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Unerupted Dentition Secondary to Congenital Osteopetrosis in the Osborne-Mendel Rat[1] (38146)

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Disorders of the osseous skeleton with concomitant unerupted dentition have been described in several species. The grey-lethal (*gl*) mouse with a 21–30 day life span and unerupted dentition was introduced in 1935 (1). This was followed in 1941 by the incisorless (*ia*) rat (2) with normal longevity and transitory lack of bone resorption, since after almost complete arrest of resorption, the bone was spontaneously resorbed, and then some molars may erupt (3). The microphthalmic (*mi*) mouse, described in 1942, had normal longevity and delayed or partial eruption of teeth (4). The osteopetrotic rabbit was introduced in 1948 and had a life span of approximately 5 weeks and variable tooth eruption (5).

This report describes a new mutant rat with a skeletal disorder, apparent normal longevity, and unerupted molar and incisor dentition. The mutation has been assigned the symbol *tl* for "toothless."

The mutation occurred spontaneously in a partly inbred rat colony derived from the Osborne-Mendel colony maintained at the National Institutes of Health, Bethesda, Maryland. The affected animals have not bred, with the exception of one F_1 generation male which was backcross mated to the original dam. Additional animals have been produced by mating of heterozygous littermates. The strain/substrain designation for the proposed strain is TLOM/Ndri (6).

The toothless rats have been maintained on dry or water mixture of powdered diet[2] (7) supplemented with fatty acids.[3] The maximum adult male and female weights reached were 295 and 230 gm, respectively. Thus far, the longest life span for affected rats is for living F_2 generation animals born February 13, 1973.

The toothless rats can be differentiated from their littermates at 10 days by their smaller size, shorter snout, lack of incisors, and characteristic periorbital encrustation. The eye encrustation (Fig. 1) is similar to the involvement observed in the grey-lethal mouse (8) and the incisorless rat (2). The eye manifestation may be associated with a defective lacrimal apparatus since dye did not penetrate from the eyes to the external nares.

Soft tissue papillae covered the areas of the missing incisors, and gingival pads covered the molar areas. Upon post-mortem examination the molar teeth were occasionally exposed through the bone, though they had not erupted through the gingiva. Foramina were seen occasionally in the area of the missing incisors, but the teeth were never exposed.

The apical odontogenic tissue of the normally continuously erupting incisors continued to proliferate (Fig. 2), and in one animal formed a mandibular tumor. Histologically the tissue consisted of dentin, cementum and a suggestion of enamel. There was metaplastic replacement of the dental pulp and medullary bone cavities with dense fibrous connective tissue which communicated with the periosteum. The replacement of hemopoietic tissue with fibrous connective tissue in the jaws is

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[2] Diet 2000 without sucrose, General Biochemicals, Laboratory Park, Chagrin Falls, Ohio 44022.

[3] Nutriderm, Norden Laboratories, Lincoln, Nebraska 68501.

FIG. 1. Adult toothless rat (right) and normal littermate. Note periorbital encrustation and smaller size of the toothless rat.

similar to the observations in the grey-lethal mouse (9). Some molar teeth were deeply embedded in dense overlying cortical bone, and like the incisors, ankylosed to the bone by proliferation of apical odontogenic tissue (Fig. 3).

All bones from the toothless animals showed gross evidence of abnormal thickening upon post-mortem examination. The vertebral, deltoid, and scapular spines were thickened and pronounced. The long bones also showed evidence of torsion. Radiographically, the bones

