THE "ASSAY OF A SO-CALLED COD-LIVER OIL EXTRACT" FOR VITAMIN-A CONTENT AND CALCIFYING PROPERTIES COMPARED TO COD-LIVER OIL.*

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The cod-liver oil extract under consideration in this investigation was a commercial product described as an alcohol-soluble extract of cod-liver oil. Its principal use is in pharmaceutical preparations which are offered as substitutes for cod-liver oil. The claims made for such products are that they have growth-promoting and other properties attributable to cod-liver oil.

The beneficial effects derived from the use of cod-liver oil are generally ascribed to its high content of vitamins A and D, which are known to be necessary for growth. An abundance of vitamin A in the diet is also essential for the body to maintain a normal resistance to disease. Vitamin D has been definitely shown to be one of the factors involved in the prevention and cure of rickets. It would seem that any extract of cod-liver oil for use as the sole active ingredient in preparations, having the properties described, should at least be as potent in respect to vitamins A and D as a commercial grade of cod-liver oil. With this in mind the assay of the alcohol-soluble extract of cod-liver oil was undertaken. In order to make results as conclusive as possible all determinations were carried out in comparison with a good grade of cod-liver oil. The method used for determining the vitamin-A content was that described by Sherman and Munsell.[1] At the present time there is no simple clear-cut method of testing a substance for the presence of vitamin D. Following the technique used in other vitamin determinations it would be necessary to have a diet adequate in all food essentials except vitamin D and entirely devoid of this factor.

Vitamin D has been shown to influence bone development and in this way is involved in growth. A test then for the presence of vitamin D in any food might be the growth and bone development made by an experimental animal when the food being tested is fed in addition to a basal diet containing all food essentials except vitamin D.

Although it was recognized that the vitamin-D potency of the alcohol-soluble extract could not be determined absolutely by any technique worked out to date, it seemed feasible to attempt a comparison of the calcium-depositing power of this substance with that of cod-liver oil under like experimental conditions. If under the same conditions the extract showed no greater calcium-depositing power than a good grade of cod-liver oil its vitamin-D content certainly could not be any greater.

EXPERIMENTAL ON VITAMIN-A CONTENT.

Young albino rats reared on a diet composed of two-thirds whole wheat, one-third whole milk powder and salt in the proportion of two per cent of the whole wheat were used for these determinations. At the age of 28–29 days when the

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[1] H C. Sherman and H. E. Munsell, *Jour. A. C. S.*, 47 (1925), 1639.

animals were weaned they were placed on the vitamin-A-free diet prepared as described by Sherman and Munsell,[1] with the exception that the dried brewery yeast was increased to 10 per cent and the starch reduced to 65 per cent, making the composition of the basal diet as follows: casein (extracted as described[1]), 20 per cent; starch 65 per cent; dried brewery yeast 10 per cent; Osborne and Mendel salt mixture[2] 4 per cent; sodium chloride (table salt) 1 per cent. This amount of dried brewery yeast seems to give better results than the smaller amount of 5 per cent. One group of rats receiving 10 mg. of cod-liver oil extract six times per week was irradiated each day with the light from a mercury vapor quartz lamp at a distance of twenty inches for five minutes. All animals were autopsied for gross pathological lesions, shown by animals confined to a vitamin-A-free diet.

Tables I and II give the results of these experiments. In each litter one or more animals were designated as controls, and these received no additions to the basal ration.

TABLE I.

WEIGHT RECORD OF RATS RECEIVING GRADED PORTIONS OF COD-LIVER OIL EXTRACT AS THE SOLE SOURCE OF VITAMIN A.

Cod-liver oil extract per rat per day, mg.	Rat no.	Weight at 28–29 days, Gm.	Weight at end of fore-period, Gm.	Weight at the end of successive weeks, Gm.								Survival days.
				1.	2.	3.	4.	5.	6.	7.	8.	
	572	56	86	79	80	78	74	71	59			84
	587	56	78	78	77	73	72	70	70	57		91
	599	58	94	104	107	96	75					71
	601	53	57	56	55	53	49	56	51	45		91
0	614	47	58	65	66	59	56	56	49	45		85
	618	42	59	58	58	55	56	46				83
	773	55	73	66	74	66	54					59
	790	60	71	69	68	70	76	61	54			93
										Average		82.1
	578	52	69	73	81	78	70	65	56	51		85
	582	61	85	96	104	99	93	67				80
10	598	59	83	86	92	91	78	77	70	66		89
	786	67	86	104	109	109	91	77				88
										Average		85.5
	579	52	72	74	83	82	80	67	64	55		86
10	583	61	91	105	114	111	107	79	75			81
Rats irra-	597	62	86	99	99	97	71	73	64			83
diated	792	55	54	63	69	71	74	73	59	53		98
										Average		87.0
	772	57	62	73	81	83	83	88	87	66	65	86
	787	62	79	84	94	110	105	85	89	83	71	107
50	775	54	64	80	87	90	89	91	84	80	67	
	791	60	76	93	97	104	104	85	74			95
										Average of 3		96.0
	580	52	75	80	90	95	95	95	95	97	77	
	612	45	56	67	77	77	75	67	71	63	56	93
100	581	52	73	76	86	84	82	80	76	73	64	
	611	46	51	62	69	67	67	66	52			86
									Average of 2			89.5

