Table 1. The percentage conversion of three leucoanthocyanin preparations into cyanidin on heating with a solution of *n*-butanol in HCl.

| Temperature (°C) | Number of minutes | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 30 | 60 |
| | *Grape phlobatannin* | | | | | |
| 50 | 0.10 | 0.56 | 0.42 | 0.56 | 0.63 | 1.15 |
| 60 | 0.90 | 0.90 | | 1.90 | | 2.84 |
| 70 | 0.94 | 1.70 | 2.07 | 2.59 | 3.59 | 5.27 |
| 80 | 2.09 | 3.45 | 4.48 | 4.96 | 5.93 | 7.32 |
| 90 | 4.23 | 6.05 | 7.09 | 7.80 | 8.70 | 9.92 |
| | *Cacao* | | | | | |
| 70 | 2.00 | 2.92 | 3.87 | | 6.04 | 8.24 |
| 90 | 4.79 | 5.85* | | | | |
| | *Melacacidin* | | | | | |
| 50 | 0.25 | 0.46 | 0.61 | 0.82 | 1.14 | 1.32 |
| 80 | 0.39 | 1.08 | 1.90 | 2.33 | 3.24 | 5.84 |
| 90 | 1.83 | 2.89 | 3.88 | 4.50 | 5.63 | 7.04 |

* After 8 minutes.

Table 2. First order reaction rate constants ($K$) of conversion of three leucoanthocyanin preparations into cyanidin after heating with a solution of *n*-butanol in HCl.

| $K \times 10^4$ | | | | | E |
|---|---|---|---|---|---|
| 50°C | 60°C | 70°C | 80°C | 90°C | Cal |
| | | *Grape leucoanthocyanin* | | | |
| 5.74 | 6.30 | 16.0 | 29.5 | 84.0 | 21,000 |
| | | *Cocao leucoanthocyanin* | | | |
| | | 17.1 | | 35.1 | 23,000 |
| | | *Melacacidin leucoanthocyanin* | | | |
| | | | 9.83 | 21.4 | 12,000 |

tinidin was reported (*16*) to be about 24 percent from the monomeric leucofisetinidin and to decrease to about 7 percent for the trimeric tannin and to 5 percent for pentameric or decameric tannins. Pigman *et al.* (*10*), on the basis of qualitative observations, indicated that the reaction occurred in stages but did not specify them.

To obtain additional information on the mechanism of the conversion, the rate of conversion of several leucoanthocyanin preparations in a solution of *n*-butanol in HCl at several temperatures was determined. The reaction was followed by heating 5 ml of ethanol solution of leucoanthocyanin containing 5 to 50 mg of leucoanthocyanin preparation (*17*) with 50 ml of *n*-butanol containing 5 percent by volume of concentrated hydrochloric acid at various temperatures. Portions were removed and cooled in an ice bath and their absorbance was determined at 550 mμ in a 1-cm quartz cell in a Beckmann Model DU spectrophotometer. The concentration of cyanidin produced was obtained from the absorbance of a pure preparation of cyanidin in *n*-butanol-HCl in the range of 0 to 10 μg/ml. The concentration of leucoanthocyanin preparation usually was 0.091 mg/ml and the hydrochloric acid was approximately 0.66$N$.

The conversion into cyanidin varied with the heating conditions from 0.10 percent to almost 10 percent (Table 1). Assuming that the equivalent weight of the leucoanthocyanin equaled the molecular weight of cyanidin, we found that when the logarithm of the concentration of unconverted leucoanthocyanin was plotted against time, the graph was linear for the first 10 minutes at higher temperatures (above 60°C) and for the first 60 minutes at lower temperatures (60°C and below). The first-order specific reaction rate constants calculated from these graphs are shown in Table 2. The Arrhenius constant corresponding to these rate constants, calculated from the slope of the curve of log $K$ versus $1/T$ varies from 23,000 calories for cacao leucoanthocyanin to 12,000 for melacacidin. A preparation of leucocyanidin obtained by reducing taxifolin with borohydride in ethanol according to the procedure of Brown (*18*) yielded a compound which formed cyanidin in the cold on addition to acidified butanol. These observations indicate that the energy required for the rate-limiting step is smaller the lower the degree of polymerization of the compound tested.

