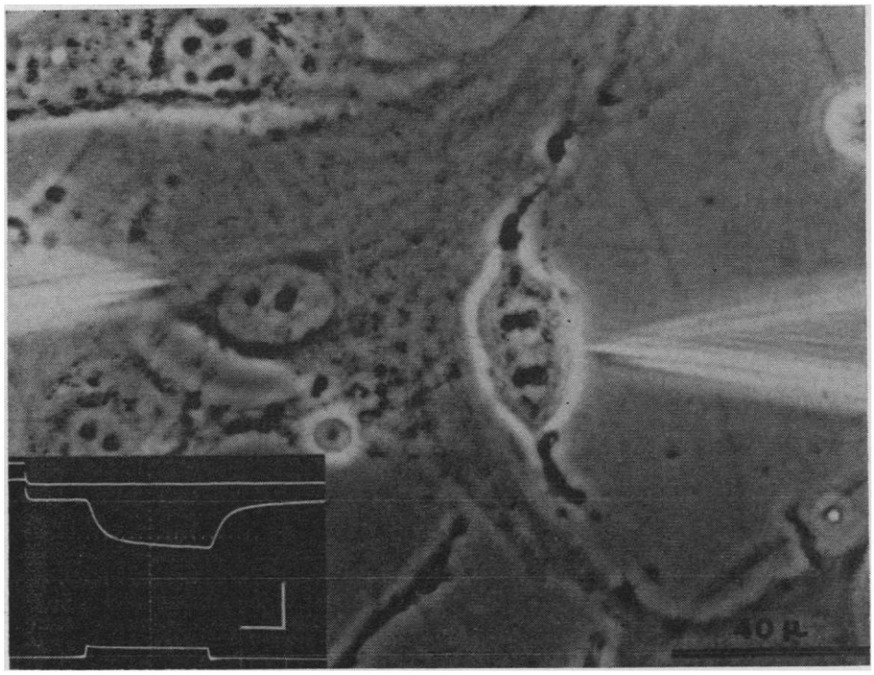

Fig. 3. Phase contrast photograph of living 3T3 cells after the coupling measurement (one day in culture). The separated chromosomes in the dividing 3T3 cell are clearly visible. (Inset) Evidence of electrical coupling. Hyperpolarizing current pulses of approximately 4×10^{-9} ampere and 56 msec were passed through the interphase cell membrane. A change in electrotonic potential was detected within the miotic cell (middle trace) but not immediately outside this cell (top trace) due to the same current pulses. Vertical white bar represents 20 mv; horizontal white bar, 20 msec. Positive calibrating pulses (see legend Fig. 1): 10 mv, 10 msec.

inside of the mitotic cell, the electrode within the interphase cell recorded symmetrical electrotonic potentials (middle trace) but did not record potential changes just outside the interphase cell (upper trace) due to these same current pulses. The resting potential of the interphase cell at the time of the coupling measurement (−5 mv) is shown by the difference between the top two voltage traces. The scanning electron micrograph of the same electrically coupled cell pair is shown in Fig. 2. The cytoplasmic extensions of the mitotic cell appear in contact with the interphase fibroblast. The long microextensions seen less clearly in Fig. 1 are characteristic features of the dividing fibroblasts that were studied, and resemble the mitotic retraction fibrils described for dividing human liver cells in vitro (*6*).

Electrical coupling between mitotic and interphase cells was also found in mouse embryonic fibroblasts (3T3) in culture. Figure 3 shows one such pair of cells. In this case, separation of the chromosomes is clearly visible in the phase contrast picture of the mitotic cell. The electrical record shows that an electrotonic potential change (middle trace) is recorded within the dividing cell due to a hyperpolarizing current pulse (lower trace) passed through the interphase fibroblast. No change was detected immediatley outside the mitotic cell (top trace).

It was not possible to directly determine whether coupling persisted throughout the division cycle since in both the chick fibroblasts and the 3T3 cells it was not possible to keep cells impaled with the microelectrodes throughout the division cycle. However, in all such cell pairs studied (well over 100), which represented cells in many phases of the mitotic cycle, including the formation of daughter cells, some degree of coupling between a mitotic cell and an adjacent interphase cell was found. Moreover, this coupling was similar to that found between adjacent interphase cells in the same cultures (*7*). It is our impression that some degree of coupling persists throughout the mitotic cycle of these cells. Our results are consistent with previous reports that cells in culture establish and maintain low-resistance junctions, and they further support the hypothesis that low-resistance junctions reflect a basic relationship between cells.

