THE EFFECT OF VITAMINS B AND G ON THE GROWTH OF PISH

by

## EDWARD SCHNEBRRGER

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### TABLE OF CONTESTS

D Tacage 7108	page
ALVIA OF LITERATURE	
history of Vitamin B	
work on Vitamin Requirements of fish	. 7
LEGIVDS	. 9
	. 12
Experiments to Control Diet	. 12
Data on Vitagin acquirements of Fish	16
Jacque Los es	37
SU PAIZ	
III Tom Clad	44

#### INTHODUCTION

The question as to whether fish require the known vitamins was raised during the summer of 1938 while working at the State Fish hutchery, Fratt, Auneae.

The spotted channel catfish (<u>letalurus punctatus</u> dafinesque) is raised by the trough methods at the Kansas Fish Hatchery. It was planned to feed the young catfish live natural foods. Daphnia and Blackfly larvae were used for this purpose. The former were raised in several

large tanks constructed for that purpose, and Blackfly larvae were collected from the drains of the ponds.

This supply of food was soon depleted and the fish had to be fed commercial foods. The first food tried was dried buttermilk powder. Shortly after the beginning of this diet the fish began to show serious pathological symptoms, and a high mortality resulted. Nervousness was the chief symptom shown by the fish. Any slight vibration or jar would cause them to throw fits, tail-spins and swim in a circle on their backs. This condition was cured by the addition of yeast, codliver meal, liver, canned tomatoes, fresh blood, lettuce and duckmeed to the diet.

The fact that a change in the diet remedied the condition of the fish would indicate that the pathological symptoms were due to some dietary deficiency, possibly the absence of a vitamin. In order to determine whether this was the case, carefully controlled experiments varying only one dietary factor at a time are needed. Since an absence of vitamin B frequently results in nervous disorders, it was regarded as possible that the symptoms noted in the fish might have been due to the lack of this vitamin. This study was undertaken in order to ascertain whether vitamins B and G are necessary for normal growth and development of fish.

This work was undertaken in collaboration with Dr.
Minna E. Jewell of the Kansas State Agricultural College,
and under the auspices of the Kansas Forestry, Fish and
Game Commission and the Department of Zoology of the Kansas
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#### REVIEW OF LITERATURE

# History of Vitamin B

Beri-beri was first known as a dietary disease in 1887 when Takaki Kanehiro, Director General of the Japanese Navy, changed the food for Japanese sailors to eliminate beri-beri.

Eijkman (1889), Director of the Hygienic Laboratory, Dutch East Indies, observed a disease in fowls which was analogous to beri-beri. After investigating into its etiology, he found that beri-beri could be produced experimentally in fowls on a diet of polished rice. He called the disease polyneuritis gallinarum. The theory he then advanced was that the starch in the cereal grain gave rise to toxins which exerted a deleterious effect upon the nervous system.

Grijns (1898) rejected the amylum-toxin theory and attributed the disease simply due to a dietary deficiency. Grijns also showed that a curative agent could be extracted from rice polishings, and that other natural foods contained this substance.

Funk (1911) separated out the active agent from rice bran by extraction with alcohol containing hydrochloric acid. This cured polyneuritus induced in birds by feeding them polished rice.

After confirming his observations the following year he named the substance vitamin. Contemporaneous with Funk's work appeared a publication by Suzuki, Shimamura and Odake (1912) on the isolation of an antineuritic substance "Organin", and another by Edie, Evens, Moore and Simpson (1912) on a similar product which they named "torulin". Tsuzuki (1912) further described a patented preparation as antiberiberin.

When Goldberger and coworkers (1925) first postulated the existence of a substance P-P, pellagri preventive, it seemed to have a slight connection with vitamin B. Later (1926) they advanced the theory that water soluble B, that is the growth promoting substance, consisted of two factors; the one an antineuritic principle for which they retained the term vitamin B in sensu strictu, and the other a pellagra preventive which they had already named P-P. The best chemical distinction made between the two was their differential behavior to heat; the vitamin B was thermolabile, the factor P-P was thermostable.

The existence of two factors was recognized by Sherman and Axtmayer (1927) who treated them as two primary vitamins rather than as subordinate parts of a vitamin complex. Accordingly they suggested vitamin P for the antineuritic thermolabile factor and suggested vitamin G for the thermostable factor.

