ample, adsorption of fibrinogen (*13*).

Although there is good correlation between the effects of fibrinogen on blood viscosity and RBC aggregation, it must be noted that these two effects were studied under different experimental conditions. The aggregate size was estimated at zero shear rate, whereas the viscosity was measured at shear rates approaching, but not equal to, zero. Because of technical limitations, microscopic observations on RBC aggregation were made on suspensions containing 0.5 rather than 45 percent RBC's. It is clearly desirable to obtain data on RBC aggregation at cell concentrations and shear rates comparable to those in viscometry.

The experiments on fibrinogen (molecular weight 340,000) have been repeated on a dextran with a high molecular weight (Dx 375, mean molecular weight 375,000, Pharamacia Corp., Uppsala, Sweden). The Dx 375 caused an increase of the viscosity of RBC suspensions preferentially at low shear rates (Fig. 1A) as well as the formation of RBC aggregates. These effects were much less pronounced when dextrans of lower molecular weight (for example, 72,000) were used (*14*). Therefore the marked effects on viscosity and aggregate size observed with fibrinogen but not with albumin are probably related to the larger molecular size of fibrinogen.

We conclude that purified autologous or heterologous fibrinogen causes RBC aggregation and a rise in viscosity preferentially at low shear rates. The shear dispersion or deformation of the RBC aggregates formed by cell-protein interactions is a major mechanism responsible for the non-Newtonian behavior of whole blood at normal cell percentages (*2*). Another factor leading to the non-Newtonian behavior of whole blood is the shear deformation of the individual RBC's (*3*), and such direct cell-cell interactions become important when the cell percentage is elevated above normal (*2*).

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9. This solution contains 0.9 g of NaCl, 0.042 g of KCl, 0.02 g of $NaHCO_3$, 0.024 g of $CaCl_2$, 0.1 g of glucose, and 20 mg of heparin per 100 ml. The *p*H is adjusted to 7.2.
10. The cell percentage was adjusted to 45 percent before viscometry, but was often found to increase by approximately 4 percent after viscometry in the samples containing fibrinogen. A correction for this change in cell percentage would lower the viscosity values slightly but would not alter the general shape of the viscosity curves.
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12. As the shear rate is reduced, the increases in "*a*" and η_{max} are almost parallel to each other and the ratio a/η_{max} is fairly constant (1.3 to 1.5 for the three shear rates shown in Fig. 2A). This ratio is also close to the corresponding ratio calculated for relation of aggregate size to fibrinogen concentration, where a/N_{max} is equal to 1.6.
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Athens Variant of Glucose-6-Phosphate Dehydrogenase

Abstract. *A variant of glucose-6-phosphate dehydrogenase (G6PD), characterized by slower than normal electrophoretic migration and associated with mild deficiency of G6PD in the red cells, was detected in two unrelated Greek males. Electrophoretic, chromatographic, and enzymologic study indicated that the new mutant is structurally different from normal G6PD (B+) and from the Mediterranean variant associated with red-cell enzyme deficiency (B−). Convincing electrophoretic separation of the new variant from the normal B+ and the Mediterranean B− enzymes was achieved only by detailed electrophoretic study in different buffer systems and conditions.*

The common type of deficiency of glucose-6-phosphate dehydrogenase (G6PD) observed in Caucasians is always associated with almost absent or very low enzyme activity in red cells (from 0 to 5 percent of normal); in electrophoretic mobility the enzyme is indistinguishable from normal B+ G6PD. This mutant, mainly found in populations of Mediterranean origin, is usually called the Mediterranean (or B−) variant of G6PD (*1*). In Greeks another variety of G6PD deficiency, associated with erythrocyte G6PD activities from 10 to 50 percent of normal, is also observed and usually referred to as the mild Greek type of G6PD deficiency (*2*). A number of Greek males having this mild type of G6PD deficiency had been studied by electrophoresis by use of either the tris-HCl (*3*) or the tris-EDTA-borate (EDTA, ethylenediaminetetraacetic acid) (*4*) buffer system; mainly they had erythrocyte G6PD migrating with the same mobility as the normal B+ enzyme (*5*). Recently the G6PD from two unrelated males, having mild deficiency of G6PD and supposedly normal electrophoretic mobility, was subjected to more detailed electrophoretic and enzymologic study. In both instances an identical, previously undescribed, G6PD mutant, differing in electrophoretic and enzymologic characteristics from normal (B+) and Mediterranean (B−) enzyme, was detected. The new mutant was not associated with hemolytic anemia. Since the first case examined originated from Athens (Greece), the new G6PD variant will be referred to as G6PD Athens.

