Substances Related to Vitamin A in the Whale Liver Oil

BY

SEIICHI ISHIKAWA, YOSHIMORI OMOTE, HIROSHI OKUDA

The maximum of absorption spectrum of whale liver oil is near 310 mμ, considerably deviating from 328 mμ, that of vitamin A. This point was pursued to isolate kitol having the absorption maximum at 290 mμ.[1] Furthermore, substances related to vitamin A such as anhydro vitamin A and subvitamin A have been confirmed in ordinary fish liver oils. Also in the whale liver oil, these vitamins may be expected being present. It is very interesting that there are some substances with the structure closely related to vitamin A. This paper is concerned with the analytical method of substances related to vitamin A, with the thermal change of whale liver oil, and with the action of maleic anhydride upon the whale liver oil.

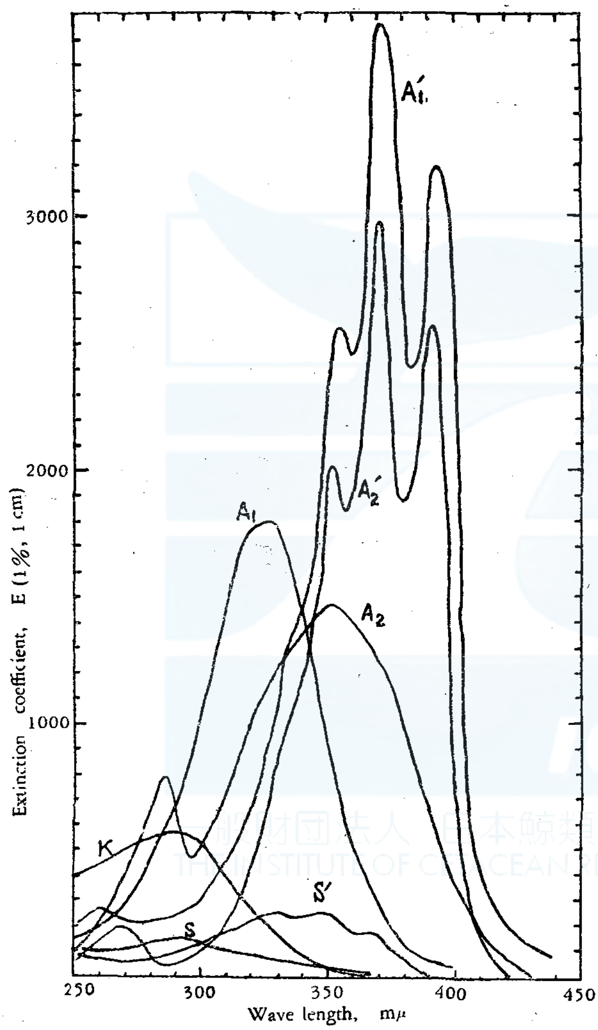

Fig. 1. Absorption spectra of vitamin A_1 (A_1), vitamin A_2 (A_2), subvitamin A (S), kitol (K), anhydrovitamin A_1 (A_1'), anhydrovitamin A_2 (A_2') and anhydrosubvitamin A (S').

Analysis of Absorption Spectrum of Whale Liver Oil.

Every substance related to vitamin A shows strong absorption between 250–400 mμ. The absorption spectra of hitherto known substances related to vitamin A are summarized in Fig. 1.[1)2)3)]

An example of absorption spectrum of whale liver oil is shown in curve S in Fig. 2. As compared with Fig. 1 deviation of the maximum of the curve S from 328 mμ, the maximum of vitamin A, to shorter wave length was considered to be due to the presence of kitol. On the assumption that additional resultant of absorption spectra of vitamin A and kitol would make the curve S, each component was calculated as shown by the curves A and K in Fig. 2. A represents the absorption spectrum of vitamin A_1, and K shows that of kitol. S' is the curve obtained by the additional composition of A and K on the figure. This method is temporarily called AK method.

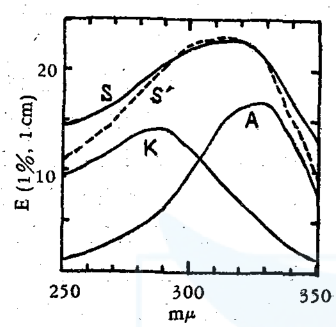

Fig. 2. Analysis of the absorption curve of whale liver oil, (S) sample, (S') resultant, (A) vitamin A, (K) kitol.

For the purpose of discussion on reliability of this method, using the same liver oil, the extinction coefficient of vitamin A at 328 mμ calculated by AK method was compared with results determined by the following two methods.

