THE DETERMINATION OF THE VITAMIN A CONTENT OF THE LIVER OIL OF THE DOG-FISH SQUALUS SUCKLII (GIRARD)

HORACE N. BROCKLESBY,

PRINCE RUPERT, 1927.

BIOLOGICAL BOARD OF CANADA

MANUSCRIPT REPORTS OF THE EXPERIMENTAL STATIONS

No. 14

Title

THE DETERMINATION OF THE VITAMIN A CONTENT OF THE LIVER OIL OF THE DOG-FISH SQUALUS SUCKLII (GIRARD)

Author

Horace N. Brocklesby.

Prince Rupert, 1927.

Determination of the Vitamin A content in the liver oil of the Dog-fish, Squalus sucklii (Girard)

The Dog-fish, Squalus sucklii (Girard) is commonly found along the North Pacific Coast of North America. In many localities it has become so numerous that it has become a pest, playing great havoc with fishermens nets, voraciously chasing and devouring salmon fingerlings and smaller food fishes. In some localities halibut fishermen pull up as much as 80% of their lines hooked with Dog-fish. Fishermen will not fish for this pest for itself as it has practically no market value. Its high content of urea tends to make it undesirable for food purposes, and up to the present, conversion of the flesh of the Dog-fish into poultry feed and fertilizer has met with indifferent success. It can be shown, however, that with proper processing this fish can be made to yield a good grade of meal. The returns from this enterprise alone, however, are not large enough to warrant paying fishermen a price which will induce them to especially set their lines for this fish. If some use could be made of the oil from the livers of this fish, it might be possible to check the increase of this species which causes so much damage to the fishing industry at large.

It was with this view in mind that the Fisheries Experimental Station (Pacific) decided to investigate the physical and chemical properties, together with the biological characteristics of this oil. The results of the physical and chemical examination will be published soon. The present paper describes the results of the biological and colorimetric examination of the oil for the Vitamin A content.

Specimens from which the oil was taken were caught by the writer during the summer of 1926 in the Strait of Georgia near the Pacific Biological Station at Nanaimo. Data on the relative weights of the liver to the weight and lengths of the fish caught as well as yield of oil to be expected have been accumulated. These data will be presented in a forth coming paper.

The oil used in the following experiments was prepared from the fresh livers. These were minced through a meat chopper, placed in a large glass beaker, and steam passed through them for about half an hour. The beaker remained covered during the heating period. The oil was then allowed to rise and was skimmed off the top. It was dehydrated with sodium sulphate, and filtered.

The biological determination of Vitamin A was done in the Department of Biochemistry of the University of Manitoba through the courtesy of Doctor A.T. Cameron, whilst the colorimetric work is being carried out at the Experimental Station at Prince Rupert.

As far as the writer is aware, the vitamin content of the liver oil from the Dog-fish, Squalus sucklii, has not been reported in the literature. Chapman (1) and also Holmes and Pigott(2) have reported on samples of the liver oil from the Dog-fish, Squalus acanthius, caught in the Atlantic. Drummond, quoted by Chapman, reports that the Vitamin A potency is equal to medicinal cod liver oil. Holmes and Pigott state that "one mg. of the dogfish liver oil under investigation contained sufficient Vitamin A to meet the needs of albino rats for growth" (2)

BIOLOGICAL ASSAY

The quantitative estimation of Vitamin A, the growth promoting accessory factor, is described by Zilva and Muira (3) and is essentially as follows:

Young rats of about 50 grams in weight and from 20 to 25 days old are placed on a diet lacking in Vitamin A but adequate in all other respects. They are maintained on such a diet until they cease to grow and develop the characteristic eye disease, xerophthalmia. The length of time that this takes varies in different litters of rats but should be within 3 to 5 weeks. It has been found that the parental diet has a great deal to do with the resistance of young rats to vitamin free rations. Therefore, rats to be used for Vitamin A work should be taken from females which have had sufficient but not an over abundance of Vitamin A in their diet. Stammers (4) ably summarizes the evidence in favour of using xerophthalmia as a criterion by which to judge the degree of avitaminosis in a rat with respect to Vitamin A. He concludes that the susceptibility of the animals and the time of incidence of the disease vary directly with the amount of Vitamin A in the oil under investigation and further that the first factor is modified by the age of the animal.

