THE TOXIC EFFECT OF FISH LIVER OILS, AND THE ACTION OF VITAMIN B.

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3 years ago in the biological assay of fish liver oils for vitamin A, it was noticed that relatively small amounts of some fish liver oils (less than 2 per cent of the diet) had a very deleterious effect, and a systematic study was initiated to determine what factor or factors of the oil were toxic or injurious.

Previously the toxic effects of cod liver oil had been noticed and efforts made to correlate the effect with hypervitaminosis of one of the fat-soluble vitamins, the excess of free fatty acids, etc. However the published results are very contradictory. Mouri-quand and Michel (1) in 1922 reported a noxious influence of large doses of cod liver oil in scurvy, especially when the diet is lacking not only in vitamin C, but also in certain amino acids and salts. In 1925 Slagswold (2) noted poisoning among cattle in Sweden by cod liver oil, stiffness, listlessness, tremors, loss of appetite, convulsions, etc. being produced and death resulting in a few hours or days; while rodents thrived on the same oil. Takahashi described a toxic effect of biosterin with paralysis in the rear extremities when fed at 10,000 times the growth-promoting dose. His results were not duplicated in the work of Drummond, Channon, and Coward (3). Von Euler and Widell (4) found that when the dosage of vitamin D increased many times, rats on marmite and lemon juice, sufficient under normal conditions, died in about 4 months. Agduhr (5) in a series of articles (1926–1929) reported the toxic effect of cod liver oil on mice. He stated that the poisonous effects are greatly influenced by the composition of the basal diet and its content of vitamins B and C, as well as warm weather and sunshine. Also that the susceptibility varies very much in different individuals of the same species and different species of animals show variations. The white rat is only susceptible in small degree.
In testing oils for vitamin A a diet was used consisting of 65 per cent corn-starch, 20 per cent alcohol-extracted casein, 4 per cent Osborne and Mendel (6) salt mixture, 1 per cent sodium chloride, and 10 per cent dried yeast. To insure sufficient vitamin D the animals were irradiated 10 minutes three times a week at 25 inches with a 110 volt, Alpine sun, quartz mercury vapor lamp. The oil to be tested was fed over a wide range in amount and it was sometimes noticed that at the higher levels after 3 or 4 weeks there was a decrease in weight; the animals grew in length of body and apparent size but, being extremely thin, finally developed neurotic symptoms and paralysis, while at lower levels the animals grew at a normal rate. As the experimental animals on large doses of cod liver oil and a basal diet containing 10 per cent yeast showed symptoms so closely resembling vitamin B deficiency the yeast content was increased in some cases, and was immediately followed by rapid increase in weight. Also the apparent toxic effect of cod liver oils was not uniform for different oils and therefore must have been due to some variable substance in the oil. The first variable factors which suggest themselves are the two known fat-soluble vitamins, A and D.

Systematic feeding experiments were made with several fish liver oils known to vary considerably in vitamin content. Two basal diets were used; one was the 10 per cent basal diet described above, and the second was the same except that it contained 18 per cent yeast. The oil was fed separately in amounts varying up to approximately 0.2 gm. per day, after the usual depletion period for vitamin A. Fig. 1 shows a few of the characteristic feeding curves obtained with two cod liver oils. Oil P1 was known to have approximately 5 times the vitamin A potency of Oil S. With Oil S on the diet containing 10 per cent yeast, animals receiving 0.0007 gm. of oil per day showed a very marked ophthalmia and other symptoms of vitamin A deficiency with slight

Fig. 1. Growth curves of rats on basal diets containing 10 per cent and 18 per cent yeast and receiving various amounts of cod liver Oils S and P1. Rats 1, 2, 9, and 10 received 0.0007 gm. of oil per day; Rats 3, 4, and 11, 0.006 gm. per day; Rats 5, 6, 12, and 13, 0.052 gm. per day; and Rats 7, 8, 14, and 15, 0.207 gm. per day of Oil S. Rats 16, 17, 24, and 25 received 0.0017 gm. per day; Rats 18, 19, 26, and 27, 0.016 gm. per day; Rats 20, 21, 28, and 29, 0.05 gm. per day; and Rats 22, 23, 30, and 31, 0.198 gm. per day of Oil P1.
growth; with larger amounts of the oil improvement in growth was obtained to almost normal growth at 0.052 gm. per day. With 0.207 gm. of oil the animals were apparently normal for 3 to 4 weeks, after which they lost weight rapidly becoming thin and at the end of 8 weeks showing the picture of vitamin B deficiency. With the same levels of oil but on a diet containing 18 per cent yeast a similar vitamin A deficiency was observed at low levels. However when fed 0.207 gm. of oil the animals showed normal rate of growth. Oil P1 gave practically normal growth for all levels of oil from 0.0017 to 0.198 gm. per day on both 10 and 18 per cent yeast.