This implied the use of vitamin B as a group name for the F and G substances. There has been a good deal of controversy as to a standard nomenclature of these factors. The committee on vitamin B terminology of the American Association of Biological Chemists (1929) have voted to recommend that (a) the term B be restricted to designate the more heat-labile antineuritic factor, (b) that the term @ be used to denote the more heat-stable, water soluble, dietary factor called P-P, pellagra preventive, factor by Goldberger and associates, and which has also to do with maintenance and growth. This is the nomen-clature that has been followed in this work.

# Work on the Vitamin Requirements of Fish

The field of nutrition and vitamin requirements of fish is relatively new. When fish, particularly trout, were first raised by the trough system, liver was almost the universal food. At that time liver could be obtained at a very low price, the fish took it readily and it seemed to be an adequate dist. More recently, however, nutritionists have found the high value of liver in the human dist, and consequently the price of liver has increased. Fish culturalists have thus been forced to use more economical foods and have encountered innumerable nutritional difficulties.

Davis and James (1924) concluded that carp and possibly trout require vitamins A, B and G.

McCay, Bing and Dilley (1927) have shown that trout need a protein level of over ten per cent for normal growth, but the addition of more than 25 per cent of protein does not give a greater or faster rate of growth. In a later paper ECCay and Dilley (1927) announce a factor which they have found in fresh meats which is essential for normal growth of trout. This factor apparently is not vitamin A, B, C, D or E. They have named it factor H. This factor is thermolabile, being destroyed in meats that have been cooked or dried. An amount as small as five per cent fresh liver added to the diets will carry enough factor H to exert a marked influence on the growth of trout. That raw meat contained growth promoting properties not found in meat extracted with alcohol and ether had already been shown by Richet (1925) and Laufberger (1926).

Davis (1927) after five years of hatchery work in the practical feeding of trout states, "In no case have we failed to get beneficial results when oil and yeast were added to the diet of rainbow trout fingerlings, while in the case of brook trout fingerlings the results have just as consistently failed to show benefit from the addition of vitamin rich foods to the diet." He states that each species of trout must be considered as an individual problem.

#### MATHODS

The common uncolored goldfish (<u>Carassius auratus</u> Linnaeus) and the spotted channel catfish (<u>Ictalurus punctatus</u> Rafinesque) were used in these experiments.

The goldfish were obtained from the Grassyforks Hatchery, Martinsville, Indiana, and the catfish were furnished by the Kansus State Fish Hatchery.

The fish were kept in wooden troughs six feet long, one foot wide and one foot deep. The water came from the College supply, and is kept to a depth of eight inches. It entered the tank through a spigot at the upper end, and was drained off the bottom of the tank at the lower end of the tank. The goldfish were separated from the catfish by a screen. About one-third of the tank was allowed to catfish and the remainder to goldfish; the goldfish occupying the space nearest the outlet.

The experiment upon goldfish was started on February 16, 1929, and the catfish on March 17, 1929. The catfish could not be obtained at the same time as the goldfish, due to weather conditions.

The fish were measured and weighed at the beginning of the experiment. Subsequently, they were weighed every week and measured every month. It was considered accessary to take both weights and lengths of the fish, since an increase in weight might be due to a deposition of fat, whereas growth should result in an increase in length. On the other hand a decrease in weight might or might not show a noticeable decrease in length. The measurements were taken of each fish from the most unterior point to the base of the caudal fin. Weights were made collectively of all the fish in a group.

The following diets were fed to the fish: Diet No. 1 - Vitamins B and G lacking

Basal ration consisting of:

Polished rice flour - 68 gas.

Alcohol extracted caesin - 18 gms.

Crisco - 6 gms.

Hogan's Salt hixture - 4 gms.

Cod liver oil - 4 gms.

Water - 150 cc.

Diet No. 2 - Vitamin 6 present, Vitamin B lacking

Basal ration plus 12 grams brewers yeast autoelaved at 15 pounds steam pressure for 150

minutes to destroy vitamin B.

Diet No. 3 - Vitamins B and G present.

Control. Basal ration plus 12 grams of fresh brewers yeast.

Diet No. 4 - Buttermilk diet.

Same as basal ration, except that caesin is replaced by 18 grams of dried buttermilk powder. To see if buttermilk contains sufficient vitamins B and G.

Diet No. 5 - Meat diet.

Same as No. 4, except that 12 grams fresh brewers yeast and 60 grams fresh ground liver were added to the diet.

