Bacteriological Studies on Freshness of Whale Meat.

(Report No. II)

By

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It was already reported in our previous paper that the degree of freshness of whale meat is proportional to the number of bacteria detected in blood and muscles, that bacteria are the chief factor in the decrease of freshness and that intestinal tracts are the main origin of the invasing bacteria. Here are the results of our further studies based in the material collected in the summer of 1948, which may justify our observations given in the previous paper.

In the summer of 1948, we collected our samples from three sei whales and one sperm whale in the same way as in the previous experiments at Ayukawa, a whaling land station in the Ojika Peninsular, Miyagi Prefecture. We examined the number of bacteria in blood and muscles on the spot and made other experiments in our laboratory in Tokyo.

1. Freshness and the number of bacteria. (a) Slide glass preparations of heart and liver blood. Slide glasses thinnly smeared with blood which had flowed from the heart and the liver respectively during the dissection were prepared, and stained with Giemsa solution, subjected to microscopic examinations with the result many bacteria, both rod and spherical, were detected. The number of bacteria in one field would indicate the approximate degree of freshness. Yet it does not make a precise standard. (b) The number of bacteria in heart blood and muscles.

Table 1

		No. 1 (sei whale)	No. 2 (sei whale)	No. 3 (sei whale)	No. 4 (sperm whale)
date of the catch		July 25	July 25	July 25	July 27
locality of the catch		E 1/2 N 178	E 3/4 N 178	E 1/2 N 181	E 1/4 N 180
temperature of air & water, air pressure		24-25°, 28°, 767	25°, 28°, 767	25°, 28°, 767	25°, 27°, 768
hours post-catch		34	26	68	24
number of bacteria	Blood(ml)	70.000	12.000	9.360.000	23.400
	muscle(gm)	9.400	8.000		
so-called freshnes		80%	80%	50%	80%

It was reported in the previous paper that the number of bacteria in heart blood and muscles obtained by aerobic culture is approximately proportional to the degree of freshness. It is now assumed that the number of the bacteria per ml. will make a standard in the determination of the degree of freshness. (Refer to table No. 1)

2. Freshness and histological degeneration of muscles and the liver. The results of histological examinations of portions of the livers and muscles from sei whales No. 1 and 2 of Table 1 will be shown in Table 2.

Due to the limited portions of the samples, bacteria filling vessels in the muscle were not detected by this experiment.

Table 2

Histological Observations

	No. 2 sei whale (26 hrs. post-catch)	No. 1 sei whale (34 hrs. post-catch)
Muscle sample	1. Shrivelling of muscle fibres medium degree; curves & ruggedness discerned.	1. Shrivelling of muslce fibres high degree; curves & ruggedness conspicuous.
	2. Muscle fibres indistinct.	2. Muscle fibres obscure.
	3. Nuclei in muscular cells discerned.	3. Nuclei in muscular cells obscure.
	4. No bacteria detected.	4. A few individual bacteria detected in connective tissue.
Liver sample	1. Formation of vacuoles marked.	1. Degeneration of vacuoles high degree.
	2. Irregularity of arrangement of liver cells medium degree.	2. Grouping of liver cells very irregular.
	3. Liver lobules disorderly.	3. Liver lobules disorderly.
	4. Porta hepatis transverse fissure, hilum disorderly.	4. Porta hepatis (transverse fissure, hilum) disorderly.
	5. Haemosiderin discerned.	5. Haemosiderin granules conspicuous.
	6. A few bacteria detected between cells.	6. Bacteria fill vessels.

3. Determination of species of the isolated bacteria. Determination of species was attempted regarding the 128 strains of bacteria isolated from blood, muscles and intestinal contents. It proved that even the bacteria isolated by anaerobic culture can grow by aerobic culture after successive transfering on culture media. So we made all the succeeding investigations of biological characters by aerobic culture.