On starch-gel electrophoresis, using a tris-EDTA-borate buffer system and the routine electrophoretic conditions described in the legend to Fig. 1, G6PD Athens migrates on the tail of the normal B+ G6PD. In a comparison of the electrophoretic behaviors of G6PD's Seattle (*6*), Athens, normal B+, and Negro A+, their relative mobilities were 92, 98, 100, and 108 percent (Fig. 1). Convincing separation of G6PD Athens from the normal B+ was achieved by high-voltage electrophoresis (Fig. 2) and by electrophoresis on phosphate buffer, *p*H 7.0 (Fig. 3). In another experiment in which starch-gel electrophoresis of G6PD's A+, B+, Athens, and Seattle was carried out in phosphate buffer, *p*H 7.0, for 20 hours at 4 volt/cm, the distances of the four dehydrogenases from the application point were: A+, 6.7 cm; B+, 5.7 cm; Athens, 4.6 cm; and Seattle, 3.9 cm.

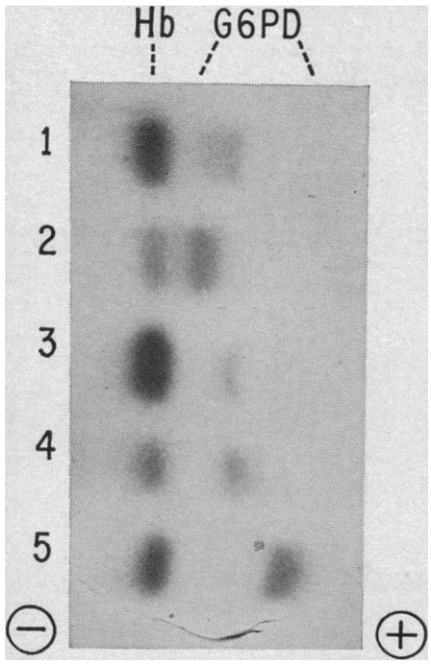

Fig. 1 (left). Electrophoresis of glucose-6-phosphate dehydrogenase on starch gel; tris-EDTA-borate buffer, *p*H 8.6. *1*, Mixture of G6PD's Athens and Seattle; *2*, G6PD Seattle; *3*, G6PD Athens; *4*, G6PD B+; *5*, G6PD A. The horizontal electrophoresis was carried out for 16 hours at 2° to 4°C; voltage, 4 volt/cm. The gels were 8 mm thick; dimensions of the trays, 22 by 14 cm. The gels were prepared with a buffer solution, containing 0.045*M* tris, 0.025*M* boric acid, and 0.001*M* EDTA, adjusted to *p*H 8.6 with HCl. After debubbling, 2.5 mg of NADP was added to the molten gels. The anode bridge solution contained 0.128*M* tris, 0.071*M* boric acid, and 0.0029*M* EDTA; the cathode bridge solution, 0.18*M* tris, 0.1*M* boric acid, and 0.004*M* EDTA; both solutions were adjusted to *p*H 8.6 with HCl. Added to the cathode compartment near the gel was 2.5 mg of NADP. The staining solution was composed of 10 ml of tris-HCl buffer (0.1*M*, *p*H 8.7), 0.1 ml of 0.1*M* $MgCl_2$, 1 ml of 3(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT tetrazolium) (2 mg/ml), 0.5 ml of G6P (20 mg/ml), 0.2 ml of NADP (10 mg/ml), and 0.05 ml of phenazinemethosulfate (5 mg/ml). The stock solutions of MTT tetrazolium, NADP, G6P, and phenazinemethosulfate were stored in a freezer. For staining of the sliced gels, a piece of filter paper (Whatman No. 3 MM) was fitted on the surface of the gel, the staining solution was poured over it, and the gel was incubated in the dark at 37°C; the color usually developed within 30 to 45 minutes of incubation. The gels were prepared for storage at 4°C by soaking for 1 to 2 hours in 75 percent methanol, washing with distilled water, absorption of the moisture with filter paper, and wrapping with plastic film.

Several other findings (Table 1) indicate that G6PD Athens is a mutant structurally different from the normal B+ enzyme; thus G6PD Athens can be distinguished from the normal B+ enzyme by its low K_m for G6P and higher utilization of 2-deoxy-G6P and deamino nicotinamide adenine dinucleotide phosphate (NADP). In addition, G6PD Athens can be separated from the normal B+ G6PD by carboxymethyl-Sephadex chromatography; G6PD Athens has been eluted with a slower rate than the normal B+ enzyme when the chromatographic conditions described by Yoshida *et al.* (*7*) were applied. Although the electrophoretic and chromatographic behavior indicates that G6PD Athens is structurally different from the normal B+ enzyme, in serologic activity the new mutant is almost the same as the normal B+ enzyme. In the quantitative neutralization test, using antiserum to normal B+ G6PD (*7*), each milliliter of antiserum neutralized an equal number of units of normal B+ G6PD and of G6PD Athens. This similarity in neutralization behavior indicates that G6PD Athens is enzymically as active as the normal B+ enzyme; consequently the enzyme deficiency in red cells results from reduction in numbers of active enzyme molecules.

