

## THE VITAMIN CONTENT OF WEST INDIAN SHARK LIVER OILS

### II.\* THE VITAMIN D CONTENT OF THE LIVER OIL OF THE SMALL BLUE SHARK.\*\*

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Liver oils from several species of shark have been investigated as to their vitamin D content and reported in the literature at different times.

Bills<sup>1</sup> in a very exhaustive study of different fish liver oils, reported that shark and dogfish oils from Newfoundland have a relative potency of 75 per cent and 3 per cent, respectively, as compared to that of Newfoundland medicinal cod liver oil.

Brocklesby<sup>2</sup> found that liver oils from dogfish caught in various localities have from less than 10 per cent to about 30 per cent the potency of medicinal cod liver oil.

Asmudson, Allardyce and Biely<sup>3</sup> investigated dogfish liver oil as a source of vitamin D for poultry. They used chicks as experimental animals and took as the index of antirachitic activity the percent ash content of the chicks at the end of the experimental period. They concluded that dogfish liver oil is a valuable source of vitamin D for poultry, but in no case as rich as medicinal cod liver oil.

Seel<sup>4</sup> investigated a number of shark liver oils, but no information as to the results obtained is known.

Schmidt-Nielson and Stene<sup>5</sup> studying the vitamin content of cartilaginous fishes came to the conclusion that sharks are not a very rich source of vitamin D.

Poulsen<sup>6</sup> finds that the oil of the cartilaginous fishes in general is poorer in Vitamin D than the oil of the cod. He tried to explain this fact on the basis that little bone formation takes place in the body of these fishes; therefore they do not need vitamin D.

\* The first article of this series was: The Vitamin A Content of West Indian Shark Liver Oil (*Carcharinus* sp.). Puerto Rico Jour. of Pub. Health and Trop. Med. 11: 158-166. 1935.

\*\* Received for publication February 3, 1937.

André and Lecoq<sup>7</sup> after examining the oil from several cartilagenous fishes, report that its potency runs from 25 per cent to 90 per cent of that of a good normal commercial liver oil.

Kawai and Yoshida<sup>8</sup> working with the liver oil of *Squalus wakiyae* Tunaka (Aburatsunosame liver oil) concluded that this oil is a very poor source of vitamin D.

Bills<sup>9</sup> reported for a shark liver oil of unknown origin and species a value of 50 international units, and for dogfish liver oils from the Pacific and Atlantic coasts, 30 and 3 international units, respectively.

One conclusion can be drawn from this review of the literature pertaining to the vitamin D content of shark liver oil, and that is, this oil and the liver oils of cartilagenous fishes in general are not very rich sources of vitamin D.

It occurred to us that since almost all these investigations had been carried out on liver oils of sharks from temperate and northern climates, it would be of interest to study the possible effect that a tropical environment might have on the vitamin D content of these oils.

The present communication deals with the investigation made of the oil from a small Blue Shark (*Carcharias milberti* Valenciennes?)<sup>10</sup> caught off the northern coast of Puerto Rico during the month of April. This animal weighed thirteen kilograms, the liver weighing a little more than one kilogram.

The oil was obtained by macerating the liver and filtering through a filter cloth. This oil was centrifuged and then placed in the ice box, where the stearine separated. The oil was decanted and filtered for a second time inside the ice box at a temperature of 8° to 10° C.

This oil has the following constants:

Specific Gravity at 25° C-----	0.9221
• Refractive Index (Abbé) at 28.5° C-----	1.4739

#### EXPERIMENTAL

The method used for determining the vitamin D content of this oil was that of Steenbock and Black<sup>11</sup>. Albino rats (coming from Winstar Institute stock), weighing about 35 grams and raised in the laboratory, were placed at the age of twenty-eight days on a basal ration containing no vitamin D, but being adequate in all other respects. This diet known

as Steenbock's No. 2965, has the following composition: 76 per cent yellow corn, 20 per cent wheat gluten, 3 per cent  $\text{CaCO}_3$  and 1 per cent  $\text{NaCl}$ . All the rats received the basal diet and distilled water ad-libitum throughout the depletion and experimental periods.

