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# THE DETERMINATION OF THE VITAMIN D IN THE LIVER OIL OF THE DOG-FISH, SQUALUS SUCKLII (GIRARD).

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HORACE N. BROCKLESBY.

PRINCE RUPERT, 1928.

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**No. 30**

**Title**

THE DETERMINATION OF THE VITAMIN D IN THE LIVER OIL OF THE DOG-FISH, SQUALUS SUCKLII (GIRARD).

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# The determination of Vitamin D in the Liver Oil of the Dog-fish, Squalus Sucklii (Girard).

Introduction.- In a previous manuscript [1] it has been shown that the liver oil of the dog-fish, Squalus sucklii, has from five to ten times the Vitamin A potency of the best grade medicinal cod liver oil. Inasmuch as medicinal and poultry oils are usually graded on their Vitamin D content, it seemed of importance to determine the content of this vitamin in dog-fish liver oil. This manuscript gives in some detail the results of the determination.

The only data published giving the actual content of the Vitamin D in dog-fish liver oil is that of C.E.Bills[2]. This author, rating medicinal cod liver oil arbitrarily at 100, places dog-fish liver oil at 3. In a private communication from Bills, it was found that the samples examined were from the Coast of Newfoundland and were two in number. Bills states that he examined also two samples from the Pacific Coast but notbeing sure of their origin did not include the results in his paper. They gave, however, about the same results.

The two oil soluble Vitamins, A and D, have been found associated in many natural products. In view of the high Vitamin A potency it would be natural to expect a relatively high potency of Vitamin D in dog-fish liver oil. However, as Doctor A.T. Cameron has pointed out in a private communication the fact of the dog-fish being an Elasmobranch might have some relation to the retention or non-retention of vitamins. As the type of food eaten by the dog-fish might possibly affect the potency of the liver oil, samples from fish caught at different times of the year and feeding on

different foods have been examined.

Methods.- All samples of oil examined were expressed from the livers of freshly caught fish. The livers were minced immediately after removal from the fish, and steamed half an hour with wet steam in a covered container. The oil was then skimmed from the top, dehydrated with sodium sulphate and filtered. In one case the oil was not dehydrated, but was cold cleared at 5 degrees C.

White rats were used for the assay. The animals used were from carefully bred stock housed in a specially built animal room. This room had two sections, one well lighted by a large window, and the other illuminated by a small electric lamp bulb only. The colony rats were kept in the lighted portion, whilst the experimental animals were kept in the dark section. The room was electrically heated and kept at approximately 25 degrees C. The cages were of the type described by Greenman and Duhring[3]. The whole stock colony was fed on Bills modification[4] of Steenbock's diet which consists of the following ingredients:

| | | | |
|---|---|---|---|
| Ground yellow corn | 76 parts | Ground Alfalfa | 2 parts |
| Linseed Oil Meal | 16 " | Iodized salt | .5 " |
| Grude Casein | 5 " | Calcium carbonate | .5 " |

To every three parts by weight of this mixture was added one part of whole dried milk (klim).
To every 500 grams of this dried mixture was added one yeast cake and the juice and rind (pulverized) of two oranges. The whole was heated with a little water in a double boiler for about 15 minutes, then about 50 grams of butter were added.

This cooking of the colony stock diet was adopted for two reasons. The first reason was that sterilization was obtained, and the second was that, as Hartwell[5] has shown, cooked diets give better results than raw diets. It has also the added advantage of making the mixture more homogenous, and the rat has less chance of picking out parts of the mixture and discarding the rest.

There are two general methods used in judging the Vitamin D potency of an oil. In both of them the experimental rats are fed on a diet high in calcium and low in phosphorous besides lacking Vitamin D. This type of diet normally produces rickets in young growing rats in about eighteen days. In the preventive type of experiment, the oil is fed concurrently with the rachitic diet, and the amount of protection given by the oil under test is decided by chemical analysis of the bones, together with radiographic and histological evidence. In the second, the curative type, the rats are allowed to develop rickets, and the smallest amount of substance under test necessary to effect a cure is determined. The commencement of healing is usually diagnosed by means of the "line test", pH of the faecal suspensions and radiography. Both methods have been used in the present investigation.

