MANUSCRIPT REPORTS OF THE BIOLOGICAL STATIONS

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Report of Work done at St. Andrews, N. B., during 1922. Part I.

Proteins of Muscle Juice of Fish.

By

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BIOLOGICAL BOARD OF CANADA

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Part I.

Proteins of Muscle Juice of Fish

Earlier studies, made with the juice from fish muscle, have shown that there are variations in the amount of the coaguable proteins due to changes occurring after death and have also suggested that certain of these proteins bear a direct relation to the changes of rigor mortis (Benson 1920). Other investigations in regard to the time till the onset of rigor and its passing have shown variations in these processes due to method of keeping (Panton 1921). The experiments, described here, were made to test these points farther.

Coaguable Proteins of Muscle Juice

The juice from the muscle was collected and handled according to the method described in the earlier paper (Benson 1920). The 5 cc. lots of juice were, therefore, heated for 5 minutes at the required temperature and the clots so formed collected on dry, weighed papers. No coagulations were made at 50° or 70°,

because, as explained in the earlier paper, these determinations were of little value for our purpose. The clots were formed at 40° and in boiling water as before, except that it was found that the inner vessel for the 40° coagulum, (where no outside source of heat was used) did not rise above 39°C after the test tubes were put in, but the bath was kept at this temperature for the later half of the period. Unfortunately this first clot may show considerable differences in amount for very small changes of temperature and time of heating. This is illustrated in Table I.

Table I.

Influence of Temperature on Formation of 40° Coagulum

Temperature of Water Bath	% of Clot Fish No. I.	Fish No. II.
Began at 40°, fell to 39°	2.46 (a)	2.74 (a)
Kept at 40° exactly, for 5 mins.	2.94 (b)	-
Began at 40°, rose to 45°	3.39 (b)	3.92 (b)
Ppt. formed in 24 hrs., 20°-24°C.		2.32 (c)

In the table, the clots (a) are those formed by the method described above. The clots (b) were obtained by adding water at 50° as required and the clot (c) was obtained by letting 5 cc. lots of juice stand over night and all day in the laboratory on a hot day. All the values here recorded for clots formed at 40°C were, however, made in the same way and are therefore comparable.

In all cases, where there was sufficient material duplicate determinations were made and gave good agreement but where only one determination could be made or smaller quantities had to be

used, these changes are noted in the tables.

Haddock muscle was again used in most of the experiments but when other fish were available, they too were tested in the same way.

In Table II. is given information in regard to the size of the fish, the conditions under which they were kept, the percentages of juice which could be squeezed from the minced muscle and the percentage of solids in the juice. The method of squeezing, caused so much loss that the figures for the percent of juice are only approximate but they are given here for the sake of comparison with the amounts of juice obtained from other species of fish, (See Table VI) and also to indicate the change in condition of the tissue due to freezing. Fish A and Fish R were carefully frozen in the cold storage plant of the Biological Station and were apparently in good condition but both gave considerably more juice than it is possible to obtain from unfrozen haddock by this method of expression. The freezing in this respect shows distinct evidence of having altered the colloidal condition of the tissue in regard to its power of holding fluids. The figures for solids of the juice as might be expected; show an inverse variation when the juice was collected immediately after the fish was thawed; but when the thawed fish was left in a refrigerator, other changes evidently occurred (See Solids of Fish A). From the experiment with Fish R there is an indication that there is more loss of material by thawing in water than in air, but this needs more investigation.

From the Fish P, different amounts of juice were deliberately squeezed out, by using differences of pressure, to see whether the

composition of the juice would be altered as a result. The percentages of solids in the juice and of the proteins coagulated at 40° and 100° (See Table III.) show that the composition of the juice is not varied by the pressure used to obtain it. With the juice from this fish should be compared that from Fish R where the percent amount is the same in the two parts of the experiment but the composition very different.

TABLE II.

Information in regard to Haddock used.