[1] H. C. Sherman and H. E. Munsell, *Jour. A. C. S.*, 47 (1925), 1639.

[2] Osborne and Mendel, *J. Biol. Chem.*, 37 (1919), 572.

TABLE II.

WEIGHT RECORD OF RATS RECEIVING GRADED PORTIONS OF COD-LIVER OIL AS THE SOLE SOURCE OF VITAMIN A.

Cod-liver oil per rat per day, mg.	Rat no.	Weight at 28–29 days, Gm.	Weight at end of fore-period, Gm.	Weight at the end of successive weeks, Gm. 1.	2.	3.	4.	5.	6.	7.	8.	Total gain, Gm.
	577	54	75	84	96	109	113	117	120	125	129	54
	586	58	67	86	91	96	98	100	109	112	113	46
1	602	49	64	73	81	89	93	99	99	105	113	49
	610	47	67	88	95	98	106	109	119	119	121	54
	.776	49	58	56	67	73	75	86	95	106	108	50
	793	52	46	51	55	57	60	69	74	77	88	42

The weight at 28–29 days is the weight at the time the young were weaned and the weight at the end of the foreperiod is that when the reserve body stores of vitamin A are judged to have been depleted and the feeding of the extract or the cod-liver oil was begun. If the animal did not live the full eight weeks, the last figure is the weight of the dead rat. Where no figure is given in the column headed "Survival," the rat lived the full eight weeks and was killed. All of the control animals and those receiving daily portions of the extract, except No. 772 and No. 580, developed the eye disease common to animals confined to a vitamin-A-free diet. The pathological lesions in the rats fed daily portions of extract were in all cases as severe as those of the controls while the rats fed cod-liver oil showed none of these lesions. The curves showing the average gains made by the groups of test animals are shown in Fig. 1.

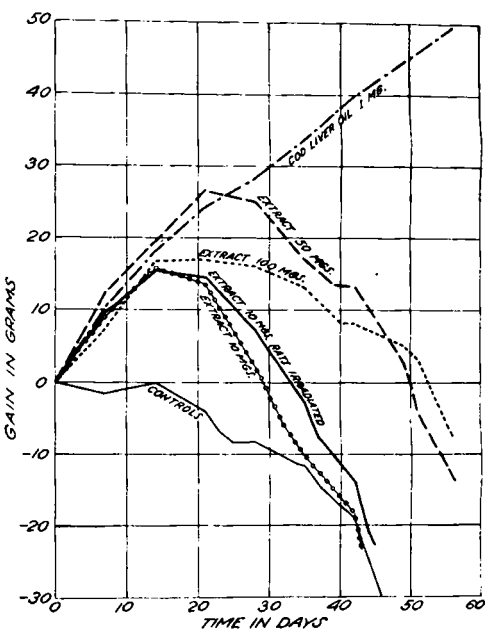

Fig. 1.—Gains in weight made during the test period by groups of rats fed cod-liver oil or the alcohol soluble extract of cod-liver oil as the sole source of vitamin A. Each curve shows the average result obtained from several tests. The quantity of cod-liver oil or extract fed to each rat six times per week is indicated on each curve. One group of rats receiving 10 mg. of the extract was irradiated with the light from a mercury vapor quartz lamp at a distance of 20 inches for 5 minutes, 6 times per week.

From the results of these experiments it is evident that 10 mg. of cod-liver oil extract per rat six times per week did not enable the rats receiving these daily portions to survive an appreciable time longer than those receiving no additions to the basal ration. When fifty mg. and one hundred mg. were used decline and death were slightly delayed, but the time was insignificant when compared with the results obtained with one mg. of cod-liver oil. The animals in this cod-liver oil group not only survived the full eight weeks but made very

nearly normal growth. No increase in growth was noted when the amount of vitamin D was made optimum by irradiating the animals with the light from a mercury vapor quartz lamp. This would indicate that the results are not vitiated by the fact that the basal diet contains little or no vitamin D.

