THE VITAMIN D CONTENT OF BURBOT LIVER OIL AND CARP, LAKE HERRING AND SUCKER OFFAL OILS

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THE VITAMIN D CONTENT OF BURBOT LIVER OIL AND CARP, LAKE HERRING, AND SUCKER OFFAL OILS

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THE VITAMIN D CONTENT OF BURBOT LIVER OIL AND CARP, LAKE HERRING, AND SUCKER OFFAL OILS

CHAPTER I

INTRODUCTION

The purpose of this experiment was to determine the vitamin D content of the oils from four fresh-water fish which abound in the waters of the Great Lakes. The oils tested were the liver oil of the burbot (Lota L. maculosa), and the offal oils of carp (Cyprinus carpio), lake herring, (Leucichthys artedi), and sucker (Cotostomus c. commersonii). It is of theoretical interest and perhaps of practical value to determine the vitamin D content of these oils.

Previous investigations have shown that fish oils vary greatly in their content of vitamin D, so that data concerning different species are of theoretical interest. This is especially true in regard to fresh-water fish oils, since few of them have been tested.

There would be a practical value to the study if it were found that these four fresh-water fish oils contained an amount of vitamin D which would make them practicable substitutes for the foreign supplies of oils which have become scarce because of the war.

Vitamin D has been found to be necessary for human beings, animals and poultry as an anti-rachitic factor. Since McCollum and his co-workers (1922a) first distinguished between the anti-rachitic factor and vitamin A in fats, considerable interest has been shown in the cause of the condition known as rickets, and the sources of vitamin D which cure or prevent it.

The exact manner in which vitamin D functions in this respect is not known. Shohl and Wolbach (1936) have studied experimental rickets in rats, and the latter author has drawn the following conclusions concerning bone histology. In normal bone, there is a continuous proliferation of cartilage cells upon the epiphyseal side, and a simultaneous degeneration of the matured cartilage cells on the diaphyseal side. In the space left by the degeneration of the cartilage cells, capillaries and osteoblasts deposit the bony matrix. In rickets, the degeneration of the cartilage cells does not occur, so that there is no invasion of the cell by the capillaries and osteoblasts to form bone. Osteoid material is deposited around the capillaries of the diaphysis, and since the proliferation of cartilage cells continues, the width of the cartilage increases, enlargement and swelling of the joints occur. When vitamin D is given, degenerated cartilage cells again appear on the diaphyseal side and calcification is renewed. Thus the necessity of vitamin D for the normal development of bone in the animal body is evident.

Guy (1923) has traced the use of cod liver oil to cure rickets through many centuries, and because fish oils still constitute one of the most important sources of vitamin D, new oils containing it may be important.

Fishermen in general would be aided if the value of the catch of burbot, carp, lake herring and sucker could be raised. According to the U. S. Department of the Interior, Fish and Wildlife Service (1940), the total catch of these fish from the Great Lakes, and the commercial value of each were as follows: burbot, 488,000 pounds valued at $5,984; carp, 5,998,000 pounds sold at $147,553; lake herring, 22,480,000 pounds valued at $486,256; and sucker, 4,398,700 pounds valued at $122,403. An increase

in the catch of the burbot which is referred to by Jordan (1908) as destructive to other more valuable fish, and the utilization of the waste of the carp, herring and sucker should raise the commercial value of the catches.

Descriptions of the fish from which the test oils were obtained have been given by Jordan (1908), and by Jordan and Evermann, (1923).

The burbot, (Lota L. maculosa), also called the ling or lawyer fish is a long fish with imbedded scales. It may average from two to three feet in length. The range of the fish is from the waters of New England through the Great Lakes to the Yukon. It is unusual because it is the only fresh-water fish which is related to the cod. Because its food is the young of other species which are considered more palatable by human beings, the burbot is considered a nuisance. The burbot itself is not valued as food.

The carp (Cyprinus carpio), is a native of the rivers of China, from whence it was imported to Europe some three hundred years ago, and then brought to this country. It is a dull, sluggish fish of brownish color which prefers tranquil waters. It is very hardy, and sometimes reaches a weight of thirty to forty pounds. Insects and vegetable matter make up its food. The carp is used as food in some parts of this country, although it is more commonly eaten in China and Europe.

The lake herring (Leucichthys artedi), is also known as the cisco. The fish has an elliptical form with compressed sides and loosely inserted, silvery scales. It is found in great numbers in the Great Lakes, and is one of the most important of the American species of fish. The flesh is considered fair eating.

The sucker, (Cotostomus c. commersonii), is a small olivaceous colored fish which is found in almost every stream east of the Rocky

Mountains. The body is rather stout, averaging from six to eighteen inches in length. The source of food is insects and small aquatic animals.

Hereafter, for convenience of expression, the fish oils which were tested will be designated by the names of borbot liver oil, carp offal oil, lake herring offal oil, and sucker offal oil.

CHAPTER II

SURVEY OF LITERATURE

Previous studies of the vitamin D content of the oils of the four fish, burbot, carp, lake herring and sucker, have been limited to burbot liver oil and carp oil.

