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Age determination of mammals by layered structure in teeth and bone

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Key to Russian Letters used in the Figures and their English Equivalents used in the Captions.

Letter	Equiv.	
А, а	A	In Figure Captions, lower case equivalent to a, b, c, d.
Б, б	B	
В, в	V	
Г, г	G	
Д	D	
З	Z	
Л	L	
Н	N	
П	P	
Р	R	
С	S	
Ц	T_s	

INTRODUCTION [page 3]

A method of accurate age determination for animals is essential for understanding many aspects of their biology. In the absence of reliable data on age we cannot establish its rate of growth, time of onset of sexual maturity, periodicity of reproduction and life span. Thus, age determination provides for establishing the intensity of reproduction and for determining the life span of individuals. As is well known, these particular questions are of cardinal importance in determining the population dynamics of a species in the sense that this was first formulated for us by S.A. Severtsov (1941).

S.A. Severtsov was the first zoologist to show that the pattern of population dynamics is a specific adaptive property, that each species of animal has its own characteristic pattern of population dynamics, that this pattern is just as characteristic for the particular species as are its morphological features, and that it is the pattern of population dynamics of the species that determines its interaction with the environment. The theory of population dynamics advanced by S.A. Severtsov has already existed for more than a quarter of a century. Verification over a period of time has proved the vitality and significance of this theory. It would be no exaggeration to say that currently the problem of population dynamics is one of the central ones in contemporary ecology. From this it is clear that a precise method of age determination is of great importance in elaborating the ecology.

Age determination of animals is also necessary in conducting many comparative anatomical and morphological investigations, investigations in the field of taxonomy, for without this we are unable to assess the uniformity of the material to be compared and the nature of age variability. Finally, age determination is also highly important for all activities in the field of population variability. Consequently, it would be difficult to overestimate the significance of a method of accurately determining the individual ages of animals with respect to theoretical planning activities.

A method of age determination of mammals is also of great practical significance as it provides an opportunity of establishing the age structure [page 4] of the population. This in turn permits an assessment to be made of the relative numerical state of the population, which is important in regulating the hunting of valuable species of animals. Particularly instructive in this regard is the example of the White Sea herd of Greenland seal. While the hunting of it was increased the stock was reduced. Aerial surveys did not halt this reduction. When the precise method of age differentiation of the herd was used it became evident that the stock was in a catastrophic situation. It became urgently necessary to consider the possibility of imposing a total ban on hunting, which was in fact done.

On the other hand, knowing the age structure of a population, it is possible to assess the numbers of harmful animals and the effectiveness of various series of measures directed against the saboteurs of the economy, and ultimately to predict mass occurrences of animals which are the carriers of diseases dangerous to man.

It is for this reason that a great deal of attention has recently been devoted to the development of methods of precisely determining the ages of mammals and, as will be seen from the ensuing presentation, up to the present time these methods have largely been developed with reference to economically valuable animals. As it is not our intention to discuss here all the proposed methods we will enumerate only the principal, most widely used ones.

One of the oldest is the method of determining the ages of animals from the degree of wear of their teeth. It is used in determining the ages of carnivores and rodents having teeth of limited growth, and of insectivores. The ages of ungulates are determined by reference to the time when the milk teeth are replaced by permanent ones and the degree of wear of the permanent teeth.

For some rodents (for example, muskrats and red voles) as a criterion of age use is made of the stage of development of the roots and the correlation between the root and crown of a tooth. At one time it was proposed to determine the age of pinnipeds from the relative width of the dental canal (Doutt, 1942). Recently, the relative width of the canine canal came to be used for age determination of land carnivores (Smirnov, 1960; Grakov, 1962).

In age determinations of individuals use is frequently made of the external structure and measurements of the skull and its components, weight of the skull, degree of fusion of the sutures of the skull, surface character of the bones of the limbs and the stage of ossification of their epiphyses, while in determining the ages of males of some species the dimensions of the penis bone are used. In order to achieve high accuracy in age determinations it is customary to use not one, but several of such age criteria.

The majority of the foregoing methods are convenient for they do not require complicated technical methods of processing the materials and some of them can be used directly under field conditions. However, they all have common basic shortcomings [page 5], for in the first place they are based on criteria which are subject to individual and frequently geographical variations; secondly, while they provide an opportunity of breaking down a series of individuals into age groups there is no possibility whatsoever of precisely determining the ages of adults and old individuals; thirdly, the majority of these methods are useful for a limited group of species.

The recently developed method of determining the ages of mammals from the weight of the crystalline lens of the eye (Lord, 1959, 1961 et al.) does not have the latter shortcoming but does have the two first ones. Thus, it was established from material on animals of known ages (Bauer et al., 1964) that while the weight of the eye lens of the fur seal increases even in old animals, individual variations within year classes, with the exception of the first and second years, exceed the dimensions of annual increases in weight.

It can be shown that the method of determining the ages of some animals from the horny ridges on claws and horns of Bovidae is evidently devoid of the first two shortcomings. This method is accurate for some Bovidae and for those mammals, the horns of which are not subject to intensive wear. However, in many mammals the horns are either poorly developed or intensively worn away.

Efforts were made to determine the ages of baleen whales from the annual markings on the horny material of the whale's baleen. Recently, widespread use has been made both here and abroad of the method of determining the ages of these animals from the number of layers in the so-called ear plugs.

Finally, we will point to yet another method of age determination from the ovaries. It was also developed on Cetacea and based on a count of the traces remaining in the ovaries of former corpora lutea associated with pregnancies. This method is also not without shortcomings. In the first place, it is useful for a small number of species, and secondly it can be used only for females engaged in reproduction. The ages of sexually immature and old males cannot be determined by this method.

The method of age determination from layered structures of teeth (dentine and cementum) and the periosteal zone of bone, to which the present work is devoted, enables a determination to be made of the age in years of each separate animal and is practically the only one providing for age determination with an accuracy of up to a year for both young and old individuals of both sexes. This method is based on a count of the layers in the tissues of teeth and bone. Since the middle of the nineteenth century information has been accumulating in literature about the existence of layering in the dentine and ridges on the roots of teeth in marine mammals (Owen, 1840-1845; Tomes, 1898 as reported by Laws, 1962; Boschma, 1938; Chapskii, 1941). At the same time, a hypothesis was advanced that in the course of a year a specific number of ridges and layers of dentine form in the teeth of marine mammals, analogous to the formation of annual rings and scales in fish. Early in the 1930's the reliable work of

Eidmann (1932) was published. The author detected [page 6] layers in the secondary dentine of the incisors of red deer, calling them annual rings, and suggested that the ages of the animals could be determined from their number. Finally, comparatively recently and independently of one another two papers were published with respect to determining the ages of pinnipeds: in the USA - by Scheffer (1950) and in Britain - by Laws (1952). Immediately following these publications there was a pronounced increase in interest in the dental structure of marine, and subsequently, land mammals.

Scheffer (1950) studied canines of fur seals Callorhinus ursinus of precisely known ages and reached the conclusion that one ridge forms on the root in the course of a year and consequently that the number of ridges is an indicator of the age of the animal. Laws (1952) described layers in the dentine of canines of the sea elephant Mirounga leonina, consisting of alternating zones of dentine with different degrees of calcification and consequently different optical densities. He showed that one such layer of dentine formed in a course of a year and therefore the number of layers of dentine equals the age of an individual, expressed in years. The papers by Laws and Scheffer provide researchers with precise material on age determination of animals and beginning in 1952 many investigations were conducted over a comparatively short period on the teeth of various species of marine mammals. As of the present time layers of dentine or dental cementum, the number of which is an indicator of the age of an animal, have been found and described in twenty-one species of pinnipeds (Laws, 1952, 1953a, 1953b, 1957, 1958, 1960a, 1962; Rasmussen, 1957; Chiasson, 1957; Mansfield, 1958a, 1958b, McLaren, 1958; Yakovenko, 1959, 1960, 1961; Popov, 1960; Mansfield and Fisher, 1960; Hewer, 1960, 1964; Fiscus, 1961; Klevezal', 1961; Kenyon and Fiscus, 1963; Scheffer and Kraus 1964; Spalding, 1964; Tikhomirov and Klevezal' 1964; Krylov, 1965; Chapskii, 1965) and in six species of toothed whales (Nishiwaki and Yagi, 1953; Mishiwaki, Hibiya, and Ohsumi, 1958; Sergeant, 1959, 1962; Berzin, 1961, 1964; Khuzin, 1961; Mishiwaki, Ohsumi and Kasuya, 1961; Kleinenberg and Klevezal' 1962; Ohsumi, Kasuya and Nishiwaki, 1963).

Soon after Laws' articles were published (1952, 1953a) papers appeared containing descriptions of similar layers of dentine and cementum in the teeth of land animals. Christian (1956) discovered layers of dentine similar to dentine layers in pinnipeds in the canines of the bat Eptesicus fuscus, and Rausch (1961) found them in canines of the black bear Ursus Americanus; Sergeant and Pimlott (1959) described annual layers in the cementum of the incisors of the elk Alces alces, and V.S.Smirnov (1960) described those in the cementum of canines of some carnivores. During the past two or three years a number of papers have appeared in which it is shown that annual layers exist in the cementum of ungulates (Mitchell, 1963; McEwan, 1965; Low and Cowan 1963; Novakowski, 1965), carnivores (Klevezal' and Kleinenberg, 1964; Mundy and Fuller, 1964; Kleinenberg and Klevezal', 1966) and rodents (Klevezal' and Kleinenberg, 1964; Van Nostrand, Stephenson, 1964; Kleinenberg and Klevezal', 1966; Klevezal' and Lavrova, 1966).

[page 7] While sufficient attention has been given to the existence of annual layering in dental tissue, especially on the part of investigators of marine mammals, a related question of interest - the existence of layering in the periosteal zone of bone and its use in determining the ages of animals - has been barely touched upon.

Appositional layers, the number of which increase with age, have long been described in the bones of poikilothermal vertebrates and used for analysing the growth and ages of these animals (a review of papers on this problem was made by Peabody (1961). V.O.Kler (1927) was the first to propose that for analysing the growth of mammals, the outer circumferential lamellae of compact bone should be used, which in his opinion, are completely homologous with the bone laminations of poikilothermal animals. Further work along these lines was done on marine mammals. K.K.Chapskii (1952) described periodical laminations in the compact bone of the lower jaw of the Greenland seal, and suggested that the age of the animals could be determined from the number of these. Subsequently, similar layers were found in the mandible of the sperm whale, the common seal, the guinea pig, and the common dolphin (Laws, 1960b) and a number of proofs were cited in regard to the annual periodicity in the formation of layers of periosteal zone of bone in marine mammals (Nishiwaki et al, 1961; Kleinenberg and Klevezal', 1962; Tikhomirov and Klevezal', 1964; Chapskii, 1965). Annual layers in bone were also discovered in land mammals. Thus, M.N. Meyer (1957) demonstrated that there are annual layers in the mandible of the small gopher Citellus pigmaeus. During the past few years such layers were found in the lower jaw of carnivores, lagomorphs and rodents (Klevezal' and Kleinenberg, 1964; Klevezal', 1965; Bernshteyn and Klevezal', 1965; Klevezal' and Lavrova, 1966).

The work being brought to the reader's attention concerns this method of accurately determining the ages of mammals from the layering of dental and bone tissues. The first chapter acquaints the reader with the morphological basis of the method and with existing concepts as to the causes of the formation of annual layers. The second chapter deals with somewhat more detailed recommendations of methodology. The third chapter is a review of the results of actual investigations related to age determinations of representatives of all orders of the mammalian fauna of the USSR. The authors do not consider this brief summary to be in any sense final. On the contrary, it can be considered only the first phase of the work. Undoubtedly, a wider use of the method will bring with it much that is new and this new information will in turn provide for a deeper insight into the morphological picture of the layering of structures and possibly a more correct evaluation of the biological meaning of processes associated with the formation of layers.

Both the published and original material used here is highly dissimilar for the separate orders. This situation must be tolerated for the time being because the collection of materials [page 8] for certain orders was attended by great difficulties, which were not always surmountable.

We embarked upon the present investigation by developing a method of determining the ages of toothed cetaceans. This work was performed on the ordinary Black Sea dolphin. One of the authors determined the age from the layering of dentine on the teeth and lower jaw, while the other made a parallel determination of the ages of the same specimens by studying the ovaries (Kleinenberg and Klevezal', 1962).

In 1964 we had already obtained our own data on age determinations of carnivores, rodents and lagomorphs (Klevezal' and Kleinenberg 1964).

In the present work, Chapters I-III and the Supplement were written by G.A. Klevezal'. S.Ye. Kleinenberg wrote the Introduction and concluding chapter and undertook the editing of the entire text.

In conclusion, the authors wish to express their deep and sincere appreciation to Professor B.S. Matveyev for his direct assistance and invariably valuable advice. We are also grateful for the consultations and systematic instructions given by Ye.A. Klebanova, N.F. Lebedkina, M.V. Mine and A.V. Yablokov.

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Chaper I. [page 9]

GENERAL DESCRIPTION OF THE ANNUAL LAYERS IN THE TISSUES OF TEETH AND BONE

Brief Description of the Morphology and Nature of Growth of the Tissues of the Teeth and the Periosteal Zone of Bone

This section contains data presented in a highly compressed form which has been extracted from abstracts and review papers. A detailed description of the macro and micro-morphology of the teeth of man and other mammals can be found in the abstracts by Lehner and Plenk (1936), Widdowson (1946) and Schour (1960). A general description of the structure of the bones is given in a monograph by A.V. Rumyantsev (1958) and in abstracts by Weinmann and Sicher (1947) and Pritchard (1956).

In the teeth of mammals the crown and the roots are distinguished. The crown protrudes from the jaw while the root of the tooth is concealed in the dental socket of the jaw. The main mass of the tooth is represented by the dentine, while the outer surface of the crown is covered with enamel and that of the root - with cementum (Fig. 1). An erupted tooth represents a thin walled dentine cap, the crown of which is already covered with enamel. Within this cap there is a papilla of soft tissue - the pulp of the tooth - containing the skeletal forming cells (odontoblasts), which account for the growth of dentine, as well as the blood vessels and nerves. As the tooth grows the walls of the cap become thicker, root formation occurs and on the root the cementum begins to be deposited.

The growth of dentine takes place from the direction of the pulp cavity (dental canal) in such a way that the previously formed layers of dentine are close to the outer walls of the tooth and the subsequently formed ones are close to the pulp cavity (Figs. 1 and 2).

In different species of mammals variations occur in the nature of increases in the length of the tooth. (In speaking about the lengthening of a tooth it is usual to have in mind lengthening of the tooth at the expense of the dentine and not at the expense of the deposition of cementum on the root). A distinction is made between constantly growing teeth and those of limited extent. In constantly growing teeth (for example the incisors of rodents, hypselodontal molar teeth of gray voles and lagomorphs) accretion of dentine in the lower part of the tooth occurs throughout the entire lifetime of the individual, whereas in the upper part of the tooth it is constantly being worn down. In teeth of limited extent growth of the lower part of the tooth at the expense [page 11] of the dentine occurs only during a specific period in the life of the individual and then ceases.

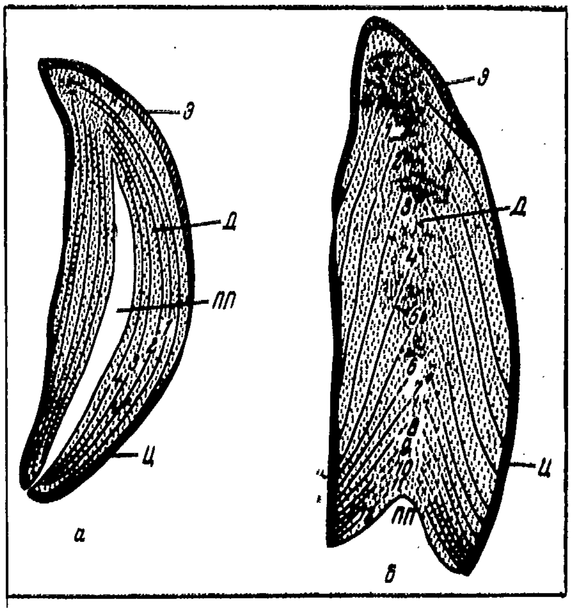


Fig. 1 [page 10]

Diagrams of longitudinal sections of canines

a - which ceased lengthening early (ribbon seal)

b - lengthening over a prolonged period (northern fur seal)

E - enamel, D - dentine, Ts - cementum, PP - pulp cavity,
NL - neonatal line; annual layers denoted by numerals.

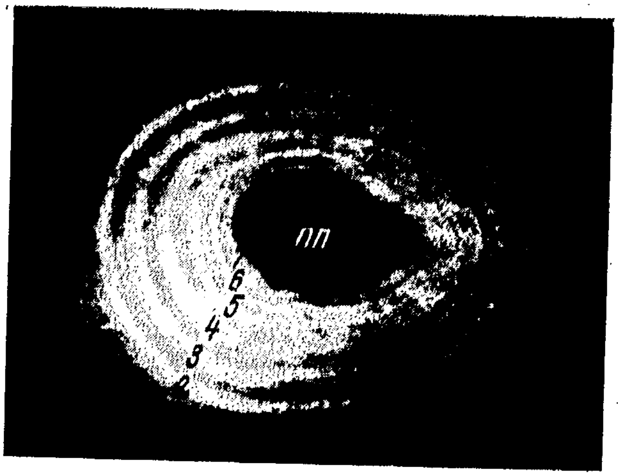


Fig. 2 [page 10]

Transverse slide of canine of ringed seal. Reflected light (X14)
PP - pulp cavity; annual layers denoted by numerals.

However, as became clear in the course of our work, teeth pertaining to the second type also, it appears, vary in the nature of their growth. Thus, canines of true seals ordinarily cease to increase in length during the second or third year while those of eared seals and walruses grow in length over a much longer period. It accordingly appears that sooner or later the teeth cease to grow in length whereas the deposition of layers of dentine within the tooth occurs in quite a different way. In true seals, for example, the ribbon seal, the pulp cavity in the lower part of the canine root is already sharply narrowed during the second year whereas in the middle parts of the tooth it is still quite wide (Fig. 1a). In the ensuing years the deposition of layers of dentine takes place in the middle of the tooth and layers are formed which roughly speaking are parallel to the longitudinal axis of the tooth. In northern fur seals the pulp cavity in the lower part of the tooth is always wide. Being filled with dentine in the upper part it seems to recede to the base of the tooth and the tooth can thus grow in length over a long period of time. The deposition of layers of dentine, moreover, seems to occur in a "herringbone" pattern, at an angle to the longitudinal axis of the tooth, whereupon this angle is sharper in young animals than in the subsequent annual layers of old animals (Fig. 1b). A similar pattern is exhibited by the growth of teeth of some toothed whales (sperm whales, belugas). We shall subsequently refer to teeth of this pattern as teeth with prolonged lengthening.

Dentine consists of mineralized fibrous organic matter, penetrated by dentine ducts - offshoots of the cells of the odontoblasts situated in the pulp cavity. In this way, the dentine itself does not contain cells. Mineralization of the growing dentine occurs as follows (Schour and Massler, 1949). A strip of organic foundation - the predentine, forms in the pulp cavity. Initially, the mineral salts in the predentine are deposited in the form of spherical blocks - calcospherites - with sectors of non-mineralized tissue between them. If mineralization does not subsequently take place then the numerous non-mineralized sectors are preserved and such dentine is called inter-globular. When further mineralization occurs and the calcospherites are situated closer to one another, with a corresponding reduction in the intervals of non-mineralized tissue, such dentine is called marmoreal. If mineralization continues to completion, the calcospherites merge, the non-mineralized spaces disappear and a dense fully mineralized dentine is formed.

The root of the tooth is covered with cementum, the thickness of which increases with the passage of time. Thickening of cementum occurs at the expense of the formation of new layers on the part of the periodontum surrounding the root of the tooth, so that the previously formed layers of cementum are situated close to the boundary of the dentine and cementum, and the subsequently formed layers, close to the outer surface of the cementum (Fig. 3). The cementum also consists of mineralized fibrous organic /page 12/ matter, but in contrast to the dentine, it incorporates cellular elements -

cementocytes (or bone corpuscles).

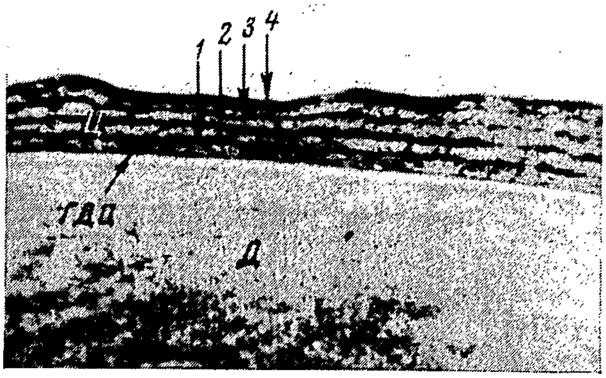


Fig. 3 [page 12]

Longitudinal section of portion of root of the canine of an arctic fox

Dye - hematoxylin (X60)

D - dentine; Ts - cementum; GDTs - boundary of dentine and cementum annual layers denoted by numerals.

A few words are in order regarding the structure of bone. Anatomically speaking, in bone, a dense substance known as the compact bone and a spongy substance the spongy bone, are distinguished. In different bones, the compact and spongy parts are shown with differing degrees of clarity. The differentiated compact bone of mammals usually consists of an intermediate osteal zone, as well as the outer and inner layers of circumferential lamellae. The osteal zone forms as a result of the reformation of the primary bone tissue. The outer circumferential lamellae are deposited as a result of appositional activity of the periosteum covering the bone on the outside and the inner circumferential lamellae - as a result of appositional activity of the endosteum, lining the inner cavity of the bone. Thus, according to the manner of their formation in the bone, periosteal, mesosteal and endosteal bone tissue are distinguished (Timofeev, 1947; Matyas, 1955). Here, we shall use the term "periosteal zone of bone" for the outer circumferential lamellae and "mesosteal zone" for the osteal zone of bone.

The thickening of a bone occurs owing to the deposition of bone tissue on the part of the periosteum - the thickening of the layer of the periosteal zone. In this way, in the periosteal zone the previously formed layers of bone are situated in its inner parts and the subsequently formed ones, close to the periphery of the bone. A process of bone resorption occurs on the part of the mesosteal zone with the result that in time the inner (initially formed) layers of periosteal zone are replaced by mesosteal bone tissue. The boundary between the mesosteal and periosteal zone of bone is emphasized by the resorption line (fig. 4).

/page 13/ Clear differentiation of the bone into periosteal, mesosteal and endosteal zones is by no means the case in all mammals. In a number of cases, for example in insectivores, Chiroptera and many rodents (as we were able to observe when studying the structure of the lower jaw), the mesosteal zone (osteal zone of bone) is either completely lacking or weakly shown. A number of special investigations have been devoted to the question of the varying degree of differentiation of the bone of mammals (Foote, 1913; Matyas, 1929, 1955; Matyas and Szabo, 1932; Ertelt, 1955; Rumyantsev, 1958; Enlow and Brown, 1958; Klebanova, 1962, 1964, 1965 et al).

The periosteal zone (outer circumferential lamellae) consists of layers of osseous lamellae, separated by adhesion lines, parallel to one another, and the outer surface of the bone (fig. 4). The term adhesion line is often applied not only to the lines separating the layers of the periosteal zone, but also to the lines delimiting the secondary osteones, as well as to the resorption lines (Weinmann and Sicher, 1947; Pritchard, 1956), since these lines are identical in their structure. The lines of adhesion in the periosteal zone were initially named by Koelliker (1889) "Ansatzlinien" - lines of deposition. In contempory English language publications they are called "resting lines" (Weinmann and Sicher, 1947; Pritchard, 1956). In Russian language publications these terms are not accepted. The lines separating the layers of the periosteal zone of bone are either called cleavage lines (Bunak and Klebanova, 1957, et al), or lines of adhesion (Svadkovskii, 1961). In future, we shall use the term "lines of adhesion."

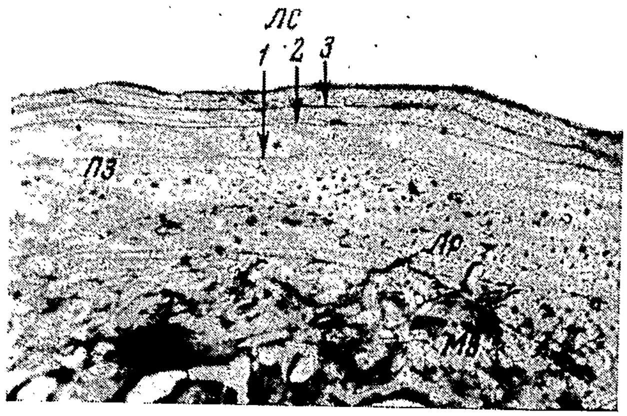


Fig. 4 /page 13/

Section of genal wall of lower jaw of beaver. Transverse section. Dye -(hematoxylin (X18).

M_z - mesosteal; P_z - periosteal zone; L_R - resorption line; L_s - adhesion line; annual layers denoted by numerals.

[page 14] The bone tissue itself of the periosteal zone, like the bone as a whole, consists of mineralized fibrous matter with numerous cellular elements - the osteocytes.

The relationship between the principal components of dentine, cementum and bone can be explained in the following table (Table 1).

Table 1. [page 14] Relationships of principal components of bone, dentine and cementum. (From Frank et al, 1960)

Component	Bone	Dentine	Cementum
Fibrous organic matter	Collagen	Collagen	Collagen
Inter-fibrillar base	Glycoprotein	Glycoprotein	Glycoprotein
Inorganic crystals	Apatite	Apatite	Apatite
Adjoining cellular elements	Fibroblasts Osteoblasts	Fibroblasts Odontoblasts	Fibroblasts Cementoblasts
Internal cellular elements	Osteocytes	Cellular offshoots	Cementocytes
Internal blood circulation system	Haversov and Folkmanov channels	Nil	Nil

Dentine, cementum and the periosteal zone of bone are similar not only from the standpoint of structure, but also in their growth pattern: in all cases, appositional growth occurs either on the part of the pulp (dentine), the periodontum (cementum), or the periosteum (bone). The essential difference between the tissues lies in the fact that in bone, processes of reformation are continuously taking place, mineral salts are not only entering the bone but are also being withdrawn from it with the result that the first layers of the periosteal zone are eventually resorbed. In dentine and cementum, normally speaking only the tissue apposition process can occur, and neither resorptions nor withdrawal of salts from already formed dental tissue takes place (Leicester, 1949; Sognnaes, 1955).

Thus, the character of formation and growth of the dentine, cementum and periosteal zone of bone ensures the preservation of the morphological peculiarities arising in the course of the growth of these tissues. In dental tissues the previously formed peculiarities of structure are preserved throughout the entire lifetime of the animal and in the periosteal zone of bones, they are preserved for a considerable period of time. One such peculiarity is the annual layers.

Annual Layers in the Dentine, Cementum and Periosteal Zone of Bone of Mammals

The morphology of annual layers /page 15/

The annual layers in dentine, cementum or the periosteal zone of bone have already been described in almost all of the pinnipeds and many of the toothed whales, as well as in a whole series of land mammals. In representatives of all nine orders of the mammalian fauna of the USSR the formation of annual layers has been observed either in the dental tissues, the periosteal zone of bone, or in dental tissues and bone (Klevezal' and Kleinenberg, 1964; Klevezal', 1966).

The table given below (Table II) gives the summary of all currently available information on the existence of annual layers in these tissues of mammals.

Besides the species listed in the table, Ye. A. Klebanova and G.A. Klevezal', (1966) have described the layers of periosteal zone of tubular bones of the limbs in a large number of species belonging to different orders (on the basis of investigations of the individual specimens from each species). Judging from the circumstantial evidence cited in this paper, these layers are annual ones, and Table II can consequently be augmented by squirrels, susliks, flying squirrels, jerboas, polecats, jungle cats, etc.

As will be seen from Table II, annual layers in dental tissue or bone exist in animals belonging to different orders and different ecological groups. They have been described in hibernating (the ordinary hamster and gray marmot) and non-hibernating rodents (the field mouse, gray rat and others), in rodents breeding once in the course of a year (beaver) and those having several litters per year (field voles and others), and in carnivores leading an aquatic (sea otter) and terrestrial form of existence (polar fox, sable). Annual layers have been found in the species of the northern hemisphere (the majority of the species listed in the table), the southern hemisphere (Antarctic seals and an inhabitant of the tropical zone (Hawaiian monk seal). Annual layers are absent from the periosteal zone of bone of bats, baleen whales and ungulates (a more detailed statement on this will be given in the corresponding sections of Chapter III), but in the case of bats and ungulates there are layers in the dentine and cementum.

The fact that the layers which were discovered are annual ones has been proved through investigations of animals of known ages (Meyer, 1957; Sergeant, 1959; Mansfield and Fisher 1960; Hewer, 1960, 1964; Rausch, 1961; Laws, 1962; Scheffer and Kraus, 1964; van Nostrand and Stephenson, 1964; Klevezal', 1965, 1966; Kleinenberg and Klevezal', 1966; Gilbert, 1966; Ransom, 1966) and also as a result of investigations of animals taken during various seasons of the year (McLaren, 1958; McEwen, 1963; Mitchell, 1963).

We know of one work in which an attempt to find annual layers in dentine and cementum proved to be in vain. /page 20/ E. Kingsmill (1962) studied the dentine and cementum of the teeth of Australian marsupials of precisely known ages.

Table II [pages 16 to 19] Annual layers in dental tissues and periosteal zone of bone in mammals (plus sign denotes presence of annual layers; minus sign - absence of layers; an ommission signifies that the particular tissue was not investigated).

Family	Species	Tissue: dentine	Tissue: cementum	Tissue: periosteal zone of bone	Author
	Order-Insectivora				
Soricidae	Common shrew *Sorex araneus*	+	+	+	Klevezal',1966; Kleinenberg and Klevezal', 1966.
	Order-Chiroptera				
Vespertilionidae	Great brown bat *Eptesicus fuscus*	+			Christian, 1956
	Large mouse-eared bat *Myotis myotis*	+	+	-	Klevezal', 1966
	Red noctule *Nyctalus noctula*	+	+	-	Same
	Order-Lagomorpha				
Ochotonidae	Long-eared pika *Ochotona roylei*			+	Bernshteyn and Klevezal', 1965
	Red pika *Ochotona rutila*			+	Same
	Order-Rodentia				
Castoridae	Beaver *Castor fiber*		+	+	Klevezal' and Kleinenberg, 1964; van Nostrand and Stephenson, 1964; Kleinenberg and Klevezal', 1966.

Family	Species	Tissue: dentine	Tissue: cementum	Tissue: periosteal zone of bone	Author
Sciurudae	Small suslik *Citellus pygmaeus*			+	Meyer, 1957
	Thin toed suslik *Spermophilopsis leptodactylus*		+	+	Klevezal', 1966
	Gray marmot *Marmota baibacina*	+	+	+	Our published data
Muridae	Field mouse *Apodemus agrarius*		+	+	Klevezal' and Lavrova, 1966
	Gray rat *Rattus norvegicus*		+	+	The same
	Tamarisk gerbil *Meriones tamariscinus*		+	+	Klevezal' 1966
	Common hamster *Cricetus cricetus*		+	+	The same
	Muskrat *Ondatra zibethica*		+	+	
	Waterrat *Arvicola terrestris*			+	
	Common vole *Microtus arvalis*			+	Klevezal' and Lavrova, 1966
	Pine vole *Microtus majori*			+	The same
	Order-Cetacea				
Physeteridae	Sperm whale *Physeter catodon*	+	+	+	Nishiwaki et al, 1958; Berzin, 1961, 1964; Ohsumi et al. 1963.
Delphinidae	Beluga *Delphinapterus leucas*	+	+		Sergeant, 1959; Khuzin, 1961, 1963.
	Pilot whale *Globicephala maelaena*	+	+		Sergeant, 1959.
	Bottle-nosed dolphin *Tursiops truncatus*	+			The same
	Common dolphin *Delphinus delphis*	+	+	+	Kleinenberg and Klevezal', 1962 Klevezal', 1966
	Blue-white dolphin *Stenella caeruleo - albus*	+			Nishiwaki and Yagi, 1953

Family	Species	Tissue			Author
		dentine	cementum	periosteal zone of bone	
Balaenopteridae	Fin whale *Balaenoptera physalus*			-	Klevezal', 1966
	Sei whale *Balaenoptera borealis*			-	The same
	Order-Carnivora				
Ursidae	Brown bear *Ursus arctos*		+		Smirnov, 1960; Mundy and Fuller, 1964
	Black bear *Ursus americanus*	+	+		Rausch, 1961
Canidae	Arctic fox *Alopex lagopus*	+	+	+	Klevezal', 1965, 1966
Mustelidae	Sable *Martes zibelina*	+	+	+	The same
	American mink *Mustela vison*		+	+	
	Sea otter *Enhydra lutris*		+		Klevezal' and Marakov, 1966
	Order-Pinnipedia				
Odobenidae	Walrus *Odobenus rosmarus*	+	+	+	Chapskii, 1952; Mansfield, 1958a; Krylov, 1965
Otaridae	Southern sea lion *Otaria byronia*	+	+		Laws, 1962
	Steller sea lion *Eumetopias jubata*	+	+		Fiscus, 1961; Spalding, 1964
	South African fur seal *Arctocephalus pusillus*	+			Rand, 1956 (according to Laws, 1962)
	Arctocephalus tropicalis	+			Laws, 1962
	South American fur seal *Arctocephalus australis*	+			The same

Family	Species	Tissue			Author
		dentine	cementum	periosteal zone of bone	
	Northern fur seal *Callorhinus ursinus*	+			Chiasson, 1957; Scheffer and Kraus, 1964
Phocidae	Common seal *Phoca vitulina*	+	+		Mansfield and Fisher, 1960; Tikhomirov and Klevezal', 1964
	Ringed seal *Pusa hispida*	+	+	+	McLaren, 1958; Tikhomirov and Klevezal', 1964
	Caspian seal *Pusa caspica*	+	+	+	Chapskii, 1965
	Baikal seal *Pusa sibirica*	+			Klevezal', 1961
	Harp seal *Pagophilus groenlandicus*	+	+	+	Chapskii, 1952; Rasmussen, 1957; Yakovenko, 1960, 1961.
	Ribbon seal *Histriophoca fasciata*	+	+		Tikhomirov and Klevezal', 1964
	Bearded seal *Erignathus barbatus*	+		+	Tikhomirov and Klevezal', 1964
	Grey seal *Halichoerus grypus*	+	+		Hewer, 1960, 1964
	Crabeater seal *Lobodon carcinophagus*	+	+		Laws, 1953a, 1957
	Leopard seal *Hydrurga leptonyx*	+	+		Laws, 1953a, 1957
	Weddell seal *Leptonychotes weddelli*	+			Mansfield, 1958b
	Ross seal *Ommatophoca rossi*	+			Laws, 1953a

Family	Species	Tissue			Author
		dentine	cementum	periosteal zone of bone	
Phocidae	Hooded seal *Cystophora cristata*	+	+		Laws, 1953a Rasmussen, 1957 Yakovenko, 1959 Popov, 1960
	Northern Elephant seal *Mirounga leonina*	+	+	+	Laws, 1953a, 1953b, 1960b, 1962
	Hawaiian monk seal *Monachus schauinslandii*	+	+		Kenyon and Fiscus, 1963
	Order-Perissodactyla				
Equidae	Wild ass *Equus hemionus*	+	+	-	Klevezal', 1966
	Order-Artiodactyla				
Cervidae	Roe deer *Capreolus capreolus*	+	+	-	The same
	Spotted deer *Cervus nippon*	+	+	-	
	Red deer *Cervus elephus*		+		Mitchell, 1953
	Caribou *Rangifer tarandus*		+		McEwen, 1965
	Elk *Alces alces*		+		Sergeant and Pimlott, 1959
	Black tailed deer *Odocoileus hermionus*		+		Low and Cowan, 1963
	White tailed deer *Odocoileus virginianus*		+		Gilbert, 1966 and Ransom, 1966
Bovidae	Bison *Bison bison*		+		Novakowski, 1965

She writes that there are layers in the dentine and the cementum but that they cannot be used for age determination as in the dentine they are irregular and in the cementum it is difficult to count them. On the basis of this work alone it would be untimely to conclude that the ages of Australian marsupials cannot be determined from the structure of the dentine and cementum. E. Kingsmill conducted her investigation only on thin sections of teeth. It is entirely possible that layers both in the dentine and the cementum could be discerned more clearly in microscopic sections of teeth, stained with hematoxylin (See Chapter II).

