The Effect of Dietary Fat on the Heat Tolerance of Goldfish (Carassius auratus)

by

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A Thesis submitted in Partial Fulfillment

of the Requirements for the Degree of

MASTER OF ARTS

in the Department of

ZOOLOGY

The University of British Columbia

August, 1948.

Accepted

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Abstract

An attempt has been made to alter the degree of unsaturation of the body fats of goldfish (Carassius auratus) and correlate these changes with any modifications of heat tolerance subsequently exhibited by the fish. The goldfish were fed three different diets each containing a fat of different degree of unsaturation. The fats used were pilchard oil (iodine value of 181.7), herring oil (iodine value of 128.4) and lard (iodine value of 66.2). Heat resistance was tested by holding the fish at a constant high temperature and observing the time to death. Variations in the ability of the groups to withstand high temperature were then compared to differences in the degree of unsaturation of their extracted fats. It was found that while diet could effect changes in the degree of unsaturation of the goldfish fats to approximately 54% of the theoretical level, and that these changes in turn modified the heat resistance of the goldfish, no quantitative relationship was established.

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INTRODUCTION

Life is maintained within a relatively narrow range of temperatures. This has attracted the attention of a great many workers, with the result that there is voluminous literature on the subject of temperature in relation to life. The observation that an extreme temperature for one organism may be an optimum or even the opposite extreme for another, has led to much work on upper and lower lethal limits for numerous plants and animals. Other work has dealt with optimum and preferred temperatures for plants and animals and vital processes. By comparison the attempts to investigate the physiological processes involved have been relatively few. However, several theories of heat death have evolved. No attempt will be made to list them all, nor will the author presume to argue their various merits. A few will be mentioned along with the generally expressed criticisms of them, in an effort to demonstrate the diversity and scope of the problem.

The various theories fall into three main classes. The first of these classes deals with interference with metabolic processes. Thus, in 1905, Winterstein suggested that when animals are subjected to high temperatures, the metabolic rate is increased so greatly that the organism is unable to supply enough oxygen to the cells and tissues. Therefore death is due to asphyxiation. Harvey (1911) working with nerve conduction in medusae observed that there is an optimum temperature for conduction, the rate falling off above and below this level, Since enzyme reactions in common with all vital processes, behave in a

similar manner, he ascribed the phenomenon of heat death to the breakdown of the enzymes. Mayer (1917) experimenting with a number of species of corals showed that their ability to stand high temperatures was roughly the same as their ability to withstand high concentrations of carbonic acid. He therefore proposed that it is the accumulation of this acid due to the increased metabolism resulting from high temperatures that causes heat death. He disagreed with the asphyxia theory on the grounds that by test, the corals can survive in the dark in the absence of oxygen for long periods. He criticized the enzyme theory because heat death is, to a limited extent, reversible and this could not be so were the inactivation of enzymes responsible.

The second class of theories developed from the experiments of Loeb and Wastenays, (1912) on the fish (Fundulus). According to these workers, the maximum temperatures the fish could stand varied with the concentration of the sea water or Ringer's solution of the surrounding medium. However, experiments with sucrose solutions demonstrated that it was not a case of simple osmosis. They therefore postulated that a rise in temperature brings about certain changes in the permeability of the surface cells of the body which result in death. They further postulated that these changes may be overcome or modified, if the temperature change is slow enough, by the salts in the blood or surrounding medium. In this manner they explain the adaptation of an organism to higher temperatures.

A third class of theories deals with the coagulation and

precipitation of the protoplasm. Thus Brodie and Richardson (1899) explained heat death on the basis of the coagulation of the protein matter of the living cell. This idea is still popular, though many workers disagree on the grounds that protein usually does not coagulate much under 50°C. while many plants and animals suffer heat death at much lower temperatures than this. Heilbrunn (1924) endorsed the view that it is a coagulation of the cell contents, but from his experiments on the coagulation of the protoplasm of the eggs of sea-urchins (Arbacia) and clams (Cuminga), felt that it is the liquifaction of the lipoids in the cell which allows the precipitation of the protoplasm. He based his assumption on the fact that, in general, organisms in a cooler habitat have more liquid fats than those in a warmer habitat. In later work he connected his calcium release theory of anaesthetic action with the liquifaction of the protoplasmic fat at high temperature. According to this composite idea when an animal is exposed to lethal heat the lipoids of the cell membrane melt, releasing the corticle calcium, this calcium in turn causing changes in the protoplasmic viscosity.

Fraenkel and Hopf (1940) in an attempt to show the relationship between the degree of unsaturation of the phosphatides and lethal temperature, bred two species of blow-fly larvae at two different temperatures and then subjected the larvae to lethal temperatures. Conflicting results were obtained. On the one hand all the larvae of both species raised at the higher temperature, not only exhibited a

gain in heat tolerance of 1°C. over those raised at the lower temperature, but also the phosphatides extracted from the former larvae had iodine values 26 units higher than the latter group. On the other hand larvae of Phormia terra nova and Calliphora erythrocephala while producing phosphatides of identical iodine number, exhibited a difference in lethal temperature of 7°C. The fact that they were of different species, however closely related, might explain the discrepancy.

In any case, it can be seen that the problem is very complex. The answer probably lies in the interaction of a number of factors. The purpose of this thesis is to explore further one of these factors, namely, the relationship between the degree of unsaturation of the protoplasmic fats and temperature tolerance.

Accordingly, goldfish (Carassius auratus) were fed diets containing fats of different degree of unsaturation, and the effect of the dietary fat on the body fats noted and correlated with any changes in the response of the animals to high temperature.

MATERIALS AND METHODS

1. Preparation of the Diets and Feeding

Three diets were developed. These were high in fat content and contained fats of different degree of unsaturation. The fats selected were pilchard oil (iodine value of 180), herring oil (iodine value of 128.4) and lard (iodine value of 66.2). Pablum, manufactured by the Mead Johnson Company of Canada Ltd., constituted the base for all the diets. The fats were bound in with lecithin in the following proportions:

Pablum - 70% by weight

Fat - 25% by weight

Lecithin - 5% by weight.

The fat and lecithin were first dissolved separately in peroxide free ether and then mixed. The solution of fat and lecithin was then poured into the Pablum, mixed thoroughly and the ether extracted in vacuo at 37°C. The resultant diets were very serviceable, the oil remaining in them for considerable time even in contact with water.

To ascertain the degree of unsaturation of the fats consumed by the fish, samples of the diets were taken immediately after they were prepared and iodine determinations were made upon the extracted fats. To determine whether any considerable changes in the fats occurred after the diets were made up the process was repeated in 7 days and again in 14 days. The results are given in table I.

TABLE I

Iodine values of fats extracted from the diets

	Iodine Values		
	0 Days	7 Days	14 Days
Pilchard Oil Diet	159	159 - 170	159
Herring Oil Diet	115	115	114.3
Solid Fat Diet	64	65	64.1

Although no appreciable changes occurred in two weeks, it was deemed advisable to make up fresh diets each week. In order to reduce the possibility of oxidation of the fats, the diets were kept in the freezing compartment of the refrigerator, except for the very few minutes during actual feeding.

The Herring and Pilchard oils were supplied by Western Chemicals Ltd., Vancouver, while the Lard was obtained from Burns Ltd., Vancouver. They were kept in a frozen condition in the cold storage plant of the Dominion Fisheries Experimental Station in Vancouver. To reduce the possibility of oxidation through frequent freezing and unfreezing of the pilchard and herring oils, a number of 50 ml. Erlenmeyer flasks were filled at one time and kept in cold storage at the station. When required, a small flask of each oil, and only as much of the frozen lard as was necessary was taken out. The small flasks of oil were subsequently unfrozen at the University, the appropriate amount of oil weighed out and the remainder put into the freezing compartment of the refrigerator at the University laboratory.

As it was impractical to feed each fish separately, an amount of diet equivalent to .3 grams per fish per day was used. This was divided into three feedings, morning, noon and night. Each feeding took approximately thirty minutes, only a small portion of food being put into each aquarium at a time, so that the animals could eat as much as possible before it sank to the bottom and disintegrated. It was necessary to change the water and clean out the aquaria once a week.

