dium was then quickly removed from the chamber; the cells were washed with 0.85 percent NaCl and fixed with 95 percent ethanol. Hydrochloric acid (1*N*, 60°C) was injected into the chambers, and the entire assemblies were immersed in a water bath at 60°C for 8 minutes. Fuelgen staining (*5*) was then executed in the chambers, and the cells were relocated by the original stage coordinates. The photographs of the stained preparations shown in Figs. 1–3 were also made with phase contrast optics and show more than one would see if ordinary optics were used. In particular, cell outlines could be readily discerned with phase contrast microscopy, giving added assurance that the cell observed after Feulgen staining was identical with the cell observed in the living state. The same preparations observed in bright field showed the usual Feulgen specific effect unambiguously.

Three pairs of photographs of cells from a human female are shown. Figures 1*a*, 2*a*, and 3*a* show living cells and Figs. 1*b*, 2*b*, and 3*b* show the same cells, respectively, after staining. The arrows in Figs. 1*a*, 2*a*, and 3*a* indicate objects observed in the living cells; these objects occupy the positions corresponding to those of the conspicuous sex chromatin masses found in the same cells after staining. Twenty-seven observations of this sort have been made with the same results, and the cells shown were selected as being representative in showing the sex chromatin mass in its most common form and position (Fig. 1), in a position away from the nuclear circumference (Fig. 2), and in a polyploid (probably tetraploid) nucleus (Fig. 3). Parallel observations of living human cells with the sex chromosome karyotypes XO, XX, XY, and XXY revealed sex chromatin masses in cells that were of XX and XXY origin but not in the others. There is little doubt that the object observed in the living cells is the sex chromatin mass.

Several hundred observations of the sort described in this report have been made of female cells of known age relative to the division in which they originated. They indicate that the sex chromatin mass observed in living cells and in stained preparations occurs only at a stage of interphase corresponding to the last few hours prior to the next division. Cells at earlier stages of interphase lack an object that can clearly be identified as a sex chromatin mass. Such cells often have one or more Feulgen positive condensations that might be identified as a sex chromatin mass but these condensations are invariably smaller than the unambiguous sex chromatin mass found at late interphase (*6*).

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27 September 1962

High-Speed Microcinematographic Studies of Blood Flow in vitro

Abstract. The macroscopically steady flow of human blood at varying hematocrit values and flow rates through small glass capillaries has been studied by following in detail the motion of individual erythrocytes. At the microscopic level the flow was found to be nonuniform and unsteady. The shape of the average velocity profile of the erythrocytes was found to be in general more blunt than the Newtonian profile, and to be affected by concentration and flow rate, particularly in the capillaries of smaller diameter.

Determination of the detailed flow characteristics of blood flowing in vitro, and the relation of these to the gross characteristics, such as the relationship of pressure and flow, is a problem of fundamental significance in blood rheology, one which has been the subject of several studies. Comprehensive critical reviews have been presented by Bayliss (*1*).

One of the most important aspects of the problem is that of obtaining information about the velocity distribution across the cross section. Investigations such as those of Thoma (*2*), of Coulter and Pappenheimer (*3*), and, more recently, of Haynes and Burton (*4*) have led to the formulation of velocity distributions on the basis of the gross characteristics of the flow or of other indirect evidence. Direct measurements of the velocity profiles have been, however, very scarce.

A systematic study of the detailed flow characteristics of blood in vitro has been undertaken, with particular regard to the measurement of the velocity of individual erythrocytes at various stations across the diameter of glass capillaries. The results described in this report are still exploratory in nature but afford some further insight into the nature of the flow and permit some comparison with the results of a very recent study by Bloch (*5*) on the detailed characteristics of blood flow in vivo.

Human blood of various hematocrits, treated with anticoagulant acid citrate dextrose solution (ACD), was caused to flow steadily at room temperature through horizontal glass capillaries 35 to 84 μ in diameter. The driving force was provided by air issuing under pressure from a reservoir devised for the purpose.

