Platelet Aggregation Produced by Collagenolysis Product Peptides[1] (35892)

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The recent observation made by Wilner *et al.* (1) of the aggregation of platelets by soluble collagen raised the question as to whether the collagen breakdown products released from soluble collagen by cutaneous collagenase activity, as previously described (2), could also affect the aggregation of platelets.

Methods. After molecular sieving and Dowex ion-exchange chromatography (2), each collagen-derived peptide fraction was desalted by dialyzing against distilled water by filtering through Amicon Diaflo membranes of 500 dalton pore size until the pH of the *effluent* was equivalent with distilled water (pH 6.8).

The salt-free collagen peptide products had a pH of 5.5 to 6.5 which was easily adjusted to pH 7.5 with 0.05 *M* Tris buffer [pH 7.5], in 0.15 *M* NaCl. The concentration of the final peptide solutions added to 1.0 ml of platelet-rich plasma were about 1 μg/0.05 ml in buffered saline.

Samples of blood were collected from Sprague-Dawley rats (weighing 250 to 400 g) by open-chest cardiac puncture. Nine milliliters of blood were withdrawn through siliconized 19-gauge needles and into pyrogen-free plastic syringes which contained 1 ml of 3.2% sodium citrate. Siliconized glassware was used throughout.

After standing at 5° for 1 hr, the blood was centrifuged in the cold for 16 min at 300*g* to collect the supernatant platelet-rich plasma. The pooled platelet-rich plasma prepared from four rats was used within 3 hr after the blood was collected. The platelet counts were about 600,000 cells/mm^3.

The 1-ml portions of platelet-rich plasma so prepared demonstrated no spontaneous aggregation with stirring at 37° in the presence of 0.05 to 0.5 ml of isotonic saline or in 0.05 *M* Tris-buffered saline [pH 7.5] for 10 min in the aggregometer.

Temperature equilibration of 1 ml of plasma from 5 to 37° required 1.5 min in the aggregometer; therefore, each peptide solution was added after a 1.5- to 2-min period of preincubation at 37°. The aggregation of these platelets at 37° was determined in the Platelet Aggregometer (Chrono-log Corporation) and the results recorded on a B & L Model VOM-5 recorder (3).

Results. These results (presented in Fig. 1) compare the effects of dilution by the buffered saline control with these effects produced by the soluble collagen product-peptides upon the aggregation of rat platelets.

All peptide products tested (B through H) demonstrated instantaneous aggregation of platelets in plasma.

Peptide D (1.05 μg/0.05 ml) was clearly the most potent material for the aggregation of platelets and this peptide was one of the least chemotactically active of the collagen peptides [shown in Table II of the Ref. (2)]. When the purified rat skin salt-soluble collagen solution (40 μg/0.05 ml) was added to platelet-rich plasma, there was a latent period of 2 sec, while when the product-peptide solutions (1 μg/0.05 ml) were added to the platelet-rich plasma, no latent period was observed.

Conclusion and Summary. Saline solutions of about 1 μg of each of seven of the eight collagen peptides released by the action of

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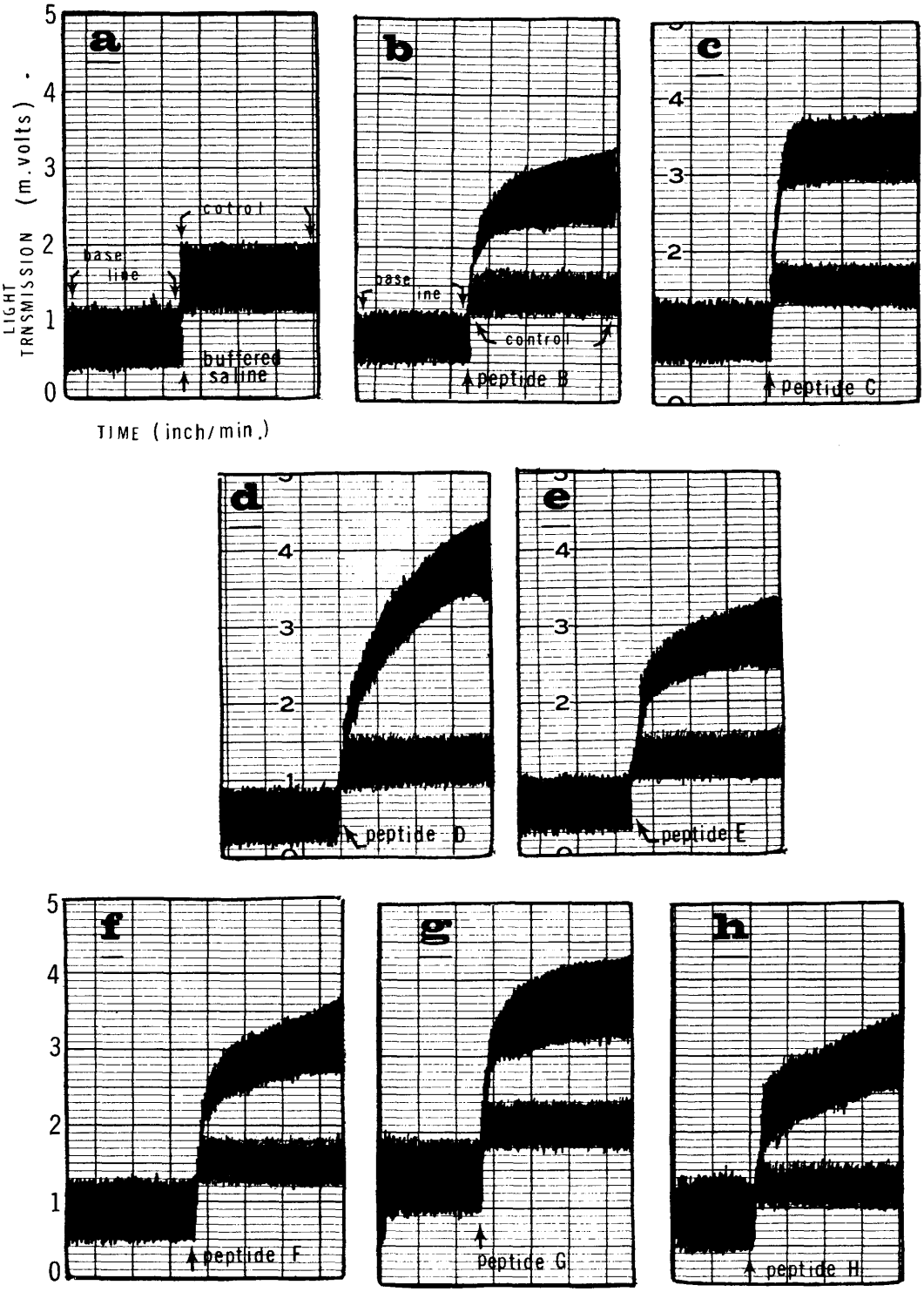

FIG. 1. Aggregometer recording of the aggregation of platelet-rich plasma in the presence of 0.05 ml of buffered saline or solutions in buffered isotonic saline of desalted collagenolysis product peptides (B through H). The quantitative contribution of the buffered isotonic saline control is indicated by the bottom curve. The arrow indicates the point of addition of the peptide (B through H).

cutaneous collagenase upon soluble collagen were capable of affecting the aggregation of platelets. Thus, at least two important cellular responses to injury (*i.e.*, PMN infiltration and platelet aggregation) can be demonstrated to be produced by the products of collagenolysis.

These findings on platelet aggregation reinforce the relative importance of collagenolysis product peptides in the overall process of inflammation.

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