Craniofacial bone remodeling in growing rats fed a low-calcium and vitamin-D-deficient diet and the influence of masticatory muscle function

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Fifty-two male growing rats were randomly divided into three groups. The first group (n = 18) received a hard deficient diet, and the second (n = 18) a soft deficient diet. The control group (n = 16) was fed the normal hard diet. At the beginning and in the middle of the 28-day experimental period oxytetracycline was injected. Two representative coronal sections of the snout and the corresponding contact microradiographs were analyzed. The bone mass of the premaxillary and nasal bones seemed to be less in the two deficient diet groups than in the normal one, due to an increased endosteal bone resorption and decreased bone formation. No difference in the bone apposition rate and pattern could be seen between the deficient hard and soft diet groups, except in the dorsal part of the premaxilla, where the bone formed in the first half of the experiment was markedly more resorbed in the deficient soft diet group during the remaining period than in the deficient hard diet group. The morphology of the sutures was influenced by the altered function, since the sutural space became narrower, and premature obliterations of the internasal suture were observed in the deficient soft diet group. In conclusion, poor bone quality was observed in the skull of rats fed a low-calcium and vitamin-D-deficient diet, with less bone mass than in normal conditions. Masticatory function was a significant factor influencing bone remodeling and sutural growth even in situations in which a metabolic bone disturbance exists. □ *Fluorescence; growth; hypocalcemia; microradiography*

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During growth the remodeling of bone is regulated by systemic and local factors. In growing individuals, vitamin D deficiency, known as rickets, causes deformities of the skull and skeleton and also a decreased resistance to mechanical stress due to disturbed osteogenesis (1, 2). To induce a similar situation in the rat, a low calcium or phosphate content or a combination of these seems to be required in addition to vitamin D deficiency, possibly due to the low requirement of vitamin D in the rat (3, 4). In animals deficient in vitamin D and calcium, an increase in bone resorption has been found in the long bones and the mandible (5–7). Slower eruption of the permanent teeth, undermineralized bone, underdevelopment of the dentin, and enamel hypoplasia were also found in the mandible of pigs with an inherited form of 1 pseudo-vitamin-D-deficiency rickets (8). Furthermore, changes in the craniofacial growth pattern have been reported in growing rats fed a low-calcium and vitamin-D-deficient diet (9, 10). This craniofacial alteration was found to be related to disturbed osteogenesis at growth sites determining the morphology of the viscerocranium in young rats (11, 12).

It was shown that low masticatory function caused alterations in the craniofacial morphology of the rat during growth (13). In normal rats with low masticatory function the remodeling pattern of individual bones differed in direction, indicating an altered tension in the fiber system of the sutures and the periosteum (14, 15). Furthermore, a recent morphometric study could verify a significantly narrower sutural space and a more parallel orientation of the bony surfaces of facial sutures in the rat after a reduced masticatory function (16).

It has also been reported that the disturbances in both craniofacial morphology and mandibular growth induced by hypocalcemia are accentuated by a low masticatory function (7, 10).

It is not yet known, however, to what extent changes in masticatory function which act during the growth period influence the bone remodeling in the craniofacial region during metabolic bone disease. Thus, the aim of the present investigation was to study the influence of masticatory muscle function on viscerocranial skeletal growth sites of young rats with experimentally induced hypocalcemia.

Materials and methods

Fifty-two male albino rats of the Sprague–Dawley strain with a mean body weight of 100 g were obtained from Anticimex, Södertälje, Sweden, and used in a longitudinal cephalometric study (10). The experimental