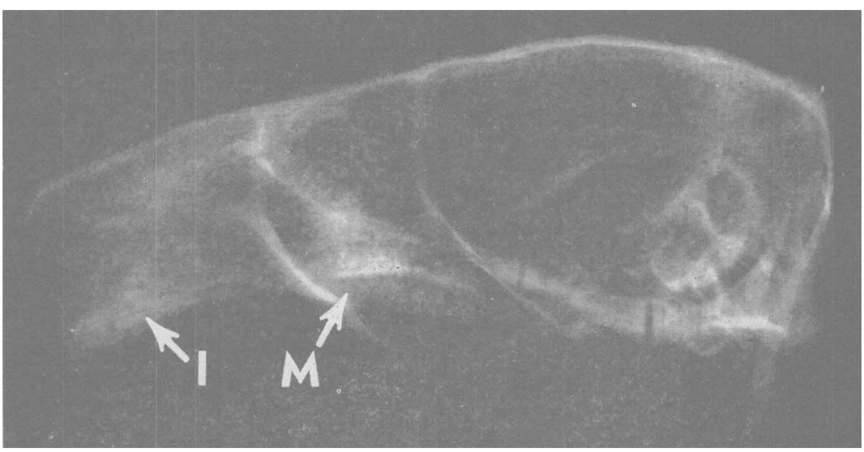

FIG. 2. Skull of F_1 generation, 183-day-old female toothless rat. Radiodensity in the maxillary incisor region (I) is due to proliferation of dentin, cementum, and enamel of the normally continuously erupting incisor. Unerupted maxillary molars are visible (*M*). Incomplete zygomatic arch was observed only in this animal.

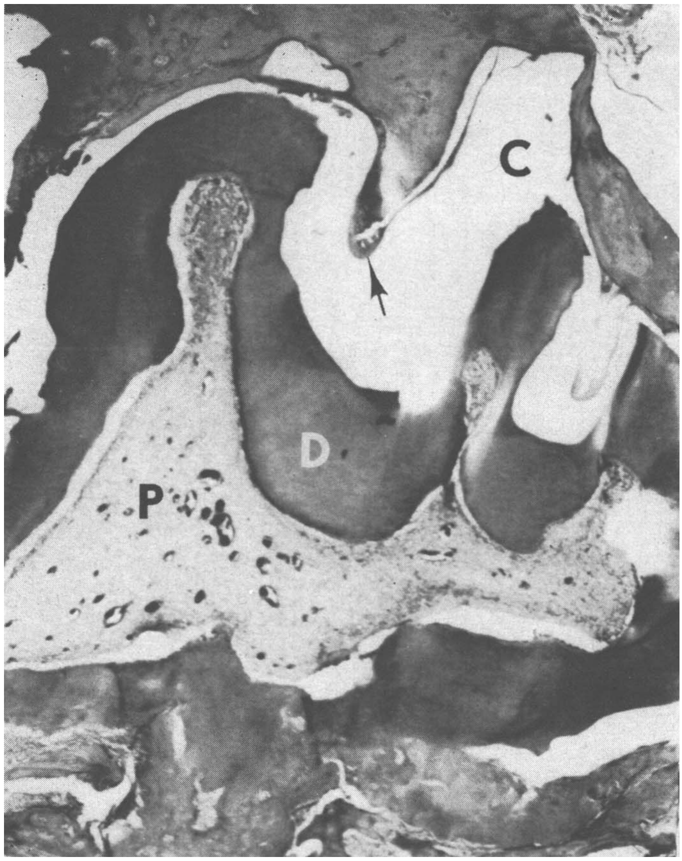

FIG. 3. Unerupted mandibular molar of 327-day-old toothless rat. Enamel cusp (C), lost during decalcification, is closely embedded in bone. The reduced enamel epithelium (arrow) persists; occasionally it contributed to dentigerous cyst, or squamous cell metaplasia and microcyst formation. Note the distorted and ankylosed roots. D = dentin, P = pulp (×10, H & E).

of control animals presented distinct cortical and medullary areas, while the bones of the mutants were dense throughout (Fig. 4).

Long and flat bones of the toothless rat presented similar histological pictures (Fig. 5). Normal compact cortical bone was replaced by irregular bone perforated by numerous communications between the periosteum and the medullary spaces. The cancellous bone consisted of a dense meshwork of trabeculae which severely reduced the amount of hemopoietic bone marrow. There was evidence of bone deposition, but no evidence of osteoclasts or resorption lacunae.

