On the Age and the Growth of Teeth in a Dolphin, (*Prodelphinus caeruleo-albus*). (I)

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Introduction

As age data are essential to the analysis of whale stocks, various methods of age determination have been tried to both whalebone and toothed whales. But a perfect one has not yet been developed, chiefly owing to the difficulty of measuring the growth of these animals in a known length of time. It is a rather old knowledge that there is seen a peculiar striation or "stripe-pattern", reminiscent of their possible relation to the age, both on the surface of the baleen plates of the whalebone whales and in the section of the teeth of the toothed whales. In the baleen plate, it is a series of those parallel grooves or "sculptures" which traverse the surface of the plate in the direction of its width; in the teeth of the toothed whales, it is those stripes found in the section of the dentine, which are arranged concentrically with the periphery of the dentine at roughly regular intervals.

It was J. T. Ruud of Norway who first proposed to utilize these "sculptures" for the age determination of whalebone whales. (1) The senior author of the present paper, who recently investigated into the rate of formation of these "sculptures" on the baleen plates, have found that the age of the whalebone whales is determinable from this structure, (2) and, applying this result, has shown that this group of whales attain sexual maturity at much older ages than hitherto believed. (3)

It is attempted in the present study to discover any relationship that may exist between the aforementioned stripes in the section of the dentine and the age of the toothed whales, in the hope that a method of age determination for these whales may be derived from the understanding of such relationship, so as to facilitate the analysis and conservation of their stocks.

Since its ultimate objective lies in contributing to the conservation of the resources of the toothed whales, this study ought to have dealt with

the sperm whale, if conditions had permitted it; for this species far exceeds the other members of the group both in economic importance and in the need for an adequate conservation measure. But this species can hardly be reared, nor is the collection of its specimens covering a wide range of young age classes so easy, because its capture is limited by regulations. On account of these situations, it was decided to use a species of dolphin (*Prodelphinus caeruleo-albus*) as the material and to secure basic knowledges on the relation between the age and the growth of teeth in toothed whales. This dolphin, quite common in the Japanese and adjacent waters, is far smaller and much easier to handle than the sperm whale.

Part of the research fund of this study was supplied by the Ministry of Education as the Grant in Aid for fundamental Scientific Research. And the members of the research group on the "Growth of Hard Tissues" led by Prof. Masahiro Okada of the Tokyo Medico-Dental University gave us plentiful cooperation and guidance during the course of the study. We wish to aknowledge these aids most gratefully. Our sincere thanks are due also to the members of the Arari Fishermen's Cooperative, Shizuoka Pref. for their cooperation in catching and investigating material animals, and to the staffs of the Mito Aquarium, Shizuoka Pref. who kindly took the labour of rearing dolphins during the experiment.

Chapter I.

Observation of the Growth of Teeth by the *Intra vitam* Staining Method with Lead Acetate

With the view of determining the rate of formation of the aforementioned stripes in the dentine of toothed whales, teeth of captured dolphins were stained *intra vitam* with lead acetate by the method of Okada and Mimura (4, 5, 6).

On May 12, 1951, about 150 dolphins were caught by the traditional "driving-in" method in Arari Bay, Shizuoka Pref., of which four, measuring 162, 164, 169 and 220 cm. in body length, were used for the experiment. These were immediately taken on board live-fish boat and brought to the Mito Aquarium after a cruise of about two and a half hours. During the cruise, smaller three dolphins were being accommodated in the live-fish tank of the boat, but the largest one was being laid on the deck and covered with wet straw mats, over which sea water was being sprinkled.

While being on board, the paste containing lead acetate was injected by means of the ordinary lumbar puncture needle into the dorsal muscle

of each dolphin about 6 cm. underneath that part of the skin about 10 cm. down from the center of the base of the dorsal fin. The paste was prepared by kneading the powdery crystals of lead acetate with the 35% solution of soluble starch which had been cooked and left to cool down and become tasty. The dose was 5 to 10 mg. of lead acetate per kg. of body weight of the dolphin, as indicated in Table 1.

Table 1. Dolphins used in the vital staining experiment with lead acetate.

