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Title

THE EFFECT OF TEMPERATURE UPON THE RATE OF DECOMPOSITION OF HADDOCK MUSCLE

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THE EFFECT OF TEMPERATURE UPON THE RATE OF DECOMPOSITION OF HADDOCK MUSCLE

by

Christine E. Rice and Ernest Hess

1.- Discussion of the general relationship of temperature to biological reactions

The relationship of temperature to biological processes is well recognized and permits more readily of quantitative investigation than do many problems in biology. Bayliss (1915) in reviewing the subject, pointed out that since vital phenomena are made up of heterogeneous systems consisting of various solid and liquid phases in addition to processes of a purely chemical nature, physical ones such as diffusion must be taken into consideration. Panton (1924) showed that for some physiological processes, at least, the temperature coefficients are probably of the same order as those for a chemical reaction when corrected for changes in the viscosity of the medium. Snyder (1911) also considers the variation in the temperature coefficients to be due to the variation in the coefficients of viscosity, the real temperature coefficient of the chemical reaction being constant. As was pointed out by Osterhout (1917), substances are continually being formed and broken down in biological systems, and the temperature coefficient of the combined reactions may be vastly different from those of the separate ones. In general, physical processes have lower coefficients than purely chemical ones, thus a chain reaction involving reactions of both types will not show a constant temperature relationship.

As stated by Van't Hoff (1884) for a rise of 10°C., the velocity of a chemical reaction is usually doubled or trebled. The temperature quotient, Q, represents the ratio between the velocity constants at the higher and lower temperature, and since determinations are ordinarily made at 10°C. intervals, the symbol Q_{10} is most generally used. The value Q_{10} for purely chemical reactions is not always constant, nor is it for purely physiological ones, there being a tendency to decrease with increase in temperature in both cases. We may assume that if two temperature characteristics apply at different parts of a

temperature range that a series of linked reactions are occurring. Although the rate of the whole reaction is governed by the slowest reaction of the series, the effect of the temperature on the velocity constants of these diverse reactions may vary, so that the controlling step in the different cases may differ in two ranges. Critical temperatures may be defined as regions in which control passes from one underlying reaction to another. For many organic and inorganic processes temperature optima are exhibited. The assimilation of carbon dioxide or the fermentation of sugars are reactions of this type. The velocity of the fermentation depends on two factors, the temperature and the amount of the enzyme. Enzymes are unstable, their instability rising with the temperature, so that although in raising the temperature the velocity of the catalyzed reaction is accelerated, at the same time the velocity of the destructive action is also increased, till finally a maximum is reached beyond which the destructive action predominates and we have a downward slope to the curve.

Kanitz (1916) reviews mathematically the relation between temperature and the velocity of reaction. The equation most generally used is the following:-

$$Q_{10} = 10^{\frac{10(\log k_1 - \log k_0)}{t_1 - t_0}}$$

where k_1 and k_0 represent the velocity of reaction at the temperature t_1 and t_0 respectively and Q_{10} the quotient for 10°C. Crozier (1925a) claims that Q_{10} is quite an imperfect means of characterizing a process. For certain vital activities which may be taken as typical, the underlying processes may be treated as systems of irreversible first order reactions (Osterhout 1914), to which may be applied the equation formulated by Arrhenius (1899) who explained the influence of temperature as being due to the conversion of inactive into active substances with the consumption of a definite amount of heat. This equation considers the velocity of an irreversible reaction to vary as the exponent

$$\frac{E}{RT}$$

where R is the gas constant, T the absolute temperature, and E or μ the gram molecular energy of activation or the critical increment of the substance (or for a bimolecular substance the sum of these energies). The quantity μ is subject to such slight variations with the temperature, the amount of catalyst, and the actual velocity of the process that it is therefore a more significant

index than Q_{10} for characterizing a process.

$$\frac{k_1}{k_0} = e^{\frac{\mu}{2} \frac{(T_1 - T_0)}{T_1 \times T_0}}$$

For the values k_1 and k_0 we may substitute respectively the reciprocals of the times $1/T_1$ and $1/T_0$ required to perform the same amount of work. This formula is used by Hecht (1919). Glaser (1924), Crozier (1924, 1925 etc.) and others. Crozier (1924a) argues that since μ means energy of activation, it is to be expected that processes governed by the same species of active molecule will yield the same value of constant μ. A preliminary survey of a large number of vital phenomena shows that they may be divided into groups according to the associated value of μ.

Przibram (1923) shows that the values of Q_{10} for organic and inorganic processes fall into four distinct classes.

1 - Q_{10}	=	1	
2 - 1.018	<	Q_{10}	< 1.6
3 - Q_{10}	=	2 to 3	(20°C.)
4 - 3.5	<	Q_{10}	< ∞

Subnormal values of Q_{10} are found in solid and liquid systems where the observed velocity is the result of two partial processes., the conversion of the solid into the liquid state and the subsequent slow diffusion governing the speed of the reaction, the velocity of diffusion being only slightly affected by temperature, Kanitz (1916).

Among the organic coefficients, Groups I and II include such phenomena as the bleaching of visual purple and the effect of beta-rays upon the eggs of Nereis; that is chemical processes in which radiant energy takes part, photochemical processes.

Group III may be divided into three sub-groups as follows:-

A-Locomotion and rhythmic action - which include such phenomena as protozoan movement, heart beat, rate of nerve impulse, respiratory movement and phagocytosis.
B-Development and duration of life - including such reactions as development of fish eggs, action of rabbit serum on B. coli and the death rate of Staphylococci in air.

C- Assimilation and dissimilation - under these he groups the rate of evolution of carbon dioxide from seeds, the oxygen consumption of Arbacia eggs and the conversion of pancreatic zymogens to enzymes.

Group IV includes such processes as the ingestion of quartz and carbon particles by amoebae, the coagulation of hemoglobin and the resistance of certain forms to heat.

II - Temperature in Relation to the Autolytic and Bacterial Decomposition of Haddock Muscle.

That decomposition and putrefaction are affected by temperature is too well known to warrant any reference to it. Both autolytic and bacterial action, the two most important agencies in the natural decomposition of plant and animal tissue, are, within certain more or less definite temperature intervals, accelerated by heat; optimum temperatures exist for both processes. Above these temperatures due to the more or less extensive alteration of the proteins, the bacteria are killed and the enzymes inactivated, the retarding influence may also be due in part to the preferential acceleration of the resynthesis of the substrate, Robertson (1908). In considering autolytic decomposition of haddock muscle we are thinking almost entirely of enzymes of a proteolytic nature. The best known enzymes whether derived from warm or cold blooded animals are most active between 35° and 45°C., Hawk and Bergein (1927). Freezing, although temporarily inhibiting their action, causes no permanent injury.

The optimum temperature for the bacterial decomposition of the haddock muscle depends upon that of the bacterial flora present, and since this is extremely varied, at different temperatures we would expect that different bacteria would predominate and that the type of reaction occurring therefore would not be entirely similar. The decay producing and putrefactive bacteria have an optimum temperature between 20° and 35°C., as also do soil and water bacteria belonging to the genera Pseudomonas, Flavobacterium and Achromobacter, most of these growing best either at 25° or 30° depending upon the species. The other genera occurring in the bacterial flora of the haddock, that is Bacillus, Escherichia, Micrococcus and Proteus types have a somewhat higher optima, 30°

in some cases, but 37° with the majority of species; that for B. mesentericus and B. vulgatus, which are found in quite large numbers in haddock slime, is given by Bergey as 30°C. Certain psychrophillic bacteria produce their best growth below 10°C., and for this reason some of them may be important in the decay of foods in cold storage. Freezing stops multiplication, due probably to the dessication; the organisms however retain their viability for a varying length of time, depending on the species. Since however in the presence of solutes in sufficiently high concentration water may be lowered considerably below the zero point without freezing, some organisms continue to multiply, although slowly, at such temperatures. From this it is evident that food stuffs cannot be kept indefinitely above the freezing of the liquids concerned.

In considering the effect of temperature upon the bacterial decomposition of the fish, three phases must be considered. (a) the effect upon the growth of the bacteria, (b) the effect upon the production of the enzymes concerned in the processes under consideration, and (c) the influence upon the rate of the enzyme reaction itself. The first may be followed by plate counts, while by determining the increase in the products of enzyme action we are arriving at a knowledge of the last and indirectly of the second. The temperature coefficient of enzyme actions is affected by the influence of temperature upon (a) the concentration of active enzyme, (b) the reaction velocity of the substrate-enzyme combination, and (c) the position of the equilibrium point. The following table from Waksman and Davison gives the temperature coefficients and increments for the action of two important proteolytic enzymes, trypsin and erepsin. A review of the literature shows that the majority of workers have found that bacterial enzymes of a proteolytic nature resemble either trypsin or erepsin most closely in their behaviour. One of us, (Rice 1927) found that B. mesentericus vulgatus, one of the organisms used in these experiments, produced also a pepsin-like enzyme.

Temperature Coefficients and Constants of Various Enzymes, Euler (1925).

