

BIOLOGICAL BOARD OF CANADA

MANUSCRIPT REPORTS OF THE BIOLOGICAL STATIONS

No. 36

Title

Rigor Mortis of Fish.

Part I. Coagulation of Proteins of Fish Muscle.

Part II. Hydrogen-Ion Concentration of Fish Muscle.

Author

C. C. Benson

BIOLOGICAL BOARD OF CANADA

Manuscript Reports of the Biological Stations

No. 36

Rigor Mortis of Fish

Report of Work Done at the Atlantic Biological Station

St. Andrews, N. B.

During 1927

By C. C. Benson

The work reported here followed two lines of study:-

- (1) Part I, which covers estimations of protein coagulated from muscle juice, squeezed from fish at different stages of rigidity;
- (2) Part II, which covers determinations of pH of fish muscle.

Part I

Coagulation of Proteins of Fish Muscle.

The studies of coagula were made as in previous years and verify results previously obtained.

As before, steaks were taken from the fish, weighed, the juice then squeezed out, filtered through muslin, the volume noted and coagula then formed. Usually 5 cc. lots of the juice were heated to 40°C, and to 100°C; the precipitated were washed, dried and weighed on previously weighed filter papers. 5 cc. lots of juice were also dried to constant weight, to obtain the total solids of the juices. Duplicate determinations were always made, but sometimes less than 5 cc. were available for

each determination, on account of the limited amount of juice. Sufficient juice was obtained from Haddock No. 14 and Haddock No. 15, but the juice from Haddock No. 2, and Haddock No. 3, was diluted before using in the cases shown. Since doing these experiments, I have learned that this treatment affects the amounts of coagulable proteins, (von Firth, Ergebnisse Physiol. Vol. 19, p. 391). In all cases, the juice, which was squeezed out, was put immediately into an ice cold water bath and kept there until used.

The results of tests are given in Tables I and II which show, in Table I, the size, etc., of the fish used and, in Table II, the results of the coagulation experiments.

It was at first, almost impossible to obtain fish sufficiently large to give the 120 cc. or so of juice needed in all for determinations at three different times on the same fish. Parts of Haddock No. 2, and Haddock No. 3 were needed for some other work, so that the amounts of juice were not sufficient to supply 5 cc. for all of the tests and the results of these two experiments are, consequently, inconclusive. Haddock No. 14 and Haddock No. 15 supplied good quantities of juice and so more satisfactory results were obtained.

Table I

Data in regard to Haddock used.

No. of Fish	Weight K.	Length cm.	Girth cm.	Caught at	by	Stunned at
2	2.33	62	31	12 n.	trawl	1.15 p.m.
3	2.02	63	30	11.15 a.m.	"	11.15 a.m.
14	2.38	63	-	11 a.m.	"	12.35 p.m.
15	2.47	65	-	11 a.m.	"	12.15 p.m.

Table II

Zero time is 1.15 p.m.

Haddock	No.	Steak Taken at	% of juice	% of solids	Coagula		40° as % of 100°	
					at 40°	at 100°		
Haddock 2	I	1/4 hr.	16%	10.35	2.22	6.60	31.7	
				10.32	2.01	6.69		
	II	4 hr.	20%	9.56	2.14	6.30	33.7	
				9.52	2.12	6.34		
	5 1/2 hrs.	III	21 hrs.	16%	9.15	2.10	6.09	34.9
					9.11	2.13	6.03	
Zero time is 11.15 a.m.								
Haddock 3	I	0 time	18%	8.47	1.80	5.43	33	
				8.55	1.90	5.46		
	II	6 1/4 hr.	16%	10.16	2.15	6.56	32	
				10.20	2.20	6.66		
	Zero time is 12.35 p.m.							
	Haddock 14	I	5 min.	16%	10.14	2.30	6.56	33.5
10.04					2.26	7.04		
3-4 hrs.		II	4 hrs.	20%	10.04	2.54	6.56	39.4
					10.02	2.62	6.52	
III		23 hrs.	15%	10.00	1.84	6.44	29.0	
				9.84	1.96	6.66		
Zero time is 12.15 p.m.								
Haddock 15	I	3/4 hr.	23%	8.34	1.94	5.16	36	
				8.26	1.88	5.18		
	2-3 1/2 hrs.	II	3 hrs.	16%	9.38	2.28	5.74	42
					9.36	2.46	5.56	
	III	22 hrs.	16%	8.74	1.88	5.16	35	
				8.66	1.82	4.94		

+ Juice diluted.

