# Effect of Glucosamine on Platelet-Collagen Reaction (32840)

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The adhesion of platelets to collagen fibers was studied by several authors using morphological and physicochemical methods to follow the reaction (1, 2, 5). Hugues and Lapiere used phase contrast microscopy to demonstrate the platelet aggregation brought about by reconstituted collagen fibers (1, 2). This aggregation of platelets induced by collagen fibers is the second phase of the hemostatic process immediately following the adhesion. The detailed mechanism of these reactions can possibly be understood by the search for substances that would activate or inhibit one or the other or both. In a previous paper, we have described an inhibitory effect produced *in vitro,* by substances normally present in connective tissue, such as hexoses and hexosamines (6). D-Glucosamine proved to be the most potent inhibitor, although the extent of the inhibition depended on the nature of the collagen preparation used in the assay. We want to present evidence for a specific interaction of D-glucosamine with tropocollagen during fibril formation impairing its aggregating activity.

*Method.* The soluble collagen preparations were obtained from calfskin and from embryonal calfskin, according to the methodology of Gross and Lapiere (7). The hexose [Dische (8)], hexosamine [Elson and Morgan (9)], hydroxyproline [Loxley and Bergmann, (10)], and nitrogen [Markham (11)] contents of these preparations are given in Table I. As shown, even after 3 succesive reprecipitations, the purified tropocollagen preparations still contain appreciable amounts of hexosamine.

Citrated platelet-rich plasma (PRP) was prepared as described previously (4, 5). The turbidimetric method of Born (3) was used with constant stirring (4, 5). A standard reaction mixture contained 0.6 ml of PRP and at zero time, 0.2 ml of the collagen suspension was added and the intensity of light transmitted recorded as a function of time at 600 mμ as described (5, 6). The collagen fiber suspension was obtained by heat precipitation of a tropocollagen solution just before the experiment, as described in (1). An S-shaped curve is obtained on plotting the increase in light transmission as a function of time. The length of the lag-phase depends of several factors, such as the amount of collagen fibers in the suspension, the platelet count in the plasma and the temperature (5). The aggregating property of the collagen–PRP supernatant was assayed by a method based on the ability of the supernatant of the collagen–PRP mixture to aggregate, in the test described above, a control citrated platelet-rich plasma (4).

TABLE I. Composition of the Collagen Preparations Used. (Results are given in μg/100 μg of dry wt.)

| Source of collagen | Embryonic calfskin | Calfskin |
|---|---|---|
| Nitrogen | 15.8 | 16.0 |
| Hydroxyproline | 13.2 | 13.0 |
| Hexose | 1.2 | 1.5 |
| Hexosamine | 3.0 | 4.5 |

*Results.* Figure 1A shows the kinetics of the aggregation reaction and the inhibition produced by D-glucosamine, with both collagen preparations described in Table I. It can be noticed that the lag-period is shorter with the embryonic calfskin collagen and the aggregation more complete as judged by the larger increase of light transmission as compared to the calfskin collagen preparations. Four mg of D-glucosamine added to collagen during the preincubation period, needed for fiber formation, produced a complete inhibition of the aggregation phenomenon. This concentration corresponds to 440 μ*M* of glucosamine/mg of collagen, or to 150 μ*M* of glucosamine/$10^9$ platelets. Two mg of glucosamine produced a 27% decrease of the

[1] Where reprints are available.

[2] Equipe CNRS No. 53.

