

Regulation of Gingival Collagenase: A Possible Role for a Mast Cell Factor¹ (37744)

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Mechanisms for the *in vivo* regulation of vertebrate collagenase activity are not clearly understood. Taylor (1, 2) reported that media from rat gingival tissue cultures were capable of degrading rat mesenteric collagen fibers and that this process occurred most readily around the mast cells of the mesentery. The findings suggested that mast cells elaborate a factor which functions *in vivo* to facilitate collagenolysis. Experiments were therefore undertaken to determine whether extracts from mast cells contain a factor which may function in the regulation of collagenase activity.

Materials and Methods. Gingival tissue was obtained from adult male rats (Sprague-Dawley) as described by Taylor (1) and incubated in culture flasks with Dulbecco's modification of Eagle's fluid with or without 2% fetal bovine serum. The culture media were harvested and replaced with fresh media daily for 1 wk. The collections were pooled and centrifuged to remove cells and debris and the supernatant was exhaustively dialyzed against 2.5 mM, Tris-HCl (pH 7.5) containing 1 mM CaCl₂. The retained medium was then lyophilized.

Rat peritoneal mast cells were obtained by a modification of the method of Uvnäs and Thon (3). Peritoneal washings from exsanguinated rats were layered over 30% Ficoll. Centrifugation carried the mast cells through the Ficoll leaving all other cells at the liquid interface. The mast cells were washed and suspended in saline buffered at pH 7.4 (approx 1,000,000 cells/ml) and strongly

sonicated. The sonicate was filtered and stored.

Mast cell extracts were also prepared from fresh and formalin fixed mastocytomas of dogs. The tissue was homogenized in Hanks' fluid, strongly sonicated, filtered to yield a clear fluid, extensively dialyzed against glass distilled water at 4° and stored.

Histological assay of collagenase activity was done by phase contrast microscopic examination of a substrate consisting of fresh rat tendon collagen fibers, teased and spread on glass slides, and incubated at 37° in tissue culture chambers with the reaction mixtures to be tested. The enzymatic breakdown of individual fibers could then be clearly discerned.

Collagenase activity was also studied by viscosimetry. Reaction mixtures contained acetic acid-extracted rat tail collagen (4), 3 mM Tris-HCl (pH 7.0) and 3 mM CaCl₂ and had final relative viscosities of 5.0. The purity and native state of collagen was established by resistance to trypsin digestion. Twenty milligrams of lyophilized gingival culture medium and 2 ml mast cell extract were used in the experiments. Viscosity changes were measured in Ostwald viscosimeters at 27°.

Samples of the collagenase degraded rat tail mixtures to be electrophoresed were brought to pH 2.0 with 2 N HCl and were subjected to thermal denaturation at 45° for 10 min (5). Disc electrophoresis as modified by Reisfeld, Lewis and Williams (6) and revised by Nagai, Gross and Piez (5) was used.

Segment long spacing aggregates were prepared from the enzyme digests according to

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TABLE I. Effect of Rat Gingival Tissue Culture Media and Mast Cell Extracts on the Viscosities of Collagen Solutions.

Reaction mixture contents ^a	% Initial viscosity ^b	
	3 hr In-cubation	24 hr In-cubation
Control (less tissue culture media)	98	98
Gingival tissue culture media	91	92
Gingival tissue culture media + mast cell extract (rat)	78	52
Mast cell extract (rat)	94	85
Gingival tissue culture media + mast cell extract (dog)	74	69
Mast cell extract (dog)	89	87
Gingival tissue culture media + TCA mast cell extract ^c	95	95
Gingival tissue culture media + heated mast cell extract ^d	91	80
Gingival tissue culture media + heated mast cell extract ^e	90	80
Gingival tissue culture media + heated mast cell extract ^f	95	83

^a Culture media used in the experiments were harvested from rat gingival tissue cultured with 2% fetal bovine serum.

^b Incubations were carried out as described in the text at 27°.

^c Mast cell extract exposed to 20% TCA.

^d Mast cell extract heated at 80° for 5 min.

^e Mast cell extract boiled for 5 min.

^f Mast cell extract boiled for 30 min.

the procedure of Gross and Nagia (7). These were stained with uranyl acetate and examined with a Hitachi 11E microscope.

