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# RELATIONSHIP BETWEEN THE VELOCITY OF ROLLING GRANULOCYTES AND THAT OF THE BLOOD FLOW IN VENULES

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SUMMARY

1. The mean velocities at which granulocytes roll along the walls of small venules have been related to their mean blood flow velocities in preparations of hamster cheek pouch and mouse mesentery. In animals anaesthetized with Nembutal, 30–200 $\mu$m venules were observed microscopically and the movement of rolling granulocytes quantitated on films. Apparent mean blood flow velocity was determined from films of embolizing platelet thrombi.

2. In four venules the velocity distribution of about 100 rolling cells was almost symmetrical about the mode, with a small proportion moving at up to three times the mode velocity. Therefore, the mean and mode velocities were very similar.

3. In two mesenteric and two cheek-pouch venules, blood flow velocity was temporarily altered during and after gentle compression with a fine glass fibre; this was associated with proportional changes in mean cell velocities.

4. In four different venules of a hamster cheek pouch, the mean velocity of rolling granulocytes increased in proportion to the mean blood flow velocity ($r = 0{\cdot}963$).

5. In thirty-six venules of ten mouse mesenteries, the velocities were proportional ($r = 0{\cdot}915$) between blood velocities of about 300 and 1000 $\mu$m/sec. Above this the velocity of the cells did not increase further.

6. The rolling of granulocytes is presumably governed by two forces, the shear force of the flowing blood and an adhesive force between the surfaces of granulocytes and vascular endothelium. Our results suggest that, within limits, the proportionality between the velocities of blood flow and rolling cells is due to shear force, the adhesive force being similar for all the cells. The results suggest that this adhesion force per granulocyte is of the order of $10^{-5}$ dynes.

## INTRODUCTION

One of the first signs of inflammation is the adhesion of granulocytes to the walls of small venules. This adhesion begins as a rolling movement of the granulocytes along the walls which is much slower than the flow of erythrocytes in the same streamlines. As inflammation progresses, increasing numbers of the rolling cells come to rest and a proportion of these migrate out of the vessels into the tissues (see Florey, 1970).

To find an explanation for these processes in biophysical and biochemical terms, we recently introduced a method for quantifying the adhesiveness between endothelium and circulating granulocytes by counting the rolling cells in small venules under various experimental conditions (Atherton & Born, 1972). With this method it was found that the rolling granulocyte count increased reversibly when different agents known to be chemotactic to granulocytes *in vitro* were applied locally to small venules. This suggested that these agents change the walls of the venules in some way so that they become selectively adhesive to circulating granulocytes which happen to touch them. Earlier observations also suggested that, in any one venule, the velocity at which the cells rolled was related to the mean velocity of the blood flow. In small venules the blood flow is laminar with very low Reynolds numbers. Under such conditions the shear force experienced by cells in contact with the vessel walls is proportional to the mean blood flow velocity (Whitmore, 1968). It seemed, therefore, that a systematic investigation of the relationship between the mean blood flow velocity and the mean velocity of rolling granulocytes might provide information about the forces responsible for the rolling phenomenon. This paper reports the results of this investigation; some of them have been communicated to the European Microcirculation Society (Atherton & Born, 1973*a*) and to the Physiological Society (Atherton & Born, 1973*b*).

## METHODS

*Preparations* were made of hamster cheek pouch and mouse mesentery as already described (Atherton & Born, 1972). The animals were anaesthetized with Nembutal (6 mg/100 g body wt.). Measurements were made only in preparations in which the blood flow in the microcirculation remained almost constant for 2–3 hr. Most experiments were completed within the first hour.

*Velocities of rolling granulocytes* were determined by filming their movement in venules through a Leitz Orthoplan microscope at a magnification of 500 with a Bolex camera at 24 or 32 ft/sec. On any one film sequence the velocities of 20 to 40 individual rolling cells were measured to establish their *mean* velocity.

*Mean blood flow velocity* was determined by filming the movement of embolizing platelet thrombi or 'white bodies' produced upstream in the venule under observation by the iontophoretic application of ADP (Begent & Born, 1970).

The films were analysed by projection through a Specto motion analyser and measurements made at a magnification of 3000.

