THE EFFECT OF VITAMIN D ON THE GROWTH OF FISH

by

Jean Alexander

B. A., Oklahoma City University, 1928

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE AGRICULTURAL COLLEGE

1929

TABLE OF CONTENTS

INTRODUCTION

This problem has been undertaken to ascertain whether spotted channel catfish (Ictalurus punctatus Rafinesque) and the common goldfish (Crassius auratus Linneaus) require vitamin D in their diets for normal growth.

So far as is known all vertebrates and some invertebrates need vitamin D. Land animals are able to meet this requirement either by their diets or by exposure to ultra violet light. Water affords very poor penetration for ultra violet rays, but some fish, such as the cod, are very rich in vitamin D. The question arises as to the way such fish acquire so much of this substance. Codfish feed largely upon smaller fish. These smaller fish live upon still smaller ones which have a menu composed mostly of diatoms. According to Pryde diatoms have been grown in steril sea water in the laboratory and it has been found that in the presence of diffuse sunlight they are capable of forming vitamins from the inorganic substances in the medium in which they grow. Fresh water algae and yeasts have the same ability.

Goldfish are able to live for a long time on diets deficient in vitamin content. It is noticeable, however, that goldfish kept in aquaria do not attain the size of those given the freedom of ponds where they are able to forage. How much the difference in growth is due to limited space and how much to improper nutrition is not known.

One of the chief problems facing fish culturalists at the present time is the provision of an adequate artificial diet for the fish in hatcheries, since it is too difficult to provide natural live food for them. In planning an artificial diet two things must be taken into consideration: the suitability of the food for fish, and the cost. All food elements essential for health and growth should be provided in the least expensive available forms. The following experiments were planned to ascertain whether vitamin D should be included in an artificial diet for fish.

REVIEW OF LITERATURE

History of Vitamin D

Rickets, now known to be a vitamin D deficiency disease is the most common disease among children in civilized communities.

It is characterized by flabby muscles, both voluntary and smooth; loose and laxed ligaments; and poor bone development. The bones growing most rapidly are affected first. Enlargements occur at the junctions of the boney parts with the cartilages. The long bones become bent and swollen at their extremities due to the absence of the zone of provisional calcification and the formation of a metaphysis. Animals with rickets have a lowered resistance and become easy victims of infections of various kinds.

This disease has long afflicted the human race. Soranus in the first century of the Christian era described its general features. Francis Glisson, in 1650, established rickets as a clinical entity. In 1885, Pommer, by means of histological studies, established the pathology of rickets. Studies of the disease in more recent times have shown much of interest concerning its causes.

Glisson (1660) attributed the disease to over eating. Jundell, in 1922, revived this theory. Heitzmann (1873) and several more recent workers give acidosis as the cause.

By 1923 there were two important theories held by scientists, namely, defective diets and deprivation of light.

Funk (1914) was the first to suggest that the cause might be defective diet. In 1918, Mellanby suggested that the cause of rickets was due to the absence of an accessory factor meaning some such substance as the vitamins. Through experimental work on puppies he reached the conclusion in 1919 that the accessory factor was vitamin A. He found that the addition of tricalcium phosphate to rachitic diets did not prevent rickets.

In 1921 McCollum, Simmonds, Shipley and Park found that diets deficient in fat-soluble and calcium phosphorus salts produced rickets.

The direct proof of the curative action of cod-liver oil, the remedy used since time immemorial for rickets, was first obtained by McCollum and his collaborators in 1921. Since Sherman, Pappenheimer and McCollum, Simmonds, Shipley and Park found that diets deficient in vitamin A alone did not produce rickets, they concluded (1921-22) that there was some factor of an organic nature in cod-liver oil which caused the lime salts to be deposited in a normal manner, and this prevented or cured rickets. The name X was given this substance which is now known as vitamin D.

In 1890, Palm noted the importance of sunlight in preventing and curing rickets. Raczynski (1912) also found sunlight important in this respect.

Buchholz (1904) used the "gluhlicht", otherwise known as the carbon filament of an electric light bulb, and obtained results in curing rickets.

The use of ultra-violet light was first made by Huldschinsky in 1919. In that same year Winkler found that x-rays had a curative value.

The name vitamin D was first given to an organic substance thought necessary for the growth of yeast by Funk and Dubin (1921). It now indicates the antirachitic factor in cod-liver oil and other natural animal fats. Vitamin D is usually found associated with fat soluble vitamin A.

In 1924 Steenbock found that substances inactive antirachitically could be made active by irradiation with ultra-violet light. Drummond taking great precautions, tried removing the traces of fat from cholesterol to destroy its pro-vitamin D qualities. But he did not purify it enough to destroy the pro-vitamin factor.

A spectograph study of cholesterol before and after irradiation with ultra violet light was made by Heilbron, Kamm and Morton in 1927. This study showed that it contained some other compound with different absorption bonds. They believed this substance to be closely related to the precursor of vitamin D.

In 1927 Rosenheim and Webster in America and Windaus in Germany found that when ergosterol ($C_{27}\ H_{42}O$) a derivity of cholesterol, was irradiated it became the highly potent anti rachitic substance found in cod-liver oil.

Ergosterol is found in the cholesterol in the human skin and there, upon irradiation by sunlight, produces the antirachitic vitamin D.

Steenbock and Drummond have recently suggested that vitamin D may be growth promoting and only exerts its full powers in this line in the presence of fat-soluble A.

Vitamin Requirements of Fish

Fish nutrition is almost a new field in which little work has been done. More experimental work has been carried on with trout than any other fish. When trout rearing was first started nearly every hatchery used liver for feeding purposes. It was low in price and seemed adequate. But in recent years the nutritional value of liver in the human diet has been recognized, and in consequence the price of liver is now too high to permit its use in feeding large quantities of fish, therefore, it has become necessary for fish culturalists to find some adequate substitute. The problem of finding such a substitute has raised some serious nutritional questions.

Davis and James (1924) found that carp and possibly trout require vitamins A, B and C for proper growth.

As a result of extensive experiments, McCay, Bing and Dilley (1927) found that trout need a protein level of over 10 per cent for normal growth, but that the addition of more than 25 per cent has no effect on the rate of growth.

These workers found that apparently the additions of vitamins A, B or D to the diets did not affect the growth of the trout, but this statement cannot be interpreted to mean that the fish do not require these vitamins because highly purified foodstuffs were not used in the diets.

McCay and Dilley in a later paper state that young trout require a factor found in raw liver that is apparently none of the known vitamins. They call this factor H.

We know from the works of Richet (1925) and Laufberger (1926), that ether extracted meat does not contain the growth promoting factor found in raw meat.

Davis (1927) in five years of hatchery work with trout breeding found that beneficial results were obtained in every case when cod-liver oil and yeast were added to the diets of rainbow trout. The brook trout on the other hand showed no benefits from the addition of these substances. It seems, therefore, that each species of fish presents an individual nutritional problem.

METHODS

For this experiment the fish used were the common uncolored goldfish (Crassius auratus Linnaeus) and the spotted channel catfish (Ictalurus punctatus Rafinesque). The goldfish were obtained from the Grassyforks Fish Hatcheries at Martinsville, Indiana. The catfish were supplied by the Kansas State Fish Hatchery at Pratt, Kansas.

The fish were kept in wooden troughs constructed for this purpose. The troughs were six feet long, one foot wide, and one foot deep. Water from a faucet flowed into each at one end and out at the other through a pipe inserted near the bottom. (Plate I). The water was from the supply of the Kansas State Agricultural College and was kept at a depth of about eight or nine inches in each trough.

