THE DEFORMABILITY AND THE WETTING PROPERTIES OF LEUCOCYTES AND ERYTHROCYTES

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A principal function of the leucocytes is phagocytosis, the removal by ingestion of foreign particles and the products of injury from the blood and tissue spaces. The process of phagocytosis involves the spreading of the leucocyte over the surface of a particle until the latter is completely enclosed. In the body, phagocytosis is enormously augmented by the deposit of serum proteins on the surface of the particle, and it is proving possible to define with some exactness the characteristics of the surface thus formed on the particle which produces a maximum tendency to phagocytosis (1). Concerning the spreading surface, the surface of the leucocyte, however, much less is known. The present study was undertaken principally with the object of gaining such information about the phagocytes and their surfaces as was possible by the interfacial technique; it was later broadened to include a study of lymphocytes, erythrocytes, and several types of leucemic cells.

Material and Methods

Rabbit Cells.—The rabbit large mononuclear* and polymorphonuclear phagocytes were obtained from sterile peritoneal exudates. The polymorphonuclear exudates were elicited by intraperitoneal injection of 0.85 per cent NaCl by a method which has been fully described (2). The mononuclear exudates were elicited by intraperitoneal injection of light paraffin oil; this method will be described in a forthcoming publication (3). Each type of cell was washed in sterile 0.85 per cent NaCl and resuspended in the same medium. Small samples of these suspensions were used for the present study, and the remainder were used for phagocytosis experiments. Both types of leucocytes functioned normally in phagocytosis. For the rabbit phagocytes we are indebted to Dr. Balduin Lucké.

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* No attempt was made at further classification of the large mononuclears by vital staining.

Human Cells.—These were obtained by pricking the finger and diluting the resulting drops of blood with Locke solution or 0.85 per cent NaCl solution. These suspensions of unwashed blood cells were used as the aqueous phase in interfacial preparations.

Mouse Cells.—For the mouse cells we are indebted to Dr. J. Furth. Mice suffering from lymphoid leucemia (4) were bled from the heart; the blood was diluted with Locke solution, and heparin, (final concentration of heparin, 1:10,000 approximately), was added to prevent clotting. The cells were washed and resuspended in Locke solution. In other instances leucemic lymph nodes were cut up in Locke solution and the cells were washed and resuspended in Locke solution.

Chicken Cells.—Chickens, normal, tuberculous, or suffering from myeloid leucemia (5) were bled from the wing vein. The blood was diluted with Locke solution and heparinized; the cells were washed and resuspended in Locke solution. Chicken myeloblasts and myelocytes similar to those used have been illustrated by Ellermann (6). For the chicken cells we are also indebted to Dr. J. Furth.

Temperature.—The experiments were conducted at room temperature.

Interfacial Technique.*—This is exceedingly simple. A droplet of 0.85 per cent NaCl or of Locke solution containing the cells and a droplet of oil are placed near each other on a carefully cleaned microscope slide. A large cover-slip is laid on top of them; this spreads both drops into films which meet along a line across the slide; the oil tends gradually to displace the water under the cover-glass; the oil-water interface therefore moves slowly in the direction of the aqueous phase. This slowly advancing interface is kept under continual microscopic observation, by the use of a mechanical stage, as it progressively overtakes the cells in the aqueous phase. Information is thus obtained by direct observation regarding the wetting of the cells by both phases at the oil-water boundary; moreover under certain conditions the cells are subjected to compressing or to stretching forces at the interface, so that information regarding their plasticity and fluidity is obtained.

Contamination of the advancing oil-water interface by protein or other adsorbable impurities sometimes causes difficulty. This effect is reduced to a minimum by keeping the test suspension as free as possible of impurities, and by making observations during the first minutes after setting up the preparations.

Experimental Results

The Observed Behavior of Various Blood Cells at the Oil-Water Interface.—Observation of various blood cells by the interfacial technique has shown that they fall into two definite categories, *i.e.*, those whose external surfaces are hydrophilic and those whose external surfaces are

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* For description and illustration of this technique, see Mudd, S., and Mudd, E. B. H., *J. Exp. Med.*, 1924, **40**, 633, 647; 1926, **43**, 127. A modified procedure permitting of more quantitative treatment has recently been developed by Nugent (Nugent, R., *J. Physic. Chem.*, 1931, in press).

relatively hydrophobic. All types of leucocytes studied fall within the hydrophilic group; the outer surfaces of mature mammalian and chicken erythrocytes are relatively hydrophobic. A further subdivision has appeared within each of these groups with respect to the resistance offered to the deforming and stretching forces at the interface.