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Shear Degradation of Fibrinogen in the Circulation

Abstract. *Plasma fibrinogen in vitro suffers a loss in clottability due to shearing. From calculation of the mass average shear to which plasma is subjected in the circulation, it is estimated that the half-life of fibrinogen is 4 days. This is in excellent agreement with reports that the half-life of fibrinogen in the circulatory system is 4 to 5 days. Hence shearing may be the mechanism for fibrinogen degradation in the circulatory system.*

Fibrinogen in plasma flowing through the circulatory system is subject to continuous shearing of various magnitude in various vessels. It has previously been observed that shearing inactivates enzymes in solution (*1*). The mass average shear for a solution passing through a cylindrical tube is

$$(\gamma\theta)_{av} = \frac{2\pi}{Q}\int_0^{R_w} R\, v\, \gamma_r\, \theta_r\, dR \quad (1)$$

where γ_r is the point shear rate; θ_r is the residence time for streamline distance R from center; v is the point velocity; Q is the flow rate in tube; R is the distance from center; and R_w is the cylinder radius.

Equation 1 may be reduced to

$$(\gamma\theta)_{av} = \frac{8}{3}\frac{L}{R_w} = \frac{16}{3}\frac{L}{D}$$

where D is equal to $2R_w$, and L is the length (see appendix). For a solution making a number of passes through a tube, the shear is

$$(\gamma\theta)^1_{av} = \frac{16}{3}\frac{L}{D} \times N \qquad (2)$$

where N is the number of passes and is determined from number of passes per unit time (N^1) times the time of flow and N^1 is the flow rate per tube volume.

The influence of shearing on fibrinogen clottability was not known, but in view of the effect on enzyme activity, it was conceivable that fibrinogen might be altered.

Fibrinogen in human plasma was sheared in a Weissenberg rheogoniom-

Table 1. Comparison of loss in clottability for fibrinogen in plasma circulated in a cylindrical tube (203 by 0.11 cm) by a finger pump with loss in clottability due to shearing in viscometer (Fig. 2).

Flow rate (cm^3/min)	No. of passes	$\gamma\theta_{av}$	Overall loss (%)	Pump loss (%)	Loss in tube (%)	Loss in viscometer for same $\gamma\theta$ (%)
4.5	50	5×10^5	3.5	2.5	1.0	1.0
4.5	80	8×10^5	6.5	4.2	2.3	2.0
4.5	130	1.3×10^6	11.0	6.5	4.5	3.8
36.0	600	6×10^6	25.0	13.0	12.0	14.0
36.0	1200	1.2×10^7	32.0	17.0	15.0	18.0
36.0	3080	3.08×10^7	42.0	23.0	19.0	26.0
36.0	7000	7×10^7	47.5	24.0	23.5	31.0

Table 2. Estimated mass average shear $(\gamma\theta)^1$ per second associated with various vessels in a 60-kg man (flow rate was 86 ml/sec). $(\gamma\theta^1/\text{sec}) = (16/3) \times 275 = 1470/\text{sec}$. From Fig. 2, $\gamma\theta$ for 50 percent loss $= 5 \times 10^5$. Time for 50 percent loss $= (5 \times 10^8)/(1.47 \times 10^3) = 3.4 \times 10^5$ seconds $\cong 4$ days.

Vessel	Blood volume $(cm)^3$	L/D	N^1 (1/sec)	$L/D \times N^1$ (1/sec)
Pulmonary artery	400	66	0.21	1.4
Pulmonary capillaries	60	100	1.42	142
Pulmonary venules	140	66	0.62	41
Pulmonary veins	700	30	0.12	3.6
Aorta	100	20	0.86	17
Systemic arteries	450	66	0.20	13
Systemic capillaries	300	100	0.28	28
Systemic venules	200	66	0.41	28
Systemic veins	2050	30	0.04	1
				275