2. Environmental Control

The experiments were carried out in two parts, a preliminary group involving 97 goldfish (Carassius auratus) procured from the Goldfish Supply Company, Stouffville, Ontario, in November 1947, and a main group involving 167 goldfish procured from the same source in May 1948.

The first of the preliminary experiments were performed during the winter of 1947 - 1948. Thirty goldfish were divided into three groups of ten fish, and the groups retained in aquaria 19" x 11.5" x 10.5". Compressed air forced through air breakers provided adequate aeration. The temperature varied with the environment from 10 - 18°C. The fish were fed the three standard diets already described.

For the last of the preliminary experiments (May and June 1948) sixty fish were divided into three groups of twenty, each group being contained in two Turtox aquaria (19" x 11.5" x 10.5"), provided with Lolag immersion heaters and Fenwal 10 amp., 115 volt, $\pm$.5°C. thermostat controls. The thermostats were set for 20°C. Since the summer temper-

ature was capable of elevating the water beyond the 20°C. mark, a cooling system was introduced. This consisted of lengths of Pyrex glass tubing (0.5" diameter) with the two ends bent at right angles forming a rectangular "U". One of these "U's" was placed diagonally in each aquarium and connected by rubber tubing to the "U's" in the adjacent aquaria. Cold water from the tap was run through the series and back into the sink. In this way a constant temperature of 20°C. ±.5°C. was maintained.

Since not all the thermostats exhibited the same degree of sensitivity, keeping each group in two aquaria allowed slight variations in the thermal history within the groups and between groups. This variable was eliminated from the main group of experiments by dividing 162 fish into three groups of 54, each being contained in one large aquarium, (32" x 16" x 20"). The thermostats selected all exhibited the same degree of sensitivity and a temperature of 20°C. ±.5°C. was maintained in each aquarium.

3. Determination of Heat Resistance

In the early experiments two methods of ascertaining the resistance of the fish to high temperatures were tried. In the first of these the fish were placed in a small aquarium and the temperature raised at a constant rate until the lethal limit was reached. This method was discarded since it was felt that there was no way to ensure identical rate of change of temperature in all cases, and any variations in this rate would almost certainly be reflected in the results obtained.

The method adopted, consisted of holding the fish at a constant

high temperature and comparing the length of survival time of the three groups, using the 50% level of mortality as the criterion. Any fish living for 14 hours at a given temperature was considered acclimatized, (Fry et al 1941). The temperature selected was in conformity with the work of Brett (1946). As in the holding tanks, compressed air was bubbled into the testing aquarium to ensure adequate oxygen content. Winkler tests were made before and after the experiments.

Using a small aquarium as a lethal chamber necessitated testing a sample from one group at a time and since the thermostats used were at best only sensitive to $\pm .5^{\circ}C$. therefore there would be slight variations in the individual tests. This variable was removed in the main group of experiments (June - August 1948) by using a large tank (4' x 2' x 3'), and dividing it into three compartments by means of two fine mesh plastic window screen partitions. Two heaters connected in series to one thermostat served to keep the water in the tank at any desired temperature while two jets of compressed air bubbled in vigorously provided adequate oxygen and circulation so that the temperature remained identical in all parts of the tank. Samples of all three groups of fish were tested simultaneously and therefore the fish for any one test were all subjected to the same temperature fluctuations.

4. Fat Extraction and Analysis

After the fish had been killed by subjecting them to high temperature, the fat was extracted. The method was the same as that used by Hunter (unpublished) with the exception that in most of the

experiments anhydrous sodium sulphate was not used to dry the macerated flesh, instead the tissue, after being chopped with scissors or a Waring blender, was dried in vacuo at about 45 - 55°C. The dried fish was then placed in the ether extraction thimbles and extracted for two hours with ether at 38°C. The advantage of this system was a reduction of the actual extraction time thus minimizing the possibility of oxidation due to any peroxides in the ether. The ether was tested for peroxides before extraction was begun but changes may have occurred during the process. Also the oils thus obtained were free from any impurities such as the anhydrous sodium sulphate which runs down the syphon carrying water with it.

Unfortunately the only means of obtaining a vacuum was from the running water in the laboratory and as the summer progressed the water pressure dropped to such a level that it became impossible to obtain sufficient vacuum to dry the fish tissues. For this reason the anhydrous sodium sulphate method was used for some of the last experiments (see appendix).

In both cases, however, each thimble full of the tissue was extracted with ether for two hours when the thimble was emptied and fresh tissue put in. This process was repeated until all the fish had been used.

The mixture of oil and ether thus obtained was placed in a round bottomed flask and the ether drawn off in vacuo at 37°C., the final oil was transferred to two-inch watch glasses and put into a

vacuum dessicator to remove any last traces of ether and water.

Iodine determinations on the oils were carried out in the manner described by Bailey (unpublished) using Wij's solution.

RESULTS

The Effect of Diet on the Unsaturation of the Extracted Fats

1. Preliminary Experiments:

Iodine determinations were not made for the winter preliminary experiments but were made for all later groups.

Before feeding was commenced in the second group of preliminary experiments (May and June 1948), six pre-diet fish were subjected to a lethal temperature (see Appendix I, Part B) and iodine determinations made on the extracted fats. The average value obtained was 114.05. Thereafter determinations were made on the fish as they were killed. A progressive desaturation of the pilchard oil group, and a saturation of the solid fat group resulted. The herring oil group showed little change. The results are tabulated in table II below and shown graphically in figure 1.

TABLE II

Iodine values of the extracted fats from goldfish fed on the three standard diets during the preliminary experiments of May and June

	Iodine Values		
Number of Days Fed	Pilchard Oil Diet (I.V.159)	Herring Oil Diet (I.V.115)	Solid Fat Diet (I.V.64)
0	114.05	114.05	114.05
12	124	112.9	90.4
21	126	115.6	-
34	136	111.4	89.2

