Zellen nacheinander ihrem Alter entsprechend in das Stadium des maximalen Zuwachses treten, zuletzt die jüngste (I), etwas früher III und II (diese beiden in der wiedergegebenen Messung mit nicht genauer, dem Alter entsprechenden Reihenfolge II–III), noch früher IV und V und schon bald nach Meßbeginn VI. Die Wachstumsintensität der ältesten Zellen (VII und VIII) derselben Zellreihe ist dagegen von Beginn der Messung an bereits in Abnahme begriffen.

Vergleicht man den maximalen Zuwachs einer Zelle mit demjenigen eines Wurzelhaares derselben Wurzel, so ergibt sich, daß die Wurzelhaare beträchtlich schneller wachsen.

E. HEITZ

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Botanisches Institut der Universität Basel, den 30. März 1950.

Summary

It is shown that by direct measuring of the subsequent cells in the growth-zone of the living root-tip of *Melandrium album* the great period of growth of each single cell in a cell-row can be observed. The maximum of the growth during 1–2 hours in a cell-row is restricted to one or two cells only. The duration of a one-cell period lasts about 6 hours.

The intensity of growth of a root-hair is greater than the maximum growth of a root-cell in the growing zone.

X-Ray Diffraction and X-Ray Absorption Studies of Immobilized Bones

Observations of MEYER, WOLFF and ROUX[1] revealed a correlation between the gross architecture of bones (structure of 1st order) and the mechanical forces acting on them. Changes in the direction of external forces are followed by changes in thickness of the compacta of the bone and by rearrangements of the trabecular net work. A correlation also exists between the amount of osseous tissue formed and the magnitude of mechanical forces. The resorption of a bone that is no longer subjected to a mechanical stimulus is an indication of this relationship. The resorption is more pronounced in the metaphysis, where the blood supply is greater[2].

The *extrinsic* factors, therefore, seem to be important for the regulation of the quantity and spacial arrangement of the osseous substance. On the other hand, it is clearly shown that bones can grow also when no external mechanical forces are present. The shape of bones developed only under the influence of *intrinsic* factors is similar to the shape developed under normal physiological conditions[3]. MURRAY and SELBY[4], who investigated bone formation in chorio-allantoic grafts of femora of 6–7 days chick embryos, express the view that after birth and in the growing and adult individual the extrinsic factors exert a greater influence than during the embryonic development (see also GLUCKSMANN[5]).

From the investigations quoted above it is clear that the external mechanical forces acting on a bone have an influence on the structure of the 1st order. Contradictory results, however, were obtained when applying the same principle to the arrangement of the Haversian systems (structure of 2nd order), to the bundles of collagen fibres embedded in the bone substance (structure of 3rd order) and to the ultrastructure (structure of 4th order). An effect of the external mechanical forces on the structures of 2nd and 3rd order was reported by several authors[1] while others found no effect[2]. Different opinions concerning the influence of external forces on the ultrastructure of bone have also been reported. HENNY and SPIEGEL-ADOLF[3] expressed the view that the function rather than the shape of the bone determines the orientation of its structure. LAMARQUE[4] reported that the ultrastructural orientation is independent of the mechanical forces acting on the bone. A similar result, although not conclusive, was reported by REED and REED[5].

The present investigation, which is a part of an extensive study on the ultrastructure and the distribution of mineral salts of different bones, is an attempt to give a definite answer to the question: Do external mechanical forces influence the ultrastructure of bone and the distribution of mineral salts of the structures of 2nd order? The ultrastructure is studied by X-ray diffraction and the composition of the Haversian systems by quantitative microradiography as developed by ENGSTRÖM[6].

Material[7]. Bones from two dogs were used for the experiments. In one 30 day old dog (A) the left anterior limb was firmly fixed to the body in a narrow cutaneous pocket, preventing any active movements of the leg. The whole leg could be considered practically inert. The dog was sacrificed six months after the operation and the bones of the inert and normal limbs were freed from soft tissues, weighed and measured (cf. Table I). The bones were fixed in alcohol. In the other dog (B) the nerves of the brachial plexus on the left side were severed, causing a complete paralysis of the corresponding limb. The muscles showed an extensive fatty degeneration at autopsy, which was performed 125 days after the operation. Lengths and weights of the bones are found in Table I.

From the middle of the diaphysis of humerus and radius on both sides of dog A longitudinal sections about 100 μ in thickness were prepared for diffraction analysis. Cross sections from the same areas were prepared for historadiography. From the fifth metacarpus on both sides of dog B longitudinal sections were prepared for X-ray diffraction analysis.

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[7] The material was kindly forwarded to us by Dr. F. VIGLIANI, Torino, who is studying the structural changes in bones developing in immobilized limbs. We wish to express our sincere thanks to Dr. F. VIGLIANI.

Table I

Lengths and weights of normally functioning and mechanically inert bones.