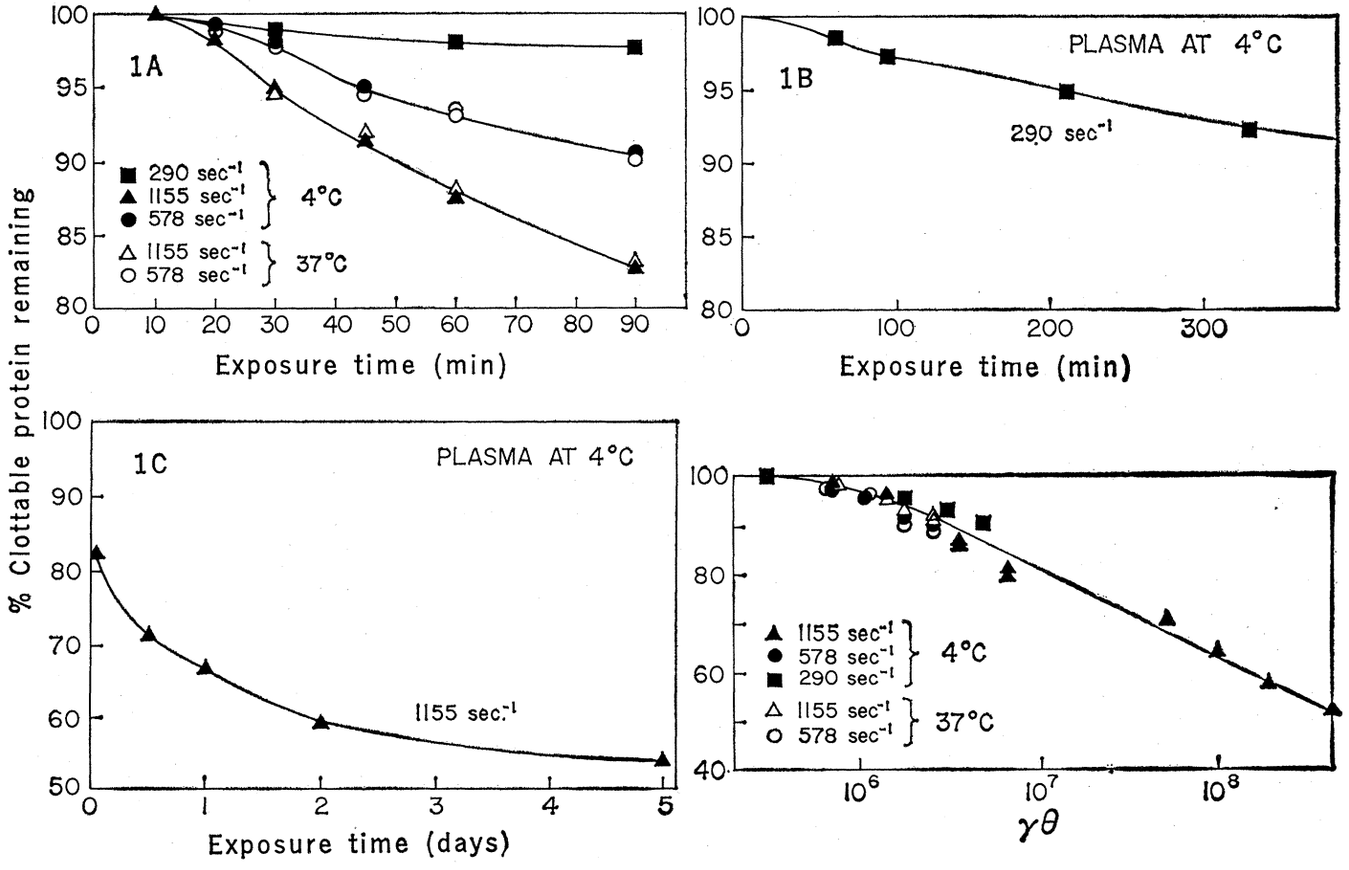

Fig. 1. (A) Clottability of plasma as a function of shear rate, exposure time, and temperature. (B) Effect of shear rate on plasma clottability at 290 sec^{-1} for relatively short periods. (C) Effect of shear rate at 1155 sec^{-1} for 5 days on plasma clottability. Fig. 2. Percentage of clottable protein remaining as a function of shear rate times exposure time at 37° and 4°C.

eter with a Couette attachment, between shear rates of 290 and 1155 sec^{-1} for various time intervals at 4° and 37°C, and tested for loss in clottability. Results were not influenced significantly by temperature. It has been found that three enzyme systems tested in a similar way were not significantly affected by surface denaturation (*1*). The plasma was prepared by passage through an ion-exchange column where calcium was removed to prevent clotting during the test (*2*).

Clottability was measured by addition of thrombin (0.2 ml, 25 unit/ml) to plasma (0.5 ml) in a buffer (1.5 ml) containing 0.15*M* ammonium acetate, 0.1*M* 6-aminocaproic acid, and 17 m*M* Ca ion. The clot that formed after the sample was incubated for 3 hours at 37°C was washed and dissolved in 40 percent urea in 0.2*N* NaOH; its optical density at 280 nm ($O.D._{280}$) was read against a urea blank. The optical density of the sample was compared with that of plasma (*3*).

