of molecular biology would suggest that destruction of proteins, DNA, or RNA might be the cause. Koffler's studies (*3*) indicated that "thermophilic" forms were able to evolve with proteins that were more thermostable than similar proteins from mesophilic forms. Thermal studies in vitro of "soluble" RNA (*4*) revealed a maximum temperature for acceptance of amino acids about 75°C. The "melting apart" of the two strands of DNA at elevated temperatures has also been widely reported. The "melting temperature" has been shown to be characteristic for each species of DNA, depending primarily on the salt concentration (*5*) and secondarily on the guanosine-cytosine content of the sample (*6*). Although the internal salt concentration of thermophilic organisms is not known, it would be expected that the melting temperature of the DNA would be in the range of 70° to 90°C (*7*).

Although the evidence is scanty, a plausible explanation for a maximum temperature is the limitation of amino acid acceptance by soluble RNA. Whatever its molecular basis, it is clear that there is a maximum temperature for active life processes. The earlier ecological reports which have been widely quoted must therefore be reinterpreted as survival without metabolism. The limiting factors which prevent life forms as we know them from evolving at boiling water temperatures is worthy of further research (*8*).

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2 October 1963

Red Blood Cells: Change in Shape in Capillaries

Abstract. *Evidence obtained from high-speed cinephotography of the microcirculation in the mesentery of the dog shows that the shape of the red blood cell is changed during its flow through capillaries from a biconcave disk to a paraboloid with a hollow bell-like center. The degree of deformation is dependent upon the velocity of flow in the capillaries. Although the effect of change in shape on the surface area of the cell is uncertain, a larger portion of the surface is brought into closer proximity to the capillary wall than in a cell that is in the form of a biconcave disk. Thus, the change in shape may have functional significance related to the exchange of respiratory gases.*

The relatively high plasticity of the mammalian red blood cell has been generally recognized. Its membrane, though apparently not highly elastic, is capable of deformation without irreversible alterations (*1*). The viscosity of the interior of the cell is probably about 30 times that of water at 38°C; this approximates the viscosity of olive oil at the same temperature (*2*). Thus the cell appears to have elastic and viscous characteristics which permit alterations in shape with the application of appropriate forces.

Although velocities of flow are higher in larger vessels, the red blood cell is subjected to a greater force that tends to distort its shape in its passage through the capillaries. The biconcave disk, as it enters a capillary, tends to be oriented with its polar axis parallel to the direction of flow, and the equatorial diameter of the disk approximates the diameter of the capillary. From measurements made with high-speed cinephotography in anesthetized dogs as described by Bloch (*3*), the velocities of red cells in mesenteric capillaries vary from 0.8 to 2 mm/sec. Thus if the plasma layer adjacent to the capillary wall is essentially stationary and the highest velocity of the plasma stream (parabolic flow) is in the center of the capillary (*4*), the center of the biconcave disk is subjected to forces acting in the direction of flow, while the peripheral edge of the cell is hindered in its forward movement by the essentially stationary ring of plasma adjacent to the luminal surface of the capillary. Such forces acting upon a deformable biconcave disk would be expected to cause it to approximate the parabolic front of the fluid system.

Changes in shape of red blood cells in vivo in animals other than the dog have been described. Palmer (*5*) in his studies of the microcirculation in the pancreas and mesentery of the rat, commented on the distortion of red cells when single rows of cells, moving through narrow vessels, are observed. He states, "the leading surface is markedly convex and the trailing surface is less convex or even concave." His photographs demonstrate this transformation when the cells are not too closely packed. With high-speed cinephotography, Bloch (*3*) studied the microcirculation in the mesenteries of frogs and rats. The published reproductions of his photographs show a tendency for the leading surface of frog erythrocytes in narrow vessels to be convex and the trailing surface concave. Brånemark and Lindström (*6*) have recently described various changes in the shape of the red blood cell in narrow circulatory passageways of modified ear chambers in rabbits. Among the changes which they describe are successive transformations from biconcave disks to contours resembling jellyfish, torpedoes, and bullets.