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## Phospholipid-Sugar Complexes in Relation to Cell Membrane Monosaccharide Transport

Abstract. *Phospholipids extracted from "ghosts" of human erythrocytes or from other sources carry substantial quantities of glucose or other monosaccharides from the dry state into highly nonpolar solvents. Various characteristics of this weak association phenomenon show suggestive parallels with known properties of the mediated sugar-transfer system in the membrane of the intact red cell.*

Much evidence has accumulated in support of the thesis that the penetration of simple sugars into various types of cells in the vertebrate body involves a transient physicochemical association of the translocated sugar molecules with some special component of the barrier (presumably the plasma membrane) at the cell surface. We have therefore tried to extract such components from the stromata of human red blood cells, in the hope of duplicating in an inanimate system some of the sugar-transport properties which have been defined for these cells. The molecules bearing the apparent reactive sites have been pictured as acting either as carriers traversing the membrane in combination with the sugar (*1*, *2*), as relayers transferring the sugar along a path of relatively fixed adjacent sites (*3*), or as modifiers rendering the sugar capable of penetrating the membrane on its own, as by inducing the formation of less hydrophilic dimers (*4*). In all of these concepts a specialized monosaccharide-accepting site of considerable sterospecificity, sensitive to a variety of defined pharmacological agents, is presumed.

Since the critical structural component of the membrane is generally considered to be the lipid layer, and since in recent years attention has been particularly directed to phospholipids as possible carrier-like participants in cation transport through cell membranes (*5*), we applied lipid solvents to the

erythrocytes or to their "ghosts" in the presence of $C^{14}$-labeled glucose, seeking to extract a complex of the carbohydrate with the membrane lipids. Such apparent complex formation was readily observed in any conventional "total lipid extract." A variety of types of fat solvent (alkanes, diethyl ether, benzene, chloroform, and so forth) can be the final vehicle for this material, but the original extraction seems to require methanol, ethanol, or other such agent presumably acting to split lipids from lipoproteins or other complex form. For nearly all of our preparations, ghosts were extracted with a mixture of hot ethanol and diethyl ether (3 : 1 by volume), the filtrate was dried under vacuum at about 50°C, and the hexane-soluble fraction of the residue was studied (Table 1). However, prolonged vacuum-oven drying, up to 95°C, did not disturb the subsequent behavior.

Because of the fairly limited number of operational sites of glucose transport in this system (*6*), we began by using the radioactive glucose at the full specific activity. However, it soon became evident that such high activity was unnecessary, since the preparations could be loaded with glucose up to approximately $5 \times 10^8$ molecules per ghost or per cell extracted (section 3 of Table 1). Preliminary contact with the cells or ghosts in a physiological medium is not required to bring about this appearance of glucose in the hexane filtrates. The apparent formation of complex occurred equally readily if the glucose was added to the crude ethanolic extract, to its filtrate, or even to an alcoholic solution of the final material dried from the hexane filtrate. Thus there is no direct evidence of the existence of the complex prior to the drying of the materials together.

Various other monosaccharides were tested in this system (including L-glucose, D-mannose, D-ribose, D-lyxose, and both enantiomorphs of xylose, galactose, and arabinose) and all behaved essentially like glucose; in contrast, the hexahydric alcohol, D-mannitol, remained almost totally insoluble in hexane under similar treatment.

The hexane-trapped radioactivity appears to represent chemically unmodified glucose held in a weak association. The free sugar is readily recovered upon access to water; thus essentially all of the label is removed if water, instead of hexane, is applied to a dried extract, and it then behaves chromatographically and enzymically like free glucose, retaining no residual lipophilic character. If however a labeled preparation in hexane is shaken with water, only a fraction of the activity readily moves into the aqueous phase, so that after an initial rapid migration in the first few minutes, continued shaking produces very little further change in the distribution. That this represents a simple equilibrium partition or dissociation is further suggested by the fact that each successive replacement of the aqueous phase with fresh water leads to a new rapid migration comparable to the first. This simple interpretation is negated, however, by the observation that the dissociation is completely independent of the glucose concentration in the aqueous phase, and by the fact that no measurable movement of labeled sugar has ever been detected in the other direction (from water into hexane), regardless of the initial load of the complex in the hexane phase (*7*). The basis of this somewhat paradoxical behavior has not yet been determined.