Results and Discussion. Rat gingival tissue culture medium containing 2% fetal bovine serum in the presence of rat mast cell extract caused a 22% reduction in the relative viscosity of a collagen solution during a 3 hr incubation. Viscosities of reaction mixtures containing the gingival tissue culture medium without mast cell factor or the mast cell extract alone remained near control levels (Table I). Similar results were obtained with dog mastocytoma mast cell preparations. Mast cell preparations (pH 7.5) lost only part of their ability to facilitate gingival collagenase on heating, but that ability was lost after

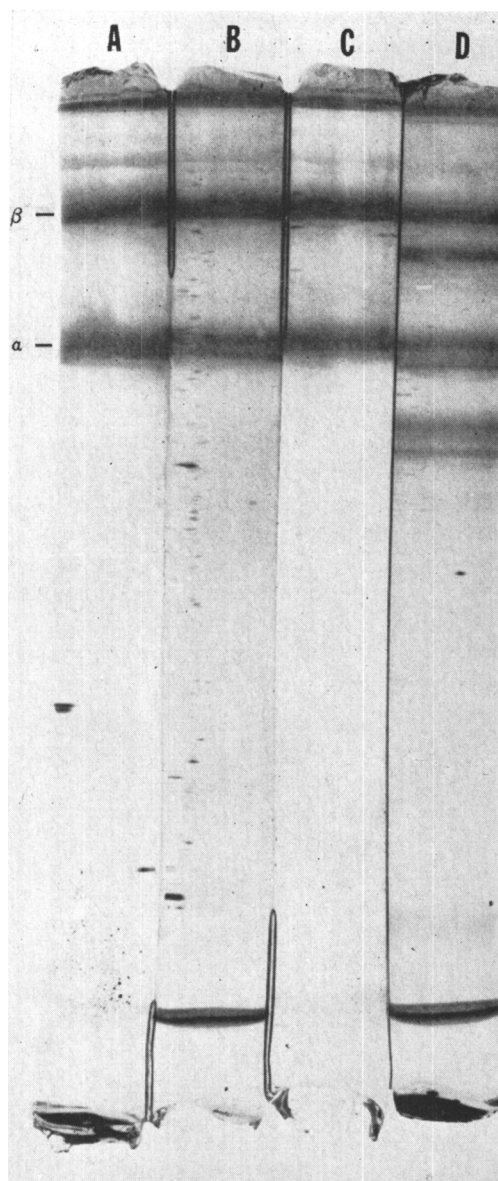


FIG. 1. Acrylamide gel electrophoresis of the products of collagen digestion by rat gingival collagenase obtained from tissue culture media containing 2% fetal bovine serum in the presence and absence of the mast cell factor after a 24 hr incubation. (A) Less enzyme control; (B) collagenase without mast cell extract; (C) mast cell extract without collagenase; (D) collagenase plus mast cell extract. Reaction mixture with collagenase and mast cell extract reduced the viscosity of the collagen solution by 48% during the 24 hr incubation.

treatment of the extract with 20% TCA

(Table I).

Electrophoretic patterns on acrylamide gels demonstrated that collagen solutions incubated 24 hr with gingival culture medium containing serum or with mast cell extract alone exhibited typical α and β chains (Fig. 1). However, reaction mixtures containing gingival culture medium with heated or non-heated mast cell extracts showed a reduction in the collagen α and β components with a concomitant appearance of collagen fragments with molecular weights lower than the α and β chains.

In the electron micrographs of the segment long spacing, prepared from the enzyme digests, aggregates of normal intact tropocollagen molecules were seen along with many $\frac{3}{4}$ length fragments that terminated distally with the β_2^2 band according to the nomenclature of Hodge and Schmitt (8). These were frequently coprecipitated with whole TC molecules in hybrid aggregates (Fig. 2). Isolated $\frac{1}{4}$ length fragments were infrequent-

ly seen. Aggregates of shorter proximal fragments similar to those described by Tokoro, Eisen and Jeffrey (9) in digests with rat skin collagenase were also seen. Figure 2 illustrates coprecipitates of intact molecules (TC) with 67% fragments.

Rat gingivae cultured in media without serum yielded tissue culture media which contained active collagenase (Table II). Addition of rat serum markedly inhibited the collagenase activity. Mast cell extract added to a reaction mixture containing gingival tissue culture media and rat serum prevented the inhibitory effect of the rat serum.