The ingredients, other than meat and yeast, were mixed together evenly, then cooked in a double boiler to form a paste or dough. This process fixed the cod liver oil and crisco in the diet so that they did not float to the surface of the water and escape in that manner. The yeast and meat were added after cooling.

The caesin was treated with alcohol by the Sherman process (1925) to remove vitamin B. This extraction was made by miss Frances harshall of the Home Economics Department of the Kansas State Agricultural College.

The salt mixture used is that of logan (1925). It has the necessary salts and minerals in proper proportions, and is composed of the following ingredients:

Ca lactate - 48.9 per cent

R2nPo4 - 29.3 per cent

NaCl - 12.8 per cent

Na citrate - 3.23 per cent

Fe citrate - 2.44 per cent

A604 - 3.32 per cent

NaI - 0.01 per cent

Fresh beef liver was ground with a food chopper and then forced through a fine screen to remove the course blood vessels. The pulp was then mixed into the cooked paste. Each diet was fed to 100 goldfish and 50 catfish.

The fish were fed twice daily with as nearly equal feeding periods between as possible. The group that ate the least was taken as a criterion as to the amount to feed all, so that all groups were fed the same amount.

#### DATA

# Experiments to Control Diet

at the beginning of the experiment buttermilk powder extracted with alcohol to remove vitamin B and G was used

as a source of proteins in diets 1, 2 and 3. In order to check the completeness of the extraction, five male white rats of the same litter were fed the same diets used for the fish. Since rat one (Table I, Fig. 1) showed as rapid a gain as did the other rats, purified caesin was substituted for the buttermilk powder thereafter. Following this change rat one on a diet free from vitamin B and G gained weight for a short period and then lost weight until it weighed less than at the beginning of the experiment. At this point Diet No. 3 with vitamin B and G was fed to the rat. It began to increase in weight.

Rat No. 2 showed normal growth (vitamin G), but no nervous disorders due to lack of vitamin B became apparent. However, as the duration of the experiment after beginning the use of purified caesin was only five weeks, the time was probably insufficient for the development of such symptoms.

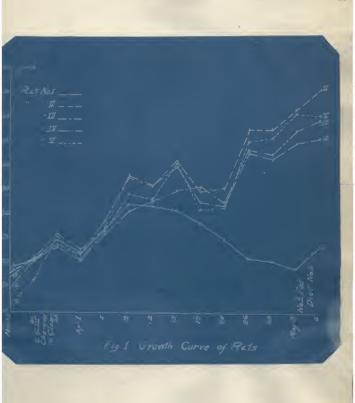
Rate Nos. 3 and 4 show approximately the same growth indicating that dried buttermilk powder contains adequate vitamin G for the growth of rats, without the addition of yeast. Rat No. 5 showed a gain over Nos. 3 and 4. Whether this gain is due to an increased protein content or to other factors present in raw meat is not determined.

The graph (fig. 1) shows fluctuations in weight of each group. This fluctuation was probably due to temperature changes. The rats were kept in an unheated basement and were subject to temperature changes. It was noted that the loss in weight coincided with periods of cool damp weather.

It was desired to still further compare purified caesin and alcohol extracted buttermilk powder, so two more rats, of the same litter, were secured and put under experiments. The larger rat A was put on the diet containing caesin and the smaller rat B was placed on the diet containing purified buttermilk. An examination of figure 2 and Table II will show that rat B gained over rat A the first week. At the end of the third week rat B began to make rapid gains while rat A lost in weight, showing that the alcohol extraction of buttermilk was not sufficient for the removal of vitamins B and G. As a result the use of alcohol extracted buttermilk for the basal ration was abandoned and only purified caesin used.