By microscopic examinations of shapes, sizes, grouping, Gram staining and motility as well as by characteristics of colonies on agar media and by gelatin liquefaction and indole reaction, typical 39 strains were selected from the above mentioned 128 strains of bacteria, and subjected to various biological examinations. As the result, six species of Gram positive spheres, three species of Gram negative spheres, five species of Gram positive rhods and ten species of Gram negative

rhods, namely twenty-four species in all, were isolated. They were identified according to Bergey's Manual of Determinative Bacteriology (5th Ed.) and other literatures: and those determined were as follows: Nine species of Micrococcuss five species of Bacillus, six species of Achromobacter, three species of Escherichia and one species of Aerobacter aerogenes. (Refer to table 3)

Table 3

The species of Bacteria Detected (Isolated in the summer of 1948)

names	samples	habitats (according to literature)
Bacillus silvaticus	muscle	soil
B. laterosporus	blood	soil, water
B. brevis	blood, muscle large and small intestines	ubiquitous
B. adhaerens	large intestfne	soil
B. novus	blood, large and small intestines	ubiquitous
Achromobacter liquefaciens	blood	water
A. butyri	blood	milk
A. candicans	large intestine	soil
A. pestifer	blood	air
A. nitrificans	blood, large intestine	soil
A. ubiquitum	blood	water, soil
Escherichia coli communis	blood	intestinal tracts of man and Vertebrata
E. acidi lactici	blood	intestinal tracts of man and Vertebrata
E. freundii	blood	water, soil, intestinal tracts of man and animals
Aerobacter aerogenes	blood	vegetables, ceveals, intestinal tracts of man and animals
Micrococcus varians	blood, muscles large intestine	sea water, water, air, milk
M. flavus	blood, small intestine	air, milk
M. epidermidis	blood, large and small intestines	skin, milk, infection
M. *luteolus*	blood	cheese
M. saccatus	blood, large and small intestines	nasal mucous membrane
M. halophilus	blood	sea water
M. perflavus	blood, muscles	water, air
M. caseolyticus	blood	udders, food stuff
M. rhodochrous	blood	water

The biological characteristic of the above mentioned bacteria coincided with those found in references in all but a few minor points, and any new species was not

detected by the present experiments. In comparison with our investigations in 1947, Micrococcus and Achromobacter were isolaled by both the previous and present experiments while Flavobacterium and Streptococcus faecalis were not detected this time. We succeeded, however, in obtaining Escherichia and Aerobacter for the first time. (Refer to Table 4)

Summary

(1) The degree of freshness is roughly proportional to the number of bacteria in blood and muscles. The number of bacteria in heart blood may be a standard in the determination of the degree of freshness. An approximate degree of freshness may be calculated by means of stained slide glass samples.

(2) In the case of a low degree of freshness, conspicuous deterioration was discerned histologically in the liver and muscles, and clumps of bacteria filling vessels were detected by microscopic examinations.

(3) Besides Micrococcus and Achromobacter which had already been isolated in 1947, Aerobacter and Escherichia were newly isolated by the present experiments. These are the species generally detected in intestinal tracts of man and animals.

It is especially worth noticing that Escherichia and Aerobacter which are generally found in intestinal tracts of man and animals were detected in blood, for it leads to the assumption that these bacteria penetrated into blood vkssels after death and distributed all over the carcass.

