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Effect of Shear Rate on Clot Growth at Foreign Surfaces

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Abstract: The hydrodynamic effect on clot growth at foreign surfaces was investigated quantitatively in vitro. Shear rates from 2 to 1,000 s^{-1} were applied to a blood sample contained in a cone and plate viscometer. Four different artificial materials were used for cone and plate combination, namely, stainless steel, polytetrafluoroethylene, polycarbonate, and polymethylmethacrylate. Evaluation of clot growth was derived from the clotting ratio (the volumetric fraction of clot in the whole blood), which was experimentally determined from the rate of increase of frictional torque between the rotating cone and the stationary plate. The results show that the clotting ratio decreases markedly as the shear rate increases to 400 s^{-1}, regardless of material used. This study demonstrates that at a shear rate of >400 s^{-1}, clot growth at foreign surfaces is considerably inhibited. **Key Words:** Blood coagulation—Hydrodynamic effect—Clot growth—Shear rate—Cone and plate viscometer—Artificial materials.

Blood coagulates when it contacts foreign surfaces, so that clot growth at least has to be inhibited to prevent clogging the blood flow path in artificial organs. The clotting process includes many stages that involve a series of enzymic reactions. With regard to clot formation on artificial materials, many studies have been conducted on the biochemical interaction between blood and the material (1). On the other hand, clot formation is also governed by a hydrodynamic effect; i.e., clots do not grow on materials where blood flows smoothly (2).

In this study, to evaluate the effect of shear rate on clot growth at foreign surfaces, relationships between the shear rate (before formation of clot) and the clotting ratio (the volumetric fraction of clot in the whole blood) were examined quantitatively in cone and plate viscometer tests.

MATERIALS AND METHODS

Test equipment

The cone and plate viscometer (Visconic ED; Tokyokeiki, Tokyo, Japan) was used to apply constant shear rates to a blood sample (Fig. 1). In this equipment, a blood sample is sheared in a constant velocity gradient field as shown in Fig. 2. When a cone whose apex P is touching a stationary plate and is rotating on the axis PZ (perpendicular to the plate) at a constant speed, a constant shear rate is applied to the blood sample throughout the space between the cone and the plate. A velocity distribution, as shown in Fig. 2b, is induced between S and Q. The shear rate G, which is calculated from the velocity distribution, is constant regardless of the value of r (distance between P and Q shown in Fig. 2) according to the following equation:

$$G = \frac{v}{d} = \frac{r\omega}{r\theta} = \frac{\omega}{\theta} \tag{1}$$

where v is the circumferential velocity at S on the circular cone, d is the distance between S and Q, ω is the angular velocity of the cone, and θ is the angle between the cone and the plate. Note that in deriving this equation and those to follow, θ is assumed to be very small.

In actual practice, the shear rate is calculated as

$$G = \frac{6N}{\phi} \tag{2}$$

where N is the rotational speed (rpm) of the cone and ϕ is the angle (°) between the cone and the plate.