No.	Body length	Date of injection	Date of death	Dose of lead acetate	Sex	Number of corpora lutea	Body weight soon after death	Remarks
P. 1	220 cm	1951. 5. 12 2.00 pm	1951. 5. 19 12.00 am	10 mg./kg.	Female	Old 1	89.8 kg	Lactating
P. 2	162	〃	1951. 5. 26 3.00 am	5 mg./kg.	Female	0	41.6 kg	Immature
P. 3	169	〃	1951. 5. 17 3.00 am	10 mg./kg.	Female	0	54.0 kg	Immature
P. 4	164	〃	1951. 5. 19 3.00 am	10 mg./kg.	Female	0	44.2 kg	Immature

Upon arrival to the Aquarium, the dolphins were released in a sea water rearing pool of about 1,000 square meters, which was the part of a small bay partitioned with net. In spite of the efforts to feed them with fresh or live squids, fresh sardine or other fresh fish, they were quite off their feed and died off within two weeks.

When they died, their teeth were fixed in a 10% formalin solution. Later, the portion containing the 20th tooth counted from the posterior end of the dental series and the adjacent alveolar bone was cut out, and decalcified by being immersed in the 0.2N hydrochloric acid saturated with hydrogen sulphide for about a week, and then cut into frozen sections. These were stained in a 0.1% solution of gold chloride, and examined under the microscope for the deposition of lead to be detected.

The line characteristic to the deposition of lead was not found in the sections from P 3, the dolphin which survived four days after 10 mg. per kg. of lead acetate was injected.

Two dolphins, P 1 and P 4, to which also 10 mg. per kg. of lead acetate was injected, survived seven days in the rearing pool. Though the deposition of lead was not detected in the sections from P 1, the line of deposited lead was found in the dentine and cement of the tooth of P 4.

P 2 survived 14 days after the injection of lead acetate. Though the dose only 5 mg. per kg., a distinct line of deposited lead was recognized in the section of its dentine close to the boundary-line between the dentine and the predentine (Fig. 1 and 2). If the dentine intervening between this line of deposited lead and the above-mentioned boundary-line is taken as

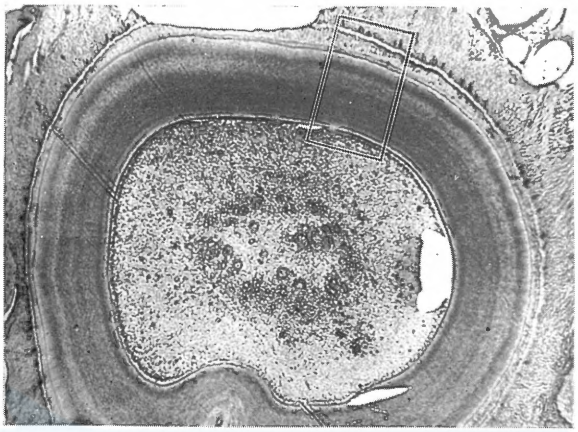

Fig. 1. Transverse section of a tooth of a dolphin (P. 1.) 14 days after the injection of lead acetate. ×20

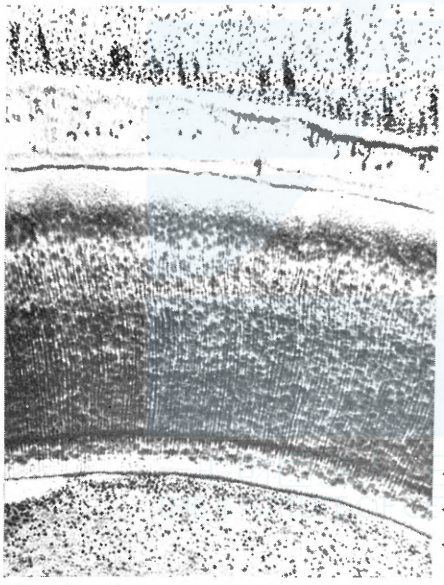

Fig. 2. Enlargement of the squarely marked part in Fig. 1. ×90
D, dentine; Pb, line of deposited lead; Per. d, predentine; Pu, pulp; C, cement.