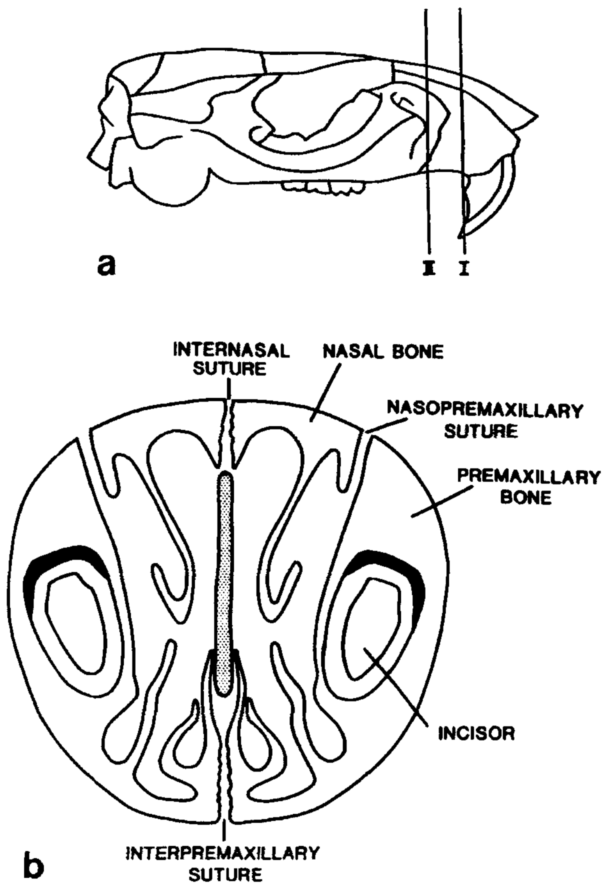

Fig. 1a. Schematic illustration of the lateral view of a rat skull. The vertical lines mark the two regions of the viscerocranium: I = anterior region; II = posterior region. 1b. Schematic illustration of a coronal section of the viscerocranium (posterior region).

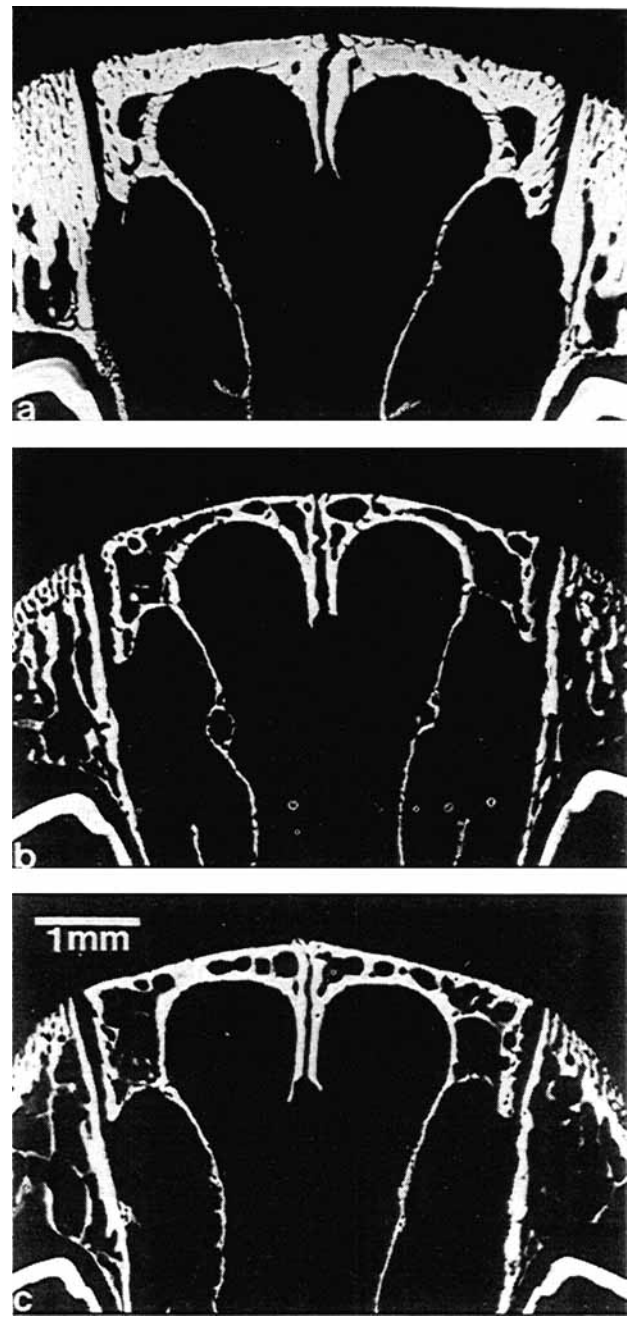

Fig. 2. Microradiographs of the superior part of the posterior region (II in Fig. 1a) of the viscerocranium from (a) the normal hard diet group; (b) the deficient hard diet group; and (c) the deficient soft diet group. Note the similarity in the overall anatomy in the different groups and a decreased bone mass in both deficient diet groups. Also, an unusually thin dentin was observed in the incisors in both deficient diet groups. (Magnification, ×20.)

protocol has been approved by the Ethics Committee of Göteborg University.