Four different materials were used for the cone

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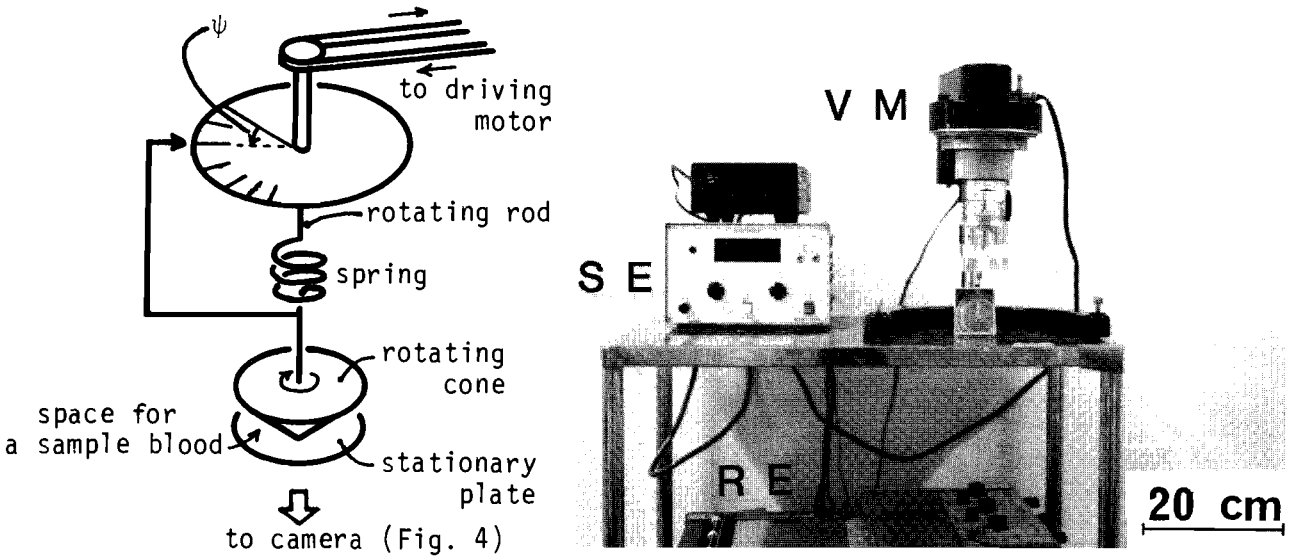


FIG. 1. Test equipment. **Left:** Schematic diagram of the cone and plate viscometer. **Right:** External appearance of the test equipment. The frictional torque is measured by the torsion angle (ψ) in the spring attached to the rotating rod in the viscometer (VM) (right). The value of the angle ψ is picked up optically every rotation by the sensor (SE) and transmitted to the recorder (RE) (right).

(radius 2.4 cm) and plate (shaped like a cup to be filled with a blood sample): stainless steel, polytetrafluoroethylene, polycarbonate, and polymethylmethacrylate (Fig. 3). To check the effect of the geometry of the space between the cone and the plate, two or three variations were made about the apex angle of the cone for each material.

Shear rates from 2 to 1,000 s^{-1} were applied to the sample blood by varying N (from 0.5 to 100 rpm) and ϕ (from 0.6 to 1.6°).

The frictional torque T between the rotating cone and the stationary plate is measured by the torsion angle ψ (Fig. 1, left) of the spring attached to the rotating rod. The value of this angle is recorded optically every rotation and transmitted to the recorder (Fig. 1, right).

Procedure

Human blood was collected from a single donor into sodium citrate (1:6.6 sodium citrate/blood; 9.8 mM final concentration in blood) and preserved for 2 weeks at 5°C before testing. To check the effect of preservation, additional tests were performed with fresh blood in the case of the polymethylmethacrylate cone and plate; canine blood collected from a single donor into sodium citrate (1:9 sodium citrate/blood; 13 mM final concentration in blood) was used immediately in this case. The blood coagulation process was initiated by adding calcium chloride (0.1:1 calcium chloride/blood; 0.25 M solution) between the cone and the plate.

Immediately after the blood sample was filled between the cone and the plate, the rotation of the cone started and a constant rotational speed was maintained for 30 min. During this period, the frictional torque was measured every rotation. In the case of the polymethylmethacrylate cone and plate, the clot formation was observed macroscopically through the transparent stationary plate at the same time.

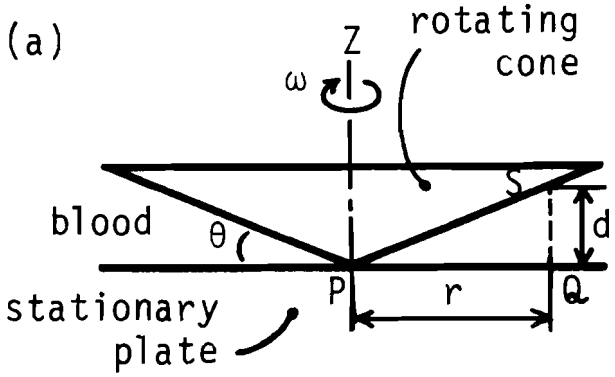
These tests were performed for the cone and plate combinations of the four kinds of materials with variations of the shear rate.