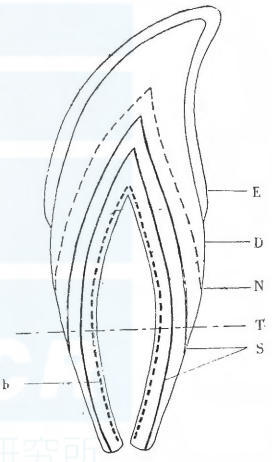

Fig. 3. Semi diagram of longitudinal section of a tooth of a dolphin.
E, enamel; D, dentine;
N, Neonatal line;
Pb, line of deposited;
T, Transverse cut;
S, Stripe.

being formed during 14 days, i. e. the period from injection to death, the whole thickness of the dentine, as measured in Fig. 2, corresponds to the formation during about 200 days. But it does not seem appropriate to draw any conclusion concerning the rate of dentine formation out of this single example, because not only this rate may vary from time to time and from one individual to another but the whole feature of the dentine is not represented adequately in the transverse section of the tooth, as is diagrammatically shown in Fig. 3. We are therefore looking forward for opportunities at which this sort of experiment can be repeated so as to reach any conclusive result.

Chapter II.

Physical Growth and the Morphological Characteristics of Teeth in this Dolphin

Along with the aforementioned experiment of the *intra vitam* staining of the teeth with lead acetate, inquiries were also made into the cause and the rate of the formation of the concentric stripes found in the section of the dentine of this dolphin. In the rooted teeth, as are possessed of by this species, the dentine formation does not proceed at constant rate throughout the life, but slows down with increasing age. Consequently, a linear relation does not hold between the thickness of the dentine and the age of its possessor, as such is the case between the body length and the age. In the dentine of this species, where the calcareous matter is deposited in the form of minute globules, there are not found in the section any growth line of short intervals, but there are recognized considerably distinct stripes being arranged concentrically with the periphery of the dentine, which are probably the relics of some changes in the physiological conditions of the respective dolpoin. Should light be thrown upon the cause and the time of the formation of these stripes, it will become possible to trace back the life history of each dolphin and thus to make a step forward toward the age determination.

General biological observations were made on the 90 dolphins (35 males and 55 females) caught by the "driving-in" method in Arari Bay, on May 13, 1951. Fifteen foetuses got in dissecting these females and another obtained in September of that year were also included in the material. Major lines of the results of this investigation are summarized below.

1. A mode was found between 120 and 150 cm. in the body length frequency (Table 2 and Fig. 4). The dolphins within this size range had

in their stomach milk only, or milk and a small amount of odontophores of squids. On the other hand, the largest foetus investigated was 103 cm. in body length. These facts suggest that the dolphins of the aforementioned size (120–150 cm.) consisted of the youngs of various stages, ranging from recently born sucklings up to the youngs in the very beginning of the weaning period.

Table. 2. Number of dolphins according to body length

Body length in cm	Male	Female	Total
120	1	0	1
130	0	1	1
140	0	2	2
150	1	1	2
160	0	0	0
170	0	0	0
180	5	1	1
190	1	2	3
200	1	4	5
210	6	5	11
220	4	16	20
230	2	12	14
240	5	10	15
250	10	1	11
260	4	0	4
270	0	0	0
Total number	35	55	90
Average length	228 cm	216 cm	221 cm
Sex ratio	38.9%	61.1%	100%

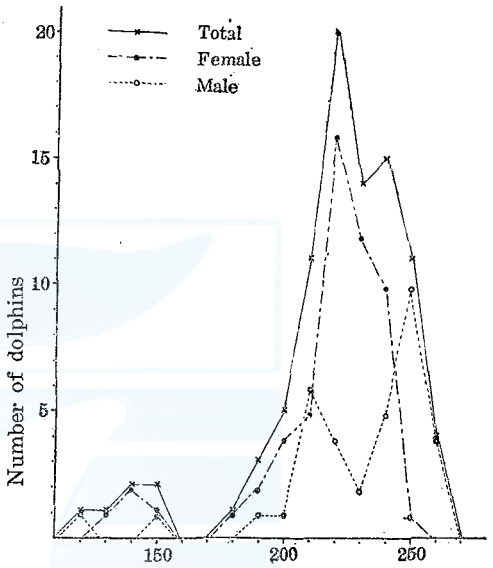

Fig. 4. Number of dolphins according to body length.

2. The number of corpora lutea in ovaries and the weight of both testes combined are plotted against the body length respectively in Fig. 5 and 6. The smallest female that had any corpus luteum in the ovary was 215 cm. in body length (Fig. 5), and the graphs suggest that in both sexes the generative organs are developed very rapidly at about this body length and upwards. It is inferred from these that the body length at the attainment of sexual maturity is about 215 cm. in this species, though the age at that time is not known. It is also suggested by the graphs that after the attainment of sexual maturity there is little increase in body length in either sex, especially in female.