The animals were randomly divided into three groups, two experimental and one control. The experimental groups were fed a diet low in calcium (0.04%) and deficient in vitamin D (Deficient diet R25, Astra-Ewos AB, Sweden), described in detail elsewhere (17). The animals in the first experimental group (n = 18) received the deficient diet in a hard-pellet form (deficient hard diet group), whereas those in the other (n = 18) received the diet in a powdered form mixed with water in standardized proportions (2:5, R25 to water) (deficient soft diet group). The animals in the control group (n = 16) were fed the normal diet (Diet R47, Astra-Ewos AB) in a hard-pellet form (normal hard diet group). All animals were fed and watered ad libitum. For the deficient soft diet group the bedding material was sifted, to remove large particles that could stimulate extra gnawing activity (13). During the 28-day experimental period the animals were weighed twice a week (10).

On days 0 and 14, oxytetracycline (10 mg/kg, Pfizer, New York) was injected. At the end of the experimental period the animals were killed with an intraperitoneal injection of an overdose of sodium barbital

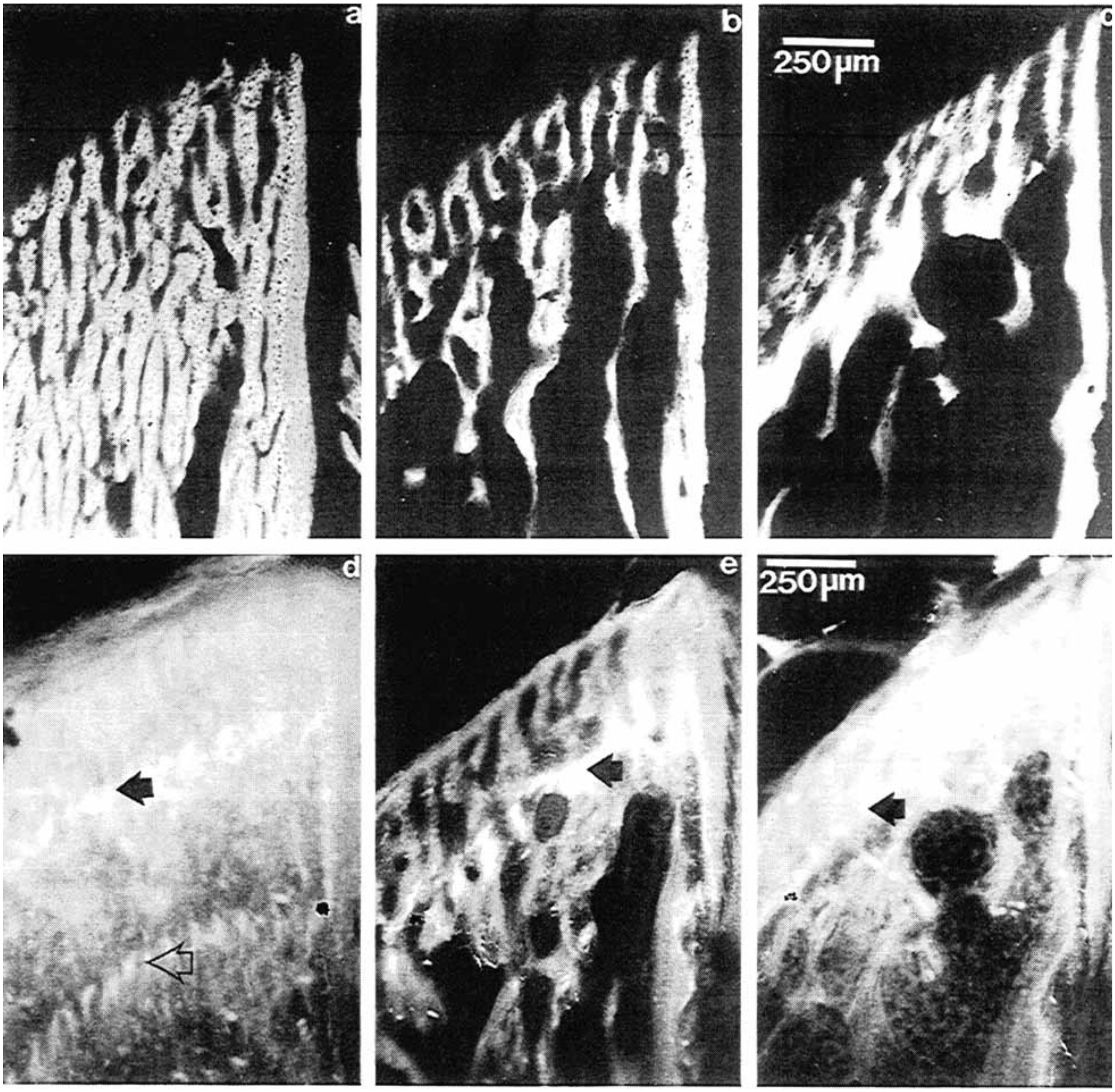

