TRYPTIC HYDROLYSIS OF FRESH AND FROZEN FISH MUSCLE

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Tryptic Hydrolysis of Fresh and Frozen Fish Muscle

Final Report.

By Jean R. Panton

During the summer of 1930, investigations were attempted to determine the difference, if any, on the digestion of fresh and frozen fish muscle by trypsin. Muscle of the haddock, cod and mackerel was examined. The fresh muscle was from fish caught in the Halifax harbour. The frozen muscle was material which had been frozen at the Fisheries Experimental Station, Halifax, by the quick brine freezing method in the making of frozen fillets. These fillets were then kept in storage in the cold rooms for varying lengths of time. This was one among several investigations to determine possible changes occurring in the muscle when freshly frozen and when stored after freezing.

Very little available information could be found on the tryptic hydrolysis of fish muscle. Bradley (1) considered that trypsin digested pig pancreas and beef kidney at a reaction so alkaline that autolysis was almost completely prohibited. He considered that there were two proteases concerned in autolysis - one digesting acid salts of protein and active between pH 7 - 3: the other present in abundance and active between pH 8 - 3. The latter protease, he considered, was unable to attack the acid salts of the protein but acted only on products formed by the first enzyme. The rate of autolysis of fresh and frozen cod muscle was compared by Callow (2). This was an extension of Fearon and Foster's work done on ox muscle. He found muscle, frozen in brine at - 19°C. for 2½ hours and then stored at -11°C for 10 days, gave practically identical results with the unfrozen control. Fearon and Foster found that brine freezing did not cause any appreciable changes in the tissues of ox muscle. Wynne (3) studied the extent and rate of hydrolysis of haddock muscle by trypsin. He found that the salting of haddock muscle reduced the rate and extent of its hydrolysis by trypsin; also, that trypsin digested smoked haddock muscle at a somewhat slower rate than fresh muscle, especially in the earlier stages. As far as is known, no records are available giving information as to rate and extent of hydrolysis of fresh fish muscle by trypsin.

The action of trypsin on protein material results in a very marked disruption of the protein molecule and at the end of the digestion a large percentage of the nitrogen of the original protein is present as nitrogen of individual amino acids. The method of investigation involved an estimation of amount of amino acids produced at intervals over an average period of 120 hours.

The Foreman (4) modification of the Sorensen formol titration method was used. This gave values for estimation of amino acids which agreed more closely with the theoretical values than did the original method of Sorensen. Foreman found that ammonia did not form ionizable compounds with phenolphthalein in alcoholic solutions containing water if the concentration of alcohol was sufficiently high. He also found that when aqueous alcoholic solutions of certain amino acids (containing about 85% alcohol) were titrated with standard alcoholic alkali, that the amino groups liberated from their "internal salt" combinations resembled ammonia in showing no basicity to phenolphthalein and the carboxyl groups were accurately estimated. Other amino acids gave low results when titrated in alcohol under these conditions. The subsequent addition of formaldehyde resulted in a disturbance of the equilibrium so that the carboxyl groups titrated quantitatively.

The titration was done in three stages..

Stage (1). Titration in water. A 5 or 10 cc. portion of the solution was titrated with N/10 alkali, using phenolphthalein.
Stage (2). Titration in alcohol. 5 or 10 cc. of the original solution was transferred to a flask or beaker of 250 cc. capacity; 10 volumes of 97% alcohol and three drops of phenolphthalein solution were added and the mixture titrated in daylight over a white plate with N/10 alcoholic soda until a light pink color was produced.
Stage (3). Titration in alcoholic formaldehyde. After obtaining the reading for stage (2), the same liquid was used for stage (3). 12.5 cc. of aqueous and neutral formaldehyde solution were added for each 50 cc. of alcohol used in stage (2), and the titration was continued to the same end point as before.

The result obtained in stage (1) gives useful information when dealing with dibasic amino acids, arginine and salts of amino acids.