3. By studying the foetal teeth of various stages of development and the teeth of postnatal individuals comparatively, it was ascertained that this species is monophyodont, i.e. with a single set of functional teeth that

is developed during the foetal period and persists all the life without being alternated. This finding confirms the proposition stated by Tomes: "No cetacean is known to develop more than one set of functional teeth." (7)–(p. 439)

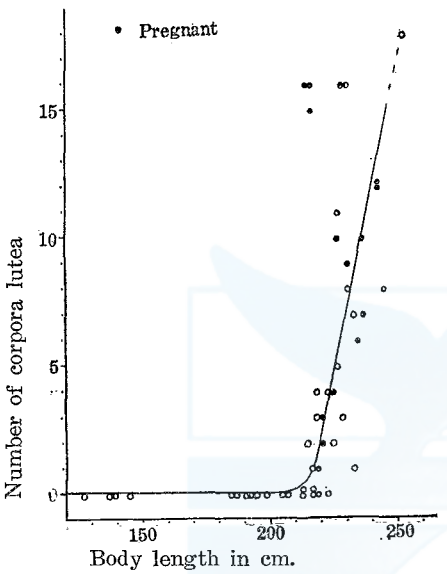

Fig. 5. Number of corpora lutea according to body length.

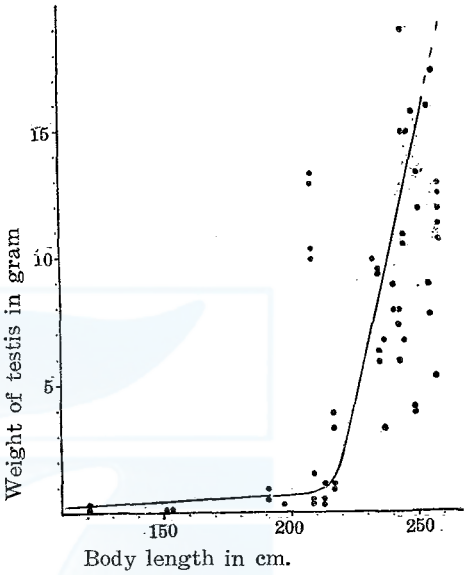

Fig. 6. Weight of testis according to body length.

The teeth of the dolphin are conical homodonts, about 50 of which are found in the dental series on each side of each jaw, though the number somewhat differs in different individuals. Their size varies slightly according to their position in the dental series. In every series, about the 20th tooth as counted from its posterior end is the largest, which is situated about the middle of the series. The tips of the teeth are exposed clear out of the gum near the posterior end of the dental series, but come to be buried in the gum towards the anterior end of the series. For this reason, the teeth are numbered more conveniently by counting them from the posterior end of the dental series.

The dentition is bilaterally symmetrical, and hardly differs in the upper and lower jaws. And all the teeth of an individual, except those implanted near the anterior end of the dental series, show the same striped pattern in the section of their dentine.

In the light of these morphological characteristics of the teeth of this species, one may reasonably designate in every individual a tooth in a fixed

position of the dental series (e. g. the 20th tooth from the posterior end of the series) as the representative of all teeth of that individual, and compare only these teeth with one another, when the teeth of different individuals are to be compared.

Chapter III.

Formation of the Neonatal Line

As was mentioned in chapter II, 15 foetuses were obtained in May, 1951. Their body length composition was: 10 under 10 cm.; 2 between 10 and 15 cm.; and one each of 30, 85 and 103 cm. Five of them were selected so as to represent each size class (Table 3), and the observation was made on the development of their teeth. For the reason stated in the same chapter, the approximately 20th in the dental series as counted from its posterior end was investigated in every case.

Table 3. Dolphin foetuses, of which the development of their teeth was observed.

No.	Body length	Date when mother killed	Sex	No.	Body length	Date when mother killed	Sex
P. 5	103 cm	1951, 5, 13	Male	P. 8	11.5	1951, 5, 13	Male
P. 6	85	〃	Female	P. 9	4.5	〃	〃
P. 7	30	〃	〃				

The longitudinal sections of the aforementioned tooth or the corresponding part of the jaw were prepared by the freezing method after decalcification, double-stained with haematoxylin and eosin, and subjected to microscopic examination.

The primitive tooth-band (primitive dental lamina), which is the thickning of the epithelium of the mouth, was found in the smallest foetus of body length of 4.5 cm. (Fig. 7). The tooth-band (dental lamina) was recognized in the 11.5 cm. long foetus (Fig. 8).

