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Age Determination of the Hare from Annual Layers in the Mandibular Bone

Oznaczanie wieku zająca bielaka na podstawie rocznych warstw kostnych żuchwy

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Ohtaishi N., Hachiya N. & Shibata Y., 1976: Age determination of the hare from annual layers in the mandibular bone. Acta theriol., 21, 11: 168—171. [With Plate II].

To establish of an exact and rapid age-determination technique for hares, the adhesion line on the mandible bones of the 14 known aged ainu hare (*Lepus timidus ainu* Barret-Hamilton, 1900) were examined. In the central part of the medial side of the mandible, the adhesion lines are regularly formed every winter.

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The establishment of an exact age-determination technique for hares and rabbits is of fundamental importance to an analysis of the age structure of a population and an estimate of the birthrate, and in consequence to forecasting population variation. Many methods have been investigated, *i.e.* using the ear length (Tiemeier & Plenert, 1964), the hind foot length (Bujalska, Caboń-Raczyńska & Raczyński, 1965), the closure and thickening of the humeral or ulnal epiphyses (Bujalska *et al.*, 1965; Connolly, Dudziński & Longhurst, 1969), the ossification of the skull and pelvis (Bujalska *et al.*, 1965), the lens weight (Bujalska *et al.*, 1965; Connolly *et al.*, 1969; Rongstad, 1966; Tiemeier *et al.*, 1964), *etc.* But all of the techniques were relative methods and did not exactly define the absolute age. For example, the method usually applied to jack rabbits was a technique using the closure of the humeral epiphysis. According to this method,

jack rabbits were divided into three age classes: under 7 to 9 months old, about 1 year old and less, and over 1 year old (Connolly *et al.*, 1969). Since Lord (1959) first reported the lens weight method for cottontail rabbits, this method has been applied for many hares and rabbits as a superior technique to the others (Connolly *et al.*, 1969; Tiemeier *et al.*, 1964). This method, however, is also a relative one, and retains the following problems: the precision of this method decreases with advancing age, and the growth curve of the lens weight is variable in each population, and the frozen lenses would be of little value in determining age (Pelton, 1970).

As for carnivorous and hooved mammals, recent investigations have established a useful method for age determination using the annual layers in the dentine or cementum. This method cannot be applied to the *Lagomorpha*, because their incisors and hapsodont molars continuously grow and cannot retain annual layers even if such layers are formed.

According to Bernstein & Klevezal (1967), so called adhesion lines are formed like those of the annual layers at the periosteal zone in the mandible of red pikas and long-eared pikas. We investigated the mandibles of known aged ainu hares (*Lepus timidus ainu* Barrett-Hamilton, 1900) (Abe, 1931), and tried to establish an exact age-determination method.

Materials were 14 ainu hares ranging from 5 months old to 7 years and 10 months old that were born and raised in pens. They were born from May to July and killed from February to October. They were body weight 2050—3000 g, head and body 470—550 mm, hind foot 140—145 mm, ear length 72—77 mm. Their measurement values of the bodies showed individual variations, but not regular age variation. Their mandible measurements also showed a similar variation.

Because it can be thought that adhesion lines run parallel with split lines and that they appear most clearly in the sections which cross the split lines at a right angle, the split lines of the hare mandible were at first investigated by the usual method. From the direction of the split lines the cutting directions were set on a mandible (Fig. 1). Bone blocks were cut out of the unfixed dry mandibles with a dental circular saw, as shown in Fig. 1. Two kinds of sections were made: one was the thin section made by the usual paraffin method, and the other was a thick one. The latter was embedded in polyestel-resin (Rigolac 2004 W obtained from Showa highpolymor Co.) and the cutting surface was first polished with a coarse-grain stone and later with aluminum No. 1000 on a glass plate by hand. Then the polished surface was decalcified with Plank-Rychlo solution for 5—15 minutes, stained with Mayer's hematoxylin, and examined under a binocular dissecting microscope with a reflected light. This time the adhesion lines looked clearer if a drop of glycerin was spilled on the surface. Though the lines do not appear so clearly, it is possible to omit the embedding procedure and to count the annual layers (adhesion lines) by using the simple method of polishing the cutting surface on a fine-grain stone.

An adhesion line is formed every winter season, and occasionaly some lines (pre-winter lines) are formed in the rapid growth period before the first winter of the hare's life. It is considered, therefore, that the lines represent a compact layer of the bone structure formed during the re-

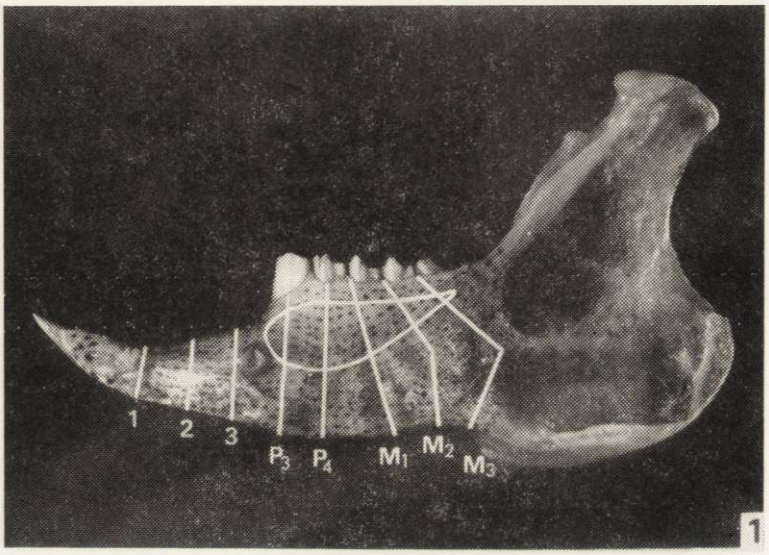
tardation of growth. Almost all parts of the periosteal zone of the mann-an-dible were observed, and the central part (the region encircled by a whititehite line in Fig. 1) of the medial side of the mandible was proved to be thethe best place for counting the annual layers.

