Prostaglandin-Induced Resorption of the Adult Rat Calvarium

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Prostaglandin E_1-containing solutions injected under the skin overlying the calvarium of adult rats produced a visible resorptive lesion within the bone in seven days. The resorptive process was characterized by fibrous replacement of bone matrix and by increased vascularity. Inflammatory cells were not apparent.

Prostaglandins are a family of naturally occurring fatty acids that participate in the inflammatory process[1-4] and exhibit hormone-like effects.[5,6] When prostaglandins are injected intradermally, they produce local inflammatory effects such as increased vascular permeability with edema and vasodilatation with erythema.[7,8] They have been identified in inflammatory exudates[9-11] and their biosynthesis is inhibited by nonsteroidal anti-inflammatory drugs.[12-15]

The prostaglandins PGE_1, PGE_2, and to a lesser extent $PGF_{2\alpha}$ stimulate resorption of cultured fetal rat bones.[16,17] In these systems E-type prostaglandins are more potent than is parathyroid hormone in producing bone resorption, although osteolytic action occurs at a slower rate. The action of the E-type prostaglandins and parathyroid hormone is accompanied by elevation of bone cyclic 3′–5′ adenosine monophosphate.[18]

When they are injected systematically, prostaglandins are rapidly metabolized[19,20] by 15 dehydrogenation and by reduction of the 13-14 double bond (Fig 1). The biologic activity of the metabolic products is reduced substantially.

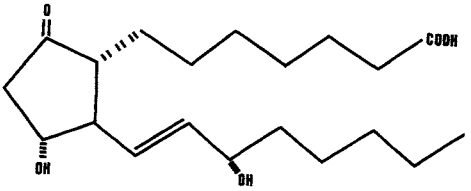
Because of the rapid inactivation and low blood levels, and since large doses of prostaglandins that are injected intraperitoneally fail to produce elevated blood calcium in parathyroidectomized rats,[16] prostaglandins do not normally participate as blood-borne humoral elements that regulate calcium metabolism like the parathyroid hormone. However, this does not preclude a role in the production of localized osteolytic lesions. Since most forms of periodontal disease are associated with inflammation and extensive loss, the participation of prostaglandins in this disease process should be considered.

The purpose of this paper is to describe morphologic alteration that is produced by prostaglandin injection over bone surfaces of the adult rat and to discuss the possible relevance of this process to periodontal disease.

Materials and Methods

The effect of prostaglandins on bone morphology was investigated in 250 gm adult male Sprague-Dawley rats. Solutions or suspensions that contained prostaglandins were injected over the calvaria.

Injections were made with a 0.25-ml tuberculin syringe by penetration of the skin with a 26-gauge, half-inch needle at a point midway between the eyes; the needle passed parallel to the skull in the direction of the calvarium. The solution was deposited over



PGE_1

FIG 1.—Chemical structure of prostaglandin E_1.

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the periosteal surface of the frontal and parietal bones—1.5 cm posterior to the junction of the zygomatic arch and the maxilla. The rats were anesthetized with light ether.

Glycerol, saline, peanut oil, and agar were tested as vehicles. Solutions for injection were made from a concentrated stock PGE_1 solution (10 $\mu g/\mu l$) that was prepared by adding 1 ml ethanol to 10 mg PGE_1.[a] Glycerol, saline, and peanut oil solutions were made by pipetting 50 μl of stock into 500 μl of vehicle. Fifty-five microliters of this solution containing 50 μg PGE_1 was injected. Control rats were injected similarly with a solution containing 50 μl of vehicle and 5 μl of ethanol. Early prostaglandin effects were studied in six animals that were killed in pairs after one, three, and five daily injections of PGE_1 in glycerol.

The use of agar as a vehicle was tested in four rats. A 1% agar solution[b] was made by heating it to 100 C. The agar was cooled to 60 C and PGE_1 in ethanol was added. The ethanol evaporated rapidly leaving a suspension of PGE_1 crystals in agar. Fifty microliters of this suspension containing 100 μg PGE_1 was injected with a warmed 0.25-ml tuberculin syringe and a 26-gauge needle over the calvaria of two rats. Two control rats were injected similarly with agar alone.