The calcified dentine was first met with in the foetus of body length of 30 cm. (Fig. 9) The other two large foetuses had more developed teeth which were almost of the same form as those of postnatal individuals. And it is worth mentioning that the dentine of the largest three foetuses stained deeply with haematoxylin and was devoid of any striped pattern (Fig. 10).

For the comparison purpose, observation was also made on the teeth of 10 postnatal individuals. Four of these were P 1 to P 4 described in

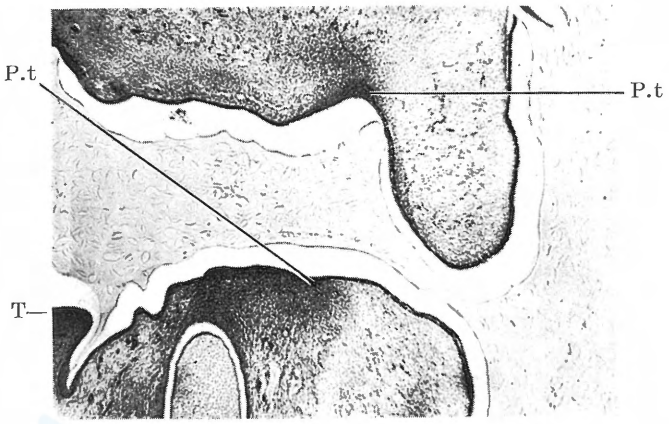

Fig. 7. Cross section of the upper and lower jaws of a 4.5 cm long foetus. Stained with haematoxylin and eosin. ×47
P.t, primitive tooth-band;
T, tongue

Fig. 8. Cross section of the upper jaw of a 11.5 cm long feotus, stained with haematoxylin and eosin. ×47
T.b, tooth-band

chapter I. The other six dolphins were selected out of those 1,050 dolphins caught at Kawana, Shizuoka Pref., on Sagami Bay on December 23, 1951, whose detailed description will be given in the next report. The approximately 20th tooth in the dental series as counted from its posterior end was taken from each dolphin, and its longitudinal sections were prepared by the freezing method after decalcification.

Fig. 9. Longitudinal section of a tooth in the upper jaw of a foetus of body Length of 30 cm, stained with haematoxylin and eosin. ×47
D, dentine.

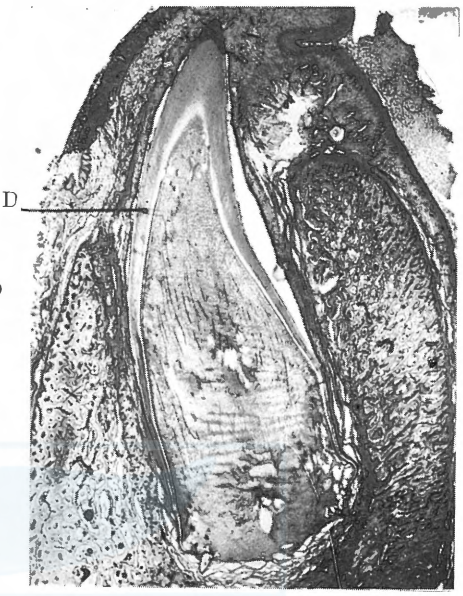

Fig. 10. Longitudinal section of a tooth in the lower jaw of a foetus (prenatal dolphin) of body length of 30 cm, stained with haematoxylin and eosin. D, dentine. ×10

In these sections, it was possible to distinguish the dentine in to three concentric parts of different structure. The innermost part is characterized by its striped appearance or "stripe-pattern", which is due to the alternate arrangement of stainable and unstainable zones, and 2 to 8 stripes (i. e. stainable zones) were seen in this part of the dentine of different individuals. The outside of this zone is bordered with a line unstainable with haematoxylin and, therefore, poorly calcified. The outermost part is characterized by its deep and homogeneous staining with haematoxylin (Fig. 11 and 12); this is the indication of sufficient and homogeneous calcification. It should be noted that this last part coincides, both in form and dimension, with the whole dentine of the 103 cm. long foetus which is regarded as being very close to birth.

If thin sections of the teeth of postnatal individuals are prepared by grinding, a peculiar line is recognized that very position in the dentine where the aforementioned line unstainable with heamatoxylin appears in decalcified sections (Fig. 13). Examination of such specimens under the polarizing microscope reveals that the arrangement of the crystals of calcareous salts differs on the two sides of this line (Fig. 14).