1. AKA' method.......To analyze the original curve assuming that it is composed of vitamin A_1, kitol, and anhydro vitamin A_1.
2. Morton-Stubbs's correction[4].

Non correction	AK method	MS correction	AKA' method
21.4	17.5	15.9	11.7

Although these corrections are less strict due to calculation on the rough assumption, they may present us far nearer value to the true vitamin A than non corrected extinction coefficient at 328 mμ in the sample, taken for that of vitamin A.

Thermal Change of Whale Liver Oil.

It has been already known that kitol produces vitamin A by thermal decomposition and that 1 mole of vitamin A is yielded from 1 mole of kitol.[1)5)] Therefore, also in case whale liver oil is heated itself, its containing kitol is expected to decompose to vitamin A. However, when liver oil is heated open in the air, the decomposition of vitamin A by oxidation makes it impossible to find newly produced vitamin A. So, first of all, whale liver oil was heated in carbon dioxide at 220°, and cod liver oil unit was measured with the lapse of time. This result is shown in Fig. 3.

When heated for 2 minutes, cod liver oil unit of the oil reached about 130 %

of that of original, while after heating for longer time, vitamin A was found to decompose. When heated under high vacuum, its absorption curve was compared as shown in Fig. 4.

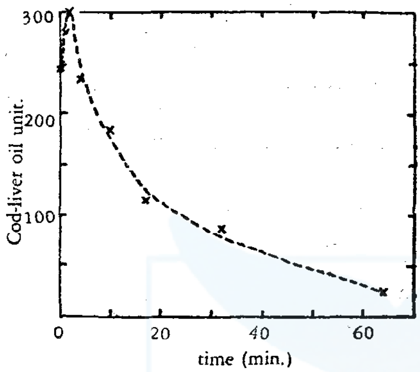

Fig. 3. Change of cod liver oil unit of the whale liver oil heated in the CO_2 tube.

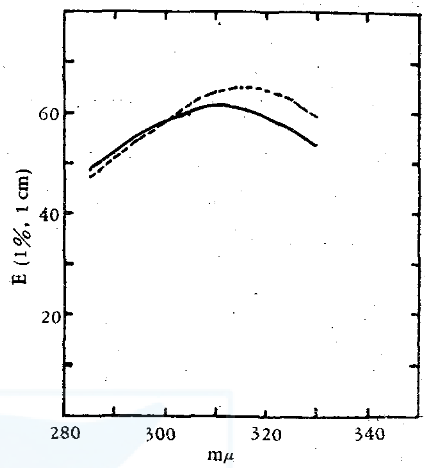

Fig. 4. Change of the absorption spectrum of whale liver oil heated in vacuum, at 220°, for 3 min.; — original sample,...... sample heated.

The extinction coefficients of vitamin A and kitol were calculated with application of AK method, on the basis of which vitamin A produced from 1 mole of

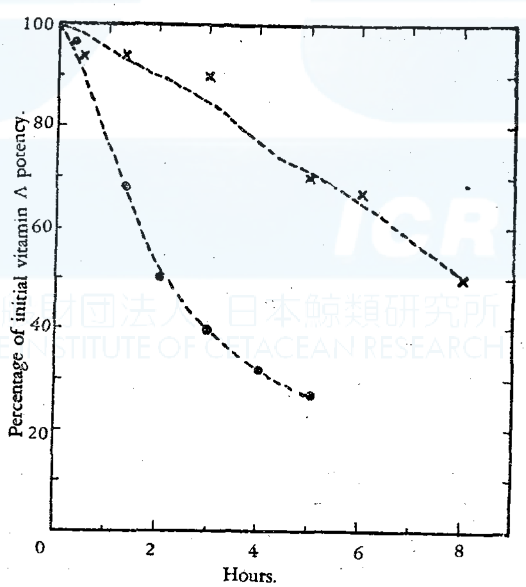

Fig. 5. Reaction of the whale liver oil vitamin A with maleic anhydride at (•) 50°C, (×) 25°C.

kitol was calculated to be 1.42 mole. Taking the decomposition of vitamin A into consideration, there is a possibility for more increase than the above figure. This contradicts with the figures previously reported that from 1 mole of kitol 1 mole of vitamin A was formed. This respect will be persued in future.

Reaction of Whale Liver Oil with Maleic Anhydride.

Baxter and others[6] stated a method to analyze vitamin A and neo-vitamin A by the action of maleic anhydride. The authors observed the reaction of whale liver oil with maleic anhydride by their method. From this result the relation between decrease of cod liver oil unit and reaction time was found as shown in Fig. 5.