Fifty catfish and one hundred goldfish were placed in each of the troughs. The goldfish and catfish were separated from each other by screen wire partitions. About one third of the trough nearest the inlet was given over to the catfish and the goldfish had the remainder of the trough towards the outlet.

Waste material was siphoned from the troughs each day. Once a week the fish were removed and the troughs scrubbed with hot water. This controlled, to some extent, the growth of fungus.

The goldfish experiment was started February 15, 1929, and the catfish March 17, 1929. Because of severe weather it was impossible to obtain both at the same time.

At the beginning of the experiment the fish were weighed and measured. Thereafter they were measured once a month and weighed each week. It was considered necessary to both weigh and measure them as an increase in weight might be due to fat deposition, and growth in length might not be accompanied by an increase in weight. The distance from the most anterior point to the base of the caudal fin, measured with a pair of dividers, was considered as the length of the fish. Weights were taken of the groups and not of the individual fish. The fish were fed the following diets:

Diet No. 1 - Vitamin D Deficient.

Yellow corn meal . . . . 68 gm.

Crisco . . . . 10 gm.

Hogan's Salt Mixture. . . 4 gm.

Ether extracted milk powder . . . . 18 gm.

Yeast . . . . 10 gm.

Grapefruit juice . . . 20-30 cc.

Water . . . . 200 cc.

Diet No. 2 - Vitamin D present.

The same as above with:

Crisco . . . . 6 gm.

Cod-liver oil . . . . 4 cc.

On May 18 the diet was changed, caesin replacing the ether extracted milk powder. The diet was again changed on May 28 when oatmeal was substituted for the yellow cornmeal.

Milk powder or casein were used as a source of protein, crisco as a source of fat, yeast as a source for vitamin B, grapefruit juice for vitamin C and cod-liver oil as a source for vitamin D. Hogan's salt mixture was used because it contains all the necessary salts and minerals in the proper proportions.

The weighed food was mixed with 200 cc. of water and cooked in a double boiler until it was a thick mush or dough. It was then allowed to cool and the yeast, grapefruit juice and cod-liver oil were added.

It was thought best to check these diets by feeding them to young white rats since with these animals a deficiency of a particular vitamin in the diet produces well known symptoms.

From April 6 until April 28 there was one rat on each of the two diets. On the latter date another rat was placed in each part of the experiment. When the ether extracted milk powder was removed from the diets, May 18, a new group of three rats was secured. Two of these were placed on diets No. 1 and 2 with casein. The third was placed on the original diet No. 1.

Another control, started by Mr. Schneberger as a control to the diet used in his studies on vitamin B and G, was continued for comparison with these experiments. This was a group of 50 catfish fed the control diet plus 60 gm. ground raw liver. During the early part of this experiment the fish were fed the diet used by Mr. Schneberger which was the same as that used by the author except that polished rice powder was used instead of corn or oatmeal, and that no grapefruit juice was administered. These same fish were continued by the author but with oatmeal instead of rice powder as the cereal.

The fish and rats were fed at as nearly equal intervals as possible, twice daily. The two groups of fish were fed equal amounts, and the rats received equal amounts. The group eating the least was taken as a criterion for the other.

DATA

Table I Growth of rats.

Date	Rat I	Rat II	Rat III
May 18	70.3	58.0	64.6
25	73.2	68.7	73.5
June 2	97.0	86.7	89.7
8	115.0	112.0	109.5
16	127.0	123.0	128.0
22	128.0	123.5	127.0
29	138.0	136.5	142.5
July 6	141.0	139.0	139.5
Total gains:	70.7	81.0	76.9

Data on Rats

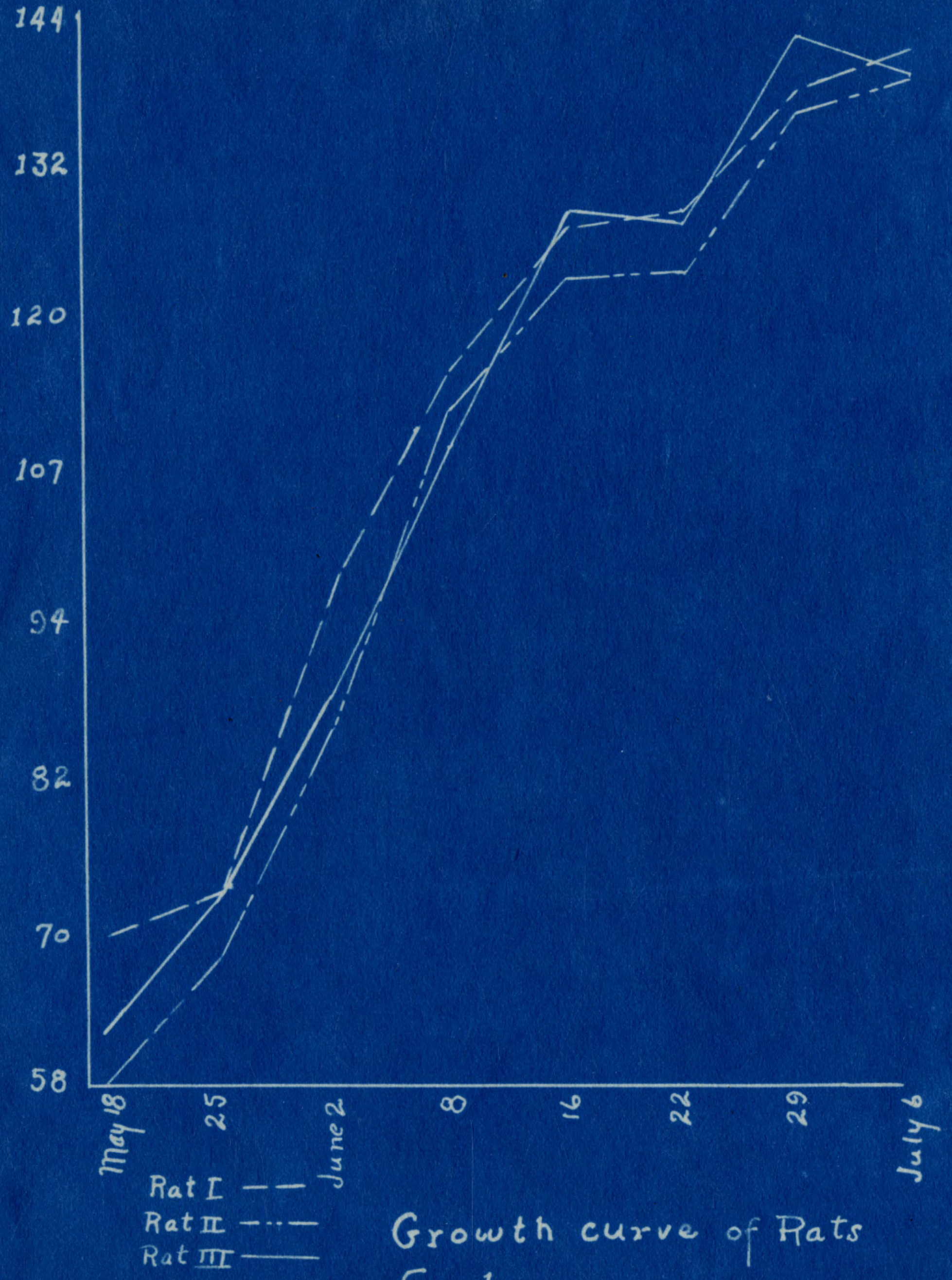
When the experiment was started ether extracted milk powder was used as a source of protein. As a check on the completeness of the extraction, two white rats, five weeks old, were fed the same diets as the fish.