Injection parameters for calvaria experiments are listed in the Table. Rats were injected daily and they were killed with an overdose of pentobarbital sodium. The calvaria from half of the rats were prepared for histological observation by fixation in a 10% w/v formaldehyde solution and they were decalcified in a 6 *N* formic acid solution with 0.3 sodium citrate for seven days. Decalcified tissues were embedded in paraffin, sectioned at 5 micrometers (μm), stained with hematoxylin and eosin, and photographed. Early effects were investigated histologically only. The remainder of the skulls were defleshed by boiling to observe macroscopic changes in the superficial bone morphology.

Prostaglandin effects on alveolar bone were also investigated. A concentrated suspension of PGE_1 in glycerol was prepared for injection in the alveolar region of two rats. The suspension was injected with a 10-μl syringe[c] that is capable of handling high viscosity liquids. Five microliters containing 50 μg PGE_1 was injected daily for seven days over the alveolar bone on the distal surface of the mandibular incisors. On the eighth day, the mandible was removed for histological processing. Control injections of glycerol were made on the contralateral side. Tissues were prepared for histology as were the calvaria.

[a] Upjohn Corp., Kalamazoo, Mich.
[b] Difco Laboratories, Detroit, Mich.
[c] Hamilton No. 701, Reno, Nev.

TABLE

SOLUTIONS INJECTED OVER THE CALVARIUM OF ADULT RATS

Daily PGE_1 Dose (μg)	No. of Injections*	No. of Rats	Vehicle
50	7	10	50 μl glycerol and
0	7	10	5 μl ethanol
50	7	2	50 μl saline and
0	7	2	5 μl ethanol
50	7	2	50 μl peanut oil and
0	7	2	5 μl ethanol
100	1	2	50 μl agar
0	1	2	
50	1	1	50 μl glycerol and
0	1	1	5 μl ethanol
50	3	1	
0	3	1	
50	5	1	
0	5	1	

* Rats were killed one day after their last injection; animals that received agar injections were killed seven days after the single injection.

Results

After seven days of repeated injection of 50 μg PGE_1 in glycerol, gross inspection of the injection site showed a localized area of highly vascular connective tissue. When the skull was boiled to remove soft tissue, a roughened area was observed on the calvarial surface beneath the site of prostaglandin injection in all the experimental rats; (Fig 2, *top*). This was not seen in rats that were injected with vehicle alone (Fig 2, *bottom*).

Low power observation of decalcified sections of prostaglandin-treated calvaria shows extensive loss of bone matrix and fibrous replacement (Fig 3, *top*). Calvaria from animals that were injected with the vehicle alone exhibited a subcutaneous fibrous reaction and some hemorrhage at the injection site; however no erosion of the bone surface was present (Fig 3, *bottom*).

