The vitamin A potency of fish liver oil and its effect on the growth of albino rat

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As shown in the previous report,¹⁾ the bio-potencies of fish and whale liver oils were different depending on the materials; some of them had the high potencies almost comparable to that of all-trans vitamin A, but some had only 70% in relative bio-potency. This difference is considered to be due to the isomerisation occurred under the various conditions by the estimation from their maleic values, and such a difference was also observed in the synthetic vitamin A. Although same result was also observed in the bio-test, but there was a trend to cause difference in the growth response depending on the dose. The experimental value²⁾ previously reported in this country was obtained at the relatively high level of vitamin A and it did not show any difference between the synthetic A and the natural A, so it is necessary to consider that the feeding effect of fish liver oil expected as the growth effect is different from the vitamin A potency and it is also necessary to compare the vitamin A potencies on the feeding level.

In this report, the bio-potencies are estimated from the maleic values of several fish liver oils by the method already described in the previous report and the results are confirmed by the growth test of albino rats same as in the previous report. Then I compared their vitamin A potencies at the feeding level by changing the dose and the feeding method of vitamin A.

The estimation of bio-potency is obtained from the relationship between the vitamin A potency obtained by the liver storage bioassay and the maleic value, this method is applied in this experiment to confirm the values.

Finally, I compared the values in this and in the previous reports with the values of vitamin A potency obtained by the various determinations.

The followings are the experimental methods, results and discussions.

Experiment I

Methods : the albino rats growth test of vitamin A and the determination of maleic values of fish liver oils are already described in the previous report.

Results : the weight increases of each group per week obtained from the curves in Fig. 1 are shown in Table 1 and the relative bio-potency calculated from these values are 80% of that of all-trans vitamin A.

Fig. 1. The growth curves of the vitamin A deficient albino rats fed the critical dose of vitamin A.

Note: Each group contains 10 animals.
Daily vitamin A doses are 1 I. U.
and 2 I. U. per head, per day.

Table 1. The weight increase and the estimated relative bio-potency for the vitamin A deficient albino rat.

Group		Total weight increase g./week	Estimated relative bio-potency
Synthetic vitamin A palmitate	1r	99.9	100%
	2r	133.3	
Natural vitamin A (fish liver oil)	1u	104.7	72
	2u	121.4	
Resulted relative bio-potency			80%

Experiment II

The result in the Experiment I is same as that in the previous report and the value is a little higher than the bio-potency estimated from the maleic value but it is different from that of all-trans vitamin A. But the growth curves for the weaning male rats of same strain without any vitamin A

deficient period shown in Fig. 2 do not cause any difference for more than five weeks at the same level of vitamin dose between them.

Experiment III

The growth of rats fed 10 I. U. and 20 I. U. of vitamin A per head per day is observed by the method used in the Experiment II. These doses correspond to about 1000 I. U. per Kg of feed which is nearly feeding level per body weight of the animal or a little lower. As described before, the growth potency should be expected as a feed and is different from true vitamin A potency. So the difference as the vitamin A source between the fish liver oil and the synthetic vitamin A should be considered that obtained

Fig. 2. The growth curves of young albino rats

Note: Vitamin A level is same as in Fig. 1.

by the dose of feeding level. I compared these values. The vitamin A doses for fish liver oil group and synthetic A group are estimated by their maleic values, and vitamin A level of the synthetic vitamin A group is limited to about 70% of that of the natural vitamin A group: the same growth effect is expected at these levels.

The experimental result shown in Fig. 3 shows that fish liver vitamin A group has a better growth effect.

The apparent vitamin A potency of the fish liver oil calculated from the average weight increase per week is 126% and it is clearly higher than that of the synthetic vitamin A.

Experiment IV

The values obtained from the growth experiment have the same trend as those estimated by the maleic values but the former is a little higher than the latter in the natural vitamin A. The growth experiment is used here for the determination of vitamin A bio-potency and for the comparison of the synthetic vitamin A oil with the natural vitamin A oil because the growth experiment has been used for these purposes in this country²⁾ But, as the equation for the estimation of vitamin A bio-potency by the maleic value is derived from the relationship³⁾ between the maleic value and the bio-potency of vitamin A obtained from the liver storage bioassay, the liver storage bioassay of vitamin A using several fish liver oils is carried out here to confirm this relationship.

Samples: molecular distilled fish liver oils (107,000 I.U. and 109,000 I.U.). Their relative bio-potencies estimated from the maleic values obtained by the method already described in the previous paper are both 74%.

Method: although many methods were already presented for the liver storage bioassay, the method of Embree et al⁴⁾ is used here.