Burbot Liver Oil. The first mention of the vitamin D content of burbot liver oil was made by McCollum, Simmonds, Becker and Shipley (1922) in a statement that the oil promoted healing in rachitic rats. Clow and Marlott (1929) in a more detailed study of the oil, estimated the potency as eight times that of cod liver oil. Branion (1931), (1934), reported a potency of two to three times that of medicinal cod liver oil, while Nelson, Tolle and Jamieson (1932) found it two to four times as potent as cod liver oil. The estimation of 400 International Units per gram of burbot liver oil was made by Holmes, Tripp and Saterfield (1941). In addition to these experiments in which the oil was tested on rachitic rats, Steenbock, Kletzien and Halpin (1932) and Haman and Steenbock (1936) report that burbot liver oil is as effective as cod liver oil for chickens. A preliminary report by Myers (1937) in which he treated infants with burbot liver oil indicates that it is an efficient anti-rachitic substance for children.

Carp Oil. The only experimental work done on carp oil was carried on by Hess, Bills, Weinstock, Honeywell and Rankin (1928). As a result of a study of the relationship of the anti-rachitic factor to reproduction of fish, they found that both the milt and roe of carp had anti-rachitic properties similar to those of the cod, and that the ovary contained even more vitamin D than the liver.

CHAPTER III

EXPERIMENTAL PROCEDURE

PREPARATION OF OILS

Sources. The oils used in this study were furnished by Dr. P. J. Schaible of the Agricultural Chemistry Experiment Station. The burbot liver oil was a sample obtained in August, 1943, from Mr. Vogelheim, a fisherman of Rogers City, Michigan. He had caught the burbot in April and had rendered the fat from the livers over an open flame. The oil was kept refrigerated until used. The carp, lake herring and sucker offal oils were extracted from the fish offal which included the viscera, heads and all waste except the scales. The carp offal oil was expressed in January, 1944, from the offal of fresh, impounded fish which were kept alive until eviscerated. The lake herring and sucker offal oils were rendered in late January and mid April, respectively, from the frozen offal of fish caught during the winter. The oils from the latter two were extracted about two months after the fish had been caught.

Extraction of test oils. The offal was autoclaved at fifteen pounds steam pressure for one-half hour, then the tissue fluids and oils were expressed. Cylinders containing the tissue fluids and oils were placed in the refrigerator overnight. The next morning, the oils were removed by pipette, heated on a steam bath, then centrifuged and the oil pipetted off. Next, the oil was filtered to remove any tissue fluid or water which might remain. The oils were then kept refrigerated at 10°C., and prepared for the assays as needed.

Preparation of oils for assay. New U. S. Pharmacopeia Reference cod liver oil containing 115 I. U. of vitamin D per gram was obtained for use as a standard. In preliminary assays upon rachitic rats, it was found that two units per day for six days caused a complete narrow line of healing designated as two plus in the metaphyseal cartilage of the distal end of the radius and ulna. The standard cod liver oil was prepared by weighing 1.7392 gm. of oil on an analytical balance into a 10 cc. volumetric flask, and diluting to 10 cc. with cottonseed oil (Wesson Oil). This amount of oil contained 200 I. U. of vitamin D, so that 0.1 cc. of the diluted solution delivered 2 I. U. The U. S. Pharmacopeia XII (1942) states that not more than 0.1 cc. of oil be given per day, so 0.6 cc. of the diluted solution was dissolved in 5 cc. of ethyl ether and incorporated in the basal rachitic diet. Bills, Honeywell, Wirick and Nussmeier (1931) found that it made no difference whether the supplement was given by mouth or in the food. Therefore the oils were all administered in the food, because of the economy in time and because it was felt that the amount given would be more accurate with only one measurement instead of six. Careful, quantitative procedure was observed so that the amount given each animal was as accurate as possible. With the first group of animals, the dilute oil was prepared with 50 gm. of the basal diet, but this amount was more than some of the animals ate, so that subsequently the oil was incorporated into 40 gm. of basal diet. The food was allowed to dry, then was given to each animal ad libitum.

The burbot liver oil and the carp, lake herring and sucker offal oils were assayed using 1.7392 gm. of each oil made up to 10 cc. with cottonseed oil (Wesson Oil). If greater healing was evidenced than that

of the positive controls receiving the U. S. P. Reference cod liver oil, the amount of fish oil was cut in half (0.8696 gm.) and diluted to 10 cc. with the cottonseed oil. All oils were dissolved in ether and mixed with either 50 or 40 gm. of the basal diet. This mixture was fed to each rat ad libitum.

CARE OF ANIMALS

Preliminary care. When young rats from the stock colony which is maintained for vitamin D assays by the Michigan State College Department of Chemistry reached a weight of 45 to 50 gm., they were placed on a rachitogenic diet of the following composition:

Yellow table corn meal	69%
Wheat gluten	25
Brewer's yeast	2
$CaCO_3$	3
NaCl	1
	100%

At the end of a three weeks period, the animals were rachitic as signified by a wabbling gait and tender, swollen joints. Weekly weight gains had been recorded, and any animal gaining less than 20 gm. in the three weeks preliminary period was discarded. The rachitic animals were transported in a covered box to the Home Economics building, and placed upon supplements immediately.