The analyses of the bones were made by the methods outlined by Chick, Korenchevsky and Roscoe [6]. The following values were usually determined; Moisture, fat, fat-free organic matter, ash, percentage of calcium calculated on the dry fat-free bone, and the ratio (A/R) of the ash residue to the amount of fat-free organic matter.

The radiographs were made with a Coolidge Dental apparatus. The regular Eastman dental films were used. The technique employed was practically the same as that recently described by Poulsson [7]. The animal was placed in a light aneasthesia and the left hind leg gently stretched until straight. A radiograph of the knee joint was then made. The pictures were taken from the anterior aspect, care being taken to keep the distance between tube and object always the same. An exposure

of 1.25 seconds was usually given.

The diagnosis was made from the negative, the latter being viewed through a piece of millimeter ruled glass illuminated by a small lamp. The width of the uncalcified band was then measured as accurately as possible. For a rat of about fifty days of age, a band of 1 mm. was judged to have a degree of rickets of 1+, 2 mm. 2+, etc. This method was not thought sufficiently accurate to judge of the degree of healing or rickets, since the new band of calcification was, in the early stages, very narrow and honeycombed, and did not give a clean, well-defined image. The radiograph has therefore been used only to decide whether the rats were suffering from a moderate degree of rickets.

The line test, so-called, was that introduced by McCollum (8). The directions given by Bills and co-workers(9) were strictly adhered to. This test was used for the histological diagnosis of the knee joint in the case of the rats used in preventive tests, with the modification that, instead of judging the healing by means of the line of provisional calcification, special attention was paid to the amount of calcification on the diaphyseal side of the cartilaginous disc. By this means the severity of disease could be established.

The faecal pHs were determined by means of a hydrogen electrode of the Hildebrand Type, in a 2% aqueous suspension. A rocking electrode of the type used by Jephcott and Bacharach(10) not being available, a rapid stirrer was used. This made it possible to obtain equilibrium in about five minutes. In all other respects the technique was that suggested by Jephcott and Bacharach.

Chemical analyses for inorganic phosphorous and calcium of the blood serum of rachitic rats were carried out in a few cases by the methods of Benedict-Theis and Clarke-Collip respectively, but, since rats had to be fed in groups in order to get enough blood for a satisfactory analysis, this means of diagnosis was abandoned.

The test oils were administered by hand, by means of dropping pipettes. These pipettes were calibrated for the oil used. The doses were usually administered just prior to the feeding time, i.e. five p.m.

During the oil testing period, a careful record was kept of the weight of the test animals, daily weighings being made. By this means the possibility of healing of rickets by starvation was eliminated. All these data have not been included, a typical example, however, is given in Table VIII.

Two rachitic diets have been used. That of Pappenheimer, McCann and Zucker[11], the first one tried, had the following composition:

| | |
|---|---|
| Patent Flour | 80.9 |
| Egg Albumin | 10.0 |
| Butter Fat | 5.0 |
| Salt Mixture | 4.1 |

Composition of Salt Mixture:

| | |
|---|---|
| KCl | 0.85 |
| $Na_2CO_3$ | 0.85 |
| $MgCO_3$ | 0.286 |
| Ca lactate | 2.000 |
| Ferric citrate | 0.1 |
| KI | 0.0002 |
| $MnSO_4$ | 0.00078 |
| NaF | 0.0024 |
| $KAl[SO_4]_2$ | 0.00024 |
| Total | 4.08962 |

The other diet used was that of Steenbock[12], and was composed of the following ingredients:

| | |
|---|---|
| Ground Yellow Corn | 76% |
| Wheat Glutin | 20% |
| Calcium Carbonate | 3% |
| Sodium Chloride | 1% |

Experimental.-

Experiment 1. This experiment was designed to test the efficiency of the Pappenheimer-McCann-Zucker diet. It was of the preventive type. Twelve rats were placed on this diet when they were 25 days old. They had access to as much of the diet, and tap water as they desired. At the end of eight weeks they were radiographed, and then chloroformed. One half of a knee joint was then examined microscopically, and the remainder of the two hind legs thoroughly cleaned and analyzed. The results of this test are summarized in Table 1.