During these tests, the blood temperature was maintained at 24°C in the room. To check the effect of the blood temperature, additional tests were performed in the case of the stainless-steel cone and plate; the blood temperature was maintained at 37°C by a constant temperature bath that was attached to the stainless-steel plate.

Determination of clotting ratio

Evaluation of clot growth was derived from the clotting ratio, this being experimentally determined by the following method. Preliminary experiments indicated that macroscopic clot formation was observed through the transparent plate (Fig. 4) within 10 min (3 min in the case of canine blood) from the start of the blood coagulation process (from the start of rotation), and that the frictional torque began to increase at the same time (Fig. 5). This increase occurs when blood flow is obstructed by clot.

The torque T (dynes • cm) is given by



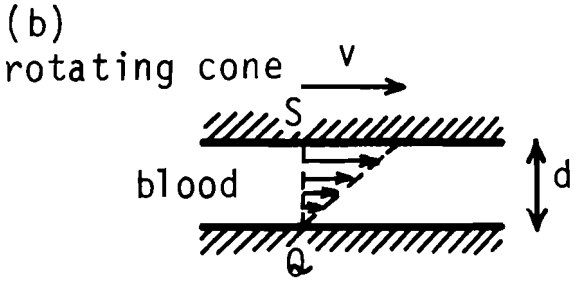


FIG. 2. Velocity gradient in a blood sample filled between a rotating cone and a stationary plate. **a:** Geometry of the space between the cone and the plate. **b:** Velocity gradient between *S* and *Q*. ω, angular velocity of the cone; *PZ*, rotational axis; θ, angle between the cone and the plate; *d*, distance between *S* (on the circular cone) and *Q* (on the plate); *r*, distance between *P* (point where the apex of the cone is touching the plate) and *Q* (on the plate); *v*, circumferential velocity at *S* on the circular cone.

$$T = \tfrac{2}{3}\,\pi\,\eta R^3 G \tag{3}$$

where η (dynes · s · cm^{-2}) is the apparent viscosity of blood, R (cm) is the radius of the cone (2.4 cm in this study), and G (s^{-1}) is the shear rate. Combining Eqs. 2 and 3,

$$T = \frac{4\pi\eta R^3 N}{\phi} \tag{4}$$

To calculate the clotting ratio by a simple equation related to a rate of increase of torque, the following approximations a–c were applied. (a) The clot is spread over the whole space between the cone and the plate in a shape like A in Fig. 6b. Then clotting ratio (CR) is given by

$$CR = \frac{\phi_0 - \phi_1}{\phi_0} = 1 - \frac{\phi_1}{\phi_0} \tag{5}$$

where ϕ_0 and ϕ_1 are angles defined in Fig. 6. (b) The flow in the clot (A in Fig. 6b) can be ignored; i.e., the angle between the cone and the plate decreases from ϕ_0 to ϕ_1 after the formation of clot. (c) The apparent viscosity of blood after the formation of clot (B in Fig. 6b) is equal to that of blood before the formation of the clot (B in Fig. 6a). That is, η is constant in Eq. 4.

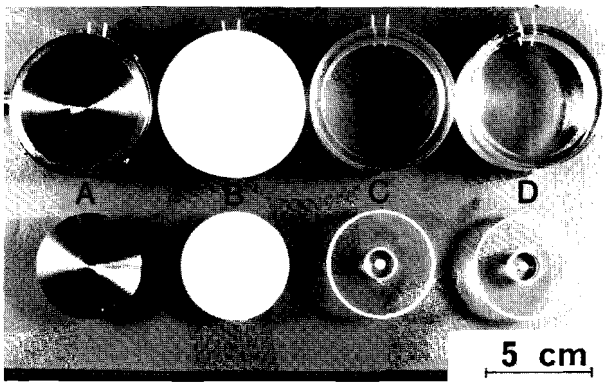


FIG. 3. Cone **(bottom)** and plate **(top)** combinations of four different artificial materials: stainless steel **(A)**; polytetrafluoroethylene **(B)**; polycarbonate **(C)**; polymethylmethacrylate **(D)**.