Media from gingival tissue cultured in the presence of bovine serum also failed to degrade freshly teased tail tendon collagen fibers (Fig. 3A). However, the same culture medium plus mast cell extract degraded collagen fibers (Fig. 3B). Rat tail tendon collagen fibers were also degraded by serum-free gingival culture medium (Fig. 4A). Addition of serum to the medium inhibited

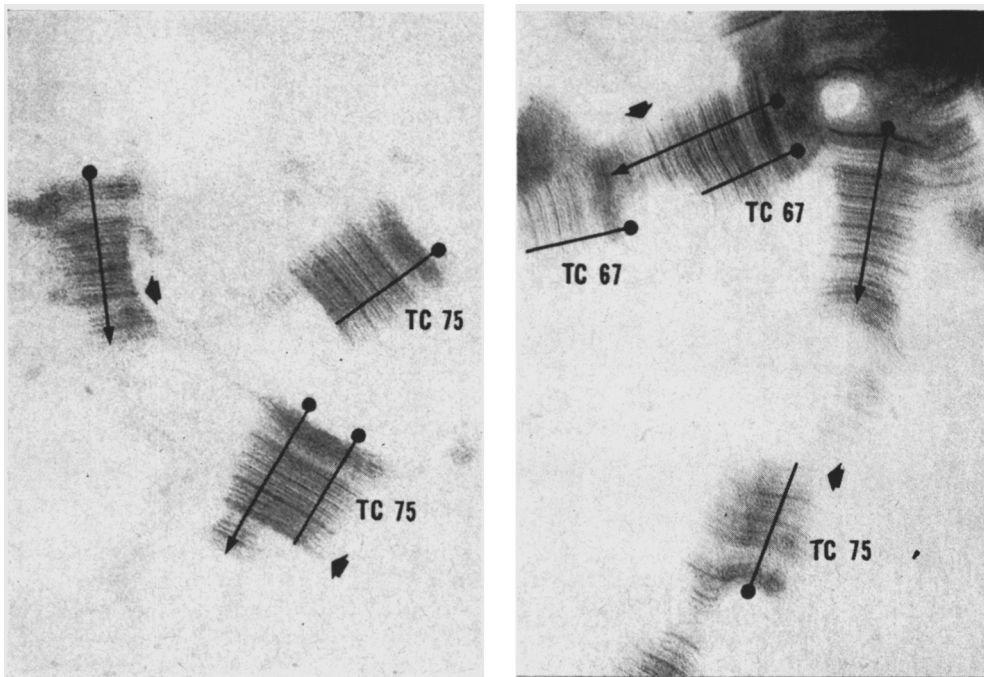


FIG. 2. Electron micrographs of segment long spacings of collagen fragments produced by action of rat gingival collagenase. Aggregates of intact normal collagen molecules are indicated by long arrows. Aggregates of $\frac{3}{4}$ length fragments labeled as TC 75 frequently coprecipitated with whole molecules. The position of the β_2^2 band marked by the heavy arrows. Shorter segments of 67% length from the "A" end designated TC 67.