Observation and recording of the behavior of erythrocytes in capillaries were made possible through the use of high-speed cinephotography. The mes-

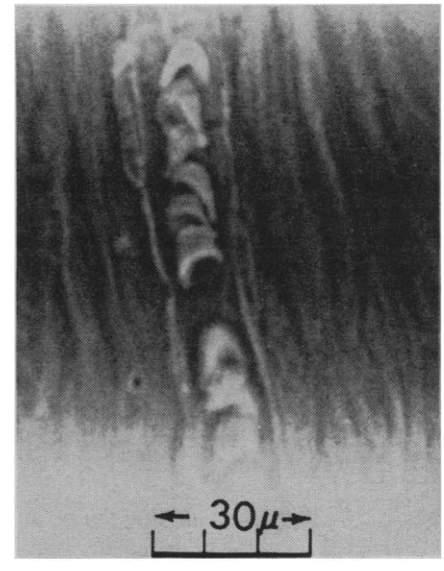

Fig. 1. Reproduction from one frame of film taken at 3200 frames per second of red blood cells passing through a capillary. Each division of the scale represents 10 microns.

enteries of anesthetized dogs were mounted in a vertical plane on a movable stage. A 1500 watt General Electric projection lamp was the light source. The light was passed through a Leitz microscope condenser, through the thin tissue of the mesentery, and into a Leitz water-immersion 90 × objective. No other lenses were used. The camera was a Beckman-Whitely Magnafax from which the lens had been removed. The exposure rate was 3200 frames per second. The film was Eastman Kodak's Ektachrome (ASA 125, tungsten). At least 5000 feet of film were used to study the capillary circulation in mesenteries of 20 or more dogs.

The characteristic shape of red cells in capillaries of mesenteries of dogs appears to be that of a thimble or a parachute. Figure 1 is representative of the transformation which occurs in the shape of red cells. The drawings in Fig. 2 depict the shape of the red cell at rest and its shape as it passes through a capillary. The outer surface of the moving erythrocyte in the capillary approximates the surface of a paraboloid. The invaginated surface probably approaches the same shape as the inner surface of a bell but, since the inner surface is only partially visible, its shape can only be surmised on the basis of the resting shape of the cell and the forces acting on it during its passage through the capillary.

Under microscopic observation and high-speed cinephotographic recording, when the field encompassed an arteriole and the arterial end of a capillary, it was noted that the biconcave red cells in the arteriole were most commonly oriented with their equatorial axes approximating a parallel position in relation to the long axis of the vessel (*7*). As a cell entered the capillary, its equatorial axis remained essentially parallel to the long axis of the arteriole, that is, its polar axis was now aligned with the direction of flow in the capillary. Almost simultaneously with their entrance into a capillary the cells were converted into paraboloids with hollow bases. This sequence is shown in Fig. 3.

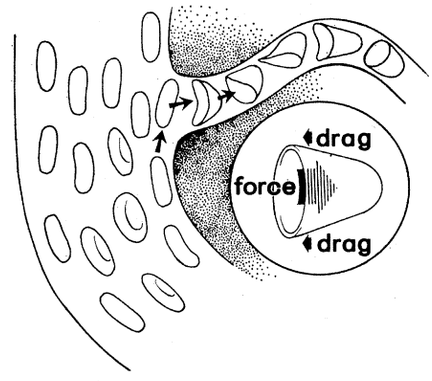

Fig. 3. Predominant flow pattern in arteriole and arteriolar end of capillary. Circle inset shows the forces acting on the red cell during its passage through the capillary.

Within limits, the velocity of flow in the capillaries appears to determine the altitude of the paraboloid. Thus, when the rate of flow increased the paraboloid became longer. When capillary flow stopped the erythrocytes immediately reverted to biconcave disks. From our observations, red cells in capillaries of the dog are charactertistically paraboloids except when flow is almost stopped or when a group of red cells pass through a capillary with their contiguous surfaces apparently in contact. As the cells entered a vessel of larger diameter than a capillary, they again reverted to biconcave disks and tended to move with their equatorial axes parallel to the vessel wall.