## RESULTS

### *Velocity distribution of rolling granulocytes in single venules*

In two preparations of mouse mesentery and in two of hamster cheek pouch, the mean velocities of approximately 100 cells were determined

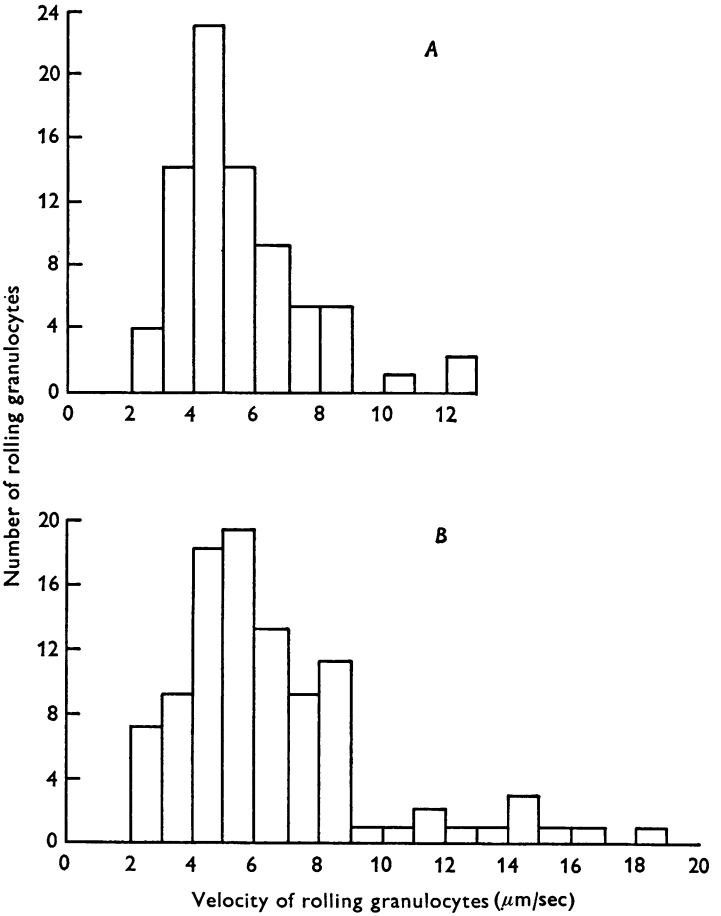


Fig. 1. Distribution of velocities of rolling granulocytes in two mouse mesentery venules, *A* and *B*, in which the mean blood flow velocities were 400 and 475 $\mu$m/sec respectively.

in single venules over periods of 3–5 min. The results (Fig. 1) were similar in all of these vessels. No cells rolled at velocities of less than about 2 $\mu$m/sec, suggesting that any cells slowed down to this velocity came to

rest. A small proportion of cells rolled up to 4 times faster than the mode velocity. The velocities of the cells were distributed steeply and almost symmetrically about this mode, and the distribution was fitted closely by a log normal plot. The mode and mean velocities were sufficiently similar to express results as mean velocities. In the following experiments, the velocities of twenty to forty individual cells were measured and the mean velocity and standard error of the mean were calculated for each point relating cell velocity to mean blood flow velocity.

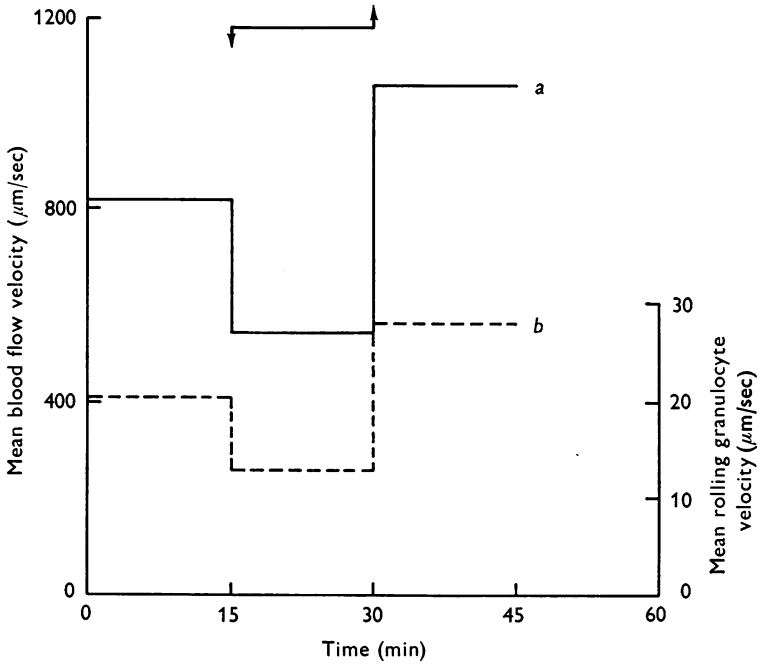


Fig. 2. Mean velocities of blood flow (*a*) and rolling granulocytes (*b*) in μm/sec before, during and after compression of a hamster cheek pouch venule (52 μm in diameter). The period of compression is indicated by the two arrows.

*Effect of artificial variations in blood flow velocity on mean granulocyte velocity in single venules*

The blood flow in venules could be decreased by gentle compression with a fine glass probe, the movements of which were accurately controlled by a micromanipulator. This was done downstream from the site of observation in order to avoid possible effects of substances released into the blood stream by the compression.

