conjunction with Fig. 1.

DISCUSSION

The most striking fact emerging from this work is that considerable differences in the electrophoretic diagrams of fish-muscle extracts occur from species to species, and that these differences are so characteristic as to 'fingerprint' any one species. These differences are not merely of degree since some quantitatively important components, particularly among the more slowly migrating group, are present in some species but completely absent from others. However, there is an overall similarity in that the components of all the diagrams may be divided into mobility groups, I-III (Jacob, 1947; Connell, 1953), whose quantitative distribution is approximately the same for all diagrams. Thus, group III always consists of one, or possibly more, rapidly migrating components making up less than 4% of the total area of the diagram; group II consists of two or more components quantitatively somewhat larger than group III, and always including component 2 with which the opacity moves. Moreover, 2 always appears in the same relative position. Group I, the slowest group, has the greatest diversity in the number and disposition of components, for example, slow and quantitatively large components (8 and 9) appear in the haddock and coalfish diagrams which are completely absent in cod and whiting diagrams.

This overall similarity has its parallel with the electrophoretic diagrams of different animal plasmas and sera which have been examined by Deutsch & Goodloe (1945) and Moore (1945), respectively. All normal animal plasmas or sera differ in detail from species to species, but are generally similar in showing a large, faster-moving albumin component followed by several smaller, slower globulin components (plus fibrinogen in the case of plasmas) which usually fall into definite mobility groups. This observation has been confirmed with a few electrophoretic analyses of fish-blood sera (Connell, unpublished). Apparently, species differences also occur in the electrophoretic properties of caseins (Dovey & Campbell, 1952).

Table 3. *N* content and pH's of extracts and duration and field strength of the experiments

Expt. no.	N content (mg./ml.)	pH	Duration (sec.)	Electrical field (v/cm.)
15/52	3.91	7.35	5580	4.95
16/52	3.91	7.35	9360	3.30
22/52	4.28	7.52	4380	4.85
39/52	2.89	7.45	2860	4.85
40/52	2.78	7.48	5520	5.03
40 <i>a</i> /52	3.00	7.50	5580	5.13
61/52	1.99	7.50	6060	4.88
61 <i>a</i> /52	2.30	7.53	5580	5.53
62/52	1.99	7.51	5400	5.70
64/52	3.56	7.51	6180	4.99
64 <i>a</i> /52	3.50	7.53	6000	5.03
65/52	3.55	7.58	4380	6.25
65 <i>a</i> /52	3.03	7.56	4380	6.25
66/52	3.03	7.61	6280	6.40
67/52	2.74	7.50	4620	6.28
68/52	2.97	7.75	3420	7.04
68 <i>a</i> /52	3.12	7.69	3720	6.89
69/52	2.85	7.70	3420	6.28
70/52	2.78	7.40	2820	6.66
71/52	4.06	7.45	3900	6.91
72/52	2.03	7.60	3960	6.28
73/52	3.01	7.60	5160	6.28
74/52	4.00	8.02	9240	5.44
75/52	2.69	8.25	4620	6.28
75 <i>a</i> /52	2.60	7.71	4860	6.28
76/52	2.74	7.58	4440	6.28
77/52	2.69	7.53	3120	7.41
80/52	2.58	7.61	4080	7.41
80 <i>a</i> /52	2.63	7.62	4200	7.41

areas and of mobilities, and where considerable overlapping occurs or where components hardly form a 'peak', the values of Table 2 must be taken as denoting only orders of magnitude. The numbering of the components is quite arbitrary and bears no relation to the designations either of Jacob (1947) or of Bosch (1951). As in a previous paper (Connell,

Whereas with sera the turbidity of the protein mixture migrates with a variable, though limited, number of components (Deutsch & Goodloe, 1945), the largest part of the turbidity of fish-muscle extracts can be associated precisely with one component, which from its position in the diagram appears to be the same in all cases. Generally speaking, the larger the amount of this component, the greater is the opacity of the extract. It has been suggested (Connell, 1953; Jacob, 1947) that this component, or others with similar mobilities, may be a soluble part of the myosin group of proteins, and also that denatured portions of the extract may be associated with it. There is also a very slight increase in turbidity at the level of group III. A curious unexplained feature in the ascending limb is the clearing of the diagram at about the level of the middle components followed by an increase of turbidity towards the δ boundary.

The diagrams of genetically closely related species, e.g. those of the genus *Gadus*, or of *Pleuronectes*, show as much variation amongst themselves as do the diagrams of unrelated species. That it is possible for related species to show special characteristics is demonstrated by the diagram of the skates and perhaps the dogfish, in which, alone among all the species examined, cathodically migrating components are present. Even at pH 8.25 (Expt. no. 75/52), component 10 of the common skate has a considerable cathodic mobility ($+1.7 \times 10^{-5} \text{ cm.}^2 \text{ v}^{-1} \text{ sec.}^{-1}$). This phenomenon may be connected with the uraemic condition of elasmobranch tissues. In addition, the skate diagrams show the greatest electrophoretic complexity yet encountered, the common-skate diagram for example, separating into twelve distinct components.