Experiment 2. Ten rats were placed on the Pappenheimer diet supplemented by daily doses of the oil under test. The experiment extended for a period of fifty days. By this time some of the rats had developed the characteristic "rachitic hop" and it was considered that any difference in the amount of protection afforded by the different doses would now be sufficiently emphasized. The rats were, therefore, radiographed and treated as in experiment 1. The results of this experiment are detailed in Table 2, together with the doses and nature of the oils administered.

Experiment 3. Only three rats were available for this test. The same method as outlined in experiment 2 was followed.

Experiment 4. The curative type of experiment was used in all subsequent work. In this experiment Steenbock's diet was fed to rats 25 days old for a period of 18 days. Daily

TABLE I.

The chemical analysis of the bones of young rats grown on a rachitic diet.

| Rat No. | Gain in Wt. | Moist % | Fat % | Organic Matter % | Ash % | A/R ratio |
|---|---|---|---|---|---|---|
| 1 | 44 gms | 51.79 | 4.11 | 27.93 | 16.21 | 0.582 |
| 2 | 55 " | 50.41 | 7.63 | 25.68 | 16.92 | 0.634 |
| 3 | 55 " | 53.38 | 1.77 | 28.79 | 16.13 | 0.561 |
| 4 | 71 " | 53.77 | 2.06 | 28.85 | 15.29 | 0.528 |
| 5 | 44 " | 41.10 | 8.26 | 31.99 | 18.80 | 0.584 |
| 6 | 48 " | 39.69 | 3.52 | 35.06 | 21.64 | 0.617 |
| 7 | 72 " | 28.32 | 4.53 | 37.40 | 29.80 | 0.796 |
| 8 | 48 " | 42.85 | 4.81 | 30.55 | 21.85 | 0.717 |
| 9 | 59 " | 16.31 | 10.81 | 41.80 | 31.10 | 0.744 |
| 10 | 74 " | 34.50 | 3.74 | 33.92 | 25.44 | 0.749 |
| 11 | 41 " | 15.72 | 8.16 | 47.41 | 31.21 | 0.658 |
| 12 | 21 " | 47.10 | 8.26 | 28.18 | 16.43 | 0.583 |

doses of the test oils were then administered for varying periods of time. The line test only was used as a means of diagnosis. The results are given in Table 4.

Experiments 5,6,7 and 8. The next four experiments were carried out as follows: 25 day old animals were placed in one large cage and fed Steenbock's diet ad. lib. for a period of eighteen days. During this preparatory period they were weighed daily in a group. The faecal pHs were determined every alternate day. At the end of the eighteenth day they were radiographed and placed in pairs in separate cages. The oils under test were now administered daily. Daily weighings and faecal pH determinations were also made. At the end of six days the animals were radiographed again and killed. The hind leg was then removed and the tibia dissected. The line test was then carried out.

In each experiment of this investigation suitable negative controls were used. In the curative tests one control was killed and autopsied at the beginning of the oil-feeding period. If, and this was invariably the case, the results of the line test showed well defined rickets, the other rats of the group whose radiographs were similar or more severe than that of the control, were considered to be ready for the test. In this way any individual variation of the rats which might have tended to produce unreliable results was eliminated.