The cell behavior at the interface will therefore be described under four types, A and B, (hydrophilic cell surface) and C and D, (relatively hydrophobic cell surface). These four types are illustrated in Fig. 1. That cell which most frequently exemplifies the type of reaction under discussion has been used in each instance; every effort has been made to have the drawings correspond as faithfully as possible to the actual appearances under the microscope. It is to be noted, however, that the lines and dots, which are black in the drawing, appear bright against a dark background in the dark-field microscope, with which most of the observations have been made.

It is to be remembered that the slow but sustained movement of the oil-water boundary in the direction of the aqueous phase is an essential feature of the experimental arrangement. In each column of Fig. 1 there is shown the sequence of events as one type of cell is overtaken by this slowly advancing interface.

Type A is most characteristically exhibited by the mononuclear phagocyte, although it is shown also in a smaller percentage of cases by the other types of leucocytes studied. In Fig. 1, A 1, the advancing interface is about to overtake a rabbit mononuclear suspended in the aqueous phase. In A 2 the oil has come into contact with a small part of the surface of the floating cell which it pushes before it. Beween A 2 and A 3 the cell is supposed to have touched the microscopic slide, to which it adheres strongly. In A 3 the general line of the advancing interface has passed beyond the cell, but the interface is retarded locally by the adherent cell, from whose surface the oil is unable to displace the water. The cell is thus subjected to a considerable pressure. One effect of this pressure is to tear the cell near its place of attachment to the glass, leaving a little granular cytoplasm behind the interface in the oil phase as shown in A 3 to A 6, inclusive. Another effect of this pressure is to press some of the cytoplasm and its enveloping membrane into rather blunt, stiff projections (A 3 to A 5,

RABBIT MONOCYTE | RABBIT POLYMORPHONUCLEAR LEUCOCYTE | RABBIT ERYTHROCYTE | CHICKEN ERYTHROCYTE

A1 Mineral oil — Advancing interface; Monocyte; Aqueous phase

B1 Mineral Oil — Advancing interface; Leucocyte; Aqueous Phase

C1 Mineral oil — Advancing interface; Normal red cell; Crenated red cell; Aqueous phase

D1 Mineral oil — Advancing interface; Normal red cell; Aqueous phase

A2 Floating cell being pushed ahead by advancing interface

B2 Floating cell being pushed ahead by advancing interface

C2 Floating cell being pushed ahead by advancing interface

D2 Cell adhering to glass; Floating cell

A3 Cell, now adherent to glass retarding interface

B3 Cell being spread along interface

C3

D3 Nucleus of disintegrated cell

A4 Oil unable to displace water from cell surface

B4 Part of disintegrated cell retarding interface

C4 Cells being drawn into lens shape in interface

D4 Nucleus adherent to glass retarding interface

A5 Droplet of water mechanically enclosed in oil

B5 Part of cell and droplet of water mechanically enclosed in oil

C5 Oil displaces water from cell surface

D5 Cell barely visible in oil

A6 Cell enclosed in droplet of water left in oil; Mineral oil; Aqueous Phase

B6 Mineral oil; Part of cell, enclosed in droplet of water, left in oil; Aqueous Phase