A phase-contrast microscope with oil-immersion objective was focused on the vertical diametral plane of the tubes. This arrangement permitted observation of sedimentation effects which would go undetected if the flow were studied in the horizontal diametral plane. The microscope was connected to a Beckman-Whitley Magnifax camera capable of operation at speeds up to 3000 frames per second. The total magnification of the process was approximately 970.

The light source was a 1400-watt alternating-current carbon arc. Although heating of the test section by the light beam could not be completely eliminated, it was reduced by interposing, between the arc and the microscope condenser, a water cell and a screen synchronized with the camera (so as to limit the exposure time to that strictly required for exposing the film). Comparison of measurements of pressure and flow made with and without heating of the objective showed an increase in the average viscosity of the flow, due to heating, of approximately 10 to 15 percent in the smaller tubes.

The films made were projected on a screen and analyzed frame by frame. In general, observation became more difficult as concentration, tube diameter, and flow velocity increased. Thus the maximum hematocrit value for which observation of the entire diametral plane was possible over a range of ve-

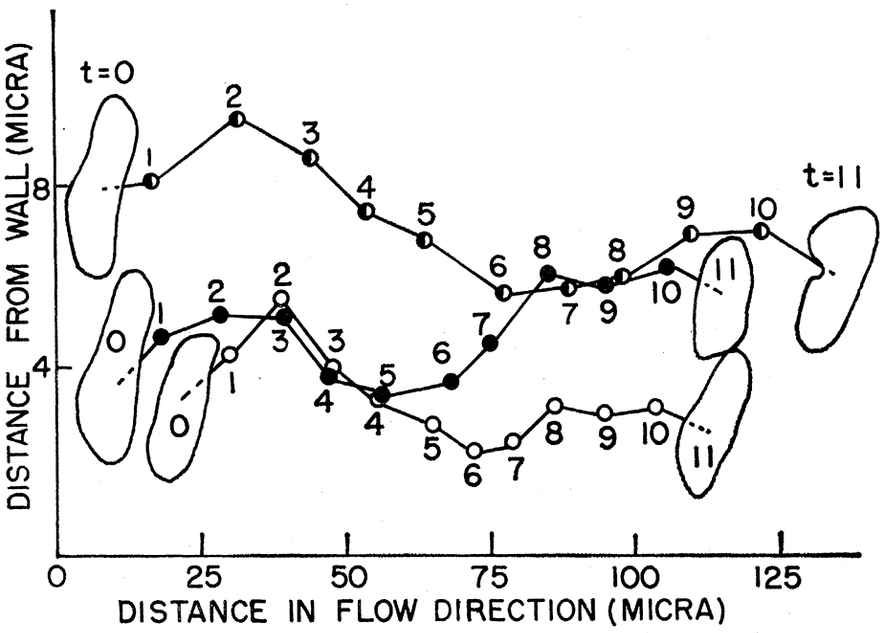

Fig. 1. Trajectories of three erythrocytes nearest to the bottom of a glass tube 39.8 μ in diameter, in a study of human blood of average hematocrit 29.5 percent. The number at each position is the time (in units of 1.33 msec) from the initial position. The cells appear distorted because the vertical and horizontal scales for the distances are different.

