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The Preparation and Vitamin A and Vitamin D Standardization of the Second U. S. P. Reference Cod Liver Oil by the U. S. P. Vitamin Advisory Board

The U. S. P. Reference Cod Liver Oil No. 2, a standard of reference for vitamin A and vitamin D, is now ready for distribution. This oil contains 1700 U. S. P. units of vitamin A and 115 U. S. P. units of vitamin D per gram. It has been prepared under the direction of the Vitamin Advisory Board and may be obtained from Dr. E. Fullerton Cook, Chairman of the U. S. P. Revision Committee, 43rd and Woodland Ave., Philadelphia, Pa.

Plans for providing this new reference cod liver oil were begun early in 1938 when it became apparent that the supply of U. S. P. reference cod liver oil was being depleted. At that time recognition was given to the principle that a standard for the assay of vitamins should preferably be the pure vitamin or a preparation made therefrom which could be readily reproduced and which might be suitable from the standpoint of stability and convenience. The International Standard for vitamin D now being distributed by the Health Organization of the League of Nations is of such a degree of purity. Some recent studies of vitamin A esters indicate that certain compounds of vitamin A may be suitable from the standpoint of stability and reproducibility as a standard for vitamin A. However, such pure preparations of vitamins A or D have not been available in sufficient quantities to supply the need for reference standards in this country and it was therefore decided to arrange for the preparation and standardization of another lot of reference cod liver oil. Owing to steadily increasing demands for samples of the reference cod liver oil for assay purposes, the stocks of the first lot of oil were depleted more rapidly than had been anticipated and it was necessary to issue samples of the new lot of oil just as soon as the standardization was completed.

In the early plans it was estimated that 200 gallons of destearinated oil would be a sufficient quantity of the new standard. With the equipment that was available for preparation of the oil it was found preferable to use a batch of 400 or 500 gallons for destearination and further processing. The fish liver oil secured was obtained from authoritative officials and is known to be exclusively the oil from the livers of *Gadus Morrhua*. This oil was obtained in lots of 120 gallons from the Norwegian Fisheries Research Station through the courtesy of Dr. Olav Notevarp, from the Fisheries Research Institute of Newfoundland through the courtesy of Dr. W. F. Hampton, and from Messrs. Crooks of London through the coöperation of Dr. J. C. Drummond. A fourth lot of 90 gallons was obtained from The Atlantic Coast Fisheries Company through the kindness of Dr. Harden F. Taylor. This specially selected oil was mixed, destearinated and packaged under carbon dioxide with relatively high pressure under the most ideal conditions obtainable and the oil has been stored under refrigeration continually from the day it was received in this country. The processing and packaging of the oil was done through the coöperation of E. R. Squibb and Sons.

In response to invitation to collaborate in the standardization of this oil more than 18 laboratories agreed to take part in the study. To these laboratories were sent samples of the oil to be standardized as well as International Standards for vitamins A and D to be used for reference in the biological assays. These assays were carried out according to the methods prescribed in the Second Supplement of the U. S. Pharmacopœia XI.

Complete reports from 18 laboratories have been received, 18 submitting results of vitamin D assays and 14 results of vitamin A assays, and interpretations of these records were used to set a value for the potency of the reference oil. A condensed summary of the reports received are presented in Tables I and II. In Table I are the vitamin A potencies assigned by each collaborator to the assay oil as well as the number of animals reported used by each of the laboratories responding. It will be noted that more than 1600 animals were used in the vitamin A assays alone. The arithmetical average of the values reported is 1787. The extreme values are 1000 and 2500. It is evident that the smaller numbers of animals were used by the laboratories reporting the highest potencies for the oil. The value 1700 was given the oil in consideration of the interpretation of the full reports submitted by the collaborators as well as in recognition of the preliminary reports of spectrophotometric collaborative studies which were yet to be completed.

Table I.—Condensed Summary of Vitamin A Collaborative Reports[a]

Laboratory No.	Vitamin A Value Assigned to Oil	Number of Animals Reported Used
1	1600	144
2	2000	86
3	1700	80
4	1475	112
5	2250	46
6	2000	158
7	1525	200
8	1800	56
9	1846	138
10	2500	40
11	2000	80
12	1000	72
13	1679	133
14	1650	320
	Average 1787.5	Total 1665

[a] The biological assay method used was that directed in the "Second U. S. P. XI Supplement" (1939), pages 132–138.

In Table II the vitamin D values assigned to the oil and the number of animals used by each laboratory are given. The average of these values is 114 and the extreme values are 100 and 130. The value of 115 units of vitamin D assigned to the reference cod liver oil seems well justified.

Table II.—Condensed Summary of Vitamin D Collaborative Reports[a]

Laboratory No.	Vitamin D Value Assigned to Oil	Number of Animals Reported Used
1	130	37
2	115	92
3	110	68
4	117	133
5	120	40
6	112	65
7	120	15
8	120	118
9	112	139
10	118	167
11	110	75
12	125	99
13	100	38
14	125	176
15	105	40
16	100	60
17	114	215
18	110	150
	Average 114.5	Total 1727

[a] The biological assay method used was that directed in the "Second U. S. P. XI Supplement" (1939), pages 132–138.

Fortunately it was possible to arrange through Dr. Chester D. Tolle, the Associate Referee on Vitamin D, an A. O. A. C. collaborative study of the new reference oil. This study, involving the comparison of the vitamin D potencies of the old and new reference oils by the A. O. A. C. chick method of assay, was conducted by 17 laboratories. The average of all vitamin D values assigned the new oil by the collaborators was 119 U. S. P. units per gram and of the 10 values ranging from 105 to 120 was 112 U. S. P. units per gram. These results were considered by the Vitamin Advisory Board since this reference standard is to be used in the chick assay of vitamin D carriers for poultry.