The histology of xerophthalmia of rats has been studied by Mori (5). He found that the lacrymal gland goes into the resting stage and ceases to produce tears. The conjunctival sac then rapidly becomes infected with bacteria. Leucocytes gather in the eye chamber which then shows a yellowish colour. Some leucocytes penetrate the outer coating of the eyeball and find their way into the conjunctival sac. This is followed by a viscous greenish exudation which tends to stick the eyelids together. Owing to the dry condition of the eye there is cornification of the external coating and this is followed by ulceration. If the ulcers penetrate the outer coating the lens may fall out.

This disease develops very rapidly and in experimentation should not be allowed to come to the stage where cornification takes place. With care, this disease in its earlier stages becomes a very sensitive indication for the presence or absence of Vitamin A in a diet.

Some authors claim that xerophthalmia precedes the cessation of growth, but in the writer's limited experience the reverse has

been true. The rats have usually ceased growing for a few days before the disease is evidenced.

The rats were weighed at least twice a week throughout the entire period of the experiment, always just prior to feeding. Near the period of cessation of growth daily examination of the rats for signs of xerophthalmia was made.

The diet used in these experiments was as follows:

20	parts	purified caseinogen
55	"	wheat starch
5	"	salt mixture #185 (6)
5	"	Fleishmans yeast
15	"	butter.

The caseinogen to be used in these experiments must be entirely freed from Vitamin A. This may either be done by digesting with ether and alcohol or by heating in a current of air at 130 degrees for 36 hours. The second method was adopted in this work. Sufficient amounts of the dry diet to last a month were made up and the yeast and butter added in the correct ratio to smaller amounts every two days. The rats were given an excess diet and distilled water ad lib. They were fed at five o'clock.

In order to protect against rickets activated cholesterol was added by the method of Drummond (7). Repurified cholesterol was dissolved in ether and placed in a quartz flask. The ether was evaporated in such a manner that the cholesterol was deposited in a thin layer more or less uniformly over the inner surface of the flask. The latter was then fitted with a tight fitting cork and glass stop-cock and evacuated with a Hy-vack pump overnight. The last traces of ether were thus removed. Whilst still in an evacuated condition, the flask was then irradiated with a Burdock quartz lamp at a distance of 20 cms. for a period of one hour and a half. During this time the flask was rotated periodically. After irradiation the cholesterol was dissolved in warm "Petrolatum" to give a 1% solution. This solution was added to the dry diet as used and was thoroughly mixed in.

When the rats showed evidence of Vitamin A starvation through both cessation of growth and appearance of xerophthalmia, they were fed varying doses of the oil under test and also with cod liver oil with which a comparison was being made. The oils were administered by hand by means of a dropping pipette, one drop being forced into the mouth of the rat whilst it was held on its back.

Comparison was made with a sample of Parke Davis Cod Liver Oil. This sample is stated to contain 12,000 units of Vitamin A per ounce. The minimum dose would then be 0.0022 gms. per day. This amount of oil was taken as one unit. Pure olive oil was mixed with the oil under test to adjust the vitamin content so that one drop would contain the desired experimental dosage.

Nine rats were used in the first experiment and were placed on the above diet when they were 20 days old. After 20 days the butter fat was replaced by Crisco, thus depriving the diet of any Vitamin A. In the meantime the rats had developed normally as shown by the growth curves. Two rats were left on the original diet as controls. Their average weights were used to construct the normal curve.

In this experiment the feeding of the Vitamin A product commenced on the 89th day of life. Three rats were used as standards, and were given 5, 2 and 1 unit per day respectively of cod liver oil. Four other rats were given 100, 10, 1 and 0.1 units of dog-fish liver oil, basing the latter units simply on the weight relation to cod liver oil. The growth curves are given in figure 1 whilst the summary of the data is given in Table 1. Individual data are given at the end of the paper.

Table 1

Disappearance of Xerophthalmia.		Increase in Weight.		Source of Vitamin.
Severity	Time	10 days	20 days	
Slight case	8 days	10 gms.	20 gms.	5 units cod-liver oil
Not definite		6 "	10 "	2 " " " "
Slight case	8 days	4 "	8 "	1 " " " "
Severe case	not cured	-2 "	3 "	0.1 " " " "
Bad case	8 days	9 "	17 "	1 unit dog-fish oil
Bad case	3 days	11 "	21 "	10 " " " "
Severe case	6 days	11 "	26 "	100 " " " "

Discussion

Examination of the data shows that in order to cure a slight case of xerophthalmia in a Vitamin A starved rat, 5 and 1 units of cod liver oil are required for a period of 8 days. In all cases where dog-fish liver oil was used, the degree of xerophthalmia was more severe. An 0.1 unit of dog-fish liver oil does not effect any cure at all.