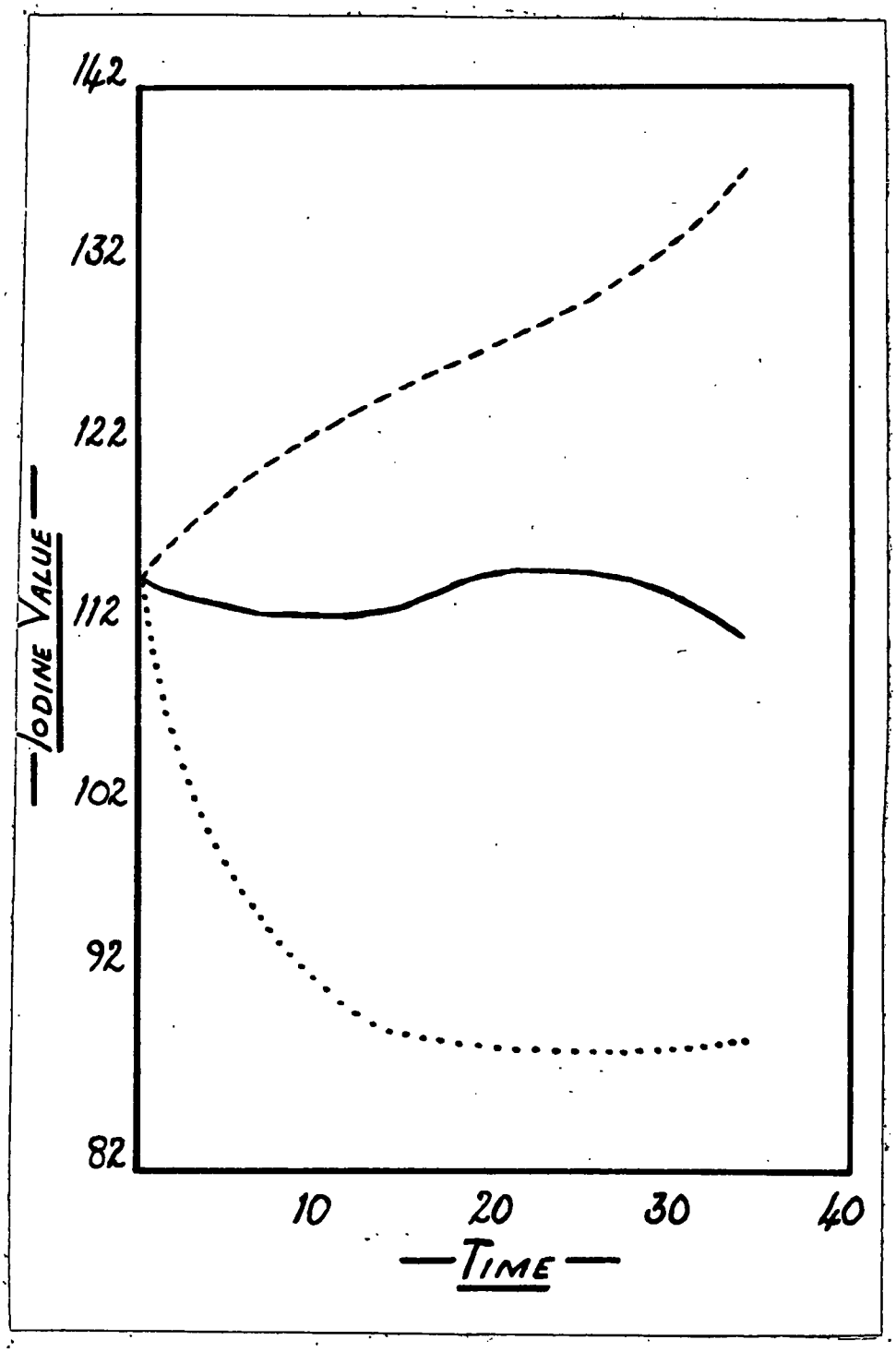

Figure 1. Graphic representation of the changes in iodine values listed in table II

----- Pilchard oil diet fish
_____ Herring oil diet fish
..... Solid fat diet fish

2. Main Group of Experiments:

As in the preceding experiments, pre-diet goldfish were killed (see Appendix I, Part C) and iodine determinations made. The average value being 112.78. The effect of the diets on the extracted fats was almost identical with that shown above, (table III and figure 2).

TABLE III

Iodine values of the extracted fats from goldfish fed on the three standard diets during the main group of experiments.

	Iodine Values		
Number of Days Fed	Pilchard Oil Diet (I.V.159)	Herring Oil Diet (I.V.115)	Solid Fat Diet (I.V.64)
0	112.78	112.78	112.78
6	122.3	115.5	93.4
29	128.2	112.6	88.4
32	133.4	112.9	86.5
37	136.1	113.1	86.4
46	137.1	113.2	91.38
55	137	110	85.8

For figure 2 regression lines (first 37 days) have been fitted to these data using the methods of Snedecor (1946). These points show that after the thirty-seventh day no particular change took place in the extracted fats of the fish fed on pilchard oil and solid fat diets. In the case of the herring oil diet, no definite change took place at any time.

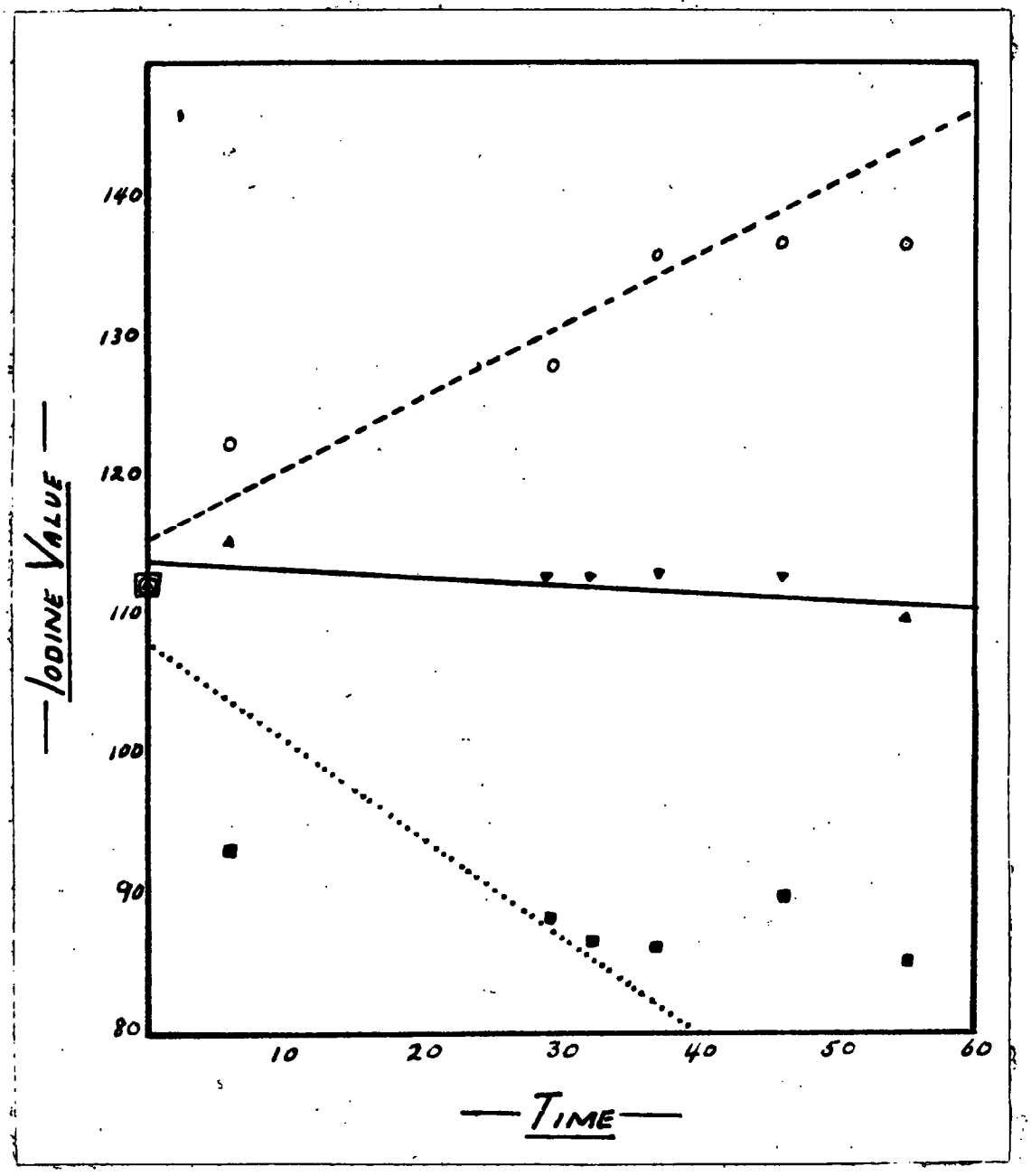

Figure 2. Regression lines fitted to the data presented in table III

o	Iodine values for pilchard oil diet	
-----	Regression line for pilchard oil diet	$b = 0.52 \pm 0.278$
▲	Iodine values for herring oil diet	
———	Regression line for herring oil diet	$b = -.032 \pm .088$
■	Iodine values for solid fat diet	
.....	Regression line for solid fat diet	$b = -0.72 \pm 0.95$

The Effect of the Dietary Fats on the ability of the Goldfish to withstand High Temperatures

1. Preliminary Experiments:

The results of the early preliminary experiments (summarized in Appendix I, Part A) showed that the resistance to heat had been modified by diet. However, thermal histories were poorly controlled and the number of fish not great enough to establish the relationship. Both these defects were remedied in later experiments.

In presenting the results of these later experiments, it was felt that, due to the small numbers of fish used in each test and the great variations among individuals of the same group to withstand heat, the results of comparable experiments could be shown to their best advantage by tabulating them collectively (see table IV). The time for 50% mortality is shown graphically in figure 3. The individual tests are summarized in Appendix I, Parts B and C.

To test further the observed differences in heat resistance, the rate of dying in each group for the first 75 minutes has been plotted in figure 4. This shows a different rate of dying for the three groups, though a consideration of the fiducial limits does not show a highly significant statistical trend.