The change in shape of red cells from biconcave disks to paraboloids as they traverse capillaries may be functionally very important in the rapid exchange of respiratory gases. The biconcave disk has been stated to be superior to a sphere as an oxygen and carbon dioxide carrier because (i) the surface available for exchange of respiratory gases is increased over that of a sphere and (ii) the mean distance of the hemoglobin from the surface is less than it is in a sphere (*8*). Using similar reasoning, a paraboloid with a deeply invaginated base brings more of the surface close to the capillary endothelium where the exchange of respiratory gases occurs; the mean distance of hemoglobin from the surface may also be less than that in a biconcave disk. Especially important may be the variability in the altitude of the paraboloid which, from our observations, is dependent upon the velocity of flow in the capillary. Thus, when rapid exchanges of respiratory gases are required at high blood velocities, the paraboloid elongates, bringing more of its volume and a larger expanse of its outer surface into approximate alignment with the endothelial surface.

The change in shape of the red cell in capillaries may also facilitate gas exchange through the effects of stress and the reorientation of the hemoglobin molecules. Ramsey and Warren (*9*) have shown that spatial disorganization of hemoglobin, on hemolysis of the red cell, increases the oxygen uptake of the suspensions for a short period. Perhaps reorientation of surface and hemoglobin may also facilitate oxygen release, thus adding to the Bohr effect.

Although the forces which act to produce and maintain the cell in the anisodiametric form are unknown (*1*), it may be that this shape is particularly adapted to paraboloidal transformation. The thin center of the disk appears to be ideally shaped to permit conversion from biconcave surfaces to convexo-concave surfaces. The thicker rim of the disk may help to stablize the structure. A change in the ratio of surface area to volume may also occur in the transformation from disk to paraboloid and, if a relative increase in surface area occurs, gas exchange may be additionally facilitated; however, available evidence suggests that the membrane is not very elastic (*1*).

Whether or not similar changes in shape occur as red blood cells pass through the pulmonary capillaries is not known. Differences in pressure and structural relationships of these vessels may modify the transformation in shape that we have observed in systemic capillaries. If a difference exists, it also may be functionally related to the direction of movement of the respiratory gases (*10*).

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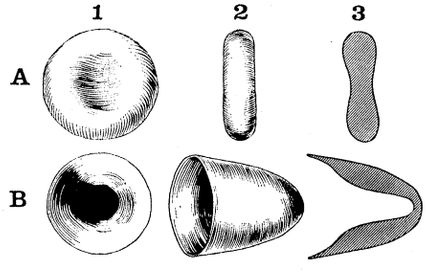

Fig. 2. (*A*) The shape of a red blood cell at rest, and (*B*) the shape of a red cell as it passes through the capillary. (Left) Cells viewed with polar axes parallel to the line of sight. (Center) Cells viewed with polar axes perpendicular to the line of sight. (Right) Cross sections through the planes of polar axes.

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New Serum Group, Gm(p)

Abstract. *The agglutination that occurs when rheumatoid arthritis serum Pond is added to erythrocytes sensitized with anti-D serum Moore is inhibited in the presence of some normal serums. The inhibitor, tentatively named Gm(p), is associated only with 7S gamma globulins and is apparently different from other previously defined serum groups. It is much more common in Caucasians than in Negroes, and probably is determined by a simple dominant gene.*

The first hereditary human γ-globulin group, Gm(a), was described by Grubb (*1*) in 1956. This description was soon followed by a number of papers describing other serum groups (*2*). A number of codominant alleles at two genetically independent loci determine two series of hereditary human γ-globulin factors: the Gm system, which includes Gm(a), Gm(b), Gm(x), Gm(c), and Gm(r), all associated only with the 7*S* γ-globulins; and the Inv system, which includes Inv(a) and Inv (b), associated with the macroglobulins and γ_{1A} globulins as well.

The frequencies of the various Gm and Inv phenotypes differ considerably in different ethnic groups (*3, 4*). The population that we studied was comprised of about 40 percent Negroes and 60 percent Caucasians.