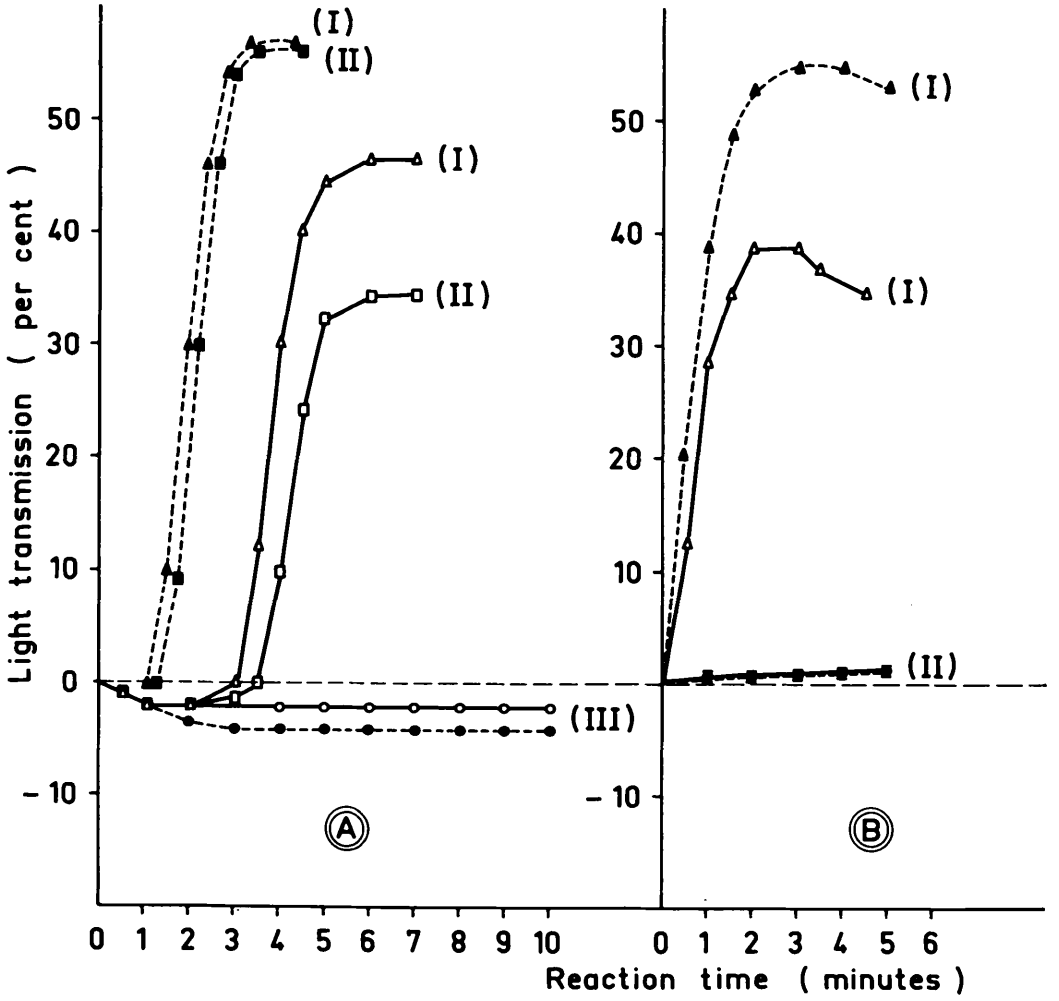


FIG. 1. A. Inhibitory effect of 2 mg (II) or 4 mg (III) of glucosamine on platelet aggregation induced by collagen (200 $\mu$g/ml = 40 $\mu$g in the reaction mixture); platelet-rich plasma: 250,00 platelets/mm$^3$, measured photometrically at 600 m$\mu$. —— Calfskin collagen in buffer (I). - - - - - Embryonic calfskin collagen in buffer I. B: Inhibitory effect on the release of platelet aggregating activity in the supernatant of the mixture collagen–platelet-rich plasma in presence of glucosamine (II) 4 mg/ml final concentration. The aggregating property released is assessed on a human citrated platelet-rich plasma (substrate).

final turbidity change and a slight increase of the lag-phase.

Figure IB shows that the platelet release phenomenon induced by collagen is also inhibited in the presence of D-glucosamine as shown by the inability of the supernatant of the platelet–collagen mixture to aggregate normal human citrated platelet-rich plasma (4, 6).

A remarkable feature of the glucosamine-inhibition is that it is stronger when glucosamine is added to the tropocollagen solution *before* the preincubation at 33°C necessary for the formation of the collagen fibrils. The longer this preincubation time, the stronger the inhibition. Table II shows the correlation between preincubation time, increase in the lag-phase of the aggregation reaction and the decrease of the intensity of the final aggregation. Only a weaker inhibition whatsoever could be observed when glucosamine was added to the polymerized collagen

TABLE II. Effect of Preincubation Time of Collagen with Glucosamine on the Platelet Aggregation Reaction.

Concentrations during the preincubation period (fibril formation): 200 $\mu$g of tropocollagen; 16 mg of D-glucosamine (= 100 $\mu M$) in 1 ml final volume, veronal buffer 0.1 $M$, pH 7.4; temperature, 33°C. Concentrations during the platelet aggregation reaction: 40 $\mu$g of collagen fibrils in suspension, 3.6 mg of D-glucosamine (20 $\mu M$) and 15 × $10^7$ platelets (2.5 × $10^5$/mm$^3$ of PRP) in 0.8 ml final volume.

| | Preincubation time of tropocollagen and glucosamine (min) | | |
|---|---|---|---|
| | 10 | 12 | 15 |
| Increase in the lag-phase before platelet aggregation (sec) | 30 | 60 | 120 |
| Inhibition of platelet aggregation (%) | 15 | 21 | 25 |

fibers or if it was added to the platelet suspension and preincubated with it, or added to the collagen–PRP mixture, during the lag-phase or during the aggregation period.

*Discussion.* These results strongly suggest that glucosamine reacts with a component of tropocollagen during the fibril formation reaction. This assumption is supported by the fact that glucosamine did not inhibit (or only weakly) platelet aggregation induced by ADP (0.4 $\mu$g/final concentration), adrenalin (1 $\mu$g/final concentration), thrombin (0.1 NIH unit/final concentration), or bovine fibrinogen (KABI) (1,2 mg/final concentration). The release of platelet aggregating activity in the presence of thrombin (6) is also not affected by glucosamine. The different sensitivities of those two collagen preparations towards D-glucosamine suggest that the carbohydrate containing impurities of collagen might be involved in the aggregation reaction.

*Summary.* The platelet aggregation induced by purified repolymerized collagen fibers is inhibited by D-glucosamine: this inhibitory effect requires a preincubation of glucosamine with tropocollagen during the polymerization of the fibers. Weak inhibition was obtained when glucosamine was added to the polymerized fibrils or to the platelets. This phenomenon seems to depend on a specific modification of the collagen fibrils polymerized in the presence of glucosamine.

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