C6 Mineral oil; Cell barely visible in oil; Aqueous Phase

D6 Mineral oil; Nucleus of disintegrated cell barely visible in oil; Aqueous Phase
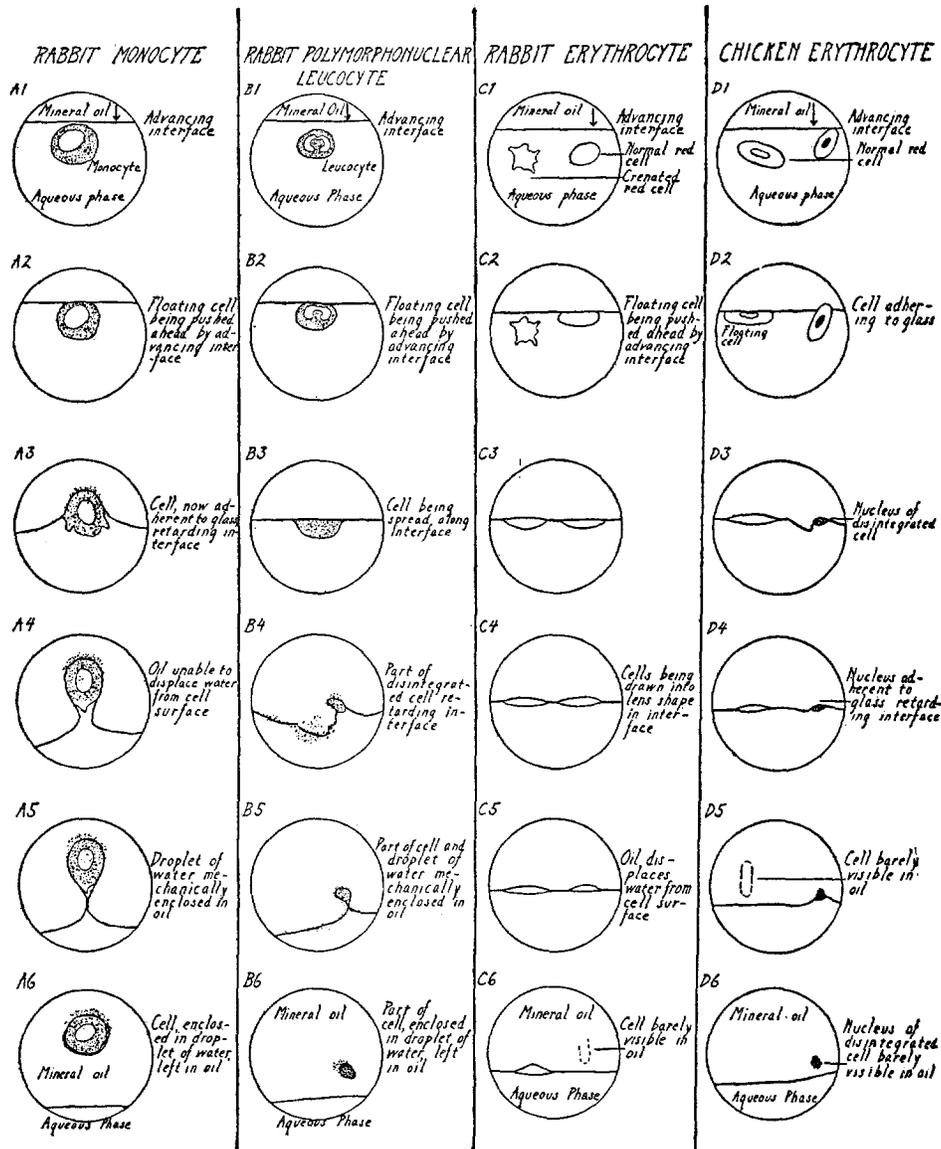
FIG. 1. The behavior of cells at an oil-water interface. Each column represents a typical sequence of events as a cell is overtaken and passed by the advancing interface. For details see text.

inclusive). In A 4 and A 5 one of these pseudopod-like projections is shown, limited by the delicate line of the surface, and filled with an optically clear cytoplasm; the granular cytoplasm has suffered little deformation in spite of the pressure upon it. Such projections, filled with optically clear cytoplasm and enveloped in the surface membrane, are frequently seen when the mononuclears are pressed upon by the advancing interface, often in more striking form than here represented. In A 4 and A 5 the interface has advanced still further, but, failing to displace the water from the cell surface, still suffers local retardation by the adherent cell. The interfaces closing in about the cell from each side are just about to make contact with each other in A 5. Between A 5 and A 6 the contact is supposed to have been made and the new interfacial line quickly returns to a minimal area, (7) leaving behind the cell still enclosed in a delicate envelope of water.

The two most striking features of this sequence are the failure of the oil to displace the water from the cell surface, and the high viscosity of the cell cytoplasm.

In Column B a rabbit polymophonuclear leucocyte is represented as floating before an advancing interface. In B 2 the oil has made contact with part of the surface and the cell is momentarily carried ahead of the interface. In B 3 the stress along the interface is tending to make the cell lenticular; the nucleus is no longer visible; whether it is broken or is merely obscured by the rapid deformation of the granular cytoplasm we do not know. Unable to withstand the interfacial stress, the cell is torn asunder; the cell detritus lowers certain local interfacial tensions, and the interface runs forward locally (B 4); the visible granules are scattered in the oil and water phases or swiftly swept out of the field along the interface like beads sliding along a string. A portion of the cell usually escapes complete disintegration; such a fragment is shown in B 4 and B 5 retarding the interface. In B 6, as in A 6, a new interfacial line has formed and contracted to minimal area, leaving behind in the oil a fragment of the disintegrated cell still enclosed in a droplet of water.

The features of the typical polymorphonuclear leucocyte (Type B), which contrast with the typical large mononuclear (Type A), are the lower viscosity of the cytoplasm of the polymorphonuclear cell and the lesser resistance of this cell to the stretching forces of the interface.

Both types of leucocyte are alike in the hydrophilic nature of the external surface. Types of behavior intermediate between A and B are of course observed; they are seen with all types of leucocytes, but have been noted as most characteristic of lymphocytes.