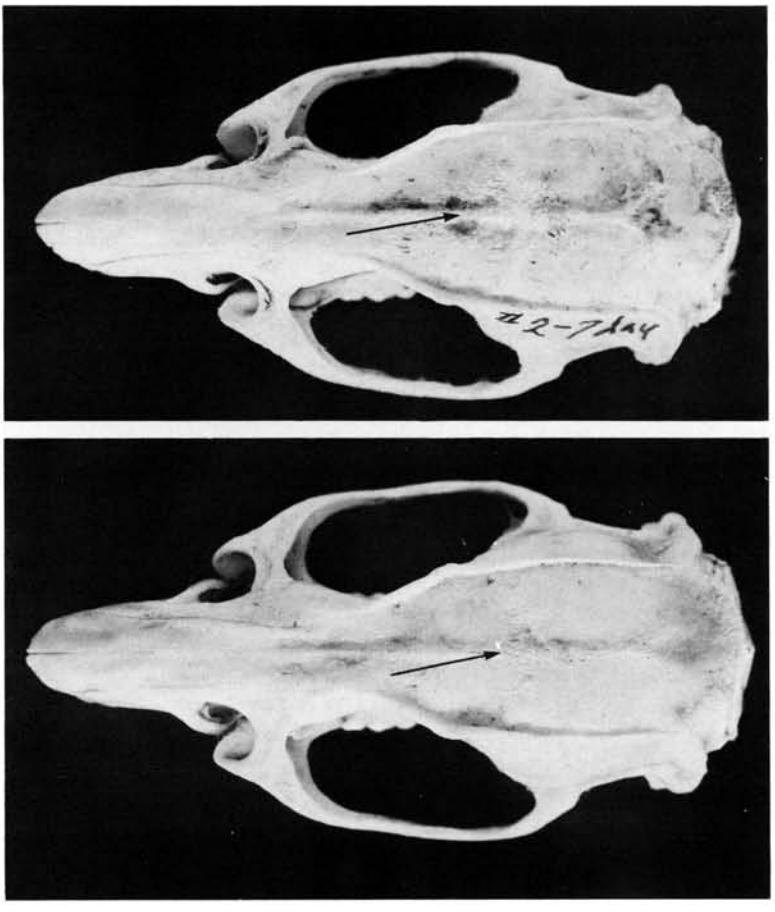

FIG 2.—Macroscopic appearance of the calvarium of an adult rat. *Top,* after repeated injection of PGE_1; *bottom,* after repeated injection of vehicle.

Microscopic examination of uninjected rat calvaria showed a dense bone matrix that was covered by a thin periosteal layer and loose subcutaneous connective tissue (Fig 4). The bone matrix had a regular lamellar structure that ran parallel to the bone surface. The matrix contained occasional small osteocytes at irregular intervals. The periosteal layer showed thin cells with flattened nuclei. The subcutaneous connective tissue contained few cellular or formed elements. Repeated injections of glycerol produced a fibrous reaction in the connective tissue, but no effect on the bone matrix was seen (Fig 5).

An altered morphology of the periosteal cells was observed after prostaglandin injection. Cells of the periosteal layer seemed enlarged one day after a single injection of 50 μg PGE_1 (Fig 6). Formerly flat nuclei seemed rounded and hyperchromatic. Areas of the bone matrix along the periosteal surface were irregular. There was an increased cellularity in the subcutaneous connective tissue layer.

During the course of repeated daily prostaglandin injections, a higher degree of bone matrix irregularity appeared. After three days of repeated injection, the laminar pattern was partially interrupted and blood vessels were more numerous in the subcutaneous connective tissue (Fig 7). In addition to the erosive pattern that is usually seen, areas were found where new growth occurred as irregular outcroppings from the original laminar surface (Fig 8). These growths occurred most often toward the periphery of the injection site.

Seven days after a single injection of 100 μg PGE_1 in agar, a bone lesion similar to the

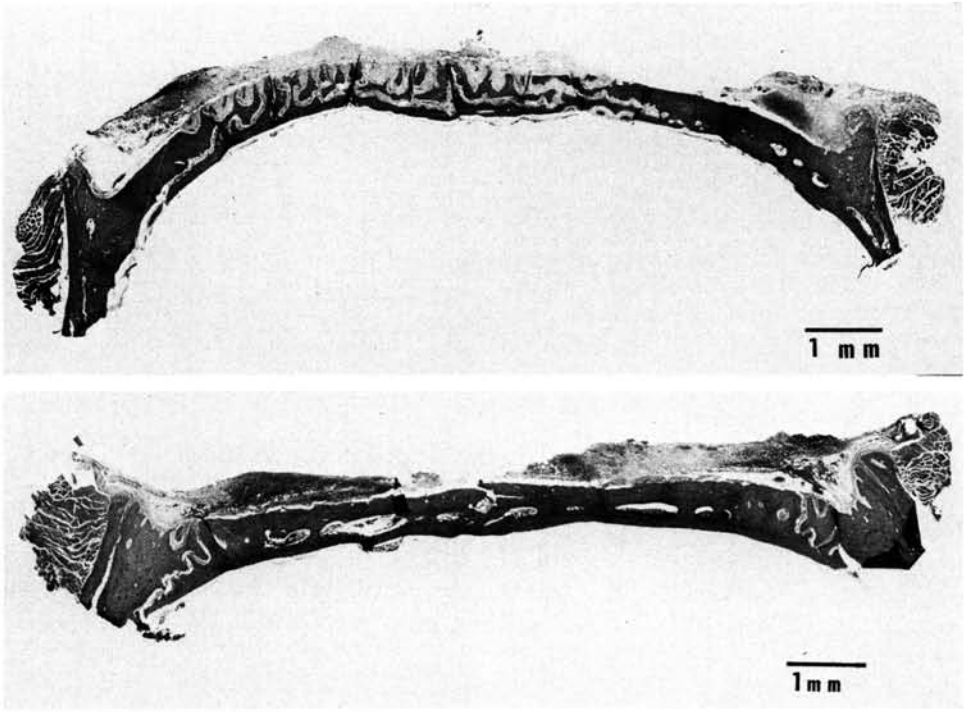

FIG 3.—Low power cross section of decalcified calvaria from prostaglandin-injected (*top*) and glycerol-injected (*bottom*) rats.