2. Main Experiments:

The immediate effect of the diet on the fish used in this section, was to increase the heat tolerance of all groups so that by the end of 29 days a temperature of 35°C. had very little effect on them.

TABLE IV

Results of Preliminary experiments carried out in May and June on the resistance to a temperature of 35°C. Fish fed for 12 to 34 days. The 50% level is indicated by /

Pilchard Oil Diet		Herring Oil Diet		Solid Fat Diet	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
11	1	1	1	22	1
25	2	13	2	25	3
37	3	17	3	27	4
40	4	20	4	30	5
42	5	27	6	40	7
43	6	30	7	57	8
50	7	32	8	58	9
53 /	9	33 /	9	62 /	10
58	10	35	10	67	11
67	11	38	11	80	12
70	12	43	12	97	13
75	13	45	13	151	14
80	14	50	14	185	15
190	15	60	16	190	16
230	16	62	17	320	17
		65	18		
Acclimatized 1		Acclimatized 0		Acclimatized 3	

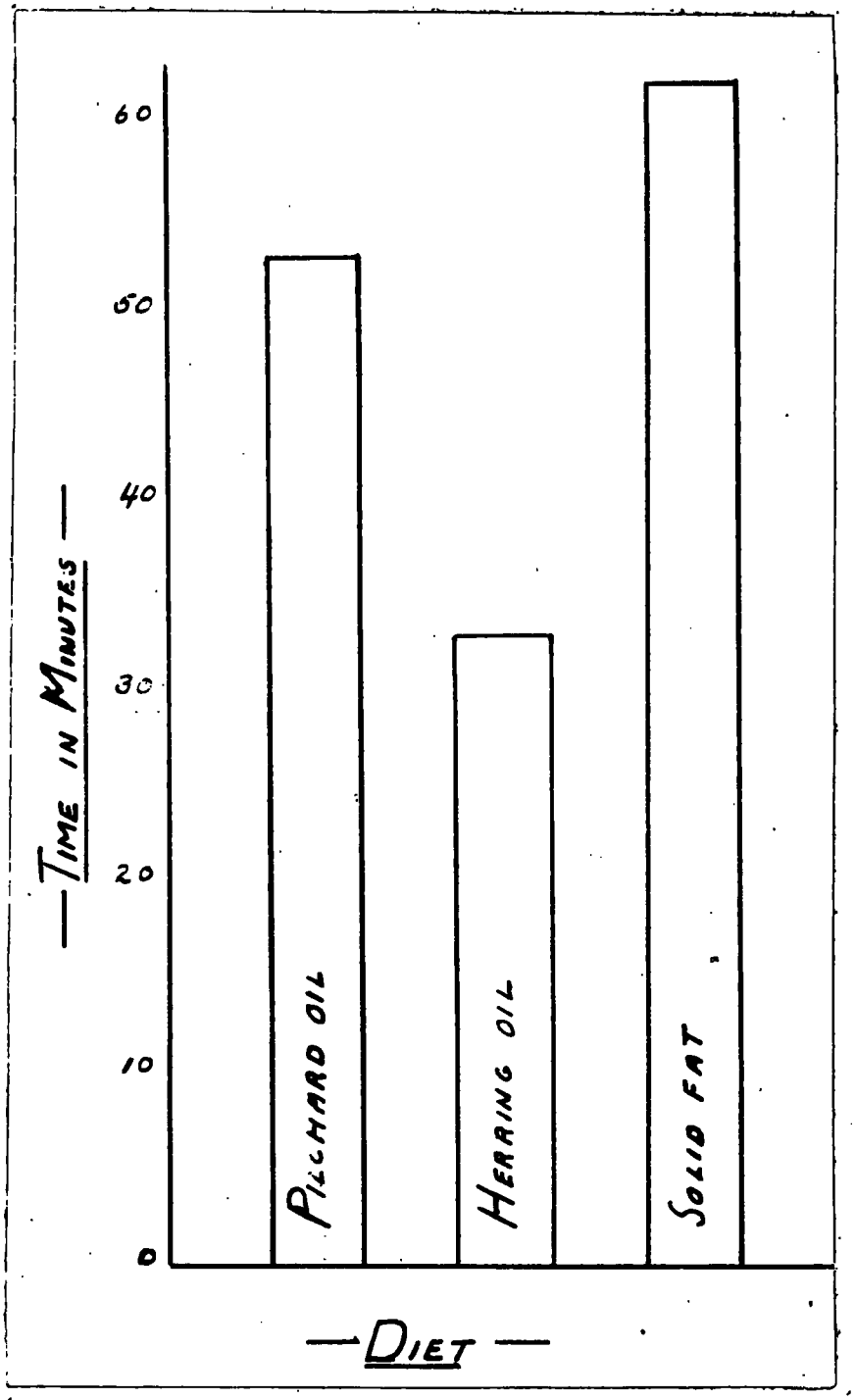

Figure 3. 50% mortality in goldfish at 35°C.

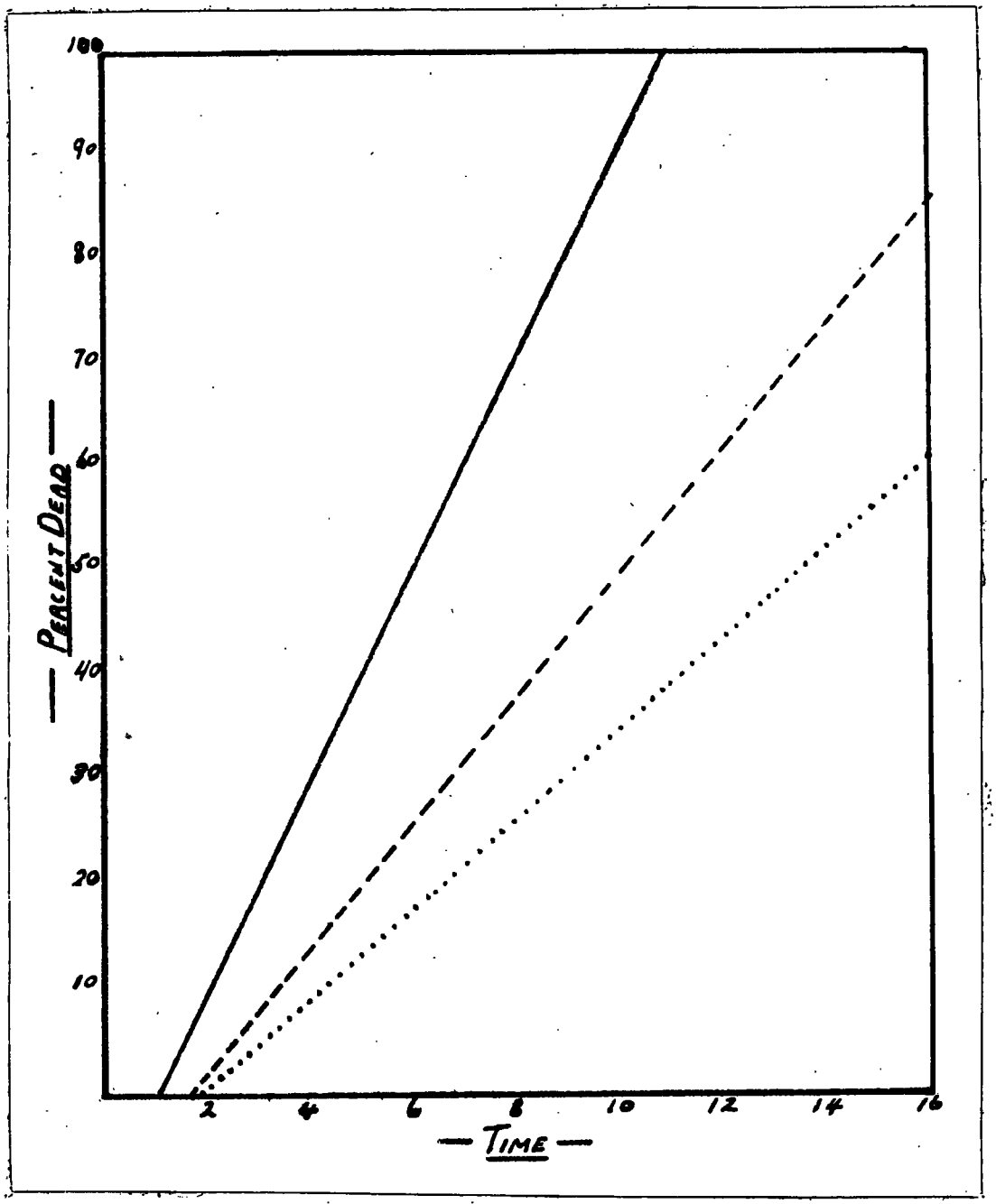

Figure 4. Regression lines for the first 75 minutes of the preliminary heat resistance tests

----- Pilchard oil diet fish $b = 5.86 \pm 1.16$
——— Herring oil diet fish $b = 7.86 \pm 0.808$
..... Solid fat diet fish $b = 4.41 \pm 0.599$

In tables V and VI are given the results of tests carried out after six days feeding and 29 days feeding respectively, showing this increase in heat resistance.