TABLE I. WEIGHTS OF RATS TO CHECK DIET

								r 3 Con	-: 1	unler	4:1	wunber	9
								B - G	i is	atter.	- 3		
Date		lacking	: 1	3 lack	ing	( D.	108	sent	::	1.k 0-	G?:	Meat	
													-
March	22:	58.5	- 5	56.0				50.0	:	54.5		43.4	
	25:	60.8		61.4		:		61.1		61.6	1	53.5	
	29:	70.0		72.9				68.8	:	67.8	:	72.9	
					in 40 M				-	-	0-40 vis 4		400
Piro	tein	of die	ts	of 1.	2	and	3	changed	to	nure	car	sein	
										P			
April	1:	64.3	:	65.7		:		64.0		61.2		61.3	
Whitz	5:	74.5		74.4		:		77.8		72.3		73.0	
	8:	84.6	2					96.0	:	82.0	:	89.0	
	12:	84.0	1	92.4				92.7	2	86.6		86.4	
	15:	82.7	:	103.0		:		100.3	:		2	101.6	
	19:	75.9	:	90.2				85.2	:	92.7		82.3	
	22:	71.3	:	91.1		2		84.0	:	85.1	2	83.2	
	26:	62.9	:	115.8		:		06.0	-	107.1		110.6	
	29:	61.4	1	116.3				04.6		105.9		112.2	
May	3:	58.5	2	124.0				10.1		115.0		122.4	
	1	0000		20200		5.	**			120.0		20000	
Gain													
April			:			2							
MAY	:	5.8		58.3		:		46.1	:	53.8		61.1	
				0000				2012		00.0		01.1	
		M	umt	per 1 1	nut	on	Di	et Numb	or :	3			
			CO 100 FG	-			-		-				
May	6:	67.2	-	132.1		9	3	12.0	2 .	119.5		121.5	
			-										



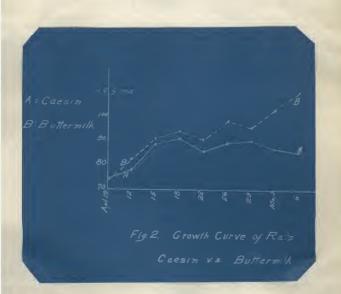


Table 11. GROWTH OF RATS
Caesin Compared to Buttermilk

		3	Rat A		Rat B
Dat	0	;	Caesin	:	Buttermilk
pril	9		71.4		73.2
Pr. a-	12	:	78.1	-	83.4
	15		88.6	8	90.8
	19	å	90.8	4	94.1
	22	- 2	85.8	3	90.2
	26	-	89.9	1	98.3
	29	2	90.2	2	95.3
ay	3	0	87.2	1	103.5
	6	\$	85.0		110.3
		ä		1	
ain			13.6		37.1

Data on Vitamin Requirements of Fish

Data were secured on five groups of 100 goldfish and five groups of fifty catfish.

Table III gives the average gain in length of the five groups of goldfish. The fish were measured every month. The fish that died during the succeeding month were measured and their lengths subtracted from the total length in order to secure an average length of the fish which survived until the next time of measuring. By subtracting this length from the average length of the next time of measuring an average growth was secured. This

method eliminated apparent growth or loss due to death of the smaller or larger fish.

Table IV gives the relate of the goldfian which were taken every week. The following graphs (Figs. 3 and 4) were drawn from Tables I and II, respectively. In the curves showing growth in length (Figs. 3 and 10), apparent increases or losses in length due to death of some of the fish are indicated by vertical broken lines. Figures 5 to 9 inclusive show the average growth in length of the five groups. The fish were arranged in groups of three mm. intervals and the curves show the number of fish in each group. Table V shows the average length gains of the catfish and Table VI shows the average gain in weight.

The average gain in length of the catfish is shown by figure 10. Figure 11 shows the average weight. The length distribution for the entire group is shown by figures 12 to 16 inclusive.

TABLE III. AVERAGE GAIN IN LENGTH OF GOLDFISH

:N :B Goldfish:a	- G : G	present	: number 3 Con- :trol. B - G :present	:Number 4 : :Butter- : :milk B-G?	
Feb. 16: March 9:	53.21: 53.91:	53.17 54.0	: 53.05 : 54.23	: 52.23	00000
Gain :	.7 :	.83	1.18	1.27	1.69
Average : length :	53.91:	54.0	: 54.204	: 53.69	55.95
April 6:	54.91:	55.34	: 55.47 : 1.266	: 54.86	56.77
Average :	:		8	:	
length : survived:	55.36:	55.36	: 05.7	: 54.70	56.77
may 4:	56.18:	56.09	: 56.36	: 55.4	57.71
Gain :	.82:	.73	.66	.70	.94
Total : Average : Gain :	2.52:	2.9	: : 3.106	: : 3.14	3.45
Per cent:	4.735:	5.454	5.854	: 6.011	6.358