Consider now the reaction types with cells of relatively hydrophobic surface properties, the mammalian and chicken erythrocytes. In C 1 a normal and a crenated rabbit erythrocyte are represented as suspended in the aqueous phase. In C 2 the oil has made contact with the normal red cell surface; in C 3 to C 5, inclusive, the red cells are drawn to lens shapes by the interfacial stress; the oil readily displaces the water from the cell surfaces and the cells slip through the interface into the oil without, or with scarcely perceptible, retardation of the interface. A cinematograph of such a sequence with human erythrocytes has been published (8). In the case of the crenated red cell the outer membrane is folded into projections. As this cell enters the interface between C 2 and C 3 the outer membrane is pulled out by the interfacial stress until the folds give way to a smooth contour, (C 3 to C 5, inclusive). This cell also finally slips through into the oil. In the oil the red cells are ordinarily not visible.

The features of this mammalian erythrocyte reaction which deserve emphasis are: first, the ease with which the water is displaced by oil from the cell surface, second, the fluidity of the cell contents; the cell is pulled into a lens by the interfacial stress as a liquid immiscible in both phases would be; and, third, the structure of the outer membrane, which may be folded into projections and stretched to a smooth contour, but which resists disruption by the interfacial stress.

In Column D are represented two chicken red cells, the one normal and floating in the aqueous phase, the other adherent to the glass. In such a preparation the red cells settle out onto the glass within a few minutes, and, as observation continues, more and more of the cells in contact with the glass are hemolysed. Such contact hemolysis by glass has been described for mammalian erythrocytes by Fenn (9). The hemolysed chicken erythrocyte appears in the dark-field to have a pale, silvery outline and a brighter, more granular nucleus than the normal cell. In Fig. 1 the chicken cell in contact with the glass is represented with the granular nucleus, intended to indicate injury, but with the bright golden outline of the unhemolysed cell.

In D 2 the floating cell is momentarily pushed ahead of the advancing interface. Between D 2 and D 3 the floating cell has turned over so that its two larger axes lie in the plane of the boundary surface; hence the cell is viewed in D 3 from its edge. Such an orientation is the usual rule when non-spheroidal objects, such as spermatozoa, spirochetes or bacterial rods, enter the interface. In D 4 this cell is slipping out of the boundary surface into the oil, with scarcely perceptible retardation of the interface.

In D 2 the water is displaced by the oil from one end of the cell adherent to the glass. Between D 2 and D 3 this cell has suddenly disintegrated with consequent local running forward of the interface. The injured nucleus of the disintegrated cell, however, markedly resists entering the oil. This nucleus is represented as having become adherent to the glass between D 3 and D 4. The further advance of the interface is retarded locally in D 5 by the adherent nucleus; however, the interface is represented as pulling over the nucleus in D 6, leaving the nucleus behind in the oil. In other instances the injured nucleus is left behind the interface still enclosed in a droplet of water.

This high resistance to wetting by the oil is believed to be a property of the granular-appearing injured nucleus, in which the protoplasm is probably coagulated. In an uninjured chicken erythrocyte, little or no structure can be seen within the nuclear membrane; when such a cell disintegrates in the interface the nucleus appears to offer comparatively little resistance to passage through the interface.

In most preparations the erythrocytes, both mammalian and avian, are invisible in the oil. Occasionally, however, for reasons that are not wholly clear, the details of the process by which the erythrocyte slips out of the interface into the oil may be made out. The erythrocyte touches and tends to adhere to the glass, the interface passes over that part of the red cell which is in contact with the glass, thus stretching the erythrocyte into a pear-shaped body with the stem attached to the glass under the oil and the base still momentarily in the interface. With further advance of the interface the base of the pear-shaped erythrocyte is pulled out of the interface into the oil and the cell there tends to contract to its usual shape.

The outer surface of the chicken erythrocyte, like that of the mammalian red cell, is relatively hydrophobic. Whether or not the outer

membrance is inherently less resistant than that of the mammalian erythrocyte to disruption by the interfacial stresses we have never been able to determine with certainty. Certainly many of the chicken erythrocytes settled out on the glass do go to pieces in the interface, but the possibility of injury by contact with the glass cannot be excluded. The injured, and probably coagulated nucleus, displays marked resistance to wetting by the oil. The behavior of these injured nuclei in the interface is very much like that of heat-injured

TABLE I

Peritoneal Exudate from Rabbit

Cell type	Test oil	No. of experiments	Reaction types, average	Remarks
			per cent	
Large mononuclears	Triolein	3	A, 95 B, 5	
" "	Tricaprylin	4	A, 90 B, 10	
" "	Tributyrin	2	A, 90 B, 10	
" "	Cyclohexane	3	A, 75 B, 25	
" "	Mineral oil	7	A, 85 B, 15	
Polymorphonuclears	Triolein	3	A, 50 B, 50	
"	Tricaprylin	6	A, 45 B, 55	
"	Tributyrin	2	A, 60 B, 40	
"	Cyclohexane	5	A, 15 B, 85	
"	Mineral oil	5	A, 15 B, 85	
Erythrocytes	Triolein	4	C 100	Interface slightly retarded
"	Tricaprylin	5	C 100	
"	Tributyrin	2	C 100	
"	Cyclohexane	3	C 100	
"	Mineral oil	2	C 100	
Lymphocytes	Mineral oil	2	A, 80 B, 20	

human polymorphonuclear leucocytes and heat-coagulated globulins (8, 10).

