Growth in width of the frontal bones after fusion of the metopic suture

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Rat frontal bones reportedly exhibit lateral surface growth many weeks after fusion of the frontal suture. This is histologically confirmed in the present study. The mean width of the cranial vault at the postorbital constriction increased by 10 % between the age of 30 and 58 days. The increase must be ascribed to periosteal growth, as the lateral, external surfaces exhibited active growth. External apposition without concomittant internal resorption causes an increase in bone thickness. The cells on the internal surfaces were in a state of rest. The study was based on a limited material; therefore, definite conclusions cannot be drawn.

Key-words: Rats, periosteum; craniology

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Periosteal growth is generally accepted as being responsible for a remodelling of the cranial vault (*Bjørk*, 1970). Its function in the increase of its dimensions is, however, unclear (*Prahl*, 1968).

It has been shown that the lateral surfaces of rat frontal bones grow up to the age of 66 days (*Cleall, Wilson & Garnett*, 1968). The posterior part of the frontal suture, on the other hand, fuses at the age of 7—11 days (*Massler & Schour*, 1951; *Moss*, 1962).

The specific aim of the present study was to test the implied possibility of studying the contribution of periosteal growth to the increase in width of the cranial vault in this particular area during a certain period of postnatal life. More generally, the study aimed at assessing the problems involved in a study of the function of periosteal growth on the changes in width of the cranial vault.

MATERIAL AND METHODS

Five litters of young rats were selected for the study. In accordance with the recommendations of *Park* (1968) each litter was reduced to five males as soon as sex could be determined. The material was thereby limited to 25 animals. They were kept in plastic cages of one litter each and received pellets (*Koppang*, 1967) and tap water ad libitum.

Between the ages of 30 and 58 days all the animals were sacrificed with intervals of one week, one animal from each litter on each occasion. One hour before a sample of five animals were put to death, two of them received intraperitoneal in-

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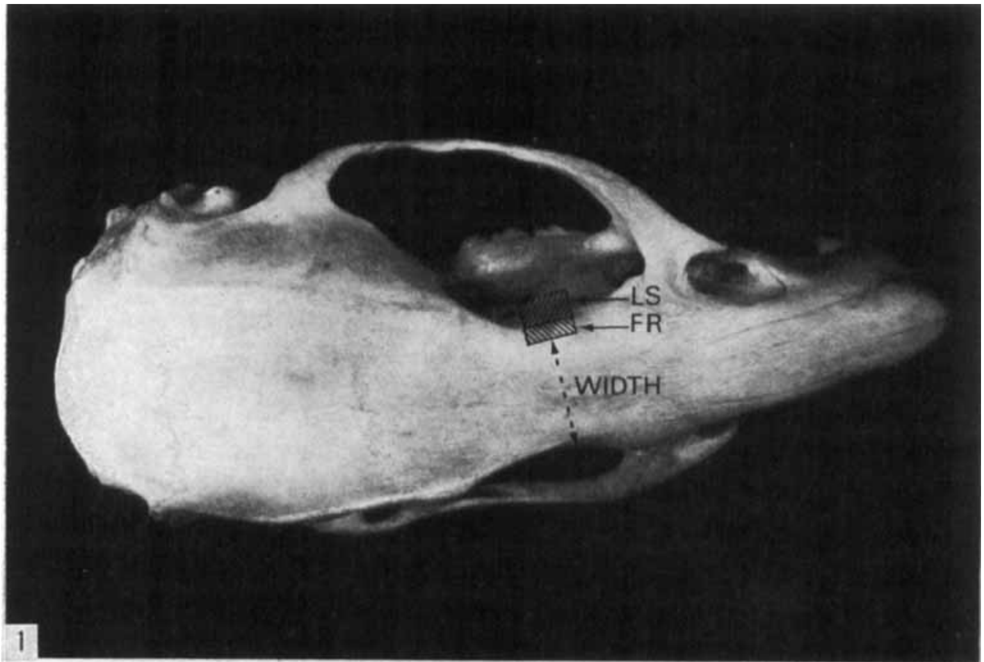