Since each rat of the first group gained practically the same amount, the diets were changed from ether extracted milk powder as a source of protein to purified caesin. A new group of three rats, six weeks old, were obtained. These were fed diets No. 1 and 2 with caesin, respectively. The third rat was continued on the original diet No. 1.

Table I and Fig. 1 show the growth of these rats. Rat I, on diet No. 1 with caesin, gained 70.7 gm. Rat II, on diet No. 2 with caesin, gained 81.0 gm., and Rat III which received the original No. 1 diet gained 76.9 gm. Rat II which received cod-liver oil gained 10.1 gm. more than Rat I on the same diet minus the cod-liver oil. Rat II gained 4.1 gm. more than Rat III on the original No. 1 diet. Rat III gained 6.0 gm. more than Rat I whose diet had caesin as a source of protein. These results, although inconclusive, would suggest that the original diets were vitamin D deficient except for the addition of cod-liver oil.

The x-ray picture of these rats (Plate I) shows the differences in bone development. In comparing the skeleton of Rat I with Rat II, which had cod-liver oil, it will be noticed that the greater trochanter shows up well defined in Rat II and shows hazily in Rat I. In Rat III these points show clearer than in Rat I, but not as plain as in Rat II. The ends of the humerus and ulna show up in the same manner. In the feet the ends of the long bones of Rat II are much better defined than the same parts of Rat I or of Rat III. By following the ribs to their connections to the sternum it will be found that in Rat II the connections show plainly, while in Rat I the connection is hazy or lost entirely. Rat III appears intermediate between Rat I and Rat II.

Rat I undoubtably had rickets, while Rat III appears to have had the disease in a much less degree, indicating that the ether extracted milk powder was deficient but probably not entirely wanting in vitamin D, whereas the purified caesin was unquestionably lacking in vitamin D.



Growth curve of Rats
Fig. 1

Table II Average weights of goldfish and catfish.

Date	Goldfish I	Goldfish II	Catfish I	Catfish II	Catfish III
Mar. 9	6.62	6.09			
Mar. 16	6.85	6.365			
Mar. 17			1.746	1.78	1.784
Mar. 23	6.66	6.506	1.64	1.68	1.64
Mar. 30	6.688	6.456	1.56	1.643	1.83
April 6	6.6	6.58	1.53	1.6	1.8
April 13	6.77	6.687	1.46	1.62	1.72
April 20	6.68	6.76	1.49	1.61	1.82
April 28	6.549	6.68	1.49	1.62	1.86
May 4	6.678	6.68	1.48	1.61	2.02
May 11			1.46	1.57	1.93
May 18			1.49	1.57	
May 25			1.46	1.53	
June 1			1.45	1.48	
June 8			1.59	1.58	2.07
June 15			1.59	1.61	2.10
June 22			1.73	1.60	2.21
June 29			1.75	1.83	2.44
July 6			1.77	1.80	2.40
Average gains	.05	.59	.024	.02	.616

Table III Goldfish and catfish lengths.

	Goldfish		Catfish		
	I	II	I	II	III
Feb. 16	53.93	53.13			
Average surviving	53.71	53.18			
Mar. 10	54.65	54.22	49.4	49.65	49.76
Average gain	.94	1.04			
Average surviving	54.88	54.45	49.4	49.79	49.76
April 6	54.88	54.80	49.16	49.72	49.76
Average gain	0.0	.35	-.24	-.07	.26
Average surviving	55.30	54.67	49.16	49.72	49.93
May 4	55.43	55.78	48.74	50.21	51.27
Average Gain	.13	1.11	-.42	.49	1.34
Average surviving			49.36	50.79	51.27
June 15			50.4	51.67	53.53
Average gain			1.04	.88	2.26
Average surviving			50.28	52.0	53.53
July 6			49.92	53.28	55.66
Average gain			-.36	1.28	2.13
Total average gain	1.07	2.50	.02	2.58	5.99