Assay period. In the Home Economics Building, the animals were housed in a room with a northeast exposure. No direct sunlight was allowed to touch animals, and to further protect them from sunlight, a screen was placed in an individual round cage with a raised screen floor. The animals were then grouped according to sex and weight, although litter control was not observed. In each group, one animal serving as a negative control,

received no supplement of fish oil; one animal acting as a positive control, was given U. S. P. Reference cod liver oil, and either two or four animals received supplements of the test oils. With the first groups of animals, 0.6 cc. of the cottonseed oil was given in the same manner as the fish oils, to the negative controls, then was discontinued for subsequent groups since no effect upon healing was evident. The basal rachitogenic diet with the incorporated oil was fed ad libitum in a small feeding cup until it was all consumed, then the basal diet was furnished until the end of the experimental period. Throughout the assay period, distilled water accessible at all times was given each animal. Care was taken to recover all food dropping onto the paper below the wire screen, so that each animal would receive the full amount of the diet supplemented with the test oil. Food records were kept to ensure that each animal ate the amount which has been deemed necessary for the assay period by the U. S. Pharmacopeia XII (1942). Weights were recorded on the first, fifth and the tenth day of the test period by weighing on a trip balance. On the tenth day, after weighing, the animals were sacrificed by killing with chloroform.

LINE TEST

Preparation of bone. The preparation of the bone and the technique of the line test is essentially that originated by McCollum, Simmonds, Shipley and Park (1922b)* The distal end of the radii and ulnae were removed from the animal, cleaned of flesh, placed on a thread labelled with the number of the animal, and placed in ethyl alcohol for a period of 48 hours to harden.

Line test. The left bone of one of the pairs was removed from the alcohol and rinsed in distilled water. The distal end of the radius and ulna were split apart and each bone was cut longitudinally with a sharp razor blade. The four sections of bone were placed in a 2% (by volume) solution of $AgNO_3$, and exposed to actinic light for approximately one minute on dark days and ten seconds on sunny days. After exposure to light, the sections of the bones were rinsed in distilled water, and placed in a small transparent glass dish and covered with distilled water until reading.

Reading of line test. A 12 power magnifying lens was used to enlarge the image of the joint. The degree of rickets was assessed by the scale of the Pharmaceutical Society of London, as given by Coward (1938) with 0 designating no healing, five intermediate steps signifying various degrees of healing and 6 showing complete healing. A sketch of each bone with the healing evidenced was made for a record.

STATISTICAL ANALYSIS

The means of the weight changes, food intakes, and healing as shown by the line test of each group of controls and test animals were analyzed for significance according to the formula: $t = \frac{M_1 - M_2 - 0}{s} \cdot \sqrt{\frac{n_1 \cdot n_2}{n_1 + n_2}}$

in which $s = \sqrt{\frac{\Sigma x - \frac{(\Sigma x)^2}{n_1} + \Sigma y - \frac{(\Sigma y)^2}{n_2} + \Sigma z - \frac{(\Sigma z)^2}{n_3}}{n_1 + n_2 + n_3 - 3}}$

CHAPTER IV

RESULTS AND DISCUSSION

RESULTS

Weight changes. Table I, which gives the mean weight changes of all groups of animals, shows that in all except one, there was a slight increase in weight ranging from 0.1 gm. gain made by the positive control group for the burbot liver oil to 7.02 gm. gained by the negative control group for the carp offal oil. The one group losing weight was that of the positive control animals for the sucker offal oil group which showed a mean loss of 1.6 gm. No significant differences in weight gains or losses were found between any one set of animals receiving the test oil and the group of controls for that test oil. The means of weight gains were small, because one group of twenty-one rats was composed of animals which were smaller, very nervous and less sturdy. Of the animals in this group, eighteen lost weight, with one remaining the same for the ten day period. The amounts lost were under 5 grams, so that the results were used. Justification for this was that four negative controls lost up to 5 grams and showed no healing. Other investigators have found that small losses do not affect the degree of healing. Coward and Key (1933) have found that a loss of 5 gm. in weight does not affect healing, and Shipley, Kinney and McCollum (1924) observed that healing did not occur when there were even larger losses of weight.

Food Intake. The U. S. Pharmacopeia XII (1942) states that a minimum of 28 grams of food should be consumed in an eight day test period. In this study, a ten day period was used, so that the requirement would be 35 grams. From Table I it can be seen that the food intake averaged from 40.3 gm.

