

On the simultaneous extraction of vitamin A-D and
vitamin B₂ complex from the liver of a fin whale
(Nagasu-kujira, *Balaenoptera physals L.*)

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Since the discovery of vitamin A in 1913, by Osborne and Mender,⁽¹⁾ studies of vitamin showed extraordinary development. Later, Mellanby,⁽²⁾ McCollum,⁽³⁾ Sherman, Pappenheimer⁽⁴⁾ and others distinguished between vitamins A and D.

Then Kawakami,⁽⁵⁾ Kawai⁽⁶⁾ and others confirmed that vitamins A and D existed in the liver of fishes widely and in rich concentrations. Material for vitamin A and D is now almost wholly supplied from the liver of aquatic animals.

It was also discovered that vitamins A and D existed richly in whale liver. Whale liver is now one of the most essential resouces of vitamin A-D oil, as its volume is very large.

In 1926, vitamin B₂ was distinguished from vitamin B, by Goldberger and others,⁽⁷⁾ and so-called "Complexity of B₂" was claimed.

Members of B₂ group is now said to contain many factors including the following: lactoflavin (riboflavin), nicotinic acid, vitamin B₆ (adermin), pantothenic acid and other factors. Then, Kuhn,⁽⁸⁾ Karrer⁽⁹⁾ and others discovered that liver contained a large amount of vitamin B₂ complex.

B₂ complex is richly contained in the liver of aquatic animal, especially, in those of tunny (Maguro; *Thunnus orientalis*), spearfish (Makajiki; *Makaira mitsukurii*), swordfish (Mekajiki; *Xiphias gladius*), bonito (Katsuo; *Katsuwonus vaganus*), fin whale (Nagasu-kujira, *Balaenoptera musculus L.*), humpback (Zato-kujira; *Megaptera nodosa Bonn*), etc.

There are many method of extracting liver oil, principal methods being as follows:

(A) Heating with steam—

Liver is heated with steam, and then the oil separated.

(B) Resolving by standing—

Liver is left standing, putrefied, resolved, and the oil separated.

(C) Vacuum method—

Liver is placed in a vacuum kettle, and the oil separated by heating with low pressure steam or hot water.

(D) Refrigeration method—

Liver is pressed while frozen, and the oil separated.

(E) Electrolytic method—

Liver cells are destroyed by passing an electric current, and then the oil separated.

(F) Extraction with organic solvents—

(i) Dry method—

Liver is heated and almost dried. And the oil extracted with organic solvents such as ether.

(ii) Resolving with caustic soda—

Liver is dissolved in caustic soda, and then the oil extracted with organic solvents, or separated by centrifuge.

At present, the last method is almost always employed because the yield of liver oil is very good, and vitamins A and D are extracted almost completely without decomposition.

The present method of preparation of liver oil is limited to the obtaining of vitamin A-D oil, and sacrificing of other effective components which are contained in the liver is unavoidable.

The author aimed at this point and tried to extract as completely as possible both the vitamins A-D and B₂ from one liver.

Vitamin A is quite stable to alkalis but is sensitive to acids, while vitamin B₂ (lactoflavin) is quite stable in strong mineral acids but is sensitive to alkalis. Vitamin A is sensitive to oxidation, but lactoflavin is quite stable to agent. On account of these conflicting properties, it is difficult to extract both A-D and B₂ completely without decomposition.

Vitamins A and D are fat-soluble vitamins while B₂ complex is water-soluble. The author, therefore, tried to extract first, B₂ complex with water, and then A-D oil from its residue. Extraction of vitamin B₂ complex on various conditions were tested to observe how the quantity of vitamin A changed by various conditions, and to discover conditions under which B₂ complex is extracted completely and decomposition of vitamin A is the least. Vitamin B₂ complex was extracted by various methods and the quantity of lactoflavin in the extract was measured. Its residue was then resolved with caustic soda, the liver oil extracted with ether, and then the

quantity of vitamin A was measured.

Considerations were given to the effect of air, acids and heat on vitamin A, and to the effect of destruction of cells, and temperature of extraction on Vitamin B₂.