Enzyme	Substrate	Temperature range	Q_{10}	μ
trypsin	Witte peptone	15-25°C.	2.3	14,830
erepsin	" "	15-25°C.	2.6	16,400

The velocity of the decomposition of the haddock muscle at the different temperatures was calculated from the increase in certain products of proteolytic cleavage during definite periods. One must remember, however, that, where the optimum growth is obtained, the organism does not necessarily bring about the maximum amount of change, less acid for instance may be produced at the end of a certain period of time by an organism kept at its optimum than one at a lower temperature, (Buchanan and Buchanan). Three Nitrogen fractions were considered, (a) Non-coagulable Nitrogen, that is the Nitrogen not precipitated by trichloracetic acid, which includes part of the peptones, the polypeptides, amino acids, ammonia and other volatile basic nitrogenous products, (b) amino and ammonia Nitrogen, and (c) volatile basic Nitrogen, including ammonia, amines, etc. Methods for determining these are dealt with in Section III.

Since during the first twenty-four hours incubation at 25°C. autolysis was found to be the most important factor in decomposition, Reed, Rice and Sinclair (1926), a preliminary experiment was carried out with fresh unautoclaved muscle to which an antiseptic had been added. The second experiment was conducted on fresh muscle to which no toluene had been added, so that both autolytic and bacterial action are included.

Due to the fact that bacterial decomposition involves a large number of processes, to avoid the additional complexity introduced by the effects of mixed bacterial cultures upon each other, pure cultures of some of the more common species occurring in the fish were employed for purposes of inoculation of sterilized minced muscle. Gee (1926) and Miss Spence (1927) report Bacillus types, particularly B. mesentericus vulgatus to be the most prevalent. The optimum temperature for this species is 37°C., but it will also be found to multiply quite rapidly at a much lower temperature. As a fourth series of experiments, a sporulating bacillus isolated from a muscle digest incubated at 0°C. and producing good growth in 48 hours on ordinary agar incubated at 0° was employed.

In Section IV the relation of temperature to all four processes is discussed mathematically.

III - Experimental Methods

(a) Preparation of Culture Flasks

Haddock fillets of freshly caught fish were ground up finely in a meat chopper, weighed into flasks, 75 grams in each flask, and 150 c.c. of a 1.5% salt solution added. The flasks were sterilized at 100° on three successive days since this was thought to produce less change in the proteins than autoclaving. The flasks were then inoculated with definite amounts of a well-shaken emulsion of the bacteria (a twenty-four hour broth-culture) and incubated at various definite temperatures for different periods.

(b) Bacterial Count

Before filtering the contents of the incubated flasks, 1 c.c. of fluid was removed from each with a sterile pipette, after stirring up the contents thoroughly by shaking and rotating the flasks. From the 1 c.c. samples appropriate dilutions were made and plated in duplicate in nutrient agar (containing 1.5% NaCl); the dilution flasks contained 1.5% saline. The plates were incubated at 35°C. and counted after 3 days incubation.

(c) Determination of Non-Coagulable Nitrogen

After the sample had been removed for the bacterial count, the liquid from the flask was decanted through a Buchner filter. To the fish remaining in the flask distilled water was added, the lumps ground up finely with a glass rod and allowed to stand for about 10 minutes before decanting. This was repeated twice and then all was emptied into the filter and again washed. The filtrate and washings were made up to 300 c.c. Aliquots were taken for the determinations of the non-coagulable Nitrogen, which were always done in duplicate so that each value recorded in the succeeding tables represents the average of two determinations.

To 25 c.c. of the filtrate 3 grams of trichloracetic acid were added, the volume made up to 100 c.c. and allowed to stand for an hour at room temperature, after which the flocculent precipitate was filtered off, well washed and a Kjeldahl determination made upon the combined filtrate and washings. Approximately 20 c.c. of crude H_2SO_4 was used for each digestion and a little $CuSO_4$ as catalyst. They were distilled into 0.2 N. acid and titrated with 0.1 N. base.

Dauphinee (1926) found that with fresh unheated muscle it was necessary, in order to extract the non-protein Nitrogen thoroughly, to heat in several portions of acidulated water before adding the trichloracetic acid. In this case however the changes brought about by heating seem to have a similar effect for the two methods give comparable results.

(d) Amino and Ammonia Nitrogen

The amino and ammonia Nitrogen were determined by means of the formol titration method of Sorensen. Ten c.c. of the original filtrate were used for each determination, five or six determinations being made upon each filtrate. For titration 0.1 N. sodium hydroxide was used and 25 c.c. formaldehyde solution (1 part Formalin to 2 parts of water).

The very faint yellow colour of the filtrate did not appreciably affect the determination of the end point in the titration.

(e) Volatile Basic Nitrogen

The volatile basic Nitrogen (Ammonia and volatile nitrogenous bases) were determined according to the method developed by Dauphinee (1926), with the modification that instead of 10 grams of the ground up fish muscle, 50 c.c. of the filtrate (corresponding to 11-12 grams of wet meat) were distilled over with alcohol (80 c.c.) after liberating the ammonia by addition of magnesium oxide. 50 c.c. of the distillate were collected in $\frac{N}{10}$ HCl, and the excess acid titrated with $\frac{N}{50}$ NaOH. A blank was run through for each batch of alcohol, and the volatile N. content subtracted from the results of the test. The determinations were carried out in duplicate in each case.

(f) Accuracy of the Determinations

In order to know how significant were the differences between values obtained for the different temperatures, it was necessary to know how great a variation might be expected between duplicate flasks receiving presumably the same inoculation and incubated for similar periods at the same temperature. Since it took some time to weigh out so many samples, the moisture content of the muscle would have changed considerably and the first sample would not be exactly comparable to the last. The fish were however kept in a covered vessel and only small amounts removed from time to time, neither was the very moist fish near the bottom of the dish used. These precautions cut

down the error from this source considerably. In sterilizing, it is evident that the heat does not affect all the flasks to the same extent since there is as much as 6% difference in some cases between the values obtained for non-coagulable and amino Nitrogen in two flasks which had not been inoculated. In spite of thorough shaking of the emulsion, it is difficult to secure an uniform inoculation, some of the bacteria being in a more active state than others and for this reason the growth and multiplication of the bacteria vary considerably.

Eight flasks were inoculated with 5 c.c. of a well-shaken emulsion of B. mesentericus vulgatus, incubated at 35° for 24 hours and a series of determinations made upon each flask. The values are recorded below, Table I.

Table I

Comparison of flasks receiving similar inoculation, and incubated for the same period and at the same temperature.

No.	Non-coagulable Nitrogen	Amino and Ammonia Nitrogen	Volatile Basic Nitrogen
	gms. per 100 gms. muscle		
1	0.725	0.1632	0.0368
2	0.727	0.1617	0.0407
3	0.732	0.1669	0.0421
4	0.724	0.1591	0.0356
5	0.781	0.1613	0.0369
6	0.881	0.1755	0.0431
7	0.752	0.1788	0.0466
8	0.738	0.1613	0.0333
Average	0.757	0.1659	0.0394

It is evident that the average deviation in the values for non-coagulable Nitrogen was 4.8%, for amino and ammonia Nitrogen 3.5% and for Volatile basic Nitrogen 9.4%. In the case of the first this is less than twice the deviation to be found between duplicate determinations.

In the determination of non-coagulable Nitrogen the principal errors are introduced in the precipitation by the trichloracetic acid and in the filtering, those introduced in the digestion and titration being less in magnitude. Below are the values obtained from a series of eight determinations made on a filtrate from one flask.

Table II

Comparison of duplicate determinations of non-coagulable Nitrogen and Volatile basic Nitrogen.

No.	Non-coagulable Nitrogen (gms. per 100 grams muscle)	Deviation from Mean	Vol. Basic Nitrogen gms. per 100 gms. muscle
1	0.728	.029	0.2538
2	0.725	.026	0.2558
3	0.656	.043	0.2587
4	0.674	-.025	0.2606
5	0.691	-.008	0.2432
6	0.717	.018	0.2536
7	0.722	.023	0.2486
8	0.683	-.016	-
Average	0.699	0.0235	0.2535

The average deviation for Non-coagulable Nitrogen is 3.36%, and for volatile basic Nitrogen it is 1.70%.

The mean deviation for the bacterial count of four samples of the same filtrate of the above flask was 10.66%, due to errors introduced in taking the sample, diluting, plating, incubating and counting, while the mean deviation of the counts of 8 flasks of similar inoculations, and the same time and temperature of incubation (same as Table I), was very much higher, amounting to about 62%, which may account for some of the irregular results of the bacterial counts in the course of the experiments.

In order to determine whether any change took place in the flasks with sterilized mixture of fish muscle and saline, due to hydrolysis of the proteins, a set of such sterile flasks were incubated at 35° and 0°C. and examined as usual, after 46 days.

Table III

	pH	Non. Coag.N.	Amino and Ammonia N.	Volatile Basic N.	Appearance
		gm. per 100 gm. fish muscle			
Initial	6.65	0.4923	0.0990	0.0278	white
46 days at 0°C.	6.55	0.4967	0.10002	0.0284	"
46 days at 35°C.	8.00	1.1399	0.3142	0.1702	yellowish brown

While little changes took place in the sterile flasks kept at 0°C., the ones incubated at 35°C. for 46 days showed considerable increases in all three forms of Nitrogen; i.e. more than twice as much non-coagulable Nitrogen, about three times as much amino Nitrogen and about six times as much volatile basic Nitrogen than initially. Results from incubation experiments with inoculated muscle emulsion over a long period would therefore be only of value if corrected for this chemical hydrolysis.