G6PD Athens can be distinguished from the Mediterranean variant associated with severe enzyme deficiency (B−) by several characteristics: the relatively slower electrophoretic mobility, especially in phosphate buffer at *p*H 7.0; the relatively lower utilization of 2-deoxy-G6P and higher utilization of deamino-NADP. The two mutants differ also in their quantitative neutralization profiles: while 1 ml of our antiserum to normal G6PD neutralizes 8 to 9 units of G6PD Athens and normal B+ enzyme, the same quantity of antiserum neutralizes only 2 to 3 units of the Mediterranean variant (*8*). Furthermore, the Mediterranean (B−) variant has been separated from the normal B+ enzyme only by calcium phosphate–gel chromatography (*8*).

A number of G6PD electrophoretic mutants not associated with chronic hemolytic anemia have been detected by their slow electrophoretic mobility; four of them, G6PD's Madison (*9*), Tel-Hashomer (*10*), Austin I (*11*), and Kerala (*12*), migrate no more than 70 to 75 percent relative to normal B+ enzyme. Four more mutants, G6PD's Seattle (*6*), D− (*13*), Austin II (*11*), and West Bengal (*12*), migrate with

Table 1. Comparison of G6PD's Athens, normal B+, and Mediterranean B−; activity in erythrocytes is expressed as percentage of normal; electrophoretic mobility, as percentage of normal G6PD migration in tris EDTA-borate buffer at *p*H 8.6 (*4*); utilization of 2-deoxy-G6P and deamino-NADP, as percentage of utilization of G6P and NADP. The K_m value was measured in 0.1*M* tris-HCl. *p*H 8.0; substrate concentration: $6.6 \times 10^{-4}M$ of G6P or 2-deoxy-G6P, $1.7 \times 10^{-4}M$ of NADP or deamino-NADP. Quantitative neutralization is the number of enzyme units neutralized by 1 ml of antiserum to normal B+ G6PD (see *6*). Chromatographic behavior, on a carboxymethyl-Sephadex column.

G6PD	Erythrocyte G6PD activity	Electrophoretic mobility	K_m ($\times 10^{-6}$) for		Utilization		Quantitative neutralization	Chromatographic behavior	*p*H optimum	Thermal lability
			NADP	G6P	2-Deoxy-G6P	Deamino-NADP				
Athens	25	98	3(2.5–6.5)*	19(16–19)*	10–15	126	8–9	Slower than normal	8.5, slightly biphasic	Slightly greater than normal
Normal B+	100	100	5.1 ± 0.7	46 ± 7	< 4	58	8–9		8.0	
Mediterranean	0–5	100	1.2–1.6†	12–26†	23–37	56	2–3	Same as normal		

* Most probable value and range of the measurement. † According to Kirkman *et al.* (*1*).