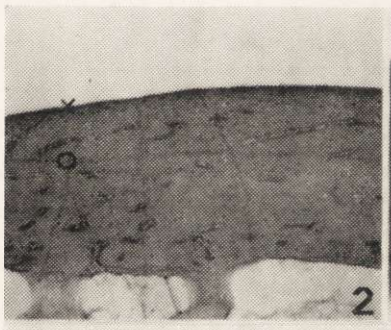
In other parts, the adhesion lines could not be counted as the annuaalual layer. The periosteal zone of the upper part of the intermediate sectioortion of the interalveolar (shown as 2 in Fig. 1) clearly showed a set of adhesioorsion lines which represented the number of winters passed by hares oldderder than one and a half yaers. Though one or two pre-winter lines disappeaapear by the second winter, they cannot be discerned from the true annuaalual adhesion lines. As for the lateral portion of the interalveolar part (fropmrom 1 to 3 in Fig. 1), the adhesion lines were also clear, but in some caseesses, the pre-winter lines remained for more than a few years, and in otheerher cases of aged hares, the lines made in the first or second winter disappeaar-ar-ed. On the under part and the inner endosteal zone of the alveolaarlar cavity of the cheek teeth (from P_3 to M_3 in Fig. 1), the layers of earlyrly years (winters) were occasionally absorbed in the older hares, and thethe pre-winter layers were not usuallly formed. The lateral part of thishis portion — the periosteal zone — is not compact and the split lines do naotnot run regularly. The adhesion lines were narrow and indistinct in thishis area. When they could be counted, the number was usually more thaaman the true age. The layers, therefore, appeared to be irregularly formeedned on this loose surface bone. On the other hand, in the central regiconion of the medial side (the encircling white line in Fig. 1), the adhesion linaesnes were regularly formed (Figs. 2, 3 & 4). A light part formed between thethe first winter line and the pre-winter line was from 150μ to 300 μ in widtth,lth, and the space between the first winter line and the second winter onneone was 40 μ—80 μ in width. Succeeding lines were formed at rather equaualual intervals of 10—30 μ each winter. Spaces between the lines at aboouiout M_2 and M_3 were narrower than those at P_3 and P_4, and the length of of the former lines was so much shorter than that of the latter, that coumt-nting the lines was occasionallly difficult in the molar region.

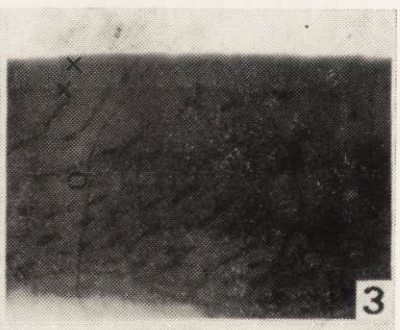
EXPLANATION TO PLATE II

Fig. 1. Photograph of the split lines of the medial side of the mandible.
White lines show the observed sections. These sections crossed the split line at at a right angle. The best region for counting the annual layers, and that which giwesives the most compact periosteal zone is encircled by a white line. The split lines showow a relatively regular direction, and there is no musclar attachment. As a routiinetine technique, lines P_3 and P_4 were cut.

Fig. 2. Transverse sections of hare mandibles in the P_4 region. 8 months old (bornorn May 20th, 1971 and killed Feb. 17th, 1972). 50.
Fig. 3. 2 years and 5 months old (May 13th, 1971 — Oct. 22th, 1973). 50×.
Fig. 4. 7 years and 10 months old (June, 1966 — Apr. 8th, 1974). 100×.
Figs. 2 & 4 are thin paraffin sections stained with hematoxylin; Fig. 3 showsows a thick section of which only the surface was finely polished ancand stained. The dark layers (×) are adhesion lines formed during the wintenter season. In February and April they are already formed (each exterior layer of of Figs. 2 & 4), but are not formed in October (Fig. 3). Figs. 2 to 4 show layers 1,, 2, 2, and 8, respectively. The dark layer (o) is also an adhesion line formed in the rapidapid growth period before the first winter. It can be discerned from the true layerers as having a 150 μ—300 μ wide light belt in the inner part of the first winter lineine. The line disappeared in the older hares (Fig. 4).







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Considering the results of each section, the section at P_3 and P_4, was best for counting the annual layers; consequently, as a routine method, we decided to use only a part between the lines of P_3 and P_4 in the encircling white line in Fig. 1. By using the surface staining method, about twenty hares can be aged in a day, and if using the simplified method without embedding, twice as many may be done.

We applied this technique to seven wild ainu hares in Hokkaido and to forty wild etigo hares (*L. bracyurus etigo* Abe, 1918), (Abe, 1931) from Niigata prefecture in Honshu. They also showed exactly the same pattern of the adhesion lines as that of the present observation. And in the case of wild hares, the adhesion lines were stained clearer than those of those raised in pens.

It is necessary to point out the following problems when applying this technique. The annual layers usually appear with regularity in the central part of the mandible, but occasionally they are partially missing on the upper or lower portion. At the uppermost region of this portion, the lines are not regularly formed and usually show more than the actual number of years. In some sections, one line is divided into two lines, or two lines are combined into one. In such cases, both sides of the sample block should be observed or be further ground until a new surface appears.

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