In order to make a comparison of the results on the several oils tested, where both male and female rats were used, the data were calculated to percentage of normal growth. Normal for both male and female under the conditions in our laboratory was obtained by averaging a large number of each respective group; average normal growth over an 8 week period after depletion of vitamin A was found to be for males 13.1 gm. per week and for females 9.8 gm. per week. The average of all rats on each level of oil was calculated and the results for four fish liver oils are plotted in Fig. 2.

The relative vitamin A potency of the four oils of Fig. 2 is cod liver Oil S 1.0, Oil L 3.0, Oil P1 5.0, and ratfish liver oil 0.3; from the curves it is very obvious that the toxic effect is not proportional to the vitamin A content. It is therefore not a hypervitaminosis due to excess of vitamin A nor a correlation between vitamins A and B, as Oil L which is 3 times as potent as Oil S showed some toxicity but considerably less than Oil S; Oil P1, 5 times as potent, showed no toxicity at the maximum level fed, while the ratfish liver oil, only 0.3 as potent in vitamin A, showed at least as great a toxicity as Oil S. With the ratfish liver oil it was impossible to obtain normal growth on a diet of 10 per cent yeast as the toxic properties of the oil were manifested long before the oil could be fed in amounts sufficient to satisfy the vitamin A requirements of the animal; however on 18 per cent yeast normal growth was readily obtained with each of the oils.

It will also be noticed, especially with Oil S and the ratfish liver oil, that at low levels of the oil in the region of restricted growth where a vitamin A unit would be obtained there is a very marked
Fig. 2. Comparison of the growth obtained with four fish liver oils in various amounts by rats on basal diets containing 10 per cent and 18 per cent yeast. Circles represent 10 per cent yeast and solid dots 18 per cent yeast.
difference in response on 10 per cent and 18 per cent yeast, so that the unit would be quite different on the two diets. That 10 per cent yeast is ample for vitamin B requirements under certain conditions is shown by the fact that normal response can be obtained with the cod liver oils, especially with Oil P₁ which gives normal growth at all levels tried. Then what interpretation is to be placed upon the statement in the u. s. v. method for determination of vitamin A, "Use sufficient dried brewers' yeast to meet the vitamine B requirements of the animal?"

Many statements are to be found in literature indicating a similar difficulty of decreased growth with increased dosage of cod liver oil, as that of Schmidt-Nielsen (7) in discussing fish liver oils in which he states that a certain oil "proved to be sufficient for a satisfactory growth, when it was applied in daily doses of 1–2 mg. The same results were also obtained with the extracted liver oil and we were disposed to conclude from this result that the content of vitamin A in both samples corresponded to 1000–500 units. This proved, however, to be quite erroneous. The extracted liver oil gives still more excellent growth, if it is applied in great dilutions e.g. in doses of 0.1 mg. a day, and the content of vitamin A is therefore in reality not 1000, but about 10,000 units." Drummond and Morton (8) in speaking of the biological assay state, "But even when the results for each group are averaged, a more serious type of discrepancy remains. This is shown clearly in the lower average rate of growth on 10 mg. of the oil J than on the dose of 7 mg. The same difficulty arises in the case of oil L." Their Oil J shows a growth of 4 gm. per week for a dose of 7 mg. and 2.6 gm. per week for a dose of 10 mg., results somewhat analogous to those we obtained with ratfish liver oil on 10 per cent yeast, where normal growth could not be induced. However, the results of Drummond et al. are for the short and variable period of 3 to 6 weeks, while all experiments in this laboratory are continued for 8 weeks.