IV - Decomposition of Haddock Muscle

(a) Autolysis at different temperatures

As was stated previously, in the decomposition of haddock muscle autolysis was found to be the most important factor during the first twenty-four hours, after which it is rapidly overshadowed by the bacterial action. Reed, Rice, Sinclair (1926). The extent of the initial autolysis also affects the growth and amount of the proteolysis carried on by certain bacteria. In an experiment to determine in how far the rate of decomposition of clam muscle by B. mesentericus vulgatus was affected by the extent to which autolysis had proceeded before inoculation with this organism, it was found that although in all cases there was an initial decrease in the amount of non-coagulable Nitrogen due to its utilization by the bacteria for growth and the production of enzymes, yet in those digests in which autolysis had been allowed to proceed farther before sterilization the curve rose again earlier than did the others. A low temperature, it is recognized, will very appreciably diminish the extent of autolytic action and will

therefore (in addition to other effects) have a retarding effect upon the growth and reactions of at least this common type of Bacillus. Callow (1925) found that at 37°C. cod muscle was practically all autolyzed after seven or eight days, but at 1° even after twelve months the change was slight. A preliminary experiment was run to determine roughly in how far the temperature did affect autolysis. The muscle was ground up, weighed into flasks containing sterile saline and a few c.c. of toluene added. The flasks were incubated at 35°, 25°, 10° and 0°. At 10° after 6 days the increase in non-coagulable Nitrogen is only slight but about the same as at 0° after 12 days incubation. The amino Nitrogen rises more rapidly, but this really represents a very small change in the total Nitrogen present. The increase at 0° is very slight. It is therefore evident that bacteria which might be active at a lower temperature would have less of these simpler products to use in their early growth, and the inhibitory effect of low temperatures upon bacteria may in part be due to this cause.

Table IV

Autolysis of Haddock Muscle at Various Temperatures.

Temperature	Time of Incubation	Soluble N.	Non-coagulable Nitrogen	Amino and Ammonia N.
		gms. per 100 gms. muscle		
35°	Initial	0.620	0.283	0.0328
"	24 hours	0.895	0.483	0.0499
"	48 hours	-.---	0.517	0.0960
25°	Initial	0.620	0.283	0.0328
"	24 hours	0.710	0.316	0.0461
"	48 hours	0.740	0.375	0.0688
10°	Initial	0.620	0.283	0.0328
"	48 hours	0.694	0.304	0.0432
"	4 days	0.723	0.320	0.0651
"	6 days	0.785	0.334	0.0744
0°	Initial	0.620	0.283	0.0328
"	4 days	-.---	0.289	0.0343
"	8 days	0.783	0.297	0.0342
"	12 days	0.852	0.346	0.0364

Determinations of the change in soluble N. were also made but the results indicated nothing of importance, the values remaining nearly constant during the experiment.

(b) Action of Mixed Cultures of Bacteria at Different Temperatures

A similar series to the above was run without toluol. The resulting decomposition was therefore due to the combined action of the autolytic enzymes of the tissue and of the bacteria included from the slime, gills and intestine of the fish itself. Platings were made from the flasks after 48 hours incubation at the different temperatures. At 35° sporulating Bacilli and Staphylococci predominated, below this temperature the principal types were Pseudomonas, Achromobacter, Flavobacterium, Staphylococci and a few Proteus. One Bacillus type was isolated from a 0° digest which gave good growth on agar after 48 hours incubation at that temperature.

Table V

Effect of a Mixed Culture of Bacteria upon Haddock Muscle incubated at various temperatures.

Temperature	Time of incubation	Soluble N.	Non-coag.N.	Amino and Ammonia N.
		gms. per 100 gms. muscle		
35°	Initial	0.563	0.288	0.0282
"	24 hours	-.---	0.477	0.0723
"	48 hours	0.916	0.701	0.1540
25°	Initial	0.563	0.288	0.0282
"	24 hours	0.912	0.372	0.0589
"	48 hours	0.829	0.571	0.1261
10°	Initial	0.563	0.288	0.0282
"	5 days	0.972	0.417	0.0913
"	7 days	1.061	0.704	0.1020
0°	Initial	0.563	0.288	0.0282
"	5 days	0.592	0.372	0.0423
"	7 days	0.841	0.391	0.0486

The results of this series of determinations are shown in Table V. It is evident that rapid decomposition has occurred in 48 hours at 25° and 35°. After 5 days at 10° the non-coagulable Nitrogen had doubled and in 7 days nearly trebled. At 0° there has been slightly more than 30% increase in this form of Nitrogen, and 50% increase in the amino and ammonia Nitrogen. It is evident that bacteria normally occurring in fish gills, intestine and slime are capable of bringing about considerable change in fish proteins at 0°, the increase in the products of the proteolytic cleavage being greater than produced by autolysis alone.

(c) Effect of B. mesentericus vulgatus at different temperatures

As a next step the effect of temperature upon the growth and proteolysis produced by pure cultures of certain bacteria was determined. Since Bacillus forms are found in greater numbers than other types of bacteria in haddock muscle, a representative of this group was chosen for the next series, B. mesentericus vulgatus which had been found by Gee (1926) and Miss Spence (1927) to be the most common and most active of these sporulating bacilli occurring in haddock muscle and slime. In addition it had been found (Rice 1927) to attack native proteins readily, which indicates its importance in the early decomposition of muscle.

Since it was necessary to sterilize the muscle previous to inoculation, the substratum acted upon by the bacteria is not exactly that which they would encounter in ordinary decomposition. Also in working with pure cultures the bacteria are developing under different conditions than normally occurring in mixed cultures, in which each type exerts certain effects upon the reactions and growth of the others. For these reasons the temperature effects noted below may not be exactly those found in normal decomposition of haddock muscle by these bacteria.

Table VI

The effect of temperature upon the decomposition produced by B. mesentericus vulgatus in haddock muscle - Series I.

Temp.	Time of Incubation	Bacterial Count	Non-coag. N.	Ammonia and amino N.	Volatile basic N.
		per c.c. fluid	Grams per 100 gram. fish muscle		
35°	Initial	75,260	0.599	0.0412	0.0261
"	24 hours	27,000,000	0.441	0.0497	0.0226
"	48 hours		0.609	0.0785	0.0358
"	4 days	122,000,000	0.841	0.0886	0.0567
25°	Initial	75,260	0.599	0.0412	0.0261
"	24 hours	1,090,000	0.350	0.0557	0.0279
"	48 hours	2,320,000	0.449	0.0656	0.0303
"	4 days	57,000,000	0.589	0.0759	0.0532
"	6 days		0.799	0.1196	0.0408?
15°	Initial	75,260	0.599	0.0412	0.0261
"	48 hours	980,000	0.450	0.0406	0.0204
"	4 days	45,500,000	0.498	0.0446	0.0218
"	6 days		0.412	0.0509	0.0213
"	8 days	6,750,000	0.445	0.0572	0.0269
5°	Initial	75,260	0.599	0.0412	0.0261
"	3 days	12,000	0.273	0.0315	0.0186
"	6 days		0.343	0.0407	0.0212
"	8 days	10.500	0.442	0.0546	0.0230
"	10 days	76,000	0.426	0.0468	0.0251
"	14 days		0.399	0.0289	0.0231

It will be noted that we have here the same initial decrease in non-coagulable Nitrogen which was found previously (Rice 1927) to be produced by this organism when acting upon clam muscle. This is due apparently to the utilization of these simpler products in the growth and metabolisms of the organism before sufficient extra-cellular enzymes have been produced to supply these necessary substances through the proteolysis of the native proteins themselves. After 24 hours at 35° and 25° the slope rises abruptly again indicating that the muscle is being rapidly hydrolyzed, but at 15° and 5° it takes 48 hours to reach its lowest point, enzyme production being less rapid evidently at these temperatures.

A similar initial decrease is shown to take place in the amounts of Amino and Ammonia N. and of the Volatile basic N., at least at the lower temperatures of 15° and 5°C., while at 35° and 25°C. the initial decrease only appears for the Volatile basic N. determination at 35°C. (24 hrs.), but possibly such a decrease may have taken place before 24 hours in the other instances.

Table VII

The effect of temperature upon the decomposition of haddock muscle by B. mesentericus vulgatus - Series II.

Temp.	Time of Incubation	Bacterial Count	Non-coag. N.	Ammonia & Amino N.	Volatile basic N.
		per c.c. fluid	grams per 100 gram muscle		
35°	Initial	410,000	0.539	0.0504	0.0270
"	24 hours	38,000,000	0.541	0.0487	0.0261
"	48 hours	40,000,000	0.840	0.0726	0.0437
"	3 days	44,000,000	0.992	0.1032	0.0670
"	4 days	27,000,000	1.090	0.1360	0.0796
"	7 days	17,000,000	1.264	0.1933	0.1287
25°	Initial	410,000	0.539	0.0504	0.0270
"	24 hours	8,400,000	0.488	0.0434	0.0234
"	48 hours	91,000,000	0.508	0.0462	0.0256
"	3 days	15,000,000	0.669	0.0466	0.0283
"	7 days	45,000,000	0.949	0.0869	0.0437
"	10 days	50,000,000	0.752	0.1044	0.0464
15°	Initial	410,000	0.539	0.0504	0.0270
"	24 hours	1,000,000	- ---	0.0410	0.0216
"	48 hours	460,000	0.498	0.0405	0.0211
"	3 days	1,300,000	0.559	0.0476	0.0227
"	7 days	2,500,000	0.756	0.0497	0.0273
"	10 days	19,000,000	0.692	0.0792	0.0324
"	14 days	34,975,000	0.619	0.1199	0.0438
5°	Initial	410,000	0.539	0.0504	0.0270
"	24 hours	170,000	0.518	0.0471	0.0194
"	48 hours	290,000	0.501	0.0430	0.0229
"	3 days	180,000	0.588	0.0383	0.0209
"	7 days	220,000	0.652	0.0396	0.0202
"	10 days	210,000	0.537	0.0600	0.0160
"	14 days	210,000	0.453	0.0687	0.0097

Series I and II show both a very rapid initial bacterial growth at 35°C., to somewhat less extent at 25°C., and with a still slower growth rate at 15°C; at 5° a reduction of bacteria took place in both cases following which the number of organisms remained almost constant (for eight days at least in Series I) throughout the experiment. This had a decided effect at 5° on the changes in Non-coagulable N. and volatile basic N, which both in both series after the initial increase, showed only a very small increase again towards the end of the experiments.

