On the Respiratory Pigments of Whale
(Studies on Whale Blood II.)

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The diving method of whale is very regular and ordinarily this may be divided into the two classes of short diving and long diving. That is, it makes a relatively shallow dive, then breathes by raising its head and back above surface of water and then makes a shallow dive again. The diving and breathing is repeated several times. This repeated dives are called surface dives. After repeating this surface dives several times, it makes a deep dive in the water. This long dive is called "sound". After one "sound", surface dives are repeated several time. The time of "sound" is relaively constant, depending on kind of whale, in case of baleen whale, it is from 7 to 10 minutes, but this exceeds 30 minutes in case it senses danger. It is especially long in case of sperm whale, which is an tooth whale. It is from 30 to 70 minutes and averages about 40 minutes.

Several explanations are given on how oxygen is supplied during such prolonged submergence.

Ommanney(1) states that the vascular networks (retia mirabilia) functions as this oxygen supply. That is, a special structure called vascular networks which are abundant in blood vessels and fats act as accessory lung and functions as strage for oxygen. However, Laurie(2) states that this is impossible and points out that from the relation between basal metabolism and lung's vital capacity, whale can be submerged for a long time. That is he calculated the lung's vital capacity of a 122,000 kg blue whale at not less than 3050 liters and that the air requirement per minute at 178.75 liters. He states that according to these calculations, it is possible for a whale to be submerged for at least 17 minutes.

As stated above, a whale makes several surface dives and then a long dive. "sound" From this fact, the author considers that the whale breathes several times during the several surface dives, by this stores a large quantity of oxygen in some part of the body and consumes it during a long submer gence. One of the storage organ may be the vascular networks as proposed by Ommanney out the author thinks that it is rather the respir-

atory pigments. The muscles of mammals which carry on a swiming life such as pinnipedias and cetaceas are darker in color than those of land mammals. Especially in the case of sperm whale, the muscles are almost black in color.

The so-called respiratory pigments are haemoglobin, myoglobin (muscle haemoglobin) and various kinds of cytochromes. All of these are pigment proteins containing iron and conbines readily with oxygen. Of the above, the molecular weight of myoglobine is 17,500[3] or about 1/4 of that of haemoglobin, about the same as that of haemoglobin in iron content, or 0.345%,[4] combining power with oxygen and carbon monoxide is much stronger than haemoglobin and also, reaction is much faster.[5] There are several kinds of cytochromes and it is believed that they act as carriers of hydrogen by the oxidation and reduction of iron within the molecules. The representative ones are *a*, *b* and *c*.

The author carried on quantitative analysis of iron in haemoglobin, myoglobin and cytochromes *a* and *c* of these respiratory pigments and determined the content of the different pigments. As control, the blood and muscle of cow and horse, which are land mammals, were used.

To determine the haemoglobin content in blood, Sahli's method using haemoglobinometer was used concurrently with the method in which iron content is quantitatively determined directly. The result of the determination shows that by Sahli's method, there is no great difference in haemoglobin content between whale and land mammals. However, the direct determination of iron indicated that it is considerably greater in whale than in land mammals. In the case of land mammals the results from Sahli's method and iron determination method coincides rather closely but in the case of whale, the iron content is greater in each case. That is, it can be assumed that there is a greater quantity of iron compounds other than in the haemoglobin state. By Barkan and Schales,[6] it was discovered that in the blood of mammals, desides haemoglobin, iron-containing organic compounds whose iron ion is readily liberated by dilute hydrochloric acid are also present. It is said that this substance has an affinity to oxygen about 4 to 10 times greater than that of haemoglobin. It can be considered that perhaps the presence of these substances is anticipated.

In regards to cytochrome *b*, the quantity was so small that it was

impossible to determine so it will be omitted. Content in cytochrome *a* and *c* did not differ greatly from those of land mammals. Thus it is assumed that it did not have any special meaning relative to oxygen storage.

Myoglobin content in whales is much greater in all cases when compared with land mammals and especially in the case of sperm whale which makes "sound", the content is about 8 times more.

From the above result, it is assumed that one reason why whales can withstand "sound" is that oxygen is stored by myoglobin within the muscles.

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Experimental

Materials used in this experiment was a sperm whale and a sei whale caught off shore of Kinkazan Island, the former 31 feet ♂ and the latter 45 feet ♂. In both cases, they were 24 hours after death.*

Materials from cow and horse used as control was obtained immediately after slaughter from the Shibaura Slaughter House in the case of heart and in the case of muscle, those on the market were used.

(1) Quantitative Determination of Haemoglobin

Blood samples in each case was obtained from the heart.

(A) Sahli's Method

According to Sahli's method, haemoglobin was transformed into acid haematin by adding $\frac{N}{10}$ HCl and colorimetric determination was made with Sahli's Haemoglobinometer.

(B) Quantitative Determination of Blood Iron

In accordance with Pincussen's method,[7] blood was decomposed with sulphuric acid and 30% H_2O_2, and comparative colorimetric determination was made of it, colored with KCNS, with standard concentration solution of Iron Alum ($Fe_2(SO_4)_3(NH_4)_2SO_4 \cdot 24H_2O$) colored with KCNS, using

* Materials were obtained in July, 1948. After this, there was a revision in law and cathing of sperm whale less than 35 feet was prohibited.

Duboscq's colorimeter.