Thus, referring to Eq. 4, T_0 (torque before formation of clot) and T_1 (torque after formation of clot) are given by

$$T_0 = \frac{4\pi\eta R^3 N}{\phi_0} \tag{6}$$

$$T_1 = \frac{4\pi\eta R^3 N}{\phi_1} \tag{7}$$

Combining Eqs. 5–7,

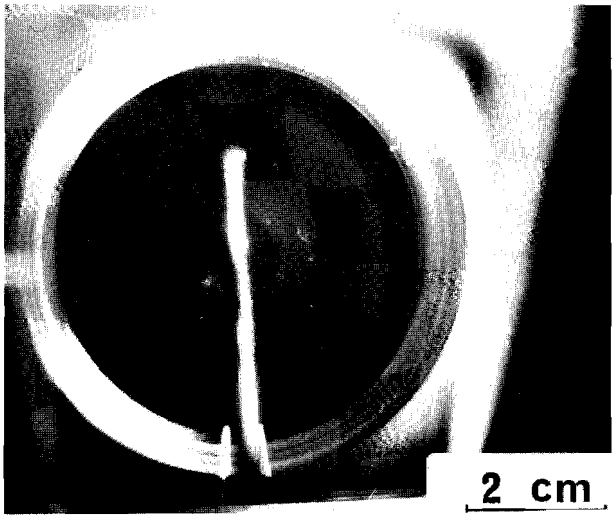


FIG. 4. Macroscopic clot formation observed through a transparent plate in the polymethylmethacrylate cone and plate test (10 min from the restart of the blood coagulation process). The arrow in the figure shows the clot (angle between the cone and the plate ϕ = 1.1°; shear rate = 550 s^{-1}; preserved human blood).

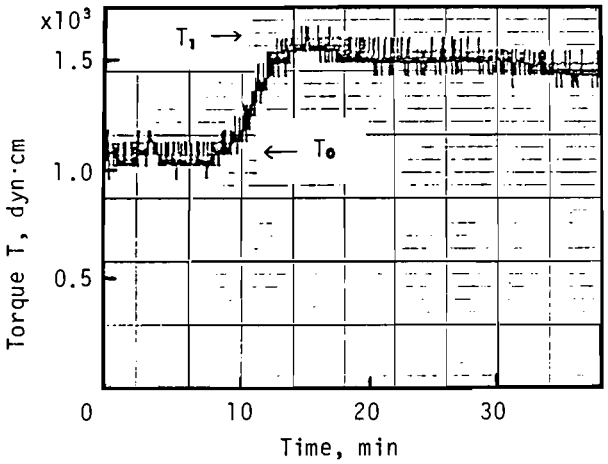


FIG. 5. Torque recording from the same test as that shown in Fig. 4 (polymethylmethacrylate cone and plate; angle between the cone and the plate $\phi = 1.1°$; shear rate = 550 s^{-1}; preserved human blood). T_0, torque before formation of clot; T_1, torque after formation of clot.

$$CR = 1 - \frac{T_0}{T_1} \quad (8)$$

Thus, the clotting ratio can be calculated by Eq. 8 where T_0 and T_1 are determined from torque data like those exemplified in Fig. 5.

RESULTS

Figure 7 shows the clotting ratio as a function of shear rate on each material, and Fig. 8 shows some examples of the visual quality of the clot formed on the polymethylmethacrylate cone and plate (fluid part is removed after test in the figure). The clotting ratio, it is seen, decreases markedly as the shear rate increases to 400 s^{-1}, independently of other factors, namely, material, ϕ (angle between the cone and the plate), blood preservation, and the blood temperature used in the tests.