Two bad cases of xerophthalmia are cured in 8 and 3 days by one and ten units of dog-fish liver oil respectively. A very severe case of xerophthalmia is completely cured in aix days by 100 units of dog-fish liver oil. This would indicate that the dog-fish liver oil is more potent for Vitamin A than cod liver oil.

More conclusive proofs are given by growth curves. One unit of dog-fish liver oil is shown to have practically the same effect as five units of cod liver oil.

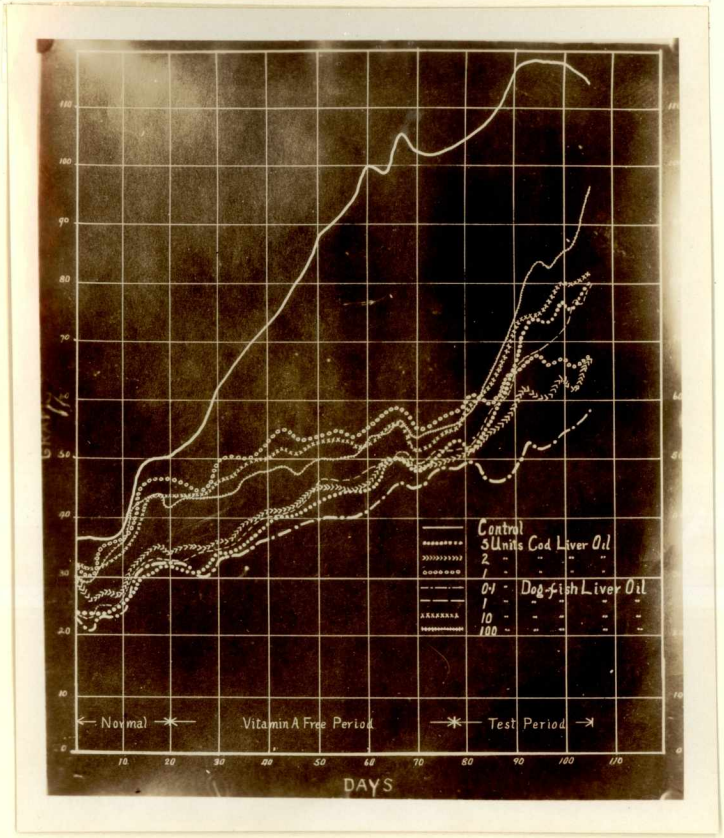
Control
5 Units Cod Liver Oil
2 -
1 -
0·1 - Dog-fish Liver Oil
1 -
10 -
100 -
Normal
Vitamin A Free Period
Test Period
DAYS

FIG. I.

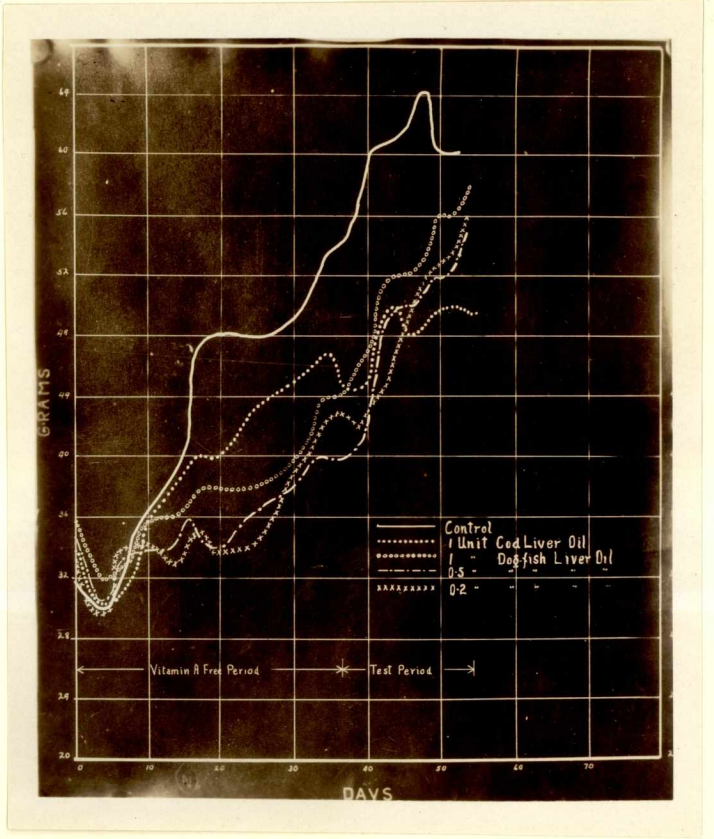
GRAMS
Control
1 Unit Cod Liver Oil
1 - Dog-fish Liver Oil
0·5 -
0·2 -
Vitamin A Free Period
Test Period
DAYS

