Table 1. Number of positive heterokaryon tests obtained with various pairwise combinations of mating type *A ad-3A* and *ad-3B* mutants and their mating type *A* F_1 progeny (from a cross to wild type) on minimal medium. Symbols: ±, weak positive response; +, vigorous positive response; −, negative response.

ad-3B mutant	*ad-3A* mutant					
	1-230-0200		1-230-0049		3-010-0194	
	Original	F_1 progeny	Original	F_1 progeny	Original	F_1 progeny
2-017-0095						
Original	−	10/18	−	14/25	−	6/27
F_1 progeny	4/25	+*	4/25	+*	7/17	+*
1-152-0068						
Original	±		−	16/24	−	7/16
F_1 progeny		+*	6/16	+*	2/17	+*
1-155-0055						
Original	±		±		−	14/16
F_1 progeny		+*		+*	21/25	+*

* At least one pairwise combination was made of heterokaryon-compatible F_1 progeny from each cross.

karyon formation with the majority of tester strains when heterozygous individually. Negative heterokaryon tests were obtained only when such mutations were present in certain doubly or multiply heterozygous combinations as shown in Table 1. Apparently the effect of heterozygosity for mutations with marginal effects is cumulative and only in some cases is the net effect extreme enough to prevent completely a positive heterokaryon test.

It is important to note that the incompatibility factor interactions found in the present experiments with relatively isogenic strains may be even more frequent among the so-called "heterokaryon-positive" derivatives of allelic mutants induced in unrelated wild-type strains as well as isolates of "isogenic" allelic mutants derived from outcrosses to other strains. The existence of such subtle interactions between incompatibility factor mutations suggests that a note of caution is advisable in the interpretation of negative heterokaryon tests. It seems possible that the basis for some of the nonlinear complementation maps (or mutants that do not map as a continuous straight line on a linear map) is the failure to recognize that apparent "functional identity" may be due to an incomplete or inadequate heterokaryon test.

The unsuspected interaction of such incompatibility factor mutations may in fact be the explanation for the greater sensitivity reported (*10*) for the pseudo-wild type (PWT) test in studies on allelic complementation. The generally accepted hypothesis for the formation of PWT's is that they result from nondisjunction of homologous chromosomes during meiosis and the formation of an unstable disomic (*11*). Thus in the PWT test, homologous chromosomes carrying nonidentical allelic mutations are present initially in the same nucleus, whereas in the heterokaryon test they are present in different nuclei in the same cytoplasm. The marked difference in sensitivity has been attributed to more favorable nuclear ratios in the heterokaryons derived from the breakdown of the disomic in PWT ascospores than in the heterokaryons obtained by direct mixing of conidia from vegetative cultures. This is undoubtedly true, but the basis for the greater sensitivity is not just a greater likelihood of forming a heterokaryon having equal numbers of two types of mutant nuclei (*10*). Even though there may be a 1 : 1 nuclear ratio in the PWT ascospore, the nuclear ratio in the hyphae emerging from opposite ends of germinating PWT ascospores may vary over a very wide range (0 to 100 percent) (*12*). The most plausible basis for the greater sensitivity of the PWT test is the elimination of any influence of heterozygosity for incompatibility factor mutations that could either prevent heterokaryon formation or cause marked departures from a 1 : 1 nuclear ratio (*9*) in the heterokaryon test. Furthermore, in at least some of the heterokaryons resulting from breakdown of the disomic, heterozygosity for very closely linked incompatibility factor mutations would even be eliminated by crossing over in addition to nondisjunction (*2*).

If the effect of heterozygosity for such mutations is always cumulative, however, then their presence in heterokaryon tests on samples of unknown mutants may be more readily detectable by the inclusion of the appropriate strain, such as 2-017-0095 or 3-010-0194, as an "incompatibility-factor control." In contrast with the other four strains studied (Table 1), all combinations of the mutations present in either of these two strains produced an effect extreme enough to completely prevent a positive test. In this way it seems possible to detect mutations with marginal effects on heterokaryon formation and to simplify the interpretation of an otherwise simple and straightforward test for allelism.