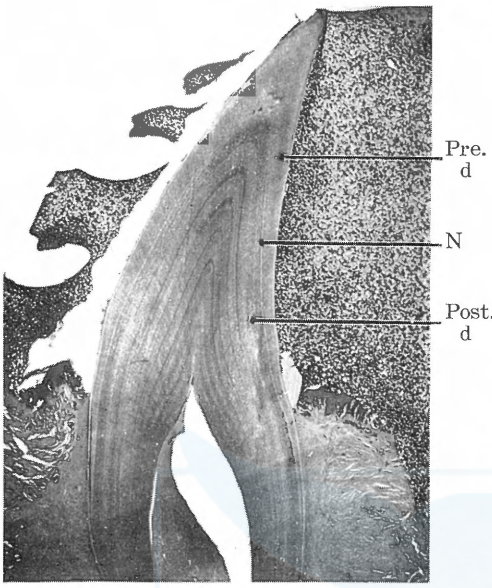

Fig. 11. Longitudinal section of a tooth in the upper jaw of a postnatal dolphin of body length of 220 cm. Stained with haematoxylin and eosin. ×10
Pre. d, prenatal dentine;
Post. d, postnatal dentine;
N, neonatal line.

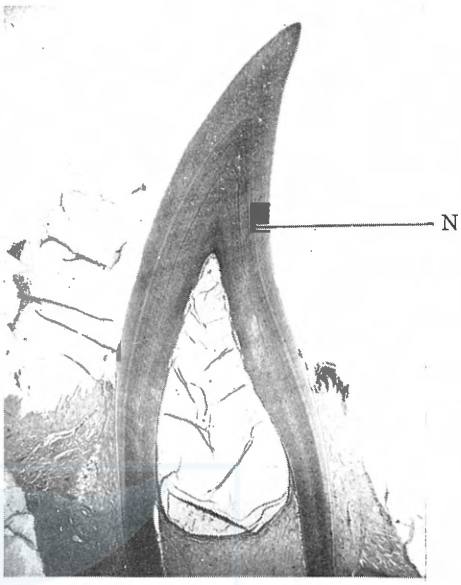

Fig. 12. Longitudinal section of a tooth in the upper jaw of a postnatal dolphin of body length of 162 cm. Stained With haematoxylin and eosin. ×10
N, neonatal line.

In the section of the root, this line is seen to extend downwards out of the dentine and terminate against the surface of the tooth. In this part the contour of the tooth is often marked with a distinct depression (Fig. 11 and 13), which is probably due to the hypoplasia (8) caused by the undernourishment.

These evidences indicate that the line in question, i. e. the line which is recognized in the longitudinal section of the dentine of the postnatal dolphin as a line unstainable with heamatoxylin and trimming the inner, striped part of the dentine, represents the thin layer of dentine which is formed during the period of undernouishment subsequent to birth. In other word, it is the neonatal line.

According to Schour (9), the neonatal line is more distinct in the enamel than in the dentine in human teeth. In the dolphin, however, it is not found in the enamel. This may be explained as that in this species the enamel formation is completed during the foetal stage,...as is indicated by the fact that the enamel of the foetal tooth is of the same dimension

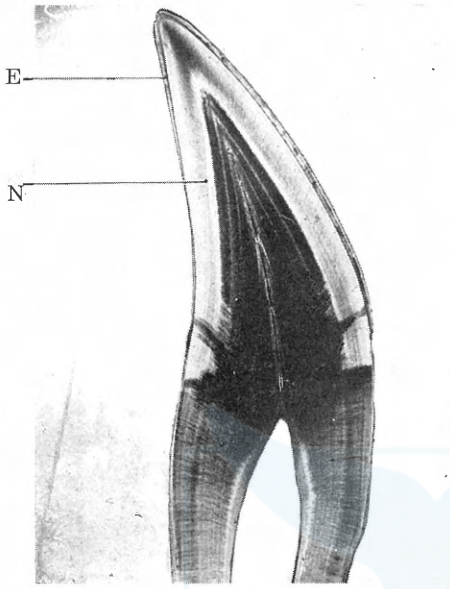

Fig. 13. Longitudinal section of a tooth in the upper jaw of a postnatal dolphin of body length of 220 cm, prepared by grinding, unstained. ×10
E, enamel; N, neonatal line