The changes of rigidity for Haddock No. 14 and Haddock No. 15 are shown in Table III, the stiffness being measured by the method of J. Pantou, (1927), described elsewhere.

Table III

Rigor Readings

(Apparatus of J.R.P. 1927)

Aug. 3, 1927		Haddock #14	Haddock #15	
Time		Reading	Reading	Temp.
12.30 p.m.	0	1.5 cm.	2.5 cm.	-
1	1/2 hr.	2	8	20.5°
1.30	1 "	4	16	"
2.00	1 1/2 hrs.	8	19	21°
2.30	2 "	12	20.5	22°
2.50	2 1/3 "	16.5	21	22 1/2°
3.12	2 3/4 "	18.5	21	"
3.30	3 "	20	21	23°
3.45	3 1/4 "	20.5	20.5	"
4.0	3 1/2 "	20.5	20	"
4.15	3 3/4 "	20.5	19.5	"
4.30	4 "	20.5	19.0	23 1/2°
4.45	4 1/4 "	19.5	18.5	23°
5.15	4 3/4 "	18.5	18	"
6.0	5 1/2 "	17.5 (dry)	17	24°
6.15	5 3/4 "	17	16	"
7.0	6 1/2 "	16	15	"
7.15	6 3/4 "	15.5	14.5	"
7.45	7 1/4 "	15	13.5	"
8.15	7 3/4 "	14.5	13	"
8.45	8 1/4 "	14 (v. dry)	12.5	22°
9.15	8 3/4 "	14	12	"
10.00	9 1/2 "	13.5	12	21°
Aug. 4, 1927				
9 a.m.	22 1/2 "	11.5	11	18.5
10 "	23 1/2 "	11.5	11	20°

With Haddock No. 14, the total solids, and coagula at 100°C were very nearly the same for the three different lots of juice, but the coagula at 40°C, varied very decidedly. The same result is seen with Haddock, No. 15 but with more variations in quantities of solids and coagula for the three lots of juice. These variations very likely result from the rough method of filtering the juice, which was used to save time. I have, therefore, calculated the 40° coagula as $\frac{1}{2}$ of those obtained at 100°C, as any differences due to the method of filtering of juice, would show equally in both these kinds of coagulations.

We see again the results obtained in earlier experiments of this kind, that the precipitate obtained by coagulation of muscle juice as a result of heating to 40°C varies in amount in relation to the degree of rigor mortis. Its amount is largest near maximum rigidity and smallest when the muscle is again relaxed, or, in the nomenclature of von Fürth, the quantity of soluble myogen fibrin is here shown to be greatest near maximum rigidity and least after the death stiffness has disappeared. Von Fürth quotes Kühne as saying that after long rigor, there is no coagulation below 43° - 45°.

Conclusions

In the condition of great stiffness of muscle after death of haddock, the protein, coagulable at 40°C (von Fürth's "soluble myogen fibrin") is in greatest amount and when relaxation follows, this substance is much less, less also than before rigor mortis was evident. The rigidity of muscle is, thus, related to changes in the nature of proteins present.

Part II.

Hydrogen-Ion Concentration of Fish Muscle.

In connection with studies of rigor mortis, it seemed worth while to try to find a satisfactory method of determining the hydrogen ion concentration of the muscle.

The Quinhydrone method has proved very satisfactory for this purpose, for it allows rapid working and can be so arranged as to cause the minimum of injury to the tissue. Both of these conditions had to be met in these experiments, for Fletcher and Hopkins (1), working with frog's muscle have shown that injury to the tissue stimulates acid production and Ritchie (2) and Simpson (3), have found for fish, that muscle glycogen soon decreases after death, and lactic acid tends to increase very quickly.