Discussion.- The results given in Table 1 show conclusively that the 12 rats used in that experiment had all developed severe rickets. Chick, Korenchevsky and Roscoe,(6) from the results of a large number of analyses, gave the follow- average figures for the analyses of bones of young rats grown up on a completely adequate and also on a rachitic diet.

TABLE II

| Rat No. | Daily Dose of Oil | Analysis of Bones | | | | | Ca % of dry fat-free bone | Histological | Radiograph |
|---|---|---|---|---|---|---|---|---|---|
| | | Moist. | Fat. | Org. Res. | Ash | A/R | | | |
| 22 | D.F.L.O. 8.7 mgms. | 58.24 | 1.53 | 27.85 | 11.53 | 0.414 | 9.35 | Advanced rickets. Not severe | Rickets. Very slight protection. |
| 23 | D.F.L.O. 8.7 mgms. | 59.50 | 1.23 | 27.70 | 11.59 | 0.418 | 10.94 | Advanced rickets. Not severe | Rickets. No protection. |
| 24 | Cod Liver Oil 17.5 mgms | 53.80 | 5.75 | 24.19 | 16.11 | 0.667 | 14.76 | Very mild rickets | Protection. Very slight sign of band. |
| 25 | Cod Liver Oil 17.5 mgms. | 53.88 | 5.48 | 24.55 | 16.00 | 0.654 | 14.59 | Mild rickets | Protected. |
| 26 | D.F.L.O. 17.5 mgms. | 57.81 | 3.08 | 24.10 | 14.85 | 0.616 | 14.08 | Slightly rachitic | Not fully protected |
| 27 | D.F.L.O. 17.5 mgms | 47.20 | 7.78 | 23.82 | 21.20 | 0.889 | 17.41 | Very mild rickets | Protected |
| 28 | D.F.L.O. 67 mgms. | 52.6 | 4.79 | 22.39 | 20.50 | 0.899 | 17.48 | Practically normal | Protected |
| 29 | D.F.L.O. 67 mgms. | 57.15 | 3.86 | 22.68 | 16.36 | 0.723 | 15.53 | Practically normal | Protected |
| 30 | None | 55.81 | 1.95 | 29.51 | 12.62 | 0.428 | 11.12 | Severe rickets | No protection |
| 31 | None | 59.98 | 1.60 | 26.78 | 11.52 | 0.431 | 11.14 | Severe rickets | No protection |

| Diet | Analysis of fresh bone | | | | Calcium % on fat free Wt. | A/R ratio. | Histology. |
|---|---|---|---|---|---|---|---|
| | $H_2O$. | Fat. | Ash. | Org.Res. | | | |
| Complete Diet | 41.0 | 2.4 | 34.0 | 22.6 | 21.5 | 1.50 | Normal. |
| McCollum 3143 diet | 60.5 | 4.25 | 11.3 | 24.0 | 12.0 | 0.468 | Severe rickets. |

These authors state that the chemical composition of rachitic bone is found to be characterized by; (1) The content of mineral ash (A) being much lower than normal, (2) the content of water being much above normal, (3) slightly increased proportions of fat and organic material (R), (4) a very low value of the A/R ratio i.e. from 0.4 to 0.8, which ratio is, in the case of normal rats, over 1.

All the findings given in Table 1, fall within the limits of the average values for rachitic animals. Consistent results were obtained when these methods were used for diagnosing the severity of rickets.

The dog-fish liver oil used in the second experiment was expressed from the livers of fresh fish caught off Malcolm Island in the Queen Charlotte Straits. The cod liver oil used was a sample bought on the open market, and was manufactured by Parke Davis Co.

The results given in Table 2 show that 17.5 mgms. per day of both the dog-fish liver oil and the cod liver oil were sufficient to afford the rats some protection from the effect of the rachitic diet, but in neither case was this fully realized. Practically complete protection was given by 67 mgms. of dog-fish liver oil, whilst 8.7 mgms. gave no protection whatsoever. The two controls had evidently developed a very severe type of rickets.