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References and Notes

1. L. Garnjobst, *Am. J. Botany* **42**, 444 (1955).
2. F. J. de Serres, *Genetics* **45**, 555 (1960).
3. Y. Suyama, K. D. Munkres, V. W. Woodward, *Genetica* **30**, 293 (1959).
4. F. J. de Serres, *Genetics* **41**, 668 (1956).
5. N. H. Giles, F. J. de Serres, E. Barbour, *ibid.* **42**, 608 (1957).
6. F. J. de Serres, *ibid.* **42**, 366 (1957).
7. ——— and R. Osterbind, *ibid.* **47**, 793 (1962).
8. L. Garnjobst, *Am. J. Botany* **40**, 607 (1953); ——— and J. F. Wilson, *Proc. Natl. Acad. Sci. U.S.* **42**, 613 (1956); B. W. Holloway, *Genetics* **40**, 117 (1955).
9. T. H. Pittenger and T. G. Brawner, *Genetics* **46**, 1645 (1961).
10. M. E. Case and N. H. Giles, *Proc. Natl. Acad. Sci., U.S.* **44**, 378 (1958).
11. T. H. Pittenger, *Genetics* **39**, 326 (1954).
12. ———, *Proc. Intern. Congr. Genet. 10th Montreal* **1958**, **II**, 218 (1959).

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Blood Clotting: The Force of Retraction

Abstract. The force of blood clot retraction was measured and found to be at least 10^4 dyne/cm^2; this is sufficient to deform many soft tissues and to allow clots to retract in vivo.

During the clotting of blood the platelets increase in size; they clump and aggregate at the interstices of the developing fibrin network (*1*). At this time the platelets exhibit rapid glycolysis and a sharp increase and subsequent fall in their content of adenosine triphosphate (ATP). Contraction follows, presumably due to the shortening of a constituent protein similar to actomyosin (*2*). This process results in marked reduction in the size of the clot (*3*). The biological significance of clot retraction is not entirely clear. Its role in hemostasis, wound healing, and in recanalization of thrombosed vessels has often been suggested (*4*). Budtz-Olsen concluded that the force of clot retraction is so weak that clot retraction does not occur at all in vivo and that this characteristic of blood represents an evolutionary vestige without usefulness in the human (*4*).

The present study was undertaken to measure the force of clot retraction in order to determine whether or not this

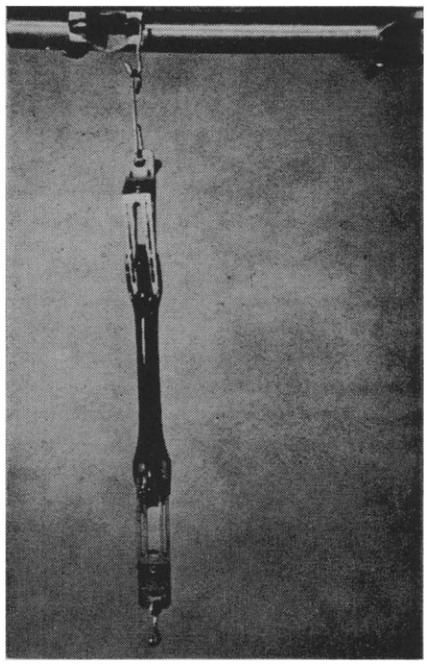

Fig. 1. The clamps and suspended clot prior to immersion in bath.

force is sufficient to effect significant deformation of the tissues to which the clot adheres, and therefore to allow retraction to occur. Previous work has been done on the mechanical properties of purified fibrin clots (*5*). However, these studies were carried out on platelet-free systems, and they are therefore not applicable to the problem of clot retraction induced by platelets.

Venous blood was collected from the forearm of normal adults in syringes of siliconized glass, nonsiliconized glass, and plastic. So long as the blood was drawn slowly and carefully there was no evident effect of the syringe wall. Nonsiliconized disposable needles were used one time. The blood was promptly transferred to nonsiliconized glass test tubes of two sizes, 13 by 125 mm or 11 by 75 mm. The blood was incubated for approximately 20 minutes at 37°C, at which time the clotting process appeared to be virtually complete. The clots were then carefully removed by rimming with a stainless steel wire, 0.5 mm in diameter. This procedure produced cylindrical clots of standard dimensions. The clots were then secured at each end by two-prong spring clamps, the tension of which was adjusted so that neither slipping nor tearing occurred (Fig. 1). The clots were suspended from above and various weights were attached to the lower clamp. The clots and the weighted clamps were then immersed in a 37°C bath of Ringer solution containing 0.08*M* glucose. At the beginning of clot suspension and 30 minutes later the intraclamp distance and the clot diameter were measured with calipers. The cross section of those clots which assumed an elliptical shape was estimated by measuring the largest and smallest diameters and by using the formula for the area of an ellipse, area $= \pi ab$, where a and b are the semimajor and semiminor axes. The force of clot retraction per unit area was calculated by dividing the sum of the half-clot weight and the weight of the lower clamp and its attachments (less a correction for the buoyancy of the entire assembly as determined by displacement) by the cross sectional area in square centimeters.

