ANALYTICAL REVIEW

Present Concepts of the Structure of the Mammalian Red Cell

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UNTIL 1936, ideas about red cell structure were based mainly on deductions made from observations in the field of permeability. Since then, they have been based mainly on the results of direct applications of physical methods, and these alone will be the subject of this review. Although it is a simplification of the real situation, we can begin by considering the structure of the red cell surface, and then go on to the structure of the interior.

Surface Structure

The surface of the mammalian red cell ghost is sufficiently thick, and its molecules are sufficiently well oriented, to give double refraction with polarized light.[1-5] When the ghost is in saline, the net birefringence is not large. Thus, in the original semiquantitative description of Schmitt, Bear, and Ponder[1] (ghosts of rabbit red cells), this was attributed to a negative birefringence, due to tangentially arranged proteins, being nearly balanced by a positive birefringence, due to radially arranged lipids. Chemical analysis shows that nearly all the lipids of the red cell can be found in the ghost; there are enough to form about two layers with a total thickness of about 20 A if the layers are continuous. The lipids were thought, in the original description, to form a highly oriented bimolecular palisade at or near the surface, while the tangentially arranged protein micelles were thought of as lying beneath the layer of lipids. Arrangements other than that of continuous lipid and protein layers have been proposed, the difficulty being that the birefringence observed is too great, and spread over too large a zone at the cell's edge, to be accounted for by only a few monolayers. There is also a tendency at present to think in terms of lipoprotein complexes rather than of sharply defined layers. One of the suggested arrangements is a discontinuous one, with lens-like lipid regions in or on a protein framework.[6,13] Another is a thin lipid layer lying on a thick hydrated protein layer arranged like corrugated paper.[4,5]

The electron microscope, applied to sections or moulages of ghosts, gives somewhat ambiguous evidence regarding the thickness of the surface layers, considerable information regarding the changes produced in them by lysins, agglutinins, etc.,[7-8] and quite uncertain information regarding fine structure. Bernhard[10] describes a herring-bone arrangement of the molecules of the surface.

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A fibrous arrangement was described in the early days of electron microscopy,[17] but the subject of the fine structure of the erythrocyte surface is still incompletely developed. The estimates of thickness range from about 100 Å to about 1000 Å, dry, the thickness of the undried structure depending, of course, on the amount of hydration assumed. Most observers agree that the thickness varies as much as 100 per cent from region to region over the surface.[9-12]

Structure of the Interior

The concentration of hemoglobin in the red cell is so great that individual molecules are within 8 Å of touching each other.[13] This physical state is such that, in some species, the withdrawal of a small amount of water leads to a paracrystalline condition[14] and is a state which can be described, with equal appropriateness, either as a viscous fluid on the verge of becoming paracrystalline, or as an expanded crystal. As might be expected, the curves for the x-ray scattering of intact red cells are intermediate between those of a solution and those of a crystal; no lines of crystal or paracrystalline structure appear (except in special cases in which the cells are actually paracrystalline), but the curves have a form which points to an arrangement of the Hb molecules, not wholly random.[15,16] The term used is "short ordering", and refers to an arrangement which, while not regular throughout, has some degree of regularity in the immediate neighborhood of any single Hb molecule.

Recent electron microscope pictures of thin sections of red cell ghosts show a loose and rather indefinite arrangement of micelles of Hb on threads.[11a] To what extent this arrangement corresponds to the interaction deduced from x-ray scattering is uncertain.

No characteristic x-ray scattering or x-ray diffraction has yet been obtained from ghosts.

Miscellaneous Observations

So far we have the picture of a highly oriented lipid and protein surface ultrastructure surrounding a quantity of Hb which has an orientation somewhat more definite than the randomness of a solution. Most observers would agree that this is a reasonable picture of the structure, the only important points still in dispute being those concerning the orientation and thickness (or degree of hydration) of the protein layer. The difficulties become apparent when we pass to details. I shall illustrate this by considering several varied aspects of the structure problem.

1. Thickness of the Surface Structure

There are four methods by means of which estimates of the thickness of the surface ultrastructure can be made.