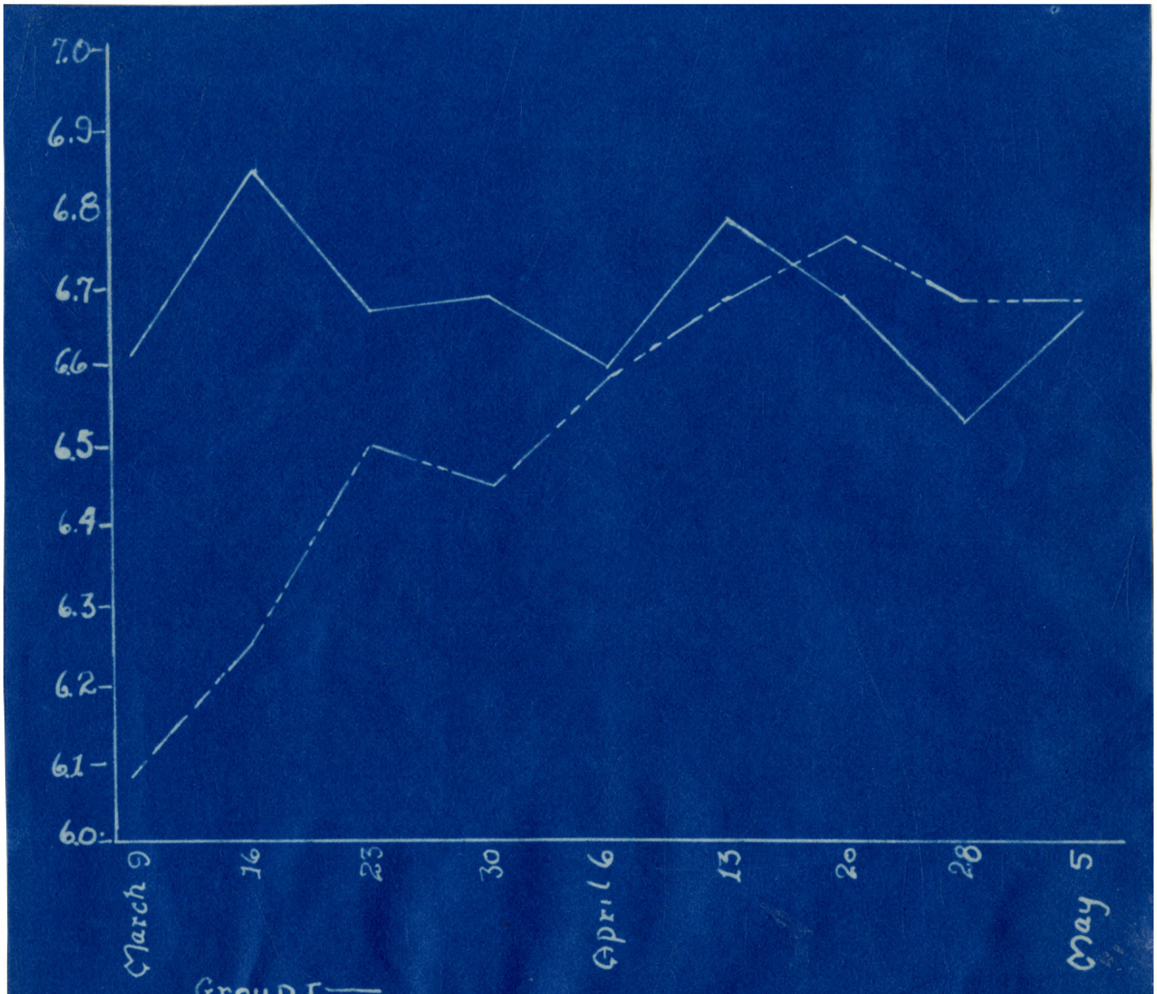


Fig. 2 Average weights of Goldfish

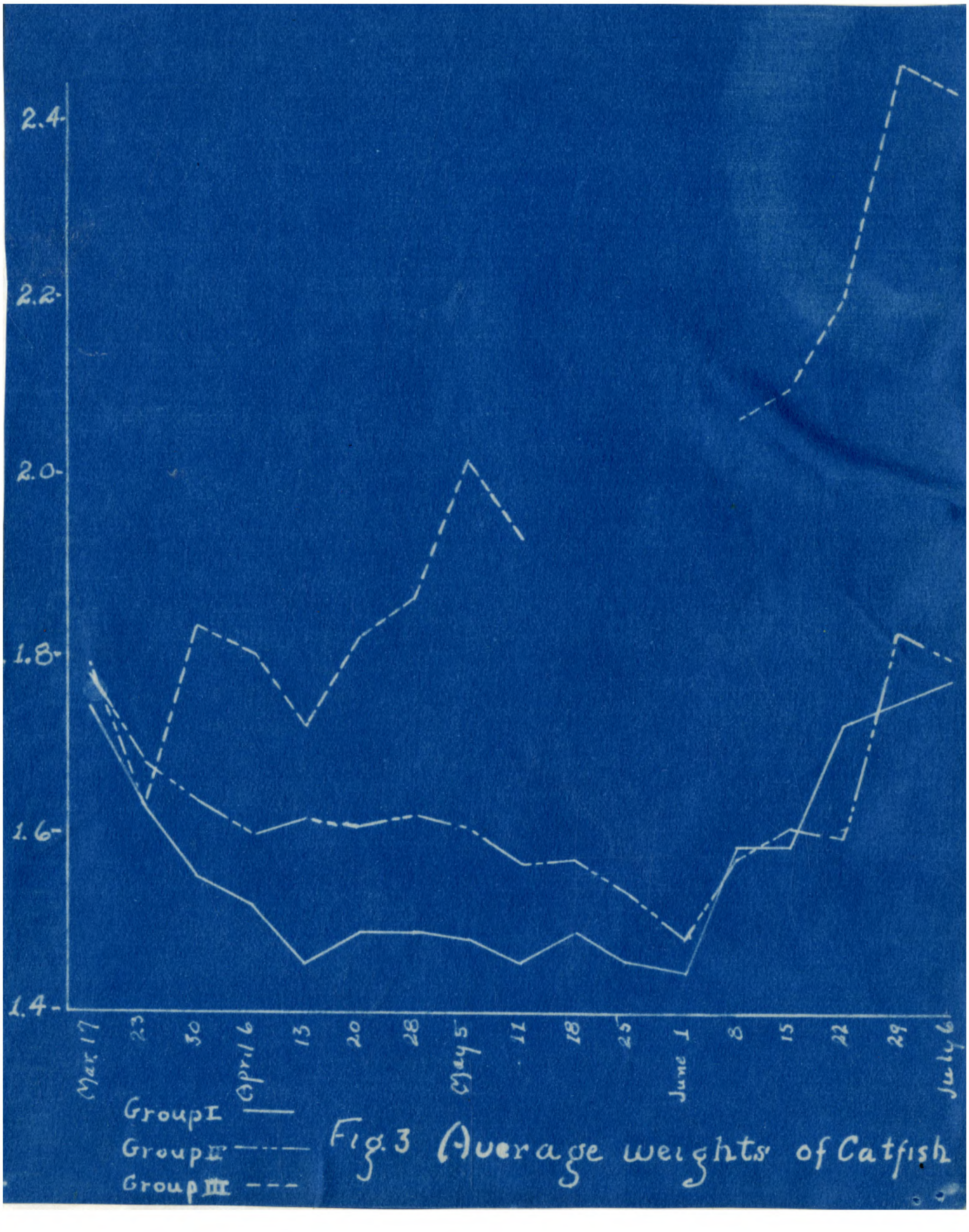


Fig. 3 Average weights of Catfish

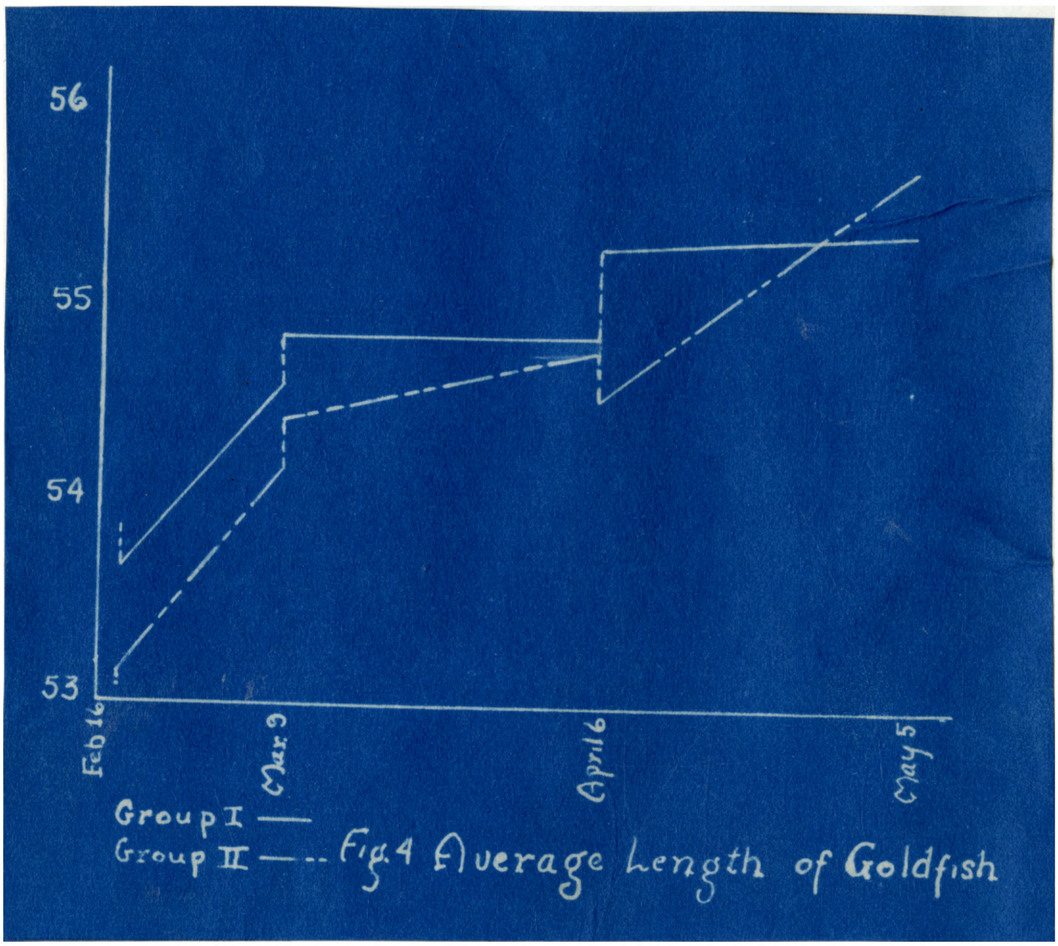
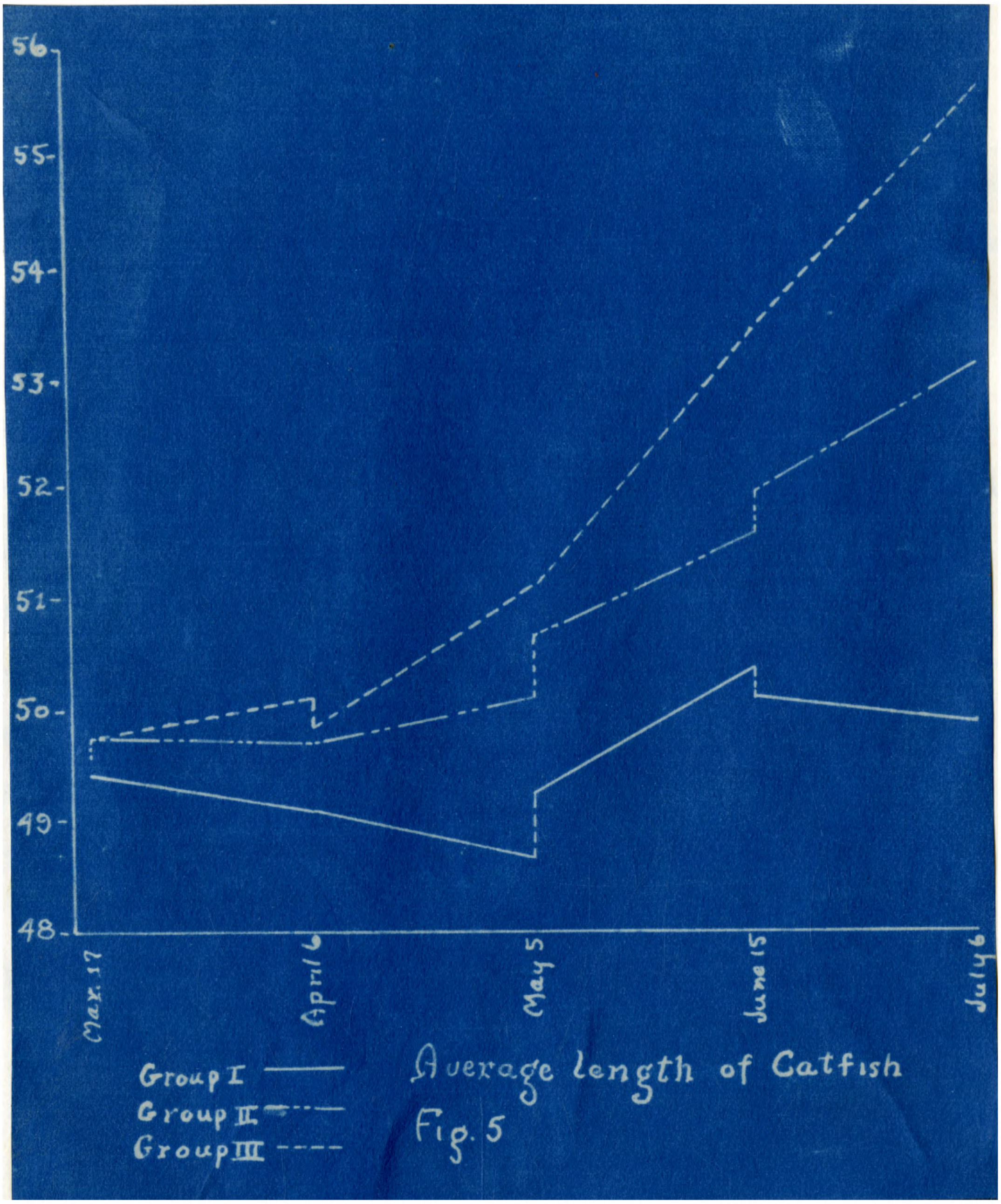


Fig. 4 Average Length of Goldfish


56
55
54
53
52
51
50
49
48
Mar. 17
April 6
May 5
June 15
July 6
Group I
Group II
Group III
Average length of Catfish
Fig. 5

Data on Fish

Data were secured from two groups of one hundred goldfish and three groups of fifty catfish. Table II gives the average gains in weight of the fish. Table III gives the average gain in length of the various groups of fish. The fish were measured once a month. The lengths of the fish that died in the interval between the dates on which the measurements were made were subtracted from the total length in order to secure an average length of the fish which survived until the next time of measuring. The average length in this way was subtracted from the average length at the next time of measuring. This eliminated apparent growth or loss due to the death of larger or smaller fish.

The graphs (Figs. 2 and 3) were drawn from Table II. Figures 4 and 5 were made from Table III.

DISCUSSION

In the goldfish experiment there has been a gain in weight in both the plus and minus vitamin D groups, as a scrutiny of Table II and Fig. 2 will show. The group

No. 1, or the minus vitamin D group, show an average gain of .05 gm. whereas an average gain of .59 gm. is shown by the plus vitamin D group. Thus the average gain in weight of the fish with vitamin D was 1000 per cent that of the fish lacking vitamin D.

Table II and Fig. 3 show the average gains in weight of the catfish. Here, also, slight gains have occurred. The minus vitamin D group (No. 1) gained an average of 0.24 gram. The plus vitamin D group (No.2) shows an average gain of .02 gm., or practically no difference at all. The third group of catfish was used as a control. This was started by Mr. Schneberger and continued by the author. The fish in this group of fish made the best average gain in weight as it gained an average of .616 gm., or 2500 per cent the average amount gained by the catfish on the meat free diets.