An annual layer in dentine and cementum consists of two bands: a wide and a narrow one, differing in their optical density and the intensity of their colouring by hematoxylin in decalcified sections. In polished micro-slides of teeth, wide non-transparent and narrow transparent bands are usually seen. In some seals all or part of the non-transparent band is occupied by interglobular dentine. Depending on whether the microslide is being examined in transient or reflected light, the separate bands of the annual layer appear as dark or light ones.[1] This can be explained by the following comparison:

Band of annual layer	Transient light	Reflected light
Transparent band	Light	Dark
Non-transparent band	Dark	Light
Inter-globular dentine	Very dark, granular	Foamy white

1 In published literature, the terms "dark" and "light" bands are sometimes used without indicating whether the investigation was made in reflected or transient light. This gives rise to confusion in the treatment of layers. Probably the term "transparent" and "non-transparent" bands are more apt in this connection as they characterize the optical density and not the colour of the bands.

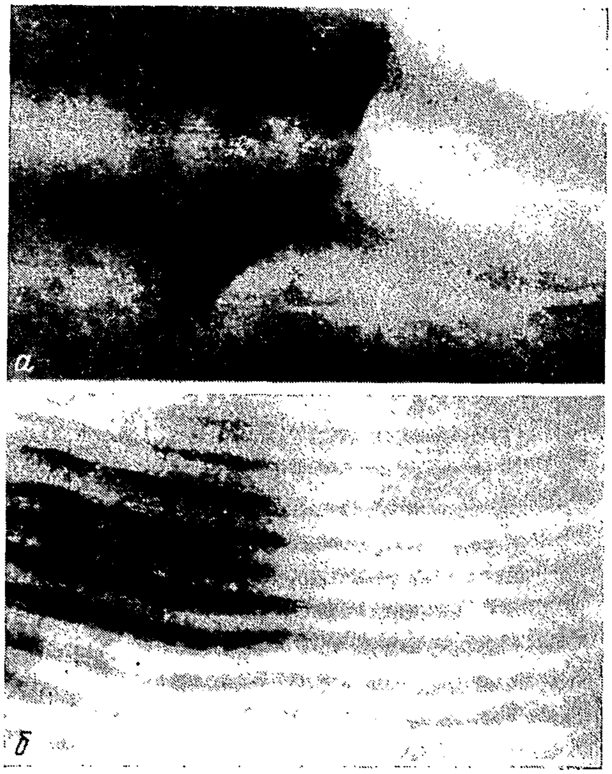


Fig. 5 [page 21]

Section of transverse microslide of dentine of the tooth of a sperm whale (a) and the cementum of canine of a hooded seal (b) Reflected light (a - magnification 80x; (b-X33). On the left - a sector treated with silver nitrate. On the right - an untreated sector. It will be seen that the silver nitrate more intensively coloured the transparent (here, dark) bands of annual layers of dentine and cementum.

In calling the bands of an annual layer "transparent" and "non-transparent" we have in mind not their absolute but only their relative optical density; a "transparent" band is more transparent than the neighbouring "non-transparent" bands just as is the case in the transparent and non-transparent zones of the otoliths of fish (Mina, 1965). It would therefore be more correct to speak about "more transparent" and "less transparent" bands of dentine and cementum, but for the sake of brevity we, along with a number of other authors, will make use of the designation "transparent" and "non-transparent" bands.

The varying optical density of the bands of an annual layer is caused by the varying content of calcium salts. Opinions differ in regard to /page 21/ which band of an annual layer is hypercalcified and which one hypocalcified. McLaren (1958), Sergeant (1959, 1962) and A.A. Berzin (1961, 1964) consider the non-transparent band to be hypercalcified. Kubota, Nagasaki, Matsumoto and Tsuboi, 1963, as well as Ohsumi, Kasuya and Nishiwaki, (1963) consider the transparent band to be hypercalcified.

Based on an analysis of available published data on the histological structure of the teeth of man and laboratory animals and on the basis of our own studies of the teeth of marine mammals (colouring with silver nitrate, providing for clarification of the relative content of calcium salts) (fig. 5) it is possible to conclude that the narrow transparent band of annual layer /page 22/ of dentine and cementum is hypercalcified relative to the wide non-transparent band (Klevezal', 1963, 1966).

In hematoxylin-stained sections of decalcified teeth an annual layer of dentine and cementum usually consists of a wide slightly coloured band and a narrow heavily-coloured one. It is considered that hematoxylin stains more heavily (after decalcification) well calcified tissues (Schour and Massler, 1949). A comparison of hematoxylin-stained slides and sections of the same teeth has shown that hematoxylin intensively stains the narrow transparent bands of dentine and cementum (Kleinenberg and Klevezal', 1966; Klevezal', 1966). This can also be regarded as evidence that the transparent band is hypercalcified relative to the non-transparent one.

The separate bands of an annual layer of cementum are distinguished perhaps not only by the relative content of calcium salts, but also by the relative content of cementocytes.

Thus, Mansfield (1958a) points out that in the dental cementum of the walrus the contrast between the transparent and non-transparent bands of an annual layer is often emphasized by the distribution of cementocytes. Mitchell (1963) writes that in the cementum of molars of the red deer the non-transparent wide band of an annual layer contains many cementocytes while the transparent one contains fewer cementocytes and more fibrous material. However, as can be ascertained in studies of the annual layers of cementum in mammals of various species, a difference in the density of cementocytes is by no means always seen.

An annual layer of the periosteal zone of bone consists of a wide band of bone tissue, separated by an adhesion line. Such adhesion lines (cleavage lines) delimit the osteons and separate the circumferential lamellae in the bones of men and laboratory animals, and here, their structure has been well studied. As distinct from bone tissue itself, they are devoid of fibrillae and consist of cohesive material nourished by calcium salts, which is more calcified than the collagen of bone tissue (Weidenreich, 1923; Weinmann and Sicher, 1947; Amprino and Engström, 1952). In the staining of decalcified cuttings with hematoxylin the lines of adhesion separating the layers of periosteal zone of bone are more heavily stained than the bands of bone tissue.

Thus the bands comprising an annual layer of dentine are distinguished only by the relative content of calcium salts while the bands of an annual layer of cementum are distinguished by the content of calcium salts and perhaps also by the density of cementocytes. In the periosteal zone of bone the lines of adhesion are distinguished from a layer of bone tissue both by the relative content of calcium salts and by the structure of the basic material. The relationship between the structure of an annual layer in tissues of teeth and bone can be explained by the following table (Table III).

Table III. [page 23] Structure of annual layer in dentine, cementum and periosteal zone of bone.

Component part of annual layer	Dentine	Cementum	Periosteal zone of bone
Wide band	Non-transparent dentine (collagenic substance with dentine ducts; weakly or normally calcified).	Non-transparent cementum (collagenic substance with large number of cementocytes; normally calcified).	Band of osseous tissue (collagenic fibres, osteocytes normally calcified).
Narrow band	Transparent dentine (collagenic substance with dentine, ducts heavily calcified).	Transparent cementum (collagenic substance with small number of cementocytes; heavily calcified).	Lines of adhesion (adhesive matter heavily calcified).

[page 23] The clearness of annual layers in various species of mammals differs. In speaking about the clearness of the layers we have in mind the mutual contrast between a band of annual layer of dentine and cementum and the clarity and correct positioning of the delimiting line of adhesion in a layer of periosteal zone of bone.

Within the separate bands of an annual layer of dentine and especially within a wide non-transparent band there are additional small streaks. Their degree of brightness varies in different species. In some cases, for example in the dentine of the arctic fox (Klevezal', 1966), the supplementary streaks are so clearly shown that they hinder determination of the boundaries between the annual layers. In teeth where the width of the annual layers sharply diminishes with age they are usually seen only in some of the primary, wider layers. In cetaceans, in which the width of the annual layers diminishes only slightly with age, they are seen in all the annual layers (for example in the sperm whale, Ohsumi et al, 1963; Berzin, 1964).

These supplementary bands also exist in annual layers of cementum in cases where the layers are quite broad, for example in beavers and wild asses (Klevezal', 1966). Occasionally, supplementary lines of adhesion are encountered in the annual layers of the periosteal zone of bone. The neighbouring annual layers are often divided not by one line of adhesion but by two closely located lines, sometimes even by a group of lines (for a description of how to avoid errors in this instance in the counting of annual layers see Chapter II).

The formation of bands of annual layers in dentine, cementum and periosteal zone of bone proceeds simultaneously in one and the same individuals (in cases when layers exist in all three tissues). In animals living more than one or two years (bats, some rodents, cetaceans, carnivores, pinnipeds and ungulates), the primary and sometimes also secondary annual layers in the dentine, cementum and periosteal zone of bone can be less clear than the later ones (Mansfield, 1958a; Laws, 1953a and 1958; Berzin, 1961; McEwan, 1963: [page 24] Klevezal', 1966). In these cases the narrow bands of primary annual layers are less transparent and less heavily stained by hematoxylin than the narrow transparent bands of the subsequent annual layers. In the periosteal zone of bone the difference in the clarity of the primary and subsequent annual layers is less pronounced, but here too, the delimiting lines of the primary annual layers can be less clear and not so heavily coloured by hematoxylin than is the case with the subsequent annual layers.

Annual layers of dentine are laid down until the moment when the entire pulp cavity is filled. The time when the pulp cavity is closed differs not only with the various species but also in the different teeth of one animal. For instance, in the case of the northern fur seal the pulp cavity in the root portion of the incisors and molars closes by the time it is four years old, whereas in the lower canine, this occurs no earlier than the eighth to the twelfth year and in the upper ones not before the fifteenth year (Scheffer and Kraus, 1964).

The growth of cementum is not limited spatially and it can therefore be laid down throughout the entire lifetime of the individual. In the various species, the overall thickness of cementum and consequently the width of the annual layers as well exhibit wide differences, a fact which is evidently connected with differences in the magnitude and character of the mechanical load on the teeth. One of the indices of the magnitude of the mechanical load on the teeth is the degree of their wear. In many species it can be seen that where there is intensive wear of the crown of a tooth the formation of broad layers of cementum occurs on the root. Such a picture is observed in the walrus (Mansfield, 1958a), sperm whale (Berzin, 1964), spotted deer, wild ass and beaver (Klevezal', 1966). Nevertheless, heavy wear does not always give rise to the formation of thick depositions of cementum. In the bearded seal the canines are so heavily worn that in old individuals they do not protrude from the alveolus or are even completely lacking, but the layering of cementum on the roots does not attain a great thickness (Tikhomirov and Klevezal', 1964). A similar picture can be seen in the molars of muskrats, which are intensively worn (Tsyganov, 1955) and at the same time have very narrow layers of cementum.

The periosteal zone attains different widths and is variously localized not only in the same bone of different species, but also in different skeletal bones of one individual. Resorption of primary annual layers also begins at different times. These differences are evidently connected with differences in the magnitude and character of the mechanical loads (Klebanova, 1953, 1954).

In the course of an investigation of different bones of the skulls of sables and ringed seals of various ages (Klevezal', 1965, 1966) it was found that depending on the character of the periosteal zone, bones can very roughly be divided into three groups: bones, where in the case of animals of different age groups the periosteal zone is either not shown or is poorly developed (malar extension of the temporal bone in sables and seals, the frontal bone, alveolar margin of the upper /page 25/ jaw and cheek-bone in sables, etc); the flat bones of the skull, wholly consisting of layers of bone tissue, divided by lines of adhesion, without the middle diploe (for example, the squamous portion of the temporal bone); bones with a well marked periosteal zone (base of the malar extension of the temporal bone of the sable, the tympanic portion of the temporal bone, palatal extension and nose membrane of the upper jaw and shaft of the lower jaw of the sable and seal). In bones of the first group annual layers are either not formed or are very rapidly replaced by mesosteal bone. In bones of the second group, due to the absence of the middle diploe it is difficult to distinguish the layers of the upper and lower surface of bone. In bones of the third group there are clearly marked annual layers. In young animals, all bones of the third group have an equal number of annual layers. In adult and especially old individuals, the number of layers in various bones differs, evidently on account of the fact that the resorption rate is not identical.

In sables and ringed seals of various ages the number of annual layers of the periosteal zone most closely corresponding to the age of the individual occurred in the lower jaw. Occupying second place was the base of the malar extension of the temporal bone in sables and the tympanic portion of the temporal bones in seals.

Laws (1953b) described layers in the tympanic portion of the temporal bone of elephant seals and proposed that they should be used for determining the age of the animals. In ringed seals, the layers in the periosteal zone of the tympanic portion of the temporal bone are well defined, but their number in seven to ten-year-old individuals was less than the age of the animal in years.

As shown by a comparison of the structure of the periosteal zone of the mandible (the sector where the periosteal zone is better developed) and the bones of the limbs (at the center of the diaphysis) in the same individuals of Greenland seal (Chapskii, 1952), large mouse-eared bat (Myotis myotis), field mouse, water rat, thin-toed suslik and ringed seal (Klebanova and Klevezal', 1966; Klevezal', 1966), the bones of the limbs are similar to the lower jaw both in the character of the periosteal zone and in the number of annual layers. In a number of species of rodents, lagomorphs and carnivores, the number of annual layers in the thigh-bone, shoulder-bone, shin-bone and radii of one individual were as a rule identical, occasionally differing by one layer (Klebanova and Klevezal', 1966).

Unquestionably, on the basis of these data it is impossible to speak of a total correspondence in the numbers of annual layers in the periosteal zone of the lower jaw and the bones of the limbs in all species of mammals. The character of the periosteal zone depends on the character of the mechanical load, which is not identical in the various species. Thus, in cetaceans in which as a result of a change in the function of the limbs the structure of the humerus has been changed, the structure of the periosteal zone of the lower jaw and humerus is not identical. For example, /page 26/ in the adult common dolphin, which has a wide periosteal zone in the lower jaw, there was no periosteal zone in the humerus and the entire bone was osteonized. It is nevertheless probable that in the majority of mammals the number of annual layers in the periosteal zone of the lower jaw and the bones of the limbs are identical. This assumption of ours is based on the above described coincidence between the number of annual layers in the lower jaw and bones of the limbs in animals of different ecological groups (we are not considering the possible causes of this coincidence since it does not enter into our task).

Annual ridges on the root of a tooth

Annual ridges consist of ring-like swellings of the lateral walls of the root of a tooth. They are directly associated with the annual layers in the dentine and are formed as a result of an uneven growth of the tooth in length (Scheffer, 1950; Mansfield, 1958a). In the large volume of material on the subject annual ridges were first described for the northern fur seal (Scheffer, 1950) and were later found in other seals (Laws, 1962), and in walruses (Mansfield, 1958a), bears (Rausch, 1961) and toothed whales (Berzin, 1961 and 1964; Khuzin, 1961 and 1963).

Annual ridges on the roots of teeth are useful for determining the ages of the majority of species of mammals for the following reasons. Since they are formed as a result of an uneven growth of the tooth in length, in teeth in which the growth is limited and has already ceased only the few ridges pertaining to the initial years are formed or the ridges are not formed at all. Thus in the sable the canine ceases to grow in length during the first year of life of the individual so that ridges are not formed on the root. In teeth with prolonged growth in length (big-eared seals, walruses, toothed whales) annual ridges form on the roots over a protracted period, but in time the depositions of cementum on the root hide the ridges, with the result that they can only be discovered on a longitudinal section of the tooth (Scheffer, 1950; Khuzin, 1961; Berzin, 1964). R.Sh. Khuzin (1961) considers that the age of the beluga whale can easily be determined from the number of these ridges to be seen in a longitudinal section of the tooth.

Thus, the number of annual ridges can be used for age determination in animals having teeth with a prolonged growth in length. In young individuals ridges can be counted on the surface of the root of a tooth cleaned of soft tissues and in old individuals - in a longitudinal section of the tooth.

Reasons for the formation of annual layers

The majority of investigators concerned with the annual layers in the teeth of marine mammals explain the formation of the annual layers as periodical changes in the rate of calcification of dental /page 27/ tissues (Laws, 1953a, 1962; McLaren, 1958; Sergeant, 1959; Carrick and Ingham, 1962, et al). They proceed from the premise that since the varying optical density of the bands of annual layers of dentine and cementum is caused by the varying content of calcium salts, these bands form as a result of a greater or lesser intake of mineral salts into the tissues. They see the cause of the periodical changes in the rate of calcification to be either in periodical changes in the intensity of feeding and the admission of vitamin D into the organism (Laws, 1953a, 1962; McLaren, 1958), or in a change in hormone activity (Carrick, Ingham, 1962; Ohsumi et al, 1963). As considerable support has been given to this interpretation by the periodical rhythm of tissue calcification established by Schour and Hoffman, (1939a) we will discuss this in more detail.

Schour and Hoffman (1939a) described the microlayers in the dentine and dental enamel of a number of animals from fish to man as being the result of the variable degree of calcification of the tissues. The width of a pair of layers, one weakly and the other strongly calcified, equalled sixteen microns in all the animals studied independently of the type of tooth. (The following mammals were studied: the giant beaver, grey squirrel, guinea pig, suslik, rabbit, cat, dog, pig, sheep, cow, macaco and man). Schour and Hoffman believe that the reason for the formation of these layers is the rhythmic changes in the rate of calcification and that the calcification process can be described as a rhythmatical formation of calcospherites which merge more or less evenly and thus give more or less calcified bands. They therefore regard the sixteen-micron calcification rhythm as a constant and general biological unit of this process. In another paper by Schour and Hoffman (1939b) it is pointed out that although the appositional growth of the organic stroma of the tooth and its calcification are separate and distinct processes, the tempos of which cannot be made the same in an experiment, existing data nevertheless indicate that usually the apposition rate and the rate of calcification are one and the same. Besides the micro-layers, a pair of which constantly equals sixteen microns, Schour and Hoffman (1939a) describe other layers - Owen's contour lines, which are formed as a result of a break in the calcification and can embrace one or several sixteen-micron pairs.

According to Schour and Hoffman (1939b) the rate of growth of the organic stroma of the tooth is from 2 to 16 microns per day depending on the type of tooth. Thus, the calcification rhythm described by them is either a diurnal one or is repeated every few days.

If the formation of annual layers is explained from the standpoint of rhythmic changes in the calcification rate it must be presumed that an annual calcification rhythm exists. However, none of the investigators explaining the formation of annual layers of dentine in marine mammals as periodical changes in the calcification rate has shown that such a rhythm occurs.

/page 28/ At the same time the formation of annual growths on dental roots which occurs as can be seen in a longitudinal section of the canine of a fur seal or tooth of a sperm whale, at the same rate as the formation of annual layers in dentine, is interpreted as seasonal differences in the rate of growth of the tooth in length (Scheffer, 1950; Mansfield, 1958a).

In considering the data of Schour and Hoffman, we assume that the small supplementary streaks within the annual layers are perhaps formed as a result of changes in the rate of tissue calcification but that the reason for the formation of annual layers of dentine and cementum lies not in periodical annual changes in the rate of calcification, but in the periodical annual changes in the growth rate of the organic stroma of the tooth (Klevezal', 1963).

A similar conclusion regarding the reason for the formation of annual layers of cementum in artiodactyla was reached by the Canadian investigators (Low and Cowan, 1963; Mitchell, 1963). Devoting their attention mainly to the varying quantity of cementocytes in the narrow and wide bands of annual layers of cementum in deer, they consider that the narrow band is formed as a result of a reduction in the accretion of cementum but do not attempt to explain the reasons for the varying degree of calcification of the bands of annual layers of cementum.

The formation of a transparent band of an annual layer of cementum, differing from the non-transparent band only in regard to the quantity of mineral salts, can only be explained on the basis of differences in the rate of tissue growth during different periods. The transparent band of an annual layer is always more narrow than a non-transparent one. In teeth with a protracted increase in length a narrow transparent band of annual layer corresponds to a reduction in the increase of the tooth in length as can be seen in the longitudinal section of a tooth (Mansfield, 1958a; Khuzin, 1963; Ohsumi et al, 1963). Evidently, a reduction occurs in the growth of the organic stroma of the tooth during the period of its formation. If we disregard changes in the rate of calcification of the tooth in the course of the year, having assumed that this is relatively constant, and consider only changes in the rate of growth of the organic stroma of the tooth, then it is evident that the bone in which growth proceeds more slowly will be more heavily calcified than the zone in which growth is faster, owing to the distribution of the same quantity of calcium salts, in the first case in a lesser amount and in the second case in a greater. It appears that the narrow hypercalcified band of an annual layer of dentine (precisely the same thing applies to cementum) is formed in just this manner. An increase in the rate of tissue calcification during the period of formation of a narrow transparent band of an annual layer is not an indispensible condition of its formation but if it occurs then it can make the band more clear.

This interpretation of the process of formation of annual layers in dentine and cementum not only enables us to explain a number of growth and structural peculiarities in dentine and cementum, which it is difficult to explain on other premises (Klevezal', 1966) but also to find a common [page 29] cause resulting in the formation of annual layers in dental tissues (and of growths on the root of a tooth) and annual layers in the periosteal zone of bone in mammals of various ecological groups.

The narrow transparent bands in annual layers of dentine and cementum and the line of adhesion distinguishing an annual layer of bone in one and the same individuals are formed simultaneously (Klevezal', 1966).

The line of coherence represents a delay in apposition of the periosteal zone of bone, i.e. a delay in the growth of a bone in thickness. This fact was established during studies of the bones of man and laboratory animals; it is not to be doubted, being mentioned in many individual and composite works (Pritchard, 1956; Sissons, 1949, 1956; Weinmann and Sicher 1947; Svadkovskiy, 1961, et al.). It can be assumed that in an annual layer of the periosteal zone of bone in mammals the line of adhesion represents the period of delay in the apposition of the bone and the wide layer of osseous tissue - the period of growth of the periosteal zone. V.O. Kler (1923, 1927, 1939) proceeded from this position in proposing that the structure of a bone be used for studying the periodicity of growth. Inasmuch as the narrow transparent bands of annual layers of dentine and cementum are similarly formed as a result of a reduction in the rate of growth of the tissues, it can be concluded that a reduction in the rate of growth of dental and bone tissues occurs annually in mammals, with the result that annual layers form in these tissues.

The structural relationships between the bands of annual layering in these tissues closely agree with this notion. Resulting from a delay in the growth of a bone a line of adhesion is formed, not containing fibrillae and cellular elements and hypercalcified in relation to the remaining bone tissue. Due to a reduction in the rate of growth of cementum a narrow transparent band is formed, containing fewer cellular elements and hypercalcified in relation to the remaining cementum. Resulting from the reduction in the rate of growth of the dentine, which does not contain cellular elements, a narrow transparent band is formed, hypercalcified in relation to the remaining dentine.

As was shown by our investigation of a number of species of mammals, in all the animals caught in the spring, the start was noted in the formation of a wide non-transparent band of the next annual layer in the dentine and cementum and wide layer in the periosteal zone of bone; in the individuals caught in summer these layers were quite wide and in those caught in the autumn they were almost completely formed. In fig. 6 there is a summation of data on the times of formation of the various bands of annual layer in dentine, cementum and the periosteal zone of bone in all of the species which were studied. Conventional designations indicate which band of an annual layer was in the process of formation during a particular period. Evidently, in all of the species studied the transparent band of an annual layer or line of adhesion is formed between /page 30/ December and February and the non-transparent band of dentine and cementum and the layer of bone tissue - during the remaining part of the year.

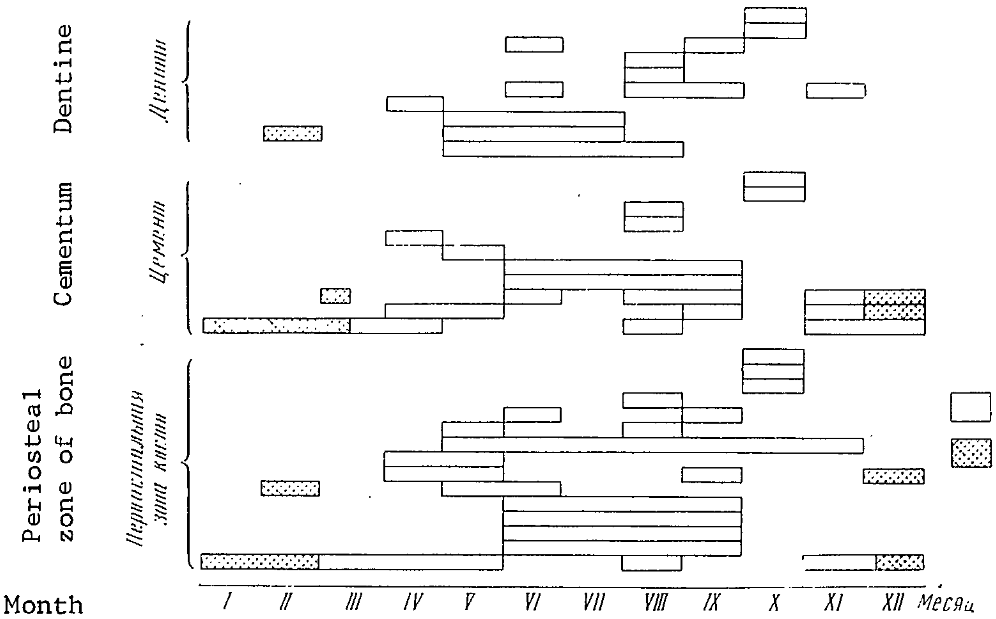


Fig. 6. /page 30/

Times of formation of individual bands of annual layers of dentine, cementum and periosteal zone of bone in various species of mammals.

1 - wide band of an annual layer; 2 - narrow transparent band of dentine and cementum, the line of coherence of the bone. A list of the cited species (reading from the bottom upwards). Periosteal zone of bone: beaver, field mouse, grey rat, common vole, pine vole, ringed seal, muskrat, long-eared pika, red pika, common hamster, common dolphin, common brown-tooth shrew, mink, sable, Arctic fox. Cementum: beaver, muskrat, wild ass, field mouse, grey rat, common hamster, large mouse-eared bat, spotted deer, common brown-tooth shrew, sable, Arctic fox. Dentine: red noctule, ribbon seal, ringed seal, large mouse-eared bat, wild ass, common brown-tooth shrew, spotted deer, common dolphin, sable Arctic fox.

It should be noted that in view of the comparatively crude techniques used in the study, the start of the formation of a new band of annual layer can probably only be noted after it attains a certain width; at the very beginning of its formation it is indistinct as it merges with the outer edge of the tissue. As the growth rate of a narrow band of an annual layer is very low the comparatively long period of its formation is evidently not fixed. This can be shown with reference to the beaver and muskrat. We studied the cementum of the muskrat in thin stained sections and the cementum of the teeth of a beaver - on the polished surface of a longitudinally sawn tooth in reflected light. In the first case we evidently /page 31/ had an opportunity of previously observing the formation of a transparent band in the outer layer of cementum. It was evidently for this reason that in the case of the muskrats caught in December a narrow band was noted on the outer edge, whereas in the case of the beavers caught in December no such band was noted.

Similar data on the time of formation of the separate bands of an annual layer are to be found in literature. The majority of authors consider that in the dentine and in the dental cementum of pinnipeds and cetaceans a transparent band of annual layer is formed in winter (Fiscus, 1961; Khuzin, 1961; Ohsumi et al., 1963; Hewer, 1964) or during the winter-spring period (Mansfield, 1958a; McLaren, 1958; Sergeant, 1959). Christian (1956) assumes that in the dentine of the large brown bat a narrow band of annual layer forms during the hibernation period. It has been established that a narrow band of annual layer is formed in the dental cementum of ungulates in winter (Sergeant and Pimlott, 1959; Low and Cowan, 1963; Mitchell, 1963; McEwen, 1963; Novakowski, 1965; Gilbert, 1966; Ransom, 1966). A similar time for the formation of a narrow band of annual layer in the cementum is indicated for Canadian beavers (van Nostrand and Stephenson, 1964).

A different opinion is held by Mundy and Fuller (1964). They found that in grizzly bears killed in the spring a transparent band was visible on the outer edge of the cementum and those killed in the autumn - a non-transparent one, and on this basis they reached the conclusion that the transparent cementum is formed in the course of the period of summer activity and the non-transparent cementum at the end of the summer. It is considered that these data could be interpreted differently: a transparent band was seen on the outer edge of the cementum of the bears killed in the spring because the deposition of the non-transparent cementum had not yet been noted; in those killed in the autumn a band of non-transparent cementum was visible on the outer edge which had been formed during the summer period. The scanty amount of published information on the time of formation of the line of adhesion delimiting the annual layers of periosteal bone also coincides with the data derived by us. In the case of the harp seal,according to K.K. Chapskii (1952) this line is formed during the winter-spring period and in the case of the lesser suslik, judging from the data of M.N. Meyer (1957) it occurs during the hibernation period.

Thus, the narrow band of an annual layer of dentine and cementum and the line of adhesion of an annual layer of the periosteal zone of bone in different species of mammals are formed during the winter period. This indicates that a delay (or retardation) of the tissue growth of dentine, cementum and bone occurs in winter.

The Canadian investigators (Low and Cowan, 1963; McEwen, 1963) associate seasonal changes in the deposition of cementum in the teeth of deer with seasonal variations in the intensity of feeding and degree of isolation. K. K. Chapskii (1952) considers that a line of adhesion separating the layers in the bone of the harp seal is formed as [page 32] a consequence of the period of starvation occurring annually with these animals.

It seems more probable that a delay or retardation of tissue growth in the dentine, cementum and periosteal zone of bone is a reflection of a general slowing down in the growth of the organism during the winter. In other words, the cause of the formation of annual layers in tissues of teeth and bone of mammals is the regular seasonal variations in the growth rate of the animals. This explains the existence of annual layers in mammals from very different ecological groups, as a cessation or retardation of growth during winter time has been noted in very different mammals.

Thus, in hibernating animals, growth is suspended during the hibernation period (Kalabukhov, 1956). In the case of rodents and small insectivores which do not fall asleep during the winter there is a retardation in the growth rate of the body and skull. (Rörig and Knoche, 1916; Kucheruk and Ryumin, 1938; Fenyuk and Sheykina, 1940; Bashenina, 1953; Scrafinski, 1955; Wasilewski, 1956a, 1956b, et al). The growth rate of rodents born in the autumn is considerably lower than in those born in the spring (Wasilewski, 1952; Mazak, 1962; Pokrovskiy, 1964).

Some data exist with regard to seasonal variations in the growth of large mammals. V.V. Dezhkin (1961) writes that young beavers increase by 1.05 kg during the first four (summer) months, that their monthly increase in weight reduces to 0.53 kg during the next eight autumn-winter months, and that it increases again to 0.61 kg during the ensuing summer. A reduction in body weight of adult beavers is noted during December and January and an increase in it during April and May (Dezhkin, 1965). The Canadian research workers (Patrick and Webb, 1960 according to Dezhkin, 1965) also report a reduction in the increase and even in the total weight of beavers during winter time. Ye. P. Knoppe (1961) points out that the growth of young elk ceases by the beginning of the winter and remains suspended for the entire cold period of the year. A suspension in growth of the animals in winter time is also noted for saigas (Bannikov et al, 1961) and wild asses (Rashek, 1965).

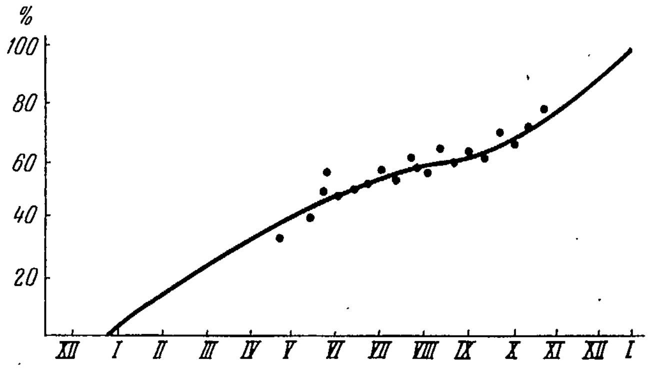
Wood, Cowan and Nordan (1962), have produced a curve of the variation in weight of black-tailed deer, identically well-nourished the year round for four years of their life. It can be seen from this curve that during the first winter the increase in weight of the animal is sharply diminished, while in subsequent years the total weight of the animals falls markedly. Based on the changes in weight, the authors assess the rate of growth of the animals and establish that the winter reduction in the growth rate applies to both male and female black-tailed deer. In the case of sexually mature animals the total rate of growth falls sharply and seasonal differences in growth rates become still more pronounced.

Evidence of a lack of growth of the skull during the winter period is provided by the "Denel' effect" - a winter reduction in skull height - discovered in insectivores and a number of rodents (Pucek, 1955, 1963, 1964; Wasilewski, 1956a, 1956b, Crowcroft and Ingles, 1959; Pucek [page 33] and Markov, 1964). The winter contraction of the skull is caused by the resorption of the bones of the cranium in the seams during the autumn; in winter the bone remains unchanged and in spring a new formation of the bone tissue begins (Pucek, 1957).

That the seasonal periodicity in the growth of dental and bone tissues is a clear reflection of periodicity in the growth of an animal can be illustrated by the following fact. As shown by the studies of beaver (Dezhkin, 1965) and black-tailed deer (Wood, Cowan and Nordan, 1962) seasonal fluctuations in the growth rate in young animals are less clearly defined than in adult ones. The legibility of primary annual layers in tissues of teeth and bone, as was noted previously, is significantly below that of subsequent ones, i.e. seasonal oscillations in the growth rate of tissues of teeth and bone are expressed less sharply during the initial years than during subsequent ones.

The degree of legibility of annual layers in different species of mammals evidently depends on the sharpness of expression of the winter retardation in the growth of the individual. As S.S. Shvarts and his colleagues pointed out, the rate of growth and degree of its dependence on a seasonal change in environmental conditions can be different even in different sub-species of one species (Shvarts et al, 1960).

Hibernating mammals have especially clear annual layers. In a hibernating common hamster neither dual nor supplementary adhesion lines were observed in the annual layers of the periosteal zone of bone, whereas both are frequently encountered in annual layers of bone in mice and voles. This evidently arises because seasonal variations in the growth rate of hibernators are more pronounced than in non-hibernators. In all remaining cases it is difficult to associate the degree of legibility of annual layers and the existence of supplementary bands within an annual layer with ecological peculiarities of the species. Thus, the aqueous, amphibious or terrestrial mode of life probably exerts no direct influence on the legibility of annual layers. Among the pinnipeds, in the majority of the species the annual layers are very clear in the dentine but there are species in which the layers are poorly expressed in dentine - the Far Eastern common seal (Tikhomirov and Klevezal', 1964), and grey seal (Hewer, 1960). In beavers, annual layers in bone are much more clearly expressed than in muskrats, although they both lead amphibious lives. Meanwhile, it has not proved possible to discern a strict relationship between the clarity of annual layers and the number of sexual cycles in the course of a year. On the one hand, in mice-like rodents and muskrats having several litters per year we have more frequently observed additional lines of adhesion in the annual layers of bone than in beavers and carnivores having one litter in the course of a year. On the other hand, the annual layers are more clear in bone and cementum of field mice than is the case with grey rats (Klevezal' and Lavrova, 1966; Klevezal', 1966), although they both have several litters a year. This question requires special study, as [page 34] data are available on the direct influence of sex hormones on growth, particularly on the growth of bone (Silberberg, M. and Silberberg, R. 1956).



a

б

Fig. 7 [page 34]

(a) Growth curve of dentine and (b) annual layers in the dentine of the tooth of a sperm whale.

a - change in the width of the non-transparent band of next annual layer of dentine in the tooth of a sperm whale. Abscissal axis - months of the year; ordinate axis - percentage ratio of width of non-transparent band of next annual layer to width of non-transparent band of preceding annual layer.

b - sector of longitudinal microslide of tooth of a sperm whale. Reflected light. Dark bands - transparent dentine, bright bands - non-transparent dentine. Boundaries of annual layers denoted as white rectangles by Ohsumi, Kasuya, and Nishiwaki. Arrow denotes supplementary streak.