Chemical analyses show that the available quantities of lipid and protein would provide a lipid layer about 40 Å thick and a layer of protein of about the same thickness, with some variation from animal to animal.[17] If, as is almost certain, some protein, as well as lipid, is lost from the ghost during washing,[9] this is a minimum value. The value for the thickness of the protein layer also excludes Hb, and the values for both lipid and protein are calculated on the

basis of dry weight.[18] Hydration would increase them in proportion to the contribution of water.

As measured with the leptoscope,[9] the thickness of the dried surface structure of the rabbit ghost produced by lysin in large volumes of hypotonic buffer is 200 to 230 Å. There is some variation with pH and from animal to animal. If the ghosts are allowed to stand in an electrolyte-poor medium, the thickness may fall to about one-half because of the leaching-out of both lipids and proteins.

The leptoscope has been used to estimate the decrease in thickness which the surface structure undergoes as it dries. The decrease is only some 25 per cent; this precludes any extensive hydration unless the structure becomes skeletonized. The greatest allowable thickness of the surface structure, as measured by this method, would be in the neighborhood of 300 Å. The reliability of the estimate of hydration is a critical point. The method has not been used to examine different kinds of ghosts in a systematic way, and the conclusion reached as regards the amount of hydration does not agree with observations[19] which show that the lipoprotein material of dried ghosts increases several times in volume when it is wetted.

Recent quantitative measurements of the retardation observed at the edges of human ghosts in glycerine[4, 5] have led to an estimate that the thickness of the birefringent structure is about 5000 Å. Such a structure, supposedly composed of looped proteins, would occupy about half the volume of the cell, would have to be highly hydrated, and would have to include Hb in its meshes. The earlier (1936) estimates of thickness, based on the surface structure being a layered body, are only semiquantitative, but are certainly smaller than this ("many times the thickness of a (40 Å) bimolecular layer"). The interest attached to thicknesses deduced from polarization optics lies in their being values for wet material. At present, however, they cannot be considered as settled because there are too many ways of interpreting the experimental data.

Electron microscopy can be used to find the dry thickness of sections of red cells and of ghosts, and the depth of defects seen in moulages. Values of from 100 Å to 800 Å have been given by different investigators who have worked with sections, measurement of the thickness of surface layers from which the Hb of the cell has shrunk away, etc.[10-12, 20] These values are certainly influenced by the way in which the ghosts are prepared; the longer they are exposed to hypotonic media, the thinner the surface structure seems to be. Moulages after shadowing give higher values (500 to 1000 Å).[7a] To convert these values into thicknesses of real structures, some value for the hydration again has to be assumed.

Several observers have remarked that the surface structure, as seen in thin sections, is not sharp on its inner side, but seems to extend, irregularly, in the direction of the interior.[10-12]

2. Volume of Ghosts

This can be measured by a combination of hematocrit and conductivity methods.[21-23] Many ghosts have larger volumes than is usually thought, and are disk-shaped bodies with volumes which range from that of the red cell itself to one-half or one-quarter that volume. The figure varies somewhat from animal to animal, still more with the method of preparation, and the measurements cannot

be trusted unless the ghosts are freshly prepared from fresh blood. Repeated washing reduces the volume, and after much washing, the ghost may have a volume one-twentieth that of the cell. Notice that, because the flat ghost tends to retain its diameter and to lose volume by becoming thinner, a surface layer 2000 Å thick would occupy about one-quarter of the cell volume, one of 500 Å would occupy one-sixteenth, and one of 100 Å, one-eightieth of the initial red cell volume. If the ghost volume is about one-half that of the cell, its volume is obviously occupied by something; this could be fluid contained within a very thin wall, a thick hydrated structure (4000 Å thick), or a combination of a thicker wall and a thinner and less hydrated structure.

3. Residual Hb

Ghosts prepared in hypotonic media tend to contain Hb in excess of that in the surrounding medium.[22, 24] When hemolysis in small (5 to 15) volumes of a hypotonic medium is followed by restoration of isotonicity, the Hb concentration in the ghost may be from 1.5 to 3 times that of the fluid around it. The mean ghost Hb may be as much as 6 Gm. per cent. This Hb must occupy space, and the space needed is about one-fifth of the original cell volume (about 18 cu. μ) if the Hb concentration is to remain below the limit of 30 Gm. per cent. A volume one-fifth that of the red cell would be contained in a strip about 1600 A thick, lying beneath the cell surface. This still does not tell us whether the residual Hb lies in an 18 cu. μ volume enclosed in a very thin wall, or whether it lies in the meshes of a thicker structure about 1600 Å thick. An explanation is needed, however, as to why it is so difficult to remove the pigment from the ghost. Its concentration can certainly be greatly reduced by sufficient washing, treatment with media at pH 9, etc., but along with this, the ghost volume becomes progressively less.