SUMMARY OF VITAMIN-A STUDIES.

The results of these tests show that 1 mg. of cod-liver oil has a higher vitamin-A content than 100 mg. of the cod-liver oil extract. The growth curves indicate, judging by the author's experience in determining the vitamin-A content of foods, that 100 mg. of the extract would contain not more than one fifth as much vitamin A as is present in 1 mg. of cod-liver oil.

CALCIFYING PROPERTIES.

The general method of procedure was to produce severe rickets in rats by feeding a low phosphorus diet and then observe the amount of calcium deposition obtained when cod-liver oil or cod-liver oil extract was added to this diet. The amount of calcium deposited was determined by the line test described by McCollum.[1] The value given to each line test was determined by comparison with the plates published by Bills.[2] A set of these plates was obtained from the Mead Johnson Company and always used for comparison when line tests were carried out. Since microphotographs could not be taken an outline drawing of the bone was made and areas corresponding to the areas blackened by silver nitrate were shaded in. In this way a permanent record of the line test given by each bone has been kept in the record book.

EXPERIMENTAL ON CALCIFYING PROPERTIES.

Preliminary experiments had shown that our rats developed rickets readily on the yellow corn ration described by Steenbock.[3] This ration is made up of yellow corn 76 per cent, wheat gluten 20 per cent, calcium carbonate 3 per cent and sodium chloride 1 per cent. For the tests litters of albino rats 28–29 days of age which had been reared on the same diet were used. Each litter was first fed a diet of one-third whole milk powder, two-thirds whole wheat and table salt two per cent of the whole wheat until they weighed approximately sixty Gm. These were then fed the yellow corn diet for twenty-one days. Such a procedure results in a uniformly severe condition of rickets in rats treated in this way.

At the end of the twenty-one days the rats of the litter were weighed and put in separate cages. One animal was killed either on this day or continued on the yellow corn diet as a control to be killed later. The other rats in the litter were fed the yellow corn diet to which cod-liver oil or cod-liver oil extract had been added. All rats were weighed at weekly intervals and a record of the food intake was kept. At the end of a previously determined number of days called the test period one rat which had had cod-liver oil and one of approximately the same weight which had had cod-liver oil extract were killed. The tibias were removed and as much of the muscle as possible cut away and the bones, properly labeled, were placed in

[1] McCollum, Simmonds, Shipley and Park, *J. Biol. Chem.*, 51 (1922), 41.

[2] Bills and McDonald, *Ibid.*, 68 (1926), 821.

[3] Steenbock and Black, *Ibid.*, 64 (1925), 263.

10 per cent formalin. In each litter a control rat was continued on the basal diet until the last pair receiving cod-liver oil and cod-liver oil extract was killed. This control served to show if any healing took place spontaneously. Later the bones were examined using the line test technique and the calcium deposited was judged as $^{-}$, $^{+}$, $^{++}$, $^{+++}$, or $^{++++}$ by comparison with the plates referred to above.

TABLE III.

SUMMARY OF EXPERIMENTS COMPARING THE VITAMIN-D CONTENT OF COD-LIVER OIL AND COD-LIVER OIL EXTRACT.

Cod-liver oil per cent of diet.	Duration of test period, days.	Number of cases.	Average intake cod-liver oil per day per 100 g. of rat, Gm.	Line test.	Cod-liver oil extract per cent of diet.	Duration of test period days.	Number of cases.	Average intake cod-liver oil extract per day per 100 g. of rat, Gm.	Line test.
0.05	5	3	0.0051	0.0	0.05	5	3	0.0048	0.0
	7	6	0.0047	0.2		7	6	0.0042	0.0
	9	11	0.0041	0.9		9	12	0.0041	1.2
	11	10	0.0042	1.4		11	10	0.0042	1.3
	13	8	0.0048	2.8		13	8	0.0046	2.5
0.1	5	10	0.0090	0.7	0.1	5	9	0.0090	0.7
	7	9	0.0086	2.4		7	10	0.0083	2.0
	9	7	0.0082	2.9		9	7	0.0082	2.9
	11	4	0.0089	3.5		11	4	0.0089	3.3
	13	2	0.0097	4.0		13	2	0.0087	3.5
0.25	5	3	0.0217	1.7	0.25	5	3	0.0206	1.0
	7	5	0.0221	2.4		7	5	0.0226	2.4
	9	4	0.0204	3.8		9	4	0.0237	3.5
	11	4	0.0249	4.0		11	5	0.0257	4.0
0.5	5	7	0.0381	2.1	0.5	5	6	0.0351	1.8
	7	6	0.0381	2.8		7	7	0.0357	3.0
	9	2	0.0325	3.0		9	2	0.0285	3.0
	11	2	0.0330	4.0		11	2	0.0286	4.0
1.0	7	1	0.0577	4.0	1.0	7	1	0.0646	3.0
	9	2	0.0760	4.0		9	2	0.0584	4.0
	11	2	0.0623	4.0		11	2	0.0658	4.0

TABLE IV.