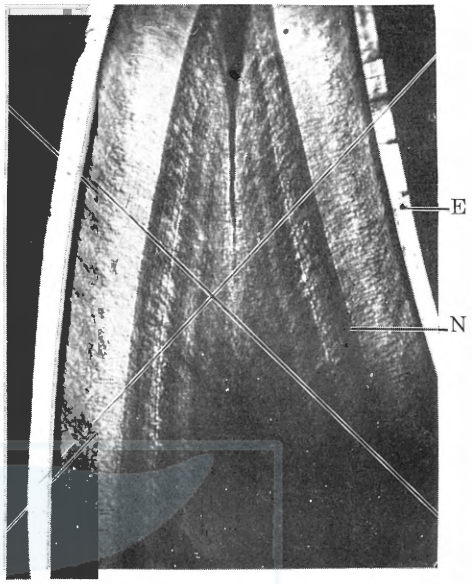

Fig. 14. Longitudinal section of a tooth in the lower jaw of a postnatal dolphin of body length of 195 cm, prepared by grinding, unstained. Observed by polarized light. ×27
E, enamel; N, neonatal line

as that of the tooth of the postnatal individual...and, therefore, is free from the influence of the birth.

Conclusions

1. By the *intra vitam* staining method consisting of the injection of lead acetate into the dorsal muscle, a layer containing the deposition of lead was produced in the dentine of the dolphin. The dose was 5 mg. of lead acetate per kg. of body weight. This layer was recognized as a distinct line in properly prepared sections of the tooth. As to the rate and cause of the formation of the striped pattern seen in the longitudinal section of the dentine, nothing more than preliminary informations were obtained, as successful observations could be made only on few individuals. This was chiefly due to the fact that the dolphins did not survived, after the injection, enough long for their teeth to grow to any considerable extent. Efforts will be made in future so that observation may be made on a considerable number of dolphins being reared for a sufficient length of time after injection.

2. So far as available data indicate, this species reach sexual maturity at the body length around 215 cm. But the data are so scarce that this must be considered as a preliminary conclusion.

3. This species is monophyodont. Its dentition is bilaterally symmetrical, and hardly differs in the upper and lower jaws. The dental series consists of the homodonts of very similar sizes. Except the very small teeth near the anterior end of the dental series, all teeth of an individual show the same striped pattern in the section of their dentine.

4. Such a striped pattern as is seen in that part of the dentine formed after the birth, does not appear in the dentine formed during the foetal period. In the longitudinal section of the teeth, the dentine is seen to be divided in these two parts by a line unstainable with haematoxylin. It was deduced that this line is the neonatal line, which is produced by the change in the nutritional conditions concurring with the transition from the foetal life to the suckling period.

5. We shall proceed further with this study by repeating the *intra vitam* staining experiments and by inquiring into the correlation between various age evidences and the striped pattern in the section of the dentine, so that an adequate age determination method may be derived.

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Errata for

"On the Age-Determination of Mystacoceti"

by M. Nishiwaki (July 1952)

p. 90, line 4. Delete "the" before "seasons."
p. 90, chapter II, line 8, "deals" should be "dealt."
p. 91, line 5, "July and August" should read "July or August."
p. 92, line 26, "year" should be "years."
p. 92, last sentence should read: Therefore, the majority of the females of the southern blue and fin Whales are considered to ovulate and conceive....
p. 94, line 2, "younger or older" should read "younger and older."
p. 94, line 7. Change "Chapter V" to "Chapter IV."
p. 95, line 2. Add "at these ages" after "the size limits."
p. 95, the 2nd line from the bottom, "photocell-ammetertype" should be "photocell-ammeter-type."
p. 101, Fig. 4-a and -b, along the abscissa, "Weight of testis in kg." should read "Weight of testes in kg."
p. 102, Table 2-b. The footnote should read: Note: (1) See the footnote (1) of Table 2-a.
p. 104, line 1. Delete "the approach of."
p. 104, line 7. Delete "the" before "breeding."
p. 107, line 18, "fastgrowing" should be "fast-growing."
p. 109, line 22. A comma should be put before, instead of after, "partly."
p. 110, line 23. Add "than" after "one" year earlier."
p. 111, line 11, "Testis" should be "Testes."
p. 111, the 7th line from the bottom, "Evalrdåets" should be "Hvalrådets."