No. of Fish	Date Caught	Length	% of Juice	% of Solids of Juice	Cond'n of fish, when samples taken: Time kept	Where kept	
A	1922 Aug. 7	71 cm.	17	12.23	2 hrs.	Lab.	very fresh
"	" "	-	17	12.38	4 "	"	Fresh
"	" "	-	30	12.5	24 "	Cold Store	Hard Frozen, minced "
"	" "	-	18	10.4	8 days	" "	Hard Frozen minced frozen
"	" "	-	22	9.5	10 "	" "	Thawed in air
"	" "	-	33	(9.9 (3))10.6	12 "	" "	Thawed; refrig. 2 dys.
R	Aug. 26	-	23	8.9	3 days	Cold Store	Thawed in water
"	"	-	23	10.9	"	"	" " air
P	Aug. 26	59 cm.	19	11.4	25 hrs.	Lab.	Rigor passed
"	" "		10	11.4	"	"	" "
M	Aug. 22	40 cm.	12	11.0	3 hrs.	Lab.	Small thin fish rigid
"	"		14	12.3	6 "	"	Rigor passing

TABLE II. (Cont'd)

S	Aug. 30	74 cm.	23.	9.1	½ hr.	Lab.	Very fresh, soft
"	"	-	21	10.1	3 "	"	Rigor beginning
"	"	-	16	9.6	5 "	"	" passing
"	"	-	18	9.1	27	"	" passed, soft
"	"	-	17	8.9	3 days Lab. & Refrig.		Decomposing
T	Sept. 2	39 cm.	13	10.5	1 hr.	Lab.	Very fresh, soft
"	"		-	10.2	2½ "	"	Rigid
V	Sept. 2	65 cm.	12	12.1	1 hr.	Lab.	Very fresh, soft
"	"		12	11.5	4½ "	"	Rigor beginning
"	"		9	-	5 "	"	Rigid
"	"		7	12.7	9 "	"	Rigor passing
K	Aug. 22		21	10.2	2 hrs.	Lab.	Very fresh, soft
"	"		13	10.0	48 "	Refrig.	

(3) Duplicates very poor, difficult to obtain good samples

In Table III. are given the percentages of proteins coagulated at 40°C and 100°C and for the sake of comparison there are reproduced here (as Table IV.) parts of the tables of the 1920 report giving information in regard to the influence of keeping on the coagulable proteins. It is much to be regretted that the coaguls formed at 40°C were not prepared in exactly the same way in both cases. The values in Table IV. (from the 1920 report) are all slightly higher than those here reported as during the work of 1922, the temperature actually rose only to 39°C. as explained above.

The Fishes 'S' and 'V' in Table III. are Fishes 'I' and '5' of Part 2 of this report. Fish T was of the same size and caught at the same time as 'Haddock 6' and these two therefore, probably reached their maximum rigidity at the same time. The tail parts of S and V and the whole of 6 were set up in the apparatus to measuring the stiffening as described below and T and the other parts of S and V were cut up and examined as described in this part of the report. S reached its greatest rigidity about four hours after death. Steaks of this fish were cut up ½ hour after catching, that is before rigor began, 3 hours after catching or before the height of rigor and 5 hours after catching or as rigor was just beginning to go off. The greatest rigidity was unfortunately missed but I was more fortunate with V (5) as the muscle was cut up just 5 hours after death, at the height of rigor. With T (6) also, a part was cut up at 2½ hours, which was the time of greatest stiffness of its companion fish.

TABLE III.

Proteins of Muscle Juice of Haddock.

No. of Fish	% Solids of Juice	Proteins as % of Juice Coagulated at		40° Coag. as % of Solids	Condition of fish
		40°	100°	%	
A	12.23	3.8	8.8	31	Very fresh (2 hrs.)
	12.38	2.1	8.2	18	Fresh (4 ")
	12.5	2.9	8.8	22	Frozen 24 hrs.
	10.4	2.5	6.9	24	" 8 days (minced frozen)
	9.5	2.0	6.1	21	Frozen, 10 days (thawed in air)
	9.9 (3) 10.6	2.0	6.5 (3) 6.8		Frozen 10 days and kept 2 days in refrig.
R	8.9	1.8	6.1	-	Frozen, thawed in water
	10.9	2.4	7.5	-	" " " air
P	11.4	2.5	7.3	-	Rigor past
	11.4	2.6	7.3	-	" "
M	11.9	(3.2) (4)	7.4	(27)	Rigid
	12.3	(2.8) (4)	7.4	(23)	Rigor passing
S	9.1	2.1	5.4	23	Very fresh
	10.2	2.4	6.8	24	Rigor beginning
	9.6	2.0	5.7	21	" passing
	9.1	1.2	5.4	13	" passed
	8.9	1.3	5.1	15	Decomposing
T	10.5	2.2 (1) 2.6 (3)	6.9 (1) 7.8 (3)	(23)	Very fresh, soft
	10.2	4.8 (1)	12.0 (1)	(47)	Rigid
V	12.1	1.9	4.6 (1)	17	Very fresh
	11.5 (1)	3.6 (1)	8.1 (1)	31	Rigor beginning
	-	3.7	7.8	-	Rigid
	12.7	3.1 (2)	7.9 (2)	25	Rigor passing
K	10.2	2.5	6.4		Very fresh, soft
	10.0	2.0	6.7		Kept 48 hours