Table 1. Carrying of glucose into hexane from dried lipid mixtures. Mixtures containing biological materials (sections 1 and 3) were extracted by the procedure described; commercial lipids (section 2) were dissolved in the same mixture of ether and alcohol, glucose was added in small volume of $H_2O$, and the mixture was handled as were the biological extracts. In each mixture, glucose included 0.7 to 2.7 $\mu$c of $C^{14}$.

| Contents of mixture | | Fraction of tracer recovered in hexane |
|---|---|---|
| Glucose (mg) | Other | |
| | *Section 1* | |
| 1.18 | $1.24 \times 10^{11}$ red cells | 0.271 |
| 1.18 | $1.24 \times 10^{11}$ "ghosts" | .351 |
| 1.18 | Medium only | .0001 |
| | *Section 2* | |
| 0.81 | 500 mg "purified lecithin" | 0.992 |
| 0.81 | 500 mg "cephalin" | .965 |
| 0.81 | 500 mg triacetin | .0008 |
| 0.81 | 500 mg olive oil | .007 |
| | *Section 3* | |
| 0.31 | $6.8 \times 10^{10}$ "ghosts" | 0.205 |
| 50.6 | $6.8 \times 10^{10}$ "ghosts" | .113 |
| 98.3 | $6.8 \times 10^{10}$ "ghosts" | .083 |
| 201 | $6.8 \times 10^{10}$ "ghosts" | 0.39 |
| 300 | $6.8 \times 70^{10}$ "ghosts" | .031 |

Chemical and physicochemical analyses showed these preparations to be largely phospholipid and implicated this as the critical ingredient underlying the formation of sugar complexes. However, no peculiarly differentiated phosphatide is suggested: the same properties are shown by any of the ordinary commercial preparations of lecithins or cephalins from various sources (section 2 of Table 1), though not by soaps or neutral lipids. Various paper chromatographic fractionations did not prove helpful as a means of identifying any distinctively active components. Most developing mixtures are sufficiently aqueous that dissociation of the complex occurs; the label behaves as the free sugar, and upon physical separation from the phospholipid becomes no longer elutable from the paper into the nonpolar solvents. Even when a highly nonpolar solvent like hexane was used as the chromatographic developer, so that a substantial portion of the sugar was at first drawn along with the migrating phospholipid near the solvent front, there was progressive dissociation as development proceeded and a continuous streak of hexane-insoluble free glucose was left behind. Since such dissociation did not appear to occur to an appreciable extent on acetylated paper or on glass fiber sheets, we turned to the silicic acid thin-layer chromatography systems designed to separate the several classes of phosphatide (*8*). The only major components of the activity were associated with the spots corresponding to the lecithins and lysolecithins, no appreciable radioactivity accompanying the phosphatidyl ethanolamines, neutral lipids, and other ingredients of the extract. (However, the higher $R_F$'s of the phosphatides not containing choline in these systems may well be responsible for this apparent restriction of complex formation to the lecithins.)

Table 2. Inactivation by DNFB of glucose uptake of red cells and complex-formation of membrane lipid and glucose. Washed human erythrocytes were incubated at 37.5°C, hematocrit value 0.32, with or without inhibitor, then diluted at the intervals indicated with plain medium, and immediately washed several times by centrifugation. Portions were then incubated at 0.32 hematocrit with 2 percent glucose, and the cell uptake at 30 minutes was chemically measured (*2*), while ghosts prepared from the remainders were extracted as in Table 1, with tracer amounts of $C^{14}$-glucose.

| Incubation | | Fraction of glucose taken into cells† | Fraction of $C^{14}$ taken into hexane |
|---|---|---|---|
| DNFB* (*M*) | Minutes | | |
| 0 | 90 | 0.219 | 0.698 |
| $1.7 \times 10^{-2}$ | 30 | .059 | .454 |
| $1.7 \times 10^{-2}$ | 60 | .051 | .252 |
| $1.7 \times 10^{-2}$ | 120 | .025 | .274‡ |

* Expressed as if distributed throughout volume of suspension. † Calculated to reach the value of 0.236 upon complete equilibration. ‡ Small diminution of effect consistently seen with prolonged treatment or elevated [DNFB].

