significant because of the variability between experiments. However, if one considers the proportion of dermatan sulfate to the total sulfated glycosaminoglycans, a pattern characteristic of Hurler's cells became apparent in every experiment. Forty percent of the total sulfated glycosaminoglycans in the Hurler's cells appeared in the fraction containing dermatan sulfate; only 23 percent of the glycosaminoglycans from normal cells appeared in this fraction. This proportional increase in dermatan sulfate in Hurler's fibroblasts would not have become evident without fractionation of the glycosaminoglycans.

We conclude that the biochemical phenotype for Hurler's syndrome, that is, an increased concentration of dermatan sulfate, persists in cells in culture, and its phenotypic expression is reinforced by inclusion of ascorbic acid in the culture medium.

Cultures of normal cells also showed consistent, albeit small, increases in dermatan sulfate concentration when grown in medium supplemented with ascorbic acid. Because a significant quantity of ascorbic acid was present in the fetal calf serum incorporated into the medium, further study is required to determine whether ascorbic acid is necessary for dermatan sulfate synthesis. In studies of whole animals, the concentration of dermatan sulfate has been reported to be lower in the aortas of scorbutic guinea pigs than in those of well-nourished animals, and a decrease in its synthesis has been observed in granulomas produced with polyvinyl sponge in guinea pigs deprived of ascorbic acid (*10*).

Since iduronic acid is a characteristic constituent of both dermatan sulfate and heparan sulfate, the glycosaminoglycans affected in Hurler's syndrome, the basic defect in Hurler's syndrome may be related to the epimerization of glucuronic acid to iduronic acid, and ascorbic acid may participate in this reaction.

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Surface Area of Human Erythrocyte Lipids: Reinvestigation of Experiments on Plasma Membrane

Abstract. *Ratios of the lipid monolayer area to the erythrocyte surface area are 2:1 at low surface pressures and approach 1:1 at collapse pressures. Unsaturated phospholipids in cholesterol-phospholipid complexes of membrane extracts resemble their saturated derivatives at collapse pressures. Area ratio and phospholipid area data are related by an equation that tests hypothetical values for molecular areas used in membrane models.*

The Davson-Danielli concept of the cell membrane as a lipid bilayer covered with protein is a generally accepted working hypothesis for membrane structure (*1*), and it is supported by the classic experiments of Gorter and Grendel (*2*), who extracted lipids from erythrocytes, spread the extract as a monomolecular film, and determined the ratio of film area to erythrocyte area. This ratio would be 2 : 1 for a bimolecular layer of lipids at the erythrocyte surface. Since a 2 : 1 ratio was found with erythrocytes from a number of mammalian species, Gorter and Grendel suggested that sufficient lipid was present for a bimolecular layer. A different structure was later proposed by Dervichian and Macheboeuf (*3*) who performed similar experiments and found 1:1 ratios which indicated a monomolecular layer at the cell surface. This discrepancy is readily explained by the fact that Gorter and Grendel measured the film area at the first detected surface pressure, whereas Dervichian and Macheboeuf assumed that the lipid film at collapse pressure more nearly resembled a membrane structure. These studies are cited in discussions of membrane structure even though they both have serious limitations in experimental design (*4*). Gorter and Grendel used a selective lipid solvent, acetone, to extract erythrocytes and presented little data on lipid recovery. Dervichian and Macheboeuf extracted an arbitrarily defined "loosely bound" lipid fraction with 10 percent ethanol in ether at room temperature. This lipid, a fraction of unknown composition, represented only 70 to 80 percent of the erythrocyte lipid. Furthermore, new surface area data are now available for erythrocytes. Gorter and Grendel, for example, assumed that the surface area for human erythrocytes was 99 μ^2 whereas recent measurements give 145 ± 8 μ^2 as the surface area (*5*).

In our study, we measured the surface pressure–area isotherms for total lipid extracts from human erythrocytes. Total lipid extracts were used because almost all red cell lipids are localized in the cell membrane (*4*). Force-area curves were then used to calculate the ratio of lipid to erythrocyte area in a reexamination of the Gorter-Grendel and Dervichian-Macheboeuf experiments. Average molecular areas for erythrocyte phospholipids at different film pressures were also calculated from lipid composition and force-area data. Experiments yield data on the molecular area which may be used in developing membrane models. We suggest that a molecular area obtained for membrane lipid at the film pressure corresponding to a bimolecular layer is undoubtedly more acceptable for the calculations than molecular areas extrapolated from synthetic lipid mixtures at arbitrary film pressures (*6*).