The average growths in length of the goldfish are shown in Table III and Fig. 4. The plus vitamin D group of fish shows an average increase of 2.50 mm. The minus vitamin D group shows an average increase of 1.07 mm. The plus vitamin D fish show an average increase in length over the minus vitamin D fish of slightly over 100 per cent.

Table III and Fig. 5 show the average increase in length of the catfish. The plus vitamin D group shows an average gain in length of 2.58 mm. and the minus vitamin D group an average gain of only .02, or a scarsely measurable amount. Thus, in length, the plus vitamin D group shows a pronounced advantage over the minus vitamin D group. The group of catfish which received raw meat in their diet shows an average gain in length of 5.99 mm., or an average increase of 10.6 per cent during the period of the experiment.

The data on weight of the fish have been influenced by two factors other than growth, namely, spawning and the death of part of the fish. In case of the goldfish there is reason to believe that the fluctuations in weight may have been caused by spawning. Since as much as 25 per cent of the total weight of a gravid female may be eggs, a few females spawning would influence the weight of the entire group. Numerous deaths occurred in both the goldfish and catfish groups. This was especially true among the catfish during the latter part of the experiment. Since the fish were weighed as groups, it has been impossible to eliminate apparent gains or losses due to the deaths of larger or smaller fish. Hence, not much reliance can be placed on the comparative weights of the groups of fish.

The length data are more accurate since length measurements were taken individually. This has made it possible to separate the growth of those fish which survived from the apparent growths caused by the deaths of smaller fish. Moreover, as growth in length necessarily implies growth in skeleton, whereas increase in weight might be due to deposition of fat or maturity of the gonads, the length data are probably more significant than the data on weight.