Results obtained in stage (2) include the reading in stage (1).

The increase in titration value in stage (3) gives an idea of the character of the amino acid and when dealing with an amino acid mixture indicates the amount of dibasic amino acids and proline present. On completing stage (3), the carboxyl groups of the total amino acids are estimated, excepting arginine. The results obtained from stages (2) and (3) gave the total amino acid groups split off during tryptic hydrolysis.

Method of Preparation of Muscle, Sampling and Titrating
(Modification of method of Wynne (3).)

Three five hundred cc. flasks, each containing 80 grams of muscle, minced in a food chopper, and 350 cc. boric acid borate buffer solution (pH 7.5) were prepared. Toluol was added to prevent bacterial action. The frozen fillets were minced frozen to avoid the leaking out of muscle juices. The flasks stood overnight at room temperature. Next morning, to two of them, 1.2 grams of pancreatin were added. The solution was readjusted to pH 7.5 when necessary. The flasks were incubated at 37°C. and at intervals samples were removed, filtered through #1 Whatman paper and titrated, according to the procedure used by Foreman (4). At the end of an experiment the pH of the remaining digest mixture was determined and was found to be lowered slightly, varying from pH 7.5 to pH 7.2.

Fresh muscle of the haddock, cod and mackerel was tested according to the method indicated and the results compared with those obtained from the frozen fillets treated in the same way. The following tables indicate the results.

TABLE I

Haddock Muscle.

Time	Fresh (Stages 2 and 3) cc. N/10 NaOH per 10 cc. sample	Time	Fresh (Stages 2 and 3) cc.N/10 NaOH per 10 cc. sample
½ hour	6.44	1 hour	8.64
24 "	14.94	20.5 "	13.62
50 "	24.22	68 "	23.64
96 "	29.64	97.5 "	29.56
121 "	30.72	119 "	31.76

TABLE I. Haddock Muscle (Cont'd.)

Frozen 7 months

Temperature of storage 0°F. to -5°F.

	(Stages 2 and 3)
Time	cc. N/10 NaOH per 10 cc. sample.
2 hours	6.14
25 "	17.20
51 "	23.56
73 "	25.26
97 "	28.58
150 "	32.52

Frozen 7 months

Temperature of storage 0°F. to -5°F.

	(Stages 2 and 3)
Time	cc. N/10 NaOH per 10 cc. sample
2 hours	5.94
25 "	18.30
51 "	24.96
73 "	28.88
97 "	31.20
150 "	33.10

Frozen 14 months

Temp. of storage 0°F, to -5°F.*

	(Stages 2 and 3)
Time	cc. N/10 NaOH per 10 cc. sample
1.5 hours	6.74
24.5 "	16.52
50 "	23.98
96.5 "	30.12
121 "	30.02

Frozen 14 months

Temp. of storage 0°F. to -5° F.*

	(Stages 2 and 3)
Time	cc. N/10 NaOH per 10 cc. sample
1.5 hours	5.34
24.5 "	17.22
50 "	24.18
96.5 "	30.02
121 "	30.74

Frozen 14 months

Temp. of storage 0°F. to 5°F. *

	(Stages 2 and 3.)
Time	cc. N/10 NaOH per 10 cc. sample
0.5 hours	7.24
50 "	24.38
77.5 "	29.42
100 "	30.98
145 "	33.58

* For three weeks, when changes were being made in the jacket of the cold storage room, this material was moved and during that time was subjected to a temperature of -5°F to -10°F.

TABLE I. Haddock Muscle (Cont'd.)

Frozen less than 24 hours

Temp. of storage 0°F. to -5°F.

Time	(Stages 2 and 3) cc. N/10aOH per 10 cc. sample
0.5 hours	6.24
24.5 "	14.76
71.5 "	25.56
96.5 "	28.28
118.5 "	27.48

Frozen less than 24 hours.

Temp. of Storage 0°F. to -5°F.