The three methods of diagnosis used in this experiment checked each other very satisfactory. According to this type of

Table III.

Dog-fish Liver Oil, Harbor October, 1927.

| Rat No. | Daily Dose | Line Test. |
|---|---|---|
| 32 | 20 mgms for 5 days | Fragmentary deposits. Grades from + to + + |
| 39 | 20 mgms. during exp. period, 24 days | Mild to moderate rickets. Very slight protection. |
| 40 | -Do- | Moderate to bad rickets. No protection. |

Table IV.

Dog-fish Liver Oil, Harbor, January, 1928.

| Rat No. | Daily Dose | Line Test. |
|---|---|---|
| 47 | Neg. control | Florid rickets. |
| 48 | C.L.O. Parke Davis 20 mgms. | Healing Degree + + + |
| 49 | D.F.L.O 20 mgms. for 5 days | Florid rickets. No sign of healing. |
| 50 | D.F.L.O. 50 mgms. for 10 days | Very slight signs of healing about + |
| 51 | D.F.L.O. .66 mgms. for 16 days | Positive healing + + + to + + + + |
| 52 | Neg. control | Florid rickets. |

exp riment, then, this dog-fish liver oil was as efficient for protection against rickets as medicinal cod liver oil. These results, however, were not substantiated when the curative type of procedure was used.

Harbour caught dog-fish were used in experiment 3. These fish were caught in the Prince Rupert Harbour during the winter of 1927-1928. The results show that 20 mgms. of this oil contained enough of Vitamin D to induce healing in 5 days as judged by the "line test". In this case the bones were also analyzed, and proved conclusively that the rat had rickets. The small amount of calcification which had taken place in the 5 days was not sufficient to effect the chemical analysis. The other two rats in this experiment, receiving the same dose as the previous rat for a period of 24 days, gave no signs of protection. The results of this experiment are rather anomalous, since they show positive results for the curative type and negative for the preventive, whereas usually the reverse is the case.

Another sample of Harbour caught fish was used in experiment 4. This was from fish caught during January, 1928. As judged by the "line test", 20 mgms. of Parke Davis cod liver oil was sufficient to induce quite positive healing in 5 days. The same amount of dog-fish liver oil for the same period of time gave no signs of healing. When 50 mgms. were fed for 10 days very slight signs were evidenced, whilst 66 mgms. for 16 days gave very positive healing. The negative controls in both cases show very bad rickets. The oil, therefore, could not be rated above 10% of the Vitamin D potency of cod liver oil.

Table 5 gives the results obtained from the type of experiment which we have now adopted as more or less standard procedure. The dog-fish liver oil fed to the experimental animals in this test

Table V.

Dog-fish Liver Oil - West Coast Vancouver Island, April, 1928.

| Rat No. | Daily Dose. | Exp time days | Radiograph Prep. period. | Radiograph Test period. | Faecal pH Averages. Prep. period. | Faecal pH Averages. Test period | Line Test | Drop in pH. |
|---|---|---|---|---|---|---|---|---|
| 58 | Neg.Control | | R+ | | 7.61 | | Mod. rickets. | |
| 59 | .36 mgms. | 6 | R++ | Healing | 7.61 | 6.98 | Healing ++to+++ | 0.63 |
| 60 | .36 " | 6 | R+ | Slight signs | 7.61 | 6.98 | Doubtful healing | 0.63 |
| 61 | Control | | R+++ | | 7.56 | | Mod. rickets ++ to+++ | |
| 62 | Control | | R++ | R++ to+++ | 7.56 | 7.20 | Severe rickets | |
| 63 | 72 mgms. | 6 | R++ | Healing | 7.80 | 6.81 | Healing++ to+++ | 0.79 |
| 64 | 72 " | 6 | R++ | Healing | 7.80 | 6.81 | Healing++ | 0.79 |
| 65 | .108 " | 6 | R++ | Healing | 7.69 | 6.70 | Healing + to ++ | 0.99 |
| 66 | .108 " | 6 | R+ | Healing | 7.69 | 6.70 | Healing++ | 0.99 |
| 67 | .126 " | 6 | R++ | Healing | 7.58 | 6.53 | Healing +++ | 1.05 |
| 68 | .126 " | 6 | R++ | Healing | 7.58 | 6.53 | Healing++ to+++ | 1.05 |
| 69 | .10 " | 6 | R++ | R +++ | 7.31 | 7.17 | Bad rickets. No sign of healing | 0.14 |
| 70 | 10 " | 6 | R++ | R+++ | 7.31 | 7.17 | Doubtful signs of healing | 0.14 |
| 71 | 50 " | 6 | R++ | R+++ | 7.31 | 7.17 | Bad rickets. No sign of healing. | 0.14 |