TABLE V

Results of six days feeding of standard diets on the resistance of the goldfish used in the Main Experiments to a temperature of 35°C. Six fish per group.

Pilchard Oil Diet		Herring Oil Diet		Solid Fat Diet	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
52	1	28	1	74	1
67	2	55	2	85	2
106	3	75	3		
195	4	170	4		
Acclimatized 2		Acclimatized 2		Acclimatized 4	

TABLE VI

Results of 29 days feeding of standard diets on the resistance of the goldfish used in the Main Experiments to a temperature of 35°C. Eight fish per group

Pilchard Oil Diet		Herring Oil Diet		Solid Fat Diet	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
		1	2		
		70	3		
Acclimatized 8		Acclimatized 5		Acclimatized 8	

Therefore a number of experiments were performed (summarized in Appendix I, Part C) to select a suitable temperature for the remainder of the experiments. The temperature finally adopted was 36°C.

As in the last section the results of all the experiments at 36°C. are presented collectively in table VII. It is to be noted at this point, however, that all these tests were made after feeding had been continued for 46 days or more. This is significant since, as has already been pointed out, there is a leveling off of the iodine values after the thirty-seventh day. For this reason it is felt that these results are more significant than the preceding ones. The time for 50% mortality is presented in figure 5. Figure 6 shows the rate of dying for the first 75 minutes, using the same method as in the previous series.

As in the first experiments the results show a difference in the ability of the three groups to withstand high temperature. While a consideration of the fiducial limits does not show a significant difference in the case of the pilchard and herring oil diets, it does show a significant difference in the case of the solid fat diet.

The Effect of Diet on Behaviour in respect to Temperature

Although it is difficult to assess variations in behaviour, it was observed that in most of the tests performed the initial reaction to heat was more violent in the case of the fish fed herring oil than in the fish fed pilchard oil. Both these groups displayed more initial distress than the fish fed on the solid diet. On four occasions herring

TABLE VII

Results of the main group of experiments on the resistance to a temperature of 36°C. Fish fed 46 days or more. 50% level indicated by /

Pilchard Oil Diet		Herring Oil Diet		Solid Fat Diet	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
8	1	1	1	8	2
11	2	7	2	9	3
16	3	8	3	18	4
18	4	10	4	27	5
20	7	12	6	30	6
25	11	14	7	33.5	8
27	13	15	9	36	9
30	14	20	10	38	10
33.5 /	15	21	11	42.5	11
35	18	22	12	50	12
37	20	24	13	55	13
42.5	22	33	14	58	14
45	24	35 /	16	60 /	16
46	25	37	18	62	17
60	26	39	20	72	18
65	27	40	21	77	19
77	28	48	24	80	20
82	29	49	27	85	21
137	30	57	28	110	22
		80	30	190	23
				195	24
				420	25
				540	27
Acclimatized 0		Acclimatized 0		Acclimatized 3	

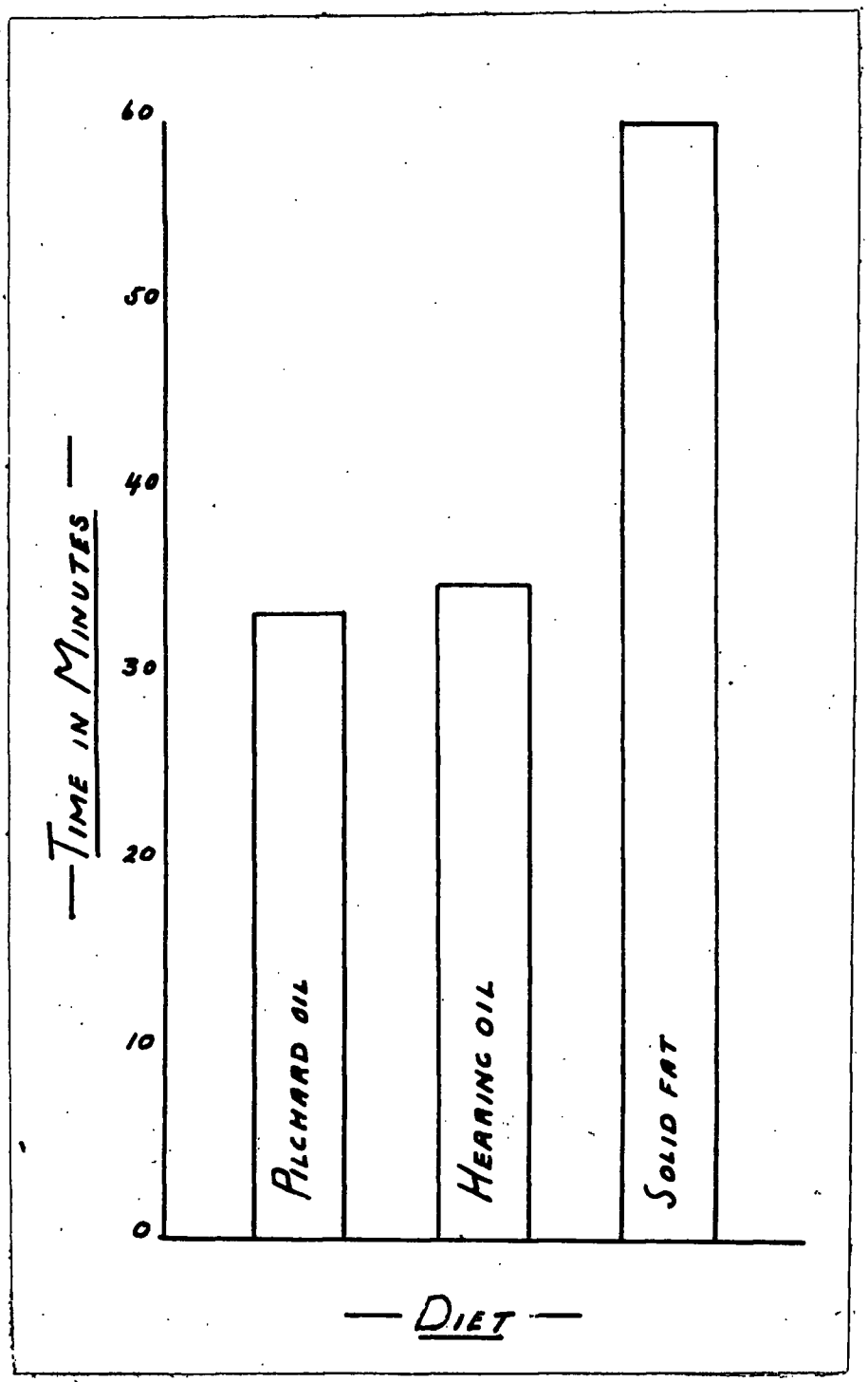

Figure 5. 50% mortality in goldfish at 36°C.