Time	(Stages 2 and 3.) cc. N/10 NaOH per 10 cc. sample
0.5 hours	7.14
24.5 "	15.44
71.5 "	26.28
96.5 "	31.38
118.5 "	31.50

By examination of Table I, and of Graph I, it will be seen that the total digestibility of haddock muscle by trypsin shows no variation whether the muscle be fresh or frozen and whether the length of storage of the frozen material be less than 24 hours or as long as 14 months.

TABLE II.

Cod Muscle.

Fresh

Time	(Stages 2 and 3) cc. N/10 NaOH per 10 cc. sample
1 hour	7.32
18.5 "	23.74
41.5 "	27.46
88.5 "	31.40
138.5"	32.50
184.5"	32.30

Fresh

Time	(Stages 2 and 3.) cc. N/10 NaOH per 10 cc. sample
1 hour	8.54
18.5 "	24.34
41.5 "	30.06
88.5 "	31.60
138.5"	--
184.5"	32.80

TABLE II. Cod Muscle (Cont'd.)

Frozen 2 years.

Kept in tin box wrapped in waxed paper.

Temp. of storage -10°F.

Time	(Stages 2 and 3.) cc. N/10 NaOH per 10 cc. sample
0.5 hours	6.04
24.5 "	18.32
68 "	26.16
120.5 "	30.00

Frozen 2 years.

Kept in tin box wrapped in waxed paper.

Temp. of storage -10°F.

Time	(stages 2 and 3.) cc. N/10 NaOH per 10 cc. sample
0.5 hours	6.82
24.5 "	19.52
68 "	28.38
120.5 "	29.50

Frozen 2 years.

Kept in tin box wrapped in wax paper.

Temp. of storage -10°F.

Time	(Stages 2 and 3.) cc. N/10 NaOH per 10 cc. sample.
0.5 hours	6.74
22.5 "	16.78
75.5 "	28.38
119.5 "	32.68

By an examination of Table II and Graph II, it will be seen that fresh or frozen cod muscle tend to maintain equivalent total digestibility values when exposed to the action of trypsin over a period of 120 hours.

TABLE III.

Mackerel Muscle.

Fresh

Time	(Stages 2 and 3.) cc. N/10 NaOH per 10 cc. sample
0.5 hours	7.00
24.5 "	23.98
69.5 "	35.08
97.5 "	37.78
140 "	37.70

Fresh

Time	(Stages 2 and 3.) cc. N/10 NaOH per 10 cc. sample
0.5 hours	8.04
24.5 "	23.98
69.5 "	37.78
97.5 "	38.50
140 "	40.30

Frozen 5 weeks

Temp. of storage 0°F. to -5°F.

1 hours	7.04
25 "	25.44
37 "	28.76
66 "	35.26
85.5 "	36.26
131 "	37.58
180 "	37.16

Frozen 5 weeks

Temp. of storage 0°F. to -5°F.

1 hours	7.14
25 "	25.24
37 "	29.26
66 "	35.66
85.5 "	37.98
131 "	40.38
180 "	39.78

Frozen 5 weeks

Temp. of storage 0°F. to -5°F.

1 hours	8.14
27 "	22.84
48.5 "	31.56
78 "	35.06
95 "	36.58
118 "	37.58

Frozen 5 weeks

Temp. of storage 0°F. to -5°F.

1 hours	8.04
27 "	24.74
48.5 "	29.46
78 "	35.06
95 "	36.34
118 "	37.06

By an examination of Table III and Graph III, we see that mackerel muscle shows no difference in total digestibility by trypsin over a period of 120 hours.

By examination of Graph IV, which shows the relative rates of tryptic hydrolysis for fresh cod, haddock and mackerel muscle, a surprising difference is noted in the initial rate of hydrolysis by trypsin and also in the total digestibility over a period of 120 hours. The total digestibility of mackerel is decidedly higher than that of cod or haddock and the initial rate of digestibility of cod muscle is quicker than that of haddock or mackerel.