	Dog A				Dog B			
	Inert limb		Control limb		Inert limb		Control limb	
	Length cm	Weigth g	Length cm	Weight g	Length cm	Weight g	Length cm	Weight g
Humerus . .	10·8	15·5	10·4	25·0	10·3	15·0	10·3	31·0
Radius . . .	10·8	5·0	10·0	9·0	10·8	6·8	10·4	12·5
Ulna	13·7	7·0	13·7	10·5	12·6	6·2	12·5	13·7

The present knowledge of the appositional growth rate and the rebuilding rate of long bones in the dog[1] indicates that the period of immobilization was long enough to ensure that all material in the compacta of the bones had been laid down during the period of immobilization.

Method: Microradiography. When recording the X-ray absorption pictures of the thin ground bone specimens the procedure schematically shown in Fig. 1 was used. The section, *D*, 50 to 100 μ in thickness, was pressed against the emulsion side, *E*, of a Lippmann film. On

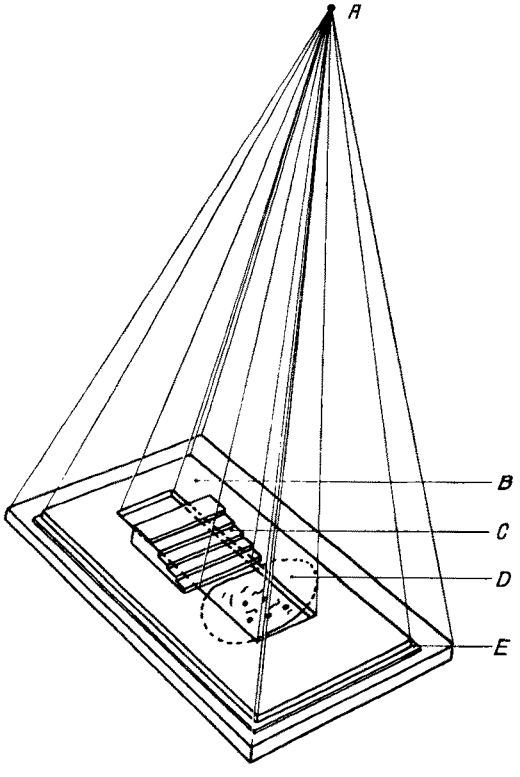

Fig. 1.

the upper surface of the metal holder, *B*, a step wedge made of collodion foils, *C*, was placed. Cf. ENGSTRÖM and LINDSTRÖM[2]. The distance from the photographic plate to the target of the X-ray tube, *A*, was about 5 cm, giving a geometrical unsharpness in a 50 μ thick sample of about 5 μ. The film emulsion had a resolving power of about 2 μ. The X-rays were generated at 5,000 volts and 30 milliamps and filtered through 1 mm Be. The time of exposure varied with the thickness of the specimen but was about 1 hour. The X-rays used had an effective wavelength of about 2·5–3 Å, lying on the short wavelength side of the K-absorption edge for calcium and giving a maximal differential absorption. The comparison between the density of the microradiographic image of the sample and the step wedge gave the X-ray absorption in different points of the sample expressed in units of the step wedge.

The thickness of the specimen may vary in different parts. However, the thickness of a Haversian system and of the neighbouring old bone can be regarded as the same, since they were only 0·1 mm apart. To get comparable values, therefore, the density of each Haversian system was divided by the density of the neighbouring old bone. The density of the primary bone was relatively constant[1]. The quotient thus obtained has the numerical value of 1 if the content of absorbing substances is the same in the Haversian system as in the fragments of old bone. The younger the Haversian system, the lower the quotient. Generally values between 0·75 and 1 are obtained. When this quotient is measured for a great number of Haversian systems and the mean is calculated, this mean is an expression of the rate of new formation of osteons. Details will be published later[1].

Diffraction. The microcamera described by CHESLEY[2] was used to record the diffractograms. The area used in the specimen was about 10 μ in diameter and was selected under the microscope. Cu Kα radiation, filtered in Ni was used.

Table II

Ratio between the content of mineral salts in primary periosteal bone and in secondary bone (Haversian systems).

Radius Dog A		Humerus Dog A	
Inert limb	Control limb	Inert limb	Control limb
0·93 ± 0·01	0·93 ± 0·01	0·92 ± 0·008	0·93 ± 0·007

Results. Table II gives the values of the quotient in density between the Haversian systems and the neighbouring fragments of old bone. The values from bones in the normal and the immobilized side are identical, indicating that the rate of new formation of Haversian systems is the same in the two instances. This finding seems to be in agreement with the identical uptake of

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radioactive phosphorus in active and inert bones[1]. In Fig. 2 photomicrographs of microradiograms from cross sections of bone from dog A are shown. The difference in absorption between the primary and the