TABLE I

AVERAGE WEIGHT CHANGES AND FOOD INTAKES
FOR RACHITIC ANIMALS FED FISH OIL SUPPLEMENTS
DURING A 10 DAY ASSAY PERIOD

Fish oil fed	No. of animals	Am't. oil given in test period	Ave. Wt. change	Standard deviation of weight change	Ave. food intake	Standard deviation of food intake
		Gm.	Gm.		Gm.	
Burbot liver oil	12	0.052	0.60 ± 0.83*	2.80	43.2 ± 2.21	7.03
Cod liver oil	12	0.104**	0.10 ± 0.82	2.83	43.7 ± 1.70	5.90
No oil	11	0.00	1.00 ± 1.20	3.65	47.9 ± 2.11	7.03
Carp offal oil	12	0.104	6.18 ± 0.90	3.10	54.3 ± 1.66	5.73
Cod liver oil	10	0.104	5.66 ± 1.80	5.70	51.2 ± 3.48	11.02
No oil	10	0.00	7.02 ± 1.90	1.86	59.2 ± 2.70	2.70
Lake herring offal oil	12	0.052	0.59 ± 1.13	3.90	44.7 ± 0.92	3.17
Cod liver oil	11	0.104	0.15 ± 0.86	2.87	44.4 ± 1.91	6.33
No oil	11	0.00	2.82 ± 1.43	4.75	47.2 ± 1.98	6.55
Sucker offal oil	11	0.104	0.60 ± 0.75	2.50	41.6 ± 1.07	3.54
Cod liver oil	5	0.104	-1.60 ± 1.12	2.50	40.3 ± 0.93	2.09
No oil	6	0.00	2.13 ± 1.43	3.50	46.7 ± 2.97	2.97

* Standard error of arithmetic mean
** 12 I. U. of vitamin D

consumed by the group given cod liver oil in the sucker offal oil test group, to 59.2 gm. eaten by the negative control group of the animals given carp offal oil. The groups of animals previously mentioned lowered the mean of all groups in which they were included, since the food intake for this group was much closer to the minimum amount. No significant differences in food intake were found between the controls and the group given the test oil.

Healing. Preliminary assaying of the burbot liver oil on six rachitic rats gave evidence that when equal amounts of cod liver oil and burbot liver oil were administered, there was more vitamin D in the burbot liver oil than in the cod liver oil, since the test oil showed a mean healing of 4.50 $\pm$ 0.23 in contrast to 2.67 $\pm$ 0.42 shown by the positive controls. The difference in healing noted is pictured in Figures 2 and 3 of Plate I. Since this was a significant difference, the amount of burbot liver oil given was cut in half. Table II shows the results of the assay of twelve animals at this level, with the animals getting the burbot liver oil showing a mean healing of 3.00 $\pm$ 0.24 in comparison to a healing of 2.86 $\pm$ 0.20 for the group of positive controls. A representative picture of the healing given by this level of burbot liver oil is shown in Figure 5 of Plate I. There was no significant difference between these means, so that may be assumed that this sample of burbot liver oil contained twice as much vitamin D as the U. S. P. Reference oil, or approximately 230 I. U. per gram.

The first group of animals given carp offal oil in the same amount as the cod liver oil given the positive controls showed a mean healing of 2.33 in contrast to 2.67 for the animals receiving cod liver oil. This difference was not statistically significant, therefore six more animals

TABLE II

AVERAGE HEALING SHOWN BY LINE TESTS
OF RACHITIC ANIMALS FED FISH OIL SUPPLEMENTS
DURING A 10 DAY ASSAY PERIOD

Fish oil fed	No. of animals	Am't. oil given in test period	Ave. Healing as shown by line test	Standard Deviation of mean of healing	Approximate I. U. of vitamin D in test oil
		Gm.			per Gm.
Burbot liver oil	12	0.052	3.00 ± 0.24 *	0.82	230
Cod liver oil	12	0.104 **	2.86 ± 0.20	0.69	
No oil	11	0.00	0.00 ± 0.00	0.00	
Carp offal oil	12	0.104	2.58 ± 0.18	0.65	115
Cod liver oil	10	0.104	2.61 ± 0.21	0.66	
No oil	10	0.00	0.00 ± 0.00	0.00	
Lake herring offal oil	12	0.052	2.35 ± 0.34	1.17	200
Cod liver oil	11	0.104	2.76 ± 0.23	0.74	
No oil	11	0.00	0.00 ± 0.00	0.00	
Sucker offal oil	11	0.104	2.68 ± 0.18	0.61	115
Cod liver oil	5	0.104	2.84 ± 0.19	0.42	
No oil	6	0.00	0.00 ± 0.00	0.00	

* Standard error for mean
** 12 I. U. of U. S. P. Reference standard cod liver oil containing 115 I. U. vitamin D per gram

PLATE I.

REPRESENTATIVE DEGREES OF HEALING IN RACHITIC RATS
ON FISH OIL SUPPLEMENTS FOR 10 DAYS

Figure 1
Negative control:
No oil.

Figure 2
Positive control:
0.104 gm. cod liver oil

Figure 3
Burbot liver oil:
0.104 gm.

Figure 4
Lake Herring offal oil:
0.104 gm.

Figure 5
Burbot liver oil:
0.052 gm.

Figure 6
Lake Herring offal oil:
0.052 gm.