lesion seen after seven days of repeated PGE_1 injection was produced (Fig 9). In this instance the injection artifact was minimal in that no area that was injected with agar alone differed substantially from the calvaria of uninjected rats. The most prominent effect was an extensive loss of bone matrix, which had been replaced by cellular connective tissue. Many blood vessels appeared in the cellular stroma surrounding isolated bone spicules. The resorbing surfaces of the bone matrix (Fig 10) were lined predominantly with uninuclear cells with dark-staining nuclei. These cells were found often in lacunae. Osteocytes seemed somewhat enlarged and prominent within the remaining bone matrix. The lamellar structure of several areas was disrupted; in some cases it departed frim the original parallel structure. Multinucleated cells were infre-

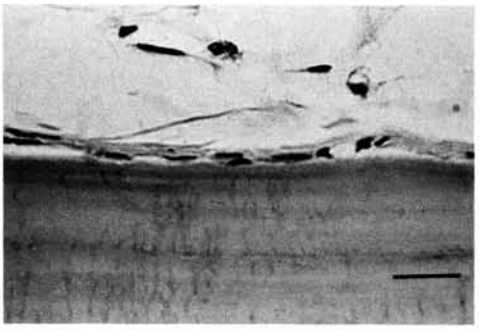

FIG 4.—Calvarial surface of uninjected rat (calibration = 50 μm).

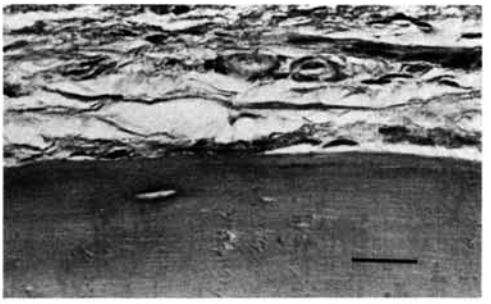

FIG 5.—Calvarial surface after seven days of repeated injection of glycerol vehicle (calibration = 50 μm).

FIG 6.—Altered periosteal morphology along calvarial surface after a single injection of PGE_1 (calibration = 50 μm).

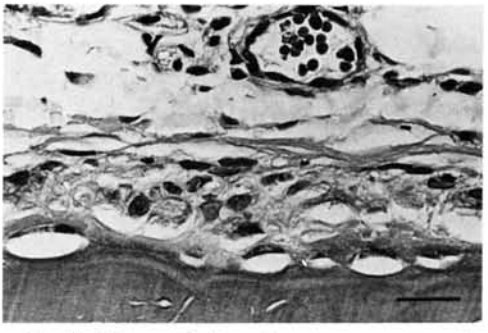

FIG 7.—Disrupted lamellar contour on calvarial surface after three daily injections of PGE_1 (calibration = 50 μm).

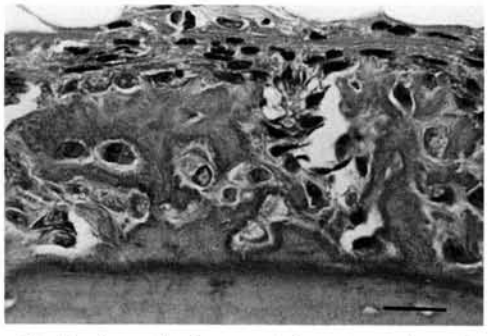

FIG 8.—Irregular bone matrix outcroppings observed in some areas on calvarial surface after five daily injections of PGE_1 (calibration = 50 μm).

