The Vitamin D Content of some New Zealand Fish Oils

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This study was undertaken to determine the vitamin D content of three New Zealand fish oils known to be rich in vitamin A (Denz & Shorland, 1934; Edisbury, Lovern & Morton, 1937); and at the same time to test the validity of the composite ashing technique in the prophylactic bone ash method, as recommended for chickens in the tentative method for vitamin D assay of the Association of Official Agricultural Chemists (1940; Griem, 1939), using rats as experimental animals.

EXPERIMENTAL

Albino rats (40–47 g. in weight and 3–4 weeks of age) were fed on Steenbock's rachitogenic diet no. 2965 for a period

defatted bones were dried and ashed to constant weight. The difference between the weight of the dry defatted bone and the corresponding ash was termed the 'organic residue' (R).

The oils tested were: (1) whole-body eel oil, *Anguilla australis* (Richardson); (2) ling liver oil, *Genypterus blacodes* (Bloch & Schn.); (3) groper liver oil, *Polyprius oxygeneios* (Bloch & Schn.) (two samples). The whole-body eel oil was obtained from short-finned migrants caught at Lakes Ellesmere and Forsythe in March 1942. The oil was extracted by boiling and pressing the eels and collecting the oil first run off. The data (Table 1) concerning the ling and groper liver oils were personally communicated by Dr F. B. Shorland, who supplied these samples and who kindly gave me access to his paper (Shorland, 1945) relating to variations in the vitamin A potency of the groper liver oils.

Table 1. *The vitamin A content and other data concerning the groper and ling liver oils*

Oil	Date when fish caught	No. of livers	Average weight of oil (g./liver)	Method of extraction	Oil content of liver (%)	Vitamin A content (%) of oil from			
						Carr-Price test			Ultra-violet absorption $E_{1\text{ cm.}}^{1\%}$
						Blue value	$E_{1\text{ cm.}}^{1\%}$ 620 m μ .	$E_{1\text{ cm.}}^{1\%}$ 580 m μ .	
Groper I	May 1939	33	145	Solvent extraction	9.52	4.14	4.10	3.96	3.98
Groper II	Sept.–Oct. 1942 and 1943	98	95	Solvent extraction	5.5	9.16	9.00	8.04	9.46
Ling	May–June 1942	Bulk	Bulk	Steamed and pressed	20.25	0.37	0.38	0.41	0.44

Note. Calculation of percentage of vitamin A is based on factors for Carr-Price test: blue value 80,000; $E_{1\text{ cm.}}^{1\%}$ 620 m μ . 5000; $E_{1\text{ cm.}}^{1\%}$ 580 m μ . 2600; ultra-violet absorption $E_{1\text{ cm.}}^{1\%}$ 328 m μ . 1600.

of 4 weeks. All comparisons were made on litter-mates. The litters were divided into seven groups (following Coward, 1938) of six to eight rats. Twice a week the doses of oil were delivered into the rats' mouths from graduated pipettes. Each animal was kept in a separate cage with a raised grid bottom, in a room protected from direct sunlight and maintained at a temperature of 65° F. After the animals had been killed the hind legs were removed and the soft tissue loosened by boiling in distilled water for 2 min. (Day, 1940; Association of Official Agricultural Chemists, 1940). Both the femur and tibio-fibula were used and all bones were uniformly cleaned.

The bones were extracted with boiling 95% ethanol for 20 hr., followed by 20 hr. with ether in a Soxhlet apparatus (Association of Official Agricultural Chemists, 1940). The literature on the extraction of fat from rat bones indicated that each investigator used a different procedure. For the extracting solvent hot or cold ethanol, either alone, or followed by ether, has been used and for varying lengths of time (Coward, 1938; Chick, Korenchevsky & Roscoe, 1926; Day, 1940; Key & Morgan, 1932; Kon & Henry, 1935). The

The initial dosage was based upon figures obtained by Cunningham (1935). In the final assay of each oil the dosage of the international standard was equivalent to 0.05, 0.1 and 0.2 i.u. daily. The lower dosage of 0.025 i.u. daily, as suggested by Coward (1938), proved, in the case of our rat colony, to be below the threshold dose required to yield any increase in percentage ash over that given by the negative controls.