The Distribution of the Reaction Types among Various Cells.—The sources and kinds of cells studied, the oils used as organic phase and the reaction types observed are set forth in Tables I to IV, inclusive. The mammalian erythrocytes all fell within Type C, and with one exception no significant differences were detected with the several oils used. The exception was triolein. The saline-triolein interface

was distinctly, although slightly, retarded in passing over each erythrocyte. This effect could plausibly be attributed to the polar double-bonds of triolein, which doubtless tend to orient themselves toward the water phase; however, we have no further evidence on this point at present.

TABLE II

Normal Human Blood Cells

Cell type	Test oil	No. of experiments	Reaction types, average	Remarks
			per cent	
Polymorphonuclears	Triolein	1	A, 75 B, 25	
"	Tricaprylin	4	A, 20 B 80	
"	Mineral oil	2	A, 10 B, 90	
Erythrocytes	Triolein	1	C 100	Interface slightly retarded
"	Tricaprylin	6	C 100	
"	Mineral oil	4	C 100	
Lymphocytes	Tricaprylin	4	A, 40 B, 60	Some intermediate reactions
"	Mineral oil	1	A, 5 B, 95	

TABLE III

Blood Cells of Lymphoid Leucemic Mouse

Source of cells	Cell type	Test oil	No. of experiments	Reaction types, average
				per cent
Leucemia blood	Polymorphonuclears	Tricaprylin	3	A, 15 B, 85
" "	"	Mineral oil	4	A, 20 B, 80
" "	Erythrocytes	Tricaprylin	4	C 100
" "	"	Mineral oil	3	C 100
" blood and lymph nodes	Lymphocytes	Tricaprylin	7	A, B, and intermediate
Leucemia blood and lymph nodes	"	Mineral oil	8	A, B, and intermediate

The chicken erythrocytes fell within Type D as already described. The majority of rabbit mononuclear phagocytes fell within Type A, the minority within Type B; the same was true for the large mononuclear leucocytes of chicken blood. In contrast to this result were

TABLE IV

Blood Cells of Chicken

Condition	Cell type	Test oil	No. of experiments	Reaction types, average	Remarks
				per cent	
Tuberculous	Large mononuclear	Tricaprylin	4	A, 70 B, 30	
"	"	Mineral oil	3	A, 70 B, 30	
Normal and tuberculous	Polymorphonuclears	Tricaprylin	11	A, 40 B, 50	
"	"	Mineral oil	7	A, 30 B, 70	
Normal, tuberculous, and leucemic	Erythrocytes	Tricaprylin	17	D 100	In 4 expts. interface retarded
"	"	Mineral oil	9	D 100	In 3 expts. interface retarded
Normal and tuberculous	Lymphocytes	Tricaprylin	8	A, 80 B, 20	An occasional intermediate reaction
"	"	Mineral oil	5	A, 75 B, 25	"
Myeloid leucemia	Myelocytes	Tricaprylin	2	A, 5 B, 95	In other experiments differentiation not so clear between myelocytes and myeloblasts
"	"	Mineral oil	2	A, 5 B, 95	
"	Myeloblasts	Tricaprylin	2	A, 95 B, 5	
"	"	Mineral oil	2	A, 85 B, 15	

those with the polymorphonuclear leucocytes of rabbit, man, mouse, and chicken. In each species the behavior of the majority of polymorphonuclear leucocytes corresponded to Type B, that of the minority to Type A. The percentages given in the tables are based on estimates, not on exact counts, and are very rough. However, the accuracy of the estimates is more than sufficient to warrant the conclusion that the polymorphonuclear leucocytes are on the average more fluid cells, less able to withstand the stretching forces of the interface, than the mononuclear leucocytes.*

Both kinds of rabbit leucocytes disintegrated in the interface in a higher percentage of cases when cyclohexane and mineral oil were used than with the other test oils. This result is in harmony with the fact that the oil-water interfacial tension, and hence the stretching force to which the cells were subjected, (see next section), is extremely high with cyclohexane and mineral oil (12).