The possibility of the yeast's containing a small amount of the growth-promoting factor measured as vitamin A was eliminated by running controls on both the 10 per cent and 18 per cent diets without added vitamin A. Ophthalmia appears at practically the same time and death occurs in the same period of time, as shown by characteristic curves in Fig. 3.
Fig. 3. Growth curves of rats on vitamin A free diets containing 10 per cent and 18 per cent yeast. Rat 260 died on the 34th day of the experimental period. Autopsy showed xerophthalmia, dilated bladder, and large pus pockets in tongue. Rat 261 died on the 42nd day of the experimental period. Autopsy showed vitamin A deficiency symptoms. Rat 282 died on the 23rd day of the experimental period. Autopsy showed vitamin A deficiency symptoms. Rat 284 died on the 30th day of the experimental period. Autopsy showed hemorrhagic lungs, pus pockets at the base of the tongue, apparent fusion of bladder and intestines, and xerophthalmia. Rat 285 died on the 19th day of the experimental period. Autopsy showed dilated bladder with hemorrhage at the base, pus pockets at the base of the tongue, and xerophthalmia. Rat 282 died on the 33rd day of the experimental period. Autopsy showed distended bladder, pus pockets in the roof of the mouth and in the base of the tongue, and xerophthalmia.
That the deleterious effect might be hypervitaminosis due to excess of vitamin D, was ruled out by feeding a series of rats 0.05 gm. of Oil S per day, which would give good growth (but which was the highest level of Oil S that would give good growth on a 10 per cent yeast diet) and by adding excess vitamin D in the form of activated ergosterol in amounts which would be equivalent to approximately 2 and 4 times the amount received with 0.2 gm. of Oil S per day, if the oil is an average oil as stated by the manufacturers. The animals showed normal growth with added ergosterol on both 10 and 18 per cent yeast. The literature on hypervitaminosis caused by high vitamin D content is somewhat contradictory but it seems the vitamin must be present in amounts equivalent to 10,000 to 100,000 times the curative dose to produce a deleterious effect. The amounts fed in the oils used in the above experiments showing toxicity could not have been of this magnitude.

After the above experiments had been completed and we were studying further the cause of the toxic effect, several papers appeared showing a similar effect. Harris and Moore (9) in 1928 studying hypervitaminosis noticed that when 15 per cent cod liver oil was substituted for 15 per cent arachis oil in a ration containing restricted allowances of vitamin B complex, a lower rate of growth was obtained and the animals had rough coats. Also in 1929 (10), they reported that the requirement for marmite is increased pro rata when the excess of cod liver oil concentrate is increased (vitamins A and D). The normal amount of marmite is inadequate with excessive dose of cod liver oil. They considered that probably an antagonistic effect is exerted between vitamin B complex and vitamin A; but they also state that the possible action of other unidentified substances is not excluded. Light, Miller, and Frey (11) in 1929 found that the acute symptoms of vitamin D overdosage can be counteracted at certain levels by liberal feedings of yeast.

In the above experiments of this paper the maximum level of cod liver oils fed was 0.2 gm. of oil per day which would average about 2 per cent of the diet. In testing food substances for vitamin B it has become common practice to add at least 2 per cent of cod liver oil to the diet to supply the required fat-soluble factors. To study the quantitative determination of vitamin B, Sherman and
Spohn (12) in 1923 used two basal diets, in the first of which vita-
mnin A was furnished by 10 per cent butter fat and in the second
by 8 per cent butter fat and 2 per cent cod liver oil; and they
found in testing milk that it required 12 cc. when cod liver oil was
added to the diet to give growth over an 8 week period approxi-
mating that given by 8 cc. without the cod liver oil. They state,
"The reason for this difference is not apparent." Sherman and
Axtmayer (13) in 1927 used 8 per cent butter fat and 2 per cent
cod liver oil in studying the multiple nature of vitamin B. Also
Hunt (14) in 1928 in testing the complex nature of vitamin B used
2 per cent cod liver oil. Croll and Mendel (15) in testing the
vitamin B content of the maize kernel used 5 per cent cod liver
oil. How different would be the results in testing for vitamin B in
the yeast used, or any food stuff, with four different basal diets
each containing 2 per cent of one of the four fish liver oils tested
above (cod liver Oils S, V, P, or ratfish liver oil). At 2 per cent
of the diet each of these oils would furnish ample vitamin A but
each would give an entirely different result for vitamin B in any
substance tested. So that vitamin B determinations with cod
liver oil of unknown toxicity to furnish vitamin A cannot be com-
pared.

Cod liver oil is not a simple mixture of neutral glycerides but
contains some phospholipids as lecithin and consequently choline,
as well as various sterols. Also in 1888 Gautier and Mourgues
(16) found cod liver oils to contain several nitrogenous bases
making up an average of 0.2 per cent of the oil. While the dis-
coverers considered these bases leucomaines dissolved from the
hepatic cells, the freshness of the livers used in preparation of
the cod liver oil must greatly influence the bases or ptomamines dis-
solved by the oil. They found butylamine, isoamylamine, hexyl-
amine, dihydrolutidine, asellin, morrhuin, and morrhuiic acid.
Isoamylamine constituting one-third of the total bases present,
was found to be a very active poison, producing rigor, convul-
sions, and death. 4 mg. produced death in a greenfinch in 3 min-
utes. 3 mg. of asellin-HCl killed a greenfinch in 14 minutes.
Hawk (17) confirmed the work of Gautier and Mourgues in 1907
and found 1.06 to 1.17 gm. of leucomaines per kilo of oil. He
identified butylamine, amyramine, hexylamine, dihydrolutidine,
and morrhuin, but found no asellin.
Fig. 4.
We demonstrated the poisonous effect of several basic amines including isoamylamine and choline-HCl when injected into the white rat, and then performed a series of experiments to test the influence of continued oral administration of small doses over a long period of time.