Table 5

Character / Species	shape	side	arrangement	Gram stain	spore	motility	surface colonies on solid media	Boulllon: turbidity	Boulllon: deposit	Boulllon: surface grows	colonies on potato	gelatin liquefaction	B.T.B milk	Indol
F 8	rod	1.3~1.5 x 2.0~4.5	single, short chain	+	+ terminal	+	yellowish brown viscid,	+	+	−	brownish white, viscid	+ stratiform liquefaction	acid coagulation	−
B 22	rod	1.0~1.2 x 2.0~3.5	single, short chain	+	+ central	+	white, round	+	++	−	greyish white, poor growth	+ crateriform	no change	−
BCIF 60	rod	0.6~1.0 x 2.0~3.5	single, scattered, in short chain	+	+ terminal	+	greyish white, viscid	+	+	−	greyish white	+ infundibuliform	acid coagulation, later peptonisation	−
C 73	rod	0.8~1.2 x 3.0~4.5	single, short chain, groups	+	+ central	−	greyish white convex, small colony	++	+	−	yellowish brown	+ crateriform	acid coagulation	−
BIC 64	rod	0.8~1.0 x 3.0~5.0	singly, pairs	+	+ terminal	+	greyish purple convex, small colony	+	+	−	greyish white	+ infundbuliform	no change	−
B 16	rod	0.6~0.8 x 1.0~1.5	in groups	−	−	+	transparent, colorless round viscid, glistening	++	++	+	brownish white, glistening	+ infundibuliform	alkali	−
B 18	rod	0.5~0.7 x 1.0~1.3	singly, pair	−	−	−	transparent, glistening, viscid	++	+++	+	greyish white, poor growth	+ infundibuliform	no change	−
C 11	rod	0.5~0.7 x 0.7~1.2	singly, pair	−	−	−	white, raised, small colony	+++	++	+++	white raised, viscid, poor growth	−	alkali	−
B 35	rod	1.2~1.5 x 1.5~2.5	singly, in groups	−	−	+	greyish yellow, raised, large colony	++	++	−	brownish white	−	no change	−
BC 33	rod	0.6~0.7 x 0.8~1.2	singly,	−	−	+	greyish white, smooth	++	++	−	white, poor growth	+ crateriform	no change	+
B 39	rod	0.8~1.0 x 1.5~2.0	singly short chains	−	−	−	white, round, raised, viscid	++	++	+	greyish white	−	acid coagulation	±
B 21	rod	0.6~0.7 x 1.0~1.3	single,	−	−	+	greyish white, round	++	+	−	greyish white	−	acid coagulation	+
B 13	rod	0.6~0.7 x 1.2~1.5	in groups	−	−	−	greyish white, round	++	+	−	greyish white,	−	acid coagulation	+
B 17	rod	0.6~0.7 x 1.0~1.3	in groups	−	−	−	greyish white, convex	++	+	−	greyish white	−	acid coagulation	+
B 9	rod	0.6~0.7 x 1.0~1.2	single,	−	−	+	greyish white	++	+	+	greyish white	−	acid coagulation	−
BCF 17	spherical	1.2	pairs, in grape-like clusters	−	−	−	white, round vescid	+	+	−	greyish white	−	acid	−
BI 13	spherical	1.0~1.2	in grape-like clusters	−	−	−	yellow, round, dry	+	+	−	whitish yellow, stunted growth	+ crateriform	acid	−
BIC 1	spherical	0.8~1.0	in groups	−	−	−	greyish white smooth, glistening	++	++	−	greyish white	−	acid→alkali	−
B 33	spherical	1.2~1.3	pair, in groups	+	−	−	orange yellow, raised, dry	+	+	−	orange yellow, dry raised	+ infundibuliform	no change	−
BIC 17	spherical	0.6~0.8	pair, botryoid	+	−	−	transparent dewdrop-shaped	+	+	−	grey semitransparent growth	+ saccate	acid	−
B 19	spherical	0.8~1.0	pair, short chain	+	−	−	transparent dewdrop-shaped	+	+	−	poor growth	−	no change	−
BF 21	spherical	1.2~1.5	pair	+	−	−	yellow, round raised	+	+	+	vivid yellow, raised glistening	+ stratiform	acid	−
B 6	spherical	1.0	pair	+	−	−	dewdrop-shaped	+	+	−	glistening, poor growth	+ saccate	coagulation later, peptonisation, acid	−
B 42	spherical	0.9~1.0	pair, in groups	+	−	−	orangered opaque, small colony	+	+	−	poor growth	−	no change	−