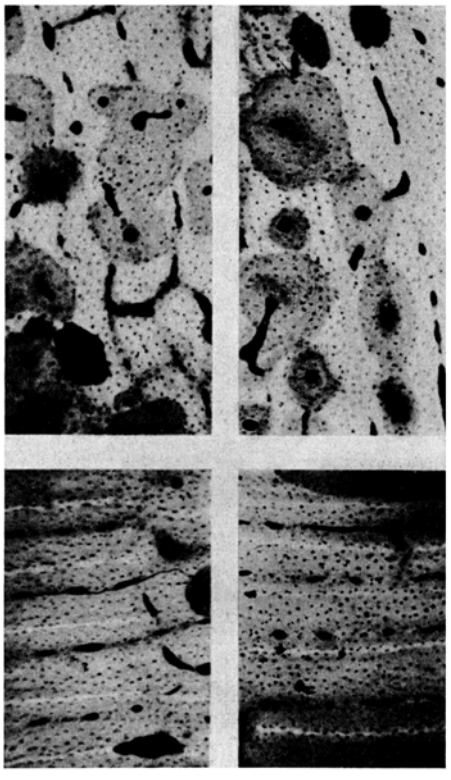

Fig. 2.

secondary bone and between different Haversian systems is apparent. When the sections were decalcified no contrast at all was obtained. Therefore the pictures show the distribution of mineral salts in the tissue.

Table III

Comparison between spacings in normally functioning and mechanically inert bones. Longitudinal sections

Ring	Spacing Å					
	Humerus Dog A		Radius Dog A		Metacarpus Dog B	
	Inert limb	Control limb	Inert limb	Control limb	Inert limb	Control limb
1			3·76	3·76	3·75	3·69
2	3·39	3·35	3·39	3·35	3·38	3·39
3			3·08	3·09	3·03	3·05
4	2·75	2·73	2·74	2·72	2·73	2·75
5			2·61	2·58	2·57	2·59
6	2·23	2·20	2·25	2·22	2·25	2·27
7			2·05	2·03	2·06	2·04
8	1·93	1·89	1·93	1·91	1·83	1·90
9	1·79	1·79	1·82	1·82	1·80	1·81
10	1·70	1·69	1·70	1·70	1·67	1·70

The results of the diffraction analysis are shown in Table III, which gives the spacings obtained from longitudinal sections. For the humerus of dog A only a few spacings are given, as the resolving power of the camera with such a small specimen to a film distance of 5·15 mm is small. The other diffractograms were taken with a specimen to a film distance of 9·85 mm. The orientation of the diffraction rings is the same in all diffractograms from longitudinal sections. In cross sections no orientation could be seen. In an extensive investigation BRANDENBERGER and SCHINZ[1] discussed the factors limiting the value of the diffraction method especially in the study of bone structures. Although the different apatite compounds possible in bone tissue show some slight difference in the X-ray diffraction picture, the results presented above tend to indicate that the ultrastructure of active as well as inactive bones is the same.

A. ENGSTRÖM and R. AMPRINO[2]

Department for Cell Research, Karolinska Institutet, Stockholm, January 15, 1950.

Zusammenfassung

Historadiographische und Röntgeninterferenzuntersuchungen ergaben, daß zwischen aktiven und immobilisierten Knochen keine Unterschiede in der Ultrastruktur und im Gehalt an Kalziumsalzen des HAVERschen Systems bestehen.

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Sur les protéines extractibles du muscle strié après traitement de la pulpe par quelques solvants organiques

Divers auteurs ont signalé la possibilité d'extraire des protéines musculaires douées de caractères particuliers, après traitement de la pulpe par des solvants organiques. C'est ainsi que STRAUB[1], en utilisant l'acétone, réussit à obtenir la G-actine et que BAILEY[2], en employant l'alcool-éther, prépara la tropomyosine.

Nous avons procédé à une étude, par la méthode d'électrophorèse (technique de TISELIUS-LONGSWORTH[3]), de la distribution des protéines dans des extraits de diverses forces ioniques obtenus après traitement de la pulpe par divers solvants organiques.

1° Si la pulpe musculaire hachée est traitée *directement par l'acétone* (3 lavages successifs avec 5 volumes d'acétone anhydre), séchée, puis *extraite par 10 volumes de KCl m* et si l'on dialyse cet extrait contre une solution contenant 0,048 m Na_2HPO_4, 0,006 m NaH_2PO_4 et 0,25 m NaCl ($p_H \sim 7{,}4$, μ 0,40), on obtient le cliché représenté fig. 1*B*, dont les gradients ont comme vitesses: à l'anode: M $-1{,}5$; A $-4{,}4$, T $-5{,}6$; à la cathode: M $-1{,}35$ et 2 autres: $-3{,}85$ et $-4{,}7 \cdot 10^{-5}$ cm/sec.

La fig. 1*A* correspond à la même pulpe, identiquement traitée par l'acétone, mais extraite ensuite avec un mélange de phosphates de μ 0,15 et de p_H 7,1. L'extrait est dialysé contre cette même solution avant l'électrophorèse.

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