For the other temperaturesno consistent relations between bacterial growth and chemical tests for proteolysis are apparent. Both changes in Amino and Ammonia N.and Volatile basic N. in Series I and II show uniformly an increase to the end at the higher temperatures (35°, 25°, 15°), while at 5° a decrease took place in both series in all three tests, (with one exception, Amino and Ammonia N.Series II), due to the inhibitive effect of this temperature on bacterial growth and enzyme-production.

In Series II, the digests at the higher temperatures have been kept longer than in the first series. It is interesting to note that at 7 days a maximum of non-coagulable Nitrogen had been reached at all temperatures, after which this form of Nitrogen decreased rapidly. The bacterial count does not show this maximum at 7 days. Examination of the 25° and 35° plates show however a greater proportion of rough and buried colonies from 7 days on; in the plates at lower temperatures the proportion of R to S colonies is greater at all stages but also shows a definite increase as digestion proceeds. Whether this dissociation phenomenon is related to this rapid decrease in non-coagulable Nitrogen is problematical. Some agency is however at work converting the simpler non-coagulable Nitrogen back into a coagulable form, as it does not seem probable that such an appreciable amount of Nitrogen has escaped in the form of volatile bases or ammonia. Proteins are resynthesized by both pepsin and trypsin. This takes place best at a lower pH (Wastenays and Borstook 1924a). The pH of these digests, however, is slightly alkaline. Temperature does affect the rate of this resynthesis as may be seen from the graph, which was also found by Borstook & Wastenays (1924b) in the resynthesis of protein by pepsin. Although the R type is undoubtedly less proteolytic than the S type and may be utilizing these simpler products in its metabolism, yet since bacteria produce enzymes in excess of their requirements, it seems hardly probable that the dissociation would affect the enzymes already produced by the S type.

In order to establish whether this rapid decrease in non-coagulable Nitrogen occurred in all cases a third series was run. The results are shown in Table VIII.

Table VIII

The effect of temperature upon the decomposition produced by B. mesentericus vulgatus in heated haddock muscle - Series III.

Temp.	Time of incubation	Non-coagulable N.
35°	Initial	0.543
"	24 hours	0.524
"	48 hours	1.045
"	6 days	2.480
"	8 days	2.420
"	12 days	1.976
25°	Initial	0.543
"	24 hours	0.260
"	48 hours	0.478
"	6 days	0.867
"	8 days	0.862
"	12 days	0.825
15°	Initial	0.543
"	24 hours	
"	48 hours	0.567
"	6 days	0.712
"	8 days	0.717
"	12 days	0.797
5°	Initial	0.543
"	48 hours	0.488
"	3 days	0.477
"	6 days	0.486
"	8 days	0.552
"	12 days	0.821

At eight days we do find a decrease in non-coagulable Nitrogen at 35° and 25°, but at 15° and 5° there is still an increase, although at 15° the curve seems to be sloping off to an equilibrium, while after 12 days at 5° the curve is still rising.

In series I the 5° digests which were the only ones continued beyond the eighth day showed a definite drop. It appears to be fairly consistent that some factor, whether dissociation of the bacteria or something else, is reversing the process of proteolysis, there being no likelihood of loss, at least in such large proportion of the Nitrogen by volatilization.

The results of series II (Table VII) in regard to the changes in Non-coagulable N are perhaps even more peculiar from other points of view than from the one mentioned above (decrease of non-coagulable N after a few days); i.e. not so much the fact that there was a decrease at all, but the fact that the maximum was reached at all four temperatures at the same time (i.e. after 7 days incubation), the extent of the proteolytic changes being less with lower temperature. This phenomenon is exactly opposite to what one would expect (Tammans' Law, see Rahn, 1916), and opposite to some of the results one of us obtained on using a mixed bacterial flora, in which a decrease in temperature decreased only the rate of decomposition, but not the extent of proteolysis. (Hess, 1931).

Table VIII however shows the expected decrease of rate of decomposition with lower temperature, with a considerable drop however of the maximal amount of non-coagulable N between 35° and 25°, but probably a fairly uniform amount of maximal decomposition for the other three temperatures (25°, 15°, 5°). It rather appears that some unknown factor must be the cause of the unexpected results of series II.

(d) Effect of Temperature upon the Decomposition brought about by Bacillus B.

A very actively proteolytic Bacillus type resembling Bacillus vulgatus was isolated from a digest of haddock muscle incubated at 0°, and producing good growth on agar at that temperature. This was therefore used as a suitable organism for another series of determinations. It was found on sub-culturing the digests between 4 to 11 days that the culture was contaminated with an Achromobacter type. Further determinations were made however to get some indication of the effect that these two cultures acting together would have upon the muscle as compared to a pure culture of the Bacillus. The results are seen in Table IX.

Table IX

The effect of a mixed culture of Bacillus B. and Achromobacter upon haddock muscle.

Temp.	Time of incubation	Bacterial Count Bacillus B.	Achromobacter	Non-coag. N.	Ammonia & Amino N.	Volatile basic N.
		per c.c. fluid		grams per 100 grams muscle		
25°	Initial	48,600	- --- ---	0.557	0.0844	0.0224
"	24 hours	300,000	- --- ---	0.741	0.1458	0.0264
"	4 days	7,000,000	22,300,000	1.026	0.1763	0.0604
"	7 days	325,000	- --- ---	1.124	0.2201	0.0565?
"	11 days	500,000	397,000,000	1.091	0.2709	0.1153
"	15 days	220,000	- --- ---	1.218	0.3035	0.1235
"	20 days	270,000	- --- ---	1.195	0.3254	0.1634
15°	Initial	48,600	- --- ---	0.557	0.0844	0.0224
"	24 hours	156,000	- --- ---	0.576	0.1177	0.0248
"	4 days	14,200,000	700,000,000	0.753	0.1204	0.0302
"	7 days	800,000	2,400,000	0.878	0.1368	0.0336
"	11 days	28,000,000	- --- ---	0.895	0.1213	0.0387
"	15 days	20,0000	- --- ---	0.232	0.1308	0.0368
"	20 days	430,000	- --- ---	1.193	0.1701	0.0527
5°	Initial	48,600	- --- ---	0.557	0.0844	0.0224
"	24 hours	48,500	- --- ---	0.553	0.0809	0.0184
"	48 hours	34,500	- --- ---	0.486	0.0928	0.0203
"	4 days	38,500	26,000,000	0.510	0.1184	0.0285
"	7 days	420,000	- --- ---	0.565	0.1022	0.0245
"	11 days	10,000	- --- ---	0.759	0.0834	
"	20 days	.600	- --- ---	0.546	0.0764	0.0294

The Achromobacter type made its appearance on the plate counts after the 4th day and disappeared again at 11 days. At 25° there was a rapid increase of Achromobacter between these days, while at 5° this type had disappeared already after 7 days.

From Table IX it can be seen that at 25° there was a rapid increase in Non-coagulable Nitrogen, which soon came to an equilibrium, while the Amino and Ammonia Nitrogen as well as the volatile basic Nitrogen increased rapidly up to the end of the experiment (20 days), indicating that at 25°C. a very rapid decomposition of peptones and polypeptides took place.

At 15°C. all three forms of Nitrogen tested increased moderately rapidly, while at 5°C. a decided decrease of Non-coagulable N. and Amino N. took place during the second half of the experiment, and only a very slight increase of volatile basic N.

The contamination of the Bacillus vulgatus cultures with Achromobacter seems to show its influence appreciably only at 25°C., which is rather interesting since this temperature is almost the optimum for Achromobacter species isolated from soil and water (Bergey). The culture of Bacillus B. was purified and used for the last series of inoculations. The results of these determinations are found in Table X.

Table X

The effect of Temperature upon the rate of decomposition of haddock muscle by Bacillus B.