was obtained from the livers of fresh dog-fish caught off the West Coast of Vancouver Island, near Ucluelet in April, 1928. The fish in this case were feeding, or had recently been feeding upon spawning herring, which were at that time running into the numerous bays and inlets of that district.

At the end of the preparatory period it will be seen that all the experimental rats had wekl defined rickets, as shown by the radiograph. The negative control which was killed at this time showed by the radiograph a degree of rickets of R+, whilst the "line test" gave a reading of moderate rickets. Practically all the other rats showed a severity of rickets as great or greater than this, therefore it was thought justifiable to assume that the rats were ready for the test. It will be noted that the faecal pHs are given as averages, that is to say, the average of the faecal pH determinations before the oil feeding, has been listed under:- "Average faecal pH of the Prep. Period"; whilst the average of the determinations during the oil feeding period are listed under:- "Average faecal pH of the Test Period". The drop in the pH is listed separately.

The drop in the faecal pH is roughly proportional to the increased doses of oil, and very doubtful healing by the radiograph and "line test" was pooduced by 36 mgms. of this oil. Healing in this case had no doubt commenced, the faecal pH dropping 0.63 of a unit. Another rat receiving 50 mgms. had however no signs of healing, whilst 10 mgms. also produced negative results. It seems that the minimum amount of oil required to give positive healing is about 72 mgms. per day for a period of six days. For this amount and over i.e. 108 and 126 mgms., positive results were obtained. The three methods of

Table VI.

| Rat | Oil | Dose | Radiograph. Prep. period | Radiograph. Test Period | pH. Prep. period | pH. Test Period. | Drop in faecal pH. | Healing by Line Test. |
|---|---|---|---|---|---|---|---|---|
| 72 | Harbor-Feb., 1928 | .46 mgms | R+++ | R+++ | 7.49 | 7.09 | | None |
| 73 | " | 46 " | R++ | R+++ | 7.49 | 7.09 | 0.40 | None |
| 74 | " | 92 " | R++ | R+++ | 7.49 | 7.26 | | None |
| 75 | " | 92 " | R++ | R+++ | 7.49 | 7.26 | 0.23 | None |
| 76 | " | 184 " | R++ | R+++ | 7.41 | 6.77 | | Slight signs about + |
| 77 | " | 184 " | R++ | R+++ | 7.41 | 6.77 | 0.64 | Doubtful healing |
| 78 | Straits of Georgia Apr.,1928 | 100 mgms | R++ | Healing | 7.41 | 7.20 | | Healing + |
| 79 | " | 100 " | R++ | " | 7.41 | 7.20 | 0.21 | Healing + |
| 80 | " | 60 " | R++ | R++ | 7.41 | 7.35 | | Slight signs? |
| 81 | " | 60 " | R+++ | Healing. | 7.41 | 7.35 | 0.06 | Healing + |
| 82 | " | 20 " | R++ | R++ | 7.41 | 7.38 | | Doubtful |
| 83 | " | 20 " | R++ | R+++ | 7.41 | 7.38 | 0.03 | Slight signs |
| 84 | " | 10 " | R++ | R++ | 7.41 | 7.30 | | Doubtful |
| 85 | " | 10 " | R++ | R+++ | 7.41 | 7.30 | 0.11 | Doubtful |
| 86 | Negative Control | | R++ | killed | 7.41 | -- | -- | Moderate rickets |
| 87 | " | | R++ | R+++ | 7.41 | 7.85 | -0.44 | Bad rickets. |