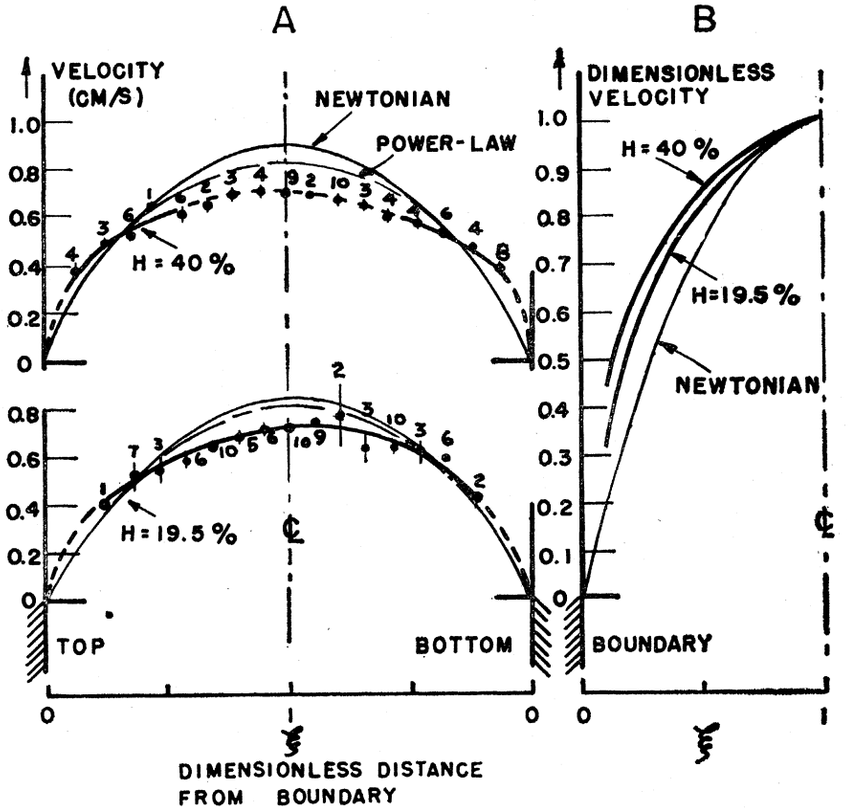

Fig. 2. Two typical velocity profiles. (A, upper profile) Hematocrit $H = 40$ percent; tube diameter $D = 41.1$ μ. (A, lower profile) $H = 19.5$ percent; $D = 37.8$ μ. The number of observations for which each point is the average is indicated; the vertical segments give the standard deviation. (B) Dimensionless plot of the profiles, averaged, for the two halves of the diameter.

locities was 40 percent in the 35- to 42-μ tubes (nominally 40-μ) and 23 percent in the 84-μ tube. At a value for hematocrit of 40 percent in the 40-μ tubes, the average flow velocities investigated ranged from 1.6 to 9.6 mm/sec, and the corresponding pressure gradients, from 0.66 to 2.53 mm-Hg/mm. At a value for hematocrit of 20 percent in the same tubes, the flow-velocity range could be extended to 1.25 mm/sec.

The results of the analysis of the motion of individual erythrocytes can be summarized as follows:

1) On a microscopic scale the flow was neither uniform nor steady; it varied from section to section along the tube and, at a given section, with time. Thus in Fig. 1, which represents results obtained in the course of a typical experiment, it may be seen that both the distances from the boundary and the velocities for three different erythrocytes varied with time.

2) The average local velocity of the erythrocytes increased from the periphery to the core of the flow. Two typical profiles are shown in Fig. 2. The dashed portions near the wall correspond to a cell-free region where the velocity profiles of the plasma are merely conjectural. The dashed portion around the center line of the upper profile indicates a region where the larger mass of erythrocytes between tube wall and diametral plane and the greater velocity made observation more difficult. It must be emphasized that the profiles are only an idealization of the actual discontinuous flow picture—an idealization that becomes increasingly meaningful as the ratio of tube diameter to cell size increases.

3) As shown in Fig. 2, usually the velocity profiles were not axially symmetric. This is attributed to sedimentation as well as to the unsteadiness of the microscopic flow.

4) In general, as shown in Fig. 2, the profiles were more blunt than a laminar Newtonian profile corresponding to the same flow rate and also more blunt than a corresponding power-law, non-Newtonian profile based on viscosity data obtained with a modified Brookfield concentric-cylinder viscometer.