Temp.	Time of inoculation	Bacterial Count	Non-coag. N.	Ammonia & Amino N.	Volatile basic N.
		per c.c. fluid	grams per 100 gm. fish muscle		
15°	Initial	24,000,000	0.345	0.0835	0.0265
"	48 hours	760,000,000	0.477	0.0910	0.0450
"	7 days	1,400,000,000	0.639	0.0871	0.0749
"	10 days	1,720,000,000	0.843	0.0917	0.0774
"	17 days	1,450,000,000	0.819	0.1748	0.1116
"	23 days	1,700,000,000	0.792	0.2472	0.1423
"	30 days	1,300,000,000	0.339	0.2606	0.1587
10°	Initial	24,000,000	0.345	0.0835	0.0265
"	48 hours	68,000,000	0.427	0.0795	0.0278
"	7 days	870,000,000	0.639	0.0871	0.0578
"	10 days	1,300,000,000	0.690	0.0789	0.0635
"	17 days	1,070,000,000	0.588	0.1518	0.0711
"	23 days	1,600,000,000	0.548	0.1633	0.0778
5°	Initial	24,000,000	0.345	0.0835	0.0265
"	48 hours	6,000,000	0.391	0.0835	0.0289
"	7 days	38,000,000	0.548	0.0767	0.0315
"	10 days	123,000,000	0.704	0.0833	0.0384
"	16 days	460,000,000	0.584	0.1715?	0.0623
"	24 days	690,000,000	0.558	0.1324	0.0685
"	31 days	380,000,000	0.636	0.1367	0.0923
0°	Initial	24,000,000	0.345	0.0835	0.0265
"	48 hours	70,000	0.468	0.0801	0.0268
"	7 days	6,200,000	0.675	0.0680	- ----
"	10 days	8,500,000	0.532	0.0781	0.0281
"	16 days	3,770,000	- ---	- ----	- ----
"	24 days	2,100,000	0.546	0.1396	0.0314

The results of this experiment show a remarkable similarity to Series II - Table VII. Here again we find uniformly a decrease of Non-coagulable Nitrogen after 10 days (7 days' incubation respectively at 0°), while the Amino and Ammonia N., as well as the Volatile basic N. increased in amount up to the end of the experiment.

V - Discussion

In order to relate these temperature effects to those of other organic reactions, the mathematical formulae referred to in Section I were applied. To do this, the velocity of the reaction during definite periods was calculated from the values found for the different forms of Nitrogen. Since the breakdown of protein into simple nitrogenous compounds such as ammonia and amines involves many processes and is catalyzed by a series of enzymes, we would expect that the temperature coefficient derived at the different stages which we are following in our experiment would vary. A definite difference is indeed shown at certain stages where one process, the breakdown from protein into peptones and polypeptides for instance, is proceeding faster than the other. As the process approaches an equilibrium the values for Q_{10}, obtained in different reactions tend to approximate to each other. We will now consider each of the experiments separately.

(a) Autolysis - From the data in Table IV the average velocity of the autolytic decomposition of the haddock during definite time interval is obtained, and the values recorded in Table XI.

Table XI

Velocity of Autolytic Decomposition of Haddock Muscle at Different Temperatures

Temperature	Time 1 day	2 days	4 days	6 days	8 days	12 days
(a) Non-coagulable Nitrogen						
35°C.	0.200	0.117	-	-	-	-
25°C.	0.033	0.046	-	-	-	-
10°C.	0.---	0.011	0.009	0.008	-	-
0°C.	-	-	0.002	-	0.002	0.005
(b) Ammonia and Amino Nitrogen						
35°C.	0.0171	0.0316	-	-	-	-
25°C.	0.0133	0.0180	-	-	-	-
10°C.	-	0.0052	0.0081	0.0069	-	-
0°C.	-	-	0.0004	-	0.0002	0.0003

It is evident that at 35°C. the greatest velocity in the initial breakdown of the proteins into peptones and polypeptides etc. is reached during the first twenty-four hours, at 25° the average velocity at the end of two days is about 1/3 of that at 35°C. The actual velocity at 25° during the 24-48 hour interval, however, is greater than that at 35°. At 10° the velocity remains low even after 6 days, while at 0° it is negligible. As measured by amino and ammonia Nitrogen the velocity at 35° is higher at 48 hours than at 24 hours, which one would expect since there are then more of the intermediate products to act upon. For the values Ki and Ko in the formulae given in Section I for the calculation of Q_{10} and μ, these velocities are substituted and the temperature quotients and increments determined for the various temperature intervals (Table XII).

Table XII

Values for Q_{10} and μ during the autolytic decomposition of haddock muscle at different temperatures.

Interval	Time	Q_{10}	μ	N-Product
(a) 35° - 25°	1 day	6.060	33,140	Non-coag. N.
35° - 25°	2 days	2.544	17,166	" " "
25° - 10°	2 days	2.595	17,064	" " "
10° - 0°	4 days	4.500	23,280	" " "
(b) 35° - 25°	1 day	1.285	4,620	Ammonia & Amino N.
35° - 25°	2 days	1.756	10,350	" " "
25° - 10°	2 days	2.288	20,980	" " "
10° - 0°	4 days	20.25	11,922	" " "

This initial high value for μ, as in XII (a), is also noted by Osterhout (1917) in several processes, and he believes it to be due to some inhibiting factor. After 48 hours in the interval 10° to 35° the initial cleavage reactions are apparently governed by the same controlling factor, but below 10° the unusually high values might indicate that the limit of normal enzyme action had been passed. In this connection Kanitz (1916) found that for only certain intervals, where life processes could continue normally, could the RGT rule be applied. Below this temperature Q_{10} was abnormally high, above it abnormally low. The low values of Q_{10}

from XII(b) would suggest that a physical process, probably diffusion, rather than a purely chemical one was the controlling reaction during the initial stages. After two days, sufficient of the soluble products of proteolysis such as peptones and polypeptides have diffused through the medium to supply the ereptic enzymes which themselves had to diffuse out from the centre of formation, with a substrate containing excess material upon which to act. Thus Q_{10} approximated to 2. Since these figures represent the results of only one experiment and not the average of several, they should not be considered more than an indication of the general trend of the process. As such they probably justify the application of mathematical formulae which would otherwise be open to question from such insufficient data.

Crozier (1925) states that for a simple chemical process μ varies from 4,000 to 35,000. For the forward movements of Paramecium Glaser (1924) found for a range of 6°-15°C. that μ was equal to 16,000, and from 16° - 40°C. to 8,000, and suggests that an oxidation may be represented by 16,000 while either modified oxidation or an hydrolysis by 8,000. In Table XII(a) the value for the temperature increment between 35° - 25° for the first 24 hour period is about twice that for the 48 hour one at the same temperature range and at 25° and 10°. In Table XII(b) judging by the μ value, the same processes are active at 35° - 25° in 2 days as are found at 10° and 0° in four days. At 25° - 10° the μ value is just double that at two days. These values for μ correspond to those reported in other biological reactions of different types. Quastel and Whethan (1924) have shown that the velocity of reduction of methylene blue by B. coli at constant temperature is a function of the pH. Crozier (1925b) suggests this as a possible reason why in biological oxidation the increment 11,500 and 16,800 are both found; the value 11.500 being rather definitely associated with the OH - catalysis. Since the pH varies only slightly during the process of autolysis of haddock muscle (Reed, Rice, Sinclair, 1926) this factor does not in any likelihood affect the μ value.

Protein decomposition by autolytic enzymes follows Van't Hoff's law, therefore, only during certain phases of the reaction; in other words only at those times and temperatures where the enzyme concentration remains constant when the formations of enzymes keep up with enzyme destruction.

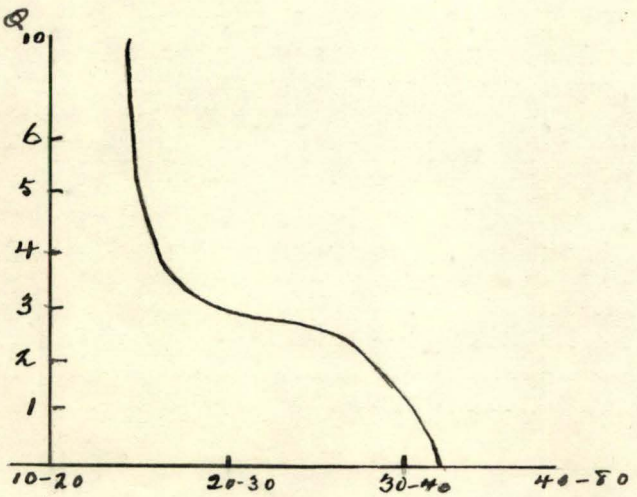
(b) Mixed Cultures of Bacteria - Several workers (Rahn, Ward Barber) have shown that bacterial growth follows Van't Hoff's law (Q_{10} equals 2-3) only within narrow limits at medium temperatures, but is not applicable over the rest of the range. The temperature coefficient for the rate of growth of bacteria is not constant

but varies from zero at the temperature maximum to infinity near the freezing point.

Marshall Ward (1895) determined the temperature coefficients of B. ramosus with the following results:-

Range	Q_{10}
14 - 24°C.	4.8
15 - 25°C.	3.4
16 - 26°C.	2.8
18 - 28°C.	2.3
21 - 31°C.	1.8
24 - 34°C.	1.2
26 - 36°C.	1.2
28 - 38°C.	0.9
30 - 40°C.	0.7 - 0.0
31 - 41°C.	0.0

The variation of the temperature coefficient may be seen in a general way from the following graph:-

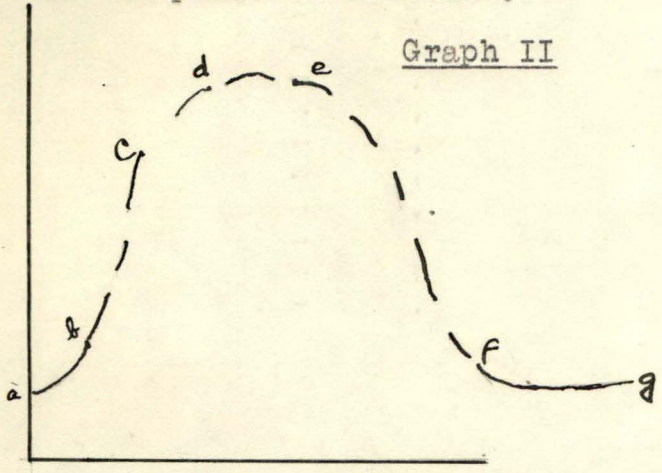


Graph I.