Note B: blood; C: colon; I: Intestine F: muscles

B milk	Indol	H_2S.	Methyl-red test	Voges-Prosk	Citrate media	Neutral red	Nitrate reduction	Katalase	Sugar decomposition: glucose	lactose	galactose	arabinose	sucrose	dextrin	mannitol	inositol	dulcitol	species	habitat (according to literature)
l coagulation	−	−	−	−	+	−	−	±	+	+	−	+	±	±	+	−	−	Bacillus silvaticus	soil
change	−	−	−	−	±	−	⧺	+	+	−	−	−	−	±	+	−	−	B. laterosporus	soil, water
l coagulation, r peptonisation	−	−	+	−	−	−	−	+	+	+	+	+	+	+	+	−	−	B. brevis	ubiquitous
l coagulation	−	+	⧺	−	±	−	−	+	+	−	+	+	+	+	+	±	−	B. adhaerens	soil
change	−	−	+	−	−	−	−	+	+	+	+	+	+	+	+	±	−	B. novus	ubiquitous
li	−	+	−	−	−	−	−	+	−	−	±	−	+	−	−	+	±	Achromobacter liquefaciens	water
hange	−	+	−	−	−	⊕	−	+	⊕	−	−	−	−	−	−	−	−	A. butyri	milk
li	−	−	−	−	⧺	−	−	+	−	−	−	−	−	−	−	−	−	A. candicans	soil
hange	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	A. pestifer	air
hange	+	⧺	−	−	+	⊕	⧺	+	⊕	−	−	−	⊕	−	−	±	−	A. nitrificans	soil
l coagulation	±	⧺	−	−	+	⊕	⧺	+	⊕	⊕	⊕	⊕	⊕	−	⊕	−	−	A. ubiquitum	water, soil
l coagulation	+	+	±	−	−	⊕	⧺	+	⊕	⊕	⊕	⊕	−	−	⊕	−	−	Escherichia coli communis	intestinal tracts of man and vertebrate
l coagulation	+	+	+	−	−	⊕	+	+	⊕	⊕	⊕	⊕	−	−	⊕	−	−	E. acidi lactici	intestinal tracts of man and vertebrate
l coagulation	+	+	+	−	±	⊕	+	±	⊕	⊕	⊕	⊕	⊕	−	⊕	±	−	E. freundii	water soil, intestinal tracts of man and animal
l coagulation	−	±	−	+	⧺	⊕	⧺	+	⊕	⊕	⊕	⊕	⊕	−	⊕	−	−	Aerobacter aerogones	intestinal tracts of man and animals, vegetables, cereal
l	−	−	−	−	−	−	⧺	−	±	±	±	−	±	−	+	−	−	Micrococcus varians	sea water, water, air, milk
l	−	−	−	−	±	−	−	−	+	±	−	−	−	−	−	−	−	M. flavus	air, milk
d→alkali	−	⧺	⧺	−	⧺	−	⧺	+	+	+	+	−	±	−	+	±	±	M. epidermidis	skin, milk; infections
change	−	−	+	−	−	−	−	−	+	−	−	−	±	−	±	−	−	M. luteolus	cheese
d	−	+	⧺	−	−	−	−	−	+	+	+	−	+	+	+	−	−	M. saccatus	nasal mucous membrane
change	−	−	⧺	−	−	−	−	−	+	+	+	−	−	+	+	−	−	M. halophilus	sea water
d	−	−	−	−	−	−	−	⧺	−	−	−	−	−	−	−	−	−	M. perflavus	water, air
gulation later, peptonisation, acid	−	+	⧺	−	−	−	−	⧺	+	+	+	+	+	+	+	−	−	M. caseolyticus	udders, food stuff
change	−	+	−	−	−	−	−	⧺	−	−	−	−	−	−	−	−	−	M. rhodochrous	water

+ acidformation ○ gas formation