Figure 7
Carp offal oil:
0.104 gm.

Figure 8
Sucker offal oil:
0.104 gm.

were given supplements of the oil. As Table II shows, the results of the twelve rats tested were that the carp offal oil gave a mean value of 2.58 ± 0.18 healing while the positive control group averaged 2.61 ± 0.21 healing. Figure 7, Plate I illustrates the healing shown by animals receiving the carp offal oil. The results of the assay showed that there is no statistically significant difference between the means of the two oils, so that it may be concluded that this sample of carp offal contains approximately 115 I. U. of vitamin D per gram.

When an equal amount of lake herring offal oil and U. S. P. Reference cod liver oil were each tested on a preliminary group of eight animals, the rats receiving the test oil evidenced an average healing of 3.25 ± 0.33, while the positive controls registered a mean healing of 2.33 ± 0.21. The degree of healing is shown in Figure 4, Plate I. This difference proved to be slightly significant in favor of the animals receiving the lake herring offal oil, consequently, a group of twelve animals were given one-half as much lake herring offal oil as cod liver oil. Table II shows that the results of this assay were that these animals showed a mean healing of 2.35 ± 0.34 in contrast to 2.76 ± 0.23 evidenced by the positive control animals. A representative drawing of the healing shown at this level is given in Figure 6, Plate I. Although this difference was not statistically significant, the degree of healing shown, 2.35, was less than the 2.76 given by the cod liver oil, and the degree of variation, ± 0.34, was the largest shown by any group, so that it seems safe to conclude that the lake herring offal oil apparently contains at least 200 I. U. of vitamin D per gram.

A group of three animals given sucker offal oil in the same amount as the cod liver oil fed the positive controls showed a mean healing of 2.3 in comparison to 2.4 for the positive controls, so nine more animals were given supplements of the test oil. One animal of this group was discarded because of a very low food intake, so that the results of the assay on eleven animals as given in Table II is that they showed a mean healing of 2.68 ± 0.18, while the positive control animals evidenced a healing of 2.84 ± 0.19. Figure 8 in Plate I shows a typical picture of the healing shown by the animals given sucker offal oil. The difference in the means of healing shown by the animals receiving the test oil was not significantly different from that shown by the animals receiving cod liver oil, so that this sample of sucker offal oil contains about the same amount of vitamin D as the standard cod liver oil or 115 I. U. per gram.

From the results of the line test on rachitic animals, it was found that the sample of burbot liver oil assayed contained 230 I. U. of vitamin D per gram of oil, while the sample of lake herring offal oil tested contained at least 200 I. U. of vitamin D per gram. The samples of carp offal oil and sucker offal oil each contained approximately 115 I. U. of vitamin D per gram.

DISCUSSION

Comparison with results published in literature. The results of this study in which the vitamin D content of burbot liver oil is about twice that of standard cod liver oil, compares with those of Branion (1934), and Nelson, Tolle and Jamieson (1932). This is lower than that of the oils tested by Clow and Marlott (1929), and Holmes, Tripp and Satterfield

(1941), for the former found the oil eight times as potent as cod liver oil, and the latter state that it contains 400 I. U. per gram, or is almost four times as potent as the standard cod liver oil. It may be that the burbot liver oil assayed in this experiment did not have a higher vitamin D content because it was rendered over an open flame which may not be the method best suited to retaining the vitamin D originally present.

Comparison with data on the vitamin D content of other fish. It is of interest to find how the vitamin D content of burbot liver oil, and the carp, lake herring and sucker offal oils compare with that of other fresh-water fish oils. This subject has not received as much investigation as the vitamin D content of the salt water fish. It was found by Bills (1927) that the oil of the fatty tissues of muddy catfish caught in the Ohio River gave a healing rated as 40 in comparison to 100 as shown by cod liver oil. The liver oil of perch from Lake Erie was assayed by Holmes, Tripp and Satterfield (1941), and found to contain about 750 U. S. P. units per gram. An anonymous article, "Fish liver as a vitamin Rich food" (1925) appearing in the Wisconsin Agricultural Experimental Station Bulletin, states that the canned livers of Lake Michigan fish had healed rickets in rats. Burbot and whitefish livers rated higher than lake trout. No data were given.

Amoung the salt water fish, the range of vitamin D content varies from none found in the gray sole and sturgeon by Bills (1927) to 54,000 I. U. per gram of blue fin tuna liver oil, as assayed by Morgan, Kimmel and Davidson (1939) and 70,000 I. U. per gram of liver oil found in the same species of fish by Holmes, Tripp and Satterfield (1941). Of the ocean fish, Bills, et

al (1935) have stated that three-fourths of all liver oils are more potent than cod in vitamin D.

Factors affecting the vitamin D content of fish oils. The amount of vitamin D in fish oils varies with the species. Bills and co-workers (1935) found that the highest concentration of the vitamin is present in the livers of the higher families of fish, such as the percomorphi which include the mackerel, tuna, sea basses and swordfish, whereas the families of lower orders are poor in D. The fish tested in this study rank in the middle of the families of fish, except for the burbot which is in the lowest order of the last family. Thus it might be expected that if the liver oils of carp, lake herring, and sucker were tested, they might rank higher than that of the burbot.