At the Second International Conference on Vitamins, held in London in June 1934 under the auspices of the Health Organization of the League of Nations, the advisability of preparing subsidiary standards for distribution within each country represented was discussed, with the view to reducing the amounts of the International Standards needed. In recognition of this view, in standardizing the U. S. P. Reference Oil No. 2 collaboration by laboratories outside of the United States was invited. The laboratory of the Pharmaceutical Society of Great Britain and the Laboratory of Hygiene, Department of Health, in Canada responded to this invitation. The reference cod liver oil now being distributed in Canada is identical with the U. S. P. Reference Cod Liver Oil No. 2 and the advantages of these two countries having a common standard are obvious.

The new reference oil was prepared in a manner identical with that used for the original oil. The original oil was destearinated, freed from moisture, placed in dry amber-colored bottles, subjected to vacuum and then charged with carbon dioxide, hermetically sealed and stored at a temperature close to zero degrees centigrade. This oil was widely used internationally as a standard both for bioassays and spectrophotometric determinations. There has been no evidence that this oil has deteriorated in any way since its original packaging. The Reference Oil No. 2 will be subjected to the same study in order to insure that there is no change in potency during its use.

Table III.—Vitamin A Value of U. S. P. Reference Cod Liver Oil No. 2 in Terms of Reference Oil No. 1 at 3000 U. S. P. Units per Gram

Laboratory No.	Determination on Raw Oil	U. S. P. Collaborative Saponification Procedure	Optional Saponification Procedure
1. Sa-40	1706	1772	1938
2. Sn-40	1446	1732	1574
3. Sl-40	1755	1841	1823
4. Sg-40	1831	....	1821
5. Si-40	1701	1393	....
6. Sh-40	1715	1640	1669
7. Sm-40	1739	1557	1607
8. Se-40	1962	1743	
9. Sf-40	1880	1968	1868
10. Sk-40	1766	1681	1919
11. Su-40	1620	1540	....
Average	1740	1687	1777

SUMMARY OF SPECTROPHOTOMETRIC STUDIES

The new Reference Cod Liver Oil was also subjected to a U. S. P. collaborative study involving the use of spectrophotometric methods for determining vitamin A. Eleven laboratories participated in this study. Determinations were made on the raw oil, on the non-saponifiable fraction of the oil prepared according to a suggested procedure, and on the non-saponifiable fraction prepared according to optional or preferred procedures. The results, in terms of

potency of the oil as calculated from the submitted data, are given in the accompanying table. The coefficient of variation in the results obtained by each of the procedures used has been determined. This value was found to be smallest for the determination on the raw oil, greater when the suggested saponification procedure was used and greatest for the optional saponification procedure results. However, the averages of the calculated potencies are in good agreement. The results of this study furnish further basis for the vitamin A value assigned the oil.

PHYSICAL FACTORS OF THE SECOND U. S. P. REFERENCE COD LIVER OIL

Dr. G. S. Jamieson, in charge of Oil, Fat and Wax Investigations, Agricultural Chemical Research Division of the Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture, has examined the new Reference Cod Liver Oil and submits the following report:

Refractive index at 25° C.	1.4771
Saponification value	188.6
Iodine number (Hanus)	172.1
Acid value	0.81
Unsaponifiable matter	0.60
Stearine (cold test)	None
Odor	Slight
Taste	Characteristic mild fishy flavor

A Phytochemical Study of the Root Bark and Fruit of *Cornus Nuttallii**

By Edward Krupski† and Louis Fischer‡

Cornus Nuttallii, commonly called Dogwood and a member of the Cornaceæ family, grows along the Pacific Coast from British Columbia to California, but thrives best in the Douglas Fir forests of the Puget Sound area. This tree was first observed by the botanist Nuttall and later named for him by Audubon (1). Torrey and Gray (2) published the first written description of this plant in their book "Flora of North America."

This tree usually grows from 20 to 30 feet high and from 6 to 8 inches in diameter. It has a dull ashy brown or reddish bark that forms thin scales on old trunks. The twigs are minutely hairy when young, later smooth, and dull red-purple in color. The leaves are deciduous, simple, opposite, three and one-half to five inches long and about one-half as wide. Button-like clusters of very small greenish-yellow flowers which ordinarily bloom in early spring are surrounded by four to six showy white bracts that are commonly taken to be the petals. The thin dry pulp of the drupes, which are bright red, and mature in clusters of 25 to 40 at the ends of the twigs, contains one or two seeds within a stony endocarp.

No record was found of *Cornus Nuttallii* ever having been used medicinally; however, *Cornus florida*, once official in the U. S. P., has been used for many years as a medicinal agent. The bark of the root and trunk of *Cornus florida* was used with some success as a substitute for Cinchona as an antiperiodic and tonic (3). Also, according to Ellis (4), the bark of *Cornus florida* was considered very valuable in the treatment of autumnal fevers; however, the active principle of neither species has been subjected to a pharmacological study in this respect.

EXPERIMENTAL

Collection of Material.—The fruits were gathered during the months of September and October in the immediate vicinity of Seattle. They were separated from the bracts and only the ripe, fully matured fruits were used in this investigation. After drying at a temperature of about 27° C., they were ground to the desired fineness.

The root bark was collected during September, in the vicinity of McCleary, Washington. After uprooting the trees, the roots were scraped clean and the bark peeled. The bark was well dried in the sun and then ground to a fine powder.

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† Submitted in partial fulfillment for a Master of Science Degree in Pharmacy.

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