RESULTS

Comparison of results obtained by composite ashing and individual bone analyses

To test the accuracy of the composite ashing technique when rats are used, both individual and composite ashing was undertaken in Exps. I, II, III and VI. The bones of the right hind legs were analyzed separately. The bones of the left hind legs from the groups of rats fed at different levels of each oil were ashed in composite samples. The average difference between single bone means and

Table 2. *Assay of the antirachitic activities of whole-body eel oil, groper liver oil (I) and ling liver oil, showing the A/R ratios* obtained by composite ashing and single bone analyses*

Dose/day	No. of rats	A/R ratio composite ashing	A/R ratio single bone mean	Difference between A/R ratios	Standard error of mean of single bones
Experiment I					
Standard { 0.025 i.u. 0.05 i.u. 0.1 i.u.	6	0.438	0.384	0.054	±0.065
	6	0.440	0.416	0.024	±0.041
	6	0.591	0.569	0.022	±0.046
Eel body oil { 0.606×10^{-3} g. 1.212×10^{-3} g. 2.424×10^{-3} g.	6	0.403	0.387	0.016	±0.033
	6	0.447	0.438	0.009	±0.027
	6	0.536	0.534	0.002	±0.052
Negative controls	6	0.429	0.409	0.020	±0.039
Experiment II					
Standard { 0.025 i.u. 0.05 i.u. 0.1 i.u.	6	0.377	0.396	0.019	±0.022
	6	0.472	0.479	0.007	±0.034
	6	0.545	0.575	0.030	±0.039
Groper liver oil (I) { 0.106×10^{-4} g. 0.211×10^{-4} g. 0.421×10^{-4} g.	6	0.482	0.473	0.009	±0.043
	6	0.751	0.713	0.038	±0.058
	6	0.799	0.867	0.068	±0.072
Negative controls	6	0.385	0.393	0.008	±0.016
Experiment III					
Standard { 0.025 i.u. 0.05 i.u. 0.1 i.u.	6	0.409	0.420	0.011	±0.018
	6	0.482	0.506	0.024	±0.044
	6	0.637	0.684	0.047	±0.070
Ling liver oil { 0.53×10^{-4} g. 1.06×10^{-4} g. 2.11×10^{-4} g.	6	0.406	0.424	0.018	±0.019
	6	0.414	0.430	0.016	±0.018
	6	0.435	0.448	0.013	±0.027
Negative controls	6	0.400	0.416	0.016	±0.025
Experiment VI					
Standard { 0.048 i.u. 0.100 i.u. 0.206 i.u.	8	0.442	0.454	0.012	±0.020
	8	0.583	0.581	0.002	±0.052
	8	0.811	0.801	0.010	±0.030
Ling liver oil { 0.34×10^{-3} g. 0.65×10^{-3} g. 1.28×10^{-3} g.	8	0.558	0.547	0.011	±0.043
	8	0.725	0.730	0.005	±0.033
	8	0.872	0.885	0.013	±0.066
Negative controls	6	0.412	—	—	—

* Ratio of weight of ash and weight of corresponding fat-free dry organic residue from bone.

composite ashing results is 0.024 (standard error (s.e.) ± 0.007). The data given in Table 2 show that the difference between the mean A/R (ash/organic residue) ratio of the single bone determinations and the A/R ratio of group ashing in no case exceeds the standard error of the mean of the single bone analyses. Therefore there is no significant difference between the A/R ratios obtained by these two methods, and results obtained by the group ashing technique are as accurate as those obtained by single bone analyses.

To illustrate the possible errors due to the ashing technique paired composite analyses were carried out in two experiments (Table 3) by ashing the bones of the right hind legs from each group of rats receiving a definite level of an oil as one composite sample, and the bones of the left hind legs as another composite sample. The average difference in A/R ratio amounted to 0.0046 (s.e. ± 0.0021). This indi-

cates that the small experimental error in the ash determination does not account in full for the apparent difference between the single bone and group determination values. This is to be expected since composite ashing gives an average weighted A/R ratio influenced by the weight of each bone, while the mean of single bone analyses gives equal significance to all bones (Loy, DeWitt & Knudsen, 1941). The effect of weighting can be gauged by totalling the weight of ash and the weight of organic residue of all the single bone analyses in any one group and determining from these the weighted A/R ratio and comparing it with the mean of the single bone analyses. In these experiments the difference between the calculated group ash value and the mean of the single bone analyses was 0.009 (s.e. ± 0.002). Therefore the apparent difference between composite ashing and individual bone determination is due partly to the small experimental

Table 3. *Assay of the antirachitic activities of whole-body eel oil and groper liver oils (I) and (II) showing the A/R ratios* obtained by composite ashing*

Dose/day	No. of rats	A/R ratio right leg	A/R ratio left leg	Difference A/R ratios
Experiment IV				
Standard { 0.05 i.u. 0.1 i.u. 0.2 i.u.	7	0.431	0.432	0.001
	7	0.552	0.544	0.008
	7	0.762	0.780	0.018
Eel body oil { 0.143×10^{-2} g. 0.286×10^{-2} g. 0.572×10^{-2} g.	7	0.392	0.391	0.001
	7	0.493	0.493	0.000
	7	0.616	0.616	0.000
Negative controls	7	0.377	0.376	0.001
Experiment V				
Standard { 0.05 i.u. 0.1 i.u. 0.2 i.u.	8	0.736	0.741	0.005
	8	0.827	0.834	0.007
	8	1.004	0.993	0.011
Groper liver oil (I) { 0.91×10^{-5} g. 1.83×10^{-5} g. 3.65×10^{-5} g.	8	0.665	0.671	0.006
	8	0.819	0.819	0.000
	8	0.994	0.992	0.002
Negative controls	6	0.658	—	—
Experiment VIII				
Standard { 0.05 i.u. 0.1 i.u. 0.2 i.u.	8	0.551	—	—
	8	0.759	—	—
	8	0.920	—	—
Groper liver oil (II) { 0.166×10^{-5} g. 0.41×10^{-5} g. 0.80×10^{-5} g.	8	0.490	—	—
	8	0.632	—	—
	8	0.858	—	—
Negative controls	7	0.544	—	—