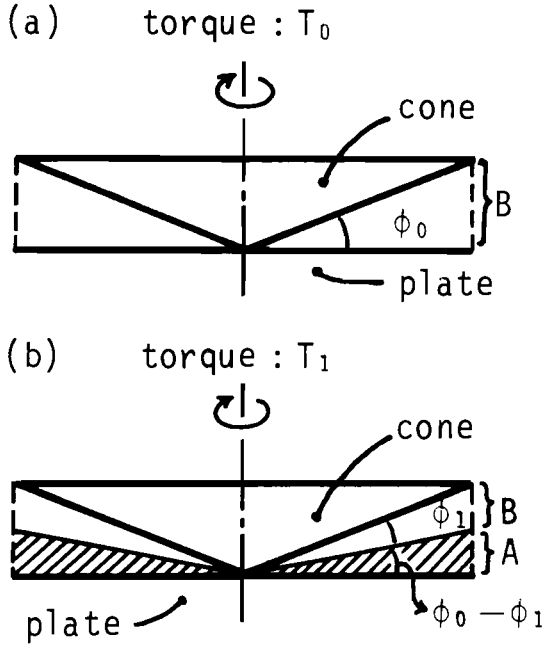


FIG. 6. Determination of clotting ratio in cone and plate viscometer tests before **(a)** and after **(b)** formation of clot. A, clot; B, blood; T_0, torque before formation of clot; T_1, torque after formation of clot.

In the shear rate range between 2 and 50 s^{-1}, the values of the clotting ratio show scatter, but they are >0.5, and the clot appears film-like (Fig. 8A, B; clot formed on the cone in Fig. 8A). In the shear rate range between 50 and 400 s^{-1}, the clotting ratio decreases markedly as the shear rate increases. In the shear rate range between 400 and 1,000 s^{-1}, the values of the clotting ratio are <0.5, and the clot shows small plots (Fig. 8C).

Relative to the artificial materials, the clotting ratio is somewhat smaller on stainless steel (Fig. 7, top left) than on polytetrafluoroethylene (Fig. 7, top right) at 200 s^{-1}.

In relation to ϕ, the data do not show a clear relationship between the clotting ratio and ϕ. For polycarbonate, the data show that the clotting ratio is bigger at 1.4 than at 0.7° at 430 s^{-1}, but smaller at 1.4 than at 0.7° at 86 s^{-1} (Fig. 7, bottom left).

In relation to blood preservation, the values of the clotting ratio in fresh canine blood are scattered but are somewhat larger than those in preserved human blood at higher shear rates on polymethylmethacrylate (Fig. 7, bottom right).

Relative to blood temperature, the clotting ratio is larger at 37 than at 24°C on stainless steel (Fig. 7, top left).

The clotting ratio was well correlated to the form of the clot observed (Fig. 8).

DISCUSSION

Evaluation of clot growth was derived from the clotting ratio in this study. The merit of this method is that clot growth can be evaluated while a constant shear rate is applied to the whole blood sample. It may be also convenient to evaluate clot growth in that the clotting ratio directly shows how much the blood flow is obstructed.

The method to determine clotting ratio in this study, however, includes some approximations, so there are some differences between the hypothesized and actual observations. First, the clot is of a more irregular shape than that in Fig. 6b, and torque sometimes increases and decreases to reveal a more complicated curve than that in Fig. 5. Thus,