While the effects of the absence of vitamin D may not be apparent in the weight of the animal it has marked effect on skeletal development, especially calcification of the bones. This is probably the explanation of the greater growth in length of the fish fed vitamin D as compared with those on the vitamin D deficient diet. The failure of both groups to make favorable gains in weight is doubtless due to the absence of some other factor not vitamin D from the diet.

The meat fed control catfish show an average increase of almost 57 per cent more in length than the average increase of the fish in the plus vitamin D group. This is further evidence that the diet of the plus vitamin D group was deficient in some factor necessary to catfish which was found in the raw meat. Whether the marked increase in both weight and length of the meat fed controls can be accounted for by the higher protein content of the diet or by the factor H of Dilley and Bing, or by some other undetermined cause is not known. The fact that the goldfish Mr. Schneberger had on the diet containing meat did not show the same degree of increase as the control catfish can be correlated with their largely vegetarian habits.

A careful examination of the x-ray picture (Plate III) of representatives of the plus and minus vitamin D groups of catfish shows little in regard to bone development as the bones are so extremely small. However, there seems to be a noticeable difference between the vertebrae of the plus group (No. 2) and the minus vitamin D group (No. 1). In the first three fish of group No. 1 the vertebrae seem rather loosely connected, while in the first three fish of the No. 2 group the vertebrae seem more compactly connected. Since the bones are so small and the connections are scarsely evidenced no more definite conclusions can be drawn.

The difference in appearance between the catfish in the different groups was quite noticeable. Those in the vitamin D minus group (No. 1) were emaciated and had bulging eyes. Those in the vitamin D plus group, although thin, were of a better color and general appearance, whereas the catfish in the meat fed control group were a normal healthy looking group. They were not only larger, plumper, and of better color, but were also less susceptible to attacks of fungi and fin and tail rot organisms. The difference in appearance of the fish of the various groups is shown in Plate IV.

In order to determine whether there was a noticeable difference in skeletal development between the plus and minus vitamin D groups, ten fish of as near the same weights as could be obtained, were taken from each group. Each was placed at 37°C in 20 cc. artificial gastric juice composed of 2.5 per cent commercial pepsin in .4 per cent hydrochloric acid. The gastric juice ate the flesh off the bones and destroyed some of the cartilage. Several days after the fish had been placed in the gastric fluid the bones were removed. PlateIV shows the difference in the appearance of the skeletons of the two groups. It was evident in removing the bones that the skeletons of the plus vitamin D group were larger and more rigidly connected than those of the minus vitamin D group.

The data obtained in this experiment would suggest that goldfish (*Crassius auratus* Linneaus)and the spotted channel catfish (*Ictalurus punctatus* Rabinesque) grow better when furnished vitamin D in their diet. The data also indicated that some factor found in raw liver is necessary for the growth and health of the spotted channel catfish.

There have been several uncontrollable factors which have entered into this experiment. The spawning of some of the goldfish has already been mentioned. Diseases have also proved a constant menace . Due to handling the fish were sometimes bruised and the mucous covering rubbed off. This afforded parasitic fungi and tail rot organisms good breeding ground and caused the deaths of many fish. Solutions of potassium permanganate were used with some success in combating the fin rot in goldfish. Another solution used with moderate success was 1 kilo of 5 parts sodium chloride and 1 part magnesium sulphate to 40 gallons of water. This treatment seemed less detrimental to the catfish. The troughs were scrubbed with hot water each week and the diseases were controlled to some extent in this way. A third factor was the age of the fish. The fish were of the hatchings of 1928. Some of the goldfish were sexually mature. It would be much better to start the experiment with fish as soon after they have absorbed the yolk sac as they can be induced to eat the experimental diet. The temperature of the water also varied during the experiment. We had no means of controlling the temperature and, during the winter and early spring, it was probably too cool to favor the growth of the fish. The time of year may also have had some effect upon the growth of the fish since, like most cold blooded animals, they probably have a rythm of activity with a period of decreased metabolism during the winter months. Where such rythms are present it is difficult to secure even the slightest growth during the period of dormancy or semidormancy. It may be that the poor growth during the early part of the experiment was due to such a condition.

SUMMARY

1. Experiments have been conducted with 200 goldfish and 150 catfish to determine whether vitamin D is necessary for their normal growth.
2. Three groups of 50 catfish each were fed respectively, the synthetic vitamin D free diet, the synthetic diet containing vitamin D, and the synthetic diet containing vitamin D plus 20 per cent raw beef liver pulp.
3. The goldfish experiment was carried on 12 weeks. The catfish were in the experiment 21 weeks.
4. Goldfish make better growth in both weight and length on a diet containing vitamin D.
5. Catfish grow better in length when furnished vitamin D in their diet, although they show no appreciable difference in weight.
6. Skeletons of catfish fed upon a diet containing vitamin D appear to be better developed and more resistant to peptic digestion than the skeletons of fish the same weight fed a diet deficient in vitamin D.
7. The control catfish whose diet contained raw liver grew better in both weight and length. The data from this

group indicate that raw liver contains some factor necessary for the normal growth of catfish.

ACKNOWLEDGMENTS

This problem was undertaken in collaboration with Dr. Minna E. Jewell of the Zoology Department of the Kansas State Agricultural College. The author wishes to thank the following for assistance in this experiment. Mr. Alva Clapp of the State Fish and Game Commission for the catfish used; Mr. Edward Schneberger for valuable aid and suggestions during the first part of the experiment; Dr. R.M. Waller for x-rays; and the Anheuser-Busch Company of St. Louis for furnishing fresh brewers yeast at regular intervals.

BIBLIOGRAPHY

Davis, H.S. and James, M.C.
1925 Some experiments in trout feeding. A progress report.
Trans. Amer. Fisheries Soc., 55:115-28

Davis, H.S.
1927 Some results of feeding experiments with trout fingerlings.
Trans. Amer. Fisheries Soc., 57:281-85

Drummond
1924 Some modern tendencies of vitamin research.
Journ. Soc. Chem. Ind., 43:908

Dutcher, R.A.; Ely, J.O.; Honeywell, H.E.
1927 Assimilation of vitamins A and D in the presence of mineral oil.
Proc. Soc. Exper. Biol. and Med., 24:953-55

Funk, C.
1914 Die Vitamine, ihre Bedeutung fur die Physiologie und Pathologie, etc.
Wiesbaden 1914

Funk, C.; Dublin, H.
1921 Vitamine requirements of certain yeast and bacteria.
Journ. Biol. Chem., 48:437

Glisson, F. (et al)
1660 De Rachitide, sive morbo puerili qui vulgo "The Rickets" dicitur, tractatus. 2. ed. London

Heilbron, I.M. ;Kamm, R.A.; Morton, R.A.
1927 The absorption spectrum of cholesterol and its biological significance with reference to vitamin D.