Fig. 3a, b, c. Microradiographs of the premaxillary bone (posterior region) from (a) the normal hard diet group; (b) the deficient hard diet group; and (c) the deficient soft diet group. Note the marked increase of the marrow spaces in both the deficient diet groups (b, c). 3d, e, f. Undecalcified sections of the premaxillary bone from (d) the normal hard diet group; (e) the deficient hard diet group; and (f) the deficient soft diet group. The white seams represent tetracycline incorporated into bone on day 0 (inner band, open arrow) and day 14 (outer band, filled arrow). Note the shorter distance between the seams and between the outer seam and the surface in the specimens from the deficient diet groups (e, f) when compared with the normal hard diet group (d). Note also that the cortical area formed between day 0 and day 14 was markedly more resorbed in the deficient soft diet group than in the deficient hard diet group (e, f).

(Mebumal®, 30 mg/kg body weight, Leo, Helsingborg, Sweden). The heads of the animals were then taken for preparation of undecalcified sections. The specimens were fixed and dehydrated in alcohol, embedded in methylmethacrylate, and sectioned serially with a Leitz saw microtome. Coronal sections of 100 µm were cut perpendicularly to the palatal plane at an interval of 200 µm. Two representative sections from every animal in all three groups were selected for the analysis, one in the anterior and one in the posterior region of the snout (Fig. 1a) (15). To study homologous representative sections from every animal, the last section before the