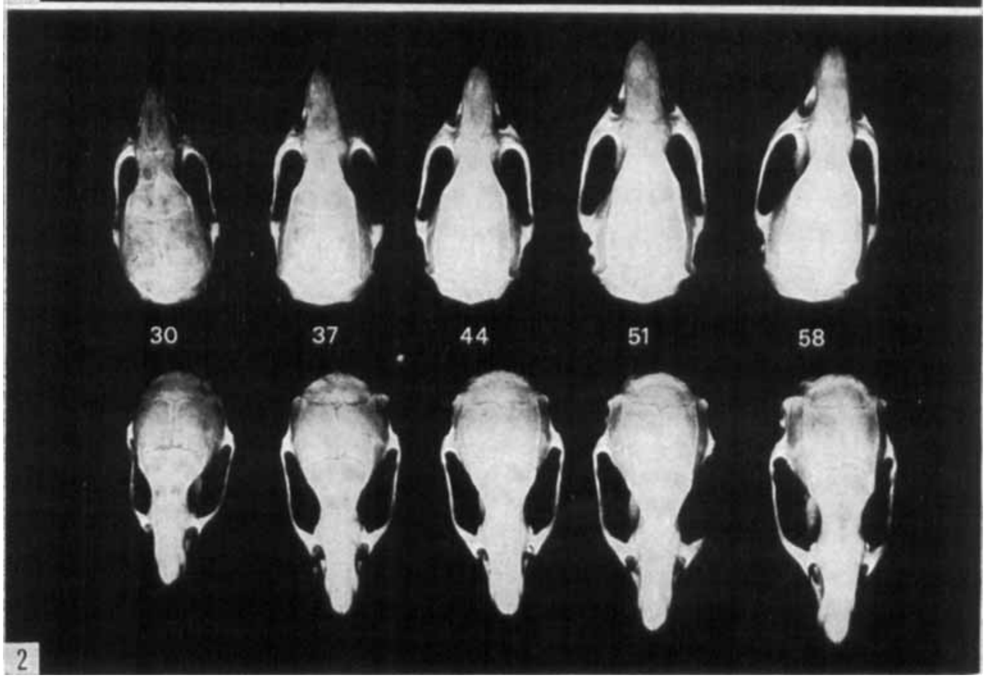


Fig. 1. Macrographs of macerated skulls showing the smallest width of the postorbital constriction. The location of the lateral surface area (LS) and of the frontal ridge (FR) are also indicated.

Fig. 2. Macerated rat skulls and age in days.

jections of tritiated thymidine (1 μCi/g B.W.), and three were injected with the corresponding volume of saline.

Two of the latter were macerated, following boiling and bleaching in 3 per cent hydrogen peroxide (Figs. 1 and 2). The smallest width of the cranial vault, in an area corresponding to the distal roots of the upper l. molars, was measured on the macerated skulls. A sliding caliper was used and read to the nearest 0.10 mm. The mean values for the two skulls from each group of sacrificed animals were calculated on the basis of double determinations.

The remaining three skulls from each group were fixed in modified Lavdowski (*Gilhuus-Moe*, 1969) for 24 hours, decalcified in 20 per cent formic acid, paraffine embedded, and serially sectioned at 5 μm in a frontal plane in an area corresponding to the distal roots of the upper first molar (Fig. 1). Most of the sections from the isotope injected animals were coated with nuclear emulsion (Kodak NTB 2^{R}), exposed in darkness for 24 days at 4°C, developed, and fixed photographically. The coated sections were counterstained with haematoxylin. Sections from the saline injected animals were stained with H—E, and some of them were coated and exposed together with the autoradiograms in order to assess background activity.

Labelled and unlabelled osteogenic cells located in the lateral periosteum of the isotope-injected animals were counted, following procedures outlined elsewhere (*Kvam*, 1972). The radioactive index (per cent labelled cells) was calculated as per cents of labelled cells on the lateral surface of the skull (Fig. 1). Labelled cells in the periosteum of the frontal ridge were counted separately (Fig. 1). At least 1500 cells were counted for each area.

The t-test was applied to estimate the difference between the values of the radioactive index of cells on the most lateral surfaces of the frontal bones and the frontal ridge of the same bones.

RESULTS

The mean values of the skull widths increased gradually from week to week, totaling an increase of 10 per cent (Table I).

In the frontal sections studied histologically there were no structures in the median plane indicating the presence or the former site of the frontal suture. Thus, the findings confirm those of *Massler & Schour* (1951) and *Moss* (1962) that the frontal suture in the rat closes early in postnatal life.