A Q_{10} of from 2-3 is given in a temperature range of 10-25° for the duration of different generations in an organism isolated from meat; growth at 0° is, however, extremely slow. (Müller 1903), Kanitz (1923) has calculated from the data of Chick (1913) and Barber (1908) that for B. coli between 22°C. and 38°, Q_{10} is equal to 2.1.

The temperature coefficient not only varies with the temperature, but also with the time. For an ideal growth curve we find that at medium temperatures Q_{10} has a changing value starting out from infinity at the beginning of the phase of adjustment, decreasing rapidly to reach 3-1.5 during the phase of logarithmic growth and further decreasing to 1.0 during the phase of negative acceleration up to the phase of crisis, and will decrease to zero after the phase of decrease.

Graph II



a-b phase of adjustment (initial stationary lag phase)
b-c Phase of logarithmic growth
c-d " of negative acceleration
d-e " of crisis
e-f " of decrease
f-g " of readjustment.

Irregularities in the growth rate such as an initial decrease of bacteria during the phase of adjustment or abnormalities during the phase of decrease will alter this change of Q_{10} from that of the ideal curve. In this experiment with mixed cultures plate counts were not made, but since the amount of change is dependent upon the number of bacteria present, at least to a large extent, we may expect to find the Q_{10} values varying in a similar manner.

From the data in Table V, the velocities in Table XIII were calculated. It is quite evident that a temperature of 35° favours the multiplication and activity of the organisms present to a marked degree. After 7 days the velocity figure at 0° is still low, although at 10° we have had a considerable increase. As previously the values Q_{10} and μ were calculated from the velocities (Table XIV).

Table XIII

Haddock muscle at different temperatures under the influences of combined autolytic and bacterial action.

Temperature	1 day	2 days	5 days	7 days
(a) Non-coagulable Nitrogen				
35°	0.189	0.207	-	-
25°	0.084	0.142	-	-
10°	0.	- -	0.026	0.0594
0°	0.	- -	0.017	0.015
(b) Ammonia and Amino Nitrogen				
35°	0.0441	0.0629	-	-
25°	0.0307	0.0489	-	-
10°	0.	- -	0.0126	0.0105
0°	-	- -	0.0028	0.0029

Except for the abnormally high value between 35-25° in the early proteolytic cleavage, all these processes are of about the same order if we may judge from the Q_{10} and μ values (Table XIV), which are all very low. Physical processes such as diffusion in all likelihood contributing to the low Q_{10} values here also. In mixed cultures however we have such a variety of processes occurring concurrently that it is surprising that even this degree of uniformity prevails. If a high Q_{10} value may be taken as an indication, 10° or below are beyond the limits of the normal change of intermediate nitrogen products to amino acids and ammonia. Or possibly again different organisms are active at these temperatures, as indeed was found on plating from the digests.

Table XIV

Mathematical Relationship of Temperature to the Decomposition of Haddock Muscle at different temperatures under the influence of combined autolytic and bacterial action.

Temp. Interval	Time	Q_{10}	μ	N.-Product
35° - 25°	1 day	2.250	29.280	Non-coagulable N.
35° - 25°	2 days	1.458	7.932	" " "
10° - 0°	5 days	1.530	6.580	" " "
10° - 0°	7 days	3.960	18.008	" " "
35° - 25°	1 day	1.436	6.662	Ammonia & Amino N.
35° - 25°	2 days	1.286	4.640	" " "
10° - 0°	5 days	4.500	23.080	" " "
10° - 0°	7 days	3.6207	19.920	" " "

(c) B. mesentericus vulgatus - It is rather more difficult to derive the relations of temperature to the proteolysis produced by this organism since we are dealing both at the beginning and the end with negative velocities, that is the substance from whose increase we are calculating our velocity values at these two periods shows a definite decrease; in which case it seemed advisable to disregard the phases of decrease and deal only with the logarithmic phase of growth. Consideration of the graphs will probably be sufficient without embodying all the velocities in separate tables.

Table XV

Velocities, Q_{10} and μ values, pertaining in the decomposition of Haddock Muscle by B. mesentericus vulgatus.

Interval	Time	$V_1 - V_0$	Q_{10}	μ	N. - product
$t_1 - t_0$	Days				
35 - 25	3	0.151 - 0.043	3.513	23,260	Non-coagulable N.
25 - 15	3	0.043 - 0.006	7.166	33,820	"
35 - 25	7	0.104 - 0.059	1.762	10,422	"
25 - 15	7	0.059 - 0.031	1.903	10,840	"
15 - 5	7	0.031 - 0.016	1.937	9,424	"
35 - 25	7	0.0204- 0.0052	3.923	6,610	Amino and
25 - 15	10	0.0054- 0.0029	1.862	10,692	Ammonia N.
15 - 5	10	0.0029- 0.0010	2.900	17,099	"
15 - 5	14	0.0050- 0.0013	3.846	23,420	"
35 - 25	7	0.0145- 0.0024	6.054	24,480	Volatile Basic N.
25 - 15	7	0.0024- 0.000043	55.81	18,740	"
35 - 25	3	14,530,000- 4,863,333	2.988	-	Bacterial Growth
25 - 15	3	4,863,333- 296,667	16.37	-	"
35 - 25	7	2,370,000- 6,370,000	0.37	-	"
25 - 15	7	6,370,000- 3,512,857	21.3	-	"
25 - 15	10	4,959,000- 1,859,000	2.66	-	"

V_1 and V_0 represent the velocities at t_1 and t_0 respectively.

The data in Table XV are derived from Table VII. In this experiment an equilibrium had been reached in all flasks after 7 days; the Q_{10} value for the change of native proteins into non-coagulable N. is at this stage equal to almost 2, and the reaction would therefore appear to follow the RGT rule. The values for μ are also approximately equal, so that all the processes are governed by the same catalysts (Crozier, 1925). At 3 days the reaction is quite different, if we may judge by the high values obtained for Q_{10} and μ. There is much less uniformity in the velocity of production of amino and ammonia Nitrogen at the lower temperatures, which may be related to the fact that, though an equilibrium seems to have been reached at the higher ranges, at these temperatures the process is still continuing upward. Volatile basic Nitrogen arises from a

very complicated and varied series of reactions and it would appear that temperature has a decidedly different effect at 35° than at 5°, if we judge by the Q_{10} value; the μ values at 35-25-15° are almost identical. Summarizing the results from the three series, we do find that during the phase of logarithmic increase and at points of equilibrium mathematical values all lie within the limits of about 2 and 3. Since the decomposition of the muscle depends upon the amount of enzyme present and this in turn depends upon the number of organisms present, we are not at the same time securing identical or even approximately identical reactions in the flasks incubated at the different temperatures, because the primary effect of the temperature is in increasing or decreasing the growth rate of the bacteria. We must therefore expect that at the same time interval we may in some cases find a quite different mathematical relation at different temperatures due to the fact that an entirely different stage in the chemical process is in progress, at higher temperatures more advanced stages being found. For this reason it would have been better in some cases to consider a constant value of each form of Nitrogen and compare the length of time taken at each temperature for this to be formed; the objection to this was, however, that the time intervals in some cases were so short at the higher temperatures, and as they had to be judged from the graphs which had insufficient points to determine very accurately the actual course of the curve, it was considered to be subject to too great error to be of any practical value.

(d) Bacillus B. - The velocities and mathematical relations were determined from Table X, and are indicated in Table XVI and XVII .

Table XVI

The velocity of decomposition of haddock muscle at different temperatures by Bacillus B.

Temp. Interval	2 days	7 days	10 days	17 days	23 days	30 days
Non.-Coag.N.						
15°	0.066	0.042	0.050	-0.003	-0.002	-0.065
10°	0.041	0.042	0.035	-0.014	-0.009	-0.0
5°	0.023	0.029	0.036	-0.015	-0.008	-0.0
0°	0.061	0.048	0.019	- -	- -	-- -

The velocities represented are the average daily velocities during the period of time elapsing between each determination. Up to 10 days the acceleration produced by temperature is positive, thereafter negative. The 30 days' figure is not very valuable, as during this period considerable hydrolysis of the proteins will have occurred even in the presence of the saline alone without the presence of enzymes. Bacillus B. does not show the initial decrease which was observed with the previous organism.

In Table XVII we have again the initially high values of Q_{10} and μ during the initial stages, decreasing during the logarithmic phase and finally reaching an almost constant value at all temperatures. The velocities obtained when calculated from increase in amino and amonia Nitrogen figures were too varied to be of sufficient significance to record here. The high Q_{10} and μ obtained from calculation of the increase in volatile basic Nitrogen are also comparative with the high values we secured when calculating the same from the previous series with B. mesentericus vulgatus.

Table XVII

Mathematical Relationship of Temperature to the Decomposition brought about by Bacillus B. at different temperatures.