With regard to the reasons for the formation of additional streaks within the annual layers of dentine and cementum it is impossible at the moment to give a clear explanation. As already stated, the small additional streaks (Owen's contour lines) are possibly associated with changes in the calcification rate of tissue, as suggested by Schour and Hoffman (1939a). At the same time there are data indicating [page 35] that large supplementary streaks, as is the case with the main bands of an annual layer, are connected with a change in the rate of growth of a tissue. Thus, Ohsumi, Kasuya and Nishiwaki (1963) have produced a curve of the change in the width of the next annual layer of the dentine of a sperm whale from April to November. The curve indicates that during the second half of the summer the accretion of dentine is somewhat retarded (fig 7a). Photographs presented in this paper depicting longitudinal microslides of teeth of sperm whales show that in the middle of an annual layer of dentine there is a narrow transparent supplementary streak, the clarity of which closely approximates that of the main transparent band of an annual layer (fig. 7b). It appears that the retardation in the accretion of dentine during the summer as noted on the curve is also a cause of the formation of this supplementary streak.

Thus, it appears that if it can be established that the main bands of an annual layer are formed as a result of seasonal changes in the rate of growth of the organism as a whole, then it can only be supposed that the largest of the supplementary streaks are formed in the same way.

Chapter II. [page 36]

PRINCIPLES OF USING THE ANNUAL LAYERS FOR AGE DETERMINATION IN MAMMALS

Selection of Tissue and the Most Useful Teeth and Bones for Age Determination

As was shown in the previous chapter, annual layers are formed in both the dentine and cementum of different teeth, as well as in the periosteal zone of bone of various skeletal bones. In determining the ages of actual species it is possible to select one of the layered structures and a specific tooth and specific bone. Some of the general approaches involved in such a choice can be clarified.

Selection of tissue for counting the layers

The value of dentine, cementum and the periosteal zone of bone as criteria of age is not uniform. The periosteal zone of bone is subject to resorption and in principle is therefore useless for determining the age of old individuals. Actually, however, in a number of species the resorption rate of a periosteal bone is so low that even in old animals all the annual layers are visible (e.g. the rodents; see Chapter III). Dentine and cementum are not subject to resorption and therefore retain all the annual layers throughout the life span. Cementum is more convenient for determining age than dentine as it is laid down not within but outside the tooth and its growth does not have spatial limitations. Moreover, inasmuch as in the majority of cases annual layers in cementum are more narrow than those in dentine, the supplementary streaks which serve as obstacles to the correct determination of the boundaries of annual layers are more weakly expressed in them.

In age determination of some species it is not fitting to speak of tissue selection, as regular annual layers exist only in one of three tissues. Thus, for age determination of lagomorphs and voles, all of whose teeth are of a constant size, only the annual layers in the periosteal zone of bone can be used (see Chapter III).

At the same time there are mammals in which annual layers are seen in all three tissues; such is the case with [page 37] insectivores, carnivores, toothed whales, pinnipeds and some rodents. In these cases, prior to formulating specific practices and establishing the relative clarity of the layers in various tissues it would first of all be better to determine the age simultaneously from the layers in the dentine, cementum and bone (or in the cementum and the bone, and dentine and bone), checking each result against the other. If the investigator is dealing with a species for which there are no data on age determination by this method, and in the selection there are no individuals of precisely known ages, such a reciprocal check will be needed. The fact is that even in those cases when a tooth is cut at the very beginning of the post-embryonic life of the individual, the first annual layer of cementum in some species is formed only during the second year of life (see the section "Rodents" in Chapter III). In the case of those species in which the layers in the cementum are sufficiently clear and the time of formation of the first layer is established it is better to use cementum for age determination as the tissue is not subject to resorption and does not have spatial limitations of growth. In some animals, for example bats (see Chapter III) the annual layers in cementum are so narrow that it is difficult to count them and the age can best be determined from the number of layers in the dentine.

Choice of the most useful teeth and bones for age determination

Annual layers are formed in dentine and cementum and are preserved in all teeth with the exception of teeth constantly increasing in length and continually being worn down. In determining age from the number of layers in dentine it is necessary to select a tooth, the pulp cavity of which is filled up with dentine very late in life. In pinnipeds and carnivores for example, such teeth are the canines. Evidently, for counting annual layers it is not necessary to use those sectors of the tooth in which the deposition of dentine occurs under the direct influence of a heavy mechanical load. Such sectors, for example, are the upper parts of the pulp cavity in the molars of rodents, where a heavy deposition of secondary dentine takes place (see the description for age determination of field mice and grey rats in the section "Rodents" in Chapter III). In such sectors numerous supplementary streaks are formed, which interfere with the correct determination of the boundaries of annual layers.

In age determination from the number of layers in the cementum it is necessary to select a tooth in which the layers are most clear. It appears that where the overall thickness of cementum on the root of the tooth is small the layers are too narrow and it is difficult to count them. But for the counting of layers no attempt should be made to use those teeth or sectors of a tooth where the thickness of cementum is very great. In this case, supplementary streaks are visible within the wide annual layers which serve to hinder age determination. The best teeth and [page 38] sectors of a tooth are those in which the layers are wide enough that they can easily be counted when subject to magnification but not so wide that numerous supplementary streaks are seen within them.

It should be remembered that in different teeth of one individual the first annual layer of cementum can begin to be laid down at different times, depending on the age at which the permanent teeth appear. Such is the case with ungulates. In this case, it is necessary for age determination to add to the number of layers of cementum the number of years, depending on when the first layer formed in the particular tooth.

A more complicated problem is the choice of a bone and sector of a bone for determining age from the number of layers in the periosteal zone. Ordinarily, for age determination of an animal from the layers in the periosteal zone of bone transverse sections of the lower jaw are used, most often those taken from the sector beyond the end of the row of teeth (Chapskii, 1952; Nishiwaki et al, 1961; Kleinenberg and Klevezal', 1962 et al).

In order that in the forthcoming presentation it should be clear as to which sectors of jaw we are talking about, in fig. 8 there are diagrams of various types of lower jaws as well as a designation of the sectors and terms used in the text.

The use of the lower jaw has the advantage that this bone is always preserved in collections and the removal of half of the lower jaw has comparatively little effect on the value of the collected materials.

At the same time the use of the lower jaw for age determination from layers in the periosteal zone of bone has an essential shortcoming. As we have already stated, the periosteal zone does not develop uniformly in various sectors of a bone. The periosteal zone in the lower jaw is not uniformly localized, not only in animals of diverse systematic and ecological groups but even in closely related animals, using similar types of food. Thus, in the lower jaw of the sable the periosteal zone attains the greatest width in the genal (outer) wall of the body of the jaw, while in the American mink this occurs on the lower (devoid of teeth) edge of the jaw (Klevezal', 1965). In all animals it is undesirable to use the sector of jaw directly adjoining the teeth for age determination as here the normal processes of apposition and resorption of the bone can be disrupted under the influence of a mechanical load on the tooth. But special difficulties arise in age determination of rodents from the structure of the lower jaw. In rodents, the bone channel in which a permanently growing incisor is located passes under the molars for the entire body of the lower jaw so that the lower wall of the jaw serves as the wall of this channel for almost the entire length. Under the influence of a heavy mechanical load on the incisor and adjacently located walls of the jaw, numerous supplementary lines of adhesion can develop in the lower wall of the jaw [page 39] of rodents as was noted in field mice and grey rats (Klevezal' and Lavrova, 1966). In voles, in which as a result of the growth of the molars the load on the incisor was less than in mice (Lebedkina, 1949) beneath the incisor channel in the wall of the jaw supplementary lines of adhesion are encountered much more rarely and are less vividly expressed (Klevezal' and Lavrova, 1966). It is therefore preferable in determining the ages of rodents to use sectors of jaw as far removed as possible from the incisor channel.

In consequence of this heterogeneous localization of the periosteal zone in the lower jaw in different species, for each species it is necessary that a sector be selected where the periosteal zone is more clearly defined, and in which the layers are most regular and their resorption rate at a minimum. For this purpose, it is necessary that in two or three adult specimens various sectors of jaw be studied successively (for example, for choosing a sector of jaw in small carnivores we compared the character of the periosteal zone, genal, lingual and lower maxillary walls in transverse cuttings of the jaw made at the beginning, in the middle and at the end of a dental row, then beyond the end of the dental row and in a cutting through the middle of the mandibular ramus (Fig. 8a). In the remaining specimens of this species only the sector acknowledged to be the best can be selected. (As to which sectors of jaw are [page 40] evidently most suitable for age determination of one species or another, we shall make a more concrete statement in the next chapter[1]).

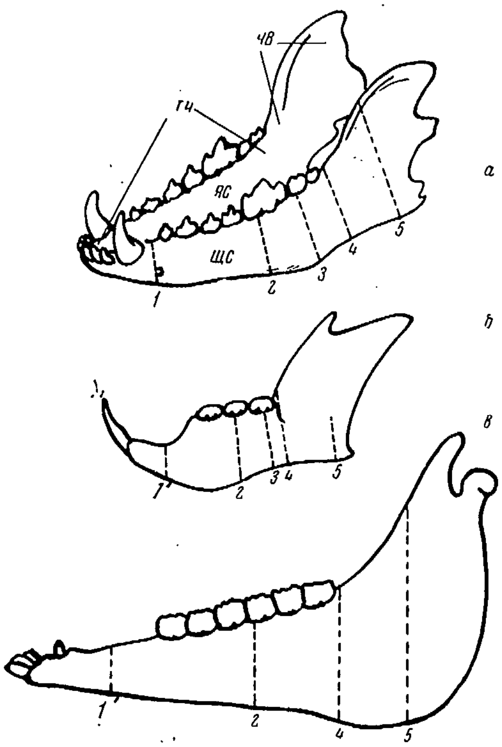


Fig. 8 [page 39]

Sketches of various types of lower jaws, showing the sectors studied.

a - sketch of jaw of a carnivore; b - the same of a rodent; c - same, of an ungulate; Tch - shaft of jaw; ChB - mandibular ramus; YaS - lingual wall of jaw; Shchs - genal wall of jaw; 1 - transverse section of jaw at beginning of dental row; 1' - the same, of diastema of jaw; 2 - the same, of jaw in middle of dental row; 3 - same, at end of dental row; 4 - same, immediately after end of dental row; 5a, 5c, - same, of mandibular ramus; 5b - same, of angular process.

[1]It must be emphasized that in determining ages of mammals from layers in the lower jaw it is necessary to make transverse and not longitudinal sections of jaw. The necessity for emphasizing this is because there is a paper (N.S. Gashev, 1966. Age determination of the northern pika. - Bulletin of MOIP. Biology Department, Vol. 71 ed. 6, pp. 24-30), the author of which studied only longitudinal sections of jaw. As he did not discover layers in all sections of the same bone he reached the incompetent conclusion that the age of the Ural Northern Pika cannot be determined from the number of layers in the periosteal zone of the lower jaw. In point of fact, the periosteal zone is frequently arranged as a band along

([1]continued)
the jaw, occupying only part of the lateral wall in the vertical plane. Naturally, in these cases not all longitudinal sections pass through the periosteal zone, so that any transverse section must of necessity embrace the periosteal zone of the jaw.

As has been shown, in the character of the periosteal zone the long bones of the limbs are similar to the lower jaw. As far as we know, as yet the structure of the periosteal zone of the bones of the limbs has not been used for large scale age determination of animals. It is possible that for a number of species, especially rodents, the long bones of the limbs will prove to be more suitable for age determination of animals than the lower jaw, as it will be more simple in them to choose a sector suitable for counting the annual layers.

Procedure for Preparing Dentine and Cementum

For age determination from the number of layers in dentine any collected material can be used. Annual layers in the dentine and cementum can be seen equally well regardless of whether the tooth is preserved in formalin, alcohol or in the dry state.

Dentine. Counting of annual layers in dentine can be done on a longitudinal or transverse section of a tooth. A longitudinal section through the middle of the tooth is used for counting the layers in teeth of any growth type, but it gives a less clear picture and is more difficult to prepare than a transverse section. If the tooth has previously ceased growing in length, counting of the layers can be done on a transverse section through the middle of the tooth, as such a section embraces all the annual layers. The transverse section of a tooth with a prolonged growth in length in which the deposition of dentine occurs at an angle to the longitudinal axis of the tooth embraces only the part of the annual layer on whatever level it was made. For age determination from the dentine of teeth of this type longitudinal sections must be made.

Frequently, annual layers of dentine are clearly visible on half-teeth (the polished surface of a longitudinally sectioned tooth) in reflected light or on thin microslides in transient light. To obtain a half-tooth - the tooth can either be cut manually with subsequent polishing of the surface of the section, or the unwanted part [page 41] of the tooth simply ground on a mechanical whet stone and the surface polished by hand. To obtain thin sections manually a lamella of the tooth must be cut out (a fret saw with a blade suitable for metal can be used) and polished first on large-grained and then on small-grained stones, clasping the lamella by a stopper. For ease of polishing the lamella can be glued to the stopper. It is probable that for this purpose a gelatine glue can be used such as that described in the section on the procedure for preparing bone. We used another method. After polishing one side of the lamella, we placed it on the smooth surface of the stopper and poured on from above celloidin cement (for this purpose, acetate film cleaned of emulsion and dissolved in acetone is suitable).

After the cement had hardened we began the polishing, polishing the tooth together with the hard cement surrounding it. The section is retained on the stopper merely by the cement encompassing it from the sides, and therefore as soon as the microslide becomes sufficiently thin it is easily withdrawn (or even falls) from its housing. (In the event of cement falling on to the lower surface of the section and fastening it to the stopper, the prepared slide can be freed by placing the stopper in acetone).

In any event, the manual method of preparing thin sections is very laborious and it is therefore better to mechanize. Fisher and Mackenzie, (1954) describe the construction of a lathe which enables a microslide to be obtained with a thickness of 100 microns in one minute. For mechanical preparation of sections it is necessary that from the moment the animal is killed the tooth be kept moist as dry teeth become brittle (Fisher and Mackenzie suggest preserving the teeth in the solution of alcohol, glycerine and thymol). A preliminary section of a lamella of the tooth is made with a jeweller's saw of carbon steel (0.020 x 3 inches, with 24 teeth to the inch), rotated at a speed of 2400 revolutions per minute using a quarter horse power motor. Polishing of the lamella is done by two identical circular finely sanded carborundum stones mounted in a bench of the lathe type. One stone is fixed to the head shaft and is rotated by the quarter horse power motor, while the other is rigidly fixed to the tail shaft; the tail shaft moves freely along the bed-plate. The stones are fitted with brass sleeves. Water enters through a tap at the top of a stationary sleeve and flows through an outlet in the bottom (it is overlapped by the tap in order to avoid losing the microslide). Below the sleeves is a special adaptation for collecting water which splashes during the polishing. A micrometer fitted to the tail shaft and a stop rigidly secured to the bed-plate enable the stonesto be placed at any desired distance from one another. The lamella of tooth is applied to the wet surface on either of the stones and the tail shaft advanced in such a way that the stones become close to the lamella on both sides. During polishing the tail shaft [page 42] is engaged with the machine and slowly moved towards the stop. In this way it is possible to prepare a section of even 25 microns in width, but Fisher and Mackenzie consider the optimum width of a slide for age determination of the harp seal to be 100 microns.

Carrick and Ingham (1962) reached the conclusion that layers of the canines of an elephant seal become more clear on half-slides if the surface is treated with silver nitrate. They placed a half-tooth overnight in a 5% solution of silver nitrate, then washed it and exposed it to the light until the time when the surface became a little more dark than necessary (exposure period did not exceed ten minutes). The tooth was then fixed in the space of ten minutes in a 10% solution of sodium thiosulphate and after an hour was rinsed in running water. As a result the annual layers appear as alternating dark and light bands.

Bow and Purday (1966) proposed a method of increasing the clarity of the layers of dentine in the teeth of a sperm whale by means of treating the half-teeth with acid. (This method is similar to the one used by Novakowski (1965) for improving the clarity of the layers in the cementum of bison, details of which are given below). They tried treating the tooth with hydrochloric, nitric and formic acids of various concentrations and established that the best result is obtained by using 5% formic acid. The surface of the half-tooth was placed in this acid for thirty hours, then washed and dried. The entire process was conducted at room temperature. As a result, dark and light bands of annual layers were seen as ridges and grooves. During treatment with acid it is difficult to establish what will be the end result as the ridges and grooves appear only after drying. If they are seen poorly the entire treatment must be repeated from the beginning.

G.A. Fedoseev (1964) considers that the ages of ringed seals can be determined more conveniently not from sections or half-teeth of a canine but from unstained sections of a decalcified tooth. He placed an entire canine in a ten-twelve per cent solution of nitric acid for approximately 24 hours or a little longer. As soon as the tooth became soft a section could be made using an ordinary razor. The advantage of this method is that it permits a large number of teeth to be treated in a short space of time without the use of complicated apparatus. As our experience has shown the counting of layers in such sections of some species of mammals (for example the ribbon seal) becomes more difficult here in that the very clearly discerned supplementary streaks hinder the correct delimitation of the annual layers.

In many mammals the annual layers in dentine are poorly seen both in half-teeth and thin sections, but show up very well in preparations stained with hematoxylin. Sergeant (1959) decalcified thin microslides of teeth of toothed whales in 70% /page 43/ alcohol and 5% hydrochloric acid and after washing them stained them with Delafield' hematoxylin. It is apparently more simple to decalcify not a thin section but the entire tooth or part of a tooth and then obtain a thin section.

For decalcifying a tooth it is entirely fitting to use one of the most widely used decalcifying agents - a 5-7% solution of nitric acid. The teeth can be decalcified either as a whole (the teeth of small mammals - together with a sector of jaw) or as a piece of same. The decalcification period varies, depending on the size and density of the tooth, from 5 to 6 hours (teeth of shrews - together with the jaw) to 1-3 days (teeth of carnivores and ungulates). The soft, decalcified tooth must be washed in running water for a period of 10-24 hours. Further treatment can be done by any acceptable histological method involving the placing in paraffin, celloidin, gelatine, etc., depending on experience and the availability of apparatus (see the instructions on microscopic techniques by G. I. Roskin (1951) and B. Romeys (1953) et al).

We used the most rapid and least laborious method - obtaining sections on a frozen microtome. The washed tooth or piece of tooth (if the entire jaw and teeth have been decalcified then a piece must be cut out with a razor after decalcification) was cut on a frozen microtome of the required flatness into 15-30 micron sections. The derived sections were placed in distilled water and then stained with Ehrlich's hematoxylin (we also tried to stain them with Karachi's hematoxylin and hemalum but found that Ehrlich's hematoxylin gives the best picture), washed in running water, passed through glycerine of increasing concentration and mounted in pure glycerine.

In such preparations the annual layers consisted of narrow intensively stained and broad weakly stained bands.

The staining period varies depending on the nature of the dye. After staining, the sections must be differentiated - placed in a 70% alcohol weighing bottle for several seconds to which from two to three drops of hydrochloric acid are added and then quickly placed in water. With differentiated stainings the layers stand out much more clearly than without differentiation. Following differentiation the sections must be washed for 10-15 minutes in running water or 30-60 minutes in frequently changed water. For this purpose, to prevent the sections from warping, after washing they must be placed first in a 25% solution and then in 50 and 75% solutions of glycerine in water. The sections are in each solution for not less than 5-10 minutes. A large section can be drawn through all the solutions with a dissecting needle, lightly curved in the middle. Very small sections, for example transverse sections of canines of bats, should preferably be replaced in a small dish, on the bottom and side walls of which are numerous openings, and the dish with the sections then transferred from the water to the dye, then into the water for washing and into the glycerine. It is preferable /page 44/ to take not one but several sections from one tooth, staining and drawing them along together and flushing them in one glass.

When mounting the preparations in glycerine so-called temporary compounds are obtained, as in time hematoxylin breaks down in glycerine. Experience has shown that such preparations are kept in a satisfactory (for determining the number of layers) state for 2 to 3 years. The advantage of temporary preparations is that if when studying a preparation it turns out that it is poorly stained or poorly washed, it can easily be withdrawn, placed in water, once again carefully washed or even restained (for which it is removed from the water and placed in alcohol and hydrochloric acid until it is fully decoloured, carefully rinsed and again stained with hematoxylin) and once more enclosed in glycerine. A shortcoming of preparations mounted in glycerine is that they can only be kept in a horizontal position on drawing boards. To obtain preparations more convenient for storage or shipment, after being drawn through the glycerines they can be enclosed in glycerine jelly or placed in glycerine and mounted (see the instructions on microscopic techniques).

Cementum. Cementum is deposited unevenly on the surface of the root of a tooth. It can be much more developed on one lateral wall of a root than on the opposite one (such is the nature of the cementum in the incisors of wild asses and spotted deer), can be laid down chiefly on the lower surface of the root. For example in beaver (Kleinenberg and Klevezal', 1966) it frequently attains a greater thickness in the inter-root cushion than on the roots (for example in the molars of deer) (Mitchell, 1963). In most cases, for age determination from the cementum of teeth it is necessary to make longitudinal sections. For correct orientation a preliminary transverse section must be made in order to ascertain whether there are any differences in the thickness of the depositions of cementum on the lateral walls of the roots. Transverse sections can be used only in those cases when the deposition of cementum on the lateral walls of the root is far less heavy than on the lower surface. In these cases (for example, in the canines of deer which have a limited growth in length) in the sections passing through the middle or upper third of the root all the annual layers will be seen.

Sometimes, annual layers in cementum are clearly seen on half-teeth examined in reflected light. We observed such a picture in beaver and spotted deer. Novakowski (1965) used the following method for improving the clarity of layers of cementum on half-teeth of bison: he placed the manually-polished surface of longitudinally cut root for two hours in a decalcifying solution (5% hydrochloric acid and 95% alcohol), then washed and dried it overnight. As a result ridges were formed due to the uneven decalcification of the non-transparent and transparent [page 45] bands of annual layer of cementum. We treated half-slides of beaver teeth in this way but found no essential improvement in the clarity of the annual layers.

Hewer (1964) counted the layers in the cementum of canines of the grey seal on thin microslides in transient light.

For counting the annual layers in the dental cementum of grizzly bears, Mundy and Fuller (1964) made a longitudinal section of a non-decalcified tooth to a thickness of 150 microns, using a specially designed machine for cutting bone and teeth (the authors give only the address of the firm which issued the machine), dehydrated the section in alcohol, clarified it in xylol and mounted it in Canadian balsam.

McEwan (1963) notes that layers of cementum were not seen in sections of caribou incisors in transient or reflected light, but stood out clearly in sections of a decalcified tooth stained with hematoxylin. We noted the same effect in the canines of the sea otter. Annual layers of cementum in the teeth of small mammals can evidently be counted only in stained sections as it is difficult to make dry sections of small teeth with the required degree of flatness. Stained preparations of cementum can be obtained by using precisely the same method as in the case of preparations of dentine (see above). As is the case with dentine, the clarity of the layers is increased if the section is over-stained somewhat and then differentiated. In these compounds the annual layers are seen as alternating narrow, heavily-stained and broad, lightly-stained bands.

Procedure for Preparing Bone Tissue.

For age determination, bones are suitable which have been preserved by many of the procedures adopted for collection purposes. We had to study bones which were freshly fixed in formalin; fixed in alcohol; dried without first removing the meat, and boiled; as well as those cleaned of meat and dried, and we did not find that the method of preservation had any influence on the clarity of the annual layers. It appears that the only unsuitable bones are those which have been whitened after removal of the meat through the use of hydrogen peroxide. We found that in most cases, after decalcification the whitened jaws of rodents became very brittle and in the preparations it could be seen that the periosteal zone had been damaged.

The lines of adhesion separating the annual layers of the periosteal zone were clearly seen both in thin sections of the bone (in transient light) and in stained compounds. A section of bone can be prepared either on a specially equipped bench for cutting and polishing bone or manually as follows: the lamella of bone is cut out and polished on increasingly fine-grained stones, being held by a cork stopper. Prior to polishing the lamella can be fixed to the stopper. For example, A.I. Doynikov (1951) used the following procedure /page 46/ in preparing sections of a human jaw. A lamella of bone was fastened to the smooth surface of the stopper by means of gelatine cement (recipe for the cement: pour 3 grams of gelatine into 25 cu.cm. of distilled water, and after swelling (40-50 minutes) slowly bring to the boil; it should be kept in a covered dish and to avoid spoilage, one or two drops of phenol should be added). The cement is heated and applied as a thin layer to the surface of the stopper, whereupon the lamella of bone is placed on it and held by a microscopic slide weighing from 50 to 100 grams. After 24 hours the rigidly cemented lamella is ready for polishing. Unsticking of the prepared section can be done by slowly heating in water. After drying and dehydration the prepared sections can be enclosed in Canadian balsam. For enclosing sections A.I. Doynikov used Shabadash's gelatine-glycerine mix: to 10 grams of 20% solution of gelatine, filtered in a filling funnel, are added 6 grams of glycerine, 4 grams of 30% solution of potassium or sodium acetate and 1-2 crystals of thymol; after cooling the mix is ready for use; for enclosing the microslide, a lump of the mixture is heated.

The use of sections for age determination is convenient in that no special apparatus is required. At the same time, the process of preparing the sections is laborious and requires special skills. Moreover, only one section is obtained from the comparatively thick lamella cut out of the bone and the main mass of the bone is lost in the course of the polishing.

It is evidently more convenient to use not sections but stained preparations of bone. The stained preparations can be obtained by the same method as was used for the stained sections of tooth (see above). Bones of small animals can be decalcified in their entirety, for as with bones of large ones it is preferable to cut out a lamella of about 0.5 cm thick from the desired sector of bone. (The cutting of bone can be done with a hand fret saw suitable for metal or even wood). Following staining with hematoxylin the section of bone must be differentiated in the event that the section is stained too heavily (very dark all over), but if the staining is normal this is not mandatory.

The Counting of Annual Layers

The counting of layers in dentine, cementum and bone can be done under binoculars or under microscope depending on the size of the object.

Layers in sections can be counted both in ordinary light and in polarized light. Thus, Chiasson (1957) found that layers of dentine on a section of the canine of a northern fur seal were more visible in polarized light than in ordinary light. The same thing was noted by Mansfield (1958b) in studying the dentine of Weddell seal canines.

For counting annual layers in the dentine of old fur seals in which the first annual layers are broad and easily counted and the latter annual layers narrow and very closely adjacent to one another /page 47/ Kubota and co-authors (1963) suggest placing longitudinal microslides of teeth (thickness of 100 microns) between glass plates and placing them in the magnifier like a negative film. In the magnified image of the tooth the first and subsequent annual layers are easily counted.

In counting the layers in the dentine of teeth of marine mammals it is usual to find the neonatal line - the line denoting the birth of the animal, and then to keep count along the narrow bands of the annual layers beginning at the periphery of the tooth and working towards the pulp cavity (fig. 1).

The approximate position of a neonatal line can be established by studying the teeth of individuals taken shortly after birth. Our experience indicates that in land mammals it is difficult to determine the neonatal line unless the animal is killed immediately after birth. Thus, in bats and carnivores in which during the first year of life almost half the thickness of the dentine of the canine is formed, within the band of dentine formed before the first winter are many supplementary streaks, which make it difficult to distinguish the neonatal line (fig. 13 b, c; 48). It is especially difficult to establish the neonatal line in the dentine of teeth of ungulates when the permanent teeth do not erupt during the first year of life of the individual. For counting the layers it is therefore easier to determine the narrow band of the first annual layer and keep count from it.

The counting of layers of cementum as well as dentine can more easily be done from the narrow bands of annual layers. In some animals (ungulates and separate species of rodents and carnivores) cementum begins to be deposited on the root of a tooth only during the second year of life of the individual and sometimes even later. It is therefore necessary to establish when the first narrow band of cementum was formed, and to count the number of annual layers from the number of narrow bands (the count is made from the inside outwards), adding, if necessary, the missing number of years.

Counting of layers in the periosteal zone of bone is more easily done from the lines of adhesion delimiting the layers. Here, it is important to find the line delimiting the first annual layer and then to keep count towards the outer edge of the bone (see fig. 4). In cases where a mesosteal zone is expressed it is necessary to differentiate the resorption line so as not to confuse it with the line of adhesion of the first year. As a rule, lines of adhesion delimiting annual layers are straight and run parallel to one another and to the outer surface of the bone. The resorption line is winding and usually runs along the outer edge of an osteal (mesosteal) zone.

In a number of species, within the annual layers supplementary streaks are seen which hinder the correct counting of the layers. In dentine and cementum the supplementary streaks with rare exceptions are less clearly seen in sections and not so heavily stained in preparations stained with hematoxylin as the main bands from which the count of the annual layers is made. With a certain amount of skill [page 48] the investigator easily detects them and omits them during the count. In the annual layers of periosteal zone of bone it is quite a different matter. Here, besides the illegible and easily distinguished supplementary lines can be seen lines which are just as bright as the main ones but are nevertheless supplementary ones, as indicated by a study of individuals of precisely known ages (an actual example of such a phenomenon is given in the section entitled "Carnivores" in Chapter III). Such lines, as far as can be judged from currently available data are encountered comparatively rarely but nevertheless they can be a cause of error in age determination. In cases when a supplementary line of adhesion exists, the normal width ratio of the annual layers is disrupted. A skilled investigator can easily note which line of adhesion separates an annual layer which is too narrow in comparison with the width of the layer characteristic for the particular year. Taken by itself, a small width of a layer merely points to the possibility that the line separating it is a supplementary one but is not proof of this. It is entirely possible that such a narrow layer can be the complete annual layer (and the line of adhesion - the main line) formed in a year when the normal growth rate was for some reason disrupted. At the present time the only method which can be proposed for distinguishing whether a particular line is supplementary or basic is to compare the relative width and numbers of annual layers in bone and dentine or cementum. As stated earlier, supplementary bands in dentine and cementum are discerned much more easily than in bone. Thus, if, when counting the layers in bone it is seen that a particular annual layer appears abnormally narrow, for precise age determination it is necessary to compare the layers not only in the bone but also in the dentine and cementum of the tooth.

Sometimes, narrow bands in dentine and cementum and adhesion lines in bone appear doubly, but nevertheless relate to one annual layer. In the cementum of molars of some rodents a narrow band of annual layer which is clearly single in the lateral walls of the tooth divides into two in its lower part. Moreover, the two streaks which are formed are sometimes separated from one another by a considerable distance. In a longitudinal cross-section of the tooth such a picture can easily be detected and recognized by following the narrow bands of the annual layers both in the lower part of the tooth and in its lateral walls. But if the annual layers are counted in a transverse section of the root and the section runs too close to the end of it, then these bands can be seen in the section as two independent rings, creating the illusion of an extra-annual layer. When determining the number of annual layers of cementum on transverse sections of a tooth it is therefore impossible to use sections from the lower part (usually the lower third) of the root.

As we stated in Chapter I, in the periosteal zone of bone in some groups of mammals the first annual layers become [page 49] resorbed in time. When the first adhesion line is fully resorbed the width ratio of the first annual layers which is normal for the given species is disrupted. Using this feature it can often be determined that a part of the annual layers has been resorbed. How many layers have been resorbed can sometimes be counted from the remnants of adhesion lines preserved between the osteons. Such remnants of adhesion lines for example can sometimes be seen in the bones of old sables (Klevezal', 1965).

Enumerated above are the difficulties that an investigator can run up against who is only just beginning to determine the ages of individuals of a particular species from the number of annual layers. Subsequently, with the acquisition of skill, they disappear as the investigator can easily determine the double and supplementary streaks and lines, resorption of the first layers, etc. Moreover, as will be seen from the contents of the next chapter, these difficulties are not encountered when determining the ages of all species.

Chapter III. /page 50/

AGE DETERMINATION IN MAMMALS OF VARIOUS ORDERS

We will examine actual data on the possibilities of using the method of age determination from the number of annual layers for species belonging to various orders of the mammalian fauna of the USSR.

It must be noted that since the layers in dentine, cementum and bone are annual ones they provide an opportunity for age determination with an accuracy of up to a year. In animals breeding once a year in definite and compressed periods (carnivores, ungulates, pinnipeds, etc.) the method can be used for determining ages with an accuracy of up to a month, after adding to the number of complete annual layers the number of months elapsed since the time of appearance of the young until the time of death of a particular individual. In animals with a protracted breeding period and which give several litters a year (many rodents, insectivores, etc.), the accuracy of this method of age determination will be plus or minus several months. Thus, in individuals born in April and caught in April of the following year the layer of bone tissue bounded on the outside by an adhesion line is actually the annual one; but in the case of individuals born in August and killed in April of the following year such a layer of bone tissue, bounded by an adhesion line, was formed in the course of eight months. As annual layers are usually counted from the narrow bands of annual layer in dentine and cementum and from the adhesion lines in bone which form during the winter period, when determining the ages of animals giving several litters a year it is more correct to speak not of the age in years but of the number of winters lived.

Order Insectivora

The annual layers were described for only one representative of the order - the common shrew <u>Sorex</u> <u>araneous</u> (Klevezal', 1966; Kleinenberg and Klevezal', 1966). The remaining species were not studied.

We had at our disposal the lower skulls of 40 specimens of the common shrew, taken during August and September in the Moscow Oblast' (material presented by M.Ya. Lavrova). Common shrews probably do not live more than two years and animals which have over-wintered are distinguished from those which have not over-wintered by the weight, condition of the sexual system and /page 51/ degree of wear of the teeth (Dunaeva, 1955). Fifteen non-wintered and twenty-five wintered individuals were selected from a large number of animals divided into age groups by M.Ya. Lavrova on the basis of these features.

Besides the bone, longitudinal sections of genal teeth were seen in sections of the rear portion of the body of the jaw, and a transverse section of the incisor root (fig. 9) in sections of its forward portion.

On the roots of all the genal teeth of non-wintered shrews there is a quite broad homogenous layer of cementum faintly stained by hematoxylin (fig. 10a). On the walls of the large incisor, the root of which passes under the premolar, there are no layers of cementum. In the over-wintered animals, the thickness of the cementum of the genal teeth shows more than a twofold increase. It consists of two faintly stained layers divided by a narrow heavily stained band (figs. 9, 10b). On the root of the incisor, on one of its lateral walls, is seen a broad layer of uniformly stained cementum (see fig. 9a).

Thus, a narrow darkly stained band in the cementum denotes that a winter has been spent. It is not formed in the cementum of the incisor because the deposition of cementum here evidently only begins during the second year of life of the individual.

The over-wintered shrews are also clearly distinguished from the non-wintered ones by the structure of the periosteal zone of the lower jaw. In non-wintered animals the bone is flat. The mesosteal zone does not occur and there are no osteons, but the central zone of bone of the shank of the lower jaw is separated by a meandering line (similar in form to a resorption line) from the outer layer of the periosteal zone, the arrangement of the fibers of which is truly parallel to the bone surface. Such a picture was seen in the upper part of the mandibular ramus (fig. 11a). In the over-wintered animals the bone structure is generally the same, but along the entire extent of the body of the jaw in the lower part of the genal wall there is a narrow layer of periosteal zone, separated from the preceding layer of periosteal zone by an adhesion line (fig. 12). A similar layer also appears in the upper part of the maxillary branch (fig. 11b). A new layer of bone runs as a band along the entire length of the body of the jaw but in the vertical plane occupies less than half of the genal walls; in the lingual one it is completely lacking.

The differences in structure of the cementum of teeth and the periosteal zone in wintered and non-wintered shrews can be seen in Table 4.

In the majority of the over-wintered individuals an annual layer was not seen in the dentine of the teeth. Probably, the main mass of dentine of the teeth is formed in the course of the first year of life, and in the second year the accretion of dentine must be so small that it does not appear in our relatively crude section. A clear annual layer of dentine can be seen only on two over-wintered specimens. In these animals the accretion of the dentine of the second year of life was comparatively [page 53] broad; perhaps they were individuals from late broods of the previous year, in which the required mass of dentine had not managed to form before the first winter.

In a cross-section of the lower part of the incisor in these specimens a narrow heavily stained band could be seen, separating a broad outer ring of dentine from a much narrower inner ring. In the non-wintered shrews such bands were lacking.