Attempts were made to use muscle, frozen with solid carbon dioxide, but without success and colorimetric determinations with extracts did not give values of sufficient exactness. Muscle, frozen in test tubes in a brine bath, was used to make cold (3°-4°C) water extracts. Extracts were made also by dropping the excised muscle into ice water and filtering. The pH_s of these extracts were taken, using Quinhydrone, but these methods were all troublesome. A few of such determinations are here shown, for the sake of comparison, in Table I, which also shows that results obtained by the procedure described below, gave similar results to those obtained with the more elaborate manipulations.

Table I

Comparison of pH determined in different ways.

Determination with trawl caught haddock.

No. of Fish	Relation to rigor	pH			Indicator B. T. B.	
		quinhydrone method on muscle	with extract	with juice		
6	before	-	6.67	-	6.4	Muscle frozen
"	during	-	6.67	-	6.5	in brine bath,
7	before	-	6.49	-	6.5	extracted with
"	during	-	6.49	-	6.5	water.
12	before	6.96	6.80			
14	before	6.70	-	-		Juice squeezed
"	during	6.54	-	6.41		from muscle.
"	after	-	-	6.50		
15	before	-	-	6.59		
"	during	6.53	-	-		
"	after	-	-	6.55		
9	before	-	6.28 6.38	-	6.4	Extract made by
"	during	6.32	6.34	-	-	plunging muscle
"	after	6.43	6.47	-	-	into ice water.
Determinations with minced beef.						
		6.27	6.25	-	H ₂ electrode with extract. 6.23	

Most of the determinations were obtained by quickly cutting pieces of muscle from the fish immediately after it was stunned, covering a spot of the muscle with the powdered Quinhydrone and inserting a stout, bare platinum electrode in order to read the voltage against a calomel electrode with saturated potassium chloride connections. The readings were regularly made with two electrodes and gave the same values; sometimes the bridges, (of 3% agar and saturated potassium chloride), were changed, and frequently readings were duplicated by changing the places where the bridges made connection, or where the Quinhydrone was placed.

In general, values duplicated well but sometimes variations were found as shown in Table, No. II. These variations are to be expected for there are indications that during rigor the musculature of the fish does not change as a whole, but rather that changes are progressive and proceed more rapidly in one place than in another.

The chief difficulty experienced was in inserting the platinum electrode too deeply and so no doubt below the part of the tissue saturated with the Quinhydrone.

The matter of temperature correction has also been troublesome for unfortunately the room temperatures during July 1927 were often high at St. Andrews, where the measurements were made, and the fish muscle was consequently in many cases cooler than was the calomel electrode, for which no means of temperature adjustment had been provided. Billman and Krarup (4) have worked out a temperature correction factor for the Quinhydrone electrode and temperature corrections for the calomel electrode are given by Clark (5). Benson (6) makes

use of a formula combining these two factors,

$$\text{pH} = \frac{0.4561 - 0.00014t_1}{0.0541 + 0.002t_1} - \frac{V}{0.0541 + 0.002t} \quad \text{volts,}$$

where V = the reading in volts, t = the temperature of the quinhydrone electrode and t_1 = the temperature of the calomel electrode. This formula has been used in calculations from the voltage readings. I have also recorded in Tables II and IV, the millivolts actually read as I have not yet been able to fully verify this correction factor for low temperatures with preparations such as these.

TRAWL CAUGHT FISH

Determinations were first made with Haddock caught by trawl, and a typical experiment of this kind is given in Table II.

Table II

Determination of pH of muscle of trawl caught haddock.
Fish No. 13; stunned when caught; max rigor (12°C) in 5 3/4 hrs.