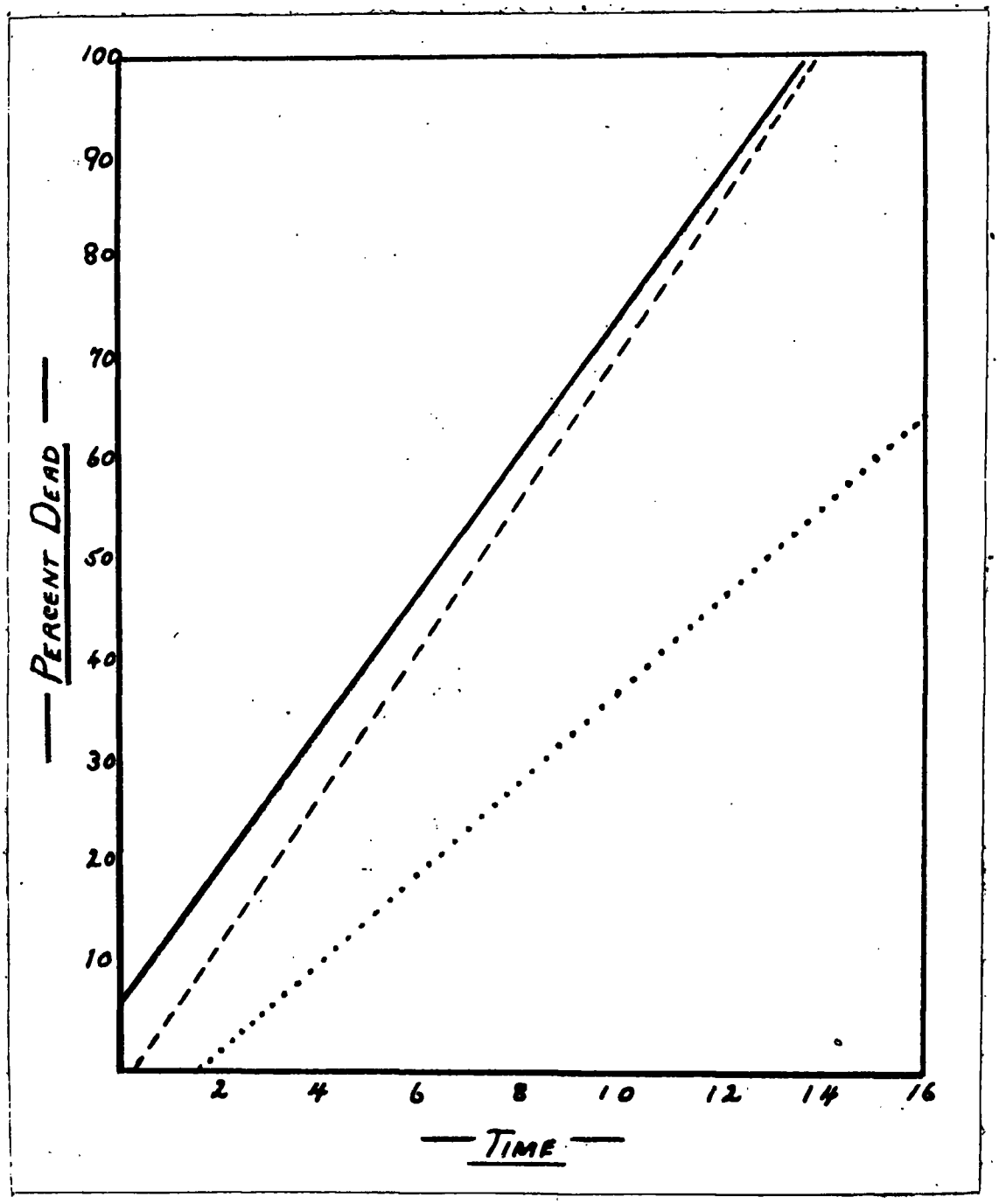

Figure 6 Regression lines for first 75 minutes of main heat resistance tests.

----- Pilchard oil diet fish b = 7.34 ± 1.32
_____ Herring oil diet fish b = 6.82 ± 1.08
..... Solid fat diet fish b = 4.42 ± 0.293

oil diet fish died of shock immediately, an occurrence which was not recorded for the other fish.

Even at room temperature, the more unsaturated fat diets produced nervous symptoms in the fish. On one occasion at least, the herring oil group became so nervous at a temperature of 18°C. as to attract the attention of strangers entering the laboratory. Subsequently two of these fish died at this temperature in a manner strikingly resembling heat death.

The reason for this behaviour is unknown but Brown (1931) noticed similar symptoms amongst pigs fed on fish oils.

DISCUSSION

Relation of Dietary to Body Fats

Four factors, temperature, sexual maturity, species and diet have been shown to influence the composition of the fats in plants and animals.

Pearson and Raper (1927) showed that the iodine values of the fatty acids produced by two species of fungi varied with the temperature at which these organisms were grown. Dean and Hilditch (1933) demonstrated that the body temperature influenced the composition of the depot fats of pigs, the outermost layers of fat being more unsaturated than the innermost. Lovern (1936) found that the tunny (Thynnus thynnus) which has a body temperature some three degrees higher than the water in which it lives, had more saturated fatty acids than other marine fish. Finally, Bailey (1936) showed that Sockeye salmon (Oncorhynchus nerka) and Pink salmon (Oncorhynchus gorbuscha) caught in the northern Pacific coast waters had more unsaturated fats than fish of the same species caught in the more southerly waters off the Fraser River.

Greene (1913) showed the progressive loss of stored fat in the Spring salmon (Oncorhynchus tshawytscha) during its spawning migration while Lovern (1934) found that in the case of the male salmon (Salmo salar) some selective mobilization of the depot fats took place during the spawning season.

Undoubtedly, species and diet are two very important factors. It is difficult to determine where the influence of one ends and the

other begins. Brockelsby (1941) discusses these factors and points out:

> " Since diet and life habits have a profound effect on the nature of the deposited fats, animals in general tend to form specific fats only insofar as their diet and habits are specific. However, any species lives on a more or less characteristic diet and the fats formed are, within certain limits, characteristic of that species. "

It seems definite that all animals possess, to a greater or lesser degree, some mechanism for modifying the ingested fats to suit the specific requirements. However, if the fat consumed varies too greatly from the normal requirements of the animal, or if the rate of ingestion is too high, the mechanism cannot successfully modify it all, and part at least of the foreign fat will be deposited relatively unchanged.

Although many controlled feeding experiments have been performed on various farm animals, with a view to altering the fats for marketing purposes, very little has been done on the controlled feeding of fish. To the author's knowledge, the work of Lovern (1938) and the experiments contained herein, constitute the only two recorded experiments in this field. For this reason the results obtained are of particular interest.

Using eels (Anguilla vulgaris) Lovern (1938) experimented with two different diets, mussels and herrings. He found that there was no appreciable modification of the eel fat toward mussel, probably because

it was not fed at a sufficiently high level. He observed some modification toward herring fat however. This latter food contained a much higher percentage of fat. The final products obtained corresponded very well to definite mixtures of eel and herring oil, but a quantitive turnover of ingested and depot fat was not demonstrated.

The present experimental results agree with those of Lovern in that diet produced modifications in the degree of unsaturation of the goldfish fats, when the ingested fats differed sufficiently from the specific varieties normally present in the fish (see tables I and II). Further, the amount of change in the case of the pilchard oil and lard diet was only approximately 54% of the theoretical. Herring oil, as has been demonstrated (figure 2), produced very little effect.

There are two possible explanations for this phenomenon. One is that since the fats were fed to the fish at too high a level for them to be converted to the specific fats, they were stored relatively unchanged in the adipose tissues, the fish meanwhile living on their specific fats previously stored. After the latter were exhausted, the fats obtained through the diets would then be converted to the specific type required for the metabolic processes. This, then, would mean that the dietary fats bear no relation to the physiological fats. This view, while conforming to the views of Lovern, is contrary to those of Rittenberg and Schoenheimer (1937) whose experiments with deuterium indicate a constant turnover between depot and body or physiological fat.

The other explanation is that, because of the nature of the

ingested fats and the rate at which they were consumed, the regulating mechanism was only capable of modifying the fats to a certain degree and it was in this form that they were stored. When the specific fats were exhausted, the ingested fats were then used in the form in which they were stored, replacing all the fats in the animal body, both depot and physiological.