Experiments were performed with human blood which was characterized by red cell count, hematocrit, and hemoglobin content. The disodium salt of ethylenediaminetetraacetic acid was the anticoagulant. Red cells were centrifuged and washed several times by resuspension in isotonic saline; the buffy layer was removed by suction. Lipids were extracted from a suspension of known cell count by adding the suspension to six volumes of a mixture of isopropyl alcohol and chloroform

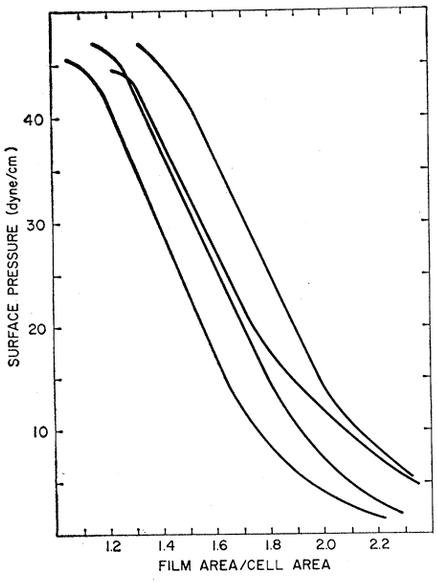

Fig. 1. Ratio of film area to erythrocyte area at increasing surface pressures.

(11 : 7, by volume) with stirring. The residue was extracted first with chloroform and then with methanol. Extracts were combined, washed with 0.01*M* KCl, and then evaporated. Lipid was dissolved in purified hexane and analyzed for phosphorus (*7*) and total cholesterol (*8*).

In surface experiments, the hexane solution was spread at the air-water interface in a teflon Langmuir trough. Water for the trough was distilled from glass into polyethylene containers. Surface pressure was measured at 26°C by the Wilhelmy plate method (*9*).

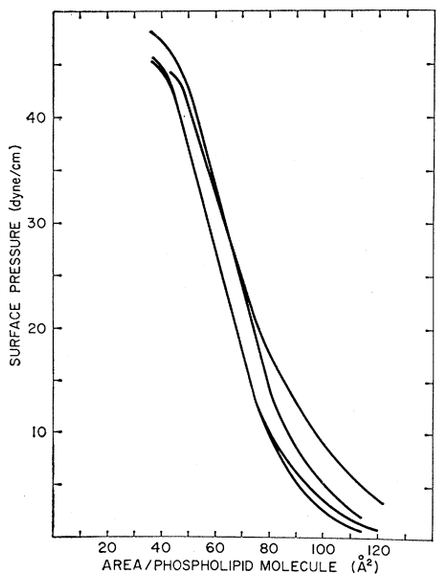

Fig. 2. Molecular areas for erythrocyte phospholipid in the cholesterol-phospholipid complex at increasing surface pressures.

Lipid extracts from five different blood samples contained 1.26 ± 0.05 × 10^{-11} mg of phosphorus per cell and 1.39 ± 0.07 × 10^{-10} mg of cholesterol per cell. These data, reported as mean ± standard deviation, correspond to higher values in a series of data on lipid recovery summarized from a number of studies (*10*). The mean cholesterol : phospholipid mole ratio for the extracts was 0.89.

Force-area curves of four lipid extracts are presented in Fig. 1, in which film areas are replaced by the ratio of film area to erythrocyte area. Erythrocyte areas used for these calculations were estimated from the cell counts and mean area per cell (*5*) of the washed suspensions extracted for lipid. The curves demonstrate that a Gorter-Grendel bilayer is possible at low surface pressures. It is apparent that the small surface area of red cells used in the original Gorter-Grendel calculations was compensated by incomplete lipid extraction. A Dervichian-Macheboeuf monolayer is approached at the collapse pressure; however, the film collapses before the area is condensed to the dimensions of a monolayer. Complete lipid extraction probably accounts for the higher ratios, 1.2 to 1.4, obtained in our study at the collapse pressure.