The number of observations with normal lymphocytes and with leucemic cells was not so large nor were the differentiations so clear that we care to give them emphasis. It was evident, however, that these cells conformed to the general leucocytic reaction patterns, and had hydrophilic surfaces.

One experiment with the immature leucocytes of a chicken with myeloid leucemia deserves passing mention. In this experiment (Table IV) the very immature cells of the granulocytic series, the myeloblasts, behaved in the interface much like rabbit mononuclears; the mature myelocytes, on the other hand, resembled the polymorphonuclear leucocytes in their interfacial behavior as in their morphology.

Analysis of Interfacial Tension Relations.—Since the progressive movement of the oil-water interface brings it successively into contact with the test cells in the aqueous phase, we need not here consider, as in colloidal aggregation problems, the factors influencing probability

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* Whether or not any given cell disintegrated in the interface depended in part upon whether it was freely suspended or adherent to the glass. The adherent cells were more frequently disintegrated than those freely suspended (*cf.* Fenn, 11). Since the mononuclears are larger than the polymorphonuclear leucocytes and readily sedimented out, this effect only serves to make the relatively greater resistance of the mononuclears to disintegration the more striking.

of collision. Let us consider, therefore, the free energy relations when the cell has reached equilibrium in the boundary surface.

When three phases which do not mix are in contact with each other, the three surfaces of separation meet in a line, straight or curved. The case under consideration is that of a cell in the interface between two immiscible liquids. Supposing the cell to be spherical and the surface uniform, the line of contact would form a circle on the surface of this sphere. Let O (Figs. 2 and 3) be a point in the line of contact, and let the plane of the paper be supposed to be normal to the line at the point O.

Let A be the aqueous phase of the preparation; let B be the organic phase, and C the disperse phase (cell). We have then three surfaces
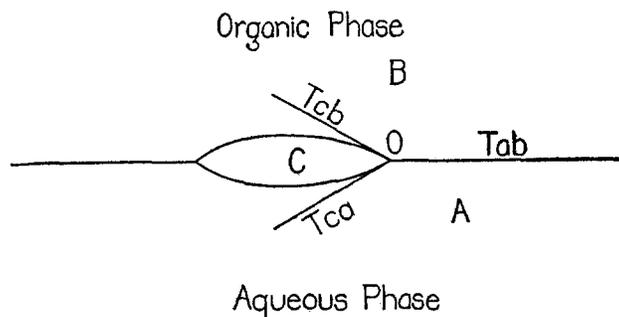
Organic Phase

FIG. 2

of separation, AB, the aqueous-organic phase interface, CA, the cell-aqueous phase interface, and CB, the cell-organic phase interface. The corresponding interfacial tensions (*i.e.*, intensity factors of the interfacial free energies) in the surfaces meeting at the point O are Tab, Tca and Tcb. Let the angle at which AB meets the tangent CA at the point O be the contact angle, θ.

If the disperse phase were a liquid it would in general be deformed by the tension in the surface AB. If $Tab > Tca + Tcb$ the liquid droplet would be spread in the interface. If $Tab < Tca + Tcb$ the droplet would assume some such lens shape as is shown in Fig. 2, the exact angles between the three surfaces of separation being determined by the values of the three interfacial tensions. Such lenses

in air-water interfaces have been measured and figured by Coghill and Anderson (13).

The interfacial technique shows that mammalian red blood cells do actually become lenses in the oil-water interface (see Fig. 1, Column C) and also illustrations from moving pictures of erythrocytes in oil-water interfaces (8). The red cell is obviously not a homogeneous liquid. Its behavior in the interface strongly suggests, however, that it is a liquid surrounded by a surface membrane which is a plastic solid or possibly a liquid of high viscosity.*

The question naturally arises as to whether the lenticular erythrocyte in the interface represents a true equilibrium of the three interfacial energies. An alternative explanation of its shape would be that the erythrocyte surface was really under a stretching force in the surface AB, (i.e., $Tab > Tca + Tcb$), but that spreading was prevented by the structure of the cell or its surface membrane. Tca and Tcb are not measurable and offer no help in answering this question. However, the often-repeated observation that the folded surfaces of crenated erythrocytes are pulled out to smooth lenses by the interfacial stress, makes us believe that the surface membrane is really under a stretching force.

The surface of the erythrocyte is evidently not strongly hydrated since it offers only a minimal resistance to passage through the interface into the oil. Nevertheless it does not pass spontaneously out of the interface into the oil. Hence, $Tca < Tcb + Tab$ and $Tcb < Tca + Tab$.

The interfacial tension relations for the erythrocytes of the chicken seem to be similar to those for mammals.