The mean velocities of blood and of rolling granulocytes were determined before, during and after compression in two hamster cheek pouch venules

and before and during compression in two mouse mesentery venules. All results were similar; an example with hamster cheek pouch is shown in Fig. 2. While the vessel was compressed the decrease in blood flow velocity was accompanied by a proportional diminution in granulocyte velocity. Afterwards the blood flow velocity was greater than before compression and the velocity of the rolling cells was increased in proportion.

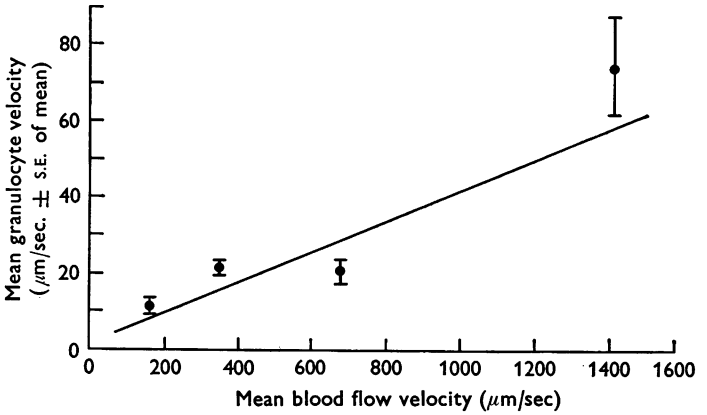


Fig. 3. Rolling granulocyte velocity (mean ± S.E. of mean) plotted against the corresponding mean blood flow velocity, both in μm/sec, in four venules of a hamster cheek pouch preparation. The correlation coefficient was 0·963.

*Relation between granulocyte and mean blood flow velocities in different venules of a vascular bed*

The mean velocity of rolling granulocytes was determined for up to four venules with varying mean blood flow velocities in each of two hamster cheek pouch and ten mouse mesentery preparations. In all, the velocities were directly proportional (Fig. 3).

*Relation between the velocities in different vascular beds*

The results of all experiments with mouse mesentery venules are shown in Fig. 4. The mean velocity of rolling granulocytes was directly proportional to mean blood flow velocity ($r$ = 0·915) when the latter varied from about 300 to about 1000 μm/sec. No results are shown for blood velocities of about 1000–1600 μm/sec because no venules were found with these flow rates. At high flow velocities, i.e. about 1600–3600 μm/sec, the mean velocities of the rolling granulocytes did not increase in proportion but remained in most venules at a value of approximately 45 μm/sec.

In an attempt to produce flow rates of between 1000 and 1600 μm/sec venules with mean blood flow velocities of about 3000 μm/sec were

partially occluded as already described. When the blood flow velocity was approximately halved, the mean granulocyte velocity was diminished in the same proportion but to values less than the 30–40 $\mu$m/sec expected for such high rates of flow.

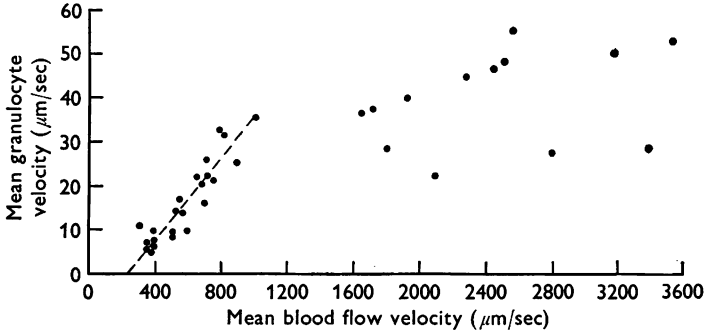


Fig. 4. Rolling granulocyte velocity (mean ± S.E. of mean) plotted against the corresponding mean blood flow velocity, both in $\mu$m/sec, in thirty-six venules of ten mouse mesentery preparations. Between blood flow velocities of about 300–1000 $\mu$m/sec, the correlation coefficient was 0·915 and the relationship was represented by the equation $y = 0{\cdot}05x - 15$, where $y$ = granulocyte velocity and $x$ = mean blood flow velocity. The interrupted line is the calculated regression line.

## DISCUSSION

The results establish three main features about the relationship between the mean velocities of blood flow in venules and the mean velocity of granulocytes that roll along their walls. First, the velocity of the rolling cells increased linearly with blood flow velocity of about 300–1000 $\mu$m/sec. Secondly, with blood flow velocities of less than 300 $\mu$m/sec, it was difficult to distinguish granulocytes from the slowly moving red cells. Thirdly, with flow velocities greater than 1600 $\mu$m/sec, rolling granulocytes were seen in considerable numbers but their mean velocities showed no further increase with the blood flow velocities. In most venules the cells moved at about 50 $\mu$m/sec but in some distinctly more slowly, i.e. around 30 $\mu$m/sec.