Table 1. The force of in vitro clot retraction in 14 tests.

Applied weight (dyne)	Clot		Force of retraction (10^3 dyne/cm^2)
	Shortening change in length/original	(cm^2)	
1390	0.11	0.53	2.7
1390	0.09	0.52	2.7
1410	0.08	0.52	2.8
2750	0.06	0.47	6.0
2780	0.08	0.47	6.0
4030	0.04	0.47	8.7
4050	0.03	0.47	8.7
2900	0.07	0.33	8.7
2900	0.04	0.33	9.0
3030	0.03	0.33	9.1
4250	0.02	0.47	9.4
4260	0.03	0.47	9.4
3050	0.04	0.33	9.5
4220	0.04	0.28	12.0

The results, summarized in Table 1, show that under the conditions of the experiment the maximum force of clot retraction induced by platelets was about 1.2×10^4 dyne/cm^2, which is equal to the pressure of about 9 mm Hg. The shortening of the clot was inversely related to the applied stretching stress. With stresses in excess of about 10^4 dyne/cm^2 clot retraction did not occur consistently.

The force of clot retraction, although small in proportion to the elastic modulus of isolated segments of such tissue as artery or vein wall, is nevertheless sufficient to effect significant distending or collapsing displacements of intact hollow structures such as stomach, bladder, lungs, and veins (*6*). It is reasonable to conclude therefore that clot retraction can occur in vivo (*7*).

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References and Notes

1. A. A. Sharp, in *Blood Platelets,* Henry Ford Hospital International Symposium (Little, Brown, Boston, 1961), p. 67.
2. E. F. Luscher, *ibid.*, p. 445.
3. C. L. Conley, *ibid.*, p. 437.
4. O. E. Budtz-Olsen, *Clot Retraction* (Thomas, Springfield, Ill., 1951).
5. J. D. Ferry and P. R. Morrison, *J. Am. Chem. Soc.* **69**, 388 (1947).
6. A. L. King and R. W. Lawton, in *Medical Physics* (Year Book Publishers, Chicago, 1950), vol. 2, p. 310; L. H. Peterson. *Mod. Concepts Cardiovas. Disease* **31**, 725 (1962).
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Four-Lead Electrical Resistance Measurements in Bridgman Anvils

Abstract. A geometry is described which permits four-lead electrical determinations of the pressure coefficient of resistance of metals in Bridgman anvils. It is also possible in this geometry to mount more than one sample and to make independent measurements on each sample simultaneously.

When Bridgman developed anvils for the determination of electrical resistance, he found it essential to run all of the necessary leads through the anvil faces (*1*). This introduced an unknown contact resistance for which no exact correction could be made. In most systems, however, the contact resistance was negligibly small compared with the total resistance. Bridgman made several attempts to run leads through the sides of the pyrophylite ring directly into the sample, but the leads were pinched off at such a low pressure that this technique was discarded.

The apparent reason for the pinching was the very large pressure gradient that exists in the gasket. In an earlier paper we, along with two associates, showed that in the 1/32-inch-wide pyrophylite ring the pressure gradient in the gasket went from a few kilobars at the outside edge to a pressure roughly 20 percent higher than the average applied load in 0.025 inch (*2*). This pressure gradient is above the shear strength of any wire, and consequently breakage by shear occurred. We have found that by inceasing the width of the ring to 3/32 inch and its thickness from 0.010 to 0.020 inch it is possible to insert electrical leads through the ring, without introducing sufficient shear to cut the wire, to an applied average load of about 200 kbar. The ring design has also been found to change the pressure-load relationships owing to the increased gasket width. Also, because of the increased thickness of the silver chloride, it is possible to