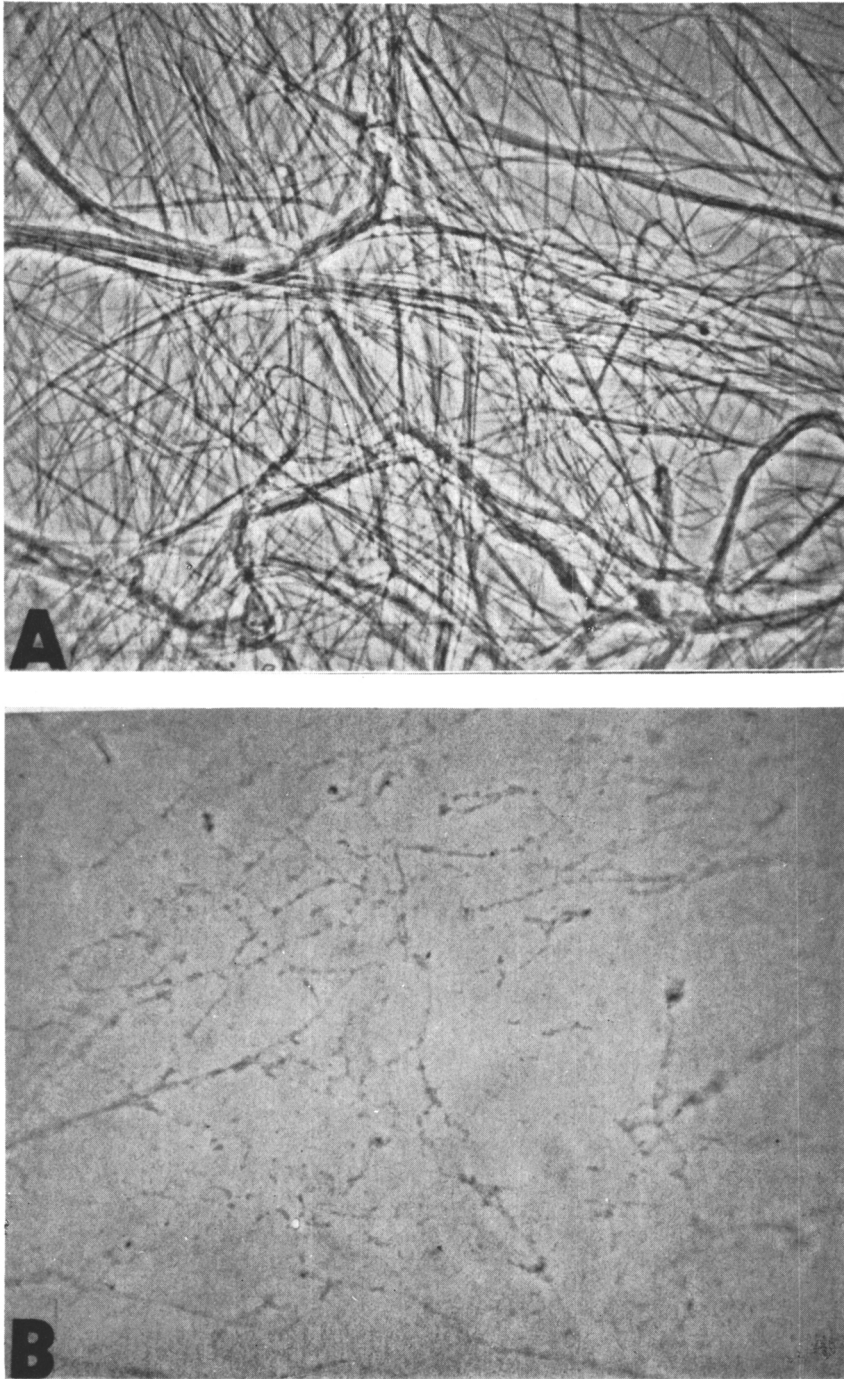


FIG. 3. Phase contrast photomicrographs of teased rat tail tendon fibers after incubation for 3 days at 37° as described in the text. (A) Incubated with fluid harvested from gingival tissue cultured in the presence of 2% fetal bovine serum (10 mg lyophilized culture medium + 1 ml Hanks' fluid). (B) Incubated in the same tissue culture medium with mast cell extract added (10 mg lyophilized culture medium + 0.5 ml mast cell extract + 0.5 ml Hanks' fluid).

