Bacteriological Studies on Freshness of Whale Meat

(Report No. 1)

Tomoichiro Akiba, Takeshi Tsuchiya,
Makoto Umehara and Yoshiharu Natsume

In using whale meat and organs as food-stuff and material of medicaments the value of their utilization depends to a large extent on their freshness. Consequently, it is of great importance to take necessary steps preventing decrease in freshness, and in other words the factors causing deterioration must be clear. However, the research along this line has been insufficient. In order to study these points it is necessary to investigate the various factors such as whether this is due only to self-digestion or autolysis after death of whale, or besides what route bacteria take to be involved in this, or what kind of bacteria invade when and how, if such bacteria are involved in this. We began this research in the summer of 1947 with this object, but our material was very scarce in 1947 due to the passed fishing season, and so these results of 1947 and a part of 1948 will be consolidated and reported as Report No. 1.

Experimental Results

1. Collection of Samples. Trips were made twice, once in August, 1947 and another in July, 1948 to Ayukawa-machi which is located at the tip of Ojika Peninsular. The determination of various kinds of bacteria, putrefaction tests, etc. in this report were carried out on these samples taken from one whale which was dissected in August 1947. As materials for our tests, its blood, muscles, contents of small and large intestines and sea water were used. Blood which flowed out from the heart during dissection, was collected with a sterilized pipette. Consequently, since dissection was not carried on under sterilized condition, the invasion of bacteria from the environments cannot be absolutely refuted. However, since a sterilized pipette was inserted deeply in a copious flow of blood, it may be assumed that bacteria contained in the blood itself were collected. As to meat, about 20 c. cm. of meat lump was cut off, placed in boiling water and boiled for about 3 to 5 minutes. After sterilizing the surface of muscle tissue it

was cut open with a scapel, and 2 or 4 gm. of muscle tissue was cut off from the center. To this, 8 or 2 cc. of physiological saline solution was added and stirred well. After standing only the meat juice was taken and used in the experiments. As to the contents of the intestines, these were cut open and the contents were collected with a sterilized pipette. This was diluted to five times with physiological saline solution and used in the experiments. The intestines were also swollen due to gas produced by fermentation of the contents at that time. Sea water was collected in front of the works and from the bay.

As culture media sea water agar media, meat infusion agar, Endo's media and cysteine glucose agar were used. Sea water agar media were used for the purpose of not losing specific sea water bacteria, cysteine agar media for anaerobic bacteria, and Endo's media for the isolation of colon type bacteria. Sea water agar media were made according to Zo Bell's method, and 0.5% of peptone, 0.01% of ferric phosphate and 1.5% of agar-agar were disolved in 1000 cc. of sea water being aged before the use.

2. Freshness and Number of Bacteria. Number of bacteria detected in blood and muscle was almost proportional to freshness and the number of bacteria also increased as the time elapsed longer after catching. However,

Table 1. Freshness and Number of Bacteria

			A 1	B 1	B 2	B 3	B 4
			Sperm Whale	Sei Whale	Sei Whale	Sei Whale	Sperm Whale
Date Whale Caught			Aug. 12	July 25	July 25	July 25	July 27
Time of elapse after catching (hours)			30	34	26	68	24
Freshness			Satis-factory (50%)	Fresh (80%)	Fresh (80%)	Satis-factory (50%)	Fresh (80%)
Number of Bacteria	Blood per ml.	Meat influsion agar media	Innume-rable	70,000	12,000	9,360,000	23,400
		Sea water agar media	Innume-rable				
	Muscle per gm	Meat influsion-agar media	4,575	9,400	8,200		
		Sea water agar media	4,425				
	Large Intestine contents per ml.	Meat influsion-agar media	Several				
		Sea water agar media	0				
	Small Intestine contents per ml.	Meat influsion-agar media	Several				
		Sea water agar media	Several				
pH		Blood		6.2	6.2	5.9	5.6
		Muscle		6.2	5.8	6.0	6.1

Note: Freshness was expressed by the visual judgement of experts.