By examination of Graph V, it is apparent that the initial rate of digestion by trypsin is definitely retarded in the case of cod muscle frozen and stored at -10°F. for 2 years. Mackerel and haddock muscle do not show this variation, but the length of time of storage in both these cases is shorter than two years.

Conclusions

1. For any one species of fish muscle, the freezing process does not alter the total digestibility of the muscle by trypsin when it is stored under suitable conditions of temperature. Cod muscle, even when stored for 2 years at -10°F. shows no marked variation in total digestibility by trypsin.

2. The relative rates of tryptic hydrolysis of the fresh muscle of haddock, cod and mackerel show definite variations over a period of 120 hours. The total hydrolysis of mackerel muscle is decidedly greater than that of cod or haddock. The initial rate of hydrolysis of cod muscle by trypsin is higher than that of haddock or mackerel.

3. The initial rate of digestion of frozen mackerel or haddock muscle shows no decrease as compared to the fresh muscle. Cod muscle which has been frozen and stored for two years at -10°F. shows a lowered initial rate of digestion as compared to fresh cod muscle.

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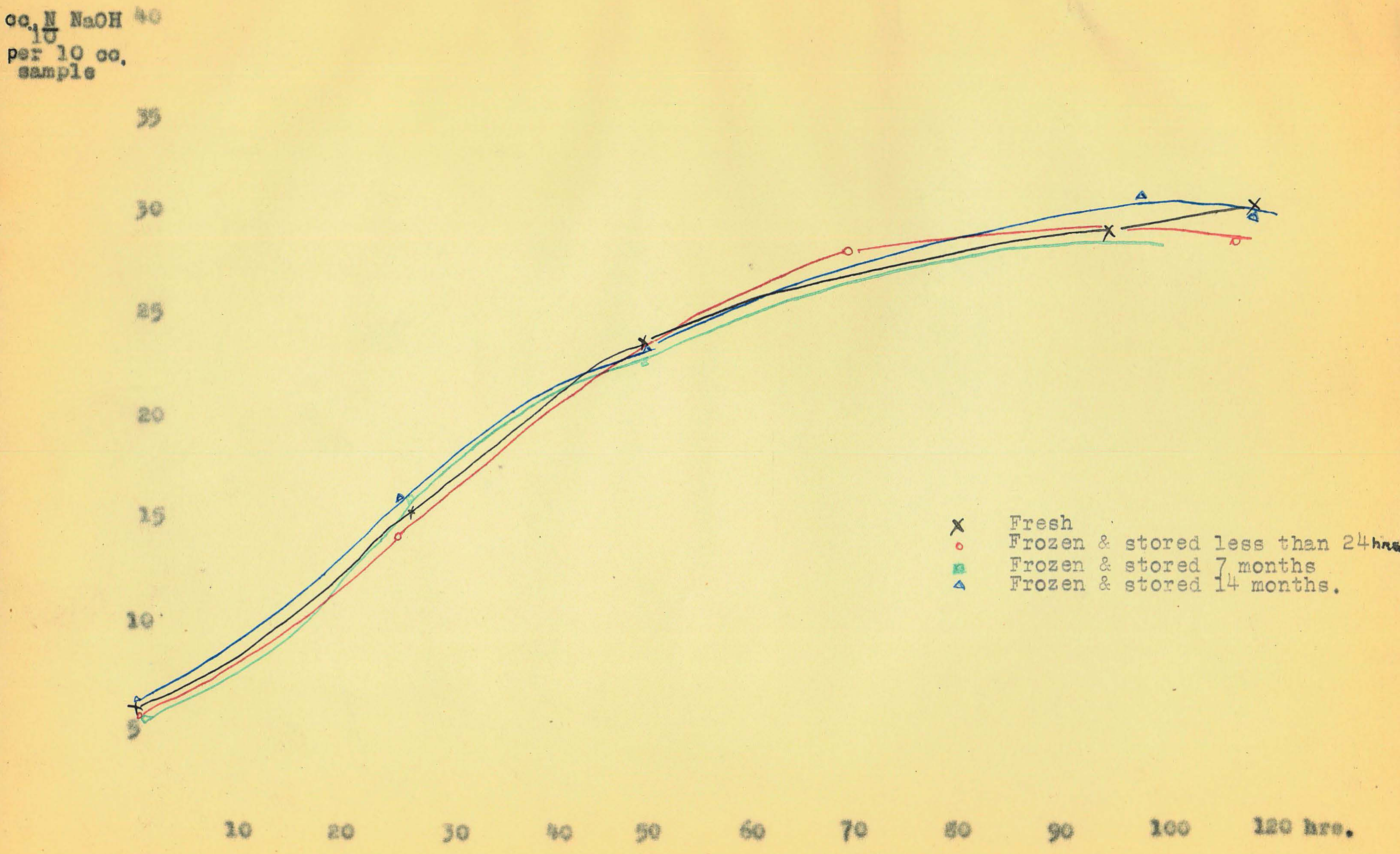
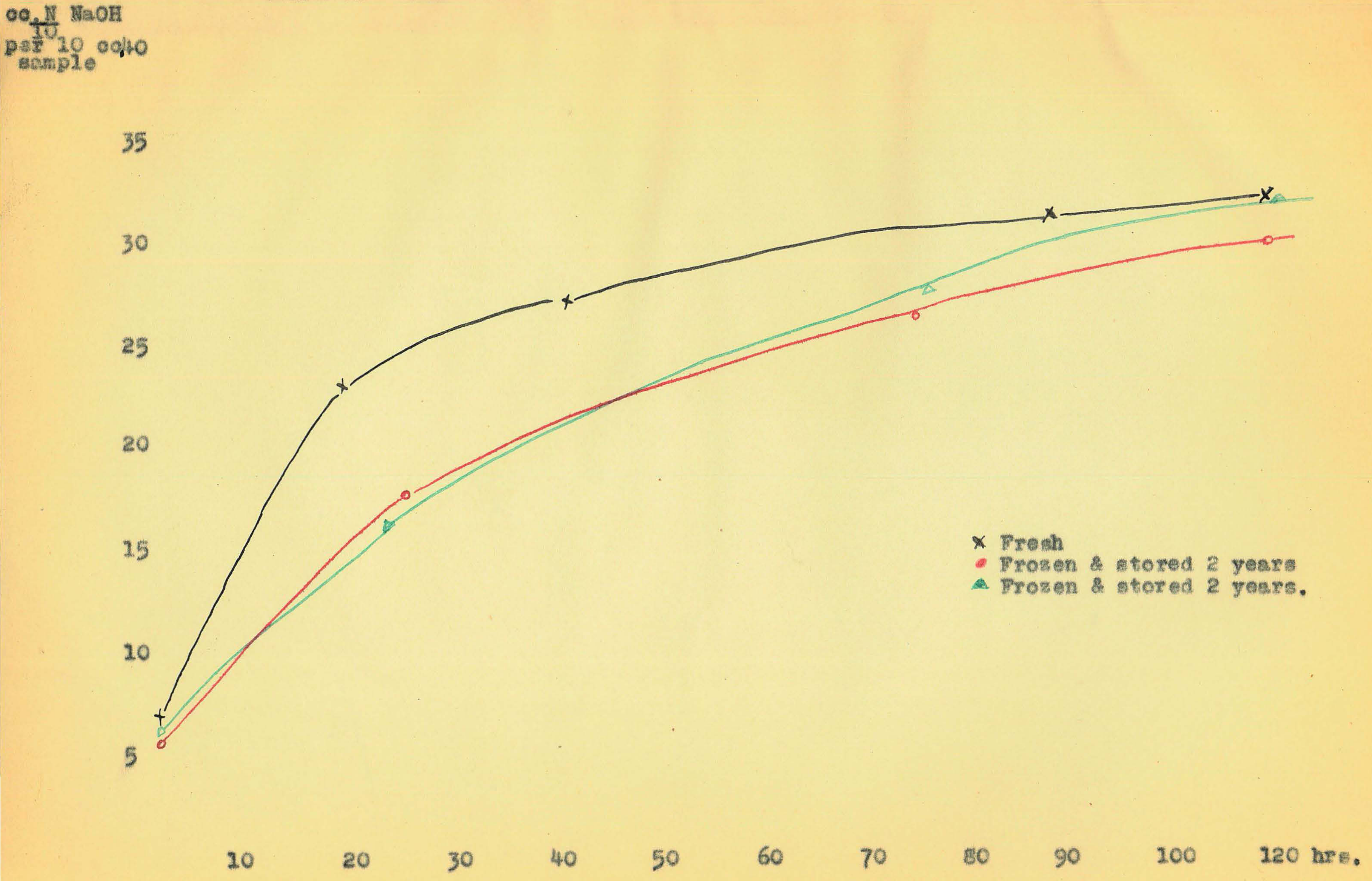
Foreman, — Biochem. J. XIV, 1920.