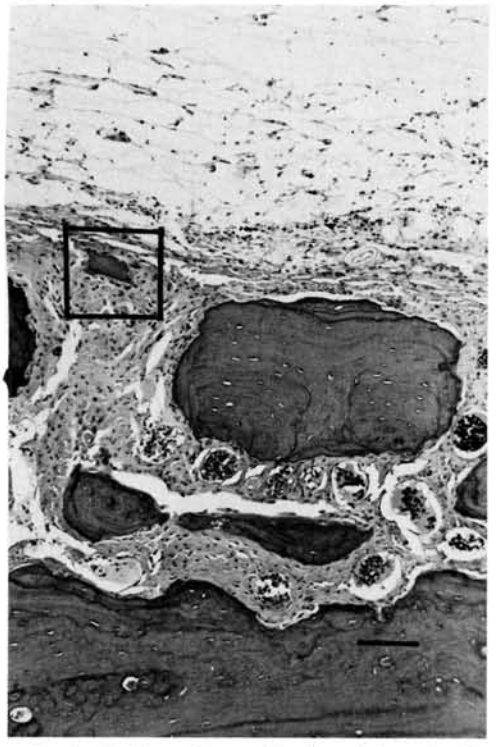

FIG 9.—Surface of rat calvarium injected with 100 μg PGE_1 in agar after seven days (calibration = 200 μm).

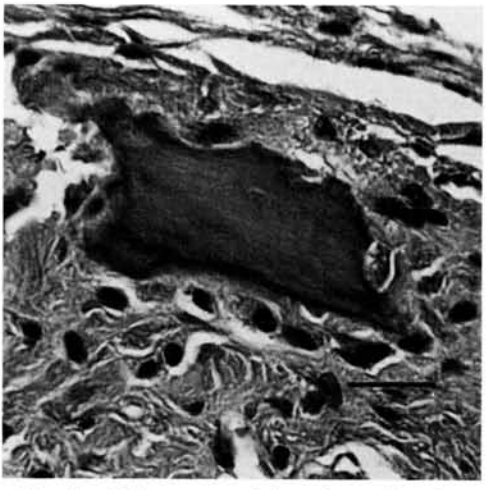

FIG 10.—Enlargement of boxed area in Figure 9. Extensive resorption of bone matrix results in formation of isolated bone spicules (calibration = 50 μm).

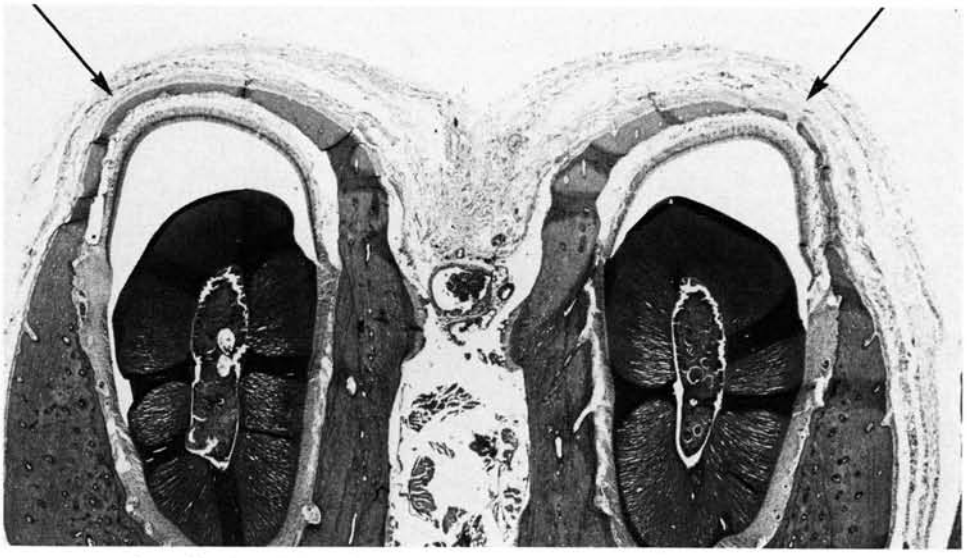

FIG 11.—Effect of PGE_1 injected over rat alveolar bone (*right arrow*) as compared with vehicle injection site (*left arrow*).

quently observed. No signs of leukocyte infiltration or exudate formation were apparent.