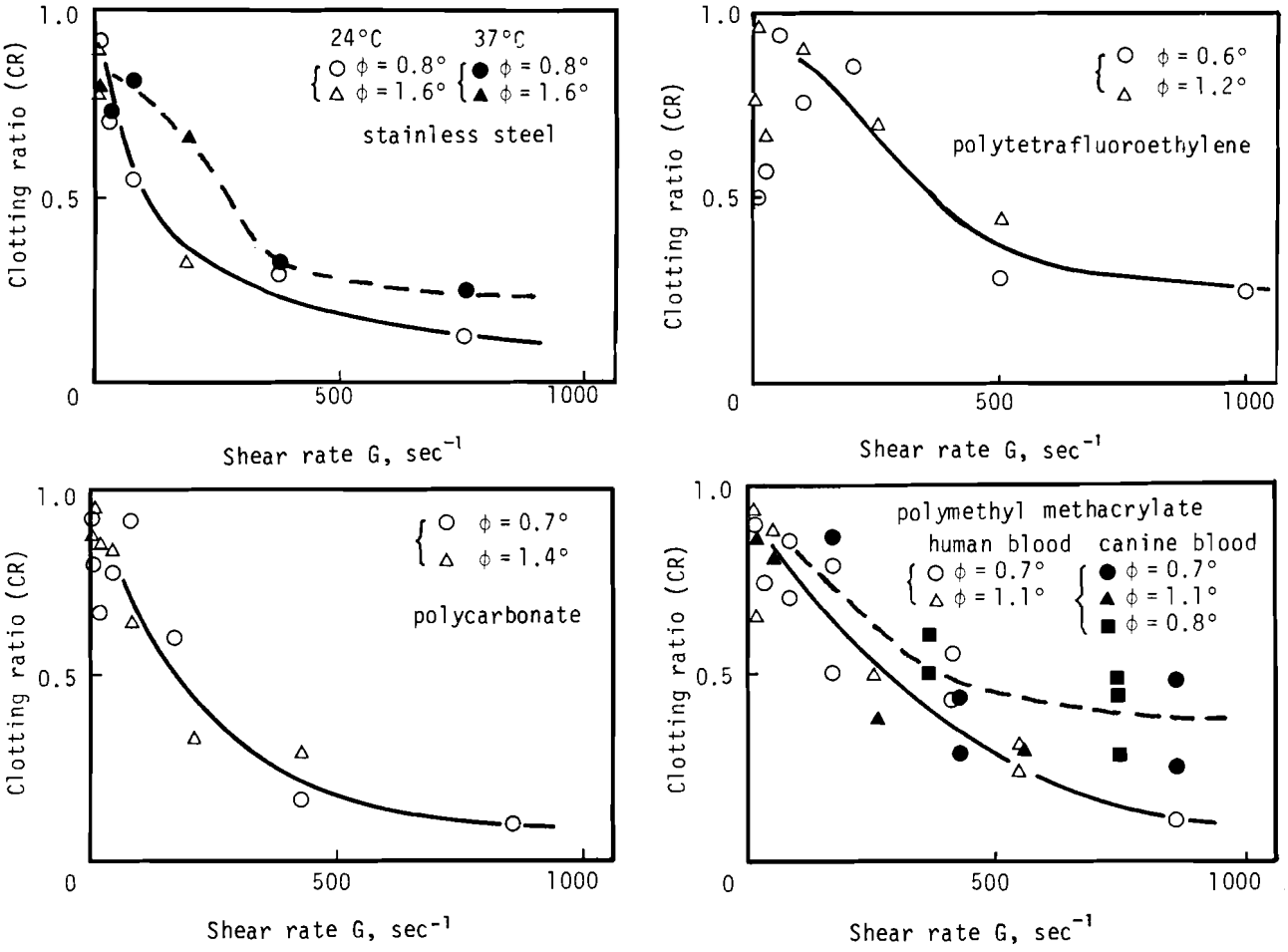


FIG. 7. Clotting ratio as a function of shear rate in blood between cone and plate made of stainless steel **(top left)**, polytetrafluoroethylene **(top right)**, polycarbonate **(bottom left)**, and polymethylmethacrylate **(bottom right)**. φ, angle between the cone and the plate.

it is sometimes difficult to determine T_1 (torque after formation of clot). This is the main reason for the fluctuations of the clotting ratio (calculated by Eq. 8) in Fig. 7. Second, blood is known to be non-Newtonian; and with reduction of the shear rate, the apparent viscosity increases steeply at a shear rate of <50 s^{-1} (3). This non-Newtonian behavior explains in part the scattering of the clotting ratio (calculated by Eq. 8) at shear rates of <50 s^{-1} in Fig. 7; i.e., the apparent viscosity of blood before the formation of clot would not be equal to that of blood after formation of the clot. At a shear rate of >50 s^{-1}, this method to determine the clotting ratio is applicable, because the apparent viscosity can be treated as a constant value (Eqs. 6 and 7).

Flow patterns in human vessels have been observed in many studies where the inside diameter of the vessel and the flow velocity or flow rate are measured (4). From these data, shear rates at the wall in the circulation are calculated to be from 60 to 800 s^{-1} for parabolic velocity profiles (5). So the range of shear rate applied to the blood in this study is typically that applied at human vessel walls. It has been determined that 200 s^{-1} is a typical shear rate at the wall of great veins (5). In this study, only four different materials were tested, but Fig. 7 shows that near 200 s^{-1} the clotting ratio depends greatly on the shear rate of each material used, so a constant shear rate condition is preferred for a comparison between materials. In this regard, the test in the cone and plate viscometer has advantages over tests in tubes.