Table 2. Characteristics of Bacteria Examined

Characteristics \ Kind of Bacteria	F 19	F 88	F 17	F 12	F 65	B 82	I 45	C 32	S 90	F 66	C 33	I 43	F 15	I 44	S 97	F 6
Shape	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	rod	rod	rod	rod	rod	rod
Size (micron)	0.9—1.3	1.1—1.5	1.7	0.8—1.1	1.1—1.7	1.4—1.6	1.5—1.8	1.3—1.5	1.5—1.6	1.5	1.1—1.3×1.7—1.9	0.8—1.0×1.8—2.0	1.0—1.5×1.8—2.0	0.8—1.0×1.8—2.0	1.0×1.8	1.4—1.5×2.1—2.2
Grouping	single, pairs, chains	single, pairs	single, pairs	single, pairs	pairs, clumps	clumps	single, pairs	single, pairs	single, pairs short chains	shrot chains	single, pairs	single, pairs	single, pairs, short chains	single, pairs gra* nular cytoplasm	single, pairs	single, pairs
Gram	−	−	+	+	+	+	+	+	+	+	−	−	−	−	−	−
Spore	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Motility	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Meat infusion agar	White, round smooth glistening	Greyish white, round, smooth glistening	Canary yellow, raised drying	Yellow, round raised	White, round, glistening	Greyish white, transparent turbid, round raised	Yellowish white, round	Reddish yellow, wrinkled, drying	White, small colonies	Transparent, colorless, small colonies, raised	Cream yellow, glistening	Cream yellow, flat	Greyish white, round, smooth	Greyish white, glistening	Greyish white, round	Greyish white, smooth
Broth: Turbidity	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Broth: Precipitate	+	+	+ yellow	+ yellow	+	+	+ yellow	+ yellow	+	−	+ yellow	+ yellow	+	+	+	+
Broth: Pellicle	−	−	−	−	−	+	+ yellow	−	−	−	−	+	−	+	−	−
Potato	Greyish White, poor growth	White	Canary yellow, poor growth	Yellowish drying	White, poor growth	Greyish white, good growth	Greyish white, poor growth	Orange yellow, wooly	White, good growth	Greyish white	Orange yellow	Orange yellow	White, viscous	Yellowish white	Slight yellowish white	Greyish white
Gelatin liquefaction	−	−	−	+	+	−	−	−	−	−	+	+	−	−	+	−
Litmus milk	acid coagulation	acid coagulation	acid coagulation	No change	No change	acid coagulation	No change	No change	No change	No change	No change	No change	acid coagulation	No change	acid coagulation	acid coagulation
Indol	+	+	+	−	−	+	−	−	−	−	−	−	−	−	+	+
H_2S	+	+	−	−	−	+	−	−	+	−	−	+	+	+	−	+
Methyl red	+	−	−	−	−	−	−	+		+	+	+	+	−	−	−
Voges-Proskauer R.	−	−	−	−	−	−	−	−		−	−	−	−	−	−	−
Citrate media	−	±	−	−	−	±	−	−		−	±	−	−	−	−	−
Neutral red Reduct.	+	+	−	−	−	+	−	−	−	−	−	+	+	−	−	−
Cysteine glucose agar (gas)	++	++	++	++	−	++	++	−	−	−	−	−	++	−	−	++
Nitrate Reduction	+	+	−	−	−	+	−	−		−	−	+	+	−	+	−
Katalase	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	−
Sugar decomposition (left is acid, rigut gas): Glucose	+ ++	+ ++	+ ++	+ ++	− −	− ++	− −	− −		− ++	− −	− −	+ ++	− −	+ −	+ ++
Lactose	− +	+ ++	+ ++	+ ++	− −	− ++	− −	− −	+ −	+ −	− −	− −	− ++	− −	− −	− ++
Saccharose	− −	+ ++	− +	+ ++	− −	+ ++	− −	+ −		+ −	+ −	− −	+ ++	− −	− −	− +
Glycogen	− −	− −	− −	− −	− −	− −	− −	− −	+ −	+ −	+ −	− −	− −	+ −	− −	− −
Xylose	+ ++	+ ++	+ ++	+ ++	− −	+ ++	− −	− −	− −	− −	− −	− −	+ ++	− −	− −	+ ++
Galactose	+ ++	+ ++	+ ++	− −	− −	+ ++	− −	− −	+ −	+ −	− −	− −	− ++	− −	− −	+ ++
Arabinose	+ ++	− ++	+ ++	− −	− +	− +	+ −	+ −	+ ++	+ −	+ −	+ −	+ ++		+ −	+ ++
Dextrin	− +	− +	− −	+ ++	− −	− +	+ +	− −	+ +	− −	+ −	− −	− +		− −	− −
Mannit	− ++	− ++	− ++	− −	− −	− ++	− −	− −	+ −	+ −	+ −	− −	+ ++		+ −	− ++
Optimum Tempt.	27	27	22	27	27	30	27	27	27	27	37	37	27	27	27	27
Range °C	22—43	22—43	22—43	22—30	22—43	22—43	22—27	22—43	22—30	22—43	27—43	22—43	22—43	22—43	22—43	22—37
Optimum pH	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Range pH	5.5—7.7	6.6—7.7	6.6—7.7	6.6—7.7	6.6—7.7	5.5—8.7	6.6—8.7	6.5—7.7	5.5—7.7	6.6—7.7	5.5—9.4	6.6—9.4	5.5—8.7	6.6—8.7	6.6—8.7	5.5—7.7
Optimum salt content	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Range pH	0.5—12	0.5—6	0.5—20	0.5—10	0.5—6	6.5—6	0.5—10	0.5—10	0.5—3	0.5—6	0.5—15	0.5—15	0.5—20	0.5—20	0.5—20	0.5—6
Name of Bacteria	Micrococcus spheroides	M. piltonensis	M. flavus	M. subcitreus	M. saccatus	M. epimetheus	M. pikowskyi	M. luteolus	M halophilus	Streptococcus foecalis	Flavobacterium fucatum	Flav. sewanense	Achromobacter ubiquitum	A. halophilum	A. Cystinovorum	A. lacticum