Table VII.

| Rat | Oil | Dose | Radiograph Prep. period | Radiograph Test period | pHs. Prep. period | pHs. Test period | Healing by "Line test" | Drop in Faecal pH |
|---|---|---|---|---|---|---|---|---|
| 88 | Negative Control | | R++ | | 7.67 | | Moderate rickets | |
| 89 | " | " | R+++ | R+++ | 7.67 | 7.68 | Bad rickets+++ | -0.01 |
| 90 | Cod Liver Oil Poultry Grade | 20 mgms. | R++ | R++ | 7.67 | 7.36 | None | .31 |
| 91 | " | 20. " | R++ | R++ | 7.67 | 7.36 | Doubtful | .31 |
| 92 | " | 10. " | R++ | R++ | 7.67 | 6.83 | None | -.16 |
| 93 | " | 10. " | R++ | R+++ | 7.67 | 6.83 | None | -0.16 |
| 94 | " | 2. " | R++ | R++ | 7.67 | 7.26 | None | .41 |
| 95 | " | 2 " | R++ | R++ | 7.67 | 7.26 | None | .41 |
| 104 | " | 40. " | R+++ | Healing | | | Positive healing++ | |
| 105 | " | 40. " | R+++ | Healing | | | Positive healing++ | |

Table VIII.

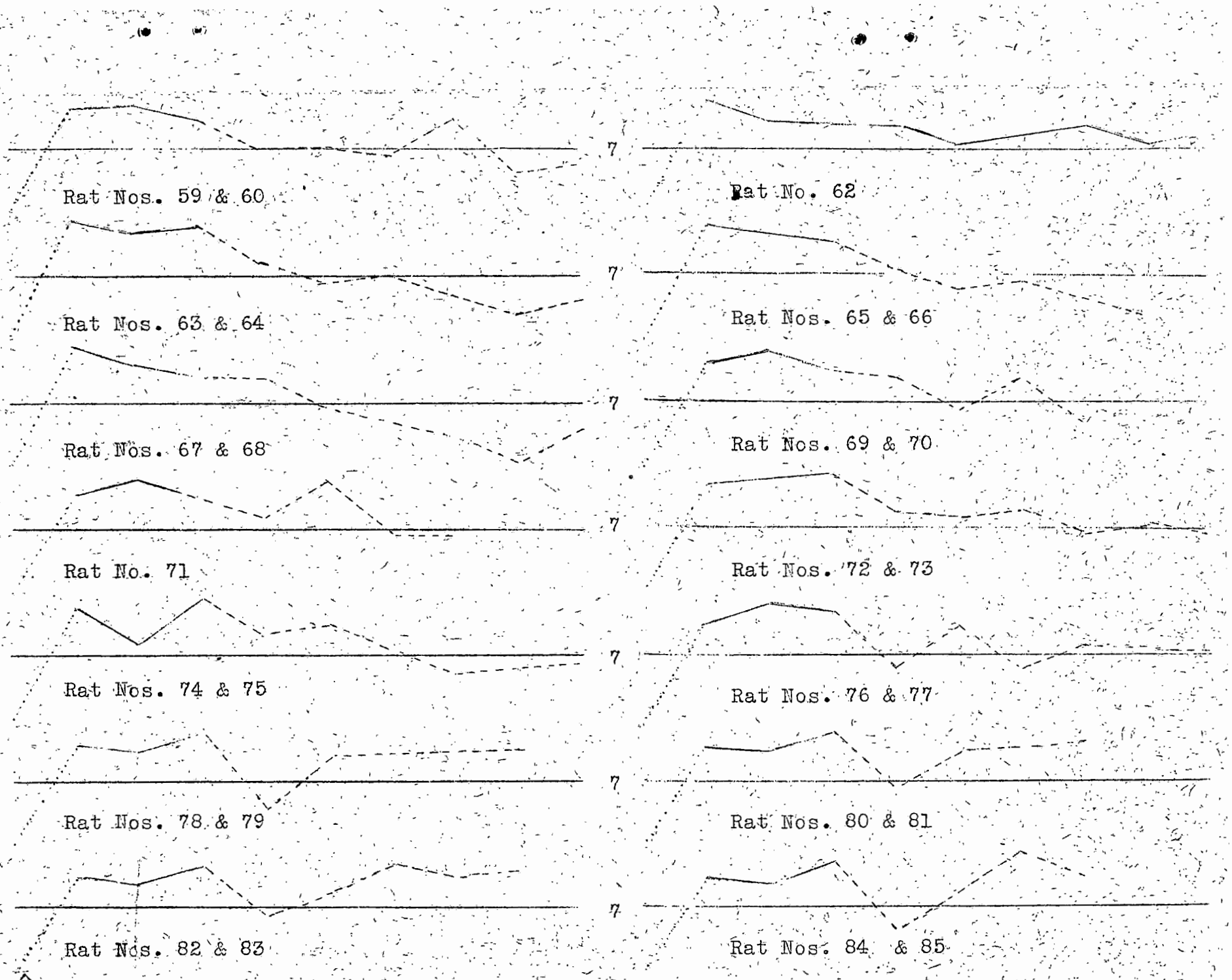
Typical Weight increases of Rats during oil feeding period.