This latter view follows more closely the experimental results in that the regression coefficient of the fish fed on the herring oil diet (figure 2) is -.032, indicating that there was an immediate and constant desaturation of the ingested fats to the specific type. It is true in this case that the ingested fats (iodine value 115) were very little different from the specific variety (iodine value 112 - 114) but the level of feeding was equally as high as in the other two cases. Again the results of the experiments on heat resistance show a relationship between the degree of unsaturation of the dietary fats and the temperature tolerance of the organism. Since it is the protoplasmic or body fats that are assumed to be involved here, no such result could be obtained were the dietary fats converted to specific fats before use by the fish.

Relation of Fats to Heat Tolerance

Figure 4 illustrates that in the Preliminary Group of experiments the order of increase of heat tolerance imparted by the diets was herring oil, pilchard oil and solid fat. The fiducial limits in this graph are listed in table 8, from the formulae:

$$l_1 = b + t.sb$$
$$l_2 = b - t.sb$$

where b = the regression coefficient
t = probability at the particular level desired
sb = standard error of b

TABLE VIII

Fiducial limits of b values for goldfish used in the preliminary experiments

Diet	Fiducial Limits of b Values	
	99% level	95% level
Herring	8.98 6.74	8.66 7.05
Pilchard	7.47 4.25	7.02 4.70
Solid	5.24 3.58	5.00 3.82

A study of the table reveals an overlapping of the b values for herring and pilchard, and pilchard and solid at the 99% level. This means that 1% of the fish in the herring oil and pilchard oil groups could have shown the same degree of heat tolerance. The same is true for the pilchard and solid fat groups. However, it is significant that overlapping does not occur between the herring and solid diet groups, indicating that the chances for a fish fed on herring oil exhibiting the same response to heat as a fish fed on lard are less than 1 in 100. A consideration of the 95% level shows that the pilchard and solid overlap but the pilchard and herring do not. Thus the chances for herring oil diet fish and pilchard oil diet fish dying at the same rate are less

than 5 in 100.

A study of figure 6 reveals that in the main group of experiments the order of dying changed in the case of the fish fed pilchard and herring oil diets. The order in these experiments was - pilchard, herring and lard. This is the order expected if the hypothesis upon which the experiments were based is correct. An examination of the fiducial limits indicates that the difference between the effects of the pilchard and herring oil diets is not statistically significant since at both the 95% level and the 99% level considerable overlapping occurs. However, in these experiments the behaviour of the fish maintained on the solid fat (lard) diet is significantly different from both the pilchard and herring oil diet fish. This is true even for the 99% level.

Thus, there is a discrepancy between the results obtained in the preliminary experiments and those of the main group as regards to the pilchard oil and herring oil diets. There are three factors that may be of importance in explaining this contradiction.

1. It will be recalled that during the preliminary experiments each group was maintained in two separate aquaria and that due to the variation in sensitivity of the thermostats, slight differences in thermal history were unavoidable. Loeb and Wastenays (1912) have pointed out that immunity to a higher temperature is lost very slowly when once acquired and that short daily exposures to a higher temperature produces a marked change in immunity. Therefore it is possible that even slight variations in thermal history might be significant.

2. This same reasoning applies to a criticism of the method of killing the fish in the preliminary experiments. Here it will be recalled a small aquarium served as a lethal tank, and each sample was tested separately. This led to variations in thermal histories in the tests themselves, which might possibly become apparent in the final results.

3. Finally, the preliminary experiments were all carried out within 34 days of the commencement of feeding. It has been shown that during this time the degree of unsaturation of the extracted fats was constantly changing which may have some bearing on the results obtained. This seems to be the most likely explanation.

In the main group of experiments the sources of variation just described were eliminated. The fact that the results of the herring oil and pilchard oil tests are not significantly different may either be attributed to the fact that the numbers of fish available for experimentation were not large enough or that while the degree of saturation of the protoplasmic fatsplays a part in heat tolerance, some other factor, or factors, are also involved. Fraenkel and Hopf (1940) concluded that an additional factor must be involved after their experiments on blow-fly larvae. Working alone Hopf (1940) showed that exposure to high temperature caused an increase in the lipoid phosphorus, inorganic phosphorous and adenyl pyro phosphate P of the Haemolymph of blow-fly larvae.

Lovern (1932-1934) analyzed the composition of the fatty acids

of a number of marine and fresh water fish and found that there were distinct differences. In 1935 the same author examined three species of fresh water crustacea and one species of marine crustacea and found the same class distinctions. This may have some bearing on the results obtained in these experiments since the goldfish, which are a fresh water species, were fed diets containing the oils of marine fishes. The fact that the iodine values changed considerably in the case of the pilchard oil diet and solid fat diet, indicates an alteration in the composition of the depot fat. However, the fact that the iodine values of the herring oil group varied very little does not necessarily imply that there was no change in the composition of the depot fats. It has been suggested that the fats were stored in a somewhat modified form in the case of the lard and pilchard oil diets. This could equally well apply in the case of the herring oil diet without causing the iodine values to change appreciably. If this were so, it may be significant in view of the results obtained in the heat resistance experiments, that the pilchard (Sardinops caerulea Girard) generally inhabits more southerly waters than the herring (Clupea pallaisii Valenciennes).

Another point of interest is the increased tolerance to a temperature of 35°C. exhibited by the fish used in the main experiments after a short period of feeding. This may be attributed to the better condition of these fish, since those used in the preliminary group had already been maintained in the laboratory for a number of months and their feeding had been unavoidably irregular. The generally improved

nutrition seems to affect the heat tolerance.

A review of the results obtained in these experiments shows that diet can be a factor in determining the type of fat produced within the fish. It also indicates the veracity of the general hypothesis that greater immunity from the effects of heat is derived from more solid dietary fats. However, these relationships are not quantitative and the degree of unsaturation of these fats is probably not the only mechanism involved in heat tolerance.

SUMMARY

1. The composition of the depot fats of goldfish may be altered by feeding fats of a different degree of saturation at a sufficiently high level.

2. This process is rapid at first but after a change of approximately 54% of the theoretical is reached very little further change occurs.

3. The degree of saturation of the dietary fats influences the heat tolerance of the fish in conformity with the general theory that the higher the melting point of the fat the greater the heat resistance of the fish. However, since some additional factor is involved which may modify this resistance, the results are not quantitative.

4. The fish oil diets modify both the normal behaviour and the initial reaction to heat. There is a general tendency for the fish oils to produce nervous symptoms.

ACKNOWLEDGEMENTS

I wish to acknowledge the interest taken in this thesis by Dr. W. A. Clemens, Head of the Department of Zoology of the University of British Columbia, and to thank Dr. W. S. Hoar, by whom this problem was suggested, for his continuous assistance and helpful criticisms. I also wish to thank Dr. B. E. Bailey of the Dominion Fisheries Experimental Station in Vancouver.

The assistance of fellow students, especially that given by I. Barrett, L. Robertson and G. Potter, has been sincerely appreciated.

APPENDIX I

PART A - Details and Results of the Winter Preliminary Experiments.

Feeding was commenced on December 18, 1947.

December 30, 1947. Experiment 1(a):

Four goldfish, one from each diet group and one control which had not been fed any special diet, were placed in an aquarium and the temperature raised 4° per hour. Results are presented in table IX below.

TABLE IX

Results of preliminary experiment 1(a)

Diet Group	Lethal Temperature
Herring	34°C.
Control	35.5°C.
Solid	36°C.
Pilchard	36.2°C.

January 2, 1948. Experiment 2(a):

Four goldfish, one from each diet group and one control, were placed in an aquarium at 34°C., left for 30 minutes and then the temperature was immediately raised to 36°C. The time of death is given in table X.

TABLE X

Results of preliminary experiment 2(a)

Diet Group	Time of Death
Herring	1 minute, 15 seconds
Solid	1 minute, 30 seconds
Control	1 minute, 30 seconds
Pilchard	2 minutes, 50 seconds

January 13, 1948. Experiment 3(a):

One goldfish from each diet group was placed in an aquarium at 9.2°C. The temperature was raised 1°C. every 90 seconds. The lethal temperature was noted and the results are given in table XI.