The part of the body of the fish from which the oil is extracted also affects the content of vitamin D in the oil. One of the most detailed studies of this subject has been made by Pugsley (1942). He tested the potency of oils from the body, liver and intestines, and found that in the pilchard the vitamin D content of the body oil was 54 I. U. per gram and of the liver, 200 I. U. per gram. The herring contained 33 I. U. per gram of vitamin D in the body oil and 250 I. U. in the liver. The same author and his assistants (1942) found that in mackerel the liver contained 1035 I. U. of vitamin D per gram of oil, while the intestines, body oil and offal oil contained respectively, 76, 20, and 51 I. U. per gram of oil. Aschehaug, Kringstad, and Lunde (1939) report that the potency of mackerel liver was 11,300 I. U. per 100 gm. of product, while the flesh rated only 85 I. U. for the same amount.

The time of year in which the fish are caught also affects the amount of vitamin D in the oils. Hess, Bills and Honeywell (1929) first found that the amount of vitamin D varied inversely with the amount of oil in the liver. Later Bills, Imboden and Wallenmeyer (1934) found that in halibut liver, the total oil content rose slowly from January to June, then increased suddenly until it was doubled in August, then slowly declined from August to January. The vitamin D content varied inversely with the oil content, for it was highest in January. Pugsley (1939) found the potency higher in July and August than in September and October when the yield of oil was higher. Supplee (1937) found that smaller, thinner fish contained more vitamin D, so that early summer catches were more potent. Since all of the fish oils tested in the present experiment were caught in the winter and very early spring months, it is probable that the vitamin D found present may be near the maximum quantity which might be found in the type of oil tested.

The method of extraction of the oils may affect the vitamin D content. Morgan, Kimmel and Davison (1939) tested several oils prepared from the same catch of sardines, but extracted in various ways. The best method for retaining the highest amounts of vitamin D was to steam autoclave the fish, press out the oils, then centrifuge to remove any other liquid present. This method of extraction was used on the carp offal, lake herring and sucker offal oils, so that it is felt that in these oils there was as little loss of the vitamin as is possible with known methods.

Possible uses for the oils. Since all of the test oils fulfill the standard set by the U. S. Pharmacopeiea XII (1942) as far as the vitamin D

content is concerned, it is possible that the oils could be used commercially as a source of vitamin D. They might all be considered as a source of vitamin D for human consumption, since burbot liver oil already has been used successfully by Myers (1931) as an anti-rachitic agent. There is a possibility that the others might be quite well used for poultry, although they would have to be tested on chickens first to find the efficacy. It was demonstrated by Bills, Massengale, and Imboden (1934) that the fish oils differ in their anti-rachitic effect when they found that the blue fin tuna liver oil was only one-sixth as effective on chickens as on rats. They felt that this was due to the fact that there are two or more forms of vitamin D present in fish oils. Hamon and Steenbock (1936) reported that burbot liver oil was as effective as cod liver oil for chickens, but further checking would have to be carried on with the other oils. In the use of these oils for poultry, it would be of special interest to test the carp offal oil, because the quantity of the catch is great, the use for them is limited, and the price is very low.

CHAPTER V.

SUMMARY AND CONCLUSIONS

Oils from burbot liver and from the offal of carp, lake herring, and sucker caught in the winter and early spring months from the Great Lakes were assayed for their vitamin D content. The burbot oil tested was a sample which had been flame rendered while the carp, lake herring, and sucker offal oils were extracted from the viscera and all other waste except the scales by autoclaving, then pressing out the oil, and centrifuging it to purify it. A total of 96 rachitic animals, 25 negative controls, 24 positive controls and 47 test animals, were used to find the vitamin D content by the use of the line test technique.

The following results were obtained:

1. The sample of burbot liver oil contained about twice as much vitamin D as the standard U. S. P. Reference cod liver oil, or about 230 I. U. per gram of oil.

2. The lake herring offal oil tested contained approximately twice as much vitamin D as the standard U. S. P. Reference cod liver oil or at least 200 I. U. of vitamin D per gram of oil.

3. The samples of carp and sucker offal oils tested contained the same amount of vitamin D as the standard U. S. P. Reference cod liver oil, or approximately 115 I. U. per gram of oil.

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APPENDIX

TABLE I.

Individual records of rachitic animals receiving no oil used as negative controls for assay of burbot liver oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
168 ♂	67.0	750	8.0	57.0	0
176 ♀	70.0	760	6.0	55.0	0
180 ♀	66.7	67.5	0.8	50.0	0
226 ♂	82.0	87.3	5.3	53.0	0
232 ♂	59.0	61.0	2.0	49.9	0
237 ♀	74.8	70.0	-4.8	38.0	0
241 ♀	69.0	70.0	1.0	48.4	0
245 ♀	64.4	67.0	2.6	46.8	0
249 ♂	70.0	67.0	-3.0	40.0	0
254 ♂	60.0	59.0	-1.0	35.0	0
260 ♀	64.6	67.7	3.1	54.5	0
M	68.0	69.8	1.81	47.96	0
σ			3.65	7.03	0
σ_m			1.10	2.11	0

TABLE II.

