

EFFECT OF PARATHYROID HORMONE AND HEPARIN
ON DENTAL DEVELOPMENT IN MICE HOMOZYGOUS
FOR THE MICROPHTHALMIA MUTATION

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Cutting of the teeth is disturbed in mice homozygous for the microphthalmia gene (mi/mi) and osteopetrosis develops. The effect of heparin and of parathyroid hormone on cutting of the teeth was studied in mi/mi mice. Administration of heparin from the 2nd to the 20th day in a dose of 5 units/g body weight was shown to stimulate cutting of the teeth in mi/mi mice. Combined administration of heparin, in the same doses, and parathyroid hormone led to eruption of practically the same number of teeth as heparin alone. This may be because the heparin level is deficient in mi/mi mice but secretion of parathyroid hormone is undisturbed.

KEY WORDS: microphthalmia mutation; parathyroid hormone; heparin; eruption of the teeth.

The microphthalmia mutation in mice in a homozygous state (mi/mi) leads to the development of a complex pathological syndrome connected with a defect of the neural crest material. One component of this syndrome is osteopetrosis and disturbed eruption of the teeth [1]. The latter is due both to a defect of the anlage of the tooth pulp itself and also with the presence of unabsorbed lamellae of bone in the jaws preventing emergence of the tooth into the oral cavity. The writers have shown that administration of parathyroid hormone or heparin in various doses to mi/mi mice promotes absorption of the bony lamellae and increases the number of erupting teeth [2, 3].

It was therefore decided to study the effect of combined administration of heparin and parathyroid hormone on eruption of the teeth in mi/mi mice.

EXPERIMENTAL METHOD

Mutant mice for the microphthalmia gene were obtained from Professor Gruneberg (England) and back-crossed six times with mice of the inbred line C57BL/Mib. The homozygotes die if kept on an ordinary diet soon after removal from the mother (on the 25th-30th day).

Eruption of the teeth was studied on the 20th day after birth in intact mice (control, series I) and in animals treated with heparin and parathyroid hormone. The substances were injected subcutaneously into the back daily from the 2nd to the 20th days of postnatal development in doses of: parathyroid hormone 0.5 unit/g body weight (series II), heparin 5 units/g (series III). In series IV the mice were injected with heparin and parathyroid hormone in the same doses simultaneously. In the experiments of series V heparin (5 units/g) was injected from the 15th to the 20th days inclusive. To prevent leaking of the solutions, the site of the injection was covered with collodion. The animals were weighed before each injection. They were killed 4-5 h after the last injection. The teeth were studied in total preparations of the skull stained

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TABLE 1. Effect of Parathyroid Hormone and Heparin on Eruption of Teeth (in %) in 20-Day mi/mi Mice When Administered from 2nd to 20th Days Inclusive

Series of expts.	Prepn. and dose (units/g)	Position of tooth	Incisor	First molar	Second molar	Third molar	All teeth
I	Control	In jaw In gum Erupted	95,0 5,0 0	37,5 62,5 0	10,0 77,5 12,5	95,0 5,0 0	59,4 37,5 3,1
II	Parathyroid hormone 0,5	In jaw In gum Erupted	30,0 50,0 20,0	0 27,5 72,5	0 10,0 90,0	75,0 15,0 10,0	26,3 25,6 48,1
III	Heparin 5	In jaw In gum Erupted	0 37,5 62,5	0 0 100	0 0 100	2,5 27,5 70,0	0,7 16,2 83,1
IV	Parathyroid hormone 0,5+ heparin 5	In jaw In gum Erupted	5,0 42,5 52,5	0 10,0 90,0	0 0 100	10,0 20,0 70,0	3,8 18,1 78,1

Legend. Ten animals used in each series of experiments. Significance of differences between number of teeth erupted, by Kolmogorov-Smirnov criterion: $P_{I-II} < 0.01$; $P_{I-III} < 0.01$; $P_{I-IV} < 0.01$; $P_{II-III} < 0.01$; $P_{II-IV} = 0.05$; $P_{III-IV} > 0.05$.

with alizarin red S by Green's method [4]. Each group consisted of 10 animals. The significance of differences was determined by the Kolmogorov-Smirnov criterion.

EXPERIMENTAL RESULTS

Eruption of the teeth was considerably disturbed in the intact mi/mi mice. At the age of 20 days only 3.1% of the teeth had erupted into the oral cavity and most were still "immured" in the jaws by the unabsorbed bone lamellae or, as in the case of most of the first and second molars, the crowns of the teeth had reached the gum (Table 1). In mice of normal genotype by this time all the teeth had erupted except the third molars.

Injection of parathyroid hormone from the 2nd to the 20th days inclusive in a dose of 0.5 unit/g daily largely restored the normal pattern of eruption of the teeth in the mi/mi mice: the number of teeth erupted into the oral cavity was increased to 48.1%. Nearly all the second molars (90%) had erupted, with most of the first molars (72.5%), but the incisors and the third molars were still held up in the gum or were "immured" by the unabsorbed bony lamellae of the jaw. This dose of parathyroid hormone was more effective in causing eruption of the teeth than a dose of 0.25 unit/g and almost as effective as a dose of 1 unit/g.

When heparin was given in a dose of 5 units/g from the 2nd to the 20th day 83.1% of the teeth erupted. The crowns of all the first and second molars were visible in the oral cavity, most incisors (62.5%) and third molars (70%) also had erupted, and only a few incisors (37.5%) and third molars (27.5%) remained in the gum, although the bony lamellae blocking the outlet of the tooth into the oral cavity were absorbed. In this series of experiments the number of erupted incisors was three times greater, and the number of molars seven times greater, than when parathyroid hormone was given.

After combined injection of the same doses of parathyroid hormone and heparin 78.1% of teeth erupted into the oral cavity. All the second and nearly all the first molars (90%) were cut. The number of erupted incisors was 2.5 times greater and the number of third molars seven times greater than when parathyroid hormone alone was given.

Administration of heparin for a short period, from the 15th to the 20th day, considerably improved eruption of the teeth in mi/mi mice. The total number of teeth erupted was increased by more than 15 times compared with the intact animals ($P < 0.01$). The second and first molars were most frequently erupted, whereas the number of erupted incisors was 2.5 times less than during the more prolonged administration of heparin. This shows that by the 15th day of development an irreversible disturbance has occurred in the mi/mi mice and eruption of these teeth does not take place in response to administration of heparin.

Injection of parathyroid hormone or heparin causes absorption of the bony lamellae preventing the tooth from erupting into the oral cavity and it considerably improves eruption of the teeth in mi/mi mice ($P < 0.01$). After administration of heparin the number of teeth erupting into the oral cavity was significantly greater than after administration of parathyroid hormone ($P < 0.01$). After combined administration of heparin and parathyroid hormone the number of erupting teeth was practically the same as when heparin alone was given ($P > 0.05$).

Combined administration of heparin and parathyroid hormone thus did not improve eruption of the teeth in mi/mi mice compared with the action of heparin. This may be because the heparin level in these mice is too low but secretion of parathyroid hormone is undisturbed, as is confirmed by the normal structure of the parathyroid glands in these mice. However, absorption of the bony lamellae of the jaw, preventing eruption of the teeth, is not observed in intact 20-day mi/mi mice as a result of the action of parathyroid hormone in a dose equivalent to normal, for heparin also participates in this process [3]. This suggests that heparin is essential for manifestation of the effects of parathyroid hormone.

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