The records of the gains in weight and food consumption per week were very uniform for all the animals throughout the experiment.

The rats were kept in individual metal cages with raised screen bottoms, and were isolated from sunlight. At the end of the four weeks depletion period, a line test was performed on the tibias of two rats chosen at random out of the twenty-six started. A mild rickets was observed to be developing. The remaining twenty-four rats were then divided into four groups of six each. One of these groups was left as the negative control group, receiving the basal ration only. Of the three remaining groups, two received daily supplements of 10 mgs. and 20 mgs. of shark liver oils, respectively. The third group was supplemented with one unit of vitamin D per day administered in the form of viosterol.

This sample of viosterol contained 10,000 U.S.P. units of vitamin D per gram. It was fed to the rats in the form of an aliquot, one cc. of which was equivalent to one U.S.P. unit of vitamin D. The present U.S.P. unit of vitamin D is equivalent to one international unit<sup>12</sup>. The diluting medium used for preparing the aliquots of viosterol and shark liver oil was petroleum ether. The volume of solution to be fed was measured by means of a pipet and emptied into a small dish containing a little of the vitamin D free basal diet. The petroleum ether was evaporated at room temperature, leaving the oil mixed with the diet. These solutions were prepared every other day, so as to prevent any destruction of the vitamin due to storage in the medium used.

The experimental period lasted three weeks. At the end of this period all the animals were killed and a line test was performed on the tibias of each. The line test was run as follows: the tibias were washed in acetone for 12 hours, hand sectioned with a sharp scalpel, stained with 1 per cent silver nitrate solution for one minute, washed with distilled water and then examined under a dissecting microscope.

The evaluation of the tibias of the animals receiving the different doses of shark liver oil was performed by compar-

ing them with those of the negative controls and the animals receiving one unit per day of vitamin D. In the latter, healing was complete, while in the former severe rickets was apparent. The animals receiving 20 mgs. of shark liver oil compared very favorably with those receiving one unit of vitamin D per day, while in those which received only 10 mgs. per day, healing was not complete.

Determinations of the Ca and P content per 100 gms. of body weight were made on five animals from each group. The results of these analyses which are reported in the following table are in accordance with the line test, i.e. the highest content of Ca and P per 100 gms. of body weight corresponds to the animals receiving one unit per day of vitamin D, while the lowest values correspond to the negative controls.

Histological sections made of the tibias from one animal out of each group confirmed the results obtained by the line test.

Supplement	Average weight of animals at time supplement was started. (gm. per rat)	Average weight of the animals at the end of the experimental period. (gm. per rat)	Average weight of diet eaten during the 7 weeks. (gm. per rat)	Average ash content per 100 gms. of body weight. (gm.)	Average Ca per 100 gms. of body weight. (gm.)	Average P per 100 gms. of body weight. (gm.)	Line Test	Histological examination	U. S. P. units per gram S. L. O.
Negative controls.....	60.8	71.5	267	2.38	0.650	0.498	—	—	.....
10 mgs. per day S. L. O..	60.8	69.0	264	2.73	0.767	0.527	+to++	+to++	.....
20 mgs. per day S. L. O..	60.8	69.9	277	2.78	0.795	0.529	+++	+++	50
One unit of vitamin D per day.....	60.8	69.6	274	2.82	0.813	0.587	+++	+++	.....

Severe rickets—  
Advanced rickets +

Mild rickets ++  
Normal +++

S.L.O.=Shark Liver Oil

#### SUMMARY

1. A bibliography of the investigations relating to the vitamin D content of the liver oil of different kinds of sharks is given.

2. The vitamin D content of the liver oil from a small blue shark caught in Puerto Rican waters is reported as 50 U.S.P. units per gram (international units).

*Acknowledgment:* The authors wish to express their thanks to Dr. S. F. Hildebrand of the Bureau of Fisheries, Washington, D. C., for the identification of the shark, and to Dr. E. Koppish for the interpretation of the histological sections of the bones.

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