Table 1. Quantity of Haemoglobin (in 100 cc of blood)

	Haemoglobin	Fe (Hb×0.336)	Fe (Pincussen Method)
Sei Whale	15.6 g	52.4 mg	84.0 mg
Sperm Whale	15.8 g	53.1 mg	95.0 mg
Spem Whale foetus	5.8 g	19.8 mg	53.8 mg
Cow	12.4 g	41.6 mg	41.0 mg
Human(29 years old)	13.0 g	43.7 mg	46.5 mg

Quantity of haemoglobin determined by Sahli's method multiplied by 0.336 should give the quantity of iron in the haemoglobin state. In the case of cow and human the quantity of iron in the haemoglobin state and the total iron content is about the same. However, in the case of whale, iron determined by Pincussen's method is much greater. The difference is great especially in the case of foetus. It is believed that this is because of the presence of larger quantity of iron other than in haemoglobin state.

(2) Quantitative Determination of Iron in Cytochrome State

In order to be accurate, the quantitative determination of cytochrome should be made by spectral analysis but in the case, it was refined considerably and iron content determined. The determination figures does not indicate absolute quantity but relative quantity determined under the same conditions. For materials the above-mentioned heart and muscles of whale, cow and horse were used.

(A) Quantitative Determination of Iron in Cytochrome *a* State[8]

A solution containing 2% Na-cholat and $\frac{1}{20}$ mol Na_2HPO_4 is, for convinience, designated as A-solution. Muscle is washed with water, chopped with a meat chopper and 1 kg taken. 3 liter of A-solution is added to this, extracted for one night at room temperature, strained with gauze and to 3 liter of the filtrate, 750 cc of a weakly ammoniac saturated ammonium sulphate soltion is added (0.2 saturation). This liquid is separated with a centrifugal separator and to the clear liquid is added more saturated ammonium sulphate solution to make it 0.5 saturation. Cytochrome *a* will precipitate out. The precipitate is placed in a centrifugal separator, dissolved in 200 cc of A-solution and made 0.2 saturation with saturated ammonium sulphate solution. This solution is left standing for 2 days in an ice room, precipitate is removed with centrifugal separator, the clear liquid is made 0.33 saturation with ammonium

sulphate, precipitate is dissolved with 100 cc of A-solution, precipitate obtained by 0.2 saturation ammonium salphate is removed, precipitate obtained by 0.33 saturation is collected and this operation is repeated once more. Finally, precipitate obtained by 0.33 saturation becomes a reddish brown, paste-like substance. This is dissolved in A-solution, the total quantity brought to 100 cc., 10 cc. of this is taken, the entire precipitate obtained by saturation with ammonium sulphate is decomposed with H_2SO_4 and H_2O_2 and iron determined by the aforementioned Pincussen's method.

Table 2. Quantitative Determination of Iron in Cytochrome *a* State (mg/kg)

	Sei Whale	Sperm Whale	Cow
Body muscle	1.29	1.91	2.28
Heart muscle	2.54	4.03	0.27

In the case of cow, the figure for body muscle was larger than for heart. In this case, it was not possible to obtain body muscle and heart from the same cow so the materials came from different cow.

(B) Quantitative Determination of Iron in Cytochrome *c* State[9][10]

The muscle sample is washed with water, chopped with a meat chopper, 2.5% trichloracetic acid is added to 1 Kg of this, stirred and extracted for about 2 hours at room temperature. This is then strained with gauze and 1 liter of the filtrate is brought to pH 7.0 with 6*N*-NaOH solution. 500 g of ammonium sulphate is added to this and left standing for one night in an ice room. This is centrifugally separated and the clear liquid brought to pH 3.7 by adding 5*N*-H_2SO_4. Precipitate preduced from this is separated with centrifuge, 20 cc. of water is added to this and dialyzed (cellophane) against 1% saline solution for 2 days. The liquid thus obtained is centlifugally separated and a dark red, clear cytochrome *c* solution is obtained. This is diluted to 100 cc., 10 cc. of this is saturated with ammonium sulphate at 60°C, the entire precipitate is decomposed with H_2SO_4 and 30% H_2O_2 solution and quantitative determination of iron made by the aforementioned method.

Table 3. Quantitative Determination of Iron Cytochrome *c* State (mg/Kg)

	Sei Whale	Sperm Whale	Blue Whale	Cow	Horse
Body muscle	0.43	0.47	0.44	0.30	—
Heart muscle	1.29	0.57	—	0.70	1.17

Cytochrome *c* is obtained by multiplying this quantity of iron by $\frac{100}{0.43}$.

(3) Quantitative Determination of Iron in Myoglobin State(4)

Sample muscle is washed with physiological saline solution, chopped with a meat chopper and 1 kg of this is taken. 1 liter of water is added to this, extracted for 1 night at room temperature and strained with gauze. *N*-NaOH solution is added to 1 liter of this solution and brought to pH 7.0, 250 cc of concentrated basic lead acetate solution is added and the precipitate produced from this is separated with centrifuge. Na_2HPO_4 solution is added to the clear liquid while maintaining it neutral with NaOH and lead is removed. 5 cc. of the clear liquid obtained by centrifuge is taken, saturated with ammonium sulphate, the entire precipitate is decomposed with H_2SO_4 and 30% H_2O_2, and colorimetric determination of iron, in the form of Rhodan-Fe, is made.

Table 4. Quantitative Determination of Iron in Myoglobin State (mg/kg)

	Sei Whale	Sperm Whale	Cow	Horse
Body muscle	31.35	151.00	18.08	—
Heart muscle	7.62	23.12	—	7.15

Myoglobin is obtained by multiplying this iron quantity by $\frac{100}{0.345}$. Compared to cow, the myoglobin content is 2 times more in sei whale, and 8—9 times more in sperm whale.

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