Individual records of rachitic animals receiving 0.104 gm. cod liver oil used as positive controls for assay of burbot liver oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
169 ♂	67.0	70.0	3.0	46.0	3
173 ♀	76.0	80.0	4.0	57.0	4
177 ♀	70.0	70.0	0.0	51.0	4
181 ♀	66.0	71.0	5.0	40.0	3
227 ♂	64.5	64.7	0.2	37.0	2
233 ♂	59.0	61.0	2.0	40.0	3
238 ♀	74.0	73.0	-1.0	49.0	3
242 ♀	65.5	65.0	-0.5	40.0	2
246 ♀	60.0	59.0	-1.0	46.8	2
250 ♂	67.2	63.0	-4.2	40.0	3
255 ♂	58.5	57.0	-1.5	40.0	2
261 ♀	64.5	60.0	-4.5	37.5	3.2*
M	66.01	66.14	0.13	43.7	2.86
σ			2.83	5.9	0.69
σ_m			0.82	1.70	0.20

* Healing calculated up to 40 Gm. intake

TABLE III.

Individual records of rachitic animals receiving 0.052 gm. burbot liver oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
170♂	65.8	70.0	4.2	56.0	3
174♀	75.2	75.6	0.4	55.0	4
178♀	68.5	66.0	-2.5	40.0	3
182♀	64.4	65.0	0.6	55.0	4
228♂	64.0	67.0	3.0	44.8	2
230♂	62.7	67.7	5.0	37.2	4.3*
239♀	70.5	73.0	2.5	33.9	2.4*
243♀	65.4	67.0	1.6	45.5	3
247♀	61.0	60.0	-1.0	40.0	2
252♂	62.5	60.0	-2.5	39.0	3
257♂	55.0	51.0	-4.0	33.0	2.4*
263♀	56.4	54.0	-2.4	39.0	3
M	64.3	64.7	0.41	43.2	3.00
σ			2.80	7.03	0.82
σ_m			0.81	2.11	0.24

* Healing calculated up to 40 gm. intake

TABLE IV.

Individual records of rachitic animals receiving no oil used as negative controls for assay of carp offal oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
102♂	61.5	69.3	7.8	55.0	0
106♂	62.2	75.0	11.8	70.0	0
110♂	63.1	80.0	16.9	62.0	0
118♂	66.6	74.0	7.4	63.0	0
122♂	72.0	83.0	11.0	64.5	0
148♂	69.0	70.0	1.0	49.5	0
150♂	64.0	74.0	10.0	60.0	0
156♀	65.2	60.0	-5.2	40.0	0
159♀	71.5	75.0	3.5	60.0	0
162♂	75.0	81.0	6.0	68.0	0
M	67.01	74.13	7.02	59.20	0
σ			5.87	8.52	0
σ_m			1.86	2.70	0

TABLE V.

Individual records of rachitic animals receiving 0.104 gm. cod liver oil used as positive controls for assay of carp offal oil.

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
101♂	59.0	65.5	6.5	47.5	2
105♂	62.0	61.0	-1.0	38.0	2.11*
109♂	63.0	80.0	17.0	49.0	2
117♂	66.0	74.0	8.0	60.0	4
121♂	69.5	82.5	13.0	76.0	2
149♂	61.0	67.0	6.0	40.0	3
152♂	70.0	76.4	6.4	50.0	3
155♀	62.0	61.0	-1.0	40.0	2
158♀	70.0	71.7	1.7	60.0	3
161♀	74.7	74.7	0.0	52.0	3
M	65.72	71.38	5.66	51.25	2.61
σ			5.70	11.02	0.66
σ_m			1.80	3.48	0.21

* Healing calculated up to 40 gm. intake

TABLE VI.

Individual records of rachitic animals receiving 0.104 gm. carp offal oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
103♀	59.0	64.0	5.0	50.0	3
107♀	61.7	74.0	12.3	58.5	3
111♀	65.0	76.0	11.0	60.0	3
119♂	68.5	78.0	9.5	60.0	2
123♂	72.5	80.0	7.5	57.0	2
146♂	67.0	73.3	6.3	56.0	3
147♂	67.0	71.0	4.0	51.5	3
151♂	64.5	70.0	5.5	53.0	3
154♂	78.0	81.8	3.8	40.0	2
157♀	67.2	70.0	2.8	49.0	3
160♀	73.8	77.7	3.9	58.0	1
163♀	76.0	78.5	2.5	59.0	3
M	68.35	74.53	6.18	54.33	2.58
σ			3.1	5.73	0.65
σ_m			0.9	1.66	0.18

TABLE VII.

