

Studies on Kitol. II. Influence of Kitol Fraction on the Determination of the International Unit of Vitamin A.

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In the previous paper*, the authors showed that the unsaponifiable matter of whale liver oil gave only the apparent absorption maximum due to the presence of a mixture of two or more substances while that of tunny liver oil gave an absorption maximum at around 328 m μ . The Vitamin A fraction (fraction B) of whale liver oil gives a maximum at around 328 m μ but due to the presence of kitol fraction (fraction A), the whale liver oil gives a very complicated absorption. Accordingly, the determination of Vitamin A of whale liver oil by a spectrophotometer does not give the correct extinction coefficient at 328 m μ , only that of an apparent value, so that determination of true Vitamin A content cannot be made by this method. This fact is borne out by the fact that with whale liver oil, results of animal test and spectrum analysis do not coincide.

The authors examined the influence of kitol fraction on Vitamin A by the use of fractions A and B separated by the authors. However, this is an intermediate report and further detailed experiments must follow since there is no direct proof that the kitol fraction obtained does not contain even a minute amount of Vitamin A, or that it does not contain other unsaponifiable matter.

39 g. of Vitamin A fraction and 14 g. of kitol fraction were obtained from 53.5 g. of unsaponifiable matter of the whale liver oil. First, the international unit of Vitamin A was determined with Vitamin A fraction and then the extinction coefficient at 328 m μ of a mixture of 14 parts kitol fraction to 39 parts Vitamin A fraction, which the international unit of Vitamin A was calculated. As a result, it was shown that the value of the international unit of Vitamin A in this case came out 12.1% higher than the true value of Vitamin A content. In other words, the true content of Vitamin A is the value obtained by subtracting 12.1% from the value obtained by spectrophotometric determination.

It follows, naturally, that the determined value of Vitamin A comes

* Cf. Report I, p. 85 of this Bulletin.

out larger than the true content of Vitamin A when the amount of kitol content becomes large compared to the amount of Vitamin A. This will show that a presence of kitol has a very great effect on the assay of Vitamin A by the spectrophotometer and therefore, points to the fact that the method of Vitamin A determination of whale liver oil must be improved.

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EXPERIMENTAL

53.5 g unsaponifiable matter were obtained from 250 g. of the liver oil of a fin whale by the method as described in the previous report. From this unsaponifiable matter, 39 g Vitamin A fraction (fraction B) and 14 g. kitol fraction (fraction A) were obtained (loss of 0.5 g.).

1) Carl Zeiss' spectrophotometer was used to determine the international unit of Vitamin A fraction (solvent—absolute alcohol).

$$\text{B fraction—} E_{1\text{cm}}^{1\%} 328\text{m}\mu = 454.5$$

therefore, this substance contains 727,000 I. U. of Vitamin A per 1 g.

2) Kitol fraction (fraction A)— $E_{1\text{cm}}^{1\%} 290\text{ m}\mu = 500$

$$E_{1\text{cm}}^{1\%} 328\text{ m}\mu = 108.7$$

therefore, this substance would look as though it contained 174,000 I. U. of Vitamin A per 1 g., but the Carr-Price reaction gives a red coloration and contains virtually no Vitamin A.

3) International unit was determined with a mixture of 39 parts Vitamin A fraction and 14 part kitol fraction (a proportion so arrived because the unsaponifiable matter of this whale liver oil contained these 2 fractions in a proportion of 39 : 14). $E_{1\text{cm}}^{1\%} 328\text{ m}\mu = 375$

therefore, this substance must contain 600,000 I. U. of Vitamin A.

However, 1 g. of this substance contains only $\frac{39}{39+14}$ g. of Vitamin A fraction and, taking that the kitol fraction does not contain any Vitamin A (by Carr-Price reaction), the true content of Vitamin A can be shown by the following equation:

$$727,000\text{ I. U.} \times \frac{39}{53} = 535,000\text{ I. U.}$$

therefore $600,000 - 535,000 = 65,000\text{ I. U.}$

Actually, 65,000 I. U. Vitamin A had been shown than was actually con-

tained, or in other words 12.1% more Vitamin A.

4) The determination of international unit was made with a mixture of an equal portion of A and kitol fraction which gave:

$$E_{1\text{cm}}^{1\%} 328 \text{ m}\mu = 35.8$$

therefore, this substance seems to contain 572,800 I. U. Vitamin A but according to the aforementioned calculations, it actually contains only 363,500 I. U. In this case, the spectrophotometric value gave 30.4% higher content of Vitamin A than was actually present.

5) International unit was determined with the unsaponifiable matter of tunny liver oil which gave:

$$E_{1\text{cm}}^{1\%} 328 \text{ m}\mu = 38.5$$

therefore, 1 g. of this substance contains 61,600 I. U. of Vitamin A.

6) International unit was determined with a mixture of 39 parts unsaponifiable matter of tunny liver oil and 14 parts kitol fraction which gave:

$$E_{1\text{cm}}^{1\%} 328 \text{ m}\mu = 50.0$$

therefore 1g. of this substance seems to contain 80,000 I. U. of Vitamin A but according to the above calculation, the real content of Vitamin A is 45,100 I. U. In this case, ca. 77.4% higher value than the actual content of Vitamin A was obtained by the spectrophotometric determination.

7) International unit was determined of an equal amount of unsaponifiable matter of tunny liver oil and kitol fraction which gave:

$$E_{1\text{cm}}^{1\%} 328 \text{ m}\mu = 59.0$$

therefore 1 g. of this substance shows a Vitamin A content of 94,400 I. U. but, according to foregoing calculation, the actual content of Vitamin A would be 30,800 I. U., if there is no presence of Vitamin A in kitol fraction. In this case, the photometric determination has given 206.5% higher value than the true content of Vitamin A.