FIG. II.

FIG. III.

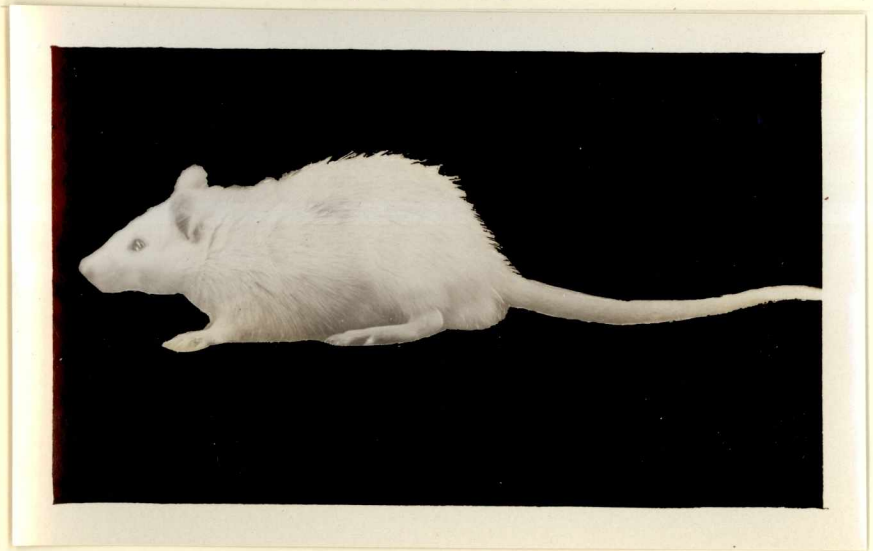

FIG. IV.

FIG. V.

Explanation of Figures.

Fig. 1. Growth curves for Experiment 1.

" 11. " " " " 11.

" 111. Rat #9 showing Vitamin A starvation.

" 1V. Rat #9 after feeding Dogfish Liver Oil.

" V. Rat #9 showing Xerophthalmia.

In experiment 2, six rats were used, two of which were controls. The experimental procedure was the same as in experiment 1, except that the rats after weaning, were placed directly on the Vitamin A free diet. The following dosages were employed: One unit cod liver oil: one unit dog-fish liver oil: 0.5 units dog-fish liver oil, and 0.2 units dog-fish liver oil. The results obtained are given in Table 11, whilst the growth curves are given in Figure 11.

Table 11.

Disappearance of xerophthalmia		Increase in Weight		Source of Vitamin A
Severity	Time	10 days	20 days	
Severe case	not cured	4 gms.	5 gms.	1 unit cod liver oil
Severe case	5 days	7 "	13 "	1 unit dogfish liver oil
Severe case	15 "	10 "	15 "	0.5 " " "
Severe case	15 "	8 "	14 "	0.2 " " " "

Discussion

This time all the rats had severe xerophthalmia. It is to be noted that one unit of cod liver oil per day failed to cure one of these cases, whilst in another case one unit of dog-fish liver oil effected a cure in 5 days. After a period of 20 days, one unit of cod liver oil had brought about an increase in weight of 5 grams, whilst one unit of dog-fish liver oil in the same time had effected an increase of 13 grams. Units .5 and .2 of dog-fish liver oil were both sufficient to cure severe cases of xerophthalmia in 15 days, and bring about gains in weight of 15 and 14 grams respectively in 20 days. Examination of the growth curves in Figure 11 will show that .2 units of dog-fish liver oil are more efficient in restoring the growth of a rat than one unit of cod liver oil.