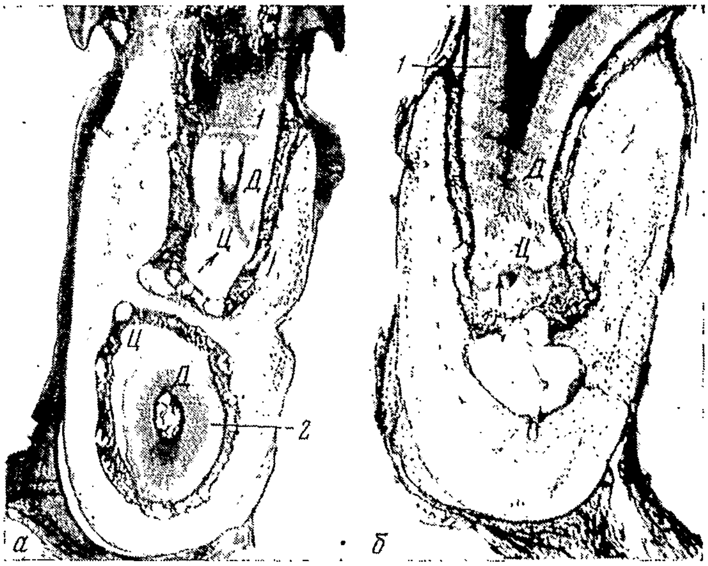


Fig. 9 [page 52] Transverse sections of lower jaw of a common shrew (a) in the forward part of the jaw; (b) in the rear part of the jaw. Staining - hematoxylin (X60). 1 - longitudinal section of genal tooth; 2 - transverse cross-section of incisor root; D - dentine; T_s - cementum; arrow denotes heavily stained bands of cementum.

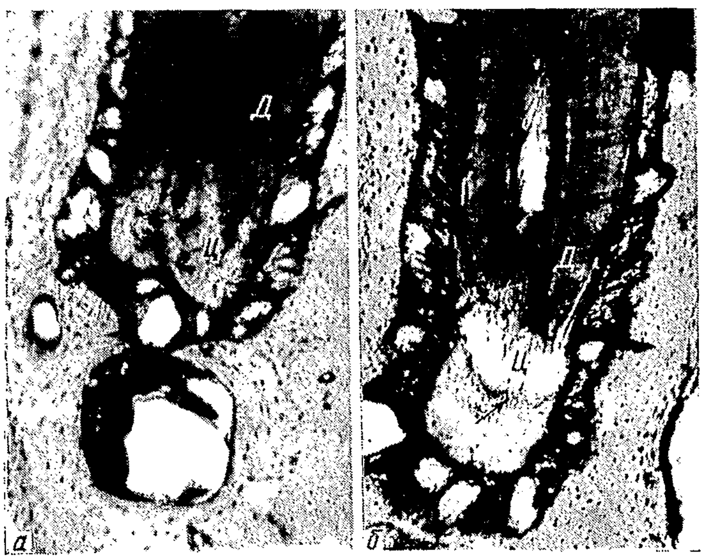


Fig. 10 [page 52] Longitudinal sections of molar teeth of (a) non-wintered and (b) over-wintered common shrews. Staining - hematoxylin (X120). For explanation of signs, see fig. 9.

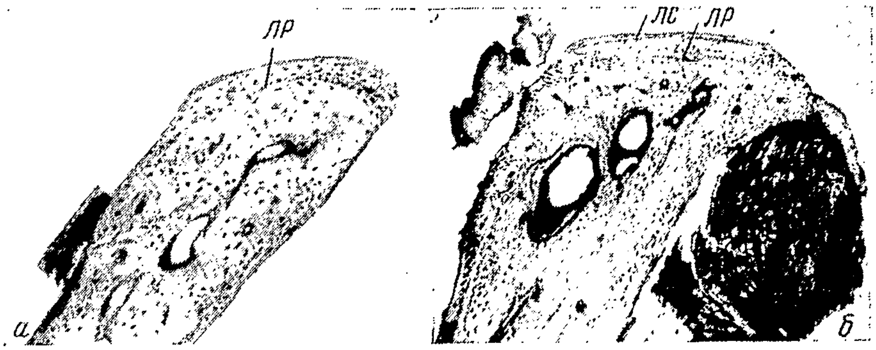


Fig. 11 [page 53]. Transverse sections of upper part of mandibular rami of lower jaw of (a) non-wintered and (b) over-wintered, common shrews. Staining - hematoxylin (X84). LR - resorption line; LS - adhesion line.

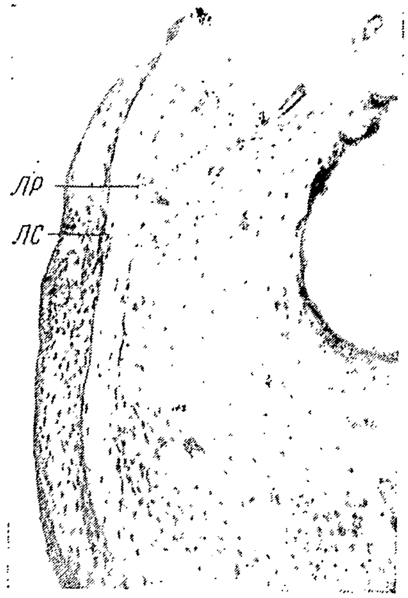


Fig. 12 [page 53]. Transverse section of lower part of genal wall of lower jaw of over-wintered common shrew. Staining - hematoxylin (X120). For explanation of signs, see fig. 11.

On the basis of these data it can be stated that the age of insectivores can be determined from the number of annual layers in the cementum /page 54/ and probably, in the dentine as well (in those species which live longer than shrews and in which the deposition of dentine occurs over a much longer period. The ages of shrews can also be determined from the annual layers in the periosteal zone of bone, but we are by no means sure that the structure of the periosteal zone of bone is suitable for age determination of long-lived insectivores (the reasons for this uncertainty will be discussed in the section headed "Chiroptera").

Table IV /page 54/

Structure of cementum and the periosteal zone of bone in non-wintered and over-wintered common shrews.

Structure of cementum and periosteal zone		Age groups		Total number studied
		Non-wintered I	wintered II	
Cementum of genal teeth	Lightly stained layer	15	-	40
	Two lightly stained layers, separated by a dark band	-	25	40
Cementum of incisor	Cementum absent	15	-	40
	Lightly stained layer of cementum	-	25	40
Periosteal zone of bone	Adhesion line absent	15	-	40
	One line of adhesion plus a new layer of bone	-	25	40

Order Chiroptera

Christian (1956) discovered layers of dentine similar to those described for pinnipeds, in a cross-section of the canines of a large brown bat (Eptesicus fuscus). He compared the number of layers in the dentine of the canines with the ages of the animals determined from the degree of wear in the teeth, and reached the conclusion that in the course of a year one layer forms in the dentine of a canine (one wide and one narrow zone).

We studied the lower jaws of 15 red noctules (Nyctalus noctula) caught during the summer in the Voronezh Oblast' (the ages of eleven of them were known as a result of marking [page 55], and lower jaws and bones of the limbs of ten large mouse-eared bats (Myotis myotis), taken in April in the Trans-Carpathians (material presented by K.K. Panyutin). The study was made on hematoxylin stained sections.

The teeth of the bats which were studied ceased lengthening early and all the annual layers are seen in both the longitudinal and transverse sections of the teeth. Distinct layers also exist in the dentine of the canines and in the dentine of the genal teeth. In all of the red noctules of precisely known ages the number of annual layers seen in the transverse sections of the canines coincided with the ages of the individuals in years (fig. 13, 14). In the same individuals the number of annual layers in the dentine of the canines and genal teeth was identical (fig. 14). In the large mouse-eared bat (adult specimens were selected, judging from their sizes and degree of wear of the teeth) from 2 to 7-8 annual layers were discovered (fig. 15).

It is interesting to note that in adult individuals the dentine seems to consist of two zones: an outer one more brightly stained with hematoxylin and an inner one, more darkly stained. The narrow heavily hematoxylin-stained band of first winter is usually located on the boundary of these zones; all the later annual layers are within the more darkly stained zone (fig. 13b,c,d; 14, 15).

Each subsequent layer is narrower than the preceding one. Starting from the fifth one the annual layers become especially narrow (fig. 13d). With such a low rate of dentine deposition the pulp cavity becomes fully closed; this probably occurs late in life. In bats caught in their eighth year of life it is still quite wide (fig. 13d).

In the wide faintly stained band of the first annual layer inner, indistinct light and dark streaks are often seen (fig. 13c). The inner streaks were not observed in the remaining layers.

Layers of cementum exist on the roots of all the teeth of bats and what are evidently annual layers are clearly visible in them. In red noctules, beginning from the second year, the cementum layers were so narrow that in our sections they merged with the single darkly stained band and it was difficult to count them. In large mouse-eared bats the width of the cementum layers not only did not diminish with age but even increased somewhat. There was an identical number of layers in the dentine and cementum of the same individuals of the large mouse-eared bat.

Annual layers were not seen in the periosteal zone of bone in all animals. A mesosteal zone of bone did not occur in the lower jaw of bats and in the bone of the same year specimens a meandering line was seen, separating the narrow outer layer of the periosteal zone. In yearlings, besides this line similar to a resorption line an adhesion line was seen, separating the first annual layer of periosteal zone. In various sectors of the jaws of individuals belonging to the older age groups [page 56] the formation of annual layers was not noted. Thus, in 3-7-year-old red noctules, 1-2 adhesion lines separating the annual layers could be seen, and in adult large mouse-eared bats - 2 to 3 such lines. In a male large mouse-eared bat having 7-8 annual layers in the dentine, three layers of bone were seen in the periosteal zone. We observed a similar picture in the thigh and shoulder bones of large mouse-eared bats.

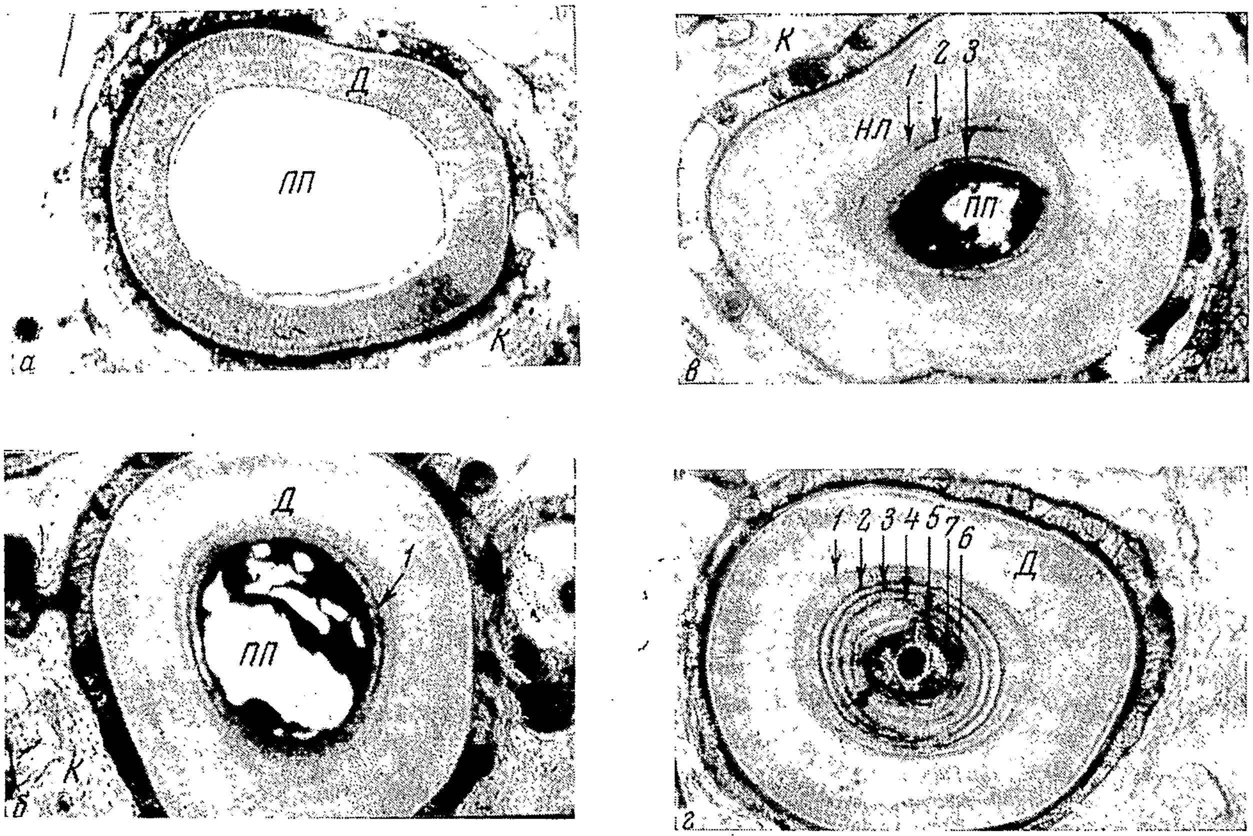


Fig. 13 [pages 56-57]. Transverse sections of canines of (a) same year specimen (b) yearling (c) three-year-old and (d) seven-year-old red noctules. pp - pulp cavity, D - dentine, NL - neonatal line.

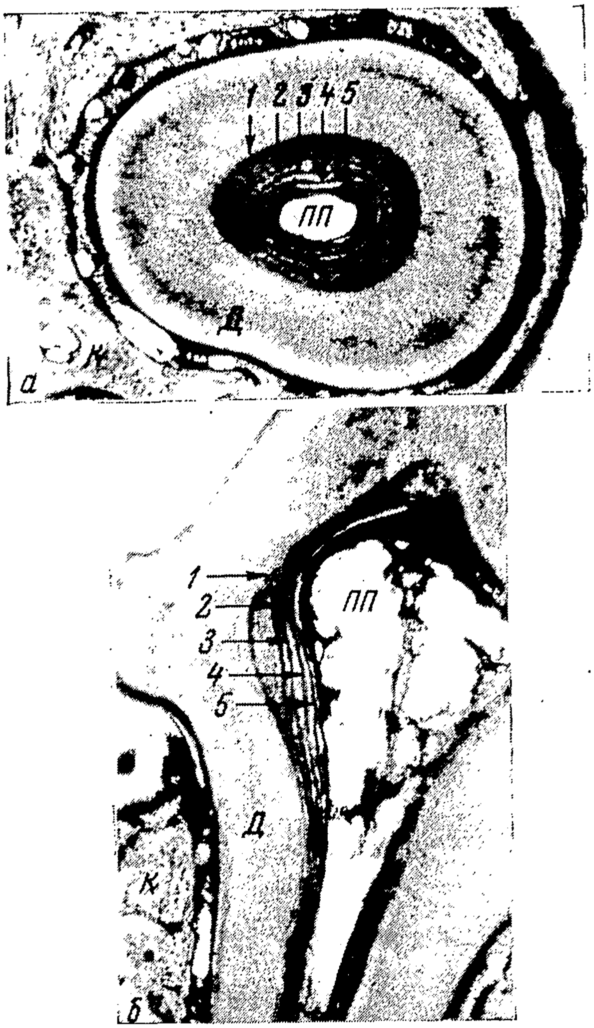


Fig. 14 [page 58]. (a) Transverse section of canine and (b) longitudinal section of molar of five-year-old red noctule. Staining - hematoxylin. (X84). For explanation see fig. 13.

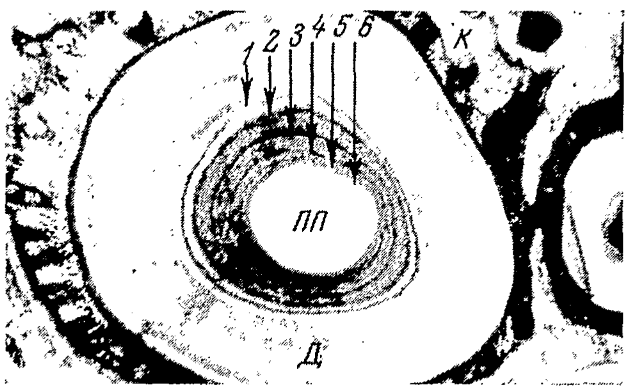


Fig. 15 [page 59]. Transverse section of canine of adult large mouse-eared bats. Staining - hematoxylin. (X84). For explanation see fig. 13.

Evidently, in bats, annual layers are formed in the periosteal zone of bone during the first two to three years of life. Later, either the growth of bone is reduced or the annual increases become so small as to appear to us to be merged with the outer edge of the bone.

/page 57/. It is probable that such growth changes in the periosteal zone of bone are also peculiar to other bats. In the periosteal zone of the lower jaw of a Daubenton's bat Myotis daubentoni studied by us, (from the collection of the Zoological Institute, Academy of Sciences of the USSR), the age of which (as determined from ringing) was not less than three years, it was possible to see only one adhesion line, separating two layers of bone tissue.

The Chiroptera, together with the insectivores, are distinguished among the other mammals by their more primitive bone structure (Enlow and Brown, 1958). It is possible that such peculiarities in the growth of the periosteal zone are associated with the primitive bone structure. (In this case, they must also be peculiar to the insectivores /page 59/. This too, compelled us in the preceding section to express doubts that regular annual layers are formed in the bone of insectivores living more than one to two years. It is possible, however, that such an earlier cessation (or retardation) of the thickening of the bone is associated with adaptation for flight, in connection with which the skeletal weight relationships formed at the beginning of the post-embryonic period must not be disturbed.

Thus, the ages of bats can be accurately determined from the number of annual layers in the dentine of teeth, and in some species, also from the number of layers in the cementum. The periosteal zone of bone in bats is unsuitable for age determination.

Order Lagomorpha

In representatives of this order the incisors and hypselodontal molars are constantly growing in length in the root part and continually being worn down on the part of the crown. Teeth of this type could not preserve annual layers even if they were formed. Therefore, the annual layers in Lagomorpha are shown only in the periosteal zone of the lower jaw (Bernshteyn and Klevezal', 1965). The bone structure was studied in hematoxylin-stained sections of red (Ochotona rutila) and long-eared (Ochotona rojlei) pikas from Tien-Shan. The precise ages of the animals was not known but young animals in their first year of life were separated from over-wintered individuals on the basis of their external features, state of the sex organs and size of the comb /? angula process- Ed./ on the lower jaw.

The best sector for counting annual layers proved to be the central part of the jaw in the area of the molars. The mesosteal /page 60/ zone in the lower jaw of pikas was not very clearly shown. In the lateral walls of the jaw it occupies the inner parts, the main mass of the walls being represented by laminated bone with tufts of collagenic fibers running parallel to the surface of the jaw. The wall of the jaw is pierced by radial circulatory channels. The resorption line is either not shown at all or weakly shown. In the outer part of the wall there is a comparatively narrow periosteal zone with adhesion lines.

In all of the young, unwintered individuals caught in summer the bone was either porous (fig. 16a) or dense, while in the outer parts there was no adhesion line. In the animals placed in the "wintered" group the bone was dense and in the outer parts of the walls could be seen from one to three adhesion lines, separating comparatively narrow layers of periosteal bone tissue (fig. 16b-d).

An analysis of the structure of the periosteal zone of bone in pikas killed during different seasons indicated that the layers of bone tissue are annual ones and the adhesion line dividing them is formed in winter. This conclusion was also confirmed by the relative numbers of animals with different quantities of adhesion lines in the periosteal zones belonging to the "wintered" group. Most of the animals had one line, a lesser number two lines, and a few individuals three lines (Bernshteyn and Klevezal', 1965). This fully agrees with the idea that in an average sample there should be more younger animals than old ones.

The rate of organization of the bones in the animals studied was either extremely slow or did not include the outer portions of the maxillary wall. Even in old individuals having three adhesion lines no traces of resorption were seen close to the first line.

Thus the ages of animals belonging to the Order Lagomorpha can be determined from the number of annual layers in the periosteal zone of bone. The number of adhesion lines in the periosteal zone indicates the number of winters lived, Depending on the length of the breeding season the age can sometimes be determined even more accurately. For example, the breeding season in both species of pika studied extends from April to August (Bernshteyn, 1964), i.e. animals born in the same year can differ in age by no more than four months. In this case, if the date of capture is known the ages of pikas can be determined from the number of adhesion lines in the bone with an accuracy of up to four months. (Bernshteyn and Klevezal', 1965).

Order Rodentia

Since various patterns of growth, wearing down of the teeth and bone structure are encountered in representatives of the rodent order, we will give separate descriptions for the species or groups of species.

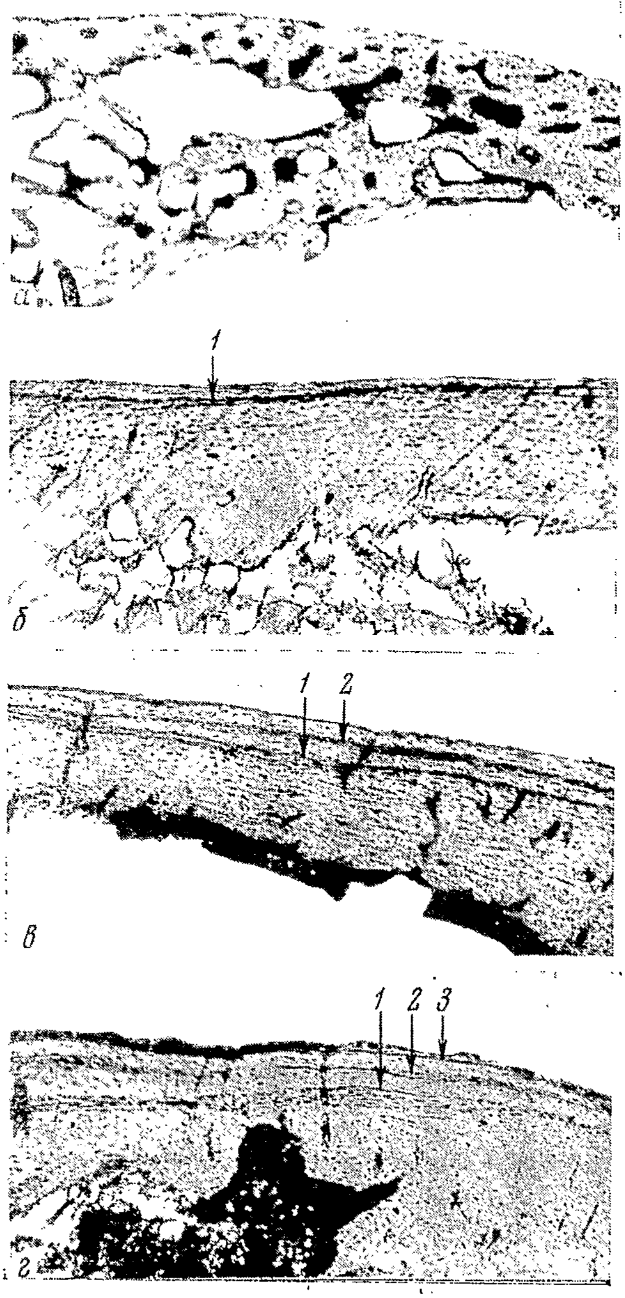


Fig. 16 [page 61]. Sectors of wall of lower jaw of (a) a young long-eared pika and of (b), (c) adult red and (d) adult long-eared pikas. Transverse sections. Staining - hematoxylin. (X60). Adhesion lines indicated by arrows, annual layers by numerals.

Beaver Castor fiber [page 62]

The beaver has molars consisting of dentine - enamel loops which are fully formed at the end of the embryonic and beginning of the post-embryonic period. The structure of the cementum and the periosteal zone of bone is therefore suitable for age determination of beavers while the structure of dentine is unsuitable.

Annual layers in the persioteal zone of the lower jaws of beavers were described by us (Klevezal' and Kleinenberg, 1964; Klevezal', 1966) on the basis of materials from 14 beavers of accurately known ages from the Voronezh animal reserve (material received from L.S. Lavrova) and 11 beavers of unknown ages from the Mordovian reserve (material received from M.N. Borodina).

The study was made on hematoxylin-stained sections. It was established that the most useful layers of the periosteal zones coincident with a minimum rate of their resorption are in the genal wall of the jaw beyond the end of the row of teeth.

In the lower jaws of beavers the mesosteal and periosteal zones are well shown together with the resorption line dividing them. In the first layer of the periosteal zone are many blood vessels, the subsequent layers being dense (fig. 17, 18, 19, 20).

The number of layers in the periosteal zone of beavers of known ages strictly corresponded with the ages of the individuals in years (fig. 17) with the sole exception of the old animals.

In old individuals the first annual layers are resorbed and replaced by osteoid bone tissue. In an eleven-year-old beaver all eleven layers were preserved in the periosteal zone, in a beaver older than fourteen years only thirteen layers remained, and in a seventeen-year-old beaver - twelve or thirteen layers. Judging from these data, resorption of the first annual layer of periosteal zone occurs at 12-13 years of age. The resorption rate can evidently differ in different individuals. Thus, among the beavers from the Mordovian reserve was one very old animal, in the periosteal zone of which eighteen layers could be counted. The broad band of the first annual layer in this case was fully resorbed, the resorption line in places closely approaching the first adhesion line, but the line itself had still not been resorbed and the second annual layer was not affected (fig. 20). We are referring here to the resorption rate only in the sector of jaw which was recognized to be the most useful one for age determination of beavers - the genal wall of the jaw beyond the end of the dental row. In other sectors the resorption rate was higher. Resorption evidently takes place most intensively in the walls of the rear incisor diastema. In one old beaver in which eighteen layers of periosteal zone were noted beyond the end of the dental row, only eight layers remained in the walls of the incisor diastem.

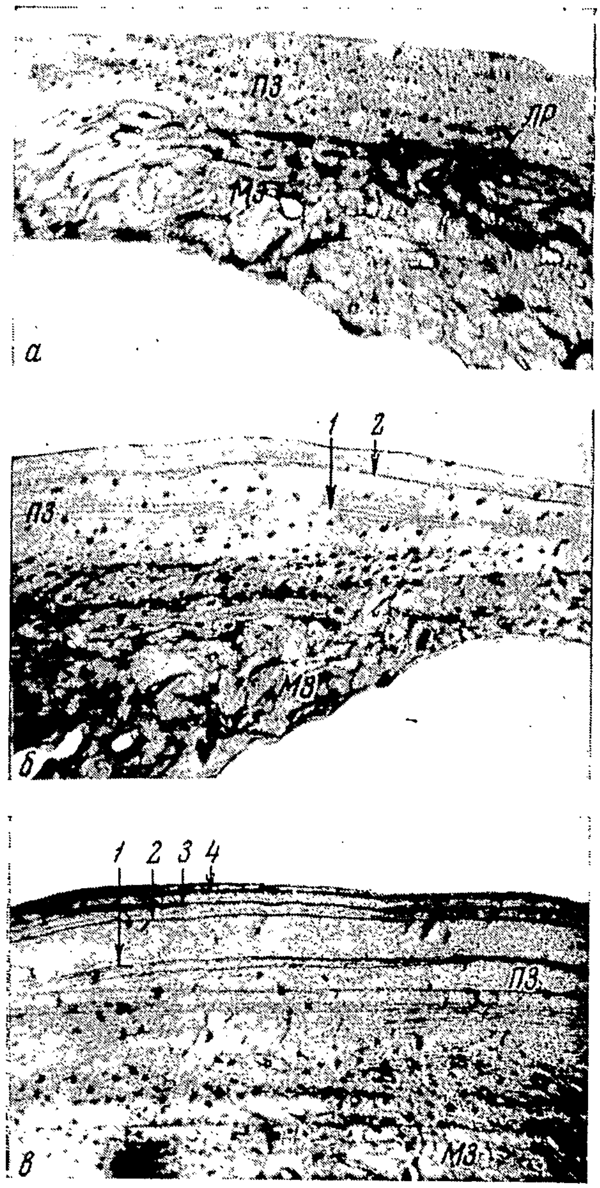


Fig. 17 [page 63]. Sectors of maxillary walls of (a) same year (b) two-year-old and (c) four-year-old beavers. Transverse sections. Staining - hematoxylin. (X18).

Mz - mesoteal zone; Pz - periosteal zone; LR - resorption line; arrows indicate adhesion lines; numerals denote annual layers.

Occasionally, in the annual layers of a periosteal zone, instead of one adhesion line, two lines form, located alongside one another /page 64/ (fig. 18), or even several contiguous lines (fig. 19). In isolated cases, in the middle of an annual layer a supplementary adhesion line was seen, which was less distinct than the main one (fig. 18, 7th annual layer).

The width of the annual layers diminishes with age. Usually the first annual layer is the widest one, the second is much narrower, and the third is narrower still (fig. 17c; 20). Beginning from the third, the subsequent 5 to 6 annual layers are of almost equal width, whereupon the width of the layers again sharply diminishes and becomes more or less constant (fig. 20). Sometimes the width of the second and third annual layers are equal, with reduction occurring from the 4th layer (fig. 18).

Annual layers in the cementum of beavers were simultaneously described for European and Canadian beavers (Klevezal' and Kleinenberg, 1964; Van Nostrand and Stephenson, 1964). Later, the method of age determination from the number of layers in the cementum was tested on a large series of beavers by V.G. Safonov (1966).

The molars of beavers become heavily worn with age and parallel to the wearing down of the crown a heavy formation of cementum occurs on the root. In very old beavers cementum comprises a large part of a molar (fig. 21).

From material on beavers, the ages of which were determined from the number of annual layers in the periosteal zone of bone (the age of one specimen was accurately known) it was established (Klevezal' and Kleinenberg, 1964; Klevezal', 1966; Kleinenberg and Klevezal', 1966), that in the cementum of all genal teeth, distinct annual layers form which are readily noted in a longitudinal half-slide of a tooth in reflected light (fig. 22). The layers were more easily seen if the section passed through the middle of the tooth perpendicular to the dentine - enamel loops.

In yearlings and two-year-old beavers cementum is deposited only along the lower edge of a tooth, at the base of which is the cavity which begins to fill up with secondary dentine. In three-year-old beavers, only a narrow channel remains of the pulp chamber and cementum begins to be deposited along the entire lower surface of the tooth. In yearlings and two-year-old beavers the layering of the cementum was seen indistinctly, the narrow dark bands being distinguished only where the light fell at a specific angle of incidence. The third annual layer, running along the entire lower surface of the tooth, was seen much better. Beginning with the fourth year annual layers of cementum, consisting of alternating wider light and narrower dark bands, become especially clear; they are deposited on the lower surface of the tooth almost perpendicular to its longitudinal axis. The pulp cavity is completely filled with secondary dentine.

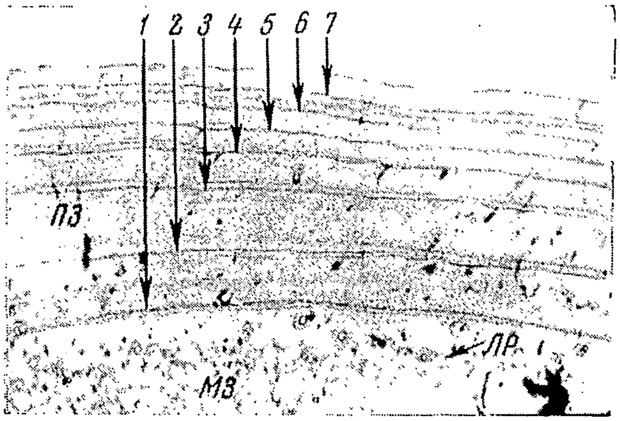


Fig. 18 [page 65]. Sector of lower maxillary wall of adult beaver. Transverse section. Staining - hematoxylin (X18). For explanation see fig. 17. The first and fourth adhesion lines are double ones. Between the sixth and seventh lines a supplementary adhesion line is visible.

Fig. 19 [page 65]. Sector of lower maxillary wall of adult beaver. Transverse section. Staining - hematoxylin (X18). The third annual layer is bounded by a group of contiguous adhesion lines (indicated by arrow).

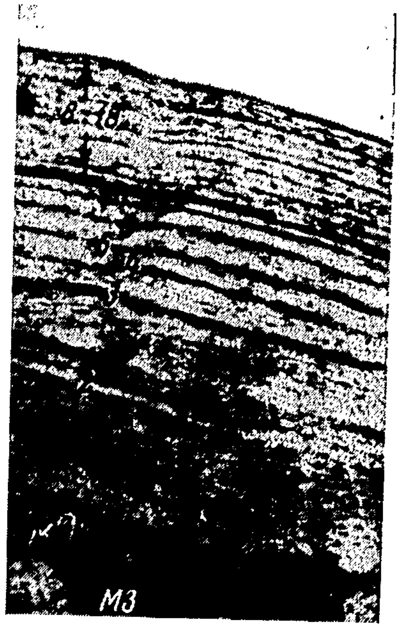


Fig. 20. /page 66/. Sector of lower maxillary wall of adult beaver. Transverse section - Staining - hematoxylin. (X60). For explanation see fig. 17.

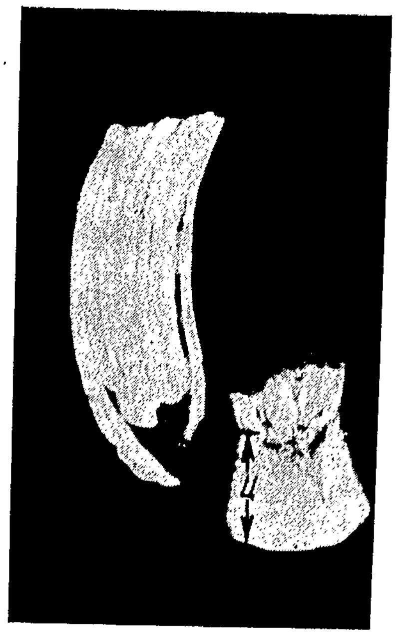


Fig. 21 /page 66/. First lower pre-molars of same year beaver (left) and old beaver (right). Longitudinal microslides. Reflected light. T_s - cementum.

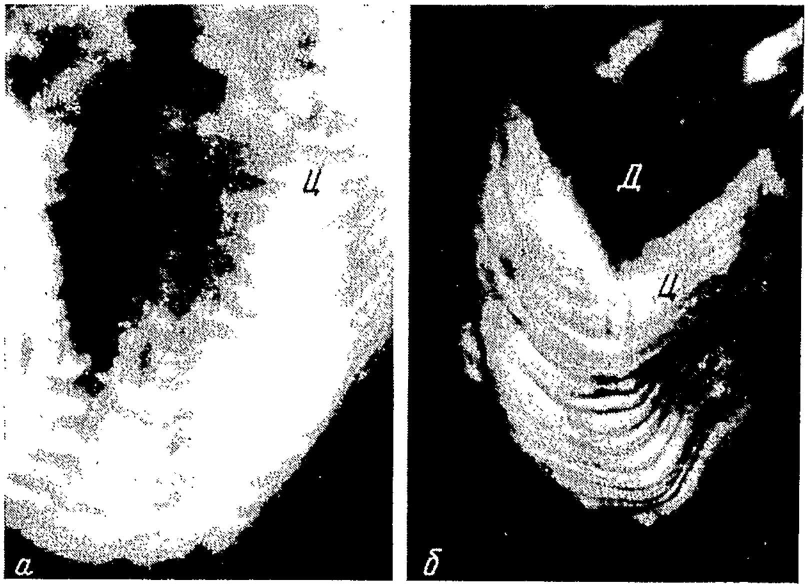


Fig. 22 /page 67/. Lower part of (a) first lower pre-molar and (b) third lower molar of old beaver. Longitudinal microslides. Reflected light. (X8). D - dentine, T_s - cementum.

The number of layers of cementum in all jaw teeth is identical but in the pre-molars the first annual layer is even more narrow than in the molars. This can be explained by the fact that the permanent pre-molar erupts last. The width of the annual layers of cementum is least of all during the first three years of life of an individual and then increases and /page 67/ remains almost constant. No marked reduction in the width of the layers with age was noted even in the cementum of an 18-year-old beaver (fig. 22).

Within the separate bands of an annual layer additional small streaks are often seen. Within a narrow dark band can be seen a still narrower light streak (fig. 22a), and within a broad light one - many narrow light and dark streaks (fig. 23).

Based on a study of 42 beavers of known ages, van Nostrand and Stephenson (1964) reached similar conclusions to ours, that "cementum is deposited in the form of annual layers throughout the lifetime of the animal". They did not find annual layers of cementum pertaining to the first and second years of life of an individual and considered the layer of cementum deposited along the entire lower surface of a tooth during the third year of life of a beaver to be the first annual layer. Moreover, a narrower band formed in winter is according to their data lighter (in reflected light) than a broad summer band. As a result of personal correspondence with van Nostrand it turned out that both we and the Canadian investigators have been calling identical bands of an annual layer of cementum "winter" and "summer" ones. In a letter of 22 October 1965, van Nostrand writes that after studying a large quantity of material he now considers that "each annual layer of cementum consists of a relatively broad summer layer and a narrower winter one, which can appear [page 68] either lighter or darker than the summer one". He also writes that in the course of determining the ages of a large number of beavers they discovered layers of cementum on the side walls of roots of teeth in beavers aged above 1½ years.