On the assumption that antimony trichloride produced blue color with only vitamin A and neo-vitamin A, the amount of neo-vitamin A was calculated to be 46.5 % of the total vitamin. This figure is approximately same as that found with common fish liver oils.[9]

The absorption spectrum of whale liver oil reacted with maleic anhydride was shown in Fig. 6. Absorption of vitamin A nearly disappeared and rather similar curve to the spectrum of kitol was found.

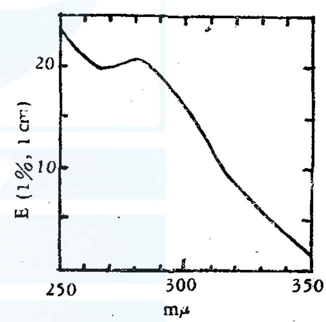

Fig. 6. Absorption spectrum of the whale liver oil reacted with maleic anhydride at room temperature.

Moreover, diene value was measured for the purpose of the reference to study the reaction of maleic anhydride upon whale liver oil and its molecular distillates. The results were shown in Table I, with comparison of cod liver oil unit.

Table I Diene Values of Whale Liver Oil and Its Molecular Distillates

	Temperature	%	Diene value		Cod liver oil unit	E (328)	A (328)
			Found	Theoretical			
Original sample			2.5	1.6	92	21.4	16.6
Distillate 1.	170°C	2.3	33.9	35.6	2105	367	360
Distillate 2.	200°C	6.2	15.1	10.9	631	120	110
Distillate 3.	210°C	7.4	3.1	5.7	345	63	58
Distillate 4.	220°C	23.5	1.4	1.8	88	19	18
Distillate 5.	230°C	17.1	1.8	0.9	41	10	9
Residue			3.0	0	—	2.9	0

Experimental Part

Experimental 1. Analysis of the Absorption Spectrum of Whale Liver Oil.

(1) AK method.........The observed curve is assumed to consist of absorption spectrum of pure vitamin A_1 and pure kitol only, additionally composed. Let absorption coefficients of sample at 290 mμ and 330 mμ, being near their absorption maxima, be represented by E (290) and E (330), and that of pure vitamin A_1 at 330 mμ in the sample, A (330), and that of pure kitol at 290 mμ, A (290). Then the following equations are obtained.

$$\left.\begin{array}{l} E(330) = A(330) + 0.293\,K(290) \\ E(290) = K(290) + 0.390\,A(330) \end{array}\right\}$$

Factor 0.293 means ratio of pure kitol, K (330)/K (290), and factor 0.390 means ratio of pure vitamin A_1, A (290) / A (330), all of which were quoted from the reference in Fig. 1. The values of A (330) and K (290) from the above equations are solved as follows:

$$\left.\begin{array}{l} A(330) = 1.13\ E(330) - 0.33\ E(290) \\ K(290) = 1.13\ E(290) - 0.44\ E(330) \end{array}\right\}\text{AK equation}$$

The way of finding A (328) from A (330) is only to multiply it by 1.018. If AK method is applied to the absorption spectrum of Antarctic whale liver oil (sample 1), we can get A (330) = 17.2, K (290) = 13.4 and A (328) = 17.5. On the basis of these figures A and K curves in Fig. 2 were plotted similarly to that of pure substance.

(2) AKA′ method.........Let absorption coefficients of sample at 290 mμ, 330 mμ and 350 mμ, be respectively represented by E (290), E (330) and E (350), that of vitamin A_1 in the sample at 330 mμ, A (330), that of kitol at 290 mμ, K (290), and that of anhydro vitamin A_1, A′ (350), and the following equations can be obtained similarly to (1).

$$\left.\begin{array}{l} A(330) = 1.462\ E(330) - 0.383\ E(290) - 0.595\ E(350) \\ K(290) = 1.139\ E(290) + 0.101\ E(350) - 0.498\ E(330) \\ A'(350) = 0.112\ E(290) + 1.304\ E(350) - 0.728\ E(330) \end{array}\right\}\text{AKA}'\text{ equation}$$

The sample 1 was analyzed with these equations as follows.

A (330) = 15.7, A (328) = 15.9, K (290) = 14.7, A′ (350) = 3.2

This fact proves the presence of anhydro vitamin A in the whale liver oil, although small amount. So, unsaponifiable matter in this liver oil was treated by chromatography. As expected, anhydro vitamin A fraction could be isolated and confirmed.