It would seem justified to conclude that the contribution of periosteal growth to the increase in width of the cranial vault may be studied explicitly in the rat during a period of postnatal growth. The findings also indicate that this contribution is considerable. However, since the study was based on small groups of animals, it could not be tested statistically. Definite conclusions can therefore not be drawn.

The histological part of the study demonstrated that the periosteal layers of the lateral, external surfaces of the frontal bones were rich in small, rounded, proliferating cells (Figs. 3—7), many of which were labelled (Figs. 5 and 6). Labelled cells were also occasionally observed close to the bone surface. On the internal surfaces of the frontal bones periosteal cells were scarce, and few of them were labelled (Fig. 7).

The external surfaces also exhibited more specific morphologic criteria of active bone formation (Figs. 5 and 6). Most cells on the internal surfaces appeared to be in a state of rest. Osteo-

Table I. *Weight of animals, skull width and radioactive index of osteoblasts*

Age in days	Saline injected animals (macerated) Weight (gms)	Skull (1) width (mm)	Isotope injected animals (sectioned) Weight (gms)	Radioactive index Lateral side (2)	Frontal ridge (3)
30	110	5.75	92	2.83	6.51
	102	5.75	96	1.97	1.93
37	162	6.05	176	1.49	3.08
	176	6.00	150	1.04	4.51
44	174	6.00	190	1.36	3.07
	198	6.10	208	1.18	4.26
51	248	6.55	224	0.78	11.45
	222	6.10	260	0.70	2.29
58	252	6.40	252	0.26	1.40
	212	6.30	270	0.13	1.60

1. Average values from repeared measurements. s.d. 0.777
2. Average cell number counted in each animal: 1577
3. Average cell number counted in each animal: 1706

clasts, indicating bone resorption, were not observed.

A comparison of sections indicated greater growth activity in the ridges of the frontal bones than in the lateral, external surfaces of the same bones. The radioactive index of the periosteal cells was generally also higher (Table I); a t-test showed that the difference was significant. ($p < 5\%$). Also, the variation in the radioactive index was larger on the ridge (1.40—11.45) than on the lateral surfaces (0.13—2.83), where it also generally decreased with age.

DISCUSSION

The histological findings support the craniometric findings in showing appreciable growth activity on the lateral, external frontal bone surfaces during the period studied. The cell population in the area in question had a radioactive index which, according to *Messier & Leblond* (1960), classifies it as a renewing or even an expanding one.

It has been suggested that internal, resorptive alterations (*Moss*, 1954) and internal appositional processes accompany the apposition on the outer surfaces of the