As we have already said, the annual layers of cementum of the first two years of life are hardly visible. It would probably be better for determining the ages of large numbers of beavers to adopt the procedure followed by the Canadian investigators: to begin counting the annual layers from the first distinct layer and add the deficient number of years. It is only necessary to take into account that the time of formation of the first layer of cementum occupying the entire lower part of a tooth can vary somewhat in beavers from different localities. (A more detailed discussion of the differences in the beginning of the formation of cementum in animals from various places is given at the end of the section "Rodents").

Field Mouse Apodemus agrarius and Grey Rat Rattus norvegicus

All representatives of the sub-family of mice have molars limited in size and deposition of cementum. According to Schour and Massler (1949), deposition of dentine in the rat occurs along the entire surface of the pulp cavity only during the first 125 days of life, after which the tooth crown wears down and only secondary dentine forms in sectors of the pulp cavity entering the crown (fig. 24). As the formation of secondary dentine in these sectors is a reaction to the tooth wear, i.e. to a heavy mechanical load, the normal periodicity in the growth of dentine is evidently disturbed here, in connection with which there are numerous supplementary bands. We therefore did not conduct studies on the existence of annual layers in the secondary dentine of rats and mice, the more so since distinct annual layers were found in the cementum and periosteal zone of bone (Klevezal' and Lavrova, 1966).

At our disposal were the lower jaws of 80 specimens of field mice and 63 specimens of grey rats, caught between June and September in the Krasnodar territory. The exact ages of the animals were not known. Based on the degree of wear of their teeth (Varshavskiy and Krylova, 1948; Varshavskiy, 1950), M.Ya. Lavrova had separated a large number of field mice and grey rats into six and five age groups respectively. The field mice included the specimens studied by us. It was considered that the field mice in age groups 5 and 6 and the grey rats in age groups 4 and 5 had clearly over-wintered, whereas the mice in group 4 and rats in group 3 were doubtful in this regard.

Fig. 23 [page 68]. Lower part of third molar of adult beaver (photographed by V.G. Safona). Longitudinal microslide. Reflected light. (X13). Streaks between annual layers indicated by arrows.

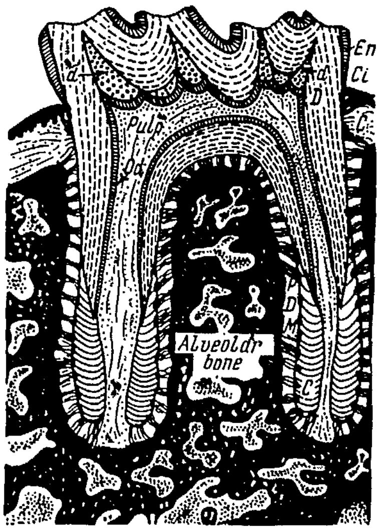


Fig. 24 [page 69]. First lower molar of white rat aged 500 days. Longitudinal section (according to Schour and Massler, 1949). Od - odontoblasts; D - dentine; d - secondary dentine; En - enamel; C - cementum; PDM - periodontal membrane.

Besides these, we had the lower jaws of three field mice of precisely known ages which had lived under laboratory conditions (material presented by N.V.Bashenina). The study was carried out on hematoxylin-stained sections.

The most undisturbed layers of periosteal bone were discovered in the sectors of the jaw not connected with the incisor channel: in field mice - on the lower edge of the jaw, at the base of the mandibular ramus immediately beyond the dental row or on the level of the last molar (fig. 8b, sector 3-4), and in grey rats - on the lower edge of the angular process (fig. 8b, sector 5).

In field mice and grey rats a mesosteal zone and resorption line are not always expressed. In grey rats a differentiated bone is encountered more frequently than in field mice. In field mice all the walls of the jaw are normally represented by lamellate bone, the inner parts of which are penetrated by blood vessels.

In the animals placed in the non-wintered group the bone was either friable (in individuals of the first age group) (fig. 25a; 26a) or already dense, but there was not a single adhesion line in it (fig. 25b; 26b). The majority of the animals placed in the wintered group had an adhesion line in the bone, separating an outer layer of bone tissue (fig. 25c; 26c-e). Three field mice which were caught when young and spent a winter in captivity each had one adhesion line in the bone /page 70/.

Table V. /page 70/. Number of adhesion lines in bones of white mice and grey rats of various age groups. /not annual groups - Ed./

Field mouse

Number of adhesion lines	Age groups						Totals
	non-wintered			doubtful	wintered		
	I	II	III	IV	V	VI	
0	5	5	10	18	4	-	42
1	-	-	-	1	14	14	29
2	-	-	-	-	6	4	10
Total number of specimens	5	5	10	19	24	18	81

Grey rat

Number of adhesion lines	Age groups					Totals
	non-wintered		doubtful	wintered		
	I	II	III	IV	V	
0	23	15	3	-	-	41
1	-	-	6	3	-	9
2	-	-	2	7	-	9
3	-	-	1	3	-	4
4	-	-	-	-	1	1
Total number of specimens	23	15	12	13	1	64

The relative proportions of animals having various numbers of adhesion lines in the bone are shown in Table V.

In the rats, the proportion of animals belonging to the various age groups appeared normal for a random sample: the majority of the individuals had not wintered at all (41 specimens), 9 had wintered once, 9 had survived two winters, 4 specimens - three winters and only 1 rat, which was caught in the settlement, had survived 4 winters.

Of the field mice, only a small numer of specimens were taken from the first three age groups as we were chiefly interested in the over-wintered individuals. There were six specimens from the fifth age group and four from the sixth age group having two adhesion lines. We are not prepared to say /page 73/ that these individuals had survived two winters although we do not exclude such a possibility. Judging from the fact that the layer of bone separated by the second adhesion line is narrower than the second annual layer in the other individuals and that the second line itself is less clear than the first (fig. 27), this line could be a supplementary one (the structure of the cementum of these individuals is also indicative of this: in the cementum of some of them a second narrow, heavily stained band was also seen, which was much less clear and bright than the first; in others, a second narrow band was completely lacking).

Age changes of the same type were observed in the genal wall of the upper half of the mandibular ramus. Thus, in grey rats the number of adhesion lines in this sector and in the angular process usually coincided. But in the wall of the mandibular ramus the adhesion lines are frequently doubled, and the layers of periosteal bone are not parallel, which hinders the counting of them.

In sectors of the mandible walls adjoining the incisor channel numerous supplementary adhesion lines are to be seen. On the lower edge of the jaw directly adjoining the incisor channel, below the first molar in some of the /page 74/ field mice of this third group which were caught in the summer there is one adhesion line, and in animals of the fourth group - 1 to 2 lines, whereas in the same individuals, on the lower edge of the jaw not adjacent to the channel, there is not a single adhesion line beyond the edge of the dental row. In all animals of the fifth and sixth group having one adhesion line in the sector of jaw beyond the end of the dental row, no less than 2 and frequently 4-5 lines were seen in the wall of the incisor channel (fig. 28). These lines are less broad and less heavily stained by the hematoxylin than the adhesion line in the wall beyond the end of the dental row.

/page 77/ Annual layers exist in the cementum of field mice and grey rats, but in field mice they are much more clearly expressed (Klevezal' and Lavrova, 1966). In field mice of the first age group the pulp cavity in the lower part of the roots of molars is still wide open and cementum is just beginning to be deposited (fig. 29a).

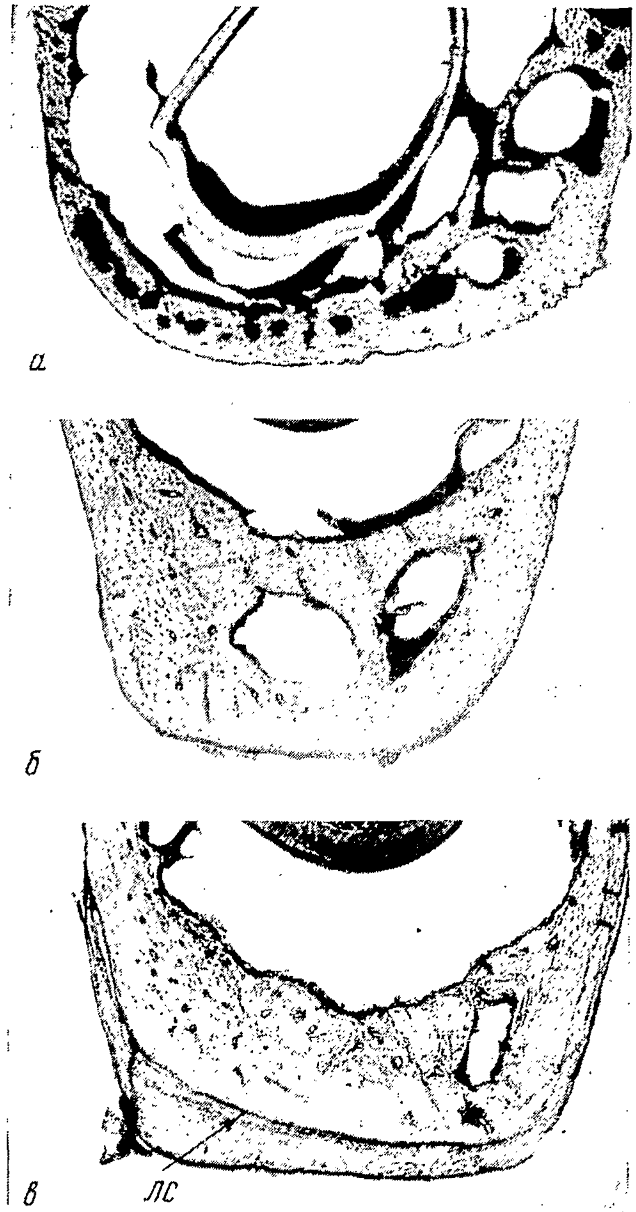


Fig. 25 /page 71/. Lower edge of body of mandible in field mice of age groups 1 (a) 4 (b) and 5 (c). Transverse sections. Staining - hematoxylin. (X60). Ls - adhesion line.

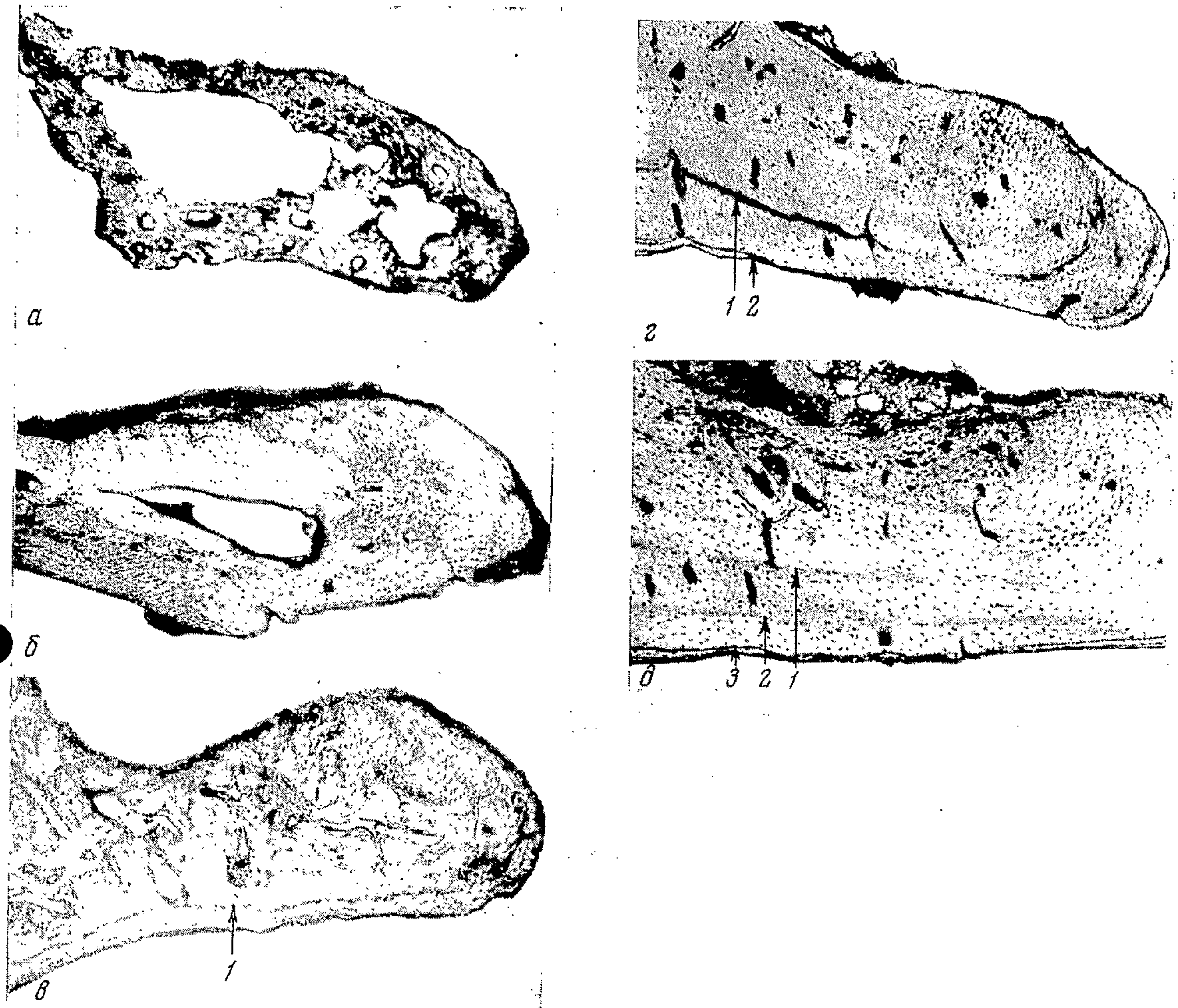


Fig. 26 [pages 72-73]. Angular processes of lower jaws in grey rats of age groups 1 (a), 2 (b), 3 (c,d) and 4 (e). Transverse sections. Staining - hematoxylin. (X60). Adhesion lines indicated by arrows. Numerals denote annual layers.

Fig. 27 [page 74]. Lower edge of body of lower jaw in field mice of age group 6. Transverse section. Staining - hematoxylin. (X60). Ls - main adhesion line; DLS - supplementary adhesion line.

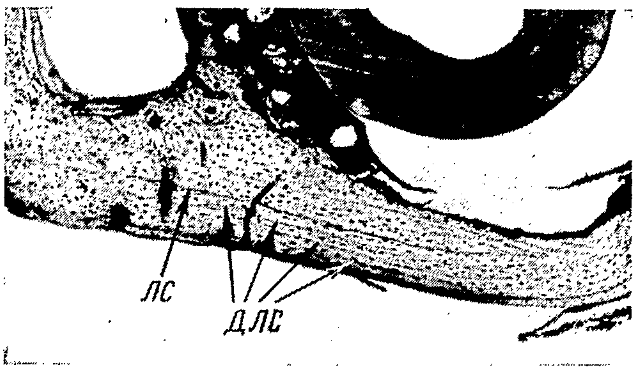


Fig. 28 [page 74]. Lower edge of body of lower jaw below incisor channel in field mice of age group 5. Staining hematoxylin. (X60) For explanation see fig. 27.

Fig. 29 [page 75]. Longitudinal sections of third molars in field mice of age groups 1 (a), 3 (b), 4 (c) and 5 (d). Staining - hematoxylin. (X60).

D - dentine; T_s - cementum; PP - pulp cavity; arrow indicates heavily stained band of cementum.

Fig. 30 [page 76]. Longitudinal section of third lower molar in field mice of age group 5. Staining - hematoxylin. (X60). DP supplementary streak. For remaining designations see fig. 29.

Fig. 31 [page 76]. Longitudinal sections of third lower molars in grey rats of age groups 3 (a) and 4 (b). Staining - hematoxylin. (X60). For explanation see fig. 29.

The width of the pulp cavity in the root part diminishes with age while the thickness of the cementum increases (fig. 29b). In adult animals belonging to the non-wintered group all of the cementum is uniformly lightly-stained by hematoxylin (fig. 29c). In the over-wintered ones a narrow heavily-stained band is seen in the cementum, dividing it into two layers (fig. 29d). The outer layer of cementum is either deposited only on the lower surface of the root or in addition, and to a lesser degree, on the side walls of the root. Occasionally, besides the main band, supplementary streaks are seen, which are not so brightly stained (fig. 30).

In the three field mice which spent the winter in the laboratory, one narrow heavily-stained band was seen in the cementum.

A complete correspondence was found between the existence of an adhesion line in bone and a narrow, heavily-stained band in the cementum of one and the same individuals. Thus, of the animals belonging to the fourth age group only one specimen had an adhesion line in the periosteal zone of bone and it alone of the fourth group had a narrow heavily-stained band in the cementum. Of the individuals in the fifth group, four of them did not have a single adhesion line in the bone, while three of them did not have a single heavily-stained band in the cementum (we did not succeed in examining the cementum of the fourth specimen as the teeth had been lost during cutting).

In the grey rats as well as the mice, layers were seen in the cementum (fig. 31) and the number of narrow, heavily hematoxylin-stained bands in the cementum coincided in the same individuals with the number of adhesion lines in the angular extension of the lower jaw. In old rats the later layers of cementum are hard to distinguish since, beginning from the third layer, they become very narrow.

Common hamster Cricetus cricetus

In hamsters, annual layers exist in the periosteal zone of bone and dental cementum (Klevezal', 1966). (For the same reasons as in the case of mice and rats the dentine was not studied).

Studies were made of the lower jaws (by means of hematoxylin-stained sections) of ten specimens caught between May and July in the Altai foothills, the ages of which were known as a result of marking, and thirteen specimens caught in May which on the basis of body dimensions, craniological features and degree of wear of the molars were placed in the adult group (material presented by Ye. V. Karaseva).

In the lower jaws of the adult individuals a clear mesosteal zone was not noted, neither was a resorption line seen. The most [page 78] regular annual layers of periosteal bone exist in the lower wall of the jaw immediately behind the end of the dental row.

In animals born the same year and caught in June the bone was still friable. In the over-wintered individuals the bone was dense, and in the outer parts of the maxillary wall adhesion lines were seen, separating the layers of bone tissue. In marked individuals the number of adhesion lines corresponded as a rule to the assumed age in years. The maximum number of annual layers - four - was found in a male which, according to the data on marking, was not less than 3 years old. The majority of the hamsters belonging to the adult group had two annual layers (fig. 32) and only four specimens had three annual layers.

Annual layers in the bones of hamsters are normally regular, and only very rarely are the adhesion lines double. We did not see any supplementary lines. The width of the second annual layer (between the first and second adhesion lines) is greater than the width of the subsequent ones. No traces were noted of resorption of an inner adhesion line in old individuals.

The cementum on the roots of molars is thin even in adult animals. In the juveniles the molars do not yet have any cementum. Unfortunately, we were unable to study the teeth of one-year-old animals. In the cementum of the two-year-old animals could be seen a narrow, heavily hematoxylin-stained band, separating a new layer of lightly-stained cementum. A comparison of the number of layers of cementum with the number of layers of periosteal zone of bone indicated that in all cases there was one layer less in the cementum than in the bone. It is possible that in hamsters, no cementum forms on the roots of teeth during the first year of life and that the deposition of cementum begins during the second year.

In hamsters having three layers of periosteal zone and two layers of cementum, the second layer of cementum is very narrow and the faintly-stained band of the following third layer is so narrow that the second narrow heavily-stained band almost merges with the outer edge of the cementum (fig.33). Annual layers in the cementum of hamsters are therefore unsuitable for counting and for age determination from the number of them.

Common vole _Microtus arvalis_ and Pine vole _Microtus majori_

In grey voles, both the incisors and molars are constantly growing in length. Therefore, in the course of research on their annual layers only the periosteal zone of bone was studied (Klevezal' and Lavrova, 1966).

Studies were made (on hematoxylin-stained sections) of the lower jaws of 63 common voles and 53 pine voles from the Krasnodar region. The material consisted of samples of a large series of voles belonging to different age groups, the approximate ages of which were determined by M. Ya. Lavrova [page 80] on the basis of external skull structure (Karaseva, 1955; Lavrova et al., 1960). The common voles were broken down into five and the pine voles into three age groups. It was assumed that the common voles of the fifth and possibly the fourth groups and the pine voles of the third group were over-wintered ones and that the animals of the remaining groups were non-wintered. The ages of three of the common voles and one pine vole were known as a result of marking.

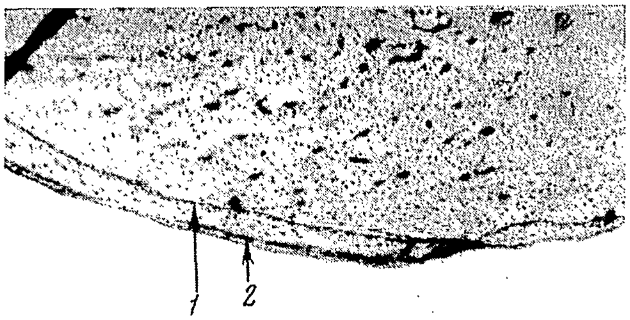


Fig. 32 /page 79/. Lower edge of body of lower jaw of common hamster belonging to the adult age group. Staining - hematoxylin. (X60). For explanation see Fig. 26.

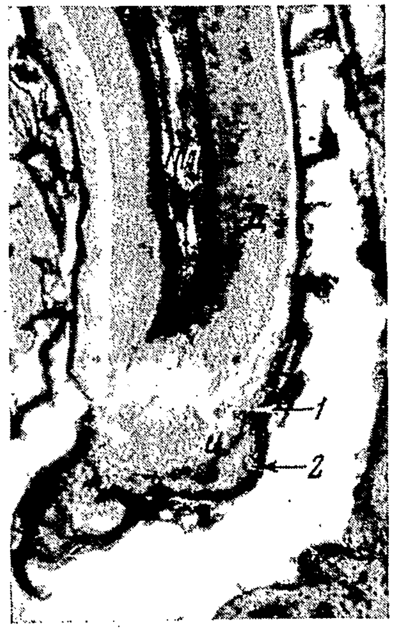


Fig. 33 /page 79/. Longitudinal section of third lower molar of common hamster belonging to the adult age group. Staining - hematoxylin. (X60). For explanation see Fig. 29.

A study of various sectors of the jaws of voles of precisely known ages indicated that for determining the ages of voles the most suitable structure of the periosteal zone of bone is on the lower edge of the jaw, in the vicinity of the last molar and in the genal wall of the mandibular ramus (on its lower part which is closer to the dental row). The jaw is transversely sectioned at the level of the last molar in such a way that the lower wall of the jaw and the upper part of the mandibular ramus also appear in the section (see fig. 8b, 3-4).

In the bones of voles there was no sign of a mesosteal zone or resorption line. In voles of the first age group the bone was friable. As age increases the bone becomes dense, but in non-wintered individuals showed no clear adhesion line (fig. 34a, b; 35a, b). Admittedly, in the sector of jaw under the incisor channel, in its middle part, there was occasionally to be seen an indistinct line, with one end normally extending to the incisor channel. In the over-wintered voles, besides such an indistinct line in the middle of the wall, closer to the outer edge of the wall there is a distinct adhesion line (sometimes several contiguous lines) separating the new layer of periosteal bone (fig. 34c,d, 35c).

The existence of adhesion lines in the bones of voles of various age groups is indicated in Table VI. Common voles did not have more than one such line (not counting the contiguous lines which were regarded as one). Three of the pine voles had two adhesion lines separating two layers of bone tissue (fig. 35d). One of them was marked as an adult in 1958 and caught in 1959. Possibly these voles had lived two winters (Klevezal' and Lavrova, 1966).

Muskrat Ondatra zibethica

In muskrats, annual layers are formed in the periosteal zone of bone and the dental cementum (the dentine was not studied) (Klevezal', 1966).

Studies were made (on hematoxylin-stained sections) of the lower jaws and in some cases the bones of the extremities of 25 animals caught in Mordovia and on Lake Balkhash in December, April - May and September (material received from S.V. Marakov, B.L. Larin and M.Ya. Lavrova). The ages of the animals were approximately determined from the stage of development of the roots and height of the crowns of the teeth. (Tsygankov, 1955).

For age determination from the bone it was convenient to study the same sectors of jaw as in the case of the grey voles. The mesosteal [page 84] zone and resorption line were well expressed. The annual layers were normally divided by as much as two or even a group of adhesion lines (fig. 36c, d), which greatly hampers the counting of the layers during age determination. Frequently, all of the layers were more clearly defined in the mandibular ramus than on the lower edge of the jaw. Similar annual layers, as indicated by a comparison of five specimens, also exist in the femur and tibia bones.

separating the layers of bone tissue. In marked individuals the number of adhesion lines corresponded as a rule to the assumed age in years. The maximum number of annual layers - four - was found in a male which, according to the data on marking, was not less than 3 years old. The majority of the hamsters belonging to the adult group had two annual layers (fig. 32) and only four specimens had three annual layers.

Annual layers in the bones of hamsters are normally regular, and only very rarely are the adhesion lines double. We did not see any supplementary lines. The width of the second annual layer (between the first and second adhesion lines) is greater than the width of the subsequent ones. No traces were noted of resorption of an inner adhesion line in old individuals.

The cementum on the roots of molars is thin even in adult animals. In the juveniles the molars do not yet have any cementum. Unfortunately, we were unable to study the teeth of one-year-old animals. In the cementum of the two-year-old animals could be seen a narrow, heavily hematoxylin-stained band, separating a new layer of lightly-stained cementum. A comparison of the number of layers of cementum with the number of layers of periosteal zone of bone indicated that in all cases there was one layer less in the cementum than in the bone. It is possible that in hamsters, no cementum forms on the roots of teeth during the first year of life and that the deposition of cementum begins during the second year.

In hamsters having three layers of periosteal zone and two layers of cementum, the second layer of cementum is very narrow and the faintly-stained band of the following third layer is so narrow that the second narrow heavily-stained band almost merges with the outer edge of the cementum (fig.33). Annual layers in the cementum of hamsters are therefore unsuitable for counting and for age determination from the number of them.

Common vole Microtus arvalis and Pine vole Microtus majori

In grey voles, both the incisors and molars are constantly growing in length. Therefore, in the course of research on their annual layers only the periosteal zone of bone was studied (Klevezal' and Lavrova, 1966).

Studies were made (on hematoxylin-stained sections) of the lower jaws of 63 common voles and 53 pine voles from the Krasnodar region. The material consisted of samples of a large series of voles belonging to different age groups, the approximate ages of which were determined by M. Ya. Lavrova [page 80] on the basis of external skull structure (Karaseva, 1955; Lavrova et al., 1960). The common voles were broken down into five and the pine voles into three age groups. It was assumed that the common voles of the fifth and possibly the fourth groups and the pine voles of the third group were over-wintered ones and that the animals of the remaining groups were non-wintered. The ages of three of the common voles and one pine vole were known as a result of marking.

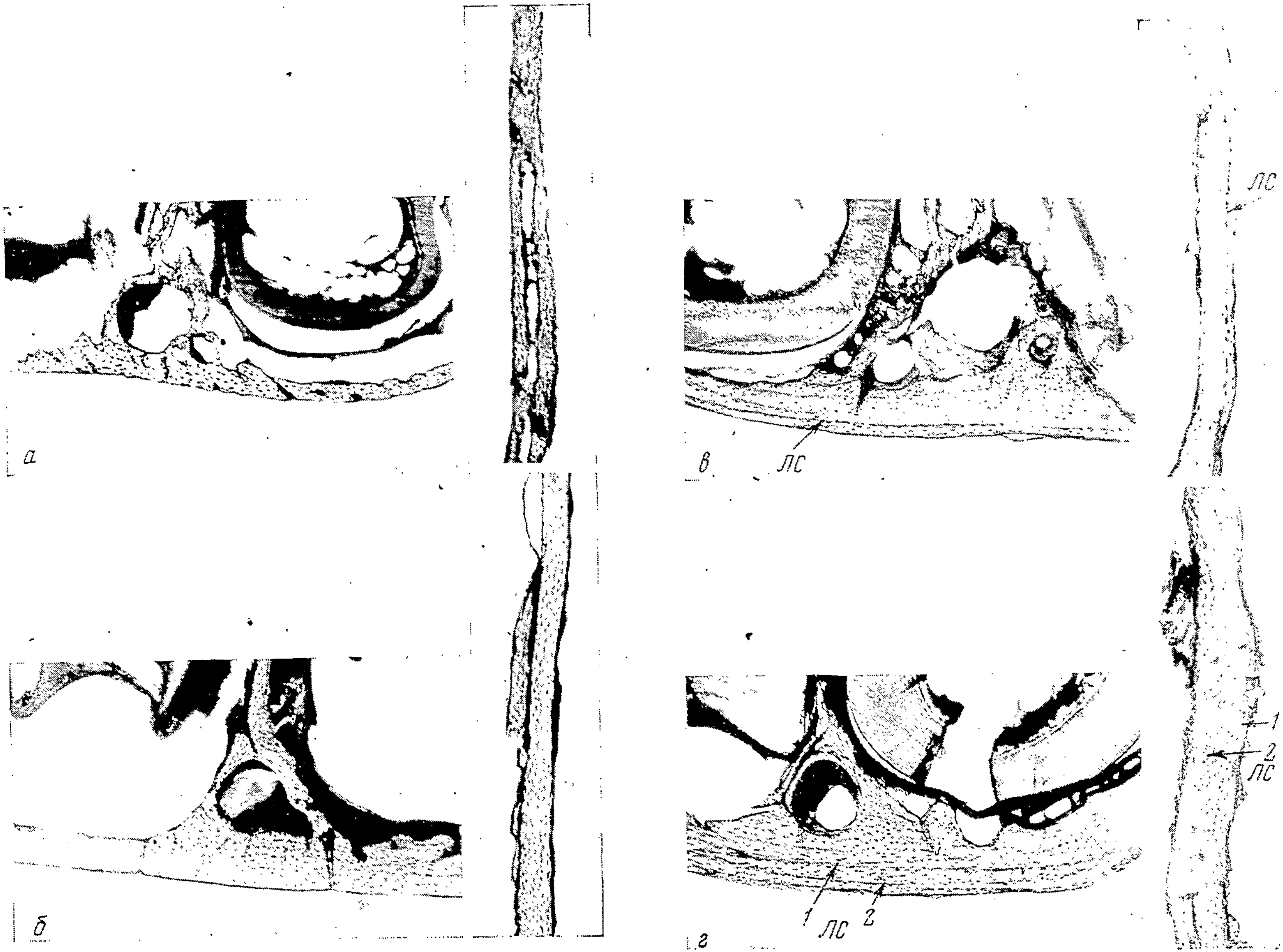


Fig. 35 [pages 82-83]. Lower edge of body of lower jaw (left) and maxillary branches (right) in pine voles of age groups 1(a), 2(b) and 3(c) and a vole, ringed as an adult and caught in the following year (d). Transverse sections. Staining - hematoxylin. (X60).

Table VI. /page 84/. Number of adhesion lines in bones of common and pine voles of different age groups. /not annual groups - Ed./

Common vole

Number of adhesion lines	Age groups				
	Non-wintered		doubtful	wintered	Total
	I + II	III	IV	V	
0	15	15	9	4	43
1	-	-	8	12	20
Total number of specimens	15	15	17	16	63

Pine vole

Number of adhesion lines	Age groups			
	non-wintered		wintered	Total
	I	II	III	
0	6	30	-	36
1	-	4	10	14
2	-	-	3	3
Total number of specimens	6	34	13	53

The fact that the layers in bone and cementum are annual ones was established both by comparison with the approximate age of the individual and by comparing the tissue structure in animals caught during different seasons (Klevezal', 1966). Thus, in an individual born the same year and caught in the autumn there was no adhesion line in the bone (fig. 36a); in a one-year-old animal caught in the autumn one adhesion line was seen far from the outer edge of the bone (fig. 36b); and in a one-year-old animal caught in December /page 86/ one adhesion line was again seen near the outer edge of the bone (fig.36c). /In fig. 36c two adhesion lines are marked and the animal is described as adult - Ed./ In an adult muskrat caught in December two double adhesion lines were seen separating two layers of bone and a third line near the outer edge of the bone (fig. 36d).

The thickness of the cementum on the roots of the teeth of muskrats is extremely small even in old individuals. The annual layers are narrow and difficult to distinguish. They are best seen not on the lower but on the side walls of a root and in the inter-root cementum cushions (fig. 37).

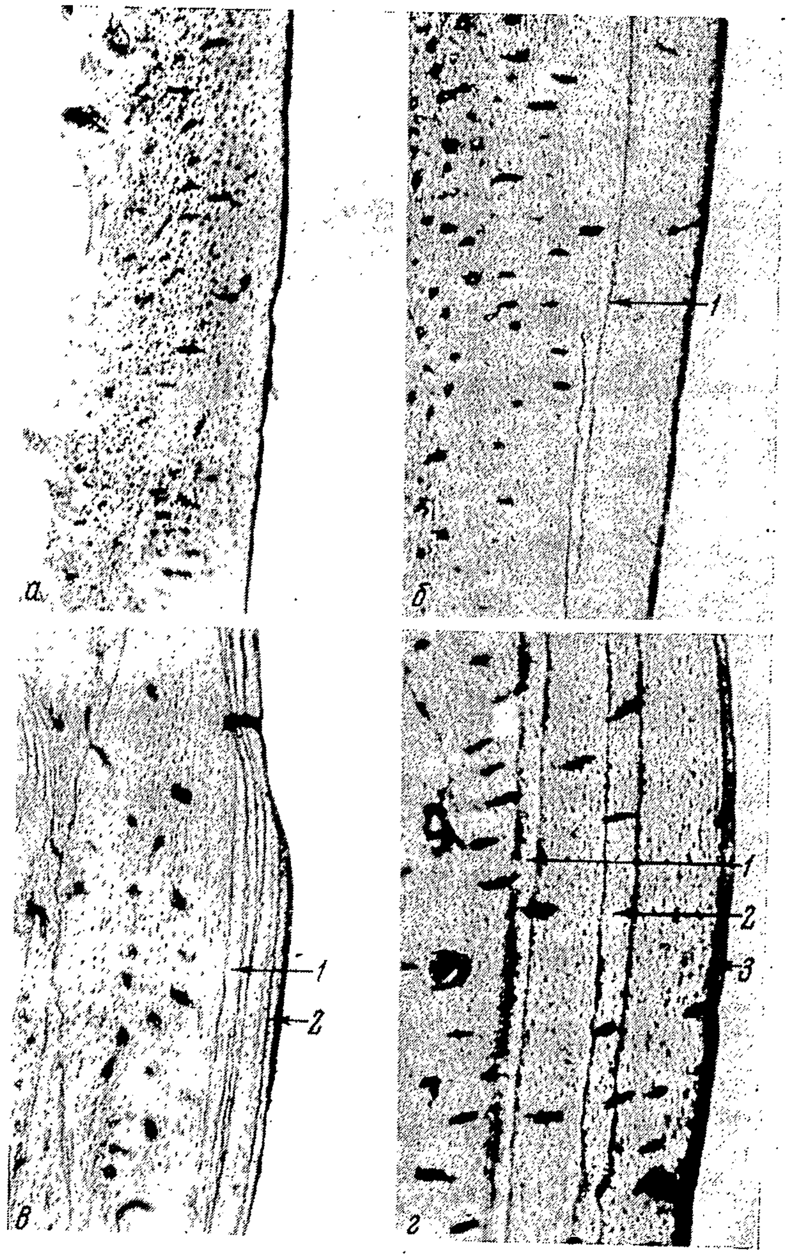


Fig. 36 [page 85]. Genal walls of maxillary branches of lower jaws in muskrats: (a) born the same year specimen; (b) one-year-old, caught in September; (c) one-year-old and (d) adult muskrat, caught in December. Transverse sections. Staining - hematoxylin. (X60). Arrows indicate adhesion lines (or groups of lines) delimiting the layers. Annual layers denoted by numerals.

Fig. 37 [page 86]. Longitudinal section of inter-root cushion of first lower molar of adult muskrat. Staining - hematoxylin. (X60).
D - dentine; Ts - cementum; P - periodontum; K - alveolar bone. Arrow indicates heavily-stained band in the cementum.

As was the case with the common hamster, in muskrats the number of annual layers in the cementum is usually one less than the number in the periosteal zone of bone, i.e. there is no formation of cementum during the first year of life. Only in two cases was the number of layers in the cementum equal to the number of layers in the bone. Possibly the muskrats were born in the spring and by the time of their first winter the cementum had already begun to form (a more detailed discussion of the problem of differences in the formation of annual layers in animals of one year of age, but from pre-spring and post-autumn litters will be found at the end of the section on rodents).