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* A solid does not undergo permanent change of shape unless the deforming stress exceeds a certain value. A liquid subjected to shearing stress, however small, undergoes continual deformation, although the rate of change of shape may be exceedingly low (14). So far as may be judged by observations of such short duration, the red cell once stretched by the interfacial tension to a smooth lens does not undergo further stretching with time and should therefore be judged to be a solid. The possibility cannot be excluded, however, that longer tension might produce further deformation.

An important paper on Surface films as plastic solids, (Wilson, R. E., and Ries, E. D., in Colloid symposium !monograph, New York, The Chemical Catalog Company, Inc., 1923, 1, 145), should be consulted in this connection.

If the disperse phase is a solid or a jelly too stiff to be readily deformed by the interfacial stresses, it cannot be pulled into a lens. In this case the line of contact of the three surfaces of separation is itself pulled over the surface of the particle until the conditions of equilibrium are satisfied.

At equilibrium $Tcb = Tca + Tab \cdot \cos \theta$.

If $Tcb > Tca + Tab$ the cell will not be in equilibrium in the interface but the line of contact will be displaced toward the surface of greatest tension until the cell is entirely enveloped in the aqueous phase. Similarly were $Tca > Tcb + Tab$ the line of contact would be displaced toward the aqueous side until the particle would be entirely enveloped in the organic phase.
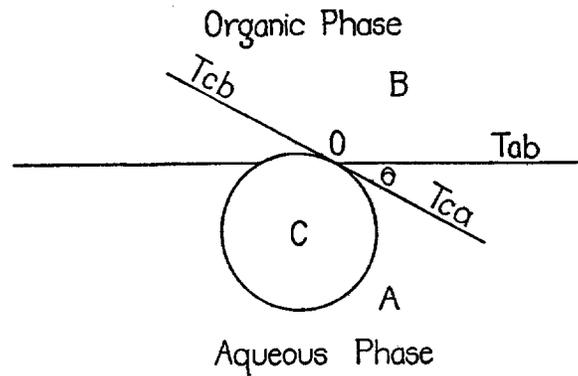
Fig. 3

Thus low tensions in the cell-water interface (Tca) tend to bring about a position of the cell in the interface such that the contact angle θ is small and the bulk of the cell is in the aqueous phase. Such a position in the interface conversely indicates a low value of Tca relative to Tcb and strongly suggests that the cell surface is hydrated. It is important to remember, however, that the equilibrium position in the interface indicates the relative values of Tca and Tcb and not the value of the cell-water interfacial energy alone; it can only be used with this reservation in mind in drawing inferences as to the hydrophobic or hydrophilic nature of the cell surface.

The equilibrium position in the interface of the rabbit large mononuclear leucocyte is approximately that just described. The indication thus given that the surface of the rabbit monocyte has strong

affinity for water is confirmed by the subsequent behavior of the cell toward the advancing interface (See Fig. 1, Column A).

By this method we have not been able to detect or infer differences in the interfacial tensions for the several types of leucocytes. The obvious differences are in the resistance to deformation of the poly-morphonuclear and mononuclear cells. The majority of polymorpho-nuclear leucocytes under the conditions of our experiments were de-formed by the interfacial stresses until their internal organization broke down and the cells were disintegrated.

Attempts to Determine Isoelectric Points of Leucocytes.—Many ef-forts were made to determine the isoelectric points of the washed leucocytes in the Northrop-Kunitz cataphoresis cell (15). Catapho-resis of the washed leucocytes suspended in various buffers presented no serious difficulties, and the direction of migration of the leucocytes regularly changed at reactions in the region of pH 4.0 to 4.4. Certain peculiarities, however, aroused suspicion. In the cataphoresis cell containing leucocytes all visible particles and cells moved with prac-tically the same velocity; in the usual cataphoresis experiment, some specks of foreign material are almost always to be seen moving at dif-ferent velocities, even in the opposite direction, to the principle objects of study. Collodion particles were therefore mixed with the leucocyte suspension and were found to move at the same rate and to reverse di-rection of migration at the same pH as the leucocytes. Collodion particles alone at pH 4.0 have a high negative charge. It was thus made evident that readily adsorbable substances were present in the suspensions of leucocytes in buffers, probably due to injury of some of the cells. Since we have thus far found no way to avoid this effect we have temporarily abandoned the attempt to determine the isoelec-tric point of the uninjured leucocyte.

A similar effect with erythrocytes in acid buffers has recently been described by Abramson (16), and has been noted by L. T. Bullock and the writer, studying red cells treated with tannin.

It would seem that such effects have been too little considered in recent experiments directed toward determination of the electrical properties of filterable viruses. When ground-up tissue containing a virus together with a mixture of substances of unknown properties is suspended in a buffer, adsorption complexes of altogether unpredictable

character may be formed. Experiments on the cataphoretic and adsorption behavior of such mixtures should be interpreted with extreme caution.