(1) Samples very small - 2 cc. only
(2) No duplicate
(3) Duplicates poor, difficult to obtain samples
(4) Coagulation period 7' 4" by mistake

TABLE IV.

Proteins of Muscle Juice of Haddock.

Data of Fish	% of Solids of Juice	Proteins of Juice Coagulated at 40°C	Proteins of Juice Coagulated at 100°C	40° Coag. as % of Solids	Condition of Fish Where kept and how long
I. (22/6/20)	-	3.6	6.6	-	Fresh. 1 hr. in Lab.
IV. (27/6/20)	11.1	3.7	7.2	34	" 2 " " "
"	10.7	1.7	6.6	16	Rigor past, 30 hrs. in Lab,
V. (30/6/20)	11.4	3.0	7.7	22	Fresh, 3 hrs. in Lab.
"	-	1.8	7.2	-	Soft, 60 hrs. in Refrig.
VII. (5/7/20)	9.5	3.0	5.7	-	Fresh
	-	2.9	4.9	-	Rigid, 8 hrs. on ice.
	-	2.3	5.7	-	Soft, 40 hrs. in Refrig.
VIII. (9/7/20)	-	3.8	6.3	-	Fresh
	-	1.5	6.2	-	Good, 24 hrs. in Refrig.
	-	1.6	6.1	-	" 48 " " "

From the Tables III. & IV. it is seen that the amounts of protein coagulated at 40°C. (von Furth's "soluble myogen-fibrin") are small when the fish is first caught and the muscle still soft, then increase probably to the time of the height of rigidity and then again decrease as the muscle begins again to soften, also that this decrease continues while the fish is kept, falling to perhaps as little as one-half the original amount, but that this fall in amount may be very much delayed by cold storage. It is thus apparent that this substance is directly related to the changes of rigidity and softening and that its quantity varies directly with these changes. As to the cause behind these variations, I have as yet no information.

Attempts were made by J. Panton (Panton 1921) to find changes in Hydrogen Ion Concentration during the rigidity and softening but without success. I have also made readings of value for pH, on the muscle juice directly and after dialyzing it through collodion sacs, but the values obtained through the whole period of the death changes were practically the same. The amounts of lactic acid present in the muscle during this period should be followed and compared with the values for pH.

In Table V are given the results of determination of hydrogen Ion concentration of juice from haddock muscle, using a series of buffers and matching colors produced by the indicators, which are given in the table. The shades of color given by the phenol red were quite definite but the yellowish tint of the juice interfered with the testing by the use of brom phenol purple.

In the tests made by Panton (1921) the method of Hirsch (1921) was used and she obtained values for pH of 6.2 and 6.3 at

intervals during 24 hours after the death of the fish and found a constancy of value as I did. She also made use of the indicator method of determination. The difference between our values may be due to the use of different preparations of indicators of buffers, although pure materials were supposedly used.

TABLE V.

Values for Hydrogen Ion Concentration

No. of Fish	pH	Indicator	Condition of fish
R	6.6	Phenol Red	Frozen 3 days, thawed in air
"	6.6	Brom Cresol (Purple)	"
	6.8)	"	" " " " in water
	6.8(	"	Which gave pH of 8.0
S	6.8	Phenol Red	Very fresh, soft
"	6.8	"	Rigor beginning
"	6.6	"	" passing
V	6.6	"	Very fresh, soft.

A few experiments were also made with other species of fish, giving results included in Table VI which agree with the information previously gathered, that the colloidal nature of the muscle of the haddock, the hake, and the cod are very different. This, too, is shown by the ease with which the muscle juice can be squeezed out and the composition of the juice so obtained. In these tables are included values obtained in 1920 and others obtained with gray fish and angle fish. The peculiarities of hake and cod which are shown here, are quite in agreement with those discussed by Jackson (1922), and are evidently due to peculiarities of colloidal condition.

TABLE VI.