Heitzmann, C.
1873 Ueber kunstliche Hervorrufung von Rhachitis und Osteomalacie.
Allg. Wien. Med. Zeitg., 18:570

Hess, A.F.; McCann, G.F.; and Pappenheimer, A.M.
1920 The failure of rats to develop rickets on a diet deficient in vitamin A.
Proc. Soc. Exper. Biol. and Med., 18:266

Hess, A.F.; McCann, G.F.; and Pappenheimer, A.M.
1921 Experimental rickets in rats. II The failure of rats to develop rickets on a diet deficient in vitamin A.
Journ. Biol. Chem., 47:395

Hess, A.F.; Unger, L.J.
1921 An interpretation of the seasonal variation of rickets.
Amer. Journ. Dis. Child, 22:186

1921 The cure of infantile rickets by artificial light and by sunlight.
Proc. Soc. Exper. Biol. and Med., 18:298

Hess, A.F.; Unger, L.J. and Pappenheimer, A.M.
1922 Experimental rickets in rats. The prevention of rickets by sunlight, by rays of the mercury vapor lamp and by the carbon arc lamp.
Journ. Exper. Med., 36:427

1921 Experimental rickets in rats. III The prevention of rickets in rats by exposure to sunlight.
Journ. Biol. Chem., 50:77

Huldschinsky, K.
1920 Die Beeinflussung der Tetanie durch Ultraviolettlicht.
Zeitschr. f. Kinderh., Orig., 26:207

Jundell, I.
1922 Pathogenesis and treatment of rickets.
Hygiea, 83:753

McCay, C.M.; Bing, F.C.; Dilley, W.S.
1927 The effect of variations in vitamin, protein, fat and mineral matter in the diet upon the growth and mortality of eastern brook trout.
Trans. Amer. Fisheries Soc., 57:240

McCollum, E.V.; Simmonds, N.; Becker, J.E. and Shipley, P.G.
1922 Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition.
Journ. Biol. Chem., 53:292

Mellanby, E.
1918 The part played by an "accessory factor" in the production of experimental rickets.
Journ. Physiol., 52:11

Mellanby, E.
1919 An experimental investigation of rickets.
Lancet, 1:407

Palm, T.A.
1890 The geographical distribution and aetiology of rickets.
Practitioner, 45:270-321

Pappenheimer, A.M. (et al).
1920 The effect of various modifications of a diet producing rickets.
Proc. Soc. Exper. Biol. and Med., 43:267

Park, E.A.
1923 The etiology of rickets.
Physiol. Reviews, 3:106

Pommer, G.
1885 Untersuchungen uber Osteomalacie und Rachitis.
Leipzig.

Pryde, John
1928 Recent advances in biochemistry
pp. 379 , ill. 38 2nd ed.
P. Blakiston's Son and Co., Philadelphia.

Rosenheim, O. and Webster, T.A.
1927 The relation of cholesterol and vitamin D.
Biochem. Journ. 21:127

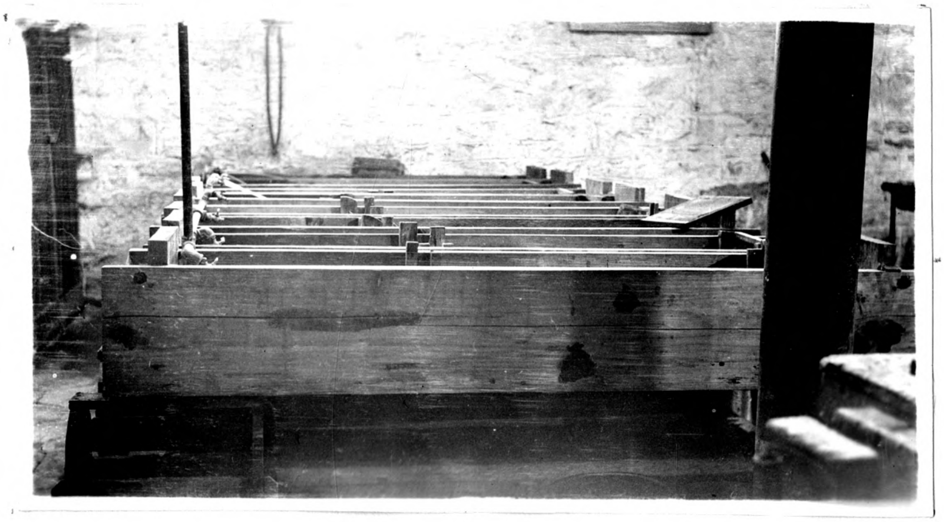
1927 The parent substance of vitamin D.
Biochem. Journ. 21:389

Plate I

a. View of the troughs in which the fish were kept showing the length and the inlet arrangements.

b. Another view of the troughs showing the outlets and water depth.

a.



b.

Plate II

X-ray picture of rats used as checks upon the diets.

Rat I had diet No. 1 with vitamin D free caesin. Shows evidence of rickets. Note indefinite outlines of greater trochanter and distal ends of tibia and metatarsals. Also of costal cartilages.

Rat II had diet No. 2 with caesin. Shows no evidence of rickets.

Rat III had the original diet No. 1 with ether extracted milk powder and purified caesin. Shows slight evidence of rickets.