4. Fragmentation of Ghosts

A good test of whether a body is hollow or solid is to fragment it, and to see whether the sum of the volumes of the fragments is much less than, or essentially the same as, that of the object. If the former, the body is probably hollow; if the latter, it is probably solid.

Ghosts, like red cells, can be fragmented by heating to 50 C., always provided that they contain some residual Hb. The sum of the volumes of the fragments is about 0.8 times the volume of the original ghost, which leads to the conclusion that the ghost is not hollow, i.e., not a very thin wall enclosing fluid.[25] If not hollow, its surface structure must be about 1600 Å thick in order to contain the residual Hb in it, and, considering the electron microscope thicknesses already discussed, the structure must be fairly well hydrated.

5. Variety of Constituents

A variety of lipids, extractable in a variety of solvents, are found in the red cell ghost in nearly the same quantities as they are found in the cell itself.[17] The protein constituents are at least as complex as the lipids. There is no uniformity as yet regarding nomenclature, nor great certainty about identification, but, using one classification, there are at least four proteins in the cell and in the

ghost: Hb, reticulin or stromin, S-protein, and others.[26, 27] The last include a globulin and an albumin (the anti-sphering substance, the existence of which is not as certain as it has been thought to be). Reticulin is a lipoprotein with long protein chains linked by ether-soluble and alcohol-soluble lipids; extraction of its ether-soluble lipids leaves elenin, which, after extraction of the alcohol-soluble lipids, leaves a protein similar to the stromatin of Jorpes.[28] Electrophoretically separated proteins, called a and b, probably correspond to reticulin, elenin, or stromatin, and to S-protein respectively.[29] Reticulin, like stromatin, is a long protein molecule which shows birefringence of flow, and which is thought to have radially arranged lipid chains. Under the electron miscroscope, elenin appears as cylindrical micelles about 1000 Å long and about 125 Å in diameter.

It is likely, in view of what is now known about lipids and proteins, that these substances exist as complexes, rather than as separate layers, in the surface structure of the cell and in the looser structure immediately below it.

6. Lipoprotein-Hb Complexes

We can use a red cell which contains an abnormal Hb with a unique physical property to show that (a) the ghost, and (b) the myelin form derived from the cell surface contain Hb in the form of a complex with lipoprotein.

The ghost obtained by hypotonic hemolysis from the red cells of persons with sickle cell anemia has the usual form, and has the usual content of residual Hb. The molecules of this abnormal Hb have the property of becoming the oriented molecules of a gel when the O_2 tension is reduced. If the reduction is effected with metabisulfite, the typical birefringent filaments of the sickle cell develop even in the ghost, provided that the concentration of residual Hb is not too small.[30]

This observation, which suggests that Hb is very closely associated with the other components of the surface structure, is not quite conclusive, but the following observation is more so. In media equilibrated with air, the red cells of sickle cell anemia, like other red cells, give rise to myelin forms on standing. These myelin forms are flexible. On reducing the O_2 tension, the red cells of sickle cell anemia become sickles, and the myelin forms derived from them become rigid.[30] If the rigidity depends on the presence of the reduced abnormal Hb, this shows that the myelin forms contain Hb, and, since the forms may be only 1000 Å or less in diameter, the Hb in them must be very intimately associated with their lipids and other proteins. The inference that the Hb exists in the form of a complex with the lipoproteins of the cell surface is almost unescapable.

Diagrammatic Representation of Red Cell Structure

The relation between the highly ordered surface ultrastructure and the less ordered, but not yet random, interior can be represented by a diagram (fig. 1).[25] The abscissa shows distances in microns along a cross-section of a quadrant of a red cell, reckoning from the center O. The upper graph shows the concentration of Hb and of lipoprotein, the former on the left ordinate and the latter on the right ordinate. The concentration of lipoprotein is very large at the surface E and very small at the interior I; the concentration of Hb is large at I, and small,

but not zero, near the surface E. Both concentrations are shown falling continuously from their high values to their low ones, the fall being steep in the region of C, situated no more, and probably considerably less, than about 4000 Å from the surface E. This value is chosen because a ghost with walls of this thickness collapsed on each other would occupy about half the volume of the cell; the steep fall of the concentrations referred to would take place somewhere within this thickness. Towards the surface, e.g., at D, the structure is predominantly lipid with a small amount of Hb oriented with respect to the oriented lipoproteins, so that Hb forms part of the surface ultrastructure.