Individual records of rachitic animals receiving no oil used as negative controls for assay of lake herring offal oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
164♂	79.0	90.0	11.0	52.0	0
176♀	70.0	76.0	6.0	55.0	0
180♀	66.7	67.5	0.8	50.0	0
184♂	60.0	70.0	10.0	46.0	0
226♂	82.0	87.3	5.3	53.0	0
237♀	74.8	70.0	-4.8	38.0	0
241♀	69.0	70.0	1.0	48.4	0
245♀	64.4	67.0	2.6	46.8	0
249♂	70.0	67.0	-3.0	40.0	0
254♂	60.0	59.0	-1.0	35.0	0
260♀	64.6	67.7	3.1	54.5	0
M	69.14	71.95	2.82	47.15	0
σ			4.75	6.55	0
σ_m			1.43	1.98	0

TABLE VIII.

Individual records of rachitic animals receiving 0.104 gm. cod liver oil used as positive controls for assay of lake herring offal oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
173♀	76.0	80.0	4.0	57.0	4
177♀	70.0	70.0	0.0	51.0	4
181♀	66.0	71.0	5.0	40.0	3
185♀	61.9	64.0	2.1	50.0	2
227♂	64.5	64.7	0.2	37.0	2.16*
238♀	74.0	76.0	2.0	49.0	3
242♀	65.5	65.0	-0.5	40.0	2
246♀	60.0	59.0	-1.0	46.8	2
250♂	67.2	63.0	-4.2	40.0	3
255♂	58.5	57.0	-1.5	40.0	2
261♀	64.5	60.0	-4.5	37.5	3.2*
M	66.19	66.37	0.15	44.39	2.76
σ			2.87	6.33	0.74
σ_m			0.86	1.91	0.23

* Healing calculated up to 40 gm. intake.

TABLE IX.

Individual records of rachitic animals receiving 0.052 gm. of lake herring offal oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
167♂	70.0	70.0	0.0	50.0	3
175♀	73.0	73.0	0.0	46.0	2
179♀	67.0	67.0	0.0	45.0	2
183♀	62.2	62.0	-0.2	44.0	4
186♀	62.0	74.0	12.0	50.0	5
229♂	64.0	65.4	1.4	47.5	1
240♀	70.0	71.7	1.7	46.8	2
244♀	65.0	67.0	2.0	47.9	2
248♀	59.5	57.0	-2.5	38.7	1.03*
251♂	63.0	60.0	-3.0	40.0	3.08*
256♂	57.8	53.5	-4.3	35.0	1.14*
262♀	60.0	60.0	0.0	44.0	2
M	64.46	65.05	0.59	44.68	2.35
σ			3.90	3.17	1.17
σ_m			1.13	0.92	0.34

* Healing calculated up to 40 gm. intake

TABLE X.

Individual records of rachitic animals receiving no oil used as negative controls for assay of sucker offal oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	healing on line test
	Gm.	Gm.	Gm.	Gm.	
232♂	59.0	67.0	8.0	49.9	0
249♂	70.0	67.0	-3.0	40.0	0
254♂	60.0	59.0	-1.0	35.0	0
260♀	64.6	67.7	3.1	54.5	0
266♂	69.5	73.0	3.5	54.0	0
270♀	69.8	72.0	2.2	47.0	0
M	65.5	67.6	2.13	46.71	0
σ			3.50	7.29	0
σ_m			1.43	2.97	0

TABLE XI.

Individual records of rachitic animals receiving 0.104 gm. cod liver oil used as positive controls for assay of sucker offal oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
253 ♂	59.0	61.0	2.0	40	3
250 ♂	67.2	63.0	-4.2	40	3
255 ♂	58.5	57.0	-1.5	40	2
261 ♀	64.5	60.0	-4.5	37.5	3.2*
271 ♀	66.3	66.5	0.2	44.0	3.0
M	63.1	61.5	-1.6	40.3	2.84
σ			2.5	2.09	0.42
σ_m			1.12	0.93	0.19

* Healing calculated up to 40 gm. intake

TABLE XII.

Individual records of rachitic animals receiving 0.104 gm. sucker offal oil

No. of rat	Initial weight	Final weight	Weight change	Food intake	Healing on line test
	Gm.	Gm.	Gm.	Gm.	
234♂	58.5	62.9	4.4	45.0	2
235♂	58.2	57.0	-1.2	40.0	3
236♂	54.5	61.0	6.5	45.8	2
253♂	61.0	62.0	1.0	47.5	3
258♂	54.0	52.0	-2.0	38.5	4.15*
259♂	52.5	52.0	-0.5	38.0	3.13*
265♀	54.5	53.5	-1.0	40.0	2
268♂	65.5	64.0	-1.5	40.0	2
269♂	60.5	61.5	1.0	45.0	2
272♀	61.0	61.0	0.0	38.0	3.16*
273♀	60.0	60.0	0.0	40.0	3
M	58.2	58.8	0.60	41.6	2.68
σ			2.50	3.54	0.61
σ_m			0.75	1.07	0.18

* Healing calculated up to 40 gm. intake.

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