| Rat No. | Weight at beginning of oil feeding period. | Days of oil feeding. | | | | |
|---|---|---|---|---|---|---|
| | | 1. | 2. | 3. | 4. | 5. |
| 72 & 73 | 121 | 118 | 122 | 122 | 125 | 123 |
| 74 & 75 | 110 | 109 | 110 | 112 | 115 | 117 |
| 76 & 77 | 105 | 104 | 103 | 104 | 105 | 108 |
| 78 & 79 | 78 | 76 | 76 | 76 | 78 | 80 |
| 80 & 81 | 84 | 80 | 80 | 81 | 81 | 84 |
| 82 & 83 | 84 | 84 | 82 | 83 | 85 | 88 |
| 84 & 85 | 83 | 83 | 83 | 83 | 87 | 84 |
| 87 | 40 | 40 | 41 | 41 | 42 | 43 |

diagnosis check tolerably well.

Another sample of oil from the Prince Rupert Harbour, February, 1928, gave very doubtful results, even when fed at the level of 184 mgms. of oil per day. An oil obtained from the livers of fish caught in the Straits of Georgia in April, 1928, shows a minimum requirement for positive healing of about 100 mgms. These results are given in Table 6.

Since dog-fish liver oil, if used at all for its medicinal properties would probably be used primarily as a poultry oil, it was thought wise to subject a sample of poultry grade cod liver oil to the same method of assay. This was done, a sample being obtained in the open market.for the purpose. The results are presented in Table 7. This table shows that 40 mgms. per day for a period of 6 days is the minimum dose of this oil necessary for positive healing. This dose is exactly twice that required for medicinal grade of cod liver oil.


7
Rat Nos. 59 & 60
Rat No. 62
Rat Nos. 63 & 64
Rat Nos. 65 & 66
Rat Nos. 67 & 68
Rat Nos. 69 & 70
Rat No. 71
Rat Nos. 72 & 73
Rat Nos. 74 & 75
Rat Nos. 76 & 77
Rat Nos. 78 & 79
Rat Nos. 80 & 81
Rat Nos. 82 & 83
Rat Nos. 84 & 85
1 pH

FAECAL pHs

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