Force-area data may be expressed in several ways. In Fig. 2, film areas (A_F) are replaced by phospholipid molecular areas (A_{PL}) which are obtained from the equation:

$$A_{PL} = (A_F - A_C \times M_C)/M_{PL}$$

The number of cholesterol and phospholipid molecules in the film, M_C and M_{PL} respectively, were estimated from the composition of the lipid extract. Since cholesterol shows little compression over a wide range of surface pressures, 38 Å² was accepted as the constant molecular area for cholesterol (*11*). Other lipids which account for approximately 5 percent of the total extract (*10*) were not included in the calculations.

Naturally occurring phospholipids are highly unsaturated and form expanded films (*4*, *11*). Egg lecithin, for example, has a cross-sectional area near 64 Å² at the collapse pressure (*12*). Hydrogenated phospholipids more nearly resemble saturated fatty acids with areas between 41 and 48 Å² per molecule or 20.5 to 24 Å² per chain at their collapse pressures. Mixtures of cholesterol and unsaturated phospholipids exhibit a well-known condensing

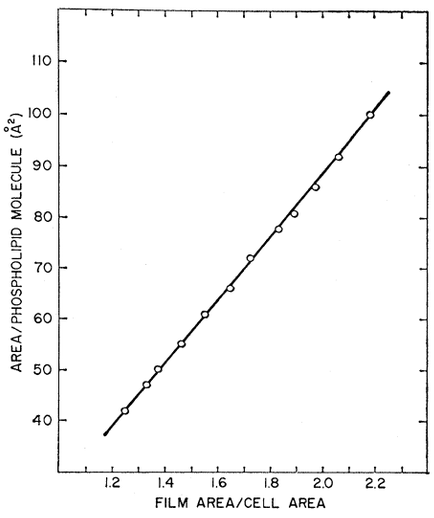

Fig. 3. Relationship between the ratio of film area to erythrocyte area and the molecular area of erythrocyte phospholipid.

effect (*4*, *11*). Erythrocyte lipids represent such a mixture, and Fig. 2 shows that the phospholipids have cross-sectional areas of 40 to 46 Å² at the collapse pressure; at this pressure they resemble their saturated derivatives. Complexes between cholesterol and membrane phospholipids are evidently capable of tightly packed structures which may compensate for fatty acid unsaturation. Thus fatty acid composition would have little effect on membrane structure if the membranes were under high surface pressures as suggested by Dervichian and Macheboeuf (*3*). If hypotheses relating membrane permeability and structure to fatty acid composition are correct (*4*, *10*), the membranes must exist under relatively low surface pressures where the lipid area is easily sufficient to form a bilayer (Fig. 1).

Experimental results are difficult to extrapolate directly from the graphs described above. However, a linear relationship exists between the ratio of film area to cell area (Fig. 1) and phospholipid molecular areas (Fig. 2). A straight line is produced when mean ratios (R) calculated at a number of different film pressures are plotted against mean areas (A_{PL}) calculated at the same film pressures (Fig. 3). This relationship which is described by the equation

$$A_{PL} = 61\,R - 34.5$$

facilitates the correlation of ratio and molecular area data. If a bilayer model for the erythrocyte membrane is assumed and it is assumed further that

membrane lipids pack in the same manner as lipids in a surface film, this equation shows that the model is satisfied by 87.5 Å^2 as the cross-sectional area for phospholipid and, therefore, 125.5 Å^2 as the unit area for the cholesterol-phospholipid complex. The data suggest that the lipid film most nearly simulates conditions in the bilayer model at a surface pressure of approximately 9 dyne/cm.