TABLE XI

Results of preliminary experiment 3(a)

Diet Group	Lethal Temperature
Herring	35.5°C.
Pilchard	36.1°C.
Solid	36.8°C.

February 16, 1948. Experiment 4(a):

The remaining fish in the three groups were tested in water at 34°C. Time to death is noted in table XII.

TABLE XII

Results of preliminary experiment 4(a)

Diet Group	Time to Death
Pilchard	5 minutes
Herring	50 minutes
Solid	80 minutes

PART B - Results of the Second Section of the Preliminary Experiments carried out in May and June, 1948

TABLE XIII

Results of experiment 1(b) on six pre-diet fish tested at 35°C.

Number Dead	Time in Minutes
1	27
4	47
6	60

TABLE XIV

Results of experiment 2(b). Six fish per diet group subjected to 35°C. Feeding time 12 days.

Pilchard Oil Diet Fish		Herring Oil Diet Fish		Solid Fat Diet Fish	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
25	1	38	1	22	1
37	2	43	2	151	2
43	3	45	3	185	3
50	4	60	4	320	4
75	5	62	5	Acclimatized	2
80	6	65	6		

TABLE XV

Results of experiment 3(b). Six fish per group subjected to 35°C. Feeding time 21 days.

Pilchard Oil Diet Fish		Herring Oil Diet Fish		Solid Fat Diet Fish	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
40	1	13	1	25	1
42	2	17	2	27	2
53	4	27	4	40	3
67	5	50	5	62	4
70	6	60	6	67	5
				97	6

TABLE XVI

Results of experiment 4(b). The remaining fish in each group, 5 pilchard, 6 herring and 8 solid subjected to 35°C. Feeding time 34 days.

Pilchard Oil Diet Fish		Herring Oil Diet Fish		Solid Fat Diet Fish	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
11	1	1	1	25	1
58	2	20	2	30	2
190	3	30	3	40	3
230	4	32	4	57	4
		33	5	58	5
		35	6	80	6
				190	7
Acclimatized 1				Acclimatized 1	

PART C - Results of Main Group of Experiments.

Feeding was commenced on June 20, 1948.

TABLE XVII

Results of experiment 1(c). Five pre-diet fish subjected to a temperature of 35°C.

Number Dead	Time in Minutes
1	15
2	21
3	29
4	50
5	110

TABLE XVIII

Results of experiment 2(c). Six fish per diet group subjected to 35°C. Feeding time 6 days.

Pilchard Oil Diet Fish		Herring Oil Diet Fish		Solid Fat Diet Fish	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
52	1	28	1	74	1
67	2	55	2	85	2
106	3	75	3		
195	4	170	4		
Acclimatized 2		Acclimatized 2		Acclimatized 4	

TABLE XIX

Results of experiment 3(c). Eight fish per diet group subjected to 35°C. Feeding time 29 days.

Pilchard Oil Diet Fish	Herring Oil Diet Fish	Solid Fat Diet Fish
All became acclimatized	2 died of shock 1 died in 70 minutes 5 became acclimatized	All became acclimatized

TABLE XX

Results of experiment 4(c). Four fish per diet group placed in the heating tank. Temperature raised 1°C. per hour until all the fish died. Feeding time 32 days.

Temperature	Number Dead Pilchard Oil	Number Dead Herring Oil	Number Dead Solid Fat
38.3	1	1	
38.8	4	2	1
39.		4	2
39.2			3
39.5			4

TABLE XXI

Results of experiment 5(c). Six fish per diet group subjected to 37°C. Feeding time 37 days.

Pilchard Oil Diet Fish		Herring Oil Diet Fish		Solid Fat Diet Fish	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
25	1	25	3	25	2
45	6	45	6	45	4
				70	6

TABLE XXII

Results of experiment 6(c). Six fish per diet group subjected to 36°C. Feeding time 46 days.

Pilchard Oil Diet Fish		Herring Oil Diet Fish		Solid Fat Diet Fish	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
16	1	1	1	33.5	2
30	2	14	2	42.5	3
33.5	3	40	3	60	5
42.5	5	49	6	195.	6
46	6				

TABLE XXIII

Results of experiment 7(c). Eight fish per diet group subjected to 36°C. Feeding time 55 days.

Pilchard Oil Diet Fish		Herring Oil Diet Fish		Solid Fat Diet Fish	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
11	1	7	1	58	1
20	2	10	2	77	2
27	3	12	3	85	3
35	4	15	4	190	4
37	5	33	5	540	6
65	6	39	7		
77	7	80	8		
137	8			Acclimatized	2

TABLE XXIV

Results of experiment 8(c). Sixteen fish per diet group subjected to 36°C. Feeding time 59 days.

Pilchard Oil Diet Fish		Herring Oil Diet Fish		Solid Fat Diet Fish	
Time in Minutes	Number Dead	Time in Minutes	Number Dead	Time in Minutes	Number Dead
8	1	8	1	8	2
18	2	12	2	9	3
20	4	15	3	18	4
25	8	20	4	27	5
27	9	21	5	30	6
35	11	22	6	36	7
37	12	24	7	38	8
45	14	35	9	50	9
60	15	37	11	55	10
82	16	48	14	62	11
		57	15	72	12
		80	16	80	13
				110	14
				420	15
				Acclimatized	1

APPENDIX II

Average Lengths, Weights and Iodine Values of Fish Used.

TABLE XXV

Fish used in second section of the preliminary experiments.

Date Killed	Experiment Number	Diet Fed	Av. Length in cm.	Av. Weight in grams	Average Iodine Value
Feb. 17, '48	1(b)	Pilchard	6.78	13.3	-
		Herring	6.74	11.06	-
		Solid	7.0	13.9	-
May 26, '48	2(b)	Pre-diet	6.8	9.25	114.045
June 8, '48	3(b)	Pilchard	6.6	10	124.
		Herring	6.7	10.1	113.7
		Solid	7.5	10.3	90.4
June 17, '48	4(b)	Pilchard	6.8	9.6	126.6
		Herring	6.8	11.5	115.5
		Solid	7.0	11.6	-
June 30, '48	5(b)	Pilchard	7.1	12.0	136.
		Herring	6.6	11.6	111.4
		Solid	7.1	11.6	88.9

TABLE XXVI

Fish used in the main group of experiments

Date Killed	Experiment Number	Diet Fed	Av. Length in cm.	Av. Weight in grams	Average Iodine Value
June 18, '48	1(c)	Pre-diet	7.1	11.1	112.78
June 26, '48	2(c)	Pilchard ≠	7.25	9.5	119.25
		Pilchard	7.	11.	124.9
		Herring ≠	7.25	11.5	118.2
		Herring	7.2	11.	113.47
		Solid ≠	7.3	12.2	95.77
		Solid	7.4	11.2	91.14
July 19, '48	3(c)	Pilchard	7.2	12.	128.25
		Herring ≠	6.7	9.4	116.35
		Herring	6.8	9.4	109.
		Solid	6.9	11.	88.41
July 22, '48	4(c)	Pilchard	7.	10.	133.45
		Herring	7.2	12.5	112.9
		Solid	7.1	12.2	86.5
July 27, '48	5(c)	Pilchard	7.2	13.1	136.1
		Herring	7.3	13.3	113.1
		Solid ≠	7.	11.	87.75
		Solid	7.2	14.	85.3
Aug. 5, '48	6(a)	Pilchard	7.6	16.	137.1
		Herring	7.1	13.	113.2
		Solid	7.3	15.5	91.38
Aug. 14, '48	7(c)	Pilchard	7.36	14.	137.
		Herring	7.26	13.75	110.86
		Solid ≠	7.5	14.2	83.14
		Solid	7.5	14.2	88.5
Aug. 18, '48	8(c)	Pilchard	7.7	17.	-
		Herring	7.6	16.5	-
		Solid	7.5	16.	-

≠ Fish that became acclimatized or showed greater resistance to heat than others in the same group sample.

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