Results: All the values obtained by this method were a little higher than those estimated from thier maleic values. The results are shown in Fig. 4 where the vertical axis indicates the relative bio-potency and the horizontal axis indicates the maleic value. The equation

Fig. 3. The growth curves of albino rats fed the vitamin A dose of the feeding level.

Notes: The average rat number is ten.

u: Fish liver oil group

r: Synthetic vitamin A group

1: Daily dose per head 10 I. U.

2: Daily dose per head 20 I. U.

The level of vitamin A dose u: r=10:7

(as determined by the U.S.P. Method)

Fig. 4. The relationship between the maleic value and the relative bio-potency (A comparison with the result of Ames)

* (%) $RP=101.6-MV \times 0.7647$ (BABA)
× $RP=99.5-0.2(MV)^2+0.000768(MV)^3$ (AMES)

under Fig. 4 was obtained from the curve by the simplification and the correction using the method of least squares.

Table 2. The amounts of vitamin A in liver and the relative bio-potencies estimated from the maleic values (1)

Group		Vitamin A in liver	Estimated relative bio-potency
Synthetic vitamin A	1r	482 I. U.	97%
	2r	1,248	
Natural vitamin A	1u	397	74
	2u	1,013	
Resulted relative bio-potency			82.7%

Notes : Each group contains 10 albino rats.
Daily dose per head 500 I. U. ...1000 I. U.
Total dose per head 1500 I. U. ...3000 I. U.

Table 3. The amounts of vitamin A in liver and the relative bio-potency estimated from the maleic values (2)

Group		Vitamin A in liver	Estimated relative bio-potency
Synthetic vitamin A	1r	83.5 I. U.	97%
	2r	273.6	
Natural vitamin A	1u	66.0	74
	2u	205.9	
Resulted bio-potency			81.0%

Notes : Daily dose per head 100I. U. ...200I. U.
Total does per head 500I. U. ...1000I. U.

A comparison of fish liver oil vitamin A potencies obtained by various determinations

As shown above in the bio-tests, the relative bio-potency of vitamin A was a little higher than that estimated by the maleic value. And the vitamin A potency of fish liver oil is shown in the relative bio-potency expressed in percentage of the bio-potency of all-trans vitamin A,

Table 4. The vitamin A potencies of fish liver oils

Materials	Spectro-photometry I. U. /g	Carr, Price Method I. U. /g	Relative bio-potency (%)		
			Growth method	Liver storage method	MV method
Distilled fish liver oil	107000	108310	80	—	73
"	109000	111540	—	83	76
"	59600	58680	—	—	65
Strong liver oil (Japan Pharm.)	9800	11500	71	—	68
"	11000	12600	*94	—	75
Fresh liver oil (average)	—	—	—	—	88
Distilled liver oil (average)	—	—	—	—	74
Buter	—	—	—	—	86

* Apparent value

Table 5. The comparison of fish liver oil vitamin A values by various determinations (I. U. /g)

Sample	U. S. P. 16	C. P.	M. V.	R. P. (%)	M. V. /U. S. P. (%)
1	111,000	120,640	89,280	74	80
2	217,000	242,600	169,800	70	78
3	111,000	127,220	91,400	78	82
4	223,000	313,400	200,560	64	90
5	430,000	479,745	374,200	78	87
6	136,100	151,180	139,000	92	102

Table 6. The comparison of vitamin A potencies estimated from the maleic values by the corrected equation with those obtained by the original method

Sample	U. S. P. 16 I. U. /g	△R. P. (%)	Corrected R. P. (%)	△Vitamin A Value by M. V. I. U.	Corrected V. A. value I. U.
1	111,000	74	83	89,280	100,000
2	217,000	70	79	169,800	192,000
3	111,000	78	85	91,400	99,800
4	223,000	64	74	200,560	231,000
5	430,000	78	85	374,200	408,000
6	136,100	92	94	139,000	142,000

we must know this value comparing with those obtained by the spectrophotometries, such as the method in the 16th edition of U. S. Pharmacopeia, frequently used now in the commercial transactions. The results of the comparison of the values obtained by the bio-tests, spectrophotometry and antimony trichloride method including the values in the previous paper are shown in Table 4 and Table 5.

Table 6 is the comparison of the values calculated by the equation to obtain the relative bio-potency from the maleic value simplified and corrected by the results of author's bio-tests with those calculated by the Ames' original equation.