Conclusions.

It seems reasonable to conclude from these results that dog-fish liver oil is a potent source of Vitamin A. Its content, weight for weight, is much greater than the standard cod liver oil preparation used. Comparison of the weight increases and length of time required to cure xerophthalmia suggests that dog-fish liver oil contains more than 5 times the potency of standard cod liver oil.

Colorimetric Determination of Vitamin A in Dogfish Liver Oil.

Many papers have appeared recently dealing with colorimetric tests for the evaluation of Vitamin A contents. The subject has been well summarized by Willimott and Wokes (8). They find that Fearon's Reagent is non-specific for Vitamin A. This is also shown by Rosenheim and Webster(9). The reaction suggested by Willimott and Wokes is that of Carr and Price (10) in which antimony trichloride is dissolved in chloroform, forming a saturated solution and this reagent added to a chloroform solution of the substance to be tested. It has been recommended that a Lovibond Tintometer be employed, using the blue glasses, as it has been shown that the blue colour developed is proportional to the Vitamin A content.

Not having a Lovibond Tintometer, a method had to be found in which an ordinary colorimeter might be used to measure the colour. The following method has been used for evaluation of the Vitamin A content of dog-fish liver oil.

The antimony trichloride solution used was a saturated one. Bakers C.P. antimony trichloride was dissolved in redistilled C.P. chloroform and allowed to stand at least 24 hours in the presence of a large excess of the crystals. For each determination the solution was shaken vigourously, and the crystals allowed to settle before the solution was drawn off. For each determination 2 ccs. were used, measured by a 2cc. pipette. The oil was run in from a 10 cc. burette with a very fine drawn-out point. From this burette the size of the different drops was practically the same within the limits of experimental error. The antimony trichloride was first run into the comparison glass, and the oil dropped into it. The two were immediately shaken and mixed. The time was taken the moment the oil was added and exactly 15 seconds later, as timed with a stop watch, the exact comparison with the standard solution was made. It was found that with practice, the oil could be added, shaken and placed in position within eight seconds. Starting at the 10th second, the change in colour was gradually followed, the final reading being taken at the 15th second. As a standard for comparison, the following solution was found satisfactory for the colours developed with the dogfish liver oil. One gram of anhydrous copper sulphate was dissolved in 100 ccs. distilled water. When dissolved, 6.65 ccs. of ammonium hydroxide (concentrated ammonia diluted 50% with water) were added. A Dubosq Colorimeter was used, employing the standard solution as the variable cup. The antimony trichloride solution was set at 60, and kept there for each reading.

The following are the results obtained by this method:

Dog-fish Liver Oil used in Biological Assay	Cod Liver Oil used in Biological Assay
3.1	0.8
3.6	0.5
3.3	0.6
3.9	0.7
3.5	0.7
3.7	0.9
Av. 3.5	Av. 0.7

Oil from the Livers of Dog-fish caught in the Prince Rupert Harbour

Dogfish Oil Jan 5/28 15 secs.	Female Dogfish Jan 5/28 15 secs.	Female Dogfish Nov 21/27 15 secs.
7.5	6.2	3.0
7.3	6.5	4.1
7.2	6.6	3.5
7.7	6.2	3.5
7.1	5.9	3.2
7.4	7.0	3.4
Av. 7.4	Av. 6.4	Av. 3.4

Female Dogfish Oct 20/27	Male Dogfish Oct 20/27
5.1	2.0
5.1	2.2
5.2	1.9
4.3	1.9
4.8	1.5
4.3	1.9
Av. 4.8	Av. 1.9

Oil from the livers of Dogfish caught in the Queen Charlotte Sound.

3.5
3.7
3.7

Av. 3.6

Cod Liver Oil from oper. market (New shipment of Squibbs)

1.7
2.1
1.6
1.9
1.4
1.5

Av. 1.7

Discussion.

The ratio of the intensities of colour developed between the dogfish liver oil and cod liver oil used in the biological assay is exactly 5 to 1 on an average. This result is remarkably close to the conclusions drawn from the preceding work. If the colour test can be taken to indicate small differences in Vitamin A potency, it becomes apparent that this potency varies in the case of dogfish. The highest figures are obtained for both male and female of January, whilst those of October and November are quite low. Until more data are collected on this question it is useless to draw conclusions. It is interesting to note that a highly purified sample of cod liver oil gave a reading of only 1.7.