As the simplest explanation of granulocytes rolling, it has been proposed (Atherton & Born, 1972) that circulating granulocytes collide randomly with venule walls and that these collisions behave elastically when the venule is normal but inelastically when it is inflamed. As a result of biochemical changes effected in the walls by inflammatory agents, granulocytes colliding with the walls experience an adhesive force as well as the shear force exerted at the walls by the blood flow. The rolling movement is then the resultant of these two forces. It is now necessary to consider

how this hypothesis could account for the newly established facts about the relation between blood flow velocities and rolling granulocyte velocities.

In small venules blood flow is laminar with very low Reynolds numbers (see Whitmore, 1968). Under such conditions the shear force or stress $\tau_0$ at the wall is given by the formula

$$\tau_0 = \frac{4\overline{V}\eta}{r_0},$$

where $\overline{V}$ = mean blood flow velocity; $\eta$ = viscosity of the blood and $r_0$ = radius of the vessel. Thus, the shear force at the wall is directly proportional to the mean blood flow velocity. This relation accounts, therefore, for the range in which the rolling granulocyte velocities are directly proportional to the mean blood flow velocities, on the assumption that the mean adhesion force was very similar for all the rolling cells. This is a reasonable assumption in view of the fact that granulocytes are very similar in size and surface properties, so far as they are known (Noseworthy, Korchak & Karnovsky, 1972).

At blood flow velocities greater than about 1600 $\mu$m/sec considerable numbers of rolling granulocytes were still seen but their mean velocities showed no further increase with blood flow velocity; indeed, in some venules their mean velocities were somewhat lower than the maximal velocity of rolling. On our hypothesis this could have one or both of two possible explanations, viz. an increase in the mean force of adhesion or the absence of a further increase in wall shear force. An increase in adhesion force is conceivable if the biochemical change causing granulocyte adhesion to endothelial lining differed in venules with low blood flow velocities from those with high blood flow velocities. As the latter are larger than the former, such a difference seems possible but unlikely. More probably the blood flow velocity profile in large venules is sufficiently different from that in smaller venules for there to be an upper limit to the shear force experienced by granulocytes in contact with the wall. A decision between these alternatives requires the determination of the blood flow velocity profiles in these vessels at high enough resolution to permit the accurate calculation of wall shear forces.

This interpretation has the interesting implication that under conditions in which the wall shear force exceeds the adhesion force, no granulocytes will be found to show the rolling behaviour. This is, for example, one possible explanation why granulocyte rolling is not observed in arterioles. These considerations suggest that our experimental results can be used to calculate the minimum wall shear force required to overcome the adhesion force and thus to provide a value for the latter. At the upper end of the

proportionality range the mean blood flow velocity gives a value for the wall shear stress of about 5 dynes/cm$^2$. It seems that this is the first time it has been possible to estimate the force of adhesion under approximately physiological conditions *in vivo*. Therefore, this value can be compared only with measurements of cell adhesion forces made under artificial conditions *in vitro*. The detachment of human erythrocytes from different artificial surfaces required a minimum critical shear stress of about 10 dynes/cm$^2$ (Hochmuth, Mohandas, Spaeth, Williamson, Blackshear & Johnson, 1972). This value was reduced to about one-half when the foreign surfaces were coated with albumin and to about one quarter when coated with either fibrinogen or fresh human plasma. These values are remarkably similar to those for the *in vivo* adhesion of granulocytes to venule walls which we have calculated from our results. Nothing quantitative is known about the relative proportion in which the surfaces of vascular endothelium and granulocytes are coated with the different plasma proteins. However, if this proportion is at all related to the relative concentrations of albumin and fibrinogen in plasma then the observation that albumin caused less diminution than fibrinogen in the minimal detachment stress for erythrocytes supports the conclusion that the shear stress experienced by granulocytes at the venule walls is about 5 dynes/cm$^2$.

Using this value it is possible to calculate the force required to detach rolling granulocytes completely from the walls. The granulocytes are approximately spherical with radii of about 5 $\mu$m; their surface area is about 300 $\mu$m$^2$ or $3 \times 10^{-6}$ cm$^2$. The shear force acting over this area is $1{\cdot}5 \times 10^{-5}$ dynes. On the assumption that this is the minimum shear stress capable of detaching a rolling granulocyte, the force causing its adhesion is of the same order of magnitude. Further elucidation of granulocyte adhesion in venules will require determination of the biochemical mechanisms responsible for this force.

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