Blood is regarded as homogeneous in this test. A possible effect of the dimensions of blood cells, e.g., can be eliminated in the experimental determination of the clotting ratio, because fluctuations of clotting ratios are within the range of experimental error in the determination of T_1 (torque after formation of clot) in Fig. 7 when the angle between the cone and the plate changes from 0.6 to 1.6°.

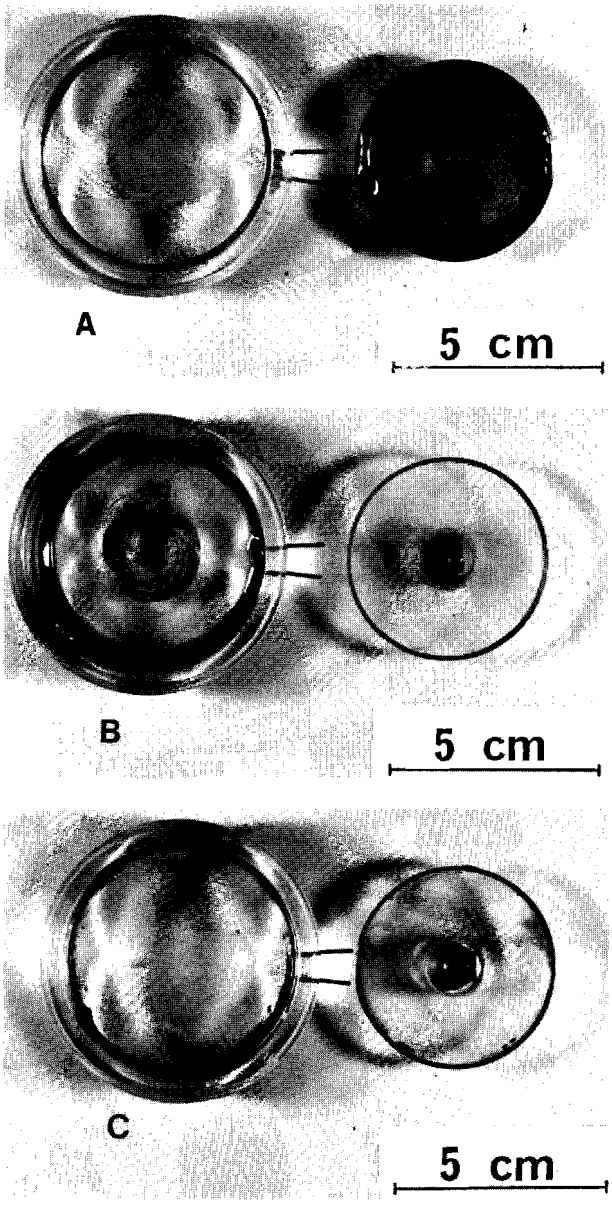


FIG. 8. Clot formed on the polymethylmethacrylate cone and plate (angle between the cone and the plate ϕ = 0.7°; preserved human blood). The fluid part is removed after the test in the figure. **A:** G = 4.3 s^{-1}, CR = 0.90; **B:** G = 43 s^{-1}, CR = 0.70; **C:** G = 430 s^{-1}, CR = 0.45. G, shear rate; CR, clotting ratio.

The time from the start of the coagulation process to clot formation was longer in preserved human blood (Fig. 5) than in fresh canine blood. This suggests that the ability of platelet aggregation might have been reduced in human blood after 2 weeks of preservation, and the effect on platelets might explain the scattering of the clotting ratio in fresh canine blood in Fig. 7 (bottom right).

Every test was performed in vitro in this study, but the effect of shear rate on clot formation may be different in vivo; i.e., when shear rate increases in vivo, flow rate sometimes increases, too. Thus, shear rate as well as the entry rate of coagulation factors should be examined at the same time in vivo. For example, Grabowski et al. (6) reported on the dependence of platelet aggregate growth rate on ADP entry rate and shear rate. In the present study, the effect of shear rate on clot growth was investigated under conditions with no addition of coagulation factors.

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