Serum chemistry and hematological findings from 99-day-old rats are presented in Tables I and II. The toothless rats and their littermates were from the F_2 generation. With exception of the calcium values, the blood chemistry values were assayed by standard techniques adapted for the Beckman DSA 560 Discrete Sample Analyzer. The calcium values were determined by standard colorimetric methods with a B and L Spectronic 20. The erythrocyte and total leucocyte counts were performed with a Coulter Counter. The leucocyte differential, hematocrit and hemoglobin were determined by standard procedures.

The excess bone deposition, observed histologically, was consistent with the highly significant increase in serum alkaline phosphatase (Table I). The calcium and phosphorus levels (Table I) were not different among the 3 groups, which was expected since the observed change was one of increased deposition and not resorption. The decreases in total protein and albumin (Table I) may be related to masticatory deficiency in the affected rats.

Associated with the decrease in hemopoietic bone marrow, there was an increase of extramedullary hemopoiesis in the liver and spleen. Splenomegaly was occasionally observed. The affected animals apparently compensated with-

FIG. 4. Radiograph of forelimb of 251-day-old normal rat (left) and 245-day-old F_1 generation, toothless rat. Note the increased radiodensity, thickness, and foreshortening of the affected limb. Scapular spine of the affected limb is also pronounced.

out manifestations of anemia, since no exceptional hematological differences were observed among the 3 groups (Table II). However, the significant increases in glutamic pyruvic transaminase and glutamic oxalacetic transaminase in the toothless rats (Table I) suggest liver involvement. The lack of increased lactic dehydrogenase and creatine phosphokinase values rules out elevated glutamic oxalacetic transaminase due to muscle damage.

The mutant skeletal disorder was diagnosed as osteopetrosis with concomitant extramedullary hemopoiesis and unerupted dentition due to lack of bone resorption. The associated eye involvement cannot be explained.

Grüneberg[10] has placed the 4 previously described animal models in order according to the severity of the bone manifestations. The grey-lethal mouse exhibits no sign of bone resorption, the micropthalmic mouse shows a little resorption in that some molars erupt, the osteopetrotic rabbit shows still more in that there are definite signs of resorption in long bones of older animals, and finally the incisorless rat shows transient osteosclerosis in that the skeletal manifestations eventually disappear completely. On the basis of severity of bone manifestations and unerupted dentition, the toothless rat appears to be equal in severity to the grey-lethal mouse. However, the true severity of bone disorder in the grey-lethal mouse is unknown since it has not been determined if the teeth would eventually erupt if the animal lived longer. If we consider both bone resorption and longevity as the bases for severity, then the order of decreasing severity would be grey-lethal mouse, osteopetrotic rabbit, toothless rat, micropthal-

TABLE I. Body Weight and Blood Chemistry Findings from 99-Day-Old Toothless Rats Compared to Normal Littermates and Controls of the Same Age.