Note. Composite analyses of both right and left legs are shown for the whole-body eel oil and groper liver oil (I).

* See footnote to Table 2.

error and partly to the fact that the two methods represent a difference in weighting.

Composite ashing is to be preferred for its greater speed and simplicity, and results obtained by this method have been accepted as of equal accuracy with the single bone determinations.

Number of animals of this breeding stock required in group to yield significant results

Motzok, Hill, Brannon & Slinger (1943) advise each investigator to determine the optimum group size for use under his particular conditions, since the variation among individual bone ash values may be expected to differ for each laboratory. Coward (1938) recommends six rats in a group for the prophylactic bone ash method. The results of Exps. I, II, III and VI were examined by the *t*-test of 'Student' (Fisher, 1938) to determine the number of animals of this stock required in a group to render significant the differences between the means of the groups receiving the equivalent of 0.05 and 0.1 i.u./day. It was found that six rats were insufficient and seven rats were needed in a group to obtain a significant result.

The vitamin D content of the oils

The potency of the oils was determined from the results of Exps. IV, V, VI and VIII (Tables 2 and 3) by plotting the A/R ratio against the value of the standard in international units and finding the points on this line corresponding to the A/R ratios obtained with the unknown oil. By plotting the A/R ratio against the dose a straight line was obtained, rendering it unnecessary to plot the A/R ratio against the logarithm of the dose (cf. Oser, 1939). The vitamin D content of the oils tested was as follows:

	i.u./g.
Whole-body eel oil	25
Ling liver oil	260
Groper liver oil (I)	5,300
Groper liver oil (II)	19,000

The vitamin D content of whole-body eel oil indicates that eel flesh is one of the few rich food sources of this vitamin. Short-finned migrant eels yield 17% of oil and therefore contain not less than 425 i.u./100 g. Consequently one serving of eel flesh would amply provide the daily vitamin D requirement of the majority of normal children and adults. From the potency of the two samples of groper liver

oil it is evident that the vitamin D content of this oil shows striking variation, which follows the same trend as the vitamin A content in the samples studied (to be discussed in the forthcoming paper by Shorland, 1945).

SUMMARY

1. Prophylactic bone ash analysis was used to determine the vitamin D content of three New Zealand fish oils. The vitamin D content in i.u./g. was found to be: whole-body eel oil, 25; ling liver oil, 260; groper liver oil (I), 5300; groper liver oil (II), 19,000. The potency of the two samples of groper liver oil shows a striking variation, and in the same direction as that of the vitamin A content of these oils.

2. Composite ashing of bones has been shown to yield results as accurate as individual ashing determinations, with rats as experimental animals, as has previously been shown for chickens. This modification of the method is recommended since it greatly reduces the time and labour required.

3. The number of animals required in a group with this particular stock of animals was found to be seven, if the results were to be statistically significant.

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The Gross Chemical Changes in the Liver in Dietetic Necrosis

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Dietetic necrosis of the liver in rats was probably first observed by Weichselbaum (1935) when he noted that rats on a low casein diet developed 'haemorrhages' into the liver. Later macroscopically similar lesions were observed, inconstantly and irregularly, in animals on various deficient diets (György & Goldblatt, 1939; Webster, 1942) and these were proved to be necroses. Recently it has been shown that such lesions can be produced with certainty, that a deficient intake of protein is the single dietary factor correlated with their occurrence, and that diets containing proteins poor in sulphur amino-acids facilitate, while supplements of such amino-acids prevent, the development of the lesion (Himsworth & Glynn, 1944a, b; Himsworth, 1945).

The histological features of the lesion are those of massive acute necrosis of the liver (Glynn & Himsworth, 1944); the gross chemical features are described below.

METHODS

The details of the diets, vitamin supplements and care of the rats have been given elsewhere (Himsworth & Glynn, 1944a). Here it need only be noted that the sources of dietary protein were casein or yeast and that, although the proportions of fat and carbohydrate varied widely in different diets, the protein intake on each was less than 500 mg./rat/day.

White Wistar and black hooded rats were used. At the beginning of experiments, their weights were 90–120 g.; on killing they weighed 50–250 g, depending on the type of