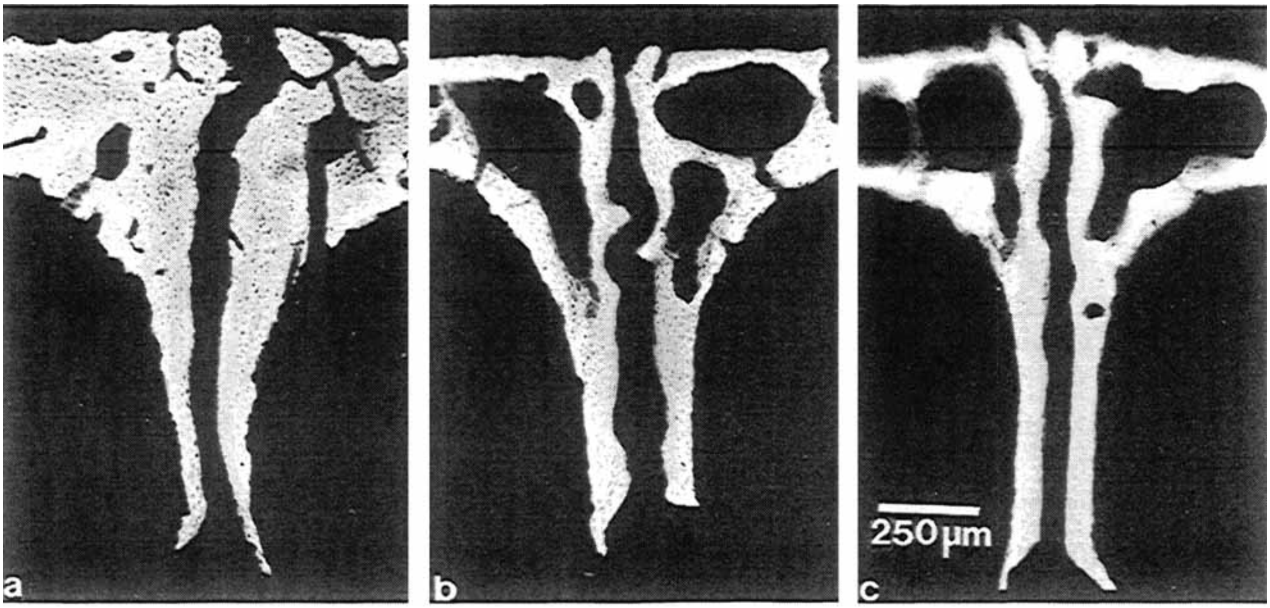

Fig. 4a, b, c. Microradiographs of the internasal suture (posterior region) from (a) the normal hard diet group; (b) the deficient hard diet group; and (c) the deficient soft diet group. Note the marked increase of the marrow spaces of the nasal bone in both the deficient diet groups (b, c). The sutural space of the deficient soft diet group (c) is narrower than that of the deficient hard diet group (b). Note also the obliterations in the uppermost region of the internasal suture in the deficient soft diet group (c).

anterior palatine foramen was used as a common basis for the selection (posterior section) and then an extra section was selected 3 mm mesially (anterior section).

The sections were examined under a fluorescence microscope as described in a previous study (15), to investigate the bone apposition pattern. Fluorescing lines marked the position of the bone apposition at the time of injection, on days 0 and 14, in specific growth sites such as sutures and cortical regions (Fig. 1b).

Contact microradiographs of the selected sections were prepared using Kodak high-resolution plates (type 1A) in a Phillips microroentgen machine with nickel-filtered copper radiation, excited at 20 kV and 20 mA at a target distance of 10 cm.

The internasal, nasopremaxillary, and interpremaxillary sutures and the cortical bone of the upper viscerocranium were examined morphologically (Fig. 1b).

Results

The effect of the deficient diet

The bone quality of the skull in both deficient diet groups was poor, with less bone mass than in the normal diet group. A considerable increase of the marrow spaces in the nasal and premaxillary bones, especially marked in areas with blood vessel channels, was present in rats fed deficient diets (Figs. 2, 3a,b,c, and 4). However, the overall anatomy of the individual bones was similar in all three groups (Figs. 2–6).

The periosteal bone apposition rate, as indicated by the distance between the tetracycline seams, was lower in the deficient diet groups (Figs. 3d–f). However, the regions with particularly high bone formation rate, such as the periosteal surface of the dorsal part of the premaxillary bone, retained this character compared with other regions. In cortical bone regions the tetracycline labeling from the injection on day 0 was frequently absent in the deficient diet groups, because this bone was resorbed during the second half of the experimental period (Figs. 3e,f).

The bony components of the sutures studied were thinner in the two deficient diet groups (Figs. 2, 4, 5, and 6), and the distance between labelings in their bony surfaces showed lower bone apposition rate than in the normal hard diet group, especially during the second part of the experimental period.

In addition to the changes in bone morphology, an unusually thin dentin was observed in the incisors in both deficient diet groups (Fig. 2).

The effect of the change in the consistency of the diet

No difference in the bone apposition rate and pattern could be seen between the deficient hard and the soft diet groups. In the dorsal part of the premaxilla, however, the bone that was formed between days 0 and 14 was resorbed markedly more in the deficient soft diet