Temp. Interval	Time	Q_{10}	μ	N. - product
15 - 5°	2 days	2.870	16,906	N-C-N
10 - 0°	7 days	0.875	5,938	" " "
15 - 5°	10 days	1.389	5,270	" " "
10 - 0°	10 days	1.851	9,528	" " "
15 - 5°	2 days	7.708	32,600	Volatile Nitrogen
10 - 0°	2 days	4.000	19,398	" "
15 - 5°	7 days	10.000	36,700	" "
15 - 5°	10 days	4.250	24,600	" "
10 - 0°	10 days	23.120	12,780	" "
15 - 5°	17 days	2.232	-	" "
10 - 0°	17 days	19.375	-	" "
15 - 10°	10 days	1.783	-	Bacterial growth
15 - 5°	10 days	17.13	-	" "
10 - 5°	10 days	164.55	-	" "
15 - 10°	23 days	1.136	-	" "
15 - 5°	23 days	2.684	-	" "
10 - 5°	23 days	6.34	-	" "

Q_{10} for bacterial growth shows uniformly higher values with decreasing temperature range, and decreasing values for the same range with increasing time.

Summary

1 - An attempt has been made to arrive at a quantitative estimate of the effect of temperature upon the decomposition of haddock muscle. This has been considered from three standpoints (a) the effect of temperature upon autolytic decomposition (b) upon the decomposition brought about by mixed cultures of bacteria normally occurring in the decomposing fish (c) upon the decomposition resulting from inoculation of sterilized muscle with pure cultures of Bacillus types very prevalent in the slime, gills and intestine of the fish itself.

2 - The progress of the decomposition was followed by determination of the increase in non-coagulable, ammonia and amino and volatile basic Nitrogen.

3 - Autolytic decomposition proceeded rapidly at 35° and 25° but much more slowly at 10° and was practically negligible at 0° even after 12 days.

4 - Although at 0° in the muscle incubated without toluene there was an increase in the products of proteolytic cleavage over that produced by autolysis alone, decomposition at this temperature was very slow.

5 - Two Bacillus types, B. mesentericus vulgatus and another closely related form produced as was to be expected their greatest change in the proteins at the higher temperatures, but also an appreciable one at 5°, and somewhat less at 0°. The former produced an initial decrease in non-coagulable Nitrogen due to the utilization of these products in the growth and production of enzymes.

6 - Temperature coefficients for these processes during the phase of logarithmic increase approximate to 2 to 3, some however, are low as has been found elsewhere for enzymatic actions, which will place them in Class III of Przibram's classification. Changes in the value of μ during the decomposition show that during the process control passes from one reaction to another, or perhaps several others in turn. Temperature increments for the same period calculated on the basis of increase in any one of the three classes of protein decomposition products are not equal so that the reactions appear to be more or less independent.

Fig. 1.

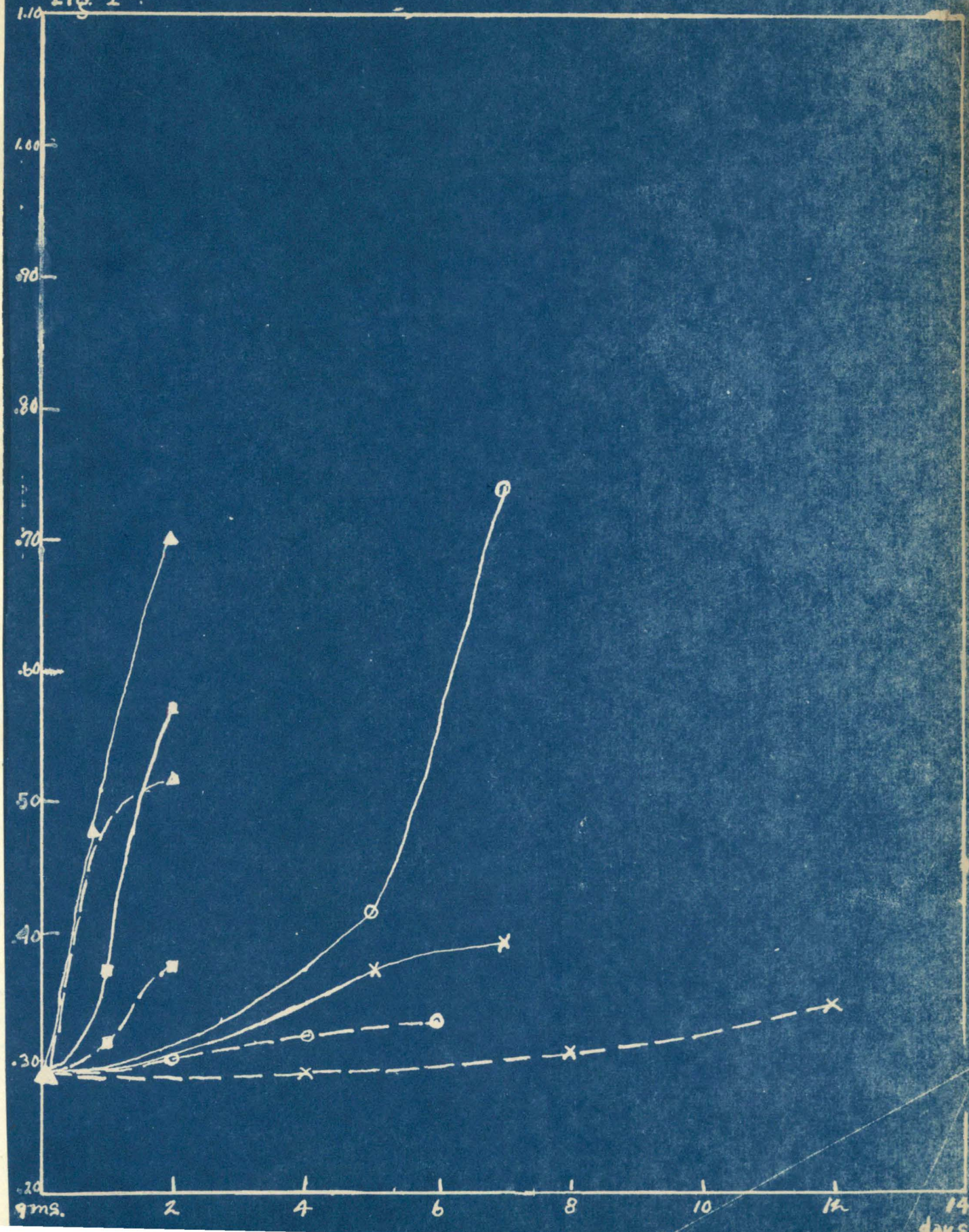


Fig. 2.

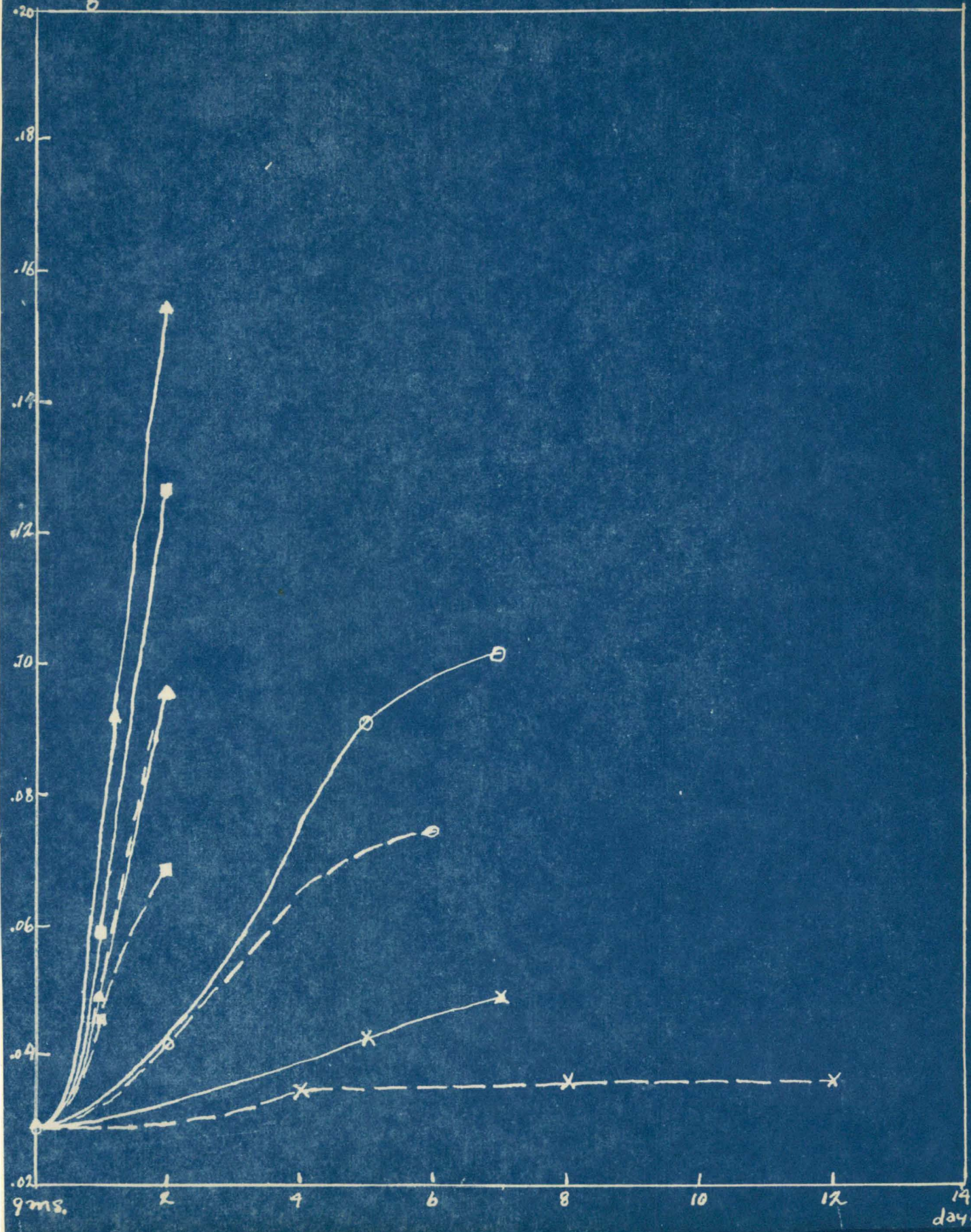


Fig. 3.