The anti-Rh serum from one individual, Moore, whose gamma globulins were grouped as Gm(a−b+x−) was used to sensitize group O D+C+E−c− erythrocytes. The Moore serum was diluted 1 : 5 in saline, and for each milliter of this dilution, 0.02 ml of packed erythrocytes were added. Sensitization was carried out at 37°C for 1 hour. The cells were then washed three times with cold saline. Two rheumatoid arthritis serums, Baxter and Pond, agglutinated the cells sensitized with Moore serum. This agglutination was inhibited by certain normal serums. The serum inhibitor has been named, tentatively, Gm(p).

Most of our studies of Gm(p) were conducted with the Pond serum, which has a titer of 1 : 1024 against erythrocytes sensitized with anti-Rh serum Moore. The titration shows a pronounced prozone.

The Pond serum, which was also Gm(a−b+x−), reacted with most of the cells sensitized with the anti-Rh serums that were used for detecting Gm agglutination. This serum was subsequently shown to have anti-Gm(a) activity in addition to anti-Gm(p). Since the donor of the anti-Rh antibody (Moore) is Gm(a−), the Gm(a) factor is not involved in inhibition of the Gm(p) system. Although the Moore serum is Gm(a−b+x−), it was not suitable for Gm(b) typing with the anti-Gm(b) reagents that we have available.

Because of its prominent prozone, the differentiation between inhibitor, Gm(p+), and non-inhibitor, Gm(p−), was determined by titrations of the agglutinator Pond at 1 : 20 to 1 : 320 in serial double dilutions. The normal sera were diluted 1 : 10 and added in equal volume to the titrated agglutinator. Titration of normal sera 1 : 2 to 1 : 32, with addition of agglutinator Pond diluted 1 : 50 in equal volume, was also used. However, because of the prozone, sharper definition was obtained by titration of the agglutinator rather than the normal sera. All tests were performed on microflocculation test slides at room temperature, with a 0.3-percent suspension of sensitized cells as described by Steinberg (*4*).

Table 1. Typing reagents used for the serum groups.

Factor	Anti-D serum	Agglutinator
Gm(a)	Bea	Deas
Gm(a)	Bea	Lipscomb
Gm(a)	Ji	Lipscomb
Gm(a)	Bks	Chapman
Gm(b)	2269	Bomb
Gm(x)	Brand	Baxter
Gm(x)	Brand	Patterson
Gm(c)	Warren	Edwards
Gm(c)	Warren	Carp
Inv(a)	Lemire	Virmontois

We tested 485 serums obtained from individuals who applied for employment at the Medical College of Virginia, from donors to the Medical College blood bank, and from parents included in the family studies described herein. Included in this number were 60 serums used as controls in our laboratory, which were typed for some of the other serum groups by use of the reagents indicated in Table 1.

The results of typing each of these 60 control normal serums for Gm(p) and five other serum groups are shown in Table 2. Chi square values were calculated in "two by two tables" to test whether Gm(p) was contingent upon Gm(x), Gm(c), or Inv(a). These values (0.67, 1.19, and 0.03, respectively, all associated with probabilities greater than 0.25) showed no dependence. The exact probability of Gm(p) and Gm(a) contingency was found to be 0.042. This rather low probability is the result of the almost total absence of the Gm(a−) type among Negroes, and probably does not indicate any relationship between Gm(p) and Gm(a). The Gm(b) types were too infrequent in our samples to permit a statistical test of Gm(p) and Gm(b) contingency.

Table 3 shows the distribution of

Table 2. Serum types of 60 normal controls.

Number of		Serum groups					
Caucasians	Negroes	Gm(a)	Gm(b)	Gm(x)	Gm(c)	Inv(a)	Gm(p)
0	1	+	+	+	+	+	−
1	1	+	+	+	−	+	−
1	1	+	+	+	−	−	+
1	3	+	+	+	−	−	−
0	2	+	+	−	+	+	−
1	7	+	+	−	−	+	+
3	7	+	+	−	−	−	+
0	11	+	+	−	−	−	−
0	6	+	+	−	−	+	−
0	5	+	+	−	+	−	−
0	3	+	+	−	+	−	+
1	0	+	−	+	−	−	+
0	1	+	−	−	−	−	−
1	0	−	+	−	−	+	+
2	1	−	+	−	−	−	+