Time since stunned	Temps.		mv	pH
	t	t ₁		
0	fish stunned			
3 min.	16°	21	53	6.85
5 "	"	"	62	6.69
9 "	17°	"	59	6.75
15 "	18°	"	64	6.66
30 "	21°	21°	64	6.69
36 "	22°	22°	72	6.51
2 1/3 hr.	"	"	65	6.63
2 1/2 "	25°	25°	60	6.72
3 "	"	"	56	6.74
4 "	"	"	67	6.52
5 1/2 "	26°	26°	63, 70	-
24 "	22	22°	72	6.51

Other experiments of a similar kind gave values for pH as shown in Table No. III. At the bottom of this Table are shown the times which the muscle took to reach maximum rigidity. These determinations were made at room temperature except in the case of Fish No. 12, which was kept in a cold chamber at 12°C. and was, therefore, slower in becoming hard. The values enclosed in brackets are those near maximum rigidity.

Table III.

pH Values for Haddock caught by Trawl.

Time	Fish No. 6	Fish No. 7	Fish No. 11	Fish No. 12	Fish No. 14	Fish No. 15	Fish No. 20
3 min.	6.67	6.54	6.64	6.56			6.67
9 "			6.94	6.75			6.74
1/2 hr.			6.62	6.62	6.70	6.59	6.66
1 "			6.64	6.51			6.72
2 + "	(6.67)	6.49	6.40		(6.54)	(6.53)	(6.68)
3 + "	6.54	(6.49)	6.40	6.44			6.57
4 + "			6.46	6.50	6.41		6.70
5 1/2 "	6.49	6.49	6.42				6.66
20+ "	5.54			6.51	6.54	6.53	
max. rigor at	3- hr.	3+ hr.		6 hr.	3 hr.	2 + hr.	3 hr.

PENNED FISH.

The muscle of fish, taken from a pen after two weeks or so of quiet, was found to have an alkaline reaction and to change as shown in Table IV.

Table IV.

Determinations of pH of Muscle of Haddock from Pen.

Time	Fish	Fish	Fish	Fish	Fish	Fish
	No. 10	No. 16	No. 17	No. 21	No. 22	No. 23
3 min.	7.30	7.09	7.16	6.92	7.28	7.30
7 "	7.31	6.91	6.94	6.80	6.94	7.10
1/3 hr.	7.28	7.03	6.88	6.74	6.87	6.96
1/2 "	7.26	6.98	6.88	6.80	6.74	6.93
1 + "	7.13	6.85	6.83	6.85	6.90	6.91
2 + "	6.88	6.75	6.74	6.74	6.81	7.00
3 "	6.86		6.94		6.71	
4 "	6.56	6.74	6.85			
7 "	6.54					
8 "	6.56					
9 "	6.48					
19 "		6.53				
Max. rigor at	16 1/2		16 hr.	(Rigor measured at 12°C.)		

From these measurements it is evident that the hydrogen-ion concentration of muscle is very different for haddock taken from the pen (rested muscle) as compared with that from trawl caught fish (fatigued muscle). As was to be expected, with penned fish, we find the muscle in alkaline condition; but with trawl caught fish, the muscle is already near its condition of maximum acidity, due no doubt to the lactic acid produced by struggling.

ACIDITY AND RIGOR

The maximum acidity seems, however, to be reached before the muscle showed marked evidence of rigor, and an examination of the variations of pH as compared with the increase and decrease of rigidity, indicates that, while the lower concentrations of acid at the time of death are associated with the slower progress of rigor mortis, this condition of stiffening does not make itself evident with any definite change of pH value.

These determinations thus fit in with the conception of rigor mortis as a process indirectly related to the accumulation of lactic acid. When one compares the pH values with conditions of rigor mortis, at corresponding times, as found by Panton (9) and others, there is little obvious relationship except that noted by Leim, Macleod and Simpson (7). This is the fact that the penned fish, which have much higher initial values for pH, pass into rigor much more slowly.

We thus see by actual measurement that the muscle of fish when

they are taken from the pen, is alkaline in reaction. The reaction changes soon to neutralite or to a faintly acid condition and then gradually grows more acid, probably to a pH of about 6.5 or possibly 6.4, though unfortunately it was not realized in time that it was wise to continue experiments long enough to see this, and the low value was only measured in two cases. With these fish, rigor was found to develop very slowly, but reached its maximum some time after the time of greatest acidity.

