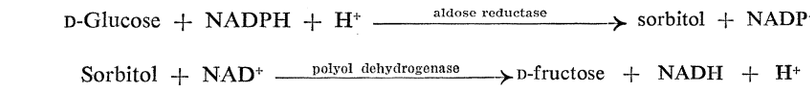

Fig. 2. Conversion of glucose to fructose via sorbitol in lens and seminal vesicle.

of marked accumulation of the products of this pathway in diabetes, has some major implications regarding the metabolism of glucose in diabetic tissues and possibly in the development of neuropathy.

Since cell membranes appear to be relatively impermeable to sorbitol and fructose, the accumulation of these substances may be due to overproduction from glucose and presumably inefficient utilization. Stewart *et al.* (*4*) claim that fructose is converted to lactate very slowly in normal sciatic nerve, and Field and Adams (*8*) showed that diabetic nerve contains no detectable glycogen. Therefore, it is probable that nerve, as opposed to liver or skeletal muscle (*9*), contains little or no specific fructokinase for initiating further glycolytic breakdown of fructose or ultimate storage as glycogen.

The additional finding of a large accumulation of intracellular glucose in diabetic nerve and cord, coupled with absent glycogen and a marked decrease in fatty acid synthesis (*10*), suggests a defect in the activation of glucose in glycolytic, oxidative, and glycogenic glucose utilization in diabetes. This would be analogous to the deficiency of specific glucokinase in diabetic liver as demonstrated by Sols *et al.* (*11*). Such a defect would then favor increased disposition of the accumulated glucose via the sorbitol pathway. Concomitantly, facilitation of glucose entry into the cell in the diabetic state is also possible. The findings of marked accumulation of glucose and the products of glucose metabolism by the sorbitol pathway is in keeping with this view.

Another possible consequence of the accumulation of sorbitol and fructose and their inability to escape from the intracellular compartment may be a harmful osmotic effect, for Kinoshita (*2*) showed that in galactosemic animals a similar process leading to accumulation of dulcitol and fructose caused water accumulation and Na^+ and K^+ alterations in the early cataractous lens. Greater amounts of cell energy are used for maintenance of osmotic equilibrium and Na^+–K^+ content. Whether a similar mechanism plays a role in some forms of diabetic neuropathy has not yet been determined.

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Plasma Membranes: Phospholipid and Sterol Content

Abstract. *The molar ratios of sterol to phospholipid in plasma membranes of five different types of rat cells range from 0.24 to 1.32. The composition of the plasma membrane of a cell has no fixed relation to that of the mitochondria. Thus the structure of cellular membranes shows both tissue and functional specificity.*

Fundamental questions raised as a result of electron microscopic studies of tissues are those of the origin of cell membranes and of the relation between structures within a single cell and between similar structures in different types of cell. Robertson (*1*) proposed that all cell membranes have a common basic structure and possibly a common origin, whereas Sjöstrand (*2*) has emphasized morphological differences. Many workers have found that nerve myelin and erythrocyte membranes have a relatively high sterol content, and more recently a similar finding was reported for intestinal microvilli (*3*). In that these all consist essentially of cell plasma membranes, we have examined a number of such membrane preparations from one species for sterol content. The sterol content is of interest because myelin is often taken as a general model for plasma membranes. We have also made comparisons with mitochondria and with liver microsomal membranes.

The following preparations were all obtained from the rat: erythrocyte ghosts (*4*), brain myelin (*5*), intestinal microvilli (*6*), muscle-cell membranes (*7*), and liver-cell plasma membranes (*8*). Mitochondria from liver, intestinal mucosa, muscle, and brain were also examined; the brain mitochondria were purified (*5*) to free them from myelin. In addition, we applied the method of Wallach and Ullrey (*9*), for obtaining Ehrlich ascites cell plasma membranes, to liver. As with the ascites cells, three suspended subfractions (numbered in order of increasing density) as well as a sediment were obtained from the crude microsomal pellet.

To ensure complete lipid extraction in the presence of sucrose, the method of Hanson and Olley (*10*) was used. Individual classes of lipids were separated by silicic acid chromatography (*11*) followed by determination of sterol (*12*) and phospholipid (*13*). Protein was estimated from the nitrogen content (micro-Kjeldahl), and the values were checked in several cases by use of the Folin-Ciocalteu reagent.

From the results (Table 1) only one finding is common to all membrane preparations—the small percentage of sterol present in the esterified form. The proportion of protein to phospholipid varies ninefold among the different plasma membranes. The largest proportion of protein was found in the intestinal microvilli, which are presumably the most active of the membranes and need more specialized proteins such as enzymes and carriers, and the smallest proportion of protein was found in myelin which presumably has lost all of its associated specialized proteins and consists only of the bare

lipoprotein framework. These results agree with those of electron microscopy of the two tissues (*14*).