Data used by a number of investigators to construct membrane and bilayer models may be evaluated by the area-ratio equation. For example, Maddy and Malcolm (*6*) recently assumed that each phospholipid molecule in a plasma membrane bilayer occupied 70 Å^2, a value obtained from the x-ray diffraction pattern of a human brain phospholipid-water system. This area is considerably less than the area required for a bilayer and indeed yields an R of 1.7. Vandenheuvel (*6*) has described a bilayer model for myelin in which phospholipid-cholesterol complexes occupy 100 Å^2, which was extrapolated from the surface area occupied by an egg lecithin–cholesterol complex at an arbitrary film pressure of 5 dyne/cm. The A_{PL}, 62 Å^2, of the Vandenheuvel model gives an R value of 1.6 in place of the predicted bilayer. Mueller *et al.* (*6*) used Gorter-Grendel and Dervichian-Macheboeuf data to calculate the average area, 25 Å^2, occupied by both cholesterol and fatty-acid chains in a bilayer. They suggested that this area may be required for bilayer stability; however, the A_{PL} is 50 Å^2 and the R is only 1.4 in their model.

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Effect of Sugars on Transport of Alanine in Intestine

Abstract. *The effect of glucose and galactose on transport of alanine by rabbit ileum has been investigated. Transmural transport and cellular accumulation of the amino acid were inhibited by the sugars, but alanine influx across the mucosal border of the cells was unaltered.*

Recent evidence (*1–4*) has indicated that D-galactose partially inhibits the transport of neutral amino acids across the wall of the small intestine and decreases the ability of the tissue to concentrate amino acids. Suggestions made regarding the mode of action of this sugar include competition for available energy (*1*), formation of the toxic metabolite galactose-1-PO_4 (*2*), and direct action on specific binding sites of a polyfunctional carrier at the brush border (*3*). However, the precise mechanism involved is unclear, and information regarding the effects of galactose on amino acid fluxes across mucosal and serosal borders of the epithelial cell is necessary in order to understand changes in transmural flux and cellular accumulation. Our experiments were designed to examine the influence of D-galactose on the influx of L-alanine from the solution bathing the mucosa into the cells.

All experiments were performed on distal ileum from male or female white rabbits maintained on a normal diet and killed by the intravenous injection of pentobarbital. The bathing solution used was a modified Krebs-Ringer-bicarbonate solution containing 140 mM sodium and 12 mM potassium (*5*). Experiments were performed at 37°C, and the solutions were constantly bubbled with a mixture of oxygen (95 percent) and carbon dioxide (5 percent). Radioactive samples were assayed in a liquid scintillation spectrometer. Transmural flux of alanine across the intestine from mucosal to serosal bathing solution was measured by the use of the chamber and methods previously described (*6*). In each experiment, two adjacent pieces of tissue were used. One piece served as a control, and the mucosal bathing solution of the other tissue contained 20 mM galactose or glucose. The tissue was bathed on both sides with Ringer solution containing 5 mM alanine. Alanine labeled with C^{14} was added to the mucosal solution and its rate of appearance in the serosal solution was determined. Influx of alanine across the brush border from mucosal solution into the cells was measured with the technique described by Fuisz *et al.* (*7*). The mucosal surface was exposed for 60 seconds to Ringer solution containing C^{14}-alanine and H^3-inulin, with or without 20 mM galactose or glucose. Influx of alanine was calculated from the uptake of C^{14} by the tissue after correction for the volume of adherent medium as determined by H^3-inulin. The effect of galactose on cellular accumulation of alanine was determined on strips of mucosa removed from the intestine and incubated in Ringer solution containing C^{14}-labeled alanine. Extracellular space was estimated in each tissue by the use of H^3-inulin. The methods for tissue preparation and the calculation of the intracellular concentration of alanine have been described (*5*).

The effects of glucose and galactose on the unidirectional transmural flux of alanine from mucosa to serosa is shown in Table 1. In each experiment, the steady state flux in the presence of sugar was lower than the control flux obtained in contiguous tissue from the same animal. On the average, the unidirectional flux of alanine was inhibited by 53 percent in the presence of 20 mM glucose and by 34 percent in the presence of 20 mM galactose. The influence of galactose on the cellular accumulation of alanine is shown in Table 2 in terms of the ratio of cellular to medium concentrations of alanine observed after a 30-minute incubation. Previous experiments (*5*) have demonstrated that this incubation time is sufficient to obtain maximum intracellular concentrations. Galactose (20 mM) caused a 32-percent reduction in the ratio of cell to medium concentration of alanine compared with the control tissue. Thus, in relative terms, the effects of galactose on the transmural flux and the steady state intracellular accumulation of alanine are quite similar. Phlorizin ($10^{-4}M$), which inhibits