In general, it can be said that annual layers in the bone and cementum of muskrats are so difficult to count that it was evidently inadvisable to use them for determining the ages of individuals, all the more so since a sufficiently accurate method is available for determining the ages of individuals of this species from the height of the crown of a tooth and stage of development of the roots (Tsygankov, 1955; Smirnov and Shvarts, 1959).

Water rat Arvicola terrestris

Studies were made of the lower jaws and bones of the limbs (on hematoxylin-stained sections) of five specimens from a random sample caught in September in Mordovia (material presented [page 87] by M.Ya. Lavrova). The bone structure was studied in the same sector of jaw which had been recognized as the most convenient for age determination in grey voles. Since the teeth of water rats show all continuous growth we did not study the dental structure.

In two of the specimens the bone was friable and in two it was dense, with one adhesion line separating a layer of bone tissue; one of them had two adhesion lines separating the layers of periosteal bone. The mesosteal zone and resorption line were not expressed.

The ages (number of winters lived) of water rats, as in the case of grey voles, can probably be determined from the number of adhesion lines dividing the layers of bone.

Tamarisk gerbil Meriones tamariscinus

Studies were made (on hematoxylin-stained sections) of the lower jaws of four specimens caught in spring in the Aral Sea region of the Kara Kum Desert (material presented by V.S. Lobachev). The ages of the animals were not known but as they were caught in the spring and were the size of adult animals it can be assumed that they had wintered at least once. In the lower jaw walls of three of them, beyond the end of the dental row was seen one adhesion line separating layers of bone tissue and situated close to the outer edge of the bone, and in one of them - two adhesion lines: the first - at a considerable distance from the outer edge of the bone and the second - not far from the edge (fig. 38).

Clear mesosteal zones and resorption lines were not noted.

In the cementum of molars of the first three gerbils there was seen one narrow heavily-stained band at the extreme outer edge of the cementum, and in the other gerbil - two such bands: the first - far from the edge and the second - on the edge itself. In connection with the fact that the roots of the teeth of tamarisk gerbils are crooked, the layers of cementum are meandering and not very clear. The dentine of the teeth was not studied.

Squirrel family Sciuridae

Up to the present time not a single study on the possibility of determining the ages of squirrels from the annual layers in the dentine, cementum and periosteal zone of bone, has been published based on a large amount of material.

M.N. Meyer (1957), in a paper on age variability of the lesser suslik Citellus pygmaeus, described layers in bone tissue in the region of the post-incisor diastema of the lower jaw and, based on material from animals of accurately known ages, demonstrated that the number of layers corresponds to the age of the animal in years.

Judging from a variety of circumstantial evidence, clear annual layers in the periosteal zone of the limb bones were discovered [page 88] in sections of the bones of individual specimens of squirrels, marmots and susliks of various species. (Klebanova and Klevezal', 1966).

Fig. 38 [page 88]. Lower edge of body of lower jaw of adult Meriones tamariscinus. Transverse section. Staining - hematoxylin. (X60). For explanation see fig. 36.

We studied an adult female thin-toed suslik Spermophilopsis leptodactylus caught in April in the Kara Kum. In hematoxylin-stained sections two adhesion lines, separating two layers of bone tissue, were seen beyond the end of the dental row. Two similar lines separating layers were seen in sections of the middle of the diaphysis of humerus and femur. A mesosteal zone in the lower jaw was not clearly expressed. In the bones of the limbs, both the mesosteal zone and resorption line were clearly seen.

In addition, we also made a preliminary study of preparations of the teeth and bones of the lower jaws of a large number of grey marmots Marmota baibacina (the preparations consisting of sections of teeth and bone, stained by hematoxylin, were presented by a zoologist of the Kirgiz anti-plague station, A.A. Mikhaylyuta). Clear layers exist in grey marmots both in the periosteal zone of bone and in the dentine and dental cementum. The ages of the marmots were not known but by analogy with the other rodents previously studied we consider these layers to be annual ones.

The most suitable material for determining the ages of marmots from the structure of the lower jaw is evidently a transverse section of jaw taken immediately beyond the end of a dental row. Judging from preliminary studies the jaw structure of the grey marmots has one striking peculiarity which we did not detect in other rodents.

In the small rodents which we studied, as well as in the hamster, muskrat and beaver, the bone becomes dense during the first year of life [page 89]. In the first annual layer in the periosteal zone of beavers there are many blood vessels, but dense bone is already being laid down for the following year. In the grey marmot, not only is the bone not dense throughout the entire first year of life, but during the second year and in individual cases the third year as well, periosteal bone forms which has numerous lacunae and blood vessels. Layers of dense periosteal zone do not begin to form until the third year of life (fig. 39).

In connection with this, the first adhesion line, separating the first annual layer and formed evidently during the first winter, is situated in the case of young individuals (one-year-olds and two-year-olds) between layers of bone which is not dense (fig. 39b). In older individuals, in which the entire bone becomes dense, this line may be fully resorbed or separate sectors of it preserved. In adult individuals, sectors of the first adhesion line are seen in the inner third of the maxillary wall and on the outside of it is a broad line of bone tissue with numerous osteons. The adhesion line of the second winter separates this broad osteonized layer of bone (the second annual layer of which is already in essence the mesosteal zone) from the subsequent typical layers of periosteal zone of bone (fig. 39c). The mesosteal zone is thus well expressed but the resorption line in indistinct. Beginning with the fourth and fifth ones, the annual layers of the periosteal zone become very narrow and are not deposited along the entire surface of the maxillary wall.

The cementum grows somewhat more intensively on the lower surfaces of the roots of the teeth than on the side walls, but nevertheless all the annual layers are seen not only in a longitudinal section but also in a transverse section when it runs ar approximately the level of one third of the length of the root from the lower edge (for information on possible errors in age determination if the section runs too low, see the preceding chapter). In one-year-olds with one adhesion line in the bone, there was seen on the roots of teeth a narrow, homogeneous, weakly hematoxylin-stained layer of cementum. In old individuals the number of layers in the cementum was one less than in the bone (fig. 40). Evidently, the cementum begins to be deposited on the roots of the teeth only during the second year of life of the individual and the first, narrow, heavily-stained band separating the first annual layer of cementum denotes the second winter of the individual.

The dentine of the molars of the grey marmot is deposited along the entire surface of the pulp cavity during the first and second years of life. Later, formation of dentine occurs on the upper projections of the pulp cavity entering into the tubercles of the tooth-crown as well as on one side of the pulp cavity of the root part of the tooth, i.e. during the third year of life "secondary" dentine begins to be deposited (as determined by Schour and Massler, 1949). Annual layers also exist in the primary and secondary dentine. Since the secondary dentine of the tooth crown as well as on one side of the pulp cavity of the root part of the tooth, i.e. during the third year of life "secondary" dentine begins to be deposited (as determined by Schour and Massler, 1949). Annual layers also exist in the primary and secondary dentine. Since the secondary dentine of the tooth crown forms, as we said earlier (see the section "field mouse and [page 91] grey rat"), under the influence of an increased mechanical load, in these sectors numerous supplementary streaks can form in the dentine and it is therefore preferable not to use them for counting annual layers for purposes of age determination. In grey marmots it is convenient to count the annual layers in the dentine in transverse sections of a root, either through the middle of it or one third of the distance from its lower end. From these sections it will be seen that secondary dentine is deposited

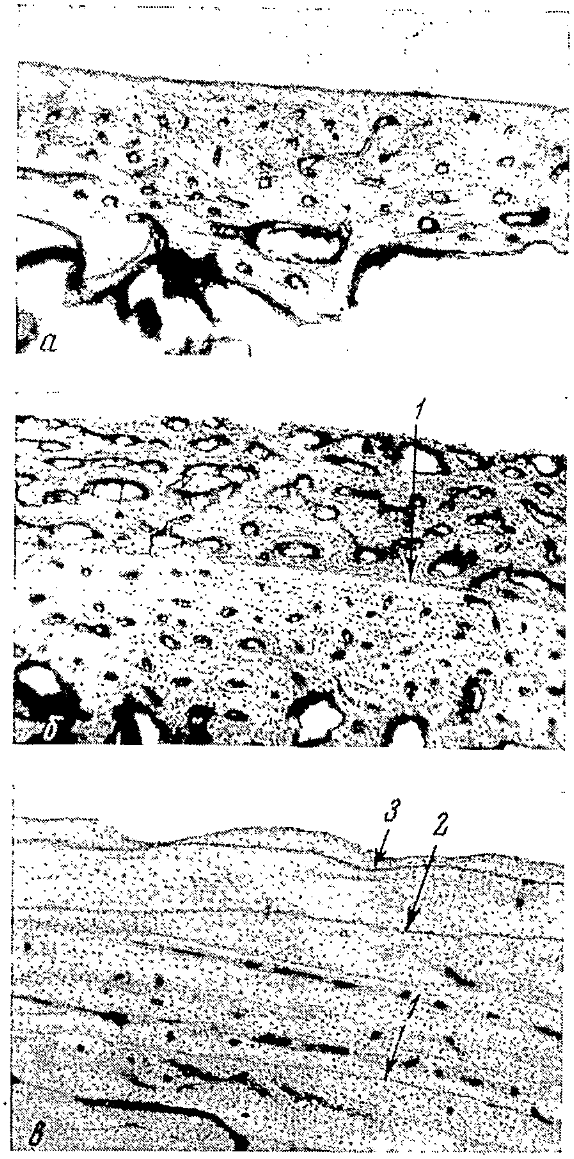


Fig. 39 [page 90]. Sectors of genal walls of lower jaws of grey marmots: (a) same year individual (b) yearling (c) one that has evidently over-wintered three times. Transverse sections. Staining - hematoxylin.

on one side of the pulp cavity of a root and has clear layers.

The narrow, heavily-stained band of the first winter delimiting the first annual layer of dentine runs in the primary dentine and is therefore seen in transverse section along the entire circumference of the tooth. It is not seen clearly in all individuals (fig. 41). The narrow band of the second winter, separating the second annual layer of primary dentine, is normally clearly seen in all individuals. It is the boundary of the primary and secondary dentine and is therefore seen only along part of the edge of the pulp cavity in the sector where secondary dentine is deposited, being followed by the formation of new layers of dentine. Sometimes, on the boundary with the secondary dentine, the narrow band of the second winter divides into two in a small sector. In the secondary dentine the annual layers are narrow and clear (fig. 41). It is better to begin counting annual layers in the dentine not from the first annual layer but the second one (from the boundary of the primary and secondary dentine) and to add one year.

Fig. 40 [page 91]. Sector of transverse section of root of third lower molar of adult grey marmot having 5 annual layers in the bone. Staining - hematoxylin. (X60). D - dentine; Ts - cementum; GDT's - boundary of dentine and cementum. Arrows indicate narrow heavily-stained bands of cementum.

Thus, the ages of grey marmots (and probably others as well) can be determined from the number of annual layers in the periosteal zone of bone and in the dentine or cementum of the teeth, but it is necessary to bear in mind: that the first adhesion line lies in the inner third [page 92] of the maxillary walls and may be fully resorbed, that the first annual layer of cementum begins to be formed during the second year of life of the individual, and that the first, narrow, heavily hematoxylin-stained band, delimiting the first annual layer of dentine, is not always seen clearly.

* * *

On the basis of the foregoing description of the annual layers in the tissues of teeth and bones of various species of rodents it is possible to conclude that the ages of all rodents can probably be determined from the number of annual layers or adhesion lines, denoting winters lived, in the periosteal zone of bone. In the majority of rodents the ages can also be determined from the number of layers in the cementum and in some of them - from the number of layers in the dentine of the molars.

Here, a very important question arises with respect to differences in the formation of annual layers in animals from early and late litters of the same year of birth.

Rodents are born with friable bones which subsequently become dense, and later, during the course of apposition of periosteal bone /page 93/ annual layers form in them. As the rate of growth of the bone during the winter period is slow, it is possible that individuals from the late autumn

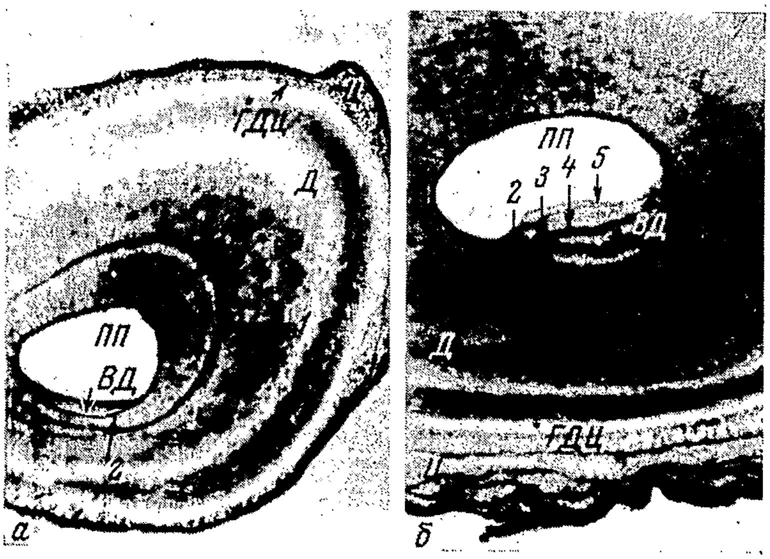


Fig. 41 /page 92/. Sectors of transverse sections of root of the third lower molars of grey marmots: with (a) two and (b) five annual layers in the bones. Staining - hematoxylin. (X60). D - dentine; VD - secondary dentine; TS - cementum; GDTs - boundary of dentine and cementum; PP - pulp cavity. Arrows indicate narrow heavily-stained bands of dentine. Annual layers denoted by numerals.

litters can winter having friable instead of dense bones.

From the example of the grey marmot we can see that the adhesion line pertaining to the first winter forms even in thin bone. It can be assumed that this also occurs in each of the other species when the individuals from the late litters winter without having dense bones.

It is quite another matter with the cementum. In those rodents in which cementum begins to be laid down on dental roots very early, individuals from early and late litters will differ not in the number of their annual layers but only in the width of the first annual layer. Thus, in field mice, cementum is already appearing on the roots of molars belonging to animals of the first age groups (aged 1 to 2 months), so that even in individuals born at the end of the breeding period a layer of cementum will manage to form before winter. In such cases, the width of the first annual layer of the cementum can be used for determining relative ages within the annual categories. Fig. 42 depicts a longitudinal section of the second [Figure legend states third - Ed.] molars of two mice which had over-wintered once and therefore had one narrow dark band in the cementum. In one of them (fig. 42a) [page 94] it is situated almost at the boundary of the dentine and cementum and the width of the band of the first year of life is very narrow. In the other (fig. 42b) this band is much wider. Evidently, this mouse was born earlier and during the first year of its life succeeded in laying down a great thickness of cementum.

Fig. 42 [page 93]. Longitudinal sections of third lower molars of field mice in 5th age group. Staining - hematoxylin. (X60). For designations see fig. 29. Explanation in text.

In rodents, on the dental roots of which cementum begins to be laid down comparatively late, sometimes even in the second year of life (for example, the hamster and muskrat), individuals from early and late litters may differ by one complete layer of cementum. If the cementum in the majority of the individuals begins to form during the first year of life, then in individuals from the later litters it cannot begin to be deposited until the following year, and thus there will be no line in their cementum denoting the first winter. If the cementum in the majority of the individuals is formed only during the second year of life, then in individuals from the earlier litters it can already begin to be laid down during the first year of life, and a line denoting the first winter, which is usually lacking in individuals of this species, will be formed. It is possible that the key to the recognition of such "extra" or "missing" layers can be the width of the first layer of cementum.

Similar differences can also be observed in individuals of those species which have one litter a year but in which the time of appearance of the young is protracted. For example, in the beavers of Byelorussia, the earliest appearance of the young was noted at the end of April and the latest appearance - at the end of July (Fomicheva, 1959). As stated earlier, the first annual layer of cementum in beavers is normally very narrow. It is possible that in the case of individuals born right at the end of the breeding period the cementum layer of the first year of life will not succeed in forming. It is also possible that in the case of individuals belonging to the northern populations, in connection with the later arrival of the young and the much shorter summer peiod, the first layer normally forms only during the second year of life of the individuals. It is accordingly possible that between different individuals from one population and individuals from different beaver populations there can also be differences in the times of appearance of the first continuous layer of cementum on the root of a tooth, which was the basis used by van Nostrand and Stephenson (1964) for counting the annual layers.

This entire question should be the subject of a special and detailed study. Meanwhile, when using cementum for age determinations of animals in which the breeding periods are protracted and the cementum layer of the first year is normally narrow, or in which the cementum appears only during the second year of life, it should be remembered that in individual cases it is possible to err by one year.

Order Cetacea

The possibilities of using the method of age determination from the annual layers in the tissues of teeth and bone are entirely different for representatives of the two sub-orders of whales.

Sub-order Odontoceti /page 95/

In toothed whales, annual layers exist in the dentine, cementum, and periosteal zone of bone (Nishiwaki et al, 1958, 1961; Sergeant, 1959; Berzin, 1961, 1964; Khuzin 1961, 1963; Kleinenberg and Klevezal', 1962; Ohsumi et al, 1963). Basically, the layers in the dentine are used for determining the ages of the animals. The teeth of the majority of toothed whales grow in length over a long period and the count is therefore made on longitudinal half-sections using reflected light or on thin sections using transient light.

The question as to how many layers of dentine form during a year in the teeth of toothed whales continues to be debated.

The periodicity in the deposition of dentine in the teeth of cetaceans was first studied in the teeth of a sperm whale Physeter catodon (Nishiwaki et al, 1958; Berzin, 1961). It was established that layers of uneven width and optical density form in proportion to the degree of deposition of dentine. The authors did not have material from animals of precisely known ages, but by comparing the number of layers in the dentine of a tooth with the length of a sperm whale and the number of corpora lutea in the ovaries they reached the conclusion that in the course of a year two wide, non-transparent bands form in the dentine, separated by narrow transparent bands, i.e. two layers. In a subsequent paper, the Japanese investigators (Ohsumi et al, 1963) took measurements of the growth of the last layer of dentine during various seasons of the year and made a count of the number of layers of dentine in the teeth of marked whales (the ages of the whales when they were marked were not precisely known). On the basis of the derived data they concluded that less than two layers, probably one, are formed annually in the dentine of the sperm whale. A.A. Berzin (1964) also discussed this question, taking into consideration the new opinion advanced by the Japanese investigators, and confirmed his previous conclusion that in the course of a year two layers form in the dentine.

Sergeant (1959), having animals of accurately known ages at his disposal, established that in the bottle-nosed dolphin Tursiops truncatus one layer of dentine forms during a year (one wide non-transparent and one narrow transparent band). He reached the same conclusion after studying the teeth of the pilot whale Globicephala melaena (Sergeant, 1959, 1962).

After examining a large series of teeth of the beluga Delphinapterus leucas, Sergeant (1959) proposed that in this species two layers of dentine form in the course of a year. At the same time R.Sh. Khuzin (1961) reached the conclusion that in the teeth of the beluga one layer of dentine forms annually.

We conducted a study of the teeth of Black Sea common dolphins Delphinus delphis (Kleinenberg and Klevezal', 1962). We made a count of the layers of dentine on longitudinal sections stained with hematoxylin (fig. 43), but inasmuch as the tooth of a saddleback dolphin ceases to lengthen early the layers can also be counted in [page 96] transverse sections. The ages of the animals (only females) were determined approximately from the number of scars left by corpora lutea of pregnancy in the ovaries (Sleptsov, 1940). After comparing the number of layers in the dentine with the assumed ages of the females we came to the conclusion that in the course of a year two layers are formed in the dentine of white-sided dolphins. This conclusion was also corroborated by comparing the number of layers in the dentine and the number of layers in the periosteal zone of the lower jaw in males and females. (Kleinenberg and Klevezal', 1962).

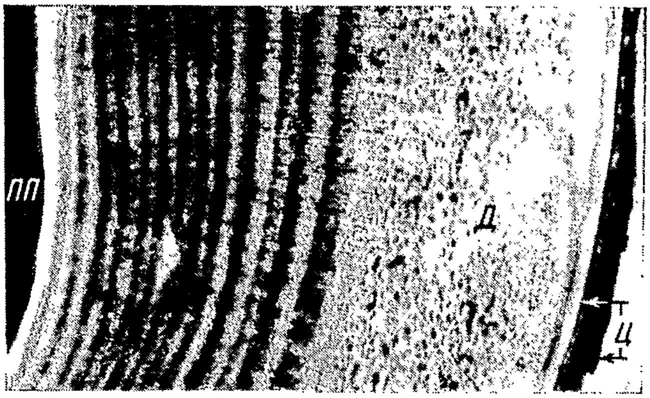


Fig. 43 [page 96]. Section of longitudinal section of tooth of saddleback dolphin. Staining - hematoxylin. (X60). D - dentine; Ts - cementum - PP - pulp cavity.

Thus, for the bottlenosed dolphin and pilot whale it was established that one layer of dentine forms annually (Sergeant, 1959, 1962) and for the common dolphin - two layers of dentine per year (Kleinenberg and Klevezal', 1962). Regarding the periodicity in the formation of layers of dentine in the sperm whale and beluga, the opinions of investigators differ. In the ensuing paragraph we will try to explain this phenomenon.

Within the wide non-transparent band of an annual layer of dentine in toothed whales, as well as in other mammals, there are supplementary streaks of varying widths and degree of clarity. Such streaks are regularly encountered in the dentine of the sperm whale (Ohsumi et al, 1963; Berzin, 1964). In the common dolphin we did not discover them, evidently on account of the small width of the annual layers themselves.

A complete layer is considered to comprise the wide, non-transparent and narrow, transparent bands, the small streaks within a non-transparent band being ignored. In the event that one of the transparent streaks within a wide, non-transparent one appears especially clearly it can be regarded either as the non-transparent band of an annual layer or assumed to be an inner streak and disregarded. It seems to us that here in particular lies the reason for the diverse opinions about the periodicity in the formation of layers of dentine in toothed whales.

[page 97] We were led to this line of reasoning by the fact that the Japanese investigators and A.A. Berzin, after making a serious detailed analysis of the periodicity in the formation of dentine in the same species (sperm whale) in approximately the same area (northern part of the Pacific Ocean), reached different conclusions. Berzin (1961, 1964) considers that two transparent and two non-transparent bands are formed in the dentine in the course of a year whereas the Japanese investigators (Nishiwaki, Ohsumi, Kasuya, 1961; Ohsumi, Kasuya, Nishiwaki, 1963) believe that one transparent band and one non-transparent band are formed annually.

After comparing the papers of these and other investigators we discovered the following. The Japanese investigators in their paper of 1963 produce photographs of sections of the teeth of the sperm whale in which the boundaries of the annual layers are denoted by white rectangles. In these photographs it will be seen that they delimit the annual layers so that within each non-transparent band of their annual layer there is one bright transparent streak. This streak is almost equal in brightness to the transparent band of an annual layer, on the basis of which the boundary between annual layers is drawn (see fig. 7b). If Berzin had likewise regarded this inner streak as the boundary of an annual layer, then with reference to the same material from which the Japanese authors have established the formation of one layer of dentine per year he could have pointed to the formation of two layers of dentine per year. We find a corroboration of this hypothesis in a paper by Berzin (1964) in which he discusses in detail the findings of Ohsumi, Kasuya and Nishiwaki (1963). He writes "In the illustration of the paper being considered, in separate instances the Japanese authors have unjustifiably identified groups of typical layers (italics ours - G.K. and S.K.) according to our methods of determination as one layer" (page 41). From this citation it is clear that Berzin regards as several complete layers what the Japanese investigators have determined to be one layer.

On the basis of the example discussed we consider in other cases too among cetacea that when the formation of two layers per year was established, there was a clear transparent streak within a wide band which was similarly taken to be the boundary of a second layer. This is especially probable in the case of our study of age variations in the dentine of the common dolphin (Kleinenberg and Klevezal', 1962), since the layers were counted in hematoxylin-stained preparations and during the staining there is an increase in the clarity both of the main bands of an annual layer and of the inner streaks.

As we have already stated in Chapter I, such clear supplementary streaks are evidently produced by some retardation in the growth of tissue during the summer period. The reason for the formation of an especially clear supplementary transparent band in separate species of toothed whales can only be imagined as follows. In some toothed whales, besides the distinct winter retardation of growth which is peculiar to all mammals and caused by some sort of common phenomena, a marked reduction occurs in the rate of tissue growth /page 98/ during the summer period under the influence of various individual factors such as a reduction in nutritional intake. This factor operates regularly and produces a regular formation of a supplementary band in the dentine.

In other species, instead of one very pronounced reduction in the rate of growth during the summer period, a series of smaller growth variations takes place as a result of which a number of indistinct supplementary streaks are formed.

Thus, we are proposing that the conclusion that in some toothed whales one layer of dentine forms in the course of a year and in others, as distinct from all the remaining mammals, two layers form, should be replaced by another conclusion: in all toothed whales one layer of dentine forms per year but in some of them within the non-transparent band of an annual layer, among the supplementary streaks there is one particularly clear streak, equal or almost equal in clarity to the main transparent band of an annual layer, whereas in other species no such inner streak is discernible.

Ordinarily, layers in the cementum of toothed whales are not used for age determination although in principle, this method of age determination is possible for a number of toothed whales. Clear layers of cementum exist in the teeth of the bottle-nosed dolphin, pilot whale, beluga and sperm whale, and the number of layers in the cementum corresponds to the number of layers in the dentine (Sergeant, 1959; Khuzin, 1961; Berzin, 1964). Layers also exist in the cementum of common dolphins but they are very narrow and difficult to count on account of the small overall thickness of the cementum (fig. 43).

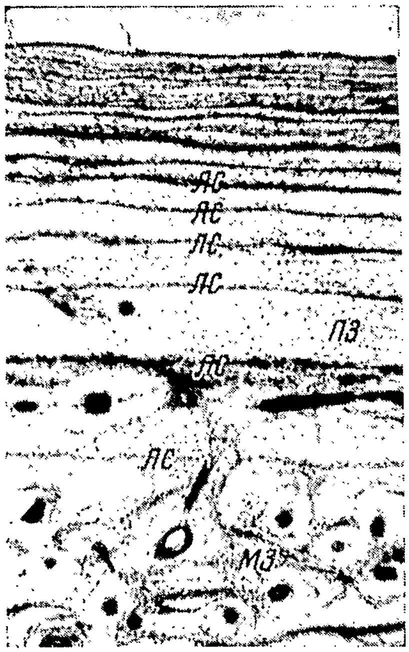


Fig. 44 [page 98]. Sector of genal wall of lower jaw of saddleback dolphin. Transverse section. Staining - hematoxylin. (X60). Mz - mesosteal zone, Pz - peridontal zone; Ls - adhesion line.

Annual layers in the periosteal zone of bone have been described in the lower jaws of a sperm whale and common dolphins (Laws, 1960; Nishiwaki et al, 1961; Kleinenberg and Klevezal', 1962). Comparison of the number of layers in the lower jaw with the number of layers in the dentine of a tooth and with the approximate age of an individual, determined from the number of scars from corpora lutea in the ovaries, indicates that one layer of bone tissue forms [page 99] in bone annually, separated by one adhesion line.

The mesosteal zone and resorption line are clearly shown in the lower jaws of the common dolphins studied by us. A transverse section of the jaw taken immediately behind the end of the dental row is most convenient for counting the annual layers. In the case of males having 10 to 11 annual layers, the first layers in this sector had not yet been affected by resorption. In one very old male in which the first layers of the periosteal zone were clearly resorbed, 18 layers were seen (fig. 44) and in other sectors of the jaw - a lesser number.

The Japanese investigators (Nishiwaki et al, 1961) point out that in Pacific Ocean sperm whales the first layers of the periosteal zone are resorbed at 13 to 16 years of age, and in one Antarctic sperm whale 18 layers were discovered.

We did not observe regular supplementary adhesion lines in the annual layers of the periosteal zone of bone in common dolphins, although such lines were seen in separate layers of separate individuals.

Thus, the ages of toothed whales can be determined from the number of layers in the dentine, cementum and periosteal zone of bone.

Sub-order Mysticeti

As baleen whales do not have teeth, annual layers can be found in them only in the periosteal zone of bone. For this purpose we studied (in polished sections and hematoxylin-stained micro-sections) sectors of the lower jaws of 5 female sei whales Balaenoptera borealis) with lengths between 13.6 and 16.4 metres and one female finback whale (Balaenoptera physalus) 20 metres in length from the Antarctic (material presented by V.A. Zemskiy). The bone structure was studied in three sectors of the jaw: in the front part of the body of the jaw immediately behind the symphysis, in the middle part of the jaw body and at the base of the maxillary branch. In each sector the genal wall, lingual wall and lower edge of the jaw were studied.

Despite the fact that there were two species of baleen whales and judging from their dimensions, they were of different ages, the bone structure was generally identical in all the specimens. The main mass of jaw cross-section in all of the sectors comprised spongeose bone, the intervals between the trabeculae being filled with fat. The density of the compact layer of the lower jaw varied in the different sectors. At the front end of the jaw the entire maxillary wall consisted of osteal bone tissue, the outer edges of the wall were friable and the osteons widely spaced; the middle part of the wall comprised a dense arrangement of osteons and towards the inner part the dense bone gradually changed to spongeose material filled with fat (fig. 45a, b). In the middle part of the jaw body the compact [page 100] wall was wider than at the front end of the jaw.

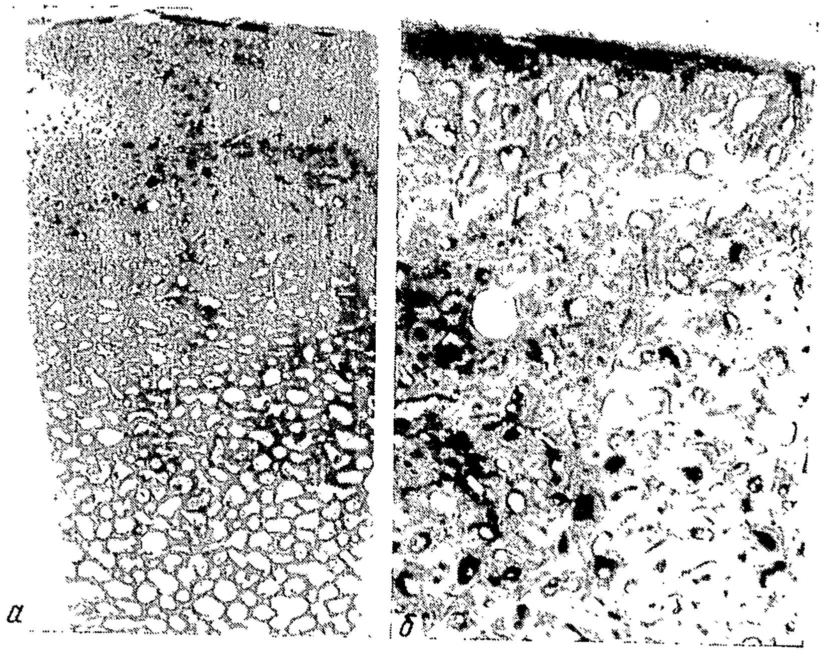


Fig. 45 [page 100]. Sector of lingual wall of front part of lower jaw of sei whale (♀ L = 16.4 meters). Transverse section. Staining - hematoxylin. a - general plan (X3); b - sector of outer edge (X13)

The main mass of the wall was very dense, and fully osteonized, the outer layers were more friable and contained numerous blood vessels, while the extreme outer layers consisted of "haloes" of bone tissue (fig. 46c). At the base of the mandibular ramus the walls of the jaw as a whole consisted of densely arranged macrocircular plates (plates of bone tissue separated by radial and circular chink-like spaces), similarly changing in their inner parts to spongeose bone (fig. 46a, b). In the fin whale there were almost no osteons here while in the sei whales the layers of the macrocircular plates were osteonized, in the small sei whales - to a lesser degree and in the large ones - to a greater degree.

In none of the cases did we detect even narrow layers of typical periosteal zone (circumferential lamellae) in the mandibular walls of the baleen whales. Neither were any adhesion lines discovered.

The only lines which we managed to find were light narrow streaks (in reflected light), noted in a dry section [page 101] of the genal wall of the jaw at the base of the maxillary branches of two sei whales.

Generally speaking, these streaks were parallel to the outer surface of the bone and divided the compactum into layers (fig. 47). In decalcified stained sections it was seen that they consisted of highly compressed rods of macrocircular plates. Whether or not they are seasonal markings of the growth of the bone cannot be established from the small /page 102/ amount of available material. They were seen in the bones of sei whales 13.6 and 14 metres long but were not noted in sei whales 16.0 and 16.4 metres long. Possibly, this can be explained by the fact that in the larger sei whales the macrocircular plates were heavily osteonized.

As far as can be judged from the small amount of available material, in baleen whales, firstly, apposition of bone tissue occurs not in the form of dense layers of periosteal bone but either as rows of macrocircular plates or in the form of friable bone tissue. Secondly, the process of bone osteonization (replacement of periosteal by mesosteal bone) occurs very intensively in a number of sectors (in this connection the bones of baleen whales are reminiscent of those of ungulates (see below).

Thus, in the bones of baleen whales we did not find the annual layers of the periosteal zone and probably the method of age determination from the number of annual layers in dental and bone tissue cannot be used for baleen whales.

Order Carnivora

In representatives of the carnivora annual layers are formed in the dentine, cementum and periosteal zone of bone.

The initial reference to two layers in the cementum of carnivores is in a paper by V.S. Smirnov (1960). V.S. Smirnov, in proposing that the ages of carnivorous mammals be determined from the relative width of the canine canal, wrote: "The cementum covering the roots of teeth in the carnivores examined by us was frequently layered. However, the number of layers does not in all cases correspond to the age of the animal. The layers of cementum are most clear in bears and it can be /page 103/ considered that each layer corresponds to a year of life. Layers of cementum have also been discovered in wolves, arctic foxes and red foxes, but not in all animals...." (page 99). Later it was pointed out on the basis of material from animals of known ages (Klevezal' and Kleinenberg, 1964; Kleinenberg and Klevezal', 1966), that in arctic foxes Alopex lagopus regular layers existed in all of the specimens studied, the number of which corresponded, as a rule, to the age of the animal.

Rausch (1961) studied Black bears Ursus americanus of known ages and established that in the canines of bears there are not only annual layers of dentine and cementum but also annual ridges on the roots. The tooth grows in length only to the sixth year and on the root of the tooth there were up to six ridges. He studied annual layers of dentine in a longitudinal section of a canine and could distinguish ten age groups from the number of annual layers. A description has been given of annual layers

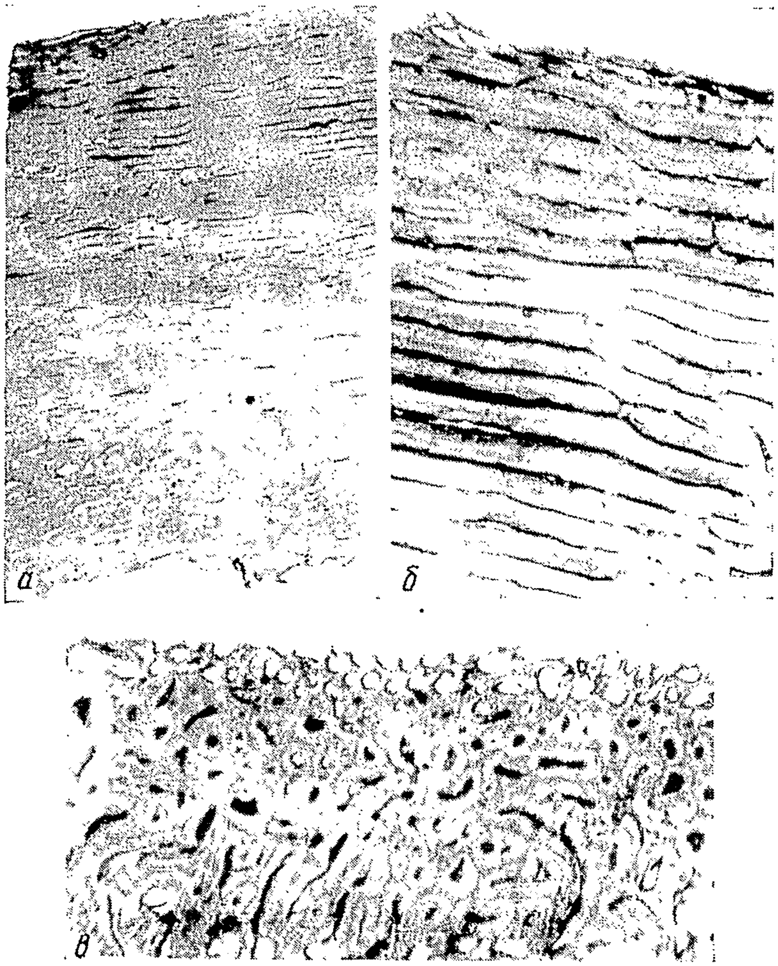


Fig. 46 [page 101]. Sector of genal wall of lower jaw (a,b,) at base of mandibular ramus (c) in middle of jaw body of fin whale (♀ L = 20 meters). Staining - hematoxylin. a - general plan (X3); b - sectors of outer edge (X13).