Discussion

In the previous report, the relative bio-potencies of fish liver oil vitamin A estimated by maleic value using the Ames' method were about 70~90%. It was discussed there that this difference is due to the isomerisation of vitamin A in fish liver oil and that the vitamin A of fish liver oil is not always inferior to the synthetic vitamin A for some fish liver oil samples showed the same potencies as all-trans vitamin A. The difference in the growth effect by the level of the dose was also observed. In this report, the growth and the liver storage experiments of albino rats were performed to confirm the relationship between the maleic value and the vitamin A potency. The values obtained in these bio-tests were a little higher than those by the Ames' original method. The simplification and the correction of the estimation equation using these results were tried. The estimated vitamin A value re-calculated

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by the gained equation was about 10% higher than that in the previous reports in the relative
bio-potency. The growth effect of fish liver oil on the feeding level of vitamin A was about
25% higher than that of the synthetic vitamin A oil.

As the true response to the growth is considered to be limited in a small range of the dose
the potency obtained here may be apparent, but this result is interesting because the vitamin
A potency as a feed is the growth effect expected at the feeding of the sufficient dose.

The potency of fish liver oil vitamin A is expressed as the percentage of that of the
synthetic vitamin A when all the vitamin A in synthetic vitamin A is supposed as of all-trans
form, but vitamin A in synthetic vitamin A oil is not all all-trans vitamin A. As it is
important to know the values obtained by the methods actually used in the commercial trans-
actions, a comparison of the values obtained by the method in the 16th edition of U. S.
Pharmacopeia, Carr-Price method and the maleic value method was performed. When the
relative bio-potency calculated from the maleic value is higher than 85%, the value obtained
by the U. S. Pharm. method is practically equal to the true potency and in the author's bio-
tests the value obtained by the spectrophotometry is considered to show the true potency when
the relative bio-potency obtained from the maleic value is 78%. This result concurs with that
of Murray et al⁵⁾ that the value obtained spectrophotometrically shows the true bio-potency of
the general fish liver oil and differs from the result of Ames et al.

It is also interesting that the relative bio-potency of vitamin A in the undistilled oil
extracted from fresh liver was very high almost equal to that of all-trans vitamin A. The
existence of the marked isomerisation of vitamin A even in the undistilled oil in the previous
paper is considered that the isomerisation has already occurred in the liver sample. From
this fact, to get the vitamin A of high relative bio-potency from fish liver, it is necessary
to use the fresh liver and to prevent the isomerisation during the extraction as well as to
consider the conditions of heat treatments such as distillation. As some of vitamin A in fish
liver oils, especially in the distilled oil samples have the relatively lower bio-potencies, the
cause of the isomerisation of vitamin A in fish liver oil will be investigated and reported
later.

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also show my appreciation here to the Japan Fish Liver Oil Manufacturer's Association, Japan
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study.

Summary

The vitamin A potency of fish liver oil was determined by the growth tests and the liver
storage tests of albino rats, and the relationship between the maleic value and the bio-potency

was confirmed.

Even in the lower level of the needed dose, the difference between the synthetic vitamin A oil and the fish liver oil was not occurred in the non deficient period.

In the feeding level, the vitamin A potency of fish liver oil was shown better than that of the synthetic vitamin A oil by their growth effects.

By the comparison of the values obtained by various methods, the relationship between the vitamin A potency to all-trans vitamin A and the value obtained by spectrophotometry was discussed.

By the corrected equation obtained from the bio-tests in this report, the vitamin A potency estimated by the maleic value was about 10% higher than that obtained by the original method, and the vitamin A potency of fish liver oil having more than 78% of relative bio-potency was equal to that obtained by the spectrophotometry.

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魚肝油ビタミン A の効力としろねずみに対する成長効果 (要旨)

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前報に示したとおり、魚肝油ビタミン A の生物効力は試料によつて種々の値を示し、効力の大きなものは全トランス型ビタミン A に匹敵するが、なかには相対生物効力にして 70% を示すものもみられた。このような異同はマレイン価から推定しえたように、種々の条件によつて生ずる異性化によると考えられ、このような異性化は合成ビタミン A についても観察された。それらの結果はしろねずみの成長試験においても認められたが、その投与量によつて差異を生ずるような傾向がみられたこと、既往の実験値は合成 A と天然 A との効力が投与量の高い所で比較され、且つ差異を生じていないことから、マレイン価と生物効力の関係をたしかめ、飼料レベルに近い投与量でのビタミン A 効力の比較を行つた。又相対生物効力によつてビタミン A の効力を示す場合、これは全トランス型ビタミン A の効力に対する % で表わされるので、種々得られた値を用いて、一般測定に用いられる吸光法による測定値との比較検討を行つた。

以上の結果より、限界投与量においても、非欠乏動物では合成ビタミン A 油、天然ビタミン A 油のビタミン A の生長効力に差を生じないこと、充分量の投与ではむしろ、天然 A 油がまさること、マレイン価から推定した効力は、Ames らの値より 10% 程度大きいこと、相対生物効力 78% 以上の魚肝油のビタミン A 値は吸光分析値と一致することをみた。

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