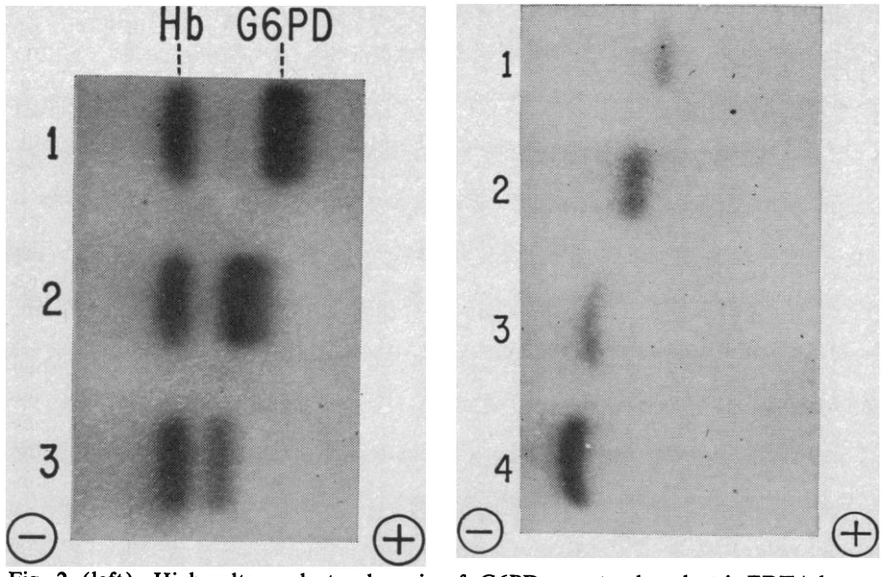

Fig. 2 (left). High-voltage electrophoresis of G6PD on starch gel; tris-EDTA-borate buffer, *p*H 8.6. *1*, G6PD A; *2*, G6PD B; *3*, G6PD Athens. The horizontal electrophoresis was carried out, on gels 2 mm thick, for 4 hours at 2° to 4°C; voltage, 10 volt/cm. For the buffer solution and staining conditions, see the legend to Fig. 1. Fig. 3 (right). Electrophoresis of G6PD on starch gels, phosphate buffer, *p*H 7.0. *1*, G6PD A; *2*, G6PD B; *3*, G6PD Athens; *4*, G6PD Seattle. Horizontal electrophoresis, on gel 8 mm thick, for 6 hours at 2° to 4°C; voltage, 4 volt/cm. Gels were prepared with 0.01*M* phosphate buffer, and 0.1*M* buffer was used for the bridge solution. Added to the molten gels, as well as to the cathode compartment near the gel, was 2.5 mg of NADP. For the staining conditions, see the legend to Fig. 1.

a mobility of 90 percent relative to normal B+ enzyme.

Enzyme G6PD D− is supposed to be identical with G6PD Seattle (*11*). Our electrophoretic findings show that G6PD Athens differs from G6PD Seattle. The K_m's for G6P and NADP, as well as for the 2-deoxy-G6P of G6PD Seattle, are very similar to those of G6PD Athens; however, utilization of deamino-NADP is definitely different: Athens, 126 percent of NADP; Seattle, 57 percent of NADP. G6PD Athens can also be distinguished from G6PD Austin II by its faster electrophoretic mobility, low activity in erythrocytes, low K_m for G6P, and high utilization of 2-deoxy-G6P and deamino-NADP. Distinction from G6PD West Bengal could be achieved on the basis of K_m for NADP, utilization of 2-deoxy-G6P, and difference in electrophoretic mobility.

The findings from the study of G6PD Athens have some bearing on further investigations of electrophoretic variation in G6PD. Mutants associated with red-cell deficiency in G6PD and normal electrophoretic mobility under routine electrophoretic conditions (especially when the widely accepted tris-EDTA-borate buffer system, *p*H 8.6, is applied) need to be studied by various electrophoretic techniques before one may consider that they do not differ from the normal enzyme (B+). Comparison of difference in electrophoretic migration between G6PD Athens and normal G6PD (B+) in phosphate buffer at *p*H 7.0 with their close similarity in mobility in tris-EDTA-borate buffer at *p*H 8.6 is particularly instructive on that point. It seems that the experience gleaned from abnormal hemoglobins applies also in the study of electrophoretic variants of G6PD. Electrophoresis of G6PD with different buffer systems and in different media will facilitate the process of distinction and characterization of new mutants.

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5. Among 14 unrelated males having mild deficiency of G6PD in erythrocytes, studied during 1964 for electrophoretic mobility of G6PD by use of tris-HCl buffer, ten had erythrocyte G6PD migrating as far as the normal enzyme, one had a fast but still-uncharacterized variant, and three had a slow-moving variant that was characterized by Kirkman and subsequently in our laboratory as G6PD Seattle. In ongoing screening electrophoretic (tris-EDTA-borate buffer) studies of Greek families, most cases of mild deficiency of G6PD show normal electrophoretic mobility. We have detected, however, several other electrophoretic variants with slightly slow or fast mobility, which will be investigated further. These findings, together with the data presented in this paper, demonstrate that the mild Greek type of deficiency of G6PD is much more heterogeneous than was originally thought.
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Oxygen Tension as a Control Mechanism in Pollen Tube Rupture

Abstract. *The decrease in stylar* pO_2 *encountered by pollen as it approaches the ovary can induce bursting of pollen tube tips. Anaerobic conditions induced a high percentage of tube tip bursting in most pollen germinated in vitro. Changes in tube tip metabolism with decreased oxygen probably sets up cell wall stress resulting in pollen tube rupture.*

In angiosperm fertilization the general growth pattern of the pollen tube down the style culminates in the rupture of the tube tip and deposition of the pollen contents into one of the synergid cells (*1*). This allows fusion of one male cell with the egg cell, and the other with the polar nuclei, forming a primary endosperm nucleus. An oxygen gradient exists in the style. Oxygen pressure, pO_2, is high in the stigma and style but suddenly decreases at the base of the style, approaching zero values in the ovary (*2*). This report evaluates the hypothesis that the decrease in style pO_2, which the pollen