TABLE IV. WEIGHTS OF GOLDFISH

	2		Į.	Averag	0	weign	ti	of Go	10	irish
Date	;	I	ô	II	*	111	:	IV	6	V
		6 60		6.68		6.65		6.309		7.093
warch 9		6.62	8							7.23
16		6.74	\$	6.857		6.94	•	6.43	0	
23		6.715	ě	6.867	å	6.959		6.38	8	6.97
_30	:	7.18	0	7.21	:	7.21	0	6.75		7.57
April 6	2	6.88	2	7.9	2	7.02	*	6.61	0	7.3
13	:	7.11	1	7.03		7.07		6.73	2 0	7.4
20		7.39	0	7.04	0.0	7.01	2	6.61		7.36
28	2	6.82	:	7.07		6.98	0	6.71	2	7.46
kay 4	:	7.08	\$	7.11	:	7.09		6.81	-	7.65
	5		3		:		2		5	
Gain	100	+.46	ő	+.50	ô	+.44	0	+.501	•	+.557
-	2		0		0		0		2	
Gain	2	+.2	:	+.05	3	+.07	:	+.20	2	+.35
-										

<sup>&</sup>quot;Diet changed to caesin.

Total gain since beginning of experiment.

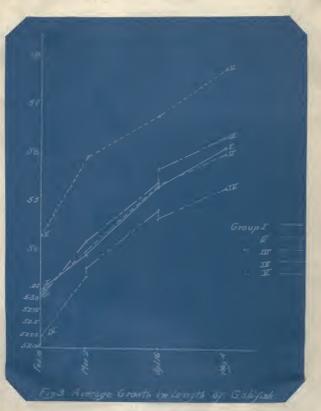
<sup>&</sup>quot;Gain made on caesin.

TABLE V. LENGTHS OF CATFISH

		amber B - B				umber 3 Con-	:Number 4	: Number
Catfis				absent			:milk B-G?	: Meat
Harch	17:	49.88	:	49.46		49.50	: 48.84	49.76
Averag length surviv		49.93	:	49.42	:	49.50	: : 48.81	49.76
April	6:	50.32	1	49.64	:	49.47	: 48.45	: 50.02
Gain	:	-39		.22		03	:36	.26
Averag Length surviv		50.32	:	49.72	:	48.83	: : 48.35	: 49.93
May	4:	51.53	2	50.79	2	50.69	: 49.16	: 51.27
Gain		1.21	1 1	1.07		1.86	: .81	1.34
Total gain		1.60		1.29		1.83	: .45	1.60

TABLE VI. AVELAGE WEIGHTS OF CATFISH

Date		I : B - G :B absent.G	absent : present:	ill :	1V :	V
march	17:	1.88:	1.82 :	1.82 :	1.706:	1.784
	23:	1.908:	.1.826 :	1.76	1.58	1.64
	30:	1.86 :	1.73 :	1.724:	1.592:	1.83
april	6:	1.88:	1.80	1.689:	1.58	1.80
	13:	1.79 :	1.76 :	1.52	1.46 :	1.72
	20:	1.877:	1.803:	1.62	1.58	1.82
	28:	1.873:	1.87	1.75	1.56	1.86
au y	4:	1.93:	1.90 :	1.81	1.57	2.02
	11:	1.93:	1.85	1.75	1.58	1.93
Gain	:	+.05:	+.03 :	07 :	226	+.146



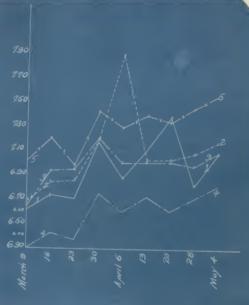
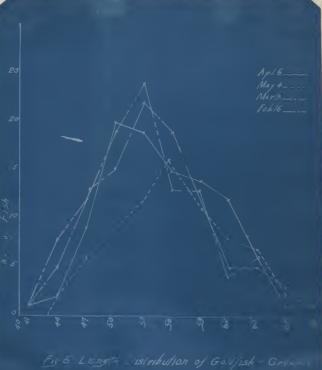
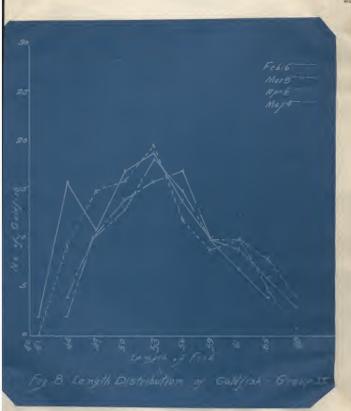