The ratios of sterol to phospholipid for myelin, erythrocyte ghosts, intestinal microvilli, muscle-cell membranes, liver mitochrondria, and muscle mitochondria agree with those already found. The ratio for intestinal mitochondria is higher than that found by Millington and Finean (*3*) but is almost identical with that of guinea pig intestinal mitochondria (*15*). Millington and Finean suggested that the ratio for microvilli should be closer to that of myelin but was affected by the presence of contaminating mitochondria. Our results do not support this view. Reports on mitochondria from rat (*16*) and guinea pig brain tissue (*17*) give much lower ratios than that in Table 1. On the other hand, the figure given by Løvtrup and Svennerholm (*16*) for liver mitochondria is also very much lower than that generally accepted. The discrepancy between our results and those of Eichberg *et al.* (*17*) is unlikely to be due to species differences. Contamination of our mitochondria with myelin seems the obvious explanation, but the ratios of phospholipid to protein for mitochondria in the two studies were the same, and the methods used to remove myelin were basically similar.

The liver plasma membranes contain more lipid than reported (*8*) but resemble the saline-extracted preparations. We used larger volumes of medium in the washing stages and this could have resulted in loss of some protein. We found a smaller but very consistent ratio of sterol to phospholipid and a much lower content of esterified sterol. The latter seems more in keeping with the other membrane analyses. Liver microsomal subfraction I corresponds to the plasma membrane fraction of ascites cells. It resembles the plasma membrane preparation of Emmelot *et al.* (*8*) in its sterol-phospholipid ratio whereas the other subfractions have the lipid composition of the total microsomal pellet and closely resemble the mitochondria. Subfraction I has slightly less protein than the preparations made according to Emmelot *et al.* and significantly more esterified sterol. This higher content of sterol esters (although small in terms of quantity) is specific to this one microsomal subfraction. From studies of chylomicron and fatty acid binding and of triglyceride hydrolysis (*18*), it appears certain that subfraction I is derived from the plasma membrane of the hepatic cell, although presumably in the form of vesicles rather than flat sheets (*8*). The nature of the other two microsomal subfractions is unknown. From their densities, we conclude that they are derived from smooth endoplasmic reticulum. They are similar in lipid composition although one has much more lipid per unit weight of protein than the other.

Table 1. Composition of rat cell membranes. Results are given as means ± S.E.M. Figures in parentheses refer to the number of experiments.

Membrane preparation	Phospholipid (mg/100 mg protein)	Sterol		
		Total (mg/100 mg phospholipid)	Esterified (% of total sterol)	Moles (per mole phospholipid*)
Erythrocyte ghosts	16.4 ± 0.3(5)	45.1 ± 0.6(6)	<0.5	0.89
Brain myelin	91.6 ± 4.5(4)	67.1 ± 4.3(4)	0	1.32
Intestinal microvilli	10.9 ± 2.5(5)	23.3 ± 2.1(6)	<1.0	0.46
Liver plasma membranes	41.9 ± 1.7(6)	13.4 ± 0.8(6)	4.1 ± 0.2(5)	.26
Muscle sarcolemma	27.8 ± 4.6(7)	12.2 ± 2.3(7)	0	.24
Brain mitochondria	45.8(2)	25.8(2)	<0.1	.51
Intestinal mitochondria	14.3(2)	30.2(2)	5.0(2)	.60
Liver mitochondria	28.2(2)	5.8 ± 0.4(4)	6.6 ± 0.9(4)	.11
Muscle mitochondria	11 (1)	7.6 ± 1.1(4)	0	.15
Liver microsomal subfraction I	30.1 ± 0.7(4)	13.7 ± 1.1(4)	13.2 ± 1.9(4)	.27
Liver microsomal subfraction II	79.6 ± 5.8(6)	6.4 ± 0.3(7)	3.8 ± 0.6(6)	.13
Liver microsomal subfraction III	35.1 ± 2.0(5)	5.5 ± 0.3(6)	7.2 ± 1.0(6)	.11

* Molecular weight is taken as 760.

The results show clearly that there are no characteristic proportions of sterol and phospholipid in the plasma membranes of rat cells. In the five preparations studied, the composition varied from 4 moles of sterol per 3 moles of phospholipid to 1 mole per 4 moles. Nor is there any fixed relation between the composition of the plasma membranes and the mitochondria of a given tissue; although if the former have a high sterol content, the level in the latter is also raised. The results agree with the proposal, made on morphological grounds, that mitochondrial and plasma membranes should be classified in different groups (*14, 19*). The findings on microsomal subfractions also support the large amount of evidence that in a given cell the basic composition of mitochondria and rough and smooth endoplasmic reticulum is the same (*15, 20, 21*). Our results do not, however, support the idea of a common repeating chemical unit in all membranes of a cell (*21*) since the plasma membrane seems so different from the major intracellular membranes. It appears that there is both tissue specificity and functional specificity in the composition and organization of cellular membranes. Hence, structures deduced for plasma membranes based on analysis of an example from only one tissue cannot be applied generally.

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