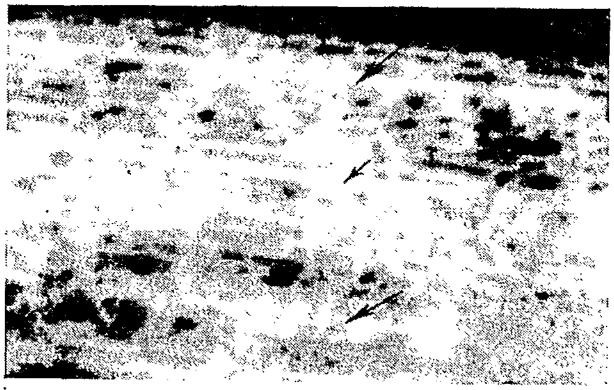


Fig. 47 [page 102]. Sector of genal wall of lower jaw at base of mandibular ramus of sei whale (♀ L = 13.6 meters). Transverse microslide. Reflected light (X8). Arrows indicate narrow light streaks.

in the cementum of grizzly bears Ursus arctos (Numdy and Fuller, 1964). Distinct layers were noted, moreover, in the cementum of the sea otter Enhydra lutris (Klevezal' and Marakov, 1966), the sable Martes zibellina, and the American mink Mustela vison (Klevezal', 1966).

We made a study of the dentine in the canines of sables of accurately known ages obtained from a State fur farm. The canines of the sable cease growing in length during the first year of life of the individual so that annual ridges do not form on the root, and counting of annual layers in the dentine can be done on transverse sections through the middle of a tooth.

The main mass of dentine of the canine is deposited during the first year of life. The layering of the subsequently formed dentine was difficult to see on thin sections in transient and reflected light. The layers stood out much better in hematoxylin-stained sections (fig. 48) but here too, the boundaries of the annual layers could not always be traced with confidence. In eleven out of twenty specimens the number of annual layers visible in stained sections was exactly equal to the age of the individual in years, while in the remainder it was equal to it within one year. The pulp cavity of the canines apparently does not become filled with dentine before 12 to 14 years of age (Klevezal', 1966).

Much more difficult to distinguish than in the case of the sables are annual layers in the dentine of canines of arctic foxes. In arctic foxes (we studied only the data for the material received from the State fur farm), within the annual layers of dentine there are many supplementary streaks of varying degree of clarity and it is therefore difficult to determine the boundary of an annual layer (fig. 49).

In the cementum of carnivores the layers are much more clear than they are in the dentine. Mundy and Fuller (1964), in determining the ages of grizzly bears, counted the layers of cementum of a third molar (so as to minimize the damage to the collected material) in sections of non-decalcified teeth in reflected light. The ages of the individuals were not known and the number of layers in the cementum were compared with the malar width of the skull. The third molar in bears erupts during the second year of life and a complete first layer of /page 104/ cementum exists only in two-year-old animals. The ages of bears can therefore be determined from the number of layers in the cementum of the third molar by adding one year.

We studied (on hematoxylin-stained sections) cementum from the teeth of arctic foxes of accurately known ages obtained from the State fur farm (Klevezal' and Kleinenberg, 1964; Kleinenberg and Klevezal', 1966). A preliminary comparison was made between the structure of the cementum of the molars and canines on the lower jaw of a six-year-old arctic fox and the cementum structure of the canines in the upper and lower jaws of five arctic foxes of known ages. In every case, the structure of the cementum and clarity and number of layers in the various teeth of one specimen were identical. Subsequently, we studied the cementum of canines only, on a stained longitudinal section through the middle of the tooth.

Fig. 48 /page 104/. Sector of transverse section of canine of 6-year-old sables. Staining hematoxylin (X60). D - dentine; Ts - cementum; PP - pulp cavity; NL - neonatal line. Numerals denote annual layers.

The cementum is much thicker in the lower part of the canine than on its lateral walls (fig. 50) but nonetheless it is deposited along the entire surface of the root and therefore the counting of layers can also be done on transverse sections made through the middle of the root.

In thirty arctic foxes studied by us the number of layers in the cementum coincided precisely with the ages of the individuals in years (fig. 50, 51, 56). The first annual layer was much clearer than the subsequent ones (fig. 56).

The layers in the cementum of the sea otter are poorly visible from thin slices and sectioned half-teeth of canines but show up well in hematoxylin-stained sections (Klevezal' and Marakov, 1966). Cementum is deposited along the entire surface of the root and the counting of layers can be done both in longitudinal and transverse sections. Layers can also be seen in the cementum of sables in hematoxylin-stained longitudinal and transverse sections but as the total width of the cementum is not great the layers must be highly magnified for counting.

Annual layers in the periosteal zone of bone of carnivores were described for arctic foxes, sables and American minks of accurately known ages obtained from a State fur farm (Klevezal', 1965).

/Page 105/ The lower jaw was studied in hematoxylin-stained sections. In all three species the periosteal zone was best developed and had the clearest layering in the sectors of the jaw body in the region of the last premolar and first molar teeth. Its thickness is not the same in different walls of the jaw. In sables it is much broader on the genal wall whereas in mink this is the case on the lower edge of the jaw. In the arctic fox, the width is either identical on both walls or greater on the lingual wall.

The overall character of periosteal layering in sables, minks, and arctic foxes is the same. In the lower jaw there is a clear mesosteal zone with osteons of varying degrees of density. It is separated from the periosteal zone by a meandering resorption line (fig. 52-54).

The number of layers of the periosteal zone was usually equal to the age of the individual in years right up to the beginning of resorption of the first annual layers (fig. 52-54). In sables, resorption affects the first adhesion line, starting from the fifth year of age and in some cases even later.

In our matérial there were neither arctic foxes nor minks aged more than six years. In the six-year-old minks and arctic foxes the first layer of the periosteal zone had not yet been resorbed (fig. 53d).

The width of the annual layer varies in different species. The width of the layers of periosteal zone of an arctic fox is less, both relatively to the transverse dimensions of the jaw and absolutely, than the width of the layers in a sable and mink of the same age. In all of the species the width of the annual layers diminishes with age. The widest one is the second annual layer - the layer situated between the first and second adhesion lines, whereas the periosteal layer of the first year, located between the resorption line and first adhesion line is much narrower than the second, probably due to the active reformation of compact bone at the beginning of the post-natal period.

Fig. 49 /page 105/. Section of transverse section of canine of adult arctic fox. Staining - hematoxylin (X60). For explanation see fig. 48. Arrows indicate assumed boundaries of annual layers.

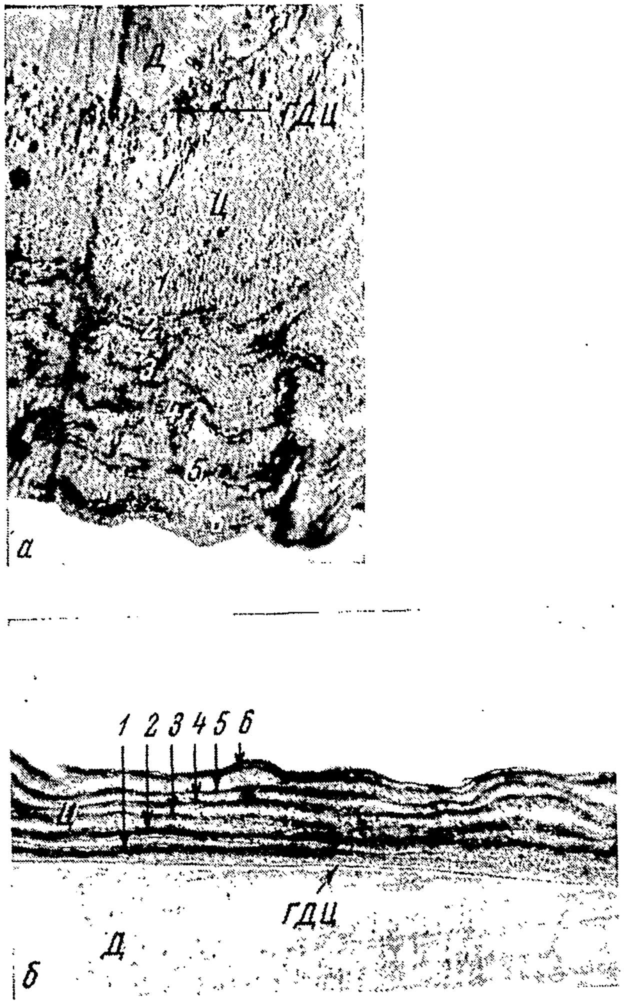


Fig. 50 /page 106/. Longitudinal section of (a) lower part and (b) lateral part of root of canine of six-year-old arctic fox. Staining - hematoxylin (X60). D - dentine; Ts - cementum; GDTs - boundary of dentine and cementum. Arrows indicate narrow, heavily-stained bands of cementum. Annual layers denoted by numerals.

Among the individuals in which the first layer had not yet been /page 106/ resorbed, in some cases (in one sable out of thirty-five, five mink out of twenty-four and two arctic foxes out of thirty), the number of layers did not correspond with the age of the animal. Some of these discrepancies can evidently be explained as due to inaccurate labelling of the material during collection (sable, arctic fox). In five mink out of twenty-four a clear supplementary adhesion line was seen, creating the illusion of an extra annual layer. It initially appeared in one-year-old animals. Evidence that this line is a supplementary one is to be found not only in a comparison between the number of adhesion lines and the age of the animal but also by comparing the widths of the annual layers.

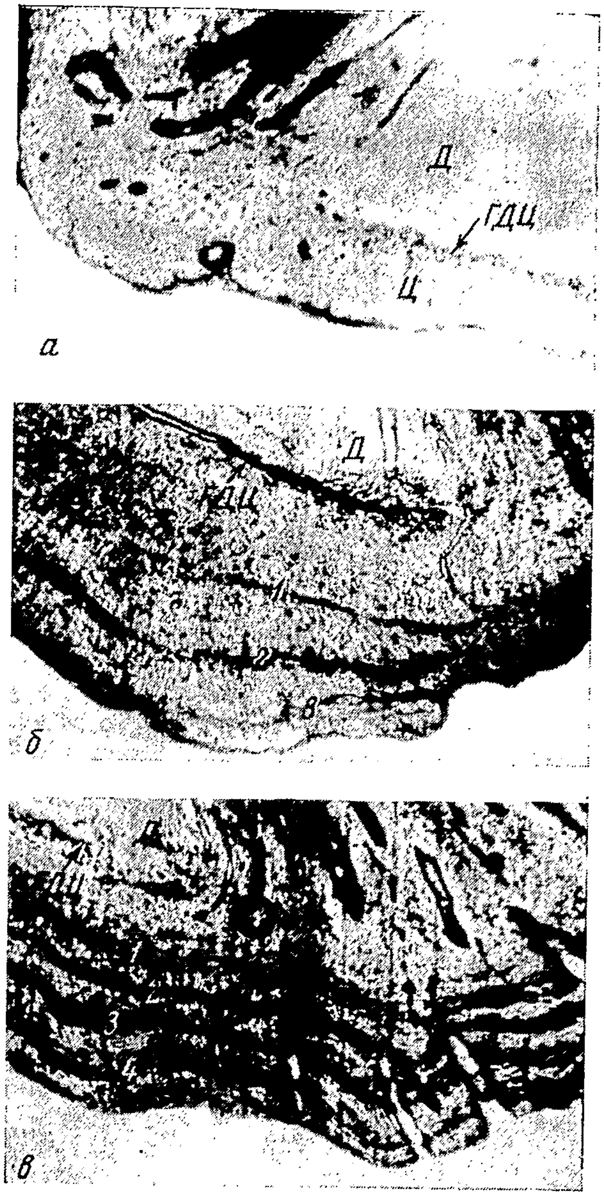


Fig. 51 [page 107]. Longitudinal sections of lower part of root of canines of arctic foxes: (a) juvenile (b) three-year-old (c) four-year-old. Staining - hematoxylin (X60). For explanation see fig. 50.

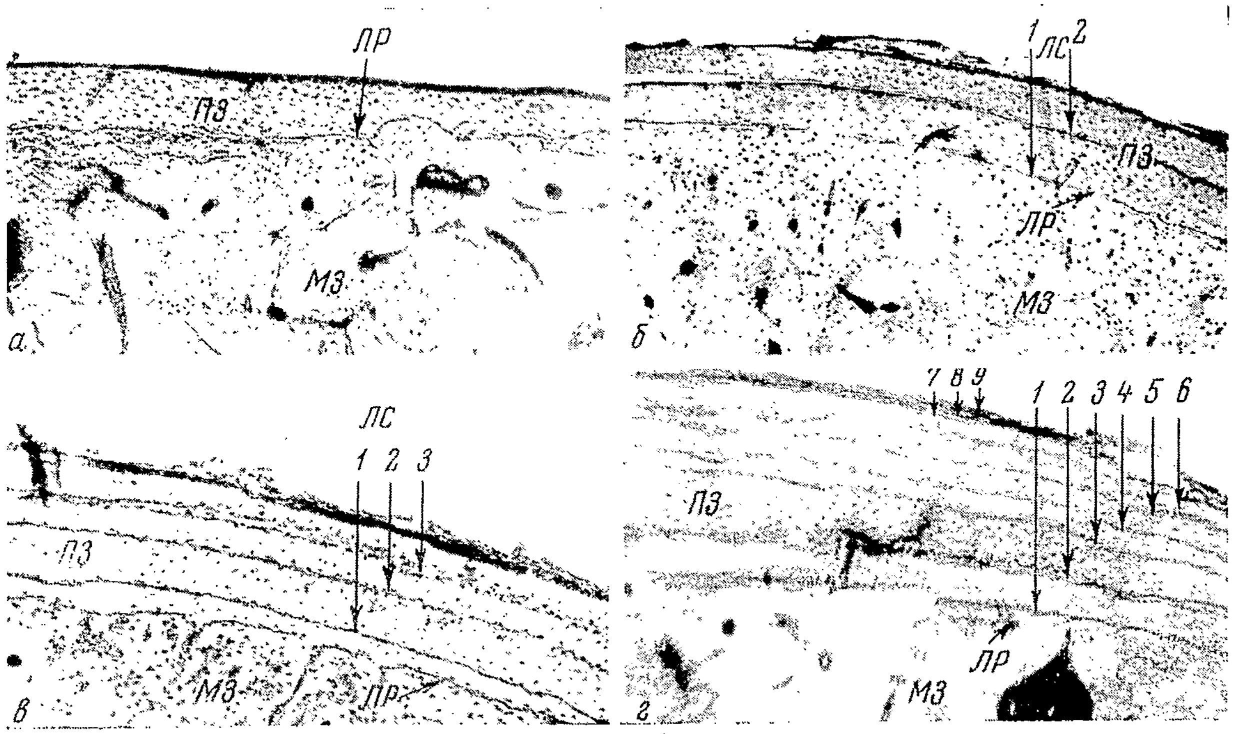


Fig. 52 /page 108/. Sectors of walls of lower jaws of sables: (a) juvenile, (b) two-year-old, (c) three-year-old, and (d) nine-year-old. Transverse sections. Staining - hematoxylin. (X60). Mz - mesosteal zone; Pz - periosteal zone; LR - resorption line; Ls - adhesion line. Annual layers denoted by numerals.

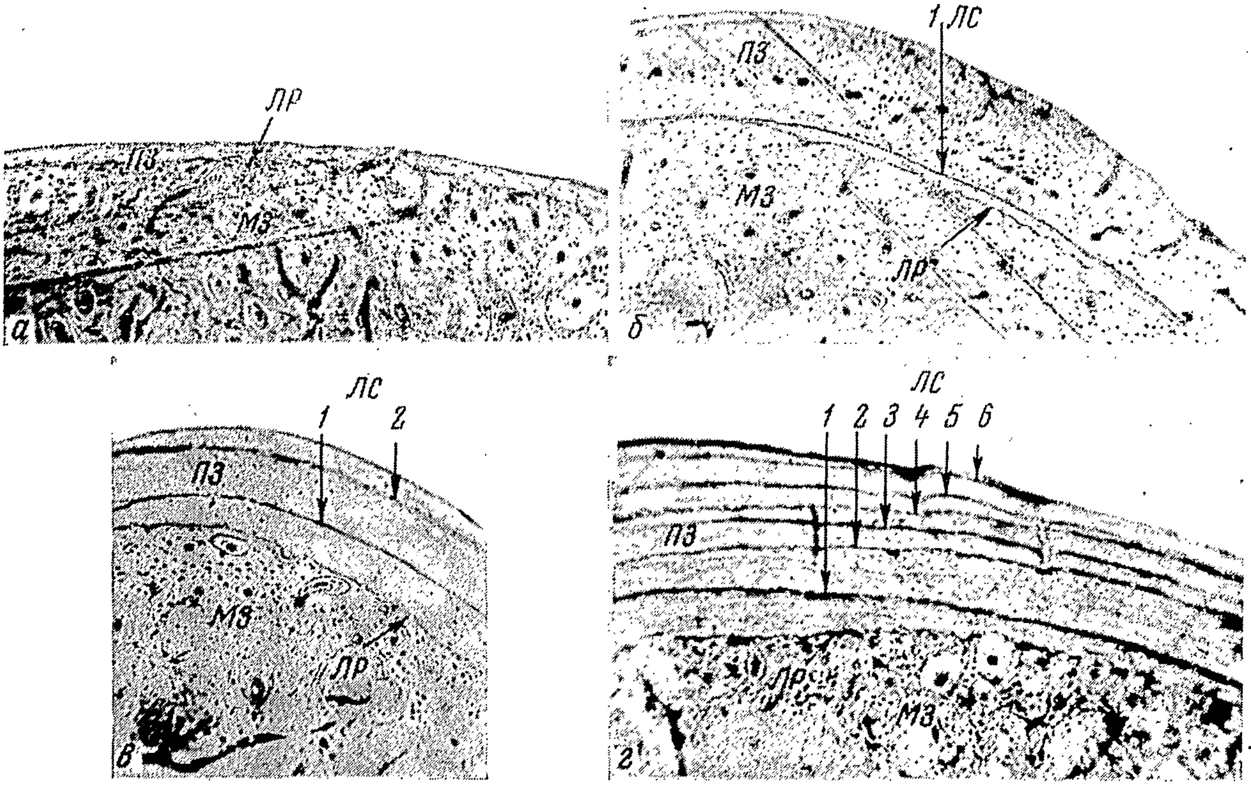


Fig. 53 /page 109/. Sector of walls of lower jaws of American mink: (a) juvenile, (b) yearling, (c) two-year-old and (d) six-year-old. Transverse sections. Staining - hematoxylin. (X60). For explanation see fig. 52.

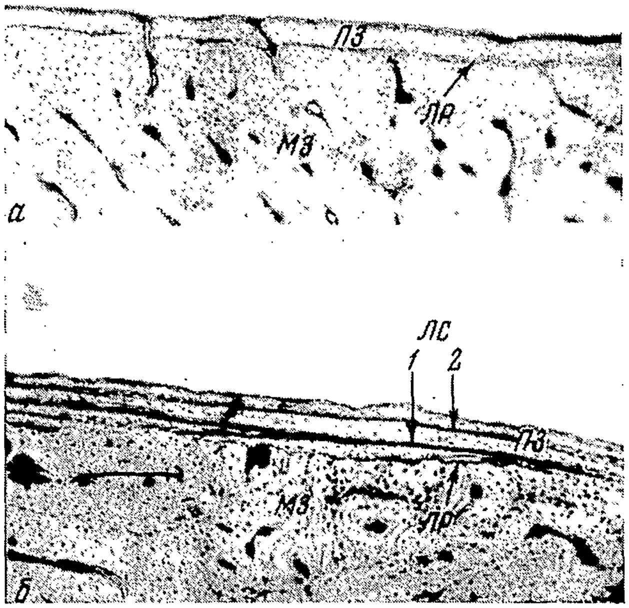


Fig. 54 [page 110]. Sectors of walls of lower jaws of arctic foxes: (a) juvenile, (b) two-year-old. Transverse sections. Staining - hematoxylin. (X60). For explanation see fig. 52.

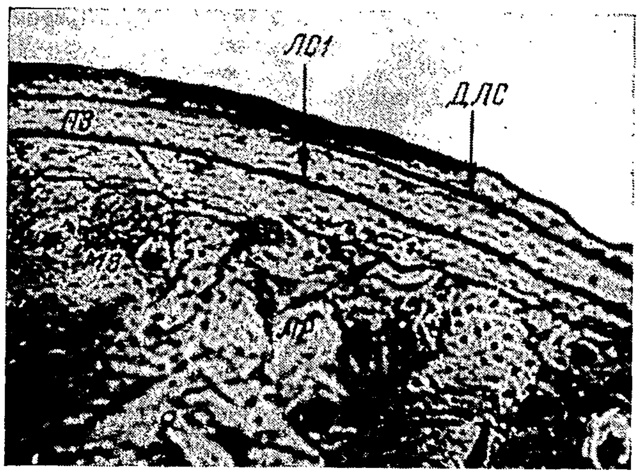


Fig. 55 [page 110]. Sector of lower jaw of one-year-old American mink. Transverse section. Staining - hematoxylin. (X60). DLS - supplementary adhesion lines; for remaining designations see fig. 52.

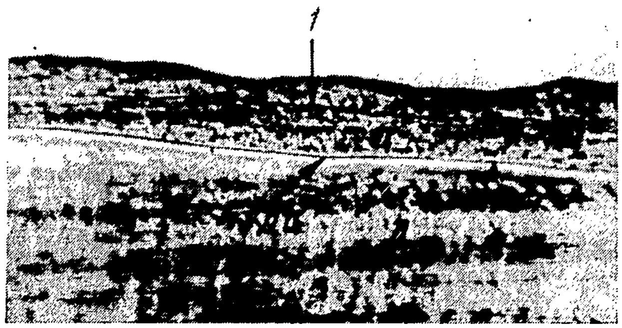


Fig. 56 /page III/. Longitudinal section of lateral part of root of canine of one-year-old arctic fox. Staining - hematoxylin. (X60). For explanation see fig. 50.

/page III/ As we have already noted, the width of the second annual layer is much greater than the width of the first one (fig. 53). If the supplementary adhesion line is considered to be the main one delimiting an annual layer, then in that case the widths of the first and second annual layer will be equal (fig. 55). (A more detailed discussion of this question will be found in Chapter II).

Sometimes, the adhesion lines delimiting the annual layers were double, separated by a very narrow streak of bone tissue (fig. 52d, second adhesion line). Occasionally, within the annual layers indistinct supplementary adhesion lines could be seen.

Distinct layers of periosteal zone, evidently annual ones, were found in the limb bones of individual specimens of a sea otter, badger, otter, wood and stone martens, sable, ermine, black pole-cat and jungle cat (Klebanova and Klevezal', 1966).

Thus the ages of representatives of the Order Carnivora can be determined not only from the number of annual layers in the dentine and cementum but also from the number of annual layers in the periosteal zone of bone.

Order Pinnipedia

Annual layers in the dentine or cementum of canines have been described in twenty-one species of pinnipeds (see Table II). A detailed analysis of the method of determining the ages of pinnipeds from the layers in dentine and cementum has been made by Laws (1962).

In the majority of pinnipeds the annual layers in the dentine are very clear and readily seen on sectioned half-canines, for example in the elephant seal Mirounga leonina (Laws, 1953a, b), ringed seal Pusa hispida (Tikhomirov and Klevezal', 1964), or on thin sections in transient light, for example in the northern fur seal [page 112] Callorhinus ursinus (Kubota et al, 1963).

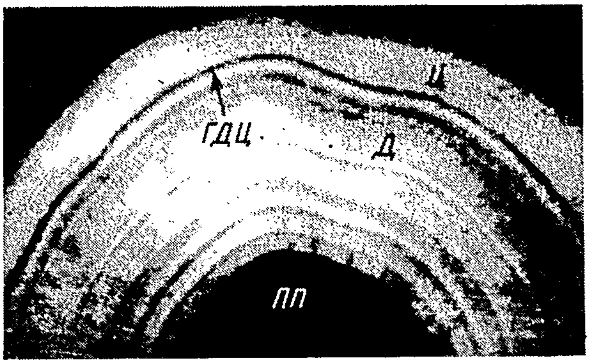


Fig. 57 [page 112]. Sector of transverse microslide of hooded seal. Reflected light. (X13). D - dentine; Ts - cementum; GDTs - boundary of dentine and cementum; PP - pulp cavity.

The canines of most of the true seals cease growing in length early and for counting the layers in the dentine it is possible to make transverse sections in the middle part of a tooth (fig. 57). In eared seals and walruses the canines grow in length over a long period and for age determination from the number of layers in the dentine it is therefore necessary to make longitudinal sections.

By studying the teeth of seals caught throughout the year (McLaren, 1958) and the teeth of seals of known ages (Laws, 1962; Scheffer and Kraus, 1964) it was shown that one layer forms in the dentine in the course of a year. Regular deposition of annual layers of dentine occurs until the entire pulp cavity is filled. In various species this occurs at various times. For example, in the ringed seal complete closing of the pulp cavity of the canine occurs not earlier than 22-24 years of age, in the ribbon seal Histriphoca fasciata - at 10 to 11 years, sometimes 14 years, and in the common largha seal Phoca vitulina largha - at 17 to 19 years (Tikhomirov and Klevezal', 1964).

Within a wide annual layer of dentine there are numerous supplementary streaks. In some seals part or all of the non-transparent band is occupied by inter-globular dentine. Inter-globular dentine exists in the annual layers of crab-eaters (Laws, 1958) and ringed seals (McLaren, 1958; Tikhomirov and Klevezal', 1964). In ribbon seals and the common largha seal it is not always seen and frequently only in the second and third annual layers. It is not always uniformly shown even in the various subspecies of one species. Thus, in the Okhotsk ringed seal there is inter-globular dentine in all the annual layers except the first, and in the ringed seal of the Barents Sea, as a rule it is not seen (Klevezal', 1966).

/page 113/ The clarity of the annual layers in young, sexually immature animals is usually less than in adult ones (Laws, 1953b; Mansfield, 1958a). For seals in the annual layers of which there is inter-globular dentine it has been noted that in the first annual layers such dentine is not shown or is less well shown than in the subsequent ones (McLaren, 1958; Tikhomirov and Klevezal', 1964).

Cementum does not attain a marked degree of development on the roots of the teeth of all species of pinnipeds. It is well developed on the roots of the canines of the grey seal Halichoerus grypus (Hewer, 1960, 1964), hooded seal Cystophora cristata (Laws, 1959), and the molars of the walrus Odobenus rosmarus (Mansfield, 1958a, Krylov, 1965). The fact that the layers in the cementum are annual ones was confirmed by studies of animals of precisely known ages (Hewer, 1960; Mansfield and Fisher, 1960), and by numerous comparisons between the number of layers in dentine and cementum.

Annual layers also exist in the periosteal zone of bone in pinnipeds, although those are not used for large-scale age determination. The first description of the periosteal zone of the lower jaw in seals of various age groups was given by K.K. Chapskii (1952) from a large volume of material on the harp seal Pagophilus groenlandicus. He also undertook a comparison between the number of layers of the periosteal zone of the lower jaw and the length of the body and skull, size of the genitalia and number of ridges on the claws and suggested that one layer of bone is formed annually. Our study of ten ringed seals and three bearded seals from the Okhotsk Sea and five ringed seals from the Barents Sea, the ages of which were determined from the annual layers in the dentine of the canines as well as comparison between the number of layers in the periosteal zone of the lower /page 114/ jaw and in the dentine and cementum of the canine of the Caspian seal (Chapskii, 1965) corroborate the suggestion put forward by Chapskii (1952).

Fig. 58 /page 113/. Sector of wall of lower jaw of ringed seal. Transverse section. Staining - hematoxylin. (X36). Annual layers denoted by numerals.

In true seals the periosteal zone is well developed in the genal and lingual walls and the lower edge of the body of the lower jaw. It is separated from the mesosteal zone by a resorption line which, however, is not always clearly shown. The resorption rate of the periosteal zone is probably not the same in different sectors of the jaw body. Thus, in a seven-year-old ringed seal in the front part of the lower jaw immediately behind the canine and in sectors of the jaw body below the middle molars the first three layers of the periosteal zone had already been resorbed, whereas in the mandibular walls beyond the end of the dental row resorption had not yet affected even the first layer. Evidently, for counting the annual layers in the periosteal zone of the lower jaw it is best to use a transverse section of a jaw beyond the end of the dental row. We cannot say at what stage in this sector complete resorption occurs of the first annual layer of the periosteal zone. The oldest individuals in our material were two fourteen-year-old ringed seals, in both of which fourteen layers were seen in the periosteal zone of the lower jaw beyond the end of the dental row (fig. 58). Sometimes, instead of one adhesion line, delimiting the annual layers, two or even three lines are seen, but in this case the layers of bone tissue located between them are very narrow - much narrower than a normal annual layer.

Thus, the ages of pinnipeds can be determined both from the number of annual layers in the dentine or dental cementum and from the annual layers in the periosteal zone of bone.

Order Perissodactyla

The literature contains no data on the existence of annual layers in the tissues of teeth and bones of Perissodactyls.

We had at our disposal individual teeth of thirteen specimens of the wild ass Equus hemionus of which the ages, and in a number of cases, sex and date of death were known. The approximate ages of some of them had been determined from the state of the dental system (using tables for determining the ages of horses) by V.N. Orlov.

The structure of the dentine was studied in transverse sections of the incisors of the lower jaw, stained with hematoxylin.

In wild asses, the probable ages of which were determined as six years and over, two zones were seen in the dentine of the permanent incisors: an outer one, in which the layers were not seen clearly and an inner one, in which there were clear layers, consisting of faintly and heavily-stained bands (fig. 59). The number of layers in the inner zone increased with an increase in the assumed ages of the animals (Table VII).

On the basis of our limited material it is difficult to conclude definitely that these layers are annual ones.

Table VII. [page 115]. Number of layers in dentine and dental cementum of adult wild asses.

No.	Sex	Age in years	Tooth	Number of layers in	
				dentine	cementum
1	♀	≈4–4,5	I_1	(2–3) *	1–2
2	♀	≈6	»	(2?)+3–4	3–4
3	♀	≈6	»	(2?)+4	4
4	♂	≈7–8	»	(2?)+8	8
5	♂	⩾11	»	(2?)+11	12–13
6	?	senex	»	(2?)+12	10–12
7	♀	≈9	»		⩾10
8	♂	≈9	»		12
9	♂	⩾11	I^2		⩾15
10	?	senex	M^1		10–11

* Assumed number of layers in outer zone of dentine given in brackets.

We do not know [page 116] when the first clear layer of dentine begins to form and a comparison with the assumed age could not give an answer to this question as the ages of the asses were established from tables for determining the ages of horses and evidently, they were very approximate. Establishing the precise relationship between the number of layers in the dentine and the age of an individual is also complicated by the fact that the permanent incisors appear at the age of 2 to 4 years, whereas the formation of layers of dentine can occur even when the tooth is still within the alveolus.

Nevertheless, by analogy with Artiodactyla (see below) we believe that the clear layers in the dentine of wild asses are are annual ones. It can be assumed that the beginning of the formation of clear layers in the dentine is associated with the onset of sexual maturity of the individual, which in wild asses occurs at 2 to 3 years of age (Heptner et al, 1961).

We studied in hematoxylin-stained sections the cementum structure of milk teeth of three wild asses, a permanent molar of one old ass and the permanent incisors of nine individuals. (The preparations of cementum of the incisors were presented to us by V.N. Orlov).

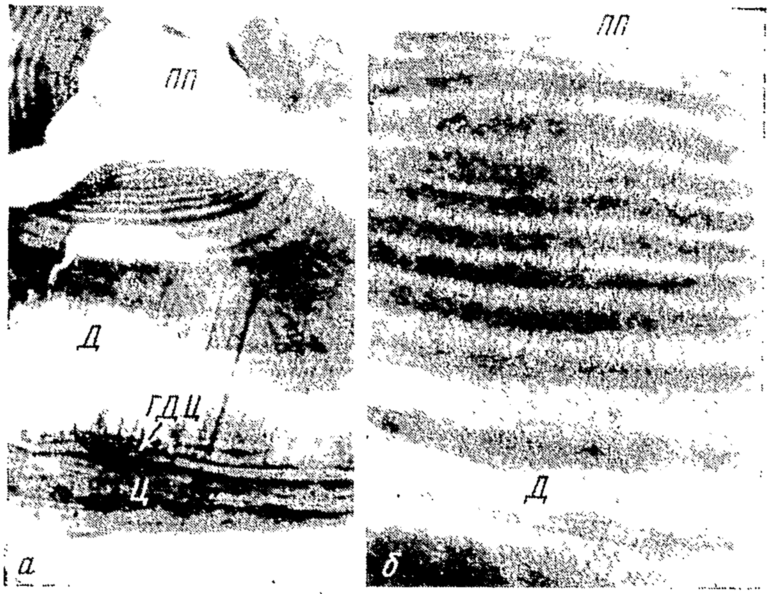


Fig. 59 /page 115/. Sector of transverse section of (a) first lower incisor and (b) inner zone of dentine of this tooth in adult wild ass. Staining - hematoxylin. (a - X13, b - X60). D - dentine; Ts - cementum; GDTs - boundary of dentine and cementum; PP - pulp cavity.

In the milk teeth of asses not more than three years old there could be seen in the lateral walls of the root a narrow, heavily hematoxylin-stained band of cementum, separating an inner, wide, lightly coloured band of cementum from an outer one. In the inter-root cushion, instead of one dark band there were several contiguous, narrow, heavily stained ones.

In the cementum of the permanent teeth clear layers were seen (fig. 60), the number of which increased with an increase in the assumed ages of the animals (Table VII).

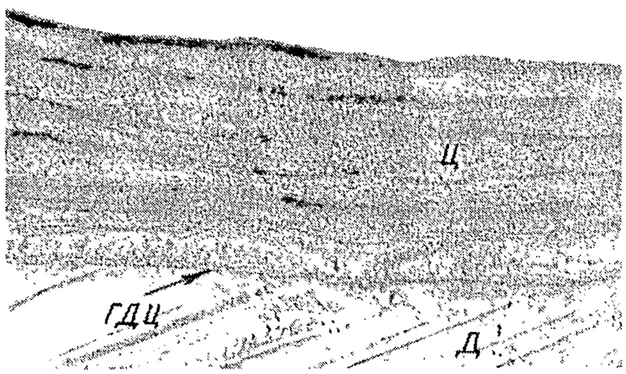


Fig. 60 /page 116/. Longitudinal section of lateral part of root of first lower incisor of adult wild ass. Staining - hematoxylin. (X60). For explanation see fig. 59.