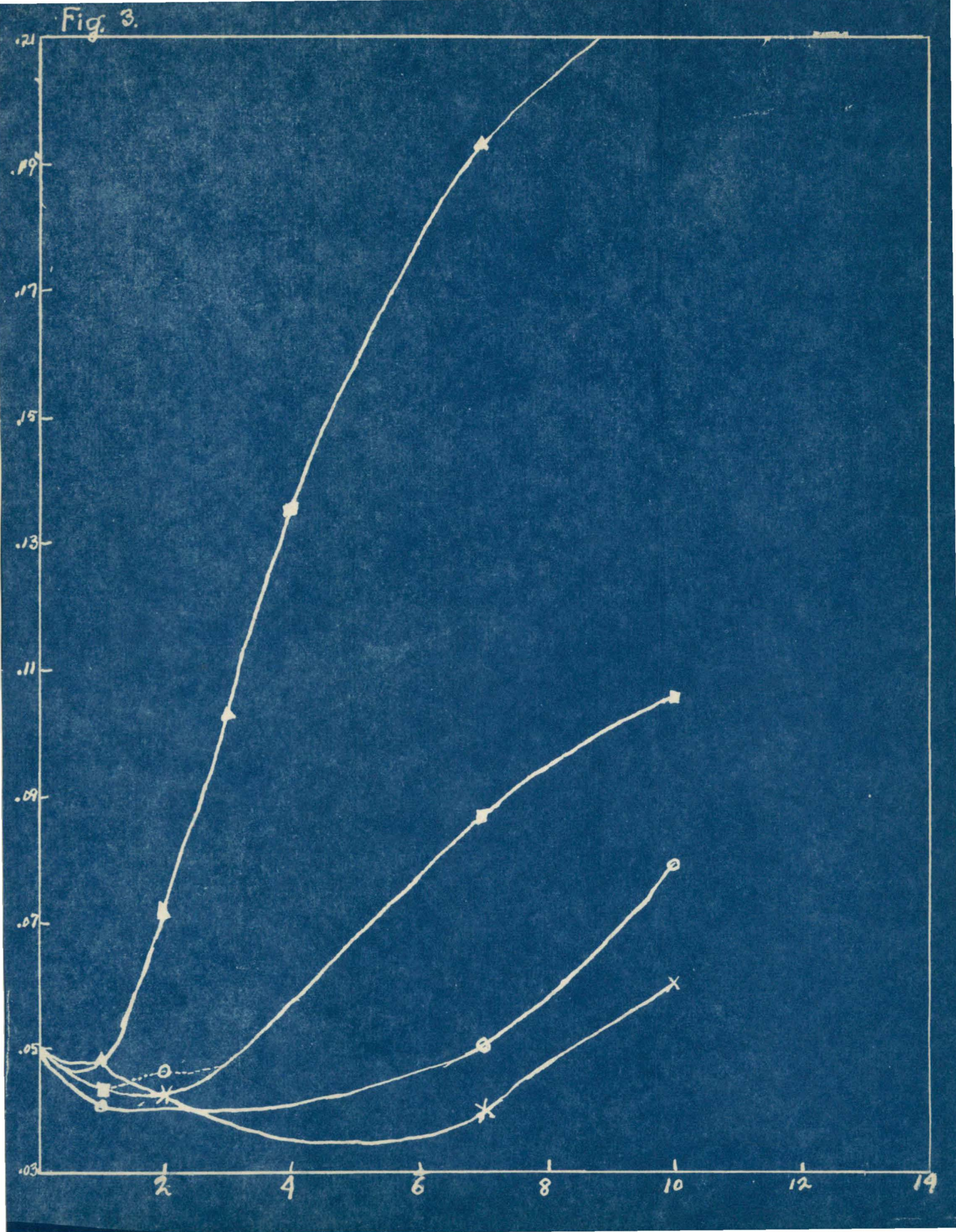
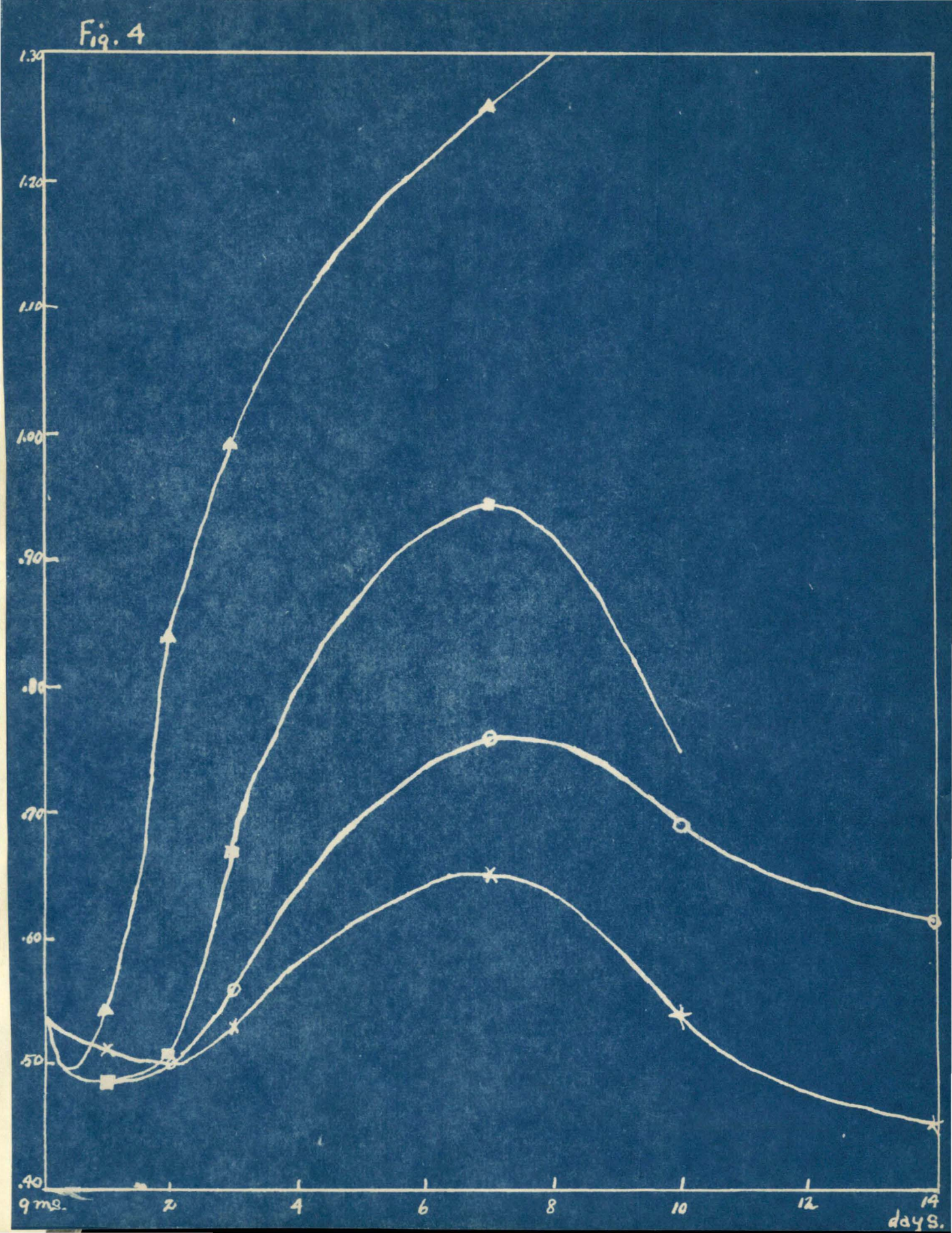


Fig. 4



The effect of Temperature upon the rate of Decomposition of Haddock Muscle

Graphs

Figure I - Autolytic and Bacterial Decomposition of Haddock Muscle at different temperatures as measured by Increase of non-coagulable Nitrogen (Data for Table 4&5).

Figure II- Autolytic and Bacterial Decomposition of Haddock Muscle at different temperatures as measured by the increase in Amino and Ammonia Nitrogen (Data for Table 4 & 5).

Figure III- Increase in Ammonia and Amino Nitrogen during the same process (Data - Table 7)

Figure IV - Increase in Non-coagulable Nitrogen during the Decomposition of Haddock by B. mesentericus vulgatus. (Data - Table 7).

▲ - 35° digest ■ 25° digest.
o - 10° digest × 0° digest.

In tables where intervals 15° and 5° are used and intervals 10° and 0°, the signs stand for 15° and for 5°.

_____ Combined autolytic and bacterial action.
_ _ _ _ _ Autolytic action alone.

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