Fin whale liver was used as material.

Experimental

Fin whale liver was minced in a meat-chopper, mixed homogeneously and 100 g of it were used for each experiment.

I. Quantitative analysis

A) Quantitative analysis of vitamin A.

Raw liver or the liver from which B₂ complex had been extracted was taken, about 100 cc of water added and warmed in a water-bath which was later brought to a boil. After the meat had coagulated, solution of 2 g NaOH dissolved in 20 cc of water was added little by little to the coagulated liver with agitation. In the meantime, about 200 cc of water was also added to it, little by little.

After the whole quantity of NaOH solution and water were added, the heating was continued until the granules of liver melted completely and turned into a liquid. This liquid was then allowed to cooled, transferred into a separating funnel, and vitamin A-D oil was extracted five times with 140 cc each of ether. The upper ethereal layer was gathered, and washed with water until the washings did not colour to phenolphthalein. After the ethereal solution was dried with anhydrous Glauber's salt, ether was distilled off, and vitamin A-D oil was obtained.

The yield of this oil was weighed, and the quantity of vitamin A in 1 g of the oil was measured with spectrophotometer by spectro-graphic method (solvent, chloroform).

$E_{1\text{ cm}}^{1\%}$ 328 millimicron = 1600⁽¹⁰⁾ was used.

International unit (I. U.) per gram multiplied by the yield of oil gives the total amount of vitamin A in 100 g of liver.

B) Quantitative analysis of vitamin B₂ (lactoflavin)

10 g of acid clay (Fuller's earth) was added to the liquid by which vitamin B₂ was extracted from 100 g of liver. By shaking this mixture vigorously, lactoflavin was adsorbed completely, and then filtered. The adsorbed earth on the filter paper was washed with a small amount of

water, and dried at 60°C. 50 cc of alcohol (94%) was added to the dried earth, the mixture shaken, filtered and the earth again dried at 60°C.

Lactoflavin was eluted from one-tenth quantity of this adsorbed earth with 20 cc of 40% alcoholic 0.25 N-NaOH solution. The residual Fuller's earth was eluted again with 20 cc of 40% alcoholic 0.1 N-NaOH solution, and the two lots of solutions were brought together. This was exactly neutralized with HCl, concentrated at 40°C under a reduced pressure, and alcohol was then distilled off. This was further concentrated to about 10 cc, and was made into a 0.5 N-NaOH solution by the addition of 5 N-NaOH solution.

This solution was measured by Kuhn's method^{(11) (12)} with Pulfrich Stufenphotometer (Filter S. 47; $E_{1\text{ cm}}^{100\gamma/\text{cc}} = 4.75$).

The value thus obtained multiplied by the gives the quantity of lactoflavin (γ) in the extract obtained from 100 g of liver.

II. Extraction of vitamin A-D oil and B₂ complex under various conditions.

(1) Quantative analysis of total vitamin A in 100 g of liver.

Total quantity of vitamin A extracted from 100 g of minced liver by the abovementioned method of resolving with caustic soda, was measured.

Vitamin A	720,000 I. U.
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(2) Extraction of vitamin B₂ complex by boiling.

100 g of liver as above was taken. 300 cc of water was added to it, and the solution was brought to pH 5.0 with the addition of phosphoric acid. This solution was boiled in a water-bath for 2 hours, filtered with filter-paper while hot. By the above-mentioned method, the quantity of vitamin A in the total amount of residue, and the quantity of lactoflavin in the filtrate were measured. In this case, owing to having boiled in acidity, the amount of vitamin A decreased greatly.

Vitamin A	550,000 I. U.
Lactoflavin	1086 γ

(3) Extraction by boiling in vacuum.

Since vitamin A is oxidized easily in acidity and on heating, the boiling was made under a reduced pressure in order to prevent decomposition. However, the degree of the heating was to such an extent that water in the water-bath was boiling although, it goes without saying that the temperature of liver solution did not reach 100°C. Other conditions were the same as above.