Young rats were weaned when 28 or 29 days of age and placed on the 10 per cent and 18 per cent yeast, vitamin A-free diets, described above. The fat-soluble factors were furnished by giving 0.0165 gm. of cod liver oil P₃ per day. Isoamylamine was dissolved in oil and fed so as to give 0.00014 and 0.00056 gm. of the base per day over an 8 week period. If an oil as found by Gautier and Mourgues contains 0.2 per cent of bases one-third of which is isoamylamine, 0.2 gm. of the oil per day would be approximately equal to 0.00014 gm. of the base per day. Fig. 4 gives the growth curves obtained. On 0.00014 gm. per day variations were found on 10 per cent yeast, as in feeding oil at high levels. The animal showing loss of weight on 0.00014 gm. and both rats on 0.00056 gm. per day and 10 per cent yeast showed paralysis, convulsions, and typical symptoms of vitamin B deficiency during the last 2 weeks of the experimental period. All rats on 18 per cent yeast and receiving isoamylamine showed normal growth.

Fig. 4 also gives the results of experiments showing the effect of small amounts of choline over an extended period of time. The conditions were the same as in the case of isoamylamine and choline hydrochloride fed at levels of 0.015, 0.033, 0.066, and 0.098 gm. per day. On 10 per cent yeast the choline causes restricted growth, while on 18 per cent yeast growth is normal. On 0.098 gm. per day and 10 per cent yeast difficulty was experienced in having the animals eat all the material offered and consequently the dose was not quantitative, however in every other case the base was quantitatively consumed. At the end of the 6th week

Fig. 4. Growth curves obtained by feeding rats basal diets containing 10 per cent and 18 per cent yeast supplemented with 0.0165 gm. per day of cod liver Oil P₃, and various amounts of toxic amines. Rats 1, 2, and 7 were controls and received no toxic substance. Rats 3, 4, 8, and 9 received 0.00014 gm. per day; Rats 5, 6, 10, and 11, 0.00056 gm. per day of isoamylamine. Rats 12 and 17 received 0.015 gm. per day; Rats 13 and 18, 0.033 gm. per day; Rats 14 and 19, 0.066 gm. per day; Rats 15, 16, 20, and 21, 0.098 gm. per day of choline hydrochloride.
Toxic Effect of Fish Liver Oils

on 0.066 gm. per day of choline-HCl the rat's hind extremities were paralyzed and it showed the symptoms of the terminal stages of vitamin B deficiency; it was then offered the 18 per cent diet and the administration of choline was continued as before. The symptoms of vitamin B deficiency immediately disappeared with rapid growth as shown in the lower right-hand corner of Fig. 4. The lower left-hand corner of Fig. 4 shows the recovery from toxemia produced by feeding high levels of cod liver Oil S on 10 per cent yeast by giving an 18 per cent yeast diet.

Further work which is nearing completion indicates that the effect of yeast is not specific for the two bases tested above. These results might explain some of the discrepancies and variations in both vitamin A and B determinations; as well as the effect of yeast or yeast extracts in counteracting an extremely high protein diet, as that described by Hassan and Drummond (18) (high protein diets are known to increase intestinal putrefaction with increased ptomaine formation), also the effect of yeast in low grade chronic infections.

SUMMARY.

1. Some cod liver oils produce symptoms similar to vitamin B deficiency when fed in relatively large amounts to animals on a diet containing as high as 10 per cent yeast.

2. The toxic effect of excess cod liver oil can be counteracted by feeding large amounts of yeast.

3. The toxic effect of large doses of some cod liver oils is not due to hypervitaminosis due to excess of vitamins A or D.

4. Continued small doses of isoamylamine in amounts which may be found in cod liver oil produce paralysis, convulsions, and lack of growth; the toxic effect may be prevented or cured by added yeast.

5. Continued small doses of choline also produce symptoms identical with vitamin B deficiency, which may be prevented or cured by added yeast.

6. The quantitative determination of vitamin A in cod liver oil is influenced by the amount of yeast in the basal diet, even when there is sufficient yeast to satisfy the vitamin B requirements under some conditions.

7. Determinations of vitamin B by methods of increase in rat
weight, where a cod liver oil of unknown toxicity is incorporated in the diet, cannot be compared.

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