To see if there was any agreement between the blue colour developed by antimony trichloride and the yellow pigment of the oil, the latter was measured in the colorimeter in comparison with the standard containing .5 grams of potassium chromate in 250 ccs. water. From a burette 5 ccs. of oil were measured into the comparison glass. This glass was set at 21 i.e. to bring the prism just above the oil and not quite touching it. The standard solution was used as the variable cup. The following are the results obtained, giving the antimony trichloride reading in one column and the relative degree of pigmentation in the other.

Ratio of Vitamin A by SC Cl_3 colour test to Pigment content.

SC Cl_3	Pigment
4.8	2.3
1.9	1.9
3.4	2.2
6.4	6.6
7.4	5.6
4.6	3.1
1.2	1.0
4.5	2.5
1.3	1.35

These figures indicate that there is evidently some relation between the amount of yellow pigment present, and the intensity of the blue colour developed by antimony trichloride. This relation will be more fully investigated at a later date.

References.

1. Chapman,A.C., Analyst, 1918, XLIII,156.
2. Holmes, A.D. and Pigott, M.G., Ind. Eng.Chem., 1925 XVII, 310.
3. Zilva, S.S. and Muira, M., Biochem. J.,1921,XV, 654.
4. Stammer, A.D., Biochem. J., 1924, XVIII, 9.
5. Mori, S., Jour. Amer. Med.Assoc., 1922, LXXIX, 197.
6. McCollum, E.V., Simmonds,N, and Parsons, H.T., J. Bio.Chem. 1921, XLVII, III.
7. Drummond,J.C., Coward,K.H., and Handy,J., Biochem.J., 1926, XIX,1068.
8. Willimott, S.G. and Wokes,F., Lancet,1927 II, 8
9. Rosenheim, O, and Webster, T.A., Biochem.J., 1926, XX, 1342.
10. Carr, F.H. and Price, E.A. Biochem.J., 1926, XX, 497.

Rat #1, receiving Vitamin complete Diet

Date	Weight	Special observations.
Nov 1		
3	37	Rat looked rather weak, substituted a healthier rat This rat used as a control. Kept on Vitamin sufficient diet Mark on left ear.
5	37	
9	37	
12	45	
15	50	
17	50	
19	50	
21	51	
25	54	
29	62	
Dec 1	64	
4	68	
7	70	
9	72	
11	74	
15	80	
17	81	
20	88	
23	90	
27	94	
30	100	
Jan 3	98	
6	106	
9	104	
13	102	
18	104	
20	100	
23	107	
26	109	
30	116	
Feb 1	118	
3	118	
6	118	
9	118	
11	119	
13	116	
15	114	

Rat #2 Vitamin A

Date	Weight	Special observations.
Nov 1	23	This rat used as a control
3	21	
5	23	Right ear
9	24	
12	28	
15	32	
17	34	
19	34	
21	36	
25	38	
29	43	
Dec 1	43	
4	45	
7	47	
9	47	
11	49	
15	52	
17	54	
20	60	
23	62	
27	67	
30	68	
Jan 3	68	
6	72	
9	70	
13	70	
18	74	
20	70	
23	83	
26	87	
30	94	
Feb 1	98	
3	104	
6	106	
9	108	
11	109	
13	112	
15	114	

Rat #3 Vitamin A - 5 units of Cod Liver Oil.

Date	Weight	Special observations.
		Placed on Vit. A deficient diet on Nov 30/26. Coat became shaggy. Rat still healthy on Dec. 30th.
Nov 1	24	
3	24	
5	24	
9	25	Centre forehead
12	28	
15	30	
17	32	
19	32	
21	32	
25	31	
29	34	
Dec 1	34	
4	35	
7	37	
9	38	
11	40	
15	40	
17	42	
20	43	
23	44	
27	44	
30	44	
Jan 3	50	
6	50	
9	48	
13	50	
18	53	Jan 18. - 0.05 ccs = 5 units cod liver oil
20	51	
23	56	
26	61	
27	63	
30	69	
Feb 1	72	
3	74	
6	73	
9	77	
11	76	
13	79	
15	80	

Rat #4 Vitamin A
2 Units of Cod Liver Oil.