it was not clearly recognized that there was some relation between freshness and hydrogen ion concentration in blood and muscle at the time of dissection, but all the reactions indicate the progressing decomposition of the sugar.

3. Indentification of Isolated Bacteria. In this paper are reported only the results of experiments as to the samples collected from a sperm whale dissected in August 1947. Result of another samples collected in July 1948 shall be reported in the second report. Identification of various bacteria was attempted as to bacteria isolated at the works and also bacteria isolated after bringing samples to our laboratory. Even the bacteria obtained by anaerobic culture grow better by aerobic culture, after successive transfer on culture media, so all the succeeding examinations of biological characters were made by aerobic culture.

Microscopic examinations were made as to the pure culture of the 57 strains to determine their shape, grouping, Gram's stain, size, motility, etc. and furthermore 19 species were selected according to shape, form and color of colonies on the media such as meat infusion agar, sea water agar media and cysteine glucose culture, and gas production, liquefaction of gelatin, hydrogen sulphide production and indol reaction. As to 19 strains the further examinations were made as following table (Table 2).

Of 19 strains another three were same strains, so that 16 species were identified according to Bergey's Manual of Determinative Bacteriology and other literatures. It was possible to determine 9 different Micrococci, 1 *Streptococcus* foecalis, 2 Flavobacteria and 4 Achromobacters.

These bacteria may generally be found in soil, air, water, etc., and although there may be few cases in which their biological characteristics do not exactly coincide with those found in references, but the principal ones coincide exactly and we could not find new species. In a case as Micrococcus epimetheus, there is a question more or less compared with the place primarily found, but there is not any reason that such bacteria must not be found in places other than where they were found, so that it may be assumed that the presence of such bacteria is probable.

4. Putrefaction Tests. When these bacteria were inoculated on pieces of whale meat and left standing at 30°C, 8 strains produced rancid irritating or light deterioration odor after 18 hours, and 14 strains after 48 hours, and the same time pH trended to alkaline.

Also, in case several bacteria being grouped according to the origines where they are found, are inoculated on the surface of a piece of meat, the rancid odor is produced faster than in the previous cases and appears after 6—12 hours.

Table 3. Deterioration Test, pH aod Odor

Time / No. of Strains	Before	After 4 hrs.	6	12	18	24	48	26
F 19	6.2	6.1	6.0	6.0	6.3	6.8	6.9	7.7
F 88	6.2	6.2	6.0	6.0	6.4	7.0	7.4	++8.2
F 15	6.3	6.1	6.0	6.0	+6.0	+5.9	++6.6	++8.2
F 17	6.2	6.1	6.3	6.3	6.3	6.4	6.5	++7.0
F 5	6.3	6.2	6.2	6.2	±6.4	+7.0	+7.4	+++8.0
F 6	6.2	6.2	6.0	6.0	6.0	6.4	++6.8	+++7.6
F 1	6.2	6.2	6.1	6.1	±6.4	+6.8	++6.8	++7.2
F 65	6.2	6.2	5.9	5.9	6.2	6.4	±6.6	+6.8
F 66	6.2	6.2	6.0	6.0	6.1	6.3	++6.3	+6.4
F 12	6.2	6.2	6.0	6.0	+6.0	+6.4	+6.4	+6.6
C 32	6.2	6.2	6.1	6.1	6.1	6.5	±7.6	+++8.0
C 33	6.2	6.1	6.3	6.3	6.1	6.0	6.1	++7.0
C 34	6.2	6.2	6.0	6.0	+6.0	+5.7	+6.2	+++6.8
B 24	6.2	6.2	6.0	6.0	±6.4	+6.8	+7.4	++8.0
B 82	6.2	6.2	6.0	6.0	6.6	7.0	+7.4	++7.7
I 43	6.2	6.2	+6.2	++6.3	++6.6	++6.9	+++7.8	+++8.0
I 44	6.2	6.2	6.2	6.2	6.0	5.8	5.8	6.6
I 45	6.2	6.2	6.0	6.0	6.0	6.2	5.8	+6.0
S 90	6.2	6.2	6.0	6.0	6.0	5.6	±6.7	+7.4
S 97	6.2	6.2	6.0	6.0	6.0	6.3	±6.5	++7.0
Control	6.2	6.2	6.2	6.2	6.2	6.3	6.3	6.6