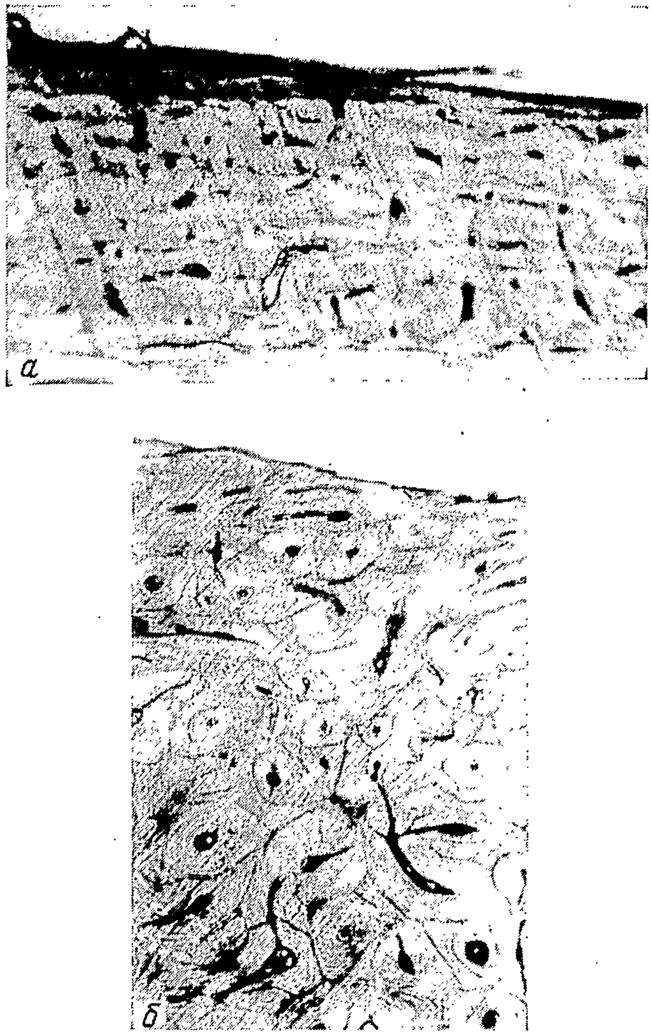


Fig. 61 [page 117]. Sector of genal wall of post incisor diastema of (a) young wild ass and (b) old wild ass. Transverse sections. Staining - hematoxylin. (X34).

[page 118] The number of layers in the cementum was approximately equal to the number of layers in the inner zone of the dentine, where the layers are clear. Evidently, the first layer of cementum begins to form when in the dentine an inner zone has already formed, in which there are probably two annual layers. But our material does not enable us to say at what age this occurs.

We did not find annual layers in the periosteal zone of bone of the wild asses. We studied the structure of the walls of the lower mandibular body in young and old asses in the area of the diastema and in the middle part of the mandibular body (on hematoxylin-stained sections).

In the young animal the main mass of the genal wall comprised reticular bone tissue and bone lamellae, separated by numerous canals of the blood vessels, on the outside of which was seen a very narrow periosteal band without adhesion lines (fig. 61a). The lingual wall was completely osteonized. In the old wild ass both the genal and lingual wall were entirely osteonized (fig. 61b).

It is probable that as in the case of the Artiodactyla (see below) in the wild ass a rapid rebuilding of the periosteal layers occurs and that the periosteal zone is preserved for a very short time.

Thus, the ages of Perissodactyla can probably be determined from the number of annual layers in the dentine and dental cementum if it is established at what age the first clear layer of dentine and first cementum layer form. The bone structure of Perissodactyla is evidently unfit for determining the ages of individuals.

Order Artiodactyla

In Artiodactyla there are clear annual layers in the dentine and dental cementum.

As early as 1932 Eidmann (1932) published a paper in which he proposed that the ages of red deer Cervus elaphus be determined from the number of "annual rings" in the secondary dentine of the incisors. As an incisor wears down, secondary dentine fills the upper part of the pulp cavity (analogous to the secondary dentine in molars of rodents, see fig. 24). Eidmann wrote that in longitudinal sectioned half-teeth of deer alternating dark and light zones of secondary dentine were seen. One dark and one light zone comprise the growth of secondary dentine in one year and the ages of the deer could therefore be determined from these annual rings. In this case, three years must be added to the number of annual rings - the time elapsed prior to the formation of the first layer of secondary dentine. As an animal becomes older, to the extent that the crown of the tooth is worn away the first few layers of the secondary dentine are also worn away. According to Eidmann's findings, the first annual ring of secondary dentine wears away [page 119] in deer aged 10 to 12 years. From that time on only the approximate age of the deer can be determined from the number of annual rings. Eidmann evidently considered that this method could be applied for determining the ages of other ungulates.

At the time when Eidmann's paper was published the method proposed by him was undoubtedly a very promising one. Today, one could hardly recommend it for determining the ages of Artiodactyla as it provides for an assessment of the precise ages of only those individuals in which the first layer of secondary dentine has not been worn away. The annual layers discovered in recent years in the cementum and normal dentine provide much greater possibilities of accurate age determination of Artiodactyla than layers in secondary dentine.

Sergeant and Pimlott (1959 described layers of cementum seen in sectioned teeth and thin slides of the incisors of the elk Alces alces, and suggested that these layers are annual ones (the ages of all the elk except one were unknown). Low and Cowan (1963) studied the incisors of black-tailed deer Odocoileus hemionus of accurately known ages (on hematoxylin-stained longitudinal sections of decalcified tooth) and established that one layer of cementum is formed annually. Accordingly, as the permanent incisor erupts at the age of approximately twelve months, the wide faintly-stained band of the first annual layer of cementum is formed during the second summer of life of an individual. McEwen (1963) described the annual layers in the cementum of incisors of caribou, Rangifer tarandus, which were readily visible in hematoxylin-stained sections. He established the periodicity of the deposition of the layers in the caribou by studying the teeth of young animals killed at different times of the year. As the deposition of cementum on the permanent incisors begins at 8 to 9 months of age i.e. during the second summer of life, for age determination one year must be added to the number of annual layers in the cementum.

Mitchell (1963) studied the teeth of Scottish red deer Cervus elaphus. As molars have larger depositions of cementum than incisors and canines, he used for counting the annual layers in the cementum a bi-sected molar in which the layers were clearly seen in reflected light, being particularly well seen in the thicker cementum "cushion" between the roots. The number of layers of cementum in the various teeth corresponded to the order of appearance of the permanent teeth. That the layers in the cementum of red deer are annual ones is shown not only by studying the cementum of deer killed at various times of the year but also by studying the cementum of two deer of precisely known ages.

Mitchell suggested that the most convenient way of determining the ages of red deer is from the number of layers in the cementum of the first lower molar. As the first non-transparent band of cementum [page 120] appears at the age of between one year, eleven months and two years, six months, he considers it necessary to add one year to the number of layers of cementum.

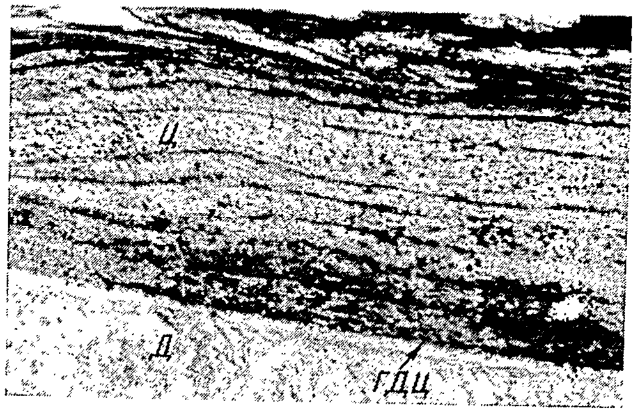


Fig. 62 [page 120]. Longitudinal section of lateral part of root of first lower incisor of spotted deer. Staining - hematovylin. (X60). For explanation see fig. 59.

Novakowsky (1965) discovered clear layers of cementum in the teeth of the bison Bison bison, which by analogy with the layers decribed in the deer he considered to be annual ones. The layers were seen on the surface of a bisected tooth partly decalcified and dried (the method is described in Chapter II). He counted the layers of cementum of the fourth lower premolar tooth and for determining the ages in years added four years to the number of layers, as the deposition of cementum in this tooth begins at 4 years of age.

Ransom (1966) and Gilbert (1966) described annual layers in the cementum of the teeth of white-tailed deer Odocoileus virginianus, having at his disposal material from animals of known ages. Ransom counted the layers on the surface of a longitudinal bisected first molar and Gilbert - those in a hematoxylin-stained longitudinal section of the first incisor. Both authors note that the non-transparent band of the first annual layer forms during the first summer of life of the individual.

When studying hematoxylin-stained longitudinal and transverse sections of the incisors and genal teeth of the spotted deer Cervus nippon we discovered similar clear layers of cementum as were described for the other deer (fig. 62).

Low and Cowan (1963) and McEwen (1963) recall that layers also existed in the dentine of the deer studied by them but they did not make a special study of these layers. We found clear layers of dentine in hematoxylin-stained transverse sections [page 121] of the incisors and cheek teeth of spotted deer Cervus nippon and a roe Capreolus capreolus of precisely known ages (fig. 63, 64). (Material presented by T.B.Sablina).

In the two-year-old roe, which was killed in August, there could be seen on the outside of the dentine of the first lower incisor, a more faintly-stained ring and on the inside a darker ring. In the inner ring there were two narrow heavily-stained bands, separating wider and faintly-stained bands - the two annual layers (fig. 64). In the dentine of the first lower incisors of five adult deer aged from 6 to 10 years could be seen a number of complete annual layers of dentine equal to the age of the animal. Permanent incisors in spotted deer replaced the milk ones at one to one and a half years of age (Mirolyubov, Ryashchenko, 1948). As the number of annual layers of dentine corresponded precisely to the age of the individual in years, the first annual layer of dentine evidently begins to appear when the tooth is still concealed in the alveolus.

In the periosteal zone of bone of Artiodactyla, as indicated by our studies, there are no annual layers from which the age of the animal can be determined.

The bone structure was studied (in slices and hematoxylin-stained sections) of the walls of the lower jaw in the area of the post-incisor diastema, the body of the jaw on a level with the dental wall and beyond the end of the dental wall, and at the base of the mandibular ramus in ten spotted deer of various ages. The ages of the deer were precisely known (material presented by T.B. Sablina). In general, the structural character of the mandibular wall was similar in the various sectors.

In young deer, in the course of appositional growth of bone, layers of macro-circular bone plates are laid down, separated by numerous circulatory canals (fig. 65a,c,), and in adult deer - layers of typical dense periosteal zone (typical outer general lamellae) (fig. 66a, b, c).

[page 122] As far as can be judged from the small volume of material at our disposal the layers deposited in the adult deer are annual ones (Klevezal', 1966). Parallel to the appositional growth, a rapid replacement occurs from within, first of the macro-circular plates and then of the dense layers of periosteal zone by osteal bone tissue. The layers of periosteal zone are therefore preserved for a very short period. The replacement of the periosteal zone by a mesosteal one proceeds more intensively in the lingual wall of the jaw than in the genal wall (fig. 65a, d). But here too, resorption takes place quite rapidly. Thus, in the genal wall of the post-incisor diastema of a six-year-old deer, quite a wide periosteal zone was seen with three adhesion lines separating the layers while in an eight-year-old deer this mandibular wall was already almost completely osteonized and in the very narrow periosteal zone could be seen not more than two annual layers (fig. 66a, b).

Fig. 63 [page 121]. Sector of transverse section of first lower premolar of eight-year-old spotted deer. Staining - hematoxylin. (X60). Numerals denote annual layers. For remaining designations see fig. 59.

A similar structure of the periosteal zone of bone also exists in the limb bones of ungulates (Klebanova and Kleveza!', 1966).

As we stated earlier a similar structure evidently exists in the periosteal zone of bone in Artiodactyla and partly in baleen whales.

The peculiarities in the structure of tubular bones of ungulates are explained by Ye. A. Klebanova (1965) as being due to their general biological peculiarities, in particular the early development in them of a capacity for movement.

Enlow (1962), following Amprino (1947) considers the varying growth rate to be an important factor in determining the structural differences in the bones of various species. According to Enlow, the amount of bone tissue deposited during a given period influences the type of bone tissue being formed. Layers of bone, separated by numerous radial and circular blood vessel canals (as was the case in the young deer in our material) are formed with the production of a large amount of bone [page 125] tissue per unit of time, and layers of dense periosteal laminated bone form when the growth of the bone is slow.

We are not going to discuss this question in detail here as it is a subject for special study. We can only conclude that when such is the nature of the age variations in the periosteal zone of bone, annual layers either do not form in it or are not preserved.

Thus, the ages of Artiodactyla can be determined from the number of layers in the dentine or dental cementum and cannot be determined from the number of layers in the periosteal zone of bone.

Fig. 64 [page 122]. Sector of transverse section of first lower incisor of two-year-old roe. Staining - hematoxylin. (X60). Numerals denote annual layers. For remaining designations see fig. 59.

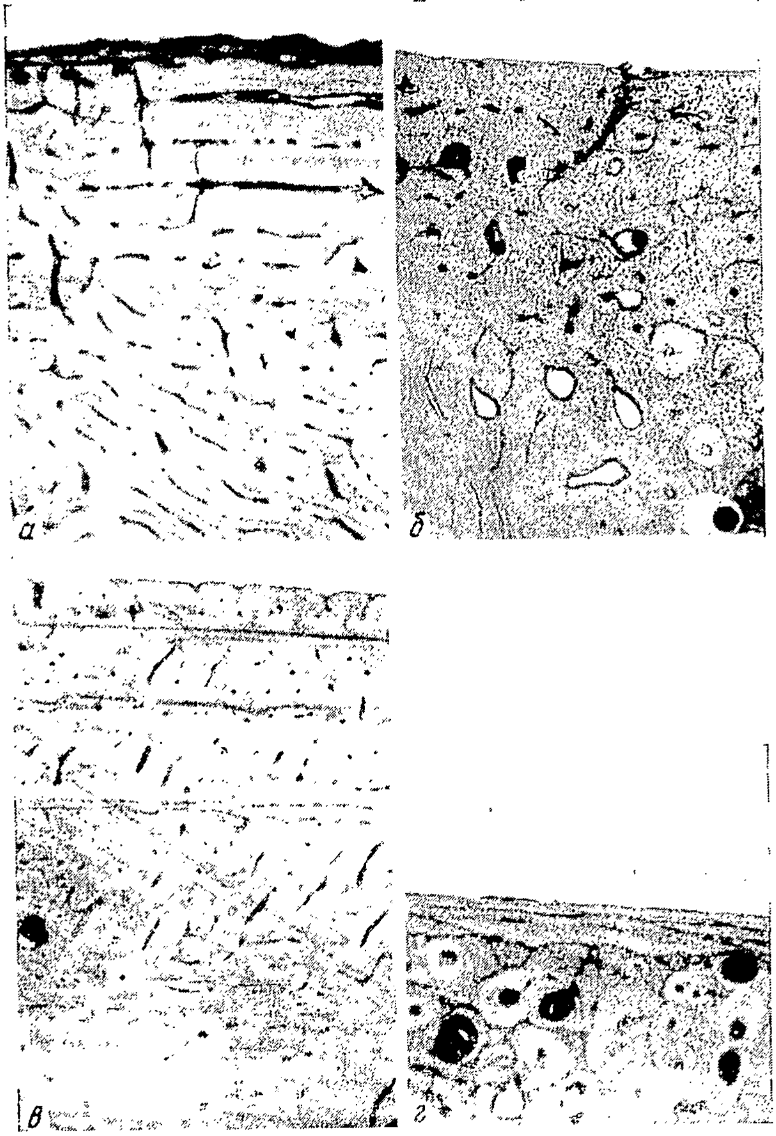


Fig. 65 [page 123]. Sectors of walls of rear incisor diastema of lower jaw of (a,b) two-year-old and (c,d) three-year-old spotted deer. a,c - genal wall; b,d - lingual wall. Transverse sections. Staining - hematoxylin (X34).

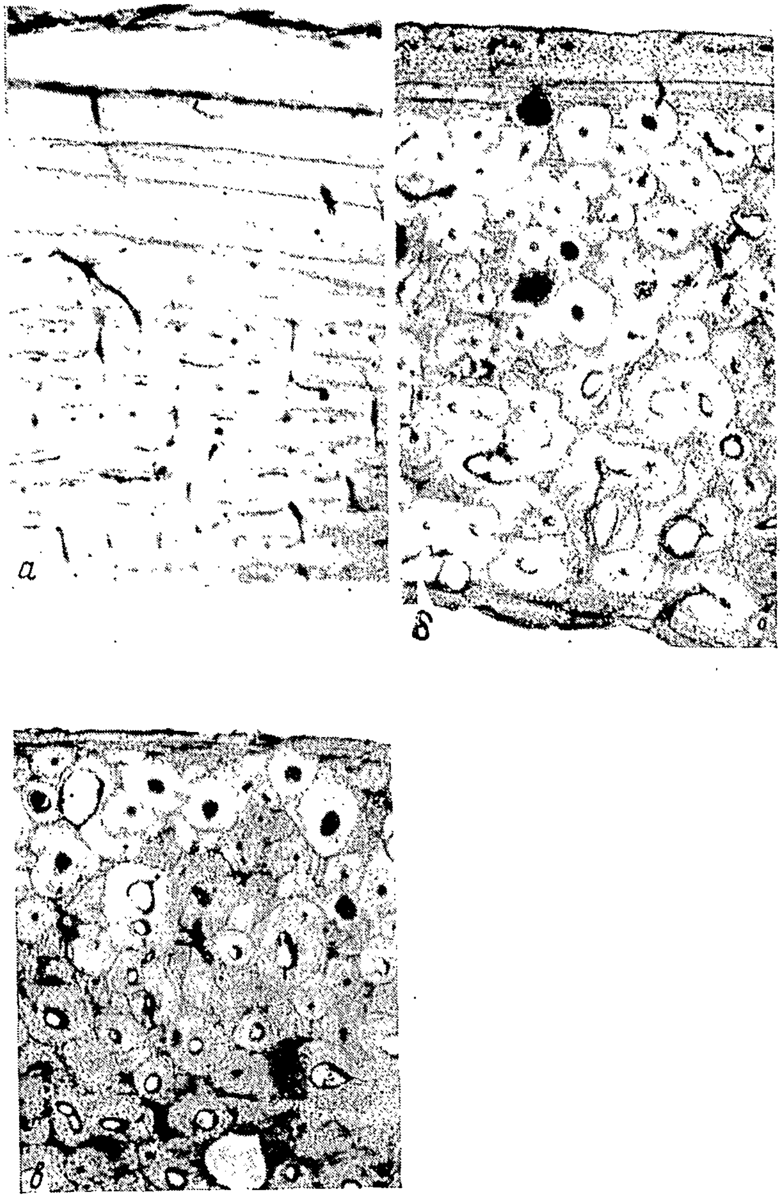


Fig. 66 [page 124]. Sectors of rear incisor diastema of lower jaw of (a) six-year-old and (b,c) eight-year-old spotted deer; a,b - malar wall, c - lingual wall. Transverse sections. Staining - hematoxylin (X34).

CONCLUSION [page 126]

The Possibilities of Using the Method

The method of determining the ages of mammals from the number of layers in the tissues of teeth and bone is the most universal and accurate one. Its universality consists in the fact that it has proved useful for determining the ages of representatives of all nine orders of the mammalian fauna of the USSR. Only in the case of one sub-order - the baleen whales - has it unfortunately proved inapplicable. At the same time, it is precisely here that an accurate and reliable method of age determination has long been vitally needed, for due to rapacious hunting, the reserves of baleen whales are on the brink of disaster. One can only hope that with more detailed study, the streaks described in Chapter III of the present work and seen in micro-sections of the mandibular walls of baleen whales will prove to be an indicator of age, whereupon the baleen whales will cease to be an exception.

At the present time, there is still not enough data to enable one to state definitely that the described method of determining the ages of mammals can be used for the tropical zone. Until recently, only one species was studied in this connection - the Hawaiian monk seal. Clear layers were found in its dental tissues (Kenyon and Fiscus, 1963). However, the geographical range of this seal embraces only the extreme northern part of the tropical zone. On the other hand, there are annual layers in the bones of some typically tropical poikilothermal animals (Peabody, 1961; Warren, 1963). Peabody (1961) associates the formation of these layers with the alternation of wet and dry periods of the year. In those places where there are no contrasting seasons, annual layers cannot form at all or else cannot form under the influence of seasonal cycles of sexual activity. It is entirely probable that a similar picture will be observed also in the case of the mammals of the tropical zone.

As we have already said, the described method of age determination of mammals is not only the most universal one but also the most accurate. Here, however, it must be stated that its degree of precision is not the same for all animals, For mammals [page 127] whose length of life is counted in years and sometimes even tens of years, which breed once a year during specific and brief periods (for example ungulates, carnivores and pinnipeds), the method is very precise. It permits the individual age of each animal to be determined to the nearest month. In animals having a long life span but in which the breeding periods are protracted, for example the toothed whales, the method provides for age determination with an accuracy of only a year.

The method is less precise for age determination of small mammals living one or two years and breeding several times in the course of a year. In such animals the method only enables one to establish whether or not a particular animal has wintered. However, even in this case the method is of great value for it enables us to say with absolute certainty what we are dealing with: an over-wintered animal, or an adult animal of a particular year of birth. In many cases the ascertaining of this fact is very important.

In some cases, the use of the method of age determination from the number of layers has proved inadvisable. Thus, in ordinary shrews which live no more than two years, there are distinct layers in the dentine, cementum and periosteal zone of bone. But the previously elaborated method of determining the ages of these animals from the size and state of the sexual system and the degree of wear of the teeth (Dunaeva, 1955) enables one to distinguish the over-wintered animals from those which have not wintered just as precisely as do the data on the structure of the dentine, cementum and bone.

In muskrats, the annual layers in both the bone and the cementum are difficult to count because the bone contains many double and even triple lines and the layers in the cementum are too narrow. The probable errors in counting connected with this, together with the errors connected with the muskrat's protracted breeding period make the method of determining the ages of muskrats from the number of layers no more accurate than the previously elaborated method of age determination from the height of the crown and degree of development of the dental roots (Tsyganov, 1955; Smirnov and Shvarts, 1959).

In general, it can be stated that for the overwhelming majority of mammals the method of age determination from the number of layers, enabling the individual age of each animal to be established, is the most accurate one. We hope that in the near future it will become the main one for determining the ages of mammals of various systematic and ecological groups. The advantage of this method over the others is so obvious that when about to make a study of a particular species, it will probably first be necessary to verify the possibility of determining the ages of these animals from the number of layers in the tissues of teeth or bone, and only after obtaining a negative result, to attempt to use other methods.

At any rate, this method became generally recognized through studies of toothed whales and pinnipeds. However, in studying [page 128] the terrestrial mammals the method has not only not yet come into general use, but for many animals has not even been worked out.

The single major shortcoming of the method of age determination from the number of layers - the somewhat complicated procedure for obtaining the preparations, in the case of a number of species - should not, in our view, be a hindrance to its extensive use. In a number of cases the layers are seen in thin sections and half-teeth, but even in those cases when it is necessary to make thin stained sections, the method of preparing them is not so complicated as to be unacceptable to zoologists and inapplicable under field conditions. After readying the appropriate apparatus and mastering the process of preparing sections the entire procedure of determining the age from the number of layers is no more complicated and difficult than for example, age determination from the weight and dimensions of the skull. In research institutions where many research workers are faced with the task of determining the age of different species of animals it may be advisable to create a special laboratory point to which material could be brought and from which the necessary preparations could be obtained for counting the layers.

Finally, we would point to several further fields in which age determinations from layering in the tissues of teeth and bone could be of invaluable help. First and foremost is the field of palaeontology in which new forms are frequently described on the basis of disconnected remains. It is precisely here that it becomes especially difficult to establish that the differences discerned between two forms are not age differences. For paleontologists, the method of determining age from the number of layers in the tissues of teeth and bone is, in our view, an extremely attractive one, as in the first place it is precisely the teeth and bones that are preserved in the excavated remains and secondly, by means of this method the precise age of an individual can be determined from a separate tooth or a separate bone. The ages of excavated poikilothermal vertebrates are currently being determined from the number of layers in the bone (Peabody, 1961). But there is currently not a single paper describing age determination of excavated mammals from the number of layers in the teeth or bone.

In archaeology, the elaboration of the method of determining the mammals (domestic and wild) used by man in the past from the bone residues found in diggings (Tsalkin, 1956) has recently been rapidly developed. The proposed method of age determination also enables these bone residues to be used for determining the age of the animal. It is believed that determining the ages of these animals could be a valuable addition to the overall picture of hunting in the past.

APPENDIX [page 129]

Determination of the Growth Rate and Age of Sexual Maturity by Means of the Layers in Teeth and Bones.

The existence of annual layers in the tissues of teeth and bone provides investigators with an opportunity of retrospectively evaluating the rate of growth of an individual during its lifetime. In transverse sections of teeth it is seen that the width of the annual layers of dentine diminishes with age with the result that sometimes, beginning with a particular annual layer, the reduction in the width is especially well marked. This suggests that the rate of deposition of the dentine runs parallel to the rate of growth of the entire organism not only in the sense of seasonal variations in the growth rate but also in the course of the entire lifetime of the individual and that the marked reduction in the deposition of dentine can be associated with a reduction in the overall growth rate of the individual with the attainment of sexual maturity.

In three species of seals it was shown (Klevezal', 1964) that there is in fact a marked reduction in the growth rate of the dentine of canines during the period corresponding to the approach of sexual maturity.

Based on an analysis of the growth rate of the dentine of the canines of twenty male and twenty female ringed seals and a similar number of specimens of ribbon seals as well as ten male and ten female largha seals of the Okhotsk Sea, the time of the approach of sexual maturity in these seals was determined. Subsequently published papers, in which on the basis of a large volume of material an analysis was made of the state of the sexual organs in various age groups of the ringed seal of the Okhotsk Sea (Fedoseev, 1964, 1965), and the ribbon seal (Shustov, 1965), indicated that in general the period of approach of sexual maturity had been correctly determined.

Thus, based on the rate of growth of the dentine it was calculated that male and female ribbon seals as a whole attained sexual maturity at three years of age, and that 27% of the females in the experiment and 47% of the males became sexually mature a year later. A.P. Shustov (1965) writes that more than half the female ribbon seals are ready for ovulation at two years of age and that all the females are ready for ovulation at three years of age; cases were noted when [page 130] the first pregnancy occurred at four years of age. Spermotozoids were found in 43% of the three-year-old males and in 90% of the four-year-old ones.

For the Okhotsk Sea ringed seals it was established from the rate of growth of the dentine that both males and females attained sexual maturity by seven years of age. But the times of approach of sexual maturity are extended; a very early pubescence can occur at five or even four years. According to the findings of G.A. Fedoseev (1964, 1965), ringed seals in the Okhotsk Sea attain sexual maturity from the fifth or sixth year of age and after seven years all the animals are sexually mature.

The slight discrepancy in the times calculated on the basis of the growth rate of the dentine of the canines and those determined by G.A. Fedoseev and A.P. Shustov is explained by the fact that in the former case there was much less material available.

In the cited paper (Klevezal', 1964) calculation was made of the rate of deposition of the dentine of canines which had previously ceased growing in length and were not subject to intensive wear. The calculation procedure was as follows.

The sections and preparations used for determining the rate of deposition of the dentine were the same as those used in determining the ages, i.e. the canines were cut across on the level of the alveolus. From all the material available only sections and preparations were selected in which the boundary between the annual layers of the dentine was readily seen. As it is practically impossible to measure the absolute quantity of dentine deposited each year its relative index was used. The amount of dentine deposited during the year was evaluated from the width of the annual layer measured along the short axis of an ellipse of micro-slide. This index gives an accurate reflection of the annual amount of dentine. The width of the annual layer of dentine in the first year of life is less than that of the second, but during the first year of life of an animal a tooth grows in length intensively so that the total amount of dentine deposited during the first year is clearly more than the amount deposited during the second year. Therefore, so as not to distort the general picture, the rate of deposition of dentine during the first year of life of an animal cannot be determined as the derived figure would clearly be understated. To make up for this, the rate of deposition of dentine during subsequent years - the seventh, eighth - etc. - turns out to be slightly higher, as the dentine is deposited over a smaller area than in the initial years (as can be seen in fig. 1a). The width of the annual layers during the remaining years fully reflects the rates of dentine deposition. Measurement of the widths of annual layers is carried out under a binocular eyepiece micrometer.

Various methods can be used for estimating age-variations in the rate of dentine deposition on the basis of alterations in the width of the annual layers. In the above-cited work (Klevezal', 1964) the rate of dentine deposition was determined from the formula for the growth rate (Shmal'hauzen, 1928)

$$C_v = \frac{\log V_2 - \log V_1}{0.4343\,(t_2 - t_1)}$$

Instead of the body measurements the total width of the dentine S was substituted in the formula. Since [page 131] the accretion of dentine for each year was being considered the magnitude of the period $(t_2 - t_1)$ equals 1. The formula takes the form of

$$C_s = \frac{\log S_{n+1} - \log S_n}{0.4343}.$$

where S_n is the amount of dentine deposited over n years (width from the outer edge of the microslide to the end of the nth annual layer), and S_{n+1} is the amount of dentine deposited over n+1 year (width of dentine from the outer edge of the microslide to the end of the n+1st annual layer). [page 132].

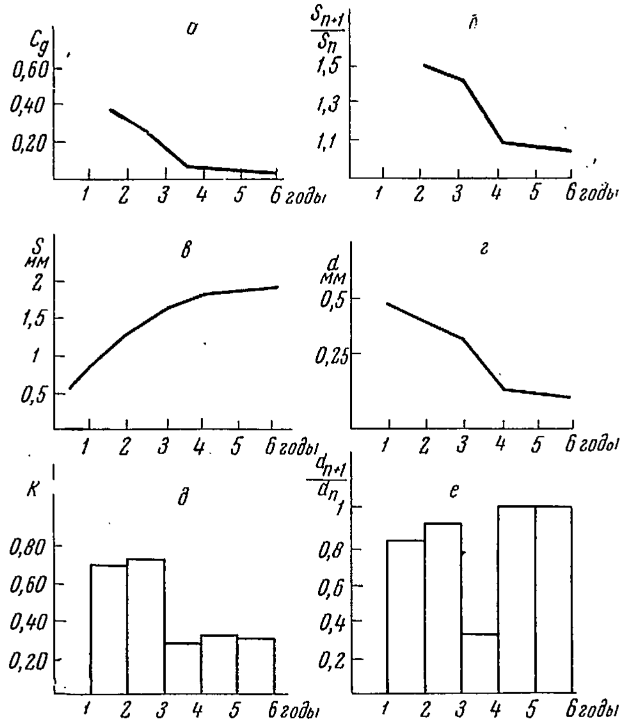


Fig. 67 [page 131]. Different ways of expressing age variations in the rate of deposition of dentine of the same tooth (canine of male ribbon seal No. 159) a - change in growth rate C_s; b - change in ratio of dentine width in a given year to its width in the following year $\frac{Sn + 1}{Sn}$ c - change in total width of dentine S; d - change in width of annual layers d; e - magnitude of growth constant K in different age groups; f - ratio of width of an annual layer for a given year to width of preceding annual layer $\frac{d_n + 1}{d_n}$ in different age group.

The rate of growth C_s pertains to the middle of the year. After obtaining numerical data a curve was constructed showing variations with age in the rate of deposition of dentine C_s, and the period of approach of sexual maturity was determined from the sharp alteration in the direction of the curve (fig. 67a). I.I. Shmal'hauzen (1928), - K = Ct. The period of approach of sexual maturity is denoted by a sharp reduction in the value of the "constant" (fig.67d).

The above formula for the rate of growth of dentine (1) represents the relationship $\frac{Sn + 1}{Sn}$, expressed logarithmically and divided by 0.4343.

Evidently, the character of the curve of age variations in the rate of deposition of dentine does not alter if along the ordinate axis is plotted not the magnitude of C_s but the magnitude of the relationship $\frac{Sn+1}{Sn}$.

The general character of age variations in the rate of deposition of dentine is also reflected by a curve showing the variation with age of the total width of the dentine and a curve of age variations in the accretion of dentine (width of the annual layers). After comparing the four curves, characterizing the age variations in the rate of deposition of the dentine of the same tooth (fig. 67a - d), it can be seen that all of them: the curve of the variation in the growth rate (fig. 67a), the curve of the variation in the ratio of the total width of the dentine in a particular year to its width in the preceding year (fig. 67b), the curve of the variation in the total width of the dentine (fig.67c), and the curve of variation in the width of the annual layers (fig. 67d) have an inflection in the fourth year. (The slight displacement to the left in the curve of the rate of growth is due to the fact that the rate of growth refers to the middle of the year for which it is computed and the indices of all the remaining curves refer to the end of the year).

For determining the period of the sharp reduction in the rate of deposition of the dentine it is also possible to calculate the ratio of the accretion of dentine for a given year to the accretion in the preceding year i.e. the ratio of the width of the n + 1st annual layer to the width of the nth annual layer. On the histogram constructed by this method (fig. 67f) the time of the reduction in the rate of growth of the dentine (the period of the approach of sexual maturity) appears even more clearly in the histogram of the "growth constant" (fig. 67e).

If more precise calculations are used and the volume of dentine deposited in the course of a year is taken into account directly, then it appears that the curve of the dentine deposition with age will change the angle of incidence even more sharply during the period of approach of sexual maturity than when calculating only on the basis of the width of an annual ring.

When calculating the growth rate of dentine in teeth with a limited but protracted growth in length (in teeth of the type shown in fig. 1b) it is evidently necessary to compute the area of the annual layer on a transverse section of tooth, since the alteration in width of an annual layer with age does not reflect the age variations in the volume of dentine deposited.

/page 133/ The foregoing discussion refers to teeth not subject to intensive wear. Evidently, in teeth with a heavy mechanical load, the annual volume of dentine deposited is greater than in teeth carrying a small load. But it is probable that in this case as well the period of the general slowing down in the growth of the animal in connection with the approach of sexual maturity is reflected in the structure of the dentine in the form of a deceleration in the rate of the deposition.

Cementum can serve as an index of alterations in the overall character of the growth of an individual during its lifetime to a lesser extent than dentine as its total thickness and the width of the annual layers are dictated by the magnitude of the mechanical load on the teeth.

In some cases, a marked reduction with age in the width of the annual layers is also observed in the cementum, which could be connected with the approach of sexual maturity. Thus, Mundy and Fuller (1964) point out that the first five annual layers of cementum of the canines of the grizzly bear are wider than the subsequent ones and that this might be connected with the period of approach of sexual maturity. But at the same time, in many species of animals the width of the annual layers of cementum not only does not increase with age but even increases somewhat as, for example, in beaver and large mouse-eared bat (Myotis myotis).

Moreover, it must be taken into consideration that, as distinct from dentine, each successive annual layer of cementum is deposited over a somewhat larger area than the preceding one and therefore even when the volume of deposited cementum is identical the width of each succeeding layer may be somewhat smaller.

The overall width of the periosteal zone of bone is evidently also determined by the magnitude of the mechanical load. But we did not observe a single instance when the width of the annual layers of periosteal zone remained constant with age or increased. In all cases, the widest ones were the first annual layers and with age the width of the layers decreased. Moreover, in animals with a long life span a period could clearly be distinguished when the width of an annual layer decreased especially sharply and later - only slightly. Thus, in ringed seal a reduction in the width of the annual layers occurs in the seventh year and in beaver - in the third or fourth year. As already stated, according to the findings of G.A.Fedoseeva (1964, 1965) all ringed seals attain sexual maturity by the seventh year. In beaver, according to L.S. Lavrov (1956), of the two-year-old females not more than 8% breed and starting from the third year and above 50-75% of them breed. These data indicate that the marked reduction in the width of the annual layers of the periosteal zone approximately coincides with the period of the approach of sexual maturity.

All this compels us to assume that while the overall width of the periosteal zone is dictated by the magnitude of the mechanical load, a change in the width of its annual layers is a reflection of age variations in the rate of growth of the animal. If this is so, then as in [page 134] the case of the dentine, from a change in the width of the annual layers of the periosteal zone we can calculate changes in the rate of growth of the animal. This is particularly valuable for studying the character of growth of those species in which dentine cannot be used by reason of peculiarities in the dental structure, for example in beaver.

The periosteal zone is a somewhat worse index of the rate of growth of an individual than dentine, as in time the first annual layers are resorbed and replaced by osteal bone.

Thus, the alteration with age of the width of the annual layers of dentine as well as, probably, the periosteal zone of bone fix the age variations in the overall growth rate of the individual, particularly the reduction in the rate of growth with the approach of sexual maturity. This provides an opportunity of assessing the rate of growth of each individual under natural conditions i.e. of obtaining information which would be difficult to collect in any other way. Such a method of determining the time of approach of sexual maturity enables one to ascertain the time of the approach of sexual maturity in individuals of a given species, even when limited to craniological materials from sufficiently old animals. This method is particularly valuable for studying fossil mammals as it is evidently impossible to establish by any other method the rate of growth and time of approach of sexual maturity in extinct species.

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