Fig. 4. Average Weight of Goldfish
Group 1 --... 2 --... 3 --... 4 ----









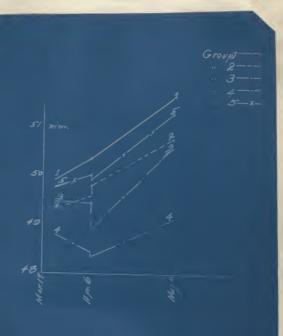
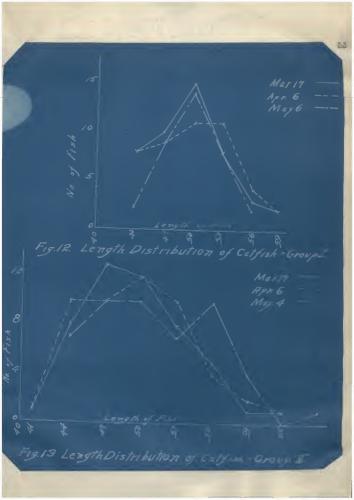


Fig. 10. Average Gast in Length of Catfish



Fig. 11. Average W- whit of Catfish



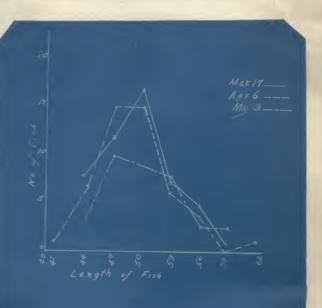


Fig. 14 Length Distribution Catfish - Group III

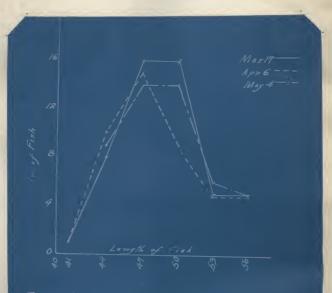


Fig 15 Length Distribution of Catfish-Group II

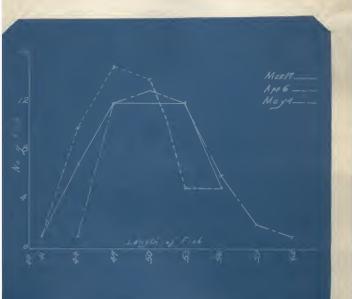


Fig. 16. Langit Distribution of Catfish Group

# DISCUSSION Goldfish

a scrutiny of Table III and figure 3 shows a slight but consistent growth of the goldfish in all groups. The goldfish on the diet without vitamin B and 6 gained the least while those on the meat diet gained the most. The group on Diet no. 2 with vitamin 6 gained slightly more than those without vitamin B or 6. The control group, or those with vitamin B and 6, made a gain of 0.486 mm. over those without vitamin B or 6, and only 0.16 mm. over those with vitamin 6, suggesting the necessity of vitamin 6 to the growth of goldfish. The group on the buttermik diet snows a gain of 0.62 mm. over the first group, indicating that there seems to be sufficient vitamin B and 6 for the growth of goldfish.

The greatest gain, however, was made by the fish on the dist containing meat. Whether this gain is due to increased protein content of the diet, the factor H of accoy and Dilley, or to some other factor has not been determined. It was noted that the fish on the diet free of vitamins B and G were more subject to fungus than the other groups, and accordingly showed a greater mortality than the other fish.

It was also noticed while nendling the fish of this group that they felt soft and flabby.

Figures 5 to 9 inclusive show the distribution of length of each group of fish at the time of measurement. A scrutiny of the graphs shows that all the groups of fish made some growth. The gain of each group is fairly uniform throughout the group, which indicates that the gain made is not the result of only a few fish making rapid gains but a result of each fish making a small gain, and growth was not restricted to the smaller or larger fish.

The value of the data of weight of goldfish (Table IV, fig. 4) is questionable, since some of the goldfish probably spawned, and inasmuch as twenty to twenty-five per cent of the weight of a gravid female may be eggs, the spawning of a few could influence the weight of the group.

There have been five uncontrollable factors in this problem. The are:

1. Age of the fish. The fish were hatched in the spring of 1928. A large percentage of the goldfish were sexually mature. It would be better to start the fish on the experiment just at the time the yolk sac is absorbed, and the fish are ready for first food.