Note: +~+++ indicate the degreee of deterioration odor.

Time / Bacterial groups	Before	After 4 hrs.	6	12	18	24	48	Oder
F group	6.2	6.2	6.4	+6.4	+6.8	++7.8	+++6.2	Putrefied odor
C group	6.2	6.2	6.2	6.4	6.5	±6.4	+6.8	Putrefied odor
B group	6.2	6.2	6.3	+6.3	+6.4	++7.0	+++8.2	Ester-like odor
I group	6.2	6.2	+6.6	+6.3	+6.5	+6.8	+++8.0	Putrefied irritating odor
S group	6.2	6.2	6.2	6.2	6.3	+6.8	+7.4	Putrefied odor
Control	6.2	6.2	6.2	6.2	6.2	6.2	6.4	

If 0.2 cc. of bacterial emulsion injected into the pieces of meat, the production of odor is even more conspicuous and also pH becomes conspicuously alkaline.

5. Invading Route of Bacteria. The route of entry of bacteria into blood and muscle has not been clarified as yet, but 4 routes of their entry

can be assumed, that is, explosion wound caused by spear, compressed air pumped into the whale after death in order to keep it afloat, respiratory tract such as lung and the intestinal tract.

In the case of entry through the explosion wound, there is considerable negative pressure in the severed vein on account of the bleeding, therefore it is understandable that some bacteria sticking to the part affected will be absorbed and reach to the heart. When pig is slaughtered and bacteria coated on the wound (in such cases, it is obvious that easily detectable bacteria are used), it is reported that the same bacteria can be found in the bone marrow, and so these facts support this theory. Whales being speared are especially different from animals being slaughtered, because the former try to escape with strenuous activity for more than one hour. More opportunity may be given for bacterial invasion due to the laboured breathing during that time. Next, in regards to pumping in high pressure air, special efforts to prevent entry of bacteria are not taken, so it can be assumed that there is a possibility of bacterial invasion. Also the cutting open the abdomen to cool can give the path of entry for bacteria. Thirdly, in the case of respiratory tract, bacteria can be discovered in the lung of human being and slaughtered pig, in which even the scraps of food are also occasionally found, so there are some scholars who say contamination of blood in heart is caused by passing through veins from the lung. If the detection of principally soil bacteria in lung of pig by Hülphers is taken into consideration, this path of entry can be naturally considered. Even with the present experiments, Micrococcus halophilus, which is a specific sea water bacteria, was detected in the sea water and muscle; also bacteria detected in blood and muscle belong chiefly to soil bacteria; and since a long time has elapsed between the time of catching and the dissection, it is considered that the above three routes of entry play considerably important parts.

However, since whales are animals living in the sea, there isn't any reason why the entry of bacteria into blood and muscle by way of intestinal tract cannot be considered. Even in the case of drowned or poisoned human beings or animals, it can be assumed that perhaps the large number of bacteria which originarily live in the intestines, enter into the blood vessels through the mucus membrane of the intestine due to the bacterial permeability of the intestines after death and to the extraordinary increase

of bacteria. In the case of whale this mode of entry may be accelerated by the air forced into the abdomen, by which the bacteria invade into the blood, and then they will circulate throughout the body and extend from the end of blood vessels into the muscle. Furthermore, from long experience, the staleness of fishes is prevented by removal of the intestine as soon as possible after death, so it may be assumed that intestinal route plays a major role. Also, from our experiments, the facts that 5 kinds of bacteria, i. e. Micrococcus piltonensis, M. epimetheus, Flavobacterium sewanense, Achromobacter ubiquitum and Achromobacter halophilum were found in the contents of the intestine, muscle and blood, and that Streptococcus faecalis which is always found in the intestine was detected in muscles, supports the theory of the intestinal tract route. We were not able to discover Bacterium coli in samples of 1947. This may be due to the fact that Bacterium coli cannot exist for long in the blood as whale is a warm blooded animal similar to pig. However, this requires further study in the future to settle this point.