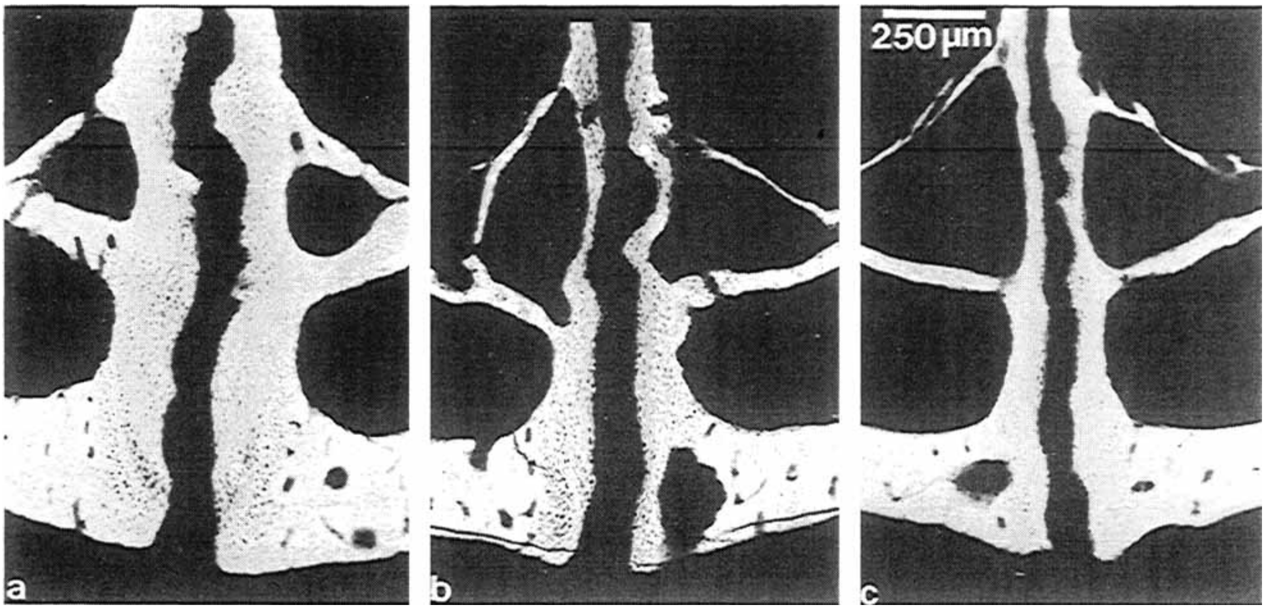

Fig. 5. Microradiographs of the inferior part of the interpremaxillary suture (posterior region) from (a) the normal hard diet group; (b) the deficient hard diet group; and (c) the deficient soft diet group. Note the narrower suture in the deficient soft diet group (c) than in the deficient hard diet group.

group during the remaining period (days 14–28) than in the deficient hard diet group (Figs. 3a–f).

In the internasal suture a narrower sutural space was noted in the deficient soft diet group than in the deficient hard diet group (Figs. 4a–c). A remarkable observation was the presence of bony obliterations in the dorsal region of the internasal suture in the deficient soft diet group (Fig. 4c), which was not found in either the normal or the deficient hard diet group.

The interpremaxillary suture was markedly narrower in the deficient soft diet group than in the deficient hard diet and the normal hard diet groups (Figs. 5a–c).

Discussion

The effect of the deficient diet

The present study, in agreement with previous reports, has shown that a low-calcium and vitamin-D-deficient diet caused a decrease in bone mass (11, 18). Furthermore, an increased bone resorption was evident in the deficient diet groups (19). These morphologic observations are consistent with an effect on bone due to hypocalcemia and the enhanced *i*-parathyroid hormone (i-PTH), which was reported in rats with this dietary disturbance (20). The decrease in bone mass was seen in both deficient diet groups, but the overall anatomy of the individual bones was similar in all three groups. In addition to the increase in the amount of resorption, the bone formation rate decreased throughout the experimental period, especially during the second half. This observation is in agreement with previous cephalometric studies, which showed that the enlargement of skull size during growth was slowed down significantly by the deficient diet (9, 10). This coincides with the observed reduction of the overall body growth (10).

The changes in the bone mass are a part of the disturbances in the hard tissue formation and resorption due to metabolic disease, as has also been seen, both in our and in previous studies, in the alteration in the structure of the dentin (8, 11).

The effect of the change in the consistency of the diet

The different consistency of the diet seemed to induce no apparent changes in the bone apposition rate and pattern, except in the dorsal part of the premaxilla, a region with a high level of bone formation, where the bone that was formed in the first half of the experimental period was markedly more resorbed in the deficient soft diet group than in the deficient hard diet group during the remaining period. The latter observation is in agreement with the findings of Lanyon et al. (19) in the turkey's ulna, that even during periods of bone loss due to calcium insufficiency, normal functional activity inhibits resorption and thus has a protective effect on bone mass, whereas absence of functional loading results in a more severe bone loss. A similar regulative role of the functional forces on cortical modeling has previously been shown in studies