SUMMARY OF LINE TESTS WITH 108 RATS FED COD-LIVER OIL.

Duration of test period, days.	Cod-liver oil—Per cent of total diet. 0.05% line test.	0.1% line test.	0.25% line test.	0.5% line test.	1.0% line test.
5	0.0	0.7	1.7	2.1	
7	0.2	2.4	2.4	2.8	4.0
9	0.9	2.9	3.8	3.0	4.0
11	1.4	3.5	4.0	4.0	4.0
13	2.8	4.0			

SUMMARY OF LINE TESTS WITH 110 RATS FED COD-LIVER OIL EXTRACT.

Duration of test period, days.	Cod-liver oil extract—Per cent of total diet. 0.05% line test	0.1% line test.	0.25% line test.	0.5% line test.	1.0% line test.
5	0.0	0.7	1.0	1.8	
7	0.0	2.0	2.4	3.0	3.0
9	1.2	2.9	3.5	3.0	4.0
11	1.3	3.3	4.0	4.0	4.0
13	2.5	3.5			

With this method over two hundred and fifty rats were used and results obtained with 0.05 per cent, 0.1 per cent, 0.25 per cent, 0.5 per cent, 1.0 per cent and 2 per cent added cod-liver oil and 0.05 per cent, 0.1 per cent, 0.25 per cent, 0.5 per cent, 1.0 per cent, 2.0 per cent and 4.0 per cent added cod-liver oil extract over test periods of 5, 7, 9, 11 and 13 days. These data are shown in Table 3. The actual consumption of cod-liver oil and cod-liver oil extract for each rat has been computed as Gm. per day per 100 Gm. of rat. The results for the controls are not given here in detail as none of the fifty-one controls representing thirty-nine litters showed any evidence of calcium deposition. In making the summaries in Tables III and IV, the line tests were given numerical values of 0, 1, 2, 3 and 4 corresponding to $^{-}$, $^{+}$, $^{++}$, $^{+++}$, and $^{++++}$ respectively. This made it possible to average the values in each group and shows more clearly the relative amount of calcification induced by varying amounts of cod-liver oil and cod-liver oil extract.

SUMMARY OF STUDIES ON CALCIFYING PROPERTIES.

The average intake of cod-liver oil extract for any one group varied little from the average intake of cod-liver oil for the corresponding group, consequently in this comparison the varying intake of cod-liver oil and cod-liver oil extract is not a factor.

These results show that the extract Gm. for Gm. has no greater calcifying properties than a good grade of cod-liver oil.

The usual method employed where the line test technique is used is to feed the food being tested until a good positive line is obtained. The method used here is likely to be more sensitive in that it is possible to determine the amount of the substance which will give a positive line test of $^{++++}$ value within much closer limits since graded quantities are fed over test periods of a definite number of days not less than 5 nor more than 13. With a larger number of determinations and smaller differences in the increasing percentages of cod-liver oil and cod-liver oil extract added a more quantitative value should be obtained for the calcifying properties of these substances.

CONCLUSIONS.

The results given above show that the alcohol soluble extract used in these tests contained a negligible amount of vitamin A and had no greater calcifying properties Gm. for Gm. than cod-liver oil.

MISBRANDED COD-LIVER OIL PREPARATIONS.

An extensive survey was made during 1927 and it is being continued by the U. S. Department of Agriculture of cod-liver oil extracts of various products found in inter-state Commerce that do not conform with the statements made by the manufacturer.

VITAMIN D IN LIVER OF DOG FISH SALMON.

Scientists at the Prince Rupert Fisheries Experimental Station of the Biological Board of Canada have been gratified to receive from Professor Asmundson, professor of poultry husbandry at the University of British Columbia, confirmation of the local stations preliminary experiments relative to the content of Vitamin D in dog fish liver oil, according to information published in the Prince Rupert News of December 3rd and forwarded to the Department of Commerce by American Consul G. Woodward at Prince Rupert.

Professor Asmundson reports, as a result of preliminary experiments that he himself has carried out, that "dog fish liver oil is equivalent in medicinal value to the best cod-liver oil on the market."