6. Putrefaction and Bacteria. It was proven that the number of bacteria will increase in blood and muscle as degree of freshness decreases. Next is that three strains of the detected bacteria do not grow at temperatures above 35°C. The majority has optimum temperatures from 27—30°C, so if the temperature of the body of a whale from the time of catching to dissection is within this range, it will be the optimum condition for increase of bacteria and their putrefaction will occur faster.

Furthermore, these bacteria grow even in a media of low salt content (1%), but growth will be better at about 3%. If the concentration is increased to 15%, growth will be greatly reduced, only 4 strains will exist at 20% and growth is checked at 25%. Therefore, putrefaction can be prevented by maintaining salt contents above 25%.

Of the 57 strains of bacteria, 38 decomposed glucose and produced large quantity of gas. The large quantity of foam observed on stale whale meat is probably due to such bacteria. If such bacteria are inoculated on a piece of whale meat and its pH is examined, at first it is about 6.2 and will become lower after 24 hours, but after 48 hours it will become 6.4—8.2 and will give off rancid, irritating or at times a mild putrified odor. These phenomena will become very marked when several strains are inoculated together, but even then the odor is not as foul as in the case of

actually putrefied fish. Also, in case of actual putrefaction proteus bacteria are sometimes detected, but in our cases there was no such advanced case of putrefaction, and the Micrococcus flavus, M. pikowskyi, M. halophilus, Strept. faecalis, Flavobacterium sewanense and Achromobacter halophilum found in our examinations coincided with those detected by other researchers.

Table 4. Species of Bacteria detected

Samples examined	Name of Bacteria	Number of strains	Place ordinarily found, according to references
Blood	Micrococcus epimetheus	2	rubber tree
	Achromobacter ubiquitum	6	soil, water
Muscle	Micrococcus spheroides	3	manure, soil
	Micrococcus piltonensis	2	manure, soil
	Micrococcus flavus	1	air, milk
	Micrococcus subcitreus	1	air, water
	Micrococcus saccatus	1	mucous of nose
	Micrococcus halophilus	3	sea water
	Streptococcus faecalis	5	animal faeces
	Flavobacterium sewanense	1	sea water
	Achromobacter ubiquitum	8	soil, water
	Achromobacter halophilum	1	sea water
	Achromobacter lacticum	1	cow's milk
Intestines	Micrococcus piltonensis	1	manure, soil
	Micrococcus epimetheus	1	rubber tree
	Micrococcus pikowskyi	1	sea water
	Micrococcus luteolus	1	cheese
	Flavobacterium fucatum	1	halibut
	Flavobacterium sewanense	1	sea water
	Achromobacter ubiquitum	7	soil, water
	Achromobacter halophilum	1	sea water
Sea watet	Micrococcus halophilum	2	sea water
	Achromobacter cystinovorum	6	soil

Conclusions

1. Freshness is decreased in proportion to the time elapsed from catching to dissection and to the number of bacteria detected in blood and muscle.

2. As shown in Table 4, 15 species of bacteria were detected in whale's blood, muscle and intestines and 2 species were found in sea water. Of the above, 6 species coincides with those reported by other researchers.

3. There are 4 possible routes for entry of bacteria into blood and muscle and it may be assumed that the intestinal tract is the most impor-

tant route.

4. Although the bacteria detected may not play the leading role in putrefaction, it is obvious that they contribute to the decrease in freshness.

5. Therefore, the most effective method to prevent decrease in freshness is to dissect and dispose of it as soon as possible after catching.

6. That the previous method of salting and freezing after dissection is effective for prevention of putrefaction, can be proven by our experimental results on arresting increase of isolated bacteria.

References

Hunter, A. C.: Bacterial groups in decomposing salmon, J. of Bact. Vol. 5, 1920.

Jensen, L. B.: Microbiological problems in the preservation of meats. Bact. Review, Vol. 8, Nr. 3, Sept. 1944.

Sanborn, J. R.: Certain relationships of marine bacteria to the decomposition of fish. J. of Bact. Vol. 19, 1930.

Takarada Michinori: Research on intestinal bacteria of fresh water fish, J. of Chiba Medical Society, Vol. 20, No. 4.

Takayama Naokiyo: Bacteriological research on decomposition of lobster, J. of Chiba Medical Society, Vol. 21, No. 9, 1943.

Weinzirl, J.: Concerning the relation of the bacterial count to the putrefaction of meat, Amer. J. of Public Health, Vol. 14, 1924.

ZoBell, C. E.: Studies on marine bacteria. J. of Marine Research. Vol. 4. Nr. 1. 1941.