As far as can be judged, the diagram of common carp is identical with that of the mirror carp (shown in Fig. 2) and both are different from any other species examined. The mirror carp is usually considered to be a variant or a 'sport' of the common carp, so it appears that for the carp such a biological change has no pronounced effect on the muscle proteins examined in this work. On the other hand, Moore (1945) has shown that large electrophoretic differences exist in the serum proteins of two strains of rat, and it is also evident that electrophoretic features of serum proteins may be transmitted from two different species to their hybrid (Brandt, Smith, Andrews & Clegg, 1952).

The diagrams of the freshwater species (pike, perch, carp) do not appear to be markedly different from those of marine species.

There is some evidence to show that age has an effect on the proportions of the electrophoretic components of cod muscle, since the faster components of a cod approximately 90 cm. in length are smaller and the slower components larger, relative

to a codling 30 cm. in length. These fish are likely to be about 6 and 2 yr. old, respectively (Graham, 1933). It is not possible to say in this case whether the absolute amounts of any one component are varying because the extractions were not exhaustive. The appearance of the cod diagram is almost identical with that of the previously published codling diagram (Connell, 1953), and the mobility of the components is hardly affected by the difference in age. One observation which may have a bearing on the difference in relative composition is the fact that the amount of myoalbumin (probably represented by group III) in embryonic tissue decreases with development (Crepax, 1952). Differences in age definitely affect the electrophoretic properties and relative proportions of the serum or plasma proteins (Koenig, Perrings & Mundy, 1949; Brandt, Clegg & Andrews, 1951; Beavan, Hoch & Holiday, 1951; Zinsser, 1952).

A number of individual species characteristics may be noted; for example, ling, angler, plaice, the skates and dogfish show a definite component 1a which migrates slightly faster than the opacity. Alone amongst the species examined group III of the common skate subdivides into two distinct components 1 and 1b. The diagrams of the angler and of the flat fishes, representing rather specialized species, appear to be little different in essence from those of other species. The high proportion of faster components of the witch diagram and their relatively higher mobility, indicates a lower average isoelectric point of the muscle proteins of this species.

The question arises whether any of the components of diagrams of different species are the same. For components which can be easily recognized from diagram to diagram, for example 1, 2 and 3, it seems likely that they are the same in the electrophoretic sense that the serum albumins of different species are the same. The serum albumins invariably occupy the same position in the electrophoretic diagram, although there are marked differences in the mobilities from species to species, even under identical conditions. This is true also of components 1, 2, 3 and possibly 4 of the muscle diagrams. In the more complex part of the diagrams, group I, it is impossible to make conclusions about identity. The most that can be said about group I is that if any of the components in it are identical then their amounts must vary enormously among the species, and that components present in one species are completely absent in others.

The extracts which have been studied in this work contain the largest proportion of the known enzymes of muscle, so that species differences in the derived electrophoretic diagrams may indicate special physiological differences between fish, for example, the fact that arginase is present in elasmobranch but normally not in teleost muscle.

SUMMARY

1. Muscle extracts of twenty different species of fish have been examined electrophoretically at 0.05*M*, pH 7.5.

2. Each species has a characteristic electrophoretic diagram, and considerable differences in mobility and composition exist between species.

3. The diagrams may, however, be still divided into definite mobility groups of approximately consistent quantitative size.

4. The diagrams of related species appear to be as dissimilar as those of unrelated species.

5. The effect of age and of species variants on the diagrams has been examined.

6. Elasmobranch muscle appears to contain proteins with unusually high isoelectric points.

7. The question of the identity of components of different diagrams has been discussed. Whatever the answer, it is certain that components which are present in some species are completely absent in others.

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Comparative Observations on the Chemical Properties and Biological Activities of L-Ascorbic and D-isoAscorbic Acids

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In a previous publication the course of oxidation of L-ascorbic acid under various conditions and in the presence of different accompanying substances was described (Leibowitz & Guggenheim, 1938*a*). It was shown that the stability of the vitamin in solution depends specifically on the nature of the accompanying substances and on the pH. It has also been shown that L-ascorbic acid exerts a detoxifying action on potassium cyanide, phenol and tetanus toxin (Leibowitz & Guggenheim, 1938*b*; Kligler, Guggenheim & Warburg, 1938). Some evidence, derived from titrimetric and cryoscopic data, indicated that the detoxifying effect results from the reversible formation of a chemical compound between the vitamin and potassium

cyanide or phenol. Other authors have reported that ascorbic acid diminishes the toxicity of various other substances such as arsphenamines, lead, sulphonamides, benzene, trinitrotoluene, and anaesthetics (Bicknell & Prescott, 1948). It has been tentatively suggested that the antiscorbutic function of L-ascorbic acid may be at least partially due to its ability to detoxify naturally produced toxic substances (Leibowitz & Guggenheim, 1938*b*).

Numerous clinical and experimental observations have indicated that ascorbic acid plays a role in certain forms of anaemia (Vilter, 1947; Dyke, Della Vida & Delikat, 1942; May, Nelson, Lowe & Salmon, 1950). Studies by Nichol & Welch (1950) and Welch, Nichol, Anker & Boehne (1951) have