Repeated injection of PGE_1 containing suspension over rat alveolar bone produced a resorptive pattern similar to that which was seen in the calvaria. A low magnification view from a section that was taken parallel to the mandible through the incisors at the injection site showed discontinuity in the alveolar plate on the side of the PGE_1 injection. Higher magnification views (Fig 12) showed that the bone at the PGE_1 injection site seemed to be partially resorbed and replaced by cellular connective tissue. This discontinuity was not seen on the side that was injected with glycerol (Fig 11).

Saline and peanut oil were inferior to glycerol as vehicles. Prostaglandin solutions in saline were not effective in producing a bone lesion. Prostaglandin solutions in peanut oil produced a bone lesion; however, animals that were injected with the vehicle alone showed a similar but smaller lesion.

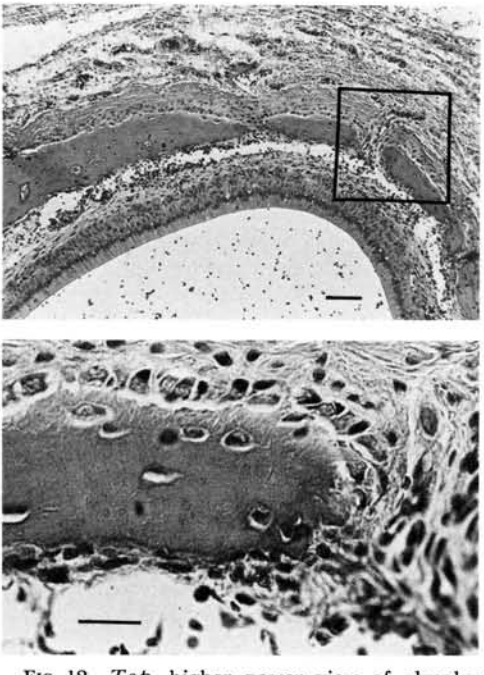

FIG 12.—*Top*, higher power view of alveolar bone discontinuity on side of PGE_1 injection (calibration = 50 μm). *Bottom*, enlargement of boxed area shows cellular detail of bone spicules apparently undergoing resorption at PGE_1 injection site (calibration = 50 μm).

Discussion

Prostaglandin administration did not mimic the periodontal disease process but there are at least three aspects in which

prostaglandin-induced bone resorption resembled that of the disease.[21] It involved the action of live cells on viable bone; resorbing surfaces were sculptured with covelike lacunae; and there was a strong suggestion of vascular component to the resorption process.

Prostaglandin-induced bone resorption differed from the histological appearance of periodontal disease in its absence of inflammatory cells. However, there is considerable evidence that oral bacteria stimulate the formation of chemotactic factors.[22] This could account for the inflammatory cell infiltration that is characteristic of periodontal disease. Leukocytes may be especially important in this connection because there is evidence that they release prostaglandins[23] and another osteolytic principle.[24]

Prostaglandins are slightly chemotactic.[3] The absence of a significant infiltration of inflammatory cells at the site of prostaglandin injection indicated a negligible chemotactic effect in the rat subcutaneous connective tissue. Furthermore, this finding indicated that bacterial inflammation was not introduced inadvertently as a result of the injection procedure.

Increased vascularity associated with bone resorption was observed commonly. Although the vasodilator action of prostaglandins is well known,[5] stimulation of new blood vessel formation with chronic administration has not been studied. Under the conditions of these experiments, it cannot be ruled out that increased vascularity could have been mediated indirectly by products of the resorptive process.

The concentration of PGE_1 that was used in this study (3 m*M*) was greater than the levels that occur in tissue. Failure of saline as a vehicle suggests that washout is relatively rapid; this is expected of a vasodilator substance.

Conclusions

A subcutaneous injection of prostaglandin E_1 over a bone surface stimulated rapid resorption of bone matrix in the adult rat. The earliest morphologic alterations observed were associated with the fibroblasts of the periosteal layer. As the resorptive process continued, areas of resorbed bone matrix developed. Cellular connective tissue containing newly formed vascular elements replaced the former bone matrix. Some areas showed signs of irregular formation of new bone. Inflammatory cells were absent and multinucleated osteoclasts were uncommon.

The rapidity and extent to which bone resorption was produced by prostaglandin injection suggests that a local synthesis of lesser amounts over a longer period of time could account for the bone loss in localized bone-wasting diseases. The recent elucidation of prostaglandins as inflammatory mediators offers a plausible hypothesis for their genesis in periodontal disease.

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