DISCUSSION AND SUMMARY

The resistance to deformation of polymorphonuclear neutrophile leucocytes under the conditions of our observations has been shown to be on the average considerably less than the resistance to deformation of large mononuclear leucocytes. It is recognized of course that the viscosity of leucocytes, as of other cells, may be markedly influenced by osmotic conditions (17), by the reaction of the suspending medium (18, 19), by temperature, or by injury (20, 21). Although the conditions of our observations were quite different from those of the body, they were nevertheless closely similar to those of simultaneous phagocytosis experiments in which the cells functioned exceedingly well (3). Moreover E. R. and E. L. Clark (22) have noted that polymorphonuclear leucocytes in the tails of living tadpoles were more fluid than the macrophages. And Goss (23) in microdissecting human polymorphonuclear neutrophiles reports that they are more fluid than the clasmatocytes and monocytes studied by Chambers and Borquist (24). Other types of leucocytes have in our experience seemed to fall between the large mononuclear and the polymorphonuclear leucocytes in their average resistance to the interfacial tensions.

The leucocyte of each type studied is surrounded by an exceedingly delicate membrane. This membrane appears under the dark-field microscope as a pale, silvery line not distinguishable by inspection alone from a simple phase boundary between two immiscible liquids. That this is a membrane, however, and not a mere interface between immiscible phases, seems certain. In the first place the cell cytoplasm and the suspending medium are not immiscible. When the cell organization is broken down by the interfacial tension the greater part of the cell contents is immediately dissolved or dispersed. Goss (23) has noted that when the membrane is torn with a microdissection needle disintegration at once spreads over the membrane and the cytoplasm undergoes profound change. Moreover it is improbable that a simple phase boundary could exist in the presence of so much protein, lipoid, and other surface active materials as are present in protoplasm; the

tendency of these substances to lower the free interfacial energy must necessarily tend to their adsorption in the interface until, if sufficient material is available at the interface, an adsorption film or membrane may be formed.

Kite (25), in a pioneer microdissection study, described the polymorphonuclear leucocyte as "naked" protoplasm. The contradiction between this statement and those just made is more apparent than real. For the capacity swiftly to form a limiting membrane between itself and other liquids is an attribute of "naked" protoplasm, as has been shown by the beautiful experiments of Chambers (20).

The present study of the wetting properties of leucocytes shows that their external membranes are hydrophilic,* a character suggesting a surface in which proteins, probably bound water and salts (27), possibly the polar radicles of soaps or fatty acids, rather than non-polar lipoid groupings, are predominantly exposed. This makes it the more remarkable that a cell of such fluidity as for instance the polymorphonuclear leucocyte, composed largely of water and of water-soluble materials, should maintain its integrity in an aqueous medium with the aid of a membrane so delicate and so mobile.† The mobility of the membrane, frequently extended in forming new pseudopodia or spreading over the surface of particles being ingested, must require constant entrance into and exit from the membrane of component materials, and their constant reorganization there. The limiting factors in the reformation of such a membrane would be the amounts of adsorbable materials available and their rates of movement up to the surface rather than the time required for orientation there, since the latter phenomenon is exceedingly rapid. Harkins (29), for instance

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* It is well known that the hydration of proteins is related to the pH of the medium. Nugent has shown that this applies also to proteins in thin surface membranes (26). Our experiments with rabbit mononuclear and polymorphonuclear cells were conducted in a faintly acid medium, i.e., 0.85 per cent NaCl in laboratory distilled water; other cells were for the most part studied both in faintly acid (0.85 per cent NaCl) and faintly alkaline (Ringer-Locke solution) suspending media.

† However protoplasm has been likened to liquid crystals by W. T. Bovie and others (28). It is not unlikely that protoplasm owes its integrity to its own internal organization as well as to differentiated surface layers.

has calculated that at a water-water vapor interface at 20°C., from the area occupied by one molecule of water, a molecule would jump out into the vapor and a vapor molecule would fall into this area of the surface 7,000,000 times in one second; the time of orientation of the water molecule he estimates to be of the order of 1/100,000,000 second or less.

The mammalian erythrocyte possesses a surface membrane capable of being folded and of withstanding tension in the interface. This has also been stretched by microdissection needles (21).

The surface of the erythrocyte, as evidenced by its wetting properties, is relatively hydrophobic, relatively non-polar in character, as compared with the leucocyte. Evidence indicating that the erythrocyte surface contains both lipoid and protein components has been summarized in earlier papers (8, 30). We have little to add here other than to point out that the wetting properties of the chicken erythrocyte surface are similar to those fully described for the mammal.

A serious source of error in certain isoelectric point determinations is discussed.

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