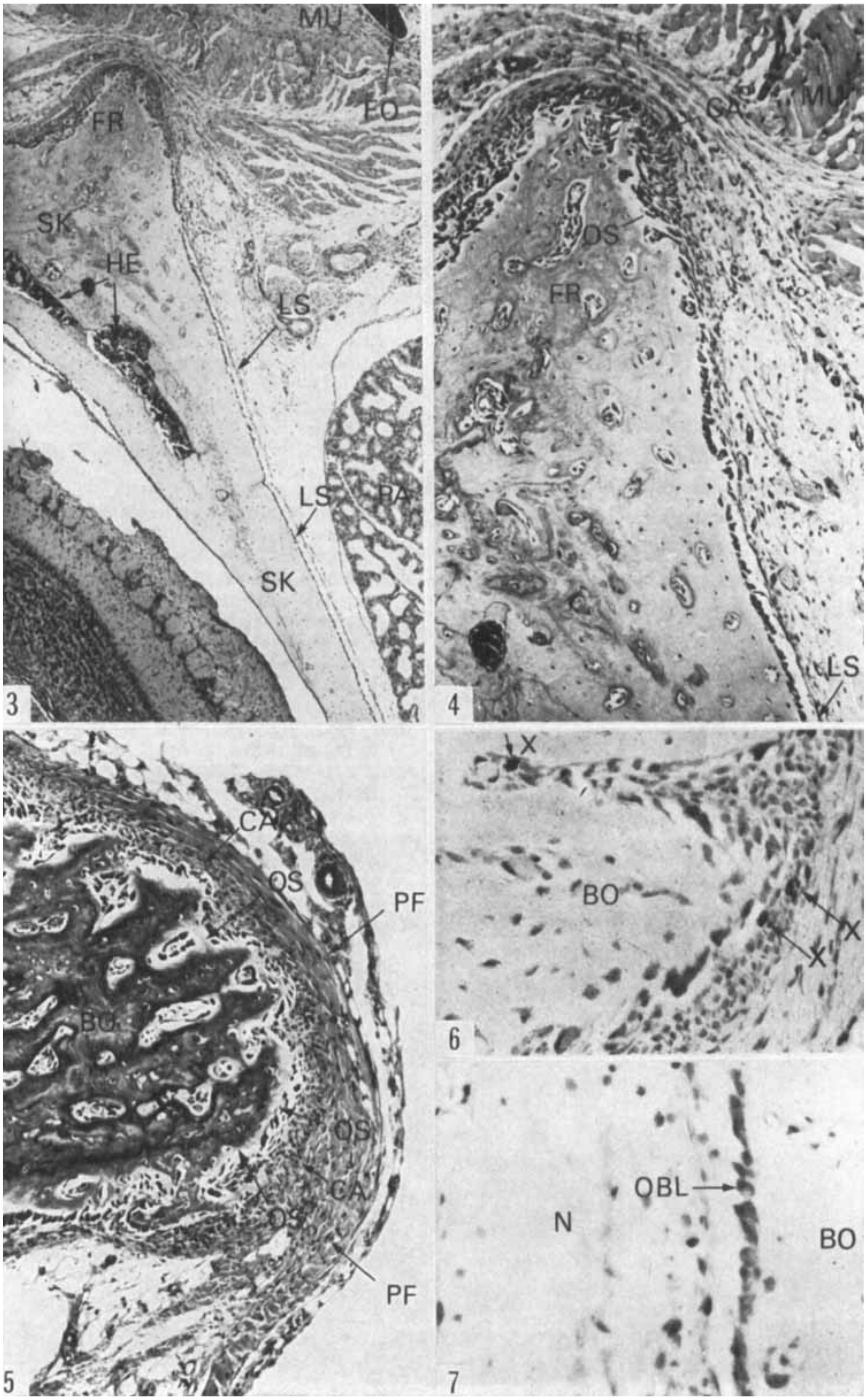
Fig. 3. Section showing part of the skull vault (SK) containing hemapoitic tissue (HE). Muscles (MU), hair follicle (FO) and parotid gland (PA) are located close to the lateral skull surface (LS) or to the frontal ridge (FR). Age of animal: 58 days. Magnification: x 20.

Fig. 4. Micrograph showing the frontal ridge (FR) and osteoid tissue (OS). The cells in the cambium layer (CA) are located beneath periosteal fibers (PF). Muscles (MU) are observed near the frontal ridge. LS = lateral bone surface. Age of animal: 58 days. Magnification: x 30.

Fig. 5. High magnification of frontal ridge showing bone trabeculae (BO) with surface covered with osteoid (OS). The cambium layer (CA) is rich in osteogenic cells situated between the bone surface and the periosteal fiber bundles (PF). Age of animal: 37 days. Magnification: x 75.

Fig. 6. Autoradiogram of external bone surface (BO) showing labelled osteoblasts (x). Age of animal: 37 days. Magnification: x 190.

Fig. 7. Endocranial bone surface (BO) with lining osteoblasts (OBL) and nerveous tissue (N). Age of animal: 58 days. Magnification: x 190.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skull vault in animals aged 30—60 days (*Massler* & *Schour*, 1951; *Cleall, Wilson* & *Garnett*, 1968). The histological findings made in the present investigation showed endocranial bone surfaces in a state of rest. The internal dimensions of the cranial vault were not measured.

External apposition without concomitant internal resorption will cause an increase in bone thickness. *Getz* (1960) found that an increase in bone thickness after completion of general growth is a characteristic feature in human cranial development. The present study indicates similar changes in the growing rat skull.

The radioactive index of the cells was significantly higher on the frontal ridges than on the lateral surfaces of the frontal bones. This index is a measure for the proliferative rate, and it denotes initial growth when osteogenic cells are assessed. A different level of radioactivity in two areas, therefore, indicates a difference in the factors which control the growth. These findings support those of *Slagsvold* (1969) that skull growth is influenced by local factors.

It is justified to believe that the function of periosteal growth in the development of the width dimensions of the rat cranial vault during a certain period of postnatal life can be investigated through a cross-sectional craniometric study.

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