Date	Weight	Special observations.
Nov 1	29	
3	26	Marking end of tail
5	27	
9	27	
12	31	
15	34	
17	35	
19	34	Placed on Vitamin A deficient diet on
21	35	Nov 20
25	35	
29	36	
Dec 1	36	
4	38	
7	40	
9	41	
11	41	
15	42	
17	44	
20	45	
23	45	
27	44	
30	46	
Jan 3	50	
6	50	
9	48	
13	48	
18	50	Jan 18 - 1 drop of 2 units cod liver oil
20	51	- has traces of xerophthalmia
23	54	clear Jan.23
26	55	
27	56	
30	60	
Feb 1	62	
3	61	
6	60	
9	64	
11	62	
13	66	
15	67	

Rat #5 Vitamin A - 1 Unit Cod Liver Oil
Age at start 30 days.

Date	Weight	Special observations. Marked left front paw
Nov 1	32	
3	30	
5	35	
9	37	
12	44	
15	46	
17	46	
19	46	Placed on Vitamin A deficient diet Nov 20/26
21	46	
25	44	
29	50	
Dec 1	50	
4	50	
7	51	
9	53	
11	55	
15	53	
17	54	
20	54	
23	55	
27	54	
30	56	
Jan 3	58	
6	59	
9	55	
13	57	
18	58	Jan 18 - 1 drop of 1 unit Cod Liver Oil Xerophthalmia in left eye, slight case
20	56	
23	59	Left eye clearing
26	61	Both eyes clear
27	62	
30	66	
Feb 1	66	
3	68	
6	66	
9	67	
11	66	
13	66	
15	67	

Rat #6 Vitamin A 0.1 unit Dogfish Liver Oil
Age at start 30 days.

Date	Weight	Special observations. Marking right front paw
Nov 1	23	
3	21	
5	24	
9	24	
12	30	
15	32	
17	32	
19	32	Placed on Vitamin A deficient diet Nov 20/26
21	32	
25	30	
29	34	
Dec 1	34	
4	34	
7	36	
9	36	
11	37	
15	35	
17	39	
20	40	
23	40	
27	40	
30	42	
Jan 3	44	
6	46	
9	45	Xerophthalmia in both eyes. Bad
13	48	
18	49	1 drop 0.1 Unit Dogfish Liver Oil
20	50	Jan 21. Xerophthalmia worse.Right eye almost closed.
23	47	No change in condition
26	46	Both eyes nearly closed
27	47	
30	50	Right eye swollen
Feb 1	53	
3	52	
6	52	Both eyes closed
9	54	Drying up. Getting a little clearer
11	55	Dry, but closed
13	57	
15	58	

Rat #7 Vitamin A
1 Unit Dogfish Liver Oil.

Date	Weight	Special observations. Marking left hind paw
Nov 1	24	
3	23	
5	25	
9	25	
12	30	
15	32	
17	33	
19	32	Nov 20. Placed on Vitamin A deficient diet
21	32	
25	34	
29	35	
Dec 1	35	
4	37	
7	38	
9	40	
11	40	
15	42	
17	43	
20	46	
23	46	
27	46	
30	48	
Jan 3	49	
6	52	
9	48	
13	52	
18	52	1 drop of 1 Unit Dogfish Liver Oil Slight xerophthalmia in both eyes
20	52	Left eye clear. Slight in right
23	55	Right eye clearing
26	60	Both eyes clear
27	61	
30	66	
Feb 1	67	
3	67	
6	69	
9	71	
11	77	
13	76	
15	80	

Rat #8 Vitamin A
10 Units Dogfish Liver Oil

Date	Weight	Special observations Marking Right hind paw
Nov 1	32	
3	31	
5	32	
9	34	
12	40	
15	44	
17	44	
19	44	Placed on Vitamin A deficient diet Nov 20/26
21	44	
25	44	
29	46	
Dec 1	47	
4	49	
7	50	
9	50	
11	52	
15	51	
17	52	
20	53	
23	53	
27	52	
30	53	
Jan 3	55	
6	56	
9	52	
13	54	
18	55	Xerophthalmia in left eye, fairly bad 1 drop of 10 units Dogfish Liver Oil
20	58	Eyes almost clear of xerophthalmia Jan 21. Both eyes clear
23	62	
26	65	Eyes normal
27	66	
30	73	
Feb 1	74	
3	74	
6	76	
9	80	Absolutely normal
11	80	
13	81	
15	82	