(3) MS method.........This method, which shows how to get corrected E (328) of vitamin A when absorption spectrum of vitamin A is affected by other irrelevant spectra, is on the basis of the assumption that other spectra can be regarded linear near the maximum of vitamin A. The wave length corresponding to 6/7

of the height of the peak in the curve of vitamin A_1 in Fig. 1 was found 311 mμ and 337 mμ. Now when the absorption coefficients of the sample at 311 mμ, 328 mμ and 337 mμ. are expressed in E (311), E (328) and E (337), corrected E (328) viz, A (328) is shown in the following equation.

A (328) = 7 E (328) − 2.42 E (311) − 4.58 E (337)………MS equation.

A (328) of sample 1 was determined 11.7 in this method.

Experiment 2. Thermal Change of Whale Liver Oil.

(1) Whale liver oil (sample 2) was put in the small test tube, the atmosphere being substituted with carbon dioxide at 10^{-2} mm, and sealed in ampoule. 8 ampoules were heated in the oil bath for 1, 2, 4, 8, 16, 32 and 64 minutes and cod liver oil units of them were determined by antimony trichloride method. The resutls are shown in Fig. 3.

(2) Two ampoules of sample 2 were similarly made, sealed immediately after degassing at 50°, under the vacuum of 10^{-4} mm, one of which was heated for 3 minutes at 220°. The absorption spectra of both ampoules were compared each other as shwon in Fig. 4. The amount of vitamin A and kitol in the above two ampoules was calculated by AK method.

	E (330)	E (290)	A (328)	K (290)
Before heating	53.17	51.81	43.80	35.15
After heating	59.08	50.80	50.80	31.41

When calculated with molecular weight of vitamin A = 286, E (328) = 1800,[7] molecular weight of kitol = 572, E (290) = 707[5], 1 g. of oil with E = 1 will contain 1.94×10^{-6} mole of vitamin A at 328 mμ and 2.47×10^{-6} mole of kitol at 290 mμ. Then the number of molecules of vitamin A produced from 1 molecule of kitol is shown in the following equation.

$$\text{Vitamin A mole} = \frac{\text{A (328) increased}}{\text{K (290) decreased}} \times \frac{1.94 \times 10^{-6}}{2.47 \times 10^{-6}}$$

$$= \frac{\text{A (328) increased}}{\text{K (290) decreased}} \times 0.786$$

The result through application of the above experiment to this equation was that 1 mole of kitol produced 1.42 mole of vitamin A.

Experiment 3. Reaction of Maleic Anhydride upon Whale Liver Oil.

(1) Sample 2 was dissolved in dry benzene to make a solution having the cod liver oil unit of about 1.0. An aliquot of 5 cc. was added to the same volume of maleic anhydride solution (10 g. maleic anhydride per 100 cc. benzene). After a

certain hours' storage in the thermostat at 25° and 50°, an aliquot was then removed to determine the cod liver oil unit.

Under the same condition, a comparative experiment was carried on for sample and benzene only instead of maleic anhydride solution. From both values, the remaining amount of vitamin A not reacted with maleic anhydride was calculated. Assuming that the initial amount of vitamin A was 100, the relation between the recovery of vitamin A and the reaction time was shown in Fig. 5. Moreover, the recovery after 16 hours' reaction at 25° was 44.5 %. According to Baxter and others,[6] under the same condition, the recovery of vitamin A palmitate was 90%, and that of neovitamin A palmitate was 5 %. Then the percentage of neovitamin A in the sample was calculated as follows.

$$\% \text{ of neovitamin A} = \frac{44.5-5}{90-5} \times 100 = 46.5\ \%$$

(2) 0.2 g. of maleic anhydride was let to react with 1 g. of sample 2 in benzene solution below 50°. When it began to show not blue but reddish purple, in antimony trichloride reaction, the absorption spectrum of the solution was searched (Fig. 6).

(3) After molecular distillation of sample 1, diene values of original sample, distillates and residual oil were measured. 5 cc. of xylene solution of maleic anhydride (about 5 %) was added to about 500 mg. of the sample and after sealing under carbon dioxide keeping at 100° for three hours, the residual maleic anhydride was titrated with alkali in the water solution. Diene value is shown in the following equation.

$$\text{Diene value} = \frac{1.269 \times \text{normal alkali in cc.}}{\text{sample in g.}}$$

As a reference, cod liver oil unit, E (328) and A (328) were also annexed in Table I.

The theoretical diene value in the table was calculated on the basis of the value of A (328), assuming that 2 moles of maleic anhydride react with 1 mole of vitamin A (viz, theroretical diene value of pure vitamin A is 178).

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