Muscle Juice from Fish, other than Haddock

Data of Fish	% of Juice	% Solids of Juice	Proteins of Juice Coag'd at 40°	100°	Condition of Fish Where kept and how long
Cod (5/8/20)		13.5 (2)	1.0(2)	4.5(2)	Rigid, 4 hrs. in bo
"			2.5	4.6	Soft, Refrig, 40 hr
Cod (2/9/22) 49 cm.	5.4	8.6	1.1(3) 1.5	4.8	Rigor past Lab. 24 hrs.
Hake (29/6/20)	4	8.2	-	-	Lab. 7 hrs.
Hake (5/7/20)	-	6.4	1.6	3.7	Frozen, spongey Ice, 24 hrs.
"	-	7.3	3.3	4.3	Frozen well Ice 24 hrs.
Hake (22/7/22) 54 cm.	9.2	7.4	0.1	3.7	Soft, Lab. & Refrig. 2 days.
Hake (26/7/22) 66 cm.	17	7.7	0.3(1) 0.5(3)	3.5	Soft, Lab. 25 hrs.
Skate (9/7/22) 139 cm.	23	11.5	?(5)	6.0	Lab. 24 hrs.
Gray fish I(17/7/22) 40 cm.	40	12.5	2.4	5.8	Lab. 5 hrs.
I. "	6	8.9(2)	4.6(1)	6.0(2)	Soft,Refrig. 27 hrs.
Gray fishII(17/7/22) 40 cm.	13	15.1	6.7	9.2	Slow, frozen - Spongey, 2 days.
II.					
Angle Fish (17/7/22)	35	-	0.3	1.8	Lab. 18 hrs.
Angle Fish (22/7/22)	30	6.8	1.0	2.9	Lab. 24 hrs.

(1) Samples very small
(2) No duplicate
(3) Duplicates poor
(5) Analysis spoiled

The fish for which information is given in this table (No. VI) were not treated in such a way as to give us information in regard to variations in composition due to condition of rigidity but it is evident that for different species of fish there are very marked variations in the nature of the muscle.

In examining cod and hake it was very difficult to squeeze out muscle juice in sufficient quantity to handle. The muscle became very gummy under pressure and so little fluid was expressed that it was impossible to make as complete determinations as were desired. The Angle fish and skate gave large amounts of juice, and left a fibrous stroma. The gray fish, too, which was examined while fresh readily gave up a watery juice but the same fish on the next day had undergone such change of colloidal condition that the squeezing formed a soft mass, almost fat like to the touch and very little juice was pressed out and this was of a creamy appearance.

The amounts of the problem coagulated at 40°, show, too, very different values from those obtained from haddock. From the more careful study which was made with the haddock, it is almost certain that all these determinations of amounts of proteins were made after the fish had passed through the various stages of rigor mortis, except in the cases of the frozen fish. Since with haddock the amounts of the 40° coagulum are small following rigidity that would partially account for the result here shown, but the precipitates obtained at 40°C from skate, from angle fish and from hake (except the well frozen specimen) were all so slight that it was very difficult to wash and accurately weigh such small amounts. They were much softer, too, and less granular

than those formed at this temperature for the juice of haddock muscle. There are very definitely differences in amounts of 'soluble myogen fibrin', in the juice collected from the muscles of different species of fish - differences, too, perhaps in its nature, and certainly also differences in colloidal condition of proteins of the muscles studies.

Conclusions.

1. The juice pressed out from the muscle of haddock shows variations in amount of protein coagulated at 40°C. (Von Furth's 'soluble myogen fibrin') during the different stages of rigor mortis and the subsequent softening. The amount of this protein is smaller before the period of rigidity, increases with the stiffening and then decreases to much smaller amounts during the relaxation of the muscle and during the following time.
2. The muscles of different species of fish react very differently to pressure. Under the conditions of these tests haddock muscle gave about 1/5 of its weight as juice but the muscle of cod and of hake gave only about 1/20 of their weight. Hake and gray fish also undergo changes on keeping which influence this relation between the amounts of liquid which can be expressed and the stroma which remains.
3. The colloidal nature of muscles of different species of fish must, therefore, be regarded as showing variations.
4. The quantities of 'soluble myogen fibrin' vary in different species of fish.

5. The freezing of fish delays the changes which are to be noted in the variations in the amounts of soluble myogen-fibrin but freezing also changes the state of the colloidal system in the muscle producing differences of relationship between the colloids present.

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