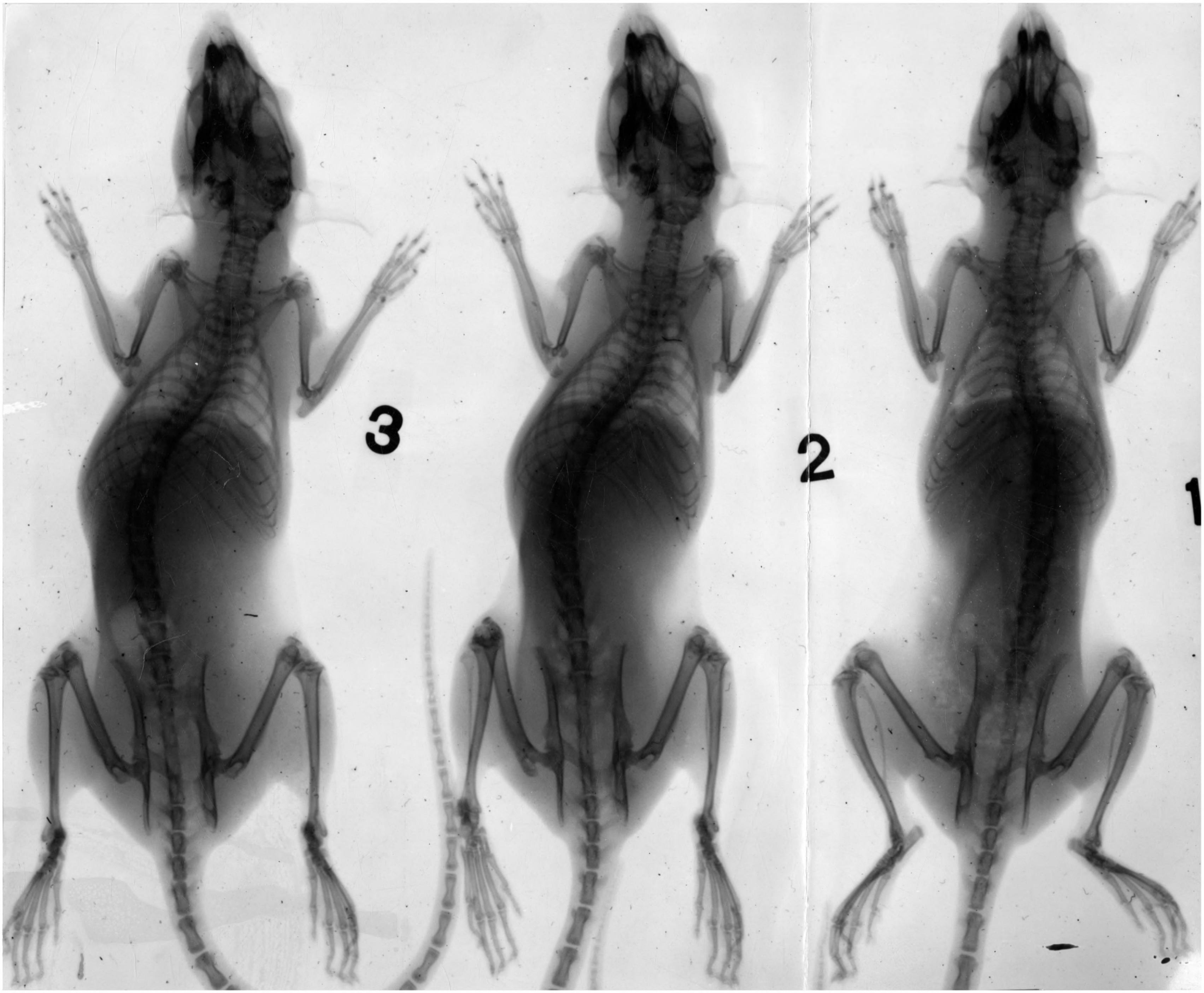

3
2
1

Plate III

X-ray picture of catfish.

Group 1 were fed diet No. 1.

Group 2 were fed diet No. 2.

Close examination shows that in group 1 the vertebrae of the first 3 fish are not as closely connected as in the first 3 fish of group 2.

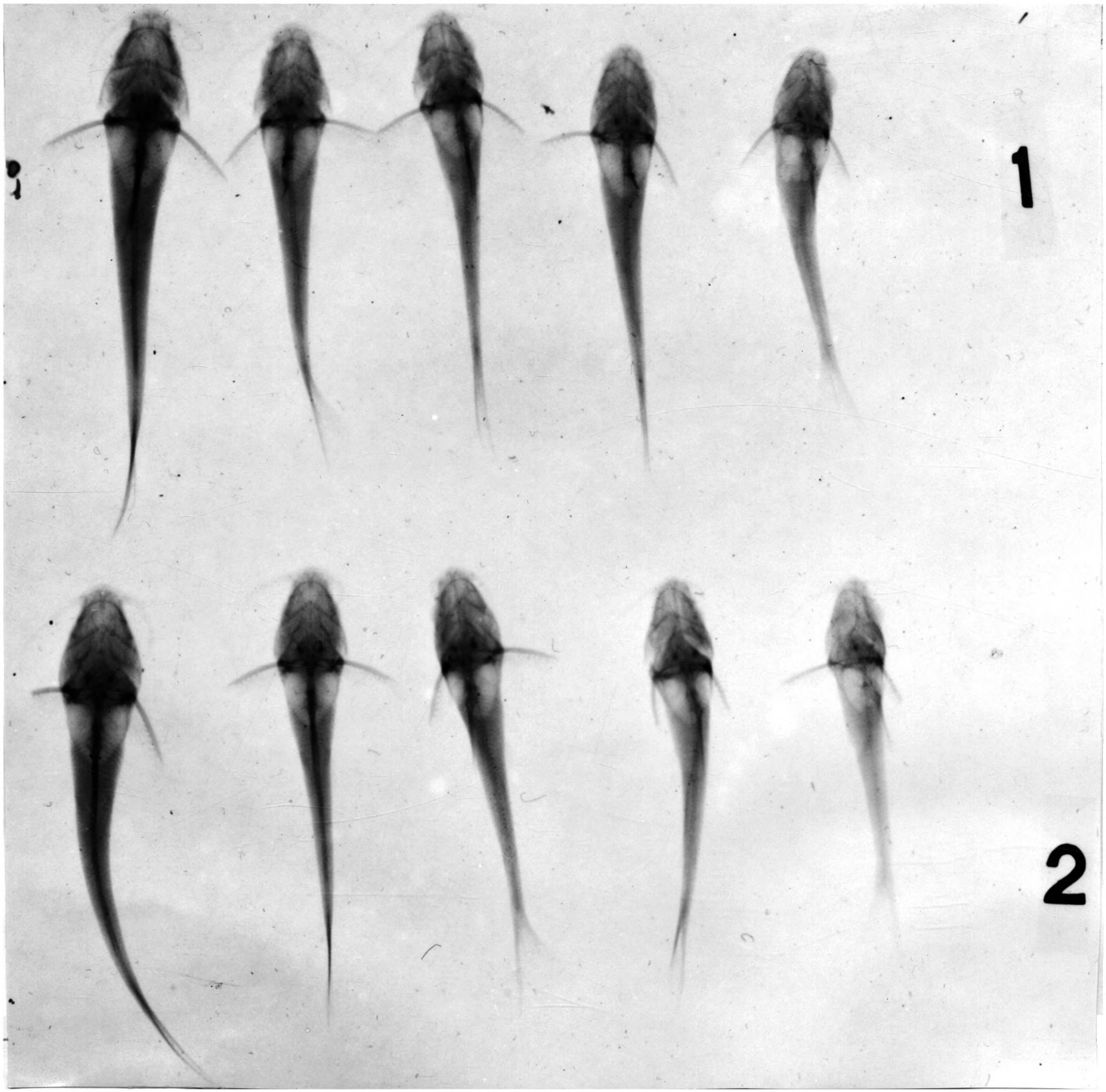

1
2

Plate IV

a. Representative catfish of the three groups in the experiment.

   1. Fish from the minus vitamin D group.

   2. Fish from the plus vitamin D group.

   3. Fish from the group receiving raw liver in their diets. Note plumper and more rounded appearance.

b. Comparison of skeletal development of the plus and minus vitamin D groups of catfish.

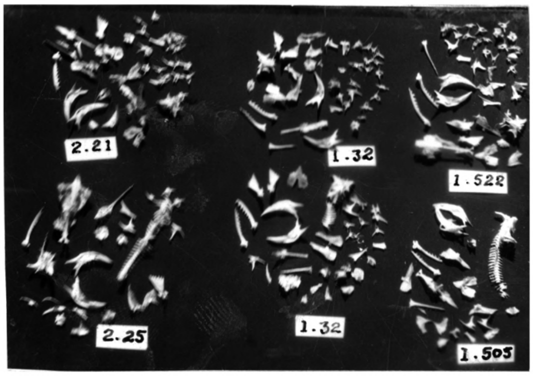
   The upper row of each photograph consists of bones from typical fish from the minus vitamin D groups.

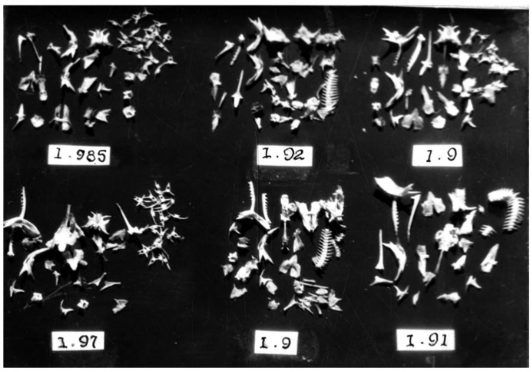
   The lower row of bones were taken from fish of approximately the same weights from the plus vitamin D group.

   Numbers under the bones represent the weight of the fish in grams.

   Note larger size of bones of control fish. Also that the vertebral column remain articulated, whereas the vertebral column of the minus vitamin D fish fall apart as the result of the same treatment.


1
2
3


2.21
1.32
1.528
2.25
1.32
1.505


1.985
1.92
1.9
1.97
1.9
1.91