An attempt was made to devise a method for the determination of the rate of peptic hydrolysis of fresh and frozen fish muscle. A modification of the method for the "Analysis of Incomplete Protein Hydrolyzates," by Wasteneys and Borsook (J. B. C. Vol LXII, No. 1, 1924) was planned. Although this method was tested, there is nothing to report until further work has been done.

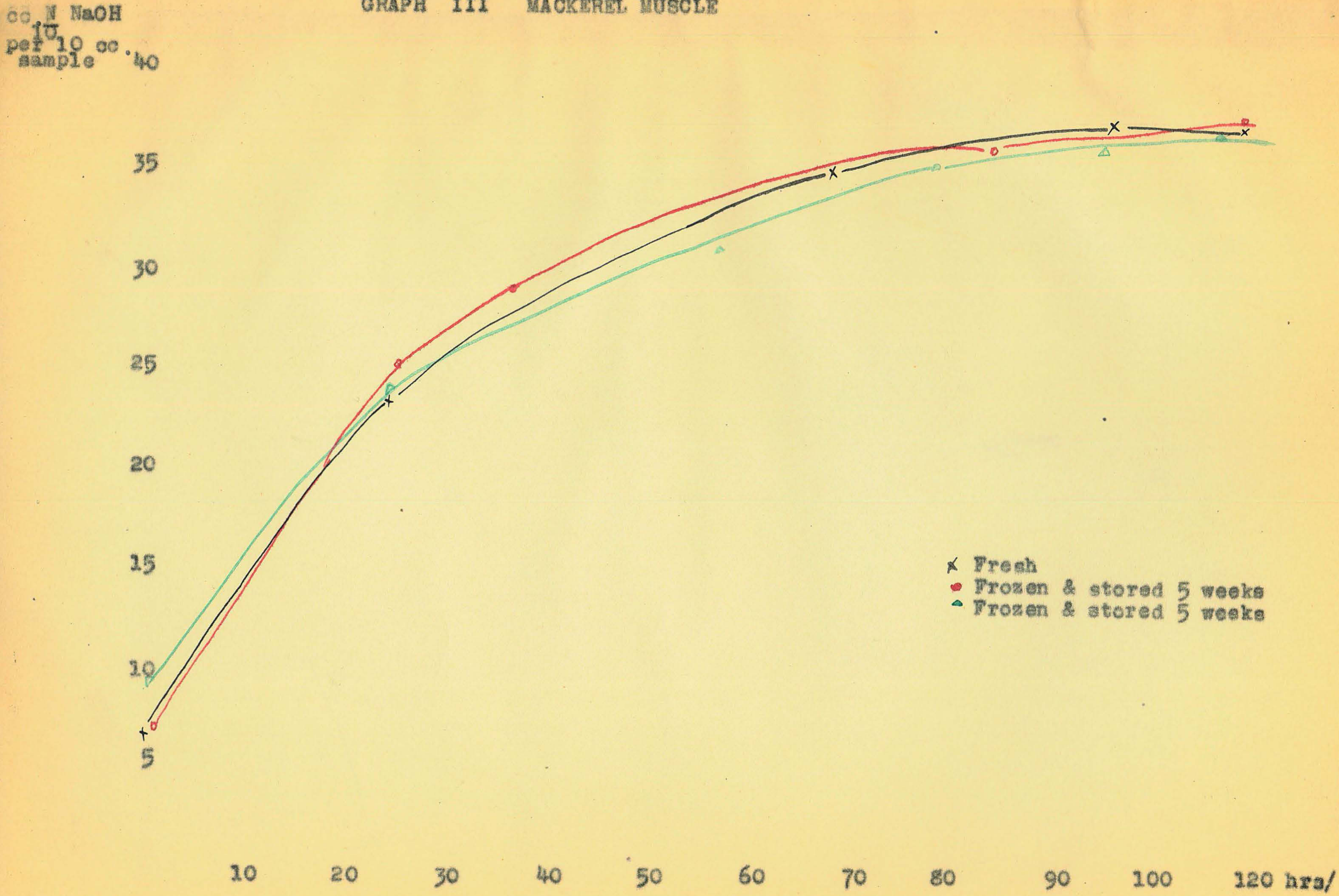
Graph I Haddock Muscle

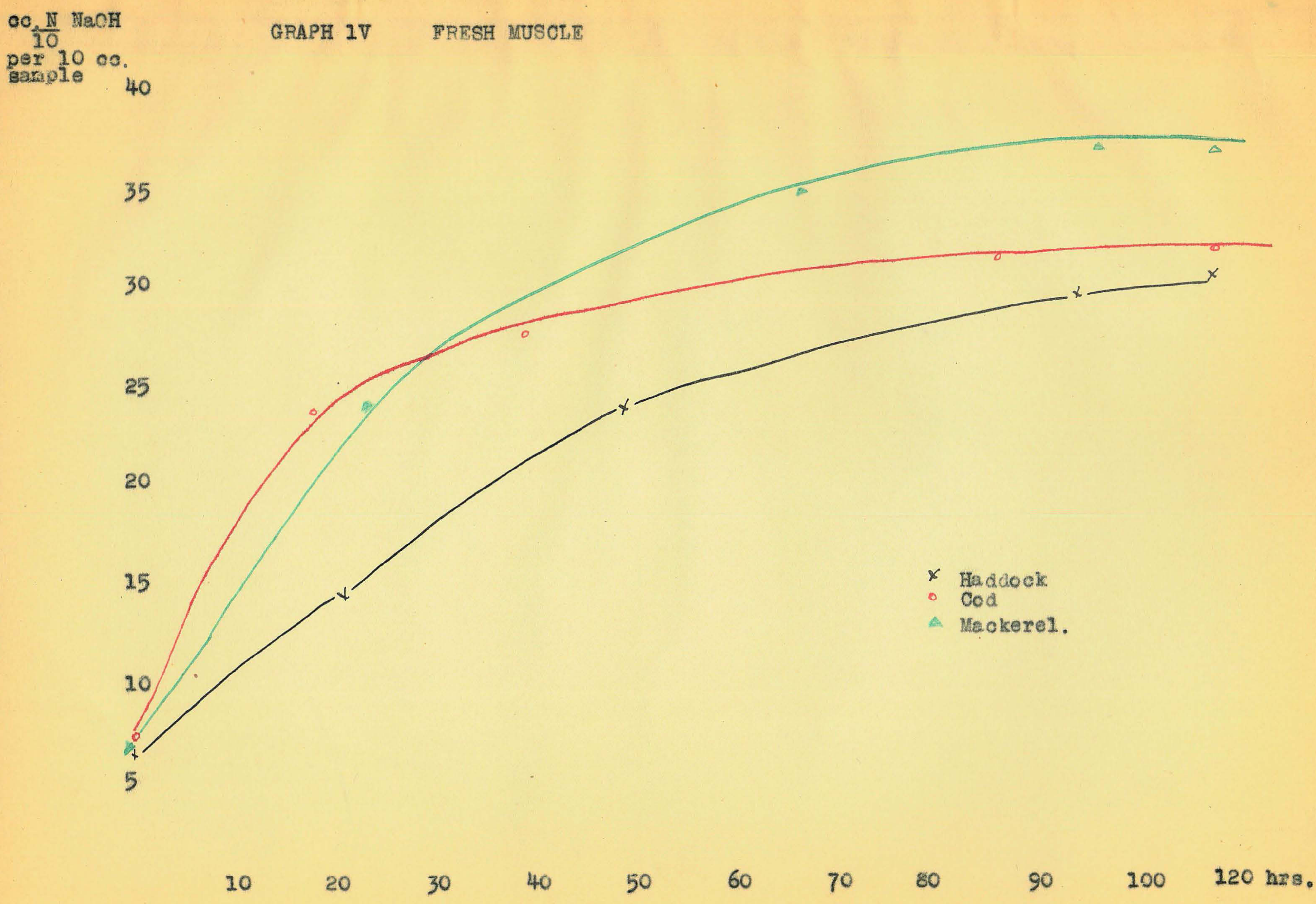


GRAPH II   COD MUSCLE



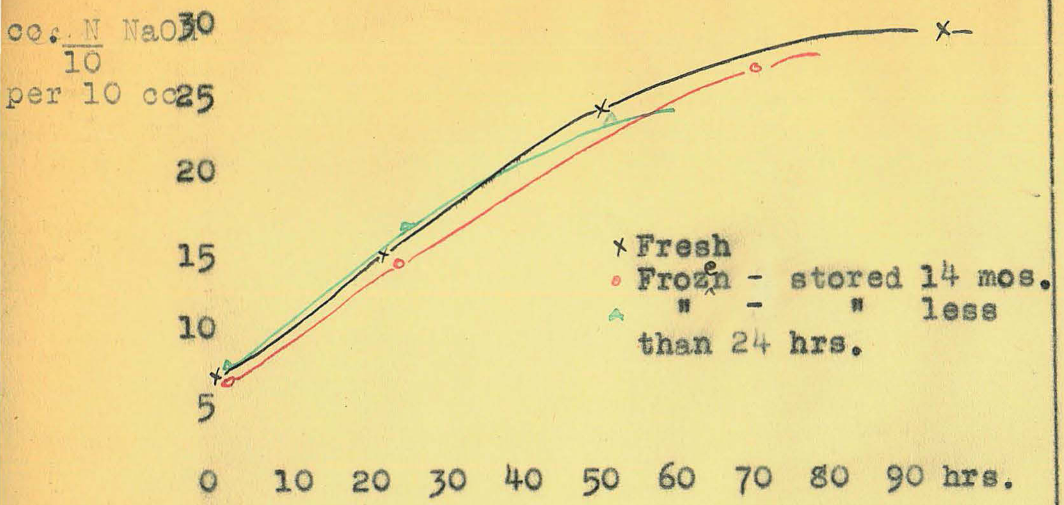
GRAPH III MACKEREL MUSCLE




cc. N/10 NaOH per 10 cc. sample
GRAPH 1V FRESH MUSCLE
40
35
30
25
20
15
10
5
10
20
30
40
50
60
70
80
90
100
120 hrs.
Haddock
Cod
Mackerel.

GRAPH V. Initial Rate of Digestion by Trypsin

Haddock Muscle.



Mackerel Muscle.