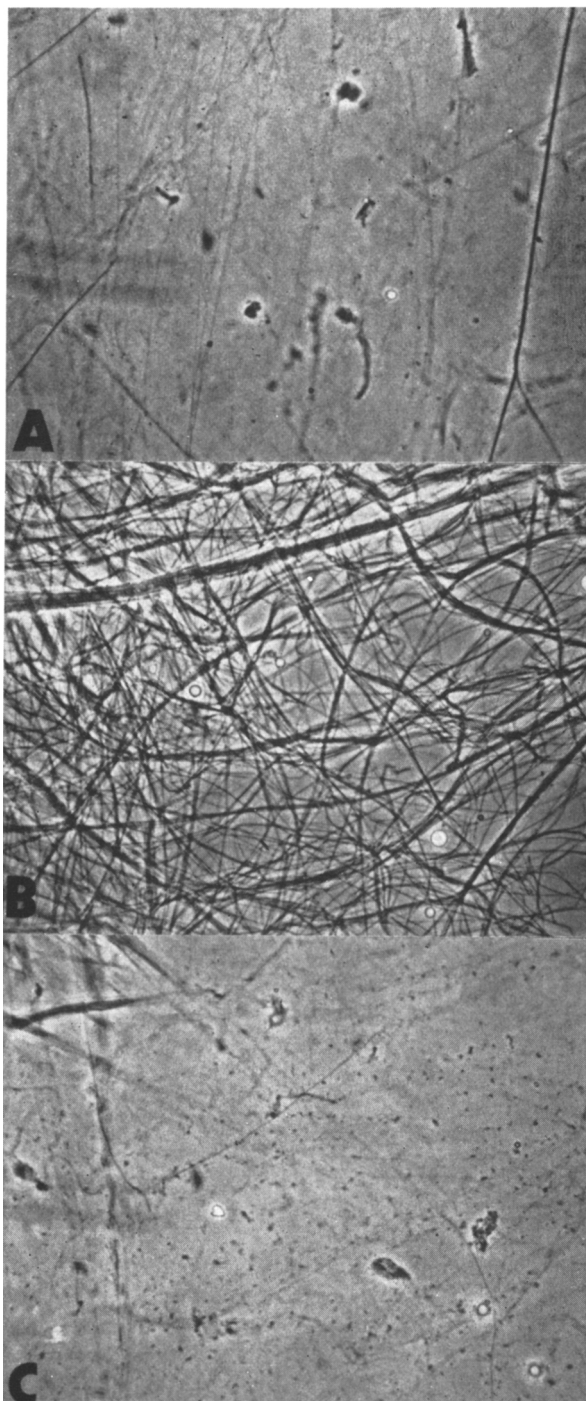


FIG. 4. Photomicrographs of teased tendon fibers incubated 4 days in fluid harvested from gingival tissue cultured without serum. (A) Incubated in the culture medium alone (5 mg lyophilized culture medium in 1 ml Hanks' fluid). (B) Incubated in the culture medium with serum added (5 mg lyophilized culture medium + 0.02 ml fetal bovine serum + Hanks' fluid to 1 ml). (C) Incubated in the culture medium with serum and mast cell extract added (5 mg lyophilized culture medium + 0.02 ml fetal bovine serum + 0.1 ml mast cell extract + Hanks' fluid to 1 ml).

TABLE II. Effect of Rat Serum and Rat Mast Cell Extract on Rat Gingival Collagenase.

Reaction mixture contents ^a	% Initial viscosity	
	A ^b	B ^c
Control (less tissue culture media)	96	92
Gingival tissue culture media	84	58
Gingival tissue culture media + rat serum ^d	93	91
Gingival tissue culture media + rat serum ^d + mast cell extract (1.0 ml)	80	73

^a Collagenase was obtained from media after culturing rat gingiva in the absence of serum.

^b Incubations were carried out at 20° as described in the text.

^c Incubations were carried out at 27° as described in the text.

^d The amount of serum added in Expt A was 0.025 ml and in Expt B, 0.03 ml.

the collagen degrading action in this system too (Fig. 4B), while the addition of mast cell extract blocked the inhibitory action (Fig. 4C).

Other experiments were carried out to determine whether gingival tissue contained an extractable collagenase which could be activated by the mast cell factor. Extracts of gingiva (50 mg tissue/ml) failed to show collagenase activity with or without mast cell extract.

Extracts of muscle, kidney, liver, intestinal mucosa and leucocytes could not substitute for mast cell extracts in the activation of gingival collagenase. This observation is consistent with the previous report that the degradation of mesenteric collagen fibers by gingival tissue culture fluid originated exclusively around the mast cells (2).

The nature of the mast cell factor has not been established; however, the active component is nondialyzable and heat stable but is removed from the extract by precipitation with 20% TCA. Neither heparin, histamine nor 5-hydroxytryptamine could substitute for mast cell factor in the activation of gingival collagenase.

The study reported here supports the suggestion that the *in vivo* control of collagenase is, in part, at the enzyme level (10, 11) and

could be controlled by a factor elaborated by mast cells. Our studies indicate that the mast cell factor acts to reverse the inhibition of collagenase by serum inhibitors. The factor was heat and formalin stable. It seems unlikely, therefore, that the mast cell factor is an enzyme which converts a procollagenase to an active enzyme, as has been described for the activation of bone (12), and leucocyte procollagenase (13).

Summary. Serum free media from rat gingival tissue cultures contained active collagenase which was inhibited by the addition of rat serum or fetal bovine serum. The inhibitory action of serum on gingival collagenase was blocked by the addition of mast cell extract. The mast cell factor was nondialyzable and heat stable but was inactivated by exposure to 20% TCA. Neither heparin, histamine and 5-hydroxytryptamine nor extracts from a variety of other tissues could substitute for the mast cell factor in the activation of gingival collagenase. A mast cell factor may participate in the *in vivo* regulation of collagenase activity.

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