	Control (C) ($N = 6$)	Littermates (LM) ($N = 6$)	Toothless (*tl*) ($N = 4$)	Comparison by Student's t-test		
	Mean ± SD	Mean ± SD	Mean ± SD	^{t}C-LM	^{t}C-*tl*	^{t}LM-*tl*
Body weight (gm)	411.30 ± 100.80	331.80 ± 85.60	146.30 ± 28.90	+ 1.47	+ 6.08[a]	+ 4.91[b]
Glucose (mg %)	100.20 ± 16.80	91.80 ± 20.80	86.30 ± 21.20	+ 0.77	+ 1.10	+ 0.40
Urea nitrogen (mg %)	32.50 ± 8.10	20.90 ± 6.40	28.70 ± 4.08	+ 2.76[c]	+ 1.00	− 2.34[c]
Total protein (gm %)	6.87 ± 0.27	6.67 ± 0.16	5.95 ± 0.44	+ 1.53	+ 3.69[b]	+ 3.09[c]
Albumin (gm %)	3.98 ± 0.29	4.28 ± 0.25	3.58 ± 0.54	− 1.94[d]	+ 1.38	+ 2.44[c]
Globulin (gm %)	2.88 ± 0.26	2.38 ± 0.21	2.38 ± 0.28	+ 3.61[b]	+ 2.91[c]	+ 0.05
Calcium (mg %)	9.20 ± 0.29	9.25 ± 0.18	9.38 ± 0.26	− 4.00	− 1.03	− 0.87
Inorganic phosphorus (mg %)	5.73 ± 0.58	5.88 ± 0.56	6.10 ± 0.75	− 0.46	− 0.83	− 0.50
Glutamic pyruvic transaminase (IU/liter)	44.0 ± 15.7	33.7 ± 4.7	66.3 ± 7.0	+ 1.54	− 3.05[c]	− 8.14[a]
Glutamic oxalacetic transaminase (30°, IU/liter)	116.7 ± 11.6	90.1 ± 8.8	209.8 ± 20.4	+ 4.46[b]	− 8.29[a]	− 11.08[a]
Lactic dehydrogenase-L (30°, IU/liter)	159.1 ± 34.6	117.3 ± 12.8	89.8 ± 22.7	+ 2.77[c]	+ 3.83[b]	+ 2.21[d]
Creatine phosphokinase (30°, IU/liter)	263.8 ± 72.7	261.0 ± 33.3	184.3 ± 56.2	− 0.09	+ 1.94[d]	+ 2.45[c]
Alkaline phosphatase (IU/liter)	27.8 ± 4.7	34.9 ± 7.3	72.6 ± 6.6	− 1.99[d]	− 11.73[a]	− 8.51[a]

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] $0.1 > P > 0.05$.

TABLE II. Hematological Findings from 99-Day-Old Toothless Rats Compared to Littermates and Controls of the Same Age.

	Controls (C) ($N = 6$) Mean ± SD	Littermates (LM) ($N = 6$) Mean ± SD	Toothless (*tl*) ($N = 4$) Mean ± SD	Comparison by Mann-Whitney test		
				U C vs. LM	U C vs. *tl*	U LM vs. *tl*
Erythrocyte (10^6/cmm)	8.33 ± 0.56	8.33 ± 0.62	7.66 ± 0.28	16.0	17.0[d]	20.0[d]
Total leucocyte (10^3/cmm)	9.18 ± 1.75	10.55 ± 1.96	11.07 ± 1.82	26.0	19.5[d]	15.0
Schilling's differential:						
Neutrophils (%)	52.66 ± 8.98	19.83 ± 11.39	16.25 ± 6.84	36.0[a]	24.0[a]	13.5
Lymphocytes (%)	39.66 ± 8.31	76.66 ± 12.32	79.25 ± 7.80	36.0[a]	24.0[a]	13.0
Monocytes (%)	5.50 ± 2.07	1.83 ± 1.16	2.50 ± 1.00	34.0[a]	22.0[b]	17.5
Eosinophils (%)	2.16 ± 0.98	1.66 ± 1.03	2.00 ± 0.81	23.0	12.5	20.0[d]
Basophils (%)	0.00	0.00	0.00			
Hematocrit (% PVC)	44.83 ± 2.85	44.08 ± 2.19	45.12 ± 1.24	22.0	13.0	16.0
Hemoglobin (gm %)	14.75 ± 0.51	15.06 ± 0.64	15.27 ± 0.17	25.0	21.0[c]	12.5

[a] $P = 0.005$.
[b] $P = 0.025$.
[c] $P = 0.05$.
[d] $0.1 > P > 0.05$.

FIG. 5. Femur diaphysis of the same toothless rat seen in Fig. 4. Note increase in trabecular bone and decrease in hemopoietic marrow. Note abnormal compact cortical bone (C) and lack of resorption. (×40, H & E).

mic mouse, and finally the incisorless rat.

Studies with grey-lethal mouse and incisorless rat have been concerned with osteopetrosis (5, 11), bone metabolism (12, 13), alkaline phosphatase (14), parathormone (15–17), bone (18–20) and tooth formation (4, 9, 21, 22), and bone resorption (23). It is anticipated that the toothless rat model will offer the advantages of both previous models, since it has the severe lack of bone resorption and unerupted dentition of the grey-lethal mouse and the normal longevity of the incisorless rat.

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