With trawl caught fish, the muscle was never alkaline, when the fish were taken from the line. The amount of the acidity varied from a pH just at the neutral point to a value of between 6.5 and 6.6. The acidity increased, but not always regularly, and the minimum values of pH did not correspond with the onset of rigidity or its maximum. The accumulation of acid seems to be initiating some other change, which brings about the stiffening.

OTHER SPECIES

In Table V, are also given results for a few determinations of pH with other species of fish, all caught by trawl.

Table V

Determinations of pH of Muscle of Fish, (trawl caught).

Time	Hake No. 2	Hake No. 3	Cod No. 1	Cod No. 2	Eel Pont	Skate No. 1	Skate No. 2
6 min.	7.06	-	-	-	-	7.12	-
1/3 hr.	7.33	-	-	-	6.53	6.82	-
1/2 "	7.22	-	-	6.97	-	6.84	-
3/4 "	7.28	-	-	-	-	-	-
1 "	7.20	-	-	-	-	6.54	6.56
2 "	7.24	-	6.96	6.76	6.54	6.65	6.50
3 "	7.16	-	6.90	-	-	6.29	-
4 + "	7.10	-	-	-	-	6.34	-
5 + "	7.22	7.17	7.08	-	6.44	6.40	-
Max. rigor at	1 1/2 hr.		2 1/2 hr.				

These values agree in general with estimations of lactic acid made by Richie (2), who noted lower values of lactic acid for cod, than for haddock, and less change in reaction with change in condition of rigidity. He found still lower values for lactic acid in hake.

Further work should be done with skate muscle.

DEGREE OF ACIDITY

In regard to actual values for pH, these are of the same order of magnitude as those found for cat and for invertebrate muscle by Furusawa and Kerridge (10), (11). Their measurements, taken immediately after death with quickly frozen muscle, varied between pH = 6.93 to 7.13; with values, after 20 - 24 hours, of 6.10 to 6.52. The pH of 6.10, for tail muscle of *Scyllium Canicula*, is lower than any of the values found for haddock muscle, where the lowest value for pH was usually about 6.40. In one case only, Haddock No. 9, Table 1, the value was as low as 6.26.

SUMMARY

1. A method is described by which it has been possible to make consecutive measurements of hydrogen-ion concentration of muscle over long periods at as frequent intervals as were needed.
2. Determinations of pH of haddock muscle, show that fatigued muscle (trawl caught) is acid at time of death, may become a little more so, but varies only slightly during the changes of rigidity. The concentration of hydrogen-ions is, therefore, not the cause of rigidity.
3. Determinations of pH with rested muscle of haddock (fish taken from a pen) showed an alkaline reaction. This soon changed to the acid side and then gradually became more acid; approaching the values found for trawl caught fish.
4. The pH of Hake muscle (2 tests) was alkaline during 5 hours after death, and never gave an acid reaction.
5. The pH of Cod muscle (2 tests) was near the neutral point and only varied slightly.
6. The muscle of Skate grew slowly more acid in one experiment, of four and half hours, while the muscle of an eel pout, which had been out of water for $3/4$ hour and apparently dead for $1/4$ hour, showed an acid value for pH during 5 hours of testing.

BIBLIOGRAPHY

- (1) W. M. Fletcher and H. G. Hopkins, (1907), *J. Physiol.*, 35, p. 247.
- (2) A. D. Ritchie, (1927), *Br. J. Exp. Biol.*, IV, p. 327.
- (3) W. W. Simpson (Ms.).
- (4) E. Billmann and I. Krarup, (1924), *J. Chem. Soc.*, 125, p. 1954.
- (5) W. M. Clark, (1927), *The Determination of Hydrogen-Ions*, 2nd ed., reprinted, p. 456.
- (6) L. E. Dawson, (1926), *Sugar.*, 26, p. 212.
- (7) A. H. Lohr, J. J. R. Macleod and W. W. Simpson, (1927), *Contrib. Can. Biol. U.S.*, III, P. 459.
- (8) A. D. Ritchie, (1925), *J. Physiol.*, IX, Proceedings, 1.
- (9) J. R. Panton (Ms.).
- (10) K. Furusawa and P.M.T. Kerridge, (1927), *Physiol.*, 53, p. 33.