Though little attention appears to have been given to this kind of phe-

nomenon in recent years (*9*), the ability of phospholipids to combine with monosaccharides to form lipid-soluble complexes was well recognized in the late 19th century, when considerable debate raged (*10*) as to whether such "jecorins" were true compounds preexisting in various tissues or were physical aggregations assembled by the extraction procedures. For the formation of the complexes studied here, drying at 50° to 60°C from mixtures containing ethanol or higher alcohols appeared to be the most effective procedure, but neither heat nor alcohol is absolutely essential to the phenomenon. In fact, dry sugars will dissolve directly into highly nonpolar solvents containing phospholipids already in solution. In our experimental system, the solubilization of glucose into a hexane vehicle proceeded very slowly at room temperatures even with rather rapid agitation, so that, with an excess of available sugar, a nearly steady rate of its appearance in the filtered solution was maintained for many hours. This rate was approximately proportional to the total amount of the dissolved phospholipid (rather than to its concentration), and the final quantity of sugar that went into solution at the steady state (requiring about 4 hours at 50° to 60°C) closely approached a 1 : 1 molecular ratio to the phospholipid present (as given by analysis for total P). With the ethanol-drying procedure, however, at least twice this amount of sugar was often carried into hexane by various phospholipids.

Doubt regarding the relevance of these observations to the red cell's sugar transport system naturally arises from the facts that (i) the extent of this sugar-complexing potentiality in the material extractable from the membrane vastly exceeds the density of the functional transport site, and (ii) the phospholipids have failed, in our experimental systems, to move perceptible amounts of sugar out of an aqueous phase into a nonaqueous phase. However, several properties of the intact cell transport system are paralleled in the phenomenon of lipid-sugar complex formation. Thus, during incubation of the erythrocytes with the "protein reagent," 1-fluoro-2,4-dinitrobenzene (DNFB), the glucose-transport capacity of the cells progressively and irreversibly deteriorates (*11*), so that after varying exposure to the agent, washing, and resuspension in ordinary medium, cells of varying residual transport capacity are obtainable. Concomitantly, there is a marked diminution in the degree to which the phospholipids extracted from the ghosts of these cells will form the hexane-soluble complex with glucose (Table 2), although the P content of the extracts is not appreciably altered. Also stilbestrol, which blocks the sugar-transport system of the intact cell in a fully reversible manner (*12*), appears to displace an equimolecular quantity of glucose from the lipid complex when it is presented during drying of the mixtures from ethanolic solution. Finally, there are small differences among the common monosaccharides with respect to the extent of their recoverability in hexane after drying with given proportions of phospholipid, which parallel substantially their larger differences with respect to affinity for the transport system in the red cells (*13*). However, this relation breaks down entirely in application to mirror-image specificity: while the transport system notably distinguishes between sugar enantiomorphic pairs, this type of specificity seems to be totally lacking in the phenomena of complex formation with extracted phospholipids.

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## Detection and Quantitation of Fallout Particles in a Human Lung

Abstract. *Portions of an adult human lung were studied by autoradiography in order to detect the presence of fallout particles. The radioactivity in the remainder of the tissue was determined with a gamma-ray spectrometer. Four particles were found and their activities were determined. From the measurement for total-fission-product activity in the lung tissue it was calculated that there were approximately 264 particles in the right lung at the time of death.*

Radioactive particles formed during the atmospheric testing of nuclear weapons have become dispersed as worldwide fallout. The presence of these particles in the body is a potentially serious source of radiation exposure in the human population. We have found nothing in the literature to show that anyone has analyzed tissue for the presence of these discrete particles in order to assess the hazard they present. However, two authors have reported the detection of fission products in the lung. In 1959, $Zr^{95}$ and $Nb^{95}$ were quantitatively measured, and $Ru^{103}$ was detected in ashed human lung tissue (*1*). In April 1962, $Cs^{137}$ and $Zr^{95}$ were measured in human lungs by external counting (*2*).

In the summer of 1962 we began work at the University of Michigan's School of Public Health to determine if it was feasible to detect individual radioactive particles in human lung tissue by autoradiographic techniques. After preliminary work had indicated that this was possible, an entire right human lung was obtained for counting and auto-