5) Comparison of the velocity profiles on a dimensionless basis was difficult because of their asymmetry and the greater uncertainty of the observations in the center-line region. However,

some broad trends could be discerned from plots such as that of Fig. 2*B*. Other things being equal, in the 40-μ tubes the profiles tended to become increasingly more blunt as the concentration increased; they also appeared to become more blunt as flow rate and diameter decreased. These effects were much less noticeable in the tubes of larger diameter, which yielded profiles less markedly different from Newtonian profiles.

No attempt was made at this stage to assess the effect of ACD on the flow characteristics observed. Measurements made with the concentric-cylinder viscometer in the shear-rate range of 13 to 170 sec^{-1} showed, however, that the ACD-treated plasma remained non-Newtonian, but the viscosity was less markedly dependent on shear rate and the values for viscosity were lower than those reported by Wells and Merrill (*7*) for untreated blood.

A brief comparison with results of previous investigations is pertinent.

The observation that there are two regions in the flow—a peripheral region containing no cells and characterized by steep velocity gradients and a core in which the cells are concentrated and the velocity gradients are flatter—is in agreement with the qualitative observations of Thoma and the predictions of Coulter and Pappenheimer.

The non-Newtonian characteristics of the velocity profiles justify the concept, proposed by Haynes and Burton, of a viscosity varying across the radius. For the smaller tube sizes investigated here, however, the concept of a viscosity coefficient is to be interpreted with caution, as an indication of an average rate of momentum transfer for a homogeneous model of the flow. The presence of the peripheral layer indicates, furthermore, that the viscosity, instead of being a smoothly increasing function, varies fairly suddenly from low values near the wall to higher values in the core. Finally, occurrence of slip at the wall cannot be entirely ruled out.

The observed characteristics of the flow, such as the statistical nature of the peripheral layer and the unsteadiness of the motion of the individual cells, are in general in close agreement with the in vivo observations of Bloch. Thus it appears that, with the proper boundary conditions, in vitro flow can indeed provide a useful model of the more complex in vivo flow (*7*).

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An Approach to the Evolution of Metabolism

Postulations that life originated by chance polymerizations of nucleotides to form macromolecules that were not only self-replicating but could also direct the formation of specific polypeptide catalysts imply that both nucleic acid and protein syntheses were antecedent to intermediary metabolism. Hypotheses more in keeping with biological concepts can be constructed from the following premises: (i) metabolic processes common to *all* cells living today evolved by stepwise accretion in primitive systems of "carriers" that were *functional* ancestors of "modern" cofactors (ADP, NAD, TPP, THF, pyridoxal phosphate, UDP, CDP, etc.); (ii) these "protocofactors" which carried units of phosphate, hydrogen, active aldehyde, C^1, etc., constituted the original "bio"-catalysts and were functional before their apoenzymes evolved; (iii) the most primitive carrier-catalysts were small molecules *directly* derived from a few simple interrelated metabolites that constituted the innermost core of the evolving metabolic system; (iv) because the evolving pattern was determined by and dependent upon these particular carrier-catalysts derived from metabolites themselves, an autocatalytic metabolite-catalyst system developed; (v) these intermediary metabolites, besides functioning as the first organic catalysts, also constituted the original "genetic" material because they were the agents which, when passed from primitive aggregates to daughter fragments, determined the types of reactions (hence the metabolic sequences) in the latter.

Modern RNA's are polymers of the carriers that finally evolved to transport units of energy (ADP) and the monomers used in synthesizing carbohydrates (UDP), lipids (CDP), and proteins (GDP).

A specific scheme, based on comparative biochemistry, will be presented to illustrate a postulated origin of modern metabolism from simple metabolites which could have simultaneously served as the original catalysts and hereditary materials.

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Inhibitor of Chymotrypsin Isolated from Potatoes

This inhibitor, purified as previously described [*Proc. Natl. Acad. Sci. U.S.* (Oct. 1962)] forms with chymotrypsin, an inactive complex which has now been crystallized. When decomposed with dilute acid the crystals yield active inhibitor which has also been crys-