Rat #9 Vitamin A
100 Units Dogfish Liver Oil

Date	Weight	Special observations.
Nov 1	30	
3	28	
5	32	
9	36	
12	42	
15	44	
17	44	
19	42	Placed on Vitamin A deficient diet Nov 20/26
21	44	
25	44	
29	44	
Dec 1	44	
4	46	
7	47	
9	47	
11	48	
15	47	
17	48	
20	50	
23	50	
27	53	
30	54	
Jan 3	54	
6	57	
9	54	
13	55	
18	56	Xerophthalmia in both eyes. Very bad case 1 drop 100 Units Dogfish Liver Oil
20	57	Both eyes clearing
23	62	Left eye clear. Right eye trace
26	66	Both eyes clear
27	67	
30	74	
Feb 1	80	
3	83	
6	82	
9	85	
11	86	
13	92	
15	96	

Rat #20 Vitamin A 1 Unit Cod Liver Oil
Placed on deficient diet immediately.

Date	Weight	Special observations.
Dec 20	34	
23	30	
27	32	
30	36	
Jan 3	38	
6	40	
9	40	
13	43	
20	45	Slight signs of xerophthalmia
23	47	Left eye almost closed
26	45	Both eyes very bad
27	44	Condition same, 1 drop Cod Liver Oil one unit
30	45	Right eye-ball broken
Feb 1	48	Both eyes swollen twice normal size
3	50	Worse
6	48	Eye ball protruding
9	49	Left eye closed, swollen. Right eye a trifle better
11	50	Fur around eyes coming out
13	49	Left eye ball gone.

Rat #21 Vitamin A 1 Unit Dogfish Liver Oil
Placed on deficient diet immediately.

Date	Weight	Special observations.
Dec 20	36	
23	32	
27	34	
30	36	
Jan 3	36	
6	38	
9	38	
13	38	
20	40	
23	44	Slight signs of xerophthalmia
26	44	Both eyes bad
27	45	Worse. 1 Unit Dogfish Liver Oil
30	47	Both eyes clearing
Feb 1	50	" " clear
3	52	
6	52	
9	56	Normal
11	56	
13	58	

Rat #22 Vitamin A 0.5 Unit Dogfish Liver Oil
Deficient diet.

Date	Weight	Special Observations.
Dec 20.	35	
23	30	
27	33	
30	34	
Jan 3	34	
6	36	
9	34	
13	36	
20	38	Xerophthalmia in both eyes
23	40	" bad in left eye and trace in right
26	40	" both eyes bad
27	40	" worse. 0.5 Unit Dogfish Liver Oil
30	43	Right eye swollen
Feb 1	48	Both eyes almost clear
3	50	Left eye clear. Right, trace
6	50	Both eyes clear. Lids still swollen
9	52	Both eyes clear. Lids trifle better
11	52	Swelling almost gone
13	55	Normal.

Rat #23 Vitamin A 0.2 Unit Dogfish Liver Oil

Date	Weight	Special Observations.
Dec 20	34	
23	30	
27	34	
30	34	
Jan 3	33	
6	35	
9	34	
13	34	
20	39	Xerophthalmia in left eye. Slight
23	42	" trace in both eyes
26	43	" both eyes fairly bad
27	42	" bad. 0.2 Units Dogfish Liver Oil
30	43	Both eyes dry. Swollen
Feb 1	44	Both clearing
3	47	" "
6	50	" " lids swollen
9	53	" clear " better
11	53	Eyes almost normal
13	56	" " "

Rat #24

Rat #25

Both Controls.

Date	Weight		Date	Weight	
Dec. 20	32		Dec. 20	34	
23	30		23	33	
27	34		27	35	
30	34		30	40	
Jan 3	38		Jan. 3	43	
6	46		6	44	
9	48		9	46	
13	48		13	44	
20	49		20	45	
23	53	Both eyes clear	23	52	Both eyes clear
26	54		26	58	
30	60		30	59	
Feb 1	61		Feb 1	63	
3	64		3	63	
6	60		6	63	
9	60		9	62	
11	60		11	61	
13	60		13	60	