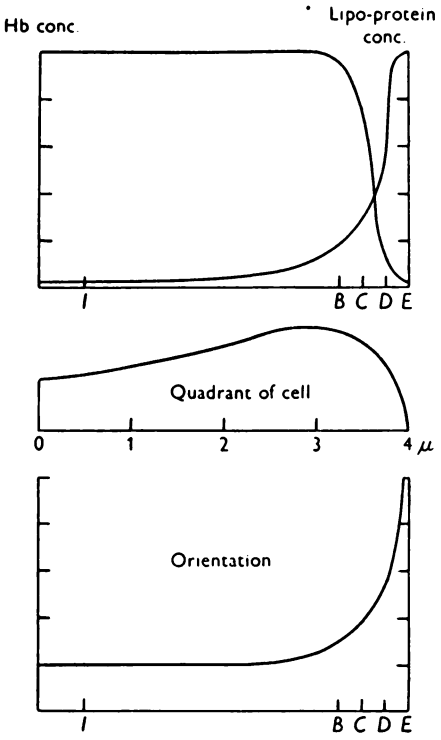

FIG. 1.—A diagrammatic representation of red cell structure. See text for explanation.

Further towards the interior, e.g., at B, the structure is now predominantly Hb, the molecules of which are still oriented, to some degree, with respect to the lipoproteins of the region BCD. In the interior I the only orientation is the short-order orientation of Hb.

The extent of the orientation in different regions is shown diagrammatically in the lower part of the figure. At the surface, the orientation of the molecules is great, and these are principally lipid. There is no objection to thinking of properties such as permeability, electrical resistance, and even shape as being properties of this region. The chemical composition and volume of the ghost, however, and probably its birefringence also, are determined by the properties

of the much thicker region BCDE. The diagram will also convey the idea that different kinds of ghosts can result from different methods of preparation. In forms of lysis which produce the least injury, the Hb at I will leave the cell but the relatively thick Hb-containing portion BCDE will be left as a ghost. Other methods of preparation, particularly if they involve prolonged washing, may cause the structure to break down at C or even at D; the resulting ghosts will be thinner, less voluminous, and will contain less Hb. After many washings, or as the result of the action of certain lytic agents, there may be nothing left except the most highly oriented material at E.

This review would be incomplete if it did not call attention to the relation between the complex ultrastructure and the complex metabolism of the red cell. The cell contains many enzyme systems,[32] some of which are concerned with the active transport of electrolytes[33, 34] and of nonelectrolytes.[35] If these transports are to be described in terms of pumps, directional pumps are required, i.e., pumps which depend for their operation on a structural element. Radioactive tracer studies have shown that the turnover of the red cell phospholipids[36] and cholesterol[37] requires only a few days, in contrast to the almost negligible turnover of Hb and the other proteins of the mature red cell. The relation between metabolism and structure may be such, indeed, that some of the energy of the former is necessary to maintain the orderliness of the latter.

Summario in Interlingua

Es presentate un revista del datos e problemas currentemente acceptate e discutite relative al structura del erythrocyta mammalian. Le resultante imagine monstra un ultrastructura superficial de lipidos e proteinas in configurationes altemente orientate. Al interior se trova un quantitate de hemoglobina con un grado de orientation alique plus determinate que le indeterminismo de un solution. In iste description general le sol aspectos de importantia nondum resolvite concerne le orientation e le grado de hydratation del strato de proteina. Nonobstante, multe problemas additional appare in un discussion plus detaliate. Istos es revidite in gruppos concernente le spissitate del structura superficial, le volumine del stroma, le hemoglobina continite residualmente in le stroma, le fragmentation del stroma, le componentes del stroma, e le complexos lipoproteinohemoglobinic. Le relation inter le orientatissime ultrastructura superficial e le minus orientate interior es representate diagrammaticamente.

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