- 2. Time of the year. Gold blooded enimals usually have a rythm of activity. Fish feed less during the winter and early spring than later in the spring. The resting or non-feeding period was more evident in the case of catfish than in that of the goldfish.
- Temperature of the water. This was probably below the optimum temperature for growth.
- e. Diseases. Frequent handling of the fish for measuring and weighing bruised the fish thus giving access to parasitic fungi and fin and tail rot organisms. The diseases were combated constantly. Copper sulfate and potassium permanganate solutions were tried with only moderate success. The best method tried was the addition of a salt mixture, consisting of five parts sodium chloride and one part magnesium sulfate. One kilogram of this salt mixture per forty gallons of water was placed in each tank every other day. In addition the tanks were thoroughly scrubbed and scalded with boiling water each week.

5. There is some evidence by post mortem that some of the fish may have aparened. In this case the eating of eggs would modify the diet of some of the fish. In case of the catfish a scrutiny of Table V and figure 10 shows no striking difference in gain in length of all the groups. The fish in group four were the smallest. These showed a slight loss for the first period, but thereafter made gains almost as rapidly as the others. This initial loss was probably due to their having been shipped from the Fish Hatchery at Fratt.

On April 6 a heavy mortality resulted in group three and a slighter mortality in groups 2, 4 and 5 as a result of the administration of a permanganate solution which was used to kill fungus growth on the goldfish. It will be noted that those catfish which survived in group three made a good gain during the last period of the experiment. These data offer no indication that the catfish require vitamins B and G. The experiments on catfish, however, are still in progress.

With regard to the weight increases of the catfish (Table VI, Fig. 11), the only interesting result is probably the increased growth of those receiving meat in the diet. These fish also had the best plumpest appearance of any of the groups. As in the case of the goldfish, the author is not prepared to say whether the apparently better growth is due to protein content of the diet, the

factor n of mcCay and Dilley or to some other factor. This question merits further investigation.

Although the weights of the catfish show considerable fluctuation from week to week, they show no such wide fluctuations as did the weights of the goldfish. As already mentioned, the weight fluctuations of the goldfish were probably influenced by the spawning of a few. Inasmuch as catfish do not spawn their first year, their weights are a more reliable criterion of growth, and even the slightly increased growth on a diet containing meat may be regarded as suggestive.

In comparing the work on goldfish and eatfish, we note that whereas in the former there is some suggestion that vitamin G is needed for optimum growth, in the latter we find no such indication. Davis (1927) found a decided difference in the response to a high vitamin diet between two fish as closely related as the brook trout and rainbow trout. In mammals it is known that some experimental animals, as the guinea pig, develop scurvy if not supplied with vitamin G; whereas, this vitamin does not appear to be necessary in the diet of the white rat.

Since the catfish belong to the order <u>Bematognathi</u> and the goldfish to the order <u>Eventognathi</u>, it would not be surprising to find that their vitamin requirements differ. The catfish furthermore differs from the goldfish in feeding habits. The goldfish is generally known as a vegetarian, while the catfish, although feeding to a small extent on vegetation, feeds largely as a carnivora. As vitamins B and G are especially abundant in many plant foods, and present in only moderate quantities in most meats, it may be that they play an important role in the diet of the vegetarian fish.

## SUMMARY

- 1. Experiments were conducted on 400 goldfish and 200 young catfish in order to determine whether vitamin B and G are necessary for growth and maintenance in these fish.
- 2. Experiments were also conducted on 100 goldfish and 50 catfish to determine the effect of the addition of fresh meat (beef liver) to control diet used in the experiments above.
- 3. Experiments on goldfish were terminated at the end of twelve weeks. The experiments on catfish were continued nine weeks and are still in progress.

- 4. The data on goldfish indicate a somewhat retarded growth (increase in length) in the diet where vitamin 6 was lacking. These fish also showed a much higher mortality from fungus infections.
- 5. The data on eatfish show no difference in growth between those having vitamin 5 and those lacking it.
- 6. In neither the goldfish or catfish is there any indication that vitamin b is necessary for normal growth. Experiments of longer duration are necessary in order to determine whether nervous disorders will develop if the diet is lacking this vitamin.
- 7. In both goldfish and catfish there is some indication that health and growth are improved by the addition of raw ment to the diet.

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	12