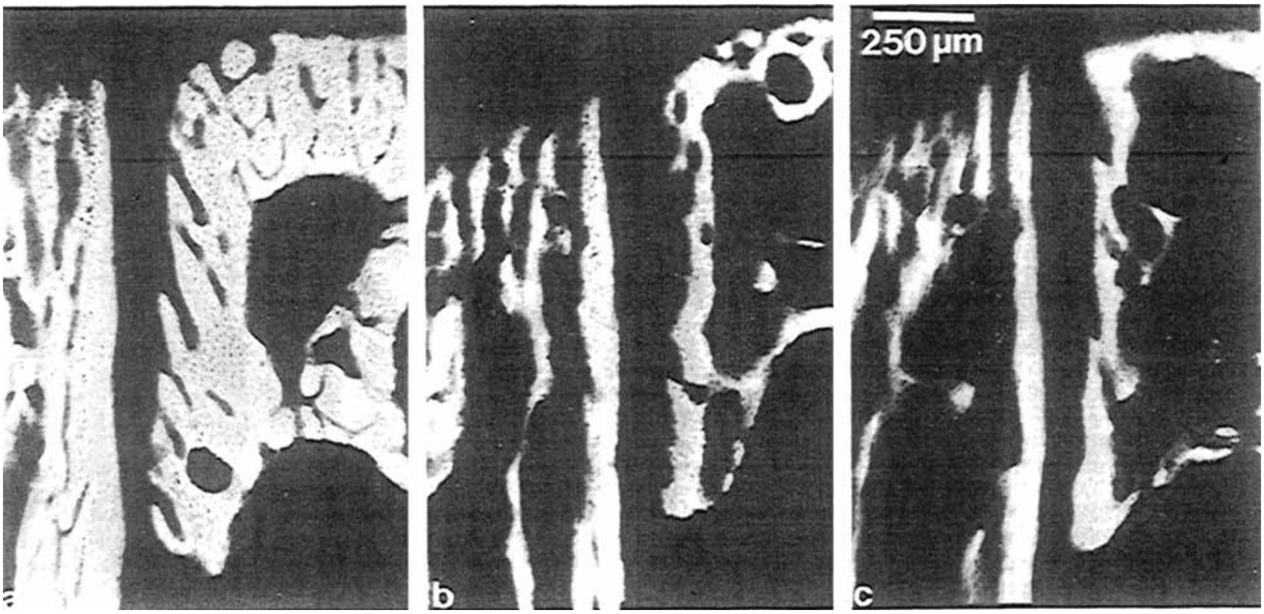

Fig. 6a, b, c. Microradiographs of the nasopremaxillary suture (posterior region) from (a) the normal hard diet group; (b) the deficient hard diet group; and (c) the deficient soft diet group.

of the effects of immobilization (22, 23) and exercise (21) on the long bones. The influence of function on bone remodeling could be attributed to inhibition of the generation of initially resorptive remodeling units, to a stimulation of restitution of the amount of bone removed during the initial resorption, or to an adaptive response with involvement of an increase in bone mass (19). The type of influence may be related to the magnitude or distribution of the functional bone strain (19).

Differences in the sutural morphology were observed between the two deficient diet groups, similar to the findings reported previously in normal rats with low masticatory function (15, 16). The narrower sutures indicated that the sutures in the low function group were subjected to less tension than in the deficient diet group with normal function. In the latter the sutural fiber bundles were stretched, as if external tension tried to pull the joint bone elements of the suture apart. According to Behrents et al. (24), who observed separation movements of the sagittal sutures during isotonic contraction of the temporal muscle in living monkeys, the bite forces could provide tensile strain sufficient to cause a separation of the sagittal suture, and that may be a major factor in the local control of osteogenesis of the sutures. The change in tension would cause an alteration in the stress distribution in the fiber system of the periosteum and the sutures. This would be consistent with the suggested model of the periosteum as a membrane highly adaptive to extrinsic factors such as muscle function (13–15, 25–27). In other words, the reduced masticatory function may have changed the direction of osseous movements during growth, and that could to some extent explain the previously reported differences in the form of the skull between deficient hard and deficient soft diet groups (10).

The possibility that the lack of attrition in animals fed the soft diet could be the initiating factor in inducing changes in bone remodeling should be considered very low, since the observed craniofacial changes were independent of the incisor attrition and eruption rate, as was found in a previous study in which the lack of attrition of rats' incisors was compensated for by grinding (28). Thus, the experimental model used should be considered a reliable one to test the influence of the masticatory muscles on the craniofacial growth and bone remodeling. In the present study a qualitative comparison was used to describe the findings, as they were observed under the microscope. The induced changes could have been further elucidated in detail by morphometric analysis of the sections. However, the present findings were based on obvious differences between the groups, which provide sufficient support to the results described.

In conclusion, poor bone quality was observed in the skull of rats fed a low-calcium and vitamin-D-deficient diet, with less bone mass than in normal conditions. Masticatory function was a significant factor influencing bone remodeling and sutural growth even in situations in which a metabolic bone disturbance exists.

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