The quantity of vitamin A in this case, was greater, but, on account of insufficient heating, the amount of lactoflavin decreased.

Vitamin A	690,000 I. U.
Lactoflavin	1022 γ

(4) Extraction by digesting.

The liver was digested in order to make the the extraction of vitamin B₂ complex better by destruction of liver cells.

100 g of liver as above, was taken, 300 cc of water and 15 cc of 90% phosphoric acid were added, and then 1 g of saccharated pepsin (J. P.) added with stirring, and kept at 40°C for 2 hours. The digested solution thus obtained was filtered, and the same processes as above were carried out on the filtrate and the residue.

In this case, the extraction of B₂ became somewhat better, but owing to higher acidity the amount of vitamin A obtained was decreased.

Vitamin A	576,000 I. U.
Lactoflavin	1150 γ

(5) Extraction by digesting in vacuo.

The digesting process, in this case, was carried out in vacuo. Other conditions were the same as above.

Vitamin A	690,000 I. U.
Lactoflavin	863 γ

The amount of vitamin A became somewhat better, but, on account of insufficient heating under reduced pressure, the yield of B₂ decreased exceedingly.

(6) Extraction by digesting and boiling.

In order to extract lactoflavin more completely, the digested liquid was boiled for 10 minutes, filtered while hot, and the residue and the filtrate were treated in the same manner as above. The condition of the digestion was the same as for (4).

Vitamin A	456,000 I. U.
Lactoflavin	1788 γ

(7) Extraction by digesting und boiling in vacuo.

In the above process, vitamin A was oxidized greatly because of boiling the solution in strong acidity. Hence the same process was carried out in vacuo.

Vitamin A	675,000 I. U.
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Lactoflavin 1563 *r*

(8) Extraction by digesting, neutralizing and boiling.

In the above process satisfactory result could not be obtained on the yields of both A and B₂. This must have been due to the fact that vitamin A was boiled in acid, and also to the reduced pressure.

The liver, accordingly, was digested by the method as in (4), then the solution exactly neutralized with NaOH solution, boiled for 10 minutes, and filtered.

Vitamin A 645,000 I. U.

Lactoflavin 1789 *r*

(9) Extraction by digesting, neutralizing and boiling in vacuo.

The operations as in (8) was carried out in vacuo.

Vitamin A 703,000 I. U.

Lactoflavin 1406 *r*

In all the methods of extractions under various conditions mentioned above, the vacuum process for the extraction of vitamin A brought a good result, but it brought a bad result on B₂. The inferior result of the extraction of B₂ was due to the failure of the temperature to reach boiling under reduced pressure.

Hence, it was thought that if the boiling was carried out under ordinary pressure without air, the result would be better. Carbon dioxide gas was used as an inert gas for this purpose.

(10) Extraction by digesting, neutralizing and boiling in carbon dioxide gas.

CO₂ gas was used instead of a vacuum, and all the processes of digestion, neutralization and boiling were carried out in CO₂ gas.

Vitamin A 711,000 I. U.

Lactoflavin 2110 *r*

The loss of vitamin A, as a result, was almost negligible, and the extraction of B₂ was better.

(11) Extraction by boiling, digesting, neutralizing and boiling in CO₂ gas.

The liver was coagulated by boiling so as to allow pepsin to act easily on proteins, and cooled to 40°C by adding water, pepsin was then added. All the process were carried out in CO₂ gas. Other conditions were the

same as for (10).

Vitamin A	711,000 I. U.
Lactoflavin	2849 γ

Summary

Conditions under which vitamin A is decomposed least and vitamin B₂ extracted completely from a liver, are as follows:

Minced liver is boiled with water in CO₂ gas, cooled to 40°C by adding water, acidified with phosphoric acid, and the liver substance is digested with protease such as pepsin at about 40°C. After digestion is completed, the solution is exactly neutralized with NaOH, boiled again and filtered. All the above process carried out in CO₂ gas.

Vitamin B₂ is obtained from this filtrate, and vitamin A-D oil from the residue.

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