The results shown in Fig. 1 indicate that fibrinogen clottability is lost with shearing. The data on the relation between shear rate and time and loss of clottability are resolved in a single curve when the loss in clottability is plotted against log $\gamma\theta$ (see Fig. 2). When $(\gamma\theta)^1$ or $(\gamma\theta)$ is less than 10^5, there is no loss in clottability of fibrinogen. At $\gamma\theta$ of 5×10^8, there is a 50 percent loss. The shearing effect is the same at 4° as at 37°C.

Plasma was also recycled by a finger pump through a cylindrical tube (203 by 0.11 cm) (*1*). The mass average shear was calculated from Eq. 2, and the loss of clottability associated with this shear was taken from Fig. 2. The loss in clottability was also determined experimentally, and the calculated loss was compared with the experimental loss after the loss due to the pump was determined. The results are in agreement (see Table 1).

The mass average shear associated with human circulation may be estimated from the L/D of the various vessels and the volume of blood in the vessels. If we use Burton's values for L/D and the volume of blood (*4*), the mass average shear per second for a 60-kg man is about 1470 per second (Table 2 and Eq. 2). The greatest shear is calculated to occur in the pulmonary capillaries.

From Fig. 2, the shear for 50 percent loss in clottability is $\gamma\theta = 5 \times 10^8$. The $(\gamma\theta)^1$ per second is 1.47×10^3 (Table 2), and the time required in the circulation for this half-life to occur is $(5 \times 10^8)/(1.47 \times 10^3) = 3 \times 10^5$ seconds or about 4 days.

There have been several reports that 50 percent of the fibrinogen in the circulation is turned over every 4 to 5 days (for example, *5*). The cause of the loss in vivo is not known. Some investigators have attributed fibrinogen turnover to constant intravascular formation of fibrin.

In view of the agreement of the half-life of fibrinogen between reported values and our values obtained by shearing degradation of fibrinogen in vitro, our results strongly suggest that shearing is the chief contributor to fibrinogen degradation in the circulation. Although the relationships derived here apply strictly to a Newtonian fluid in laminar flow, they permit an approximation to the actual conditions associated with blood flow.

Thus, shearing may also be the mechanism for reported degradation of lipoproteins, transferrin, acid α-glycoprotein, albumin, and globulins which also occurs in the circulation (*5*).

Appendix

To obtain $(\gamma\theta)_{av} = (8/3)\ (L/R_w)$ the following substitutions are made in Eq. 1 before integrating: $Q = (\pi/8\mu)\ (P/L)\ (R_w^4)$, Poiseuille's equation; $\theta_r = L/v$; and $\gamma_r = PR/4\mu L$. Here P/L is the drop in pressure per unit length and μ is the viscosity.

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Gibbon Fibrinopeptides: Identification of a Glycine-Serine Allelism at Position B-3

Abstract. *The fibrinopeptides A and B of the gibbon (an Asian ape) have been characterized and their relation to other primate types examined. An allelic situation was discovered at location B-3; two of the gibbons studied had both glycine and serine at that position, whereas four others were homozygous for glycine.*

In the course of a study on primate relationships based on molecular comparisons, we examined the fibrinopeptides of an individual gibbon (*Hylobates lar entelloides*). Gibbons are Asian apes, a group which also includes the siamang (great gibbon) and orangutan. Because of the small size and high value of these creatures, it was necessary to employ plasmapheresis to obtain enough blood plasma for this study without endangering the animal. Using previously described techniques (*1–4*), we were able to prepare fibrinogen from about 100 ml of plasma and to characterize the fibrinopeptides A and B (Fig. 1).

The gibbon peptides are closely related to other hominoid types, although the differences are numerous enough to emphasize how much more closely man is related to the African apes (chimpanzee) than to Asian apes, confirming previous immunochemical observations (*5*). The gibbon fibrinopeptide A differed from that of chimpanzee and human, whose fibrinopeptides are identical (*4*), in 2 of 16 positions. At position A-14 the gibbon has a threonine, just as do all the Old World monkeys examined to date; chimpanzees and humans have serine. At position A-10 the gibbon has a glutamic acid instead of the aspartic acid which exists in the other known primate structures. In the case of the fibrinopeptide B, there was one unambiguous amino acid difference between the gibbon and chimp-human structures, the latter having a phenylalanine at position B-5 where the gibbon has a leucine. Furthermore, an internal deletion of one residue has occurred on the gibbon line at position B-7 or B-8 (Fig. 2).