In approximate values, the following K/Ca ratios can be given as an illustration:

Nitella	15	*Ricinus* sprout	3
Yucca phloem exudate	120	*Ricinus* phloem exudate	400
Hevea latex	200		

This low calcium content, especially in the sieve tubes, has been investigated more closely. A high phosphate concentration and high pH have already been suggested as restricting calcium solubility. Recently VAN GOOR[13], at our Institute, has obtained evidence that the phloem exudate will hardly accept even very small additions of calcium. Very soon after the first minute additions, turbidity occurs, indicating precipitation. Thus the low soluble calcium content is near its ultimate limits. The governing factors, besides the pH of ca. 7.5, are thought to be high content in (organic) phosphates and the numerous organic acids.

Thus we come to consider the living transport system of the plant as able to contain only small concentrations of soluble calcium. This is the result of the necessity to transport important components to supply the growth centres. Relevant factors in this respect are the metabolically important phosphates and the large amount of organic anions. These organic anions – originating from nitrate reduction – and mainly coupled to the important potassium ion[14], could explain the high pH.

Résumé. La basse teneur en calcium et la haute teneur en potasse du latex et du suc de phloème sont en relation avec une faible viscosité, condition nécessaire à une certaine fluidité. Il semble que la concentration du calcium dans le cytosol des cellules normales est également très basse, comme on peut s'y attendre quand il s'agit d'un «symplasma». La teneur très basse en calcium dans le phloème peut être mise en relation avec la teneur très élevée en phosphates, le pH élevé et la présence de beaucoup d'acides organiques.

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Carbohydrate Components of Plasma Membrane of Transitional Epithelium of Urinary Tract

Electron microscopic and electrophoretic observations have already been presented suggesting the cellular origin of the Tamm-Horsfall glycoprotein in the cell surfaces of the transitional epithelium of the urinary system[1]. Also, it has been shown that the plasma membrane of transitional epithelium of extrarenal urinary passages (renal pelvis, ureter and urinary bladder) has a notable developed filamentous glycocalyx[2]. This was in accord with the observation of relatively high content of carbohydrates in the microsomal fraction of transitional epithelium[3].

In the present study, a plasma membrane fraction of sheep transitional epithelium of urinary bladder was isolated and various carbohydrates were assayed on paper chromatography and on polycrylamide gel electrophoresis.

Materials and methods. Transitional epithelium cells were obtained from sheep urinary bladder[4]. The cell pellet was processed for the isolation of plasma membranes[5]. Cells were resuspended in 0.02 *M tris*-HCl, pH 8 and disrupted in a Dounce homogeneizer with 5 strokes, at 4 °C. The homogenate was mixed with an equal volume of 60% sucrose, layered on 45% sucrose and centrifuged in a SW 25 rotor of a Spinco ultracentrifuge at 4000 *g* for 45 min. The uppermost layer was removed, diluted 10 times with distilled water and centrifuged at 100,000 *g* in a No. 40 rotor for 90 min. The residue was suspended in a 17% sucrose solution and layered on a discontinuous gradient of sucrose (20, 25, 30, 35, 40 and 45%) which was centrifuged for 4 h at 70,000 *g* in a SW 25 rotor. Fractions collected from the various interphases, were resuspended in distilled water. A sample of each fraction was processed for electron microscopy observation. The fraction collected between 35–40% sucrose layers was shown to contain the greatest number of plasma membrane profiles (Figure 1).

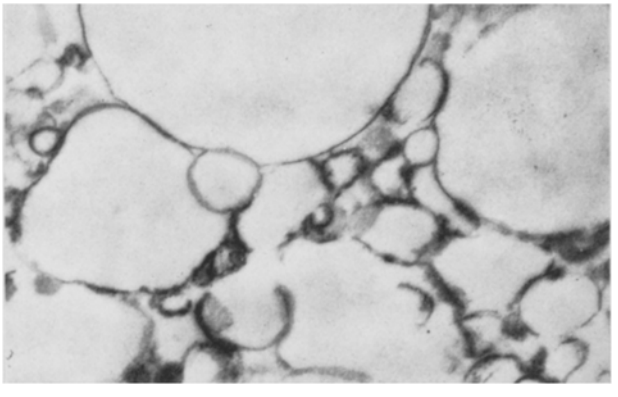

Fig. 1. Plasma membrane fraction. Profiles of membranous structures are seen. ×48,000.

The determination of the following enzyme activities were performed on samples corresponding to the 35–40% interphase: 5-nucleotidase (E.C.3.1.3.5)[6], glucose-6-phosphatase (E.C.3.1.3.9)[7], Mg-dependent adenosinetriphosphatase (E.C.3.6.1.4)[8], α-D-glucosidase (E.C.3.2.1.20), β-D-glucosidase (E.D.3.2.1.23)[9], succinic dehydrogenase

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(E.C.1.3.99.1)[10] and β-glucuronidase (E.C.3.2.1.31)[11] (Table I). In addition, the plasma membrane fraction was thoroughly dialyzed against distilled water and freeze-dried. Suspensions of 1 mg of the dried tissue in 0.1% Triton X-100 were dialyzed and assayed for proteins[12], hexoses[13], hexosamines[14] and sialic acids[15] (Table II). Chromatography of carbohydrates was carried out on Whatman paper No. 1 using a mixture of *n*-butanol-pyridine-0.1 *N* HCl (5/3/2 v.v.)[16]. Samples of 1 mg of freeze dried plasma membrane fraction were hydrolized for 150 min in 1 ml of 2 *N* HCl at 100 °C and dried under vacuum at 4 °C. Samples dissolved in 10 µl distilled water were chromatographed[17].

The plasma membrane fraction obtained was also subjected to disk electrophoresis analysis on polycrylamide gel[18,19] using 5% sodium dodecylsulphate. Gels were stained with amidoblack B and mucicarmine. Total lipids were extracted according to FOLCH et al.[20] from both total homogenates and the plasma membrane fraction. For the determination of lipid-bound sialic acid, the total lipid extract was partitioned and washed[21]. The upper aqueous phase was dialyzed 72 h against distilled water. The dialysate was concentrated to complete dryness. The residue was taken with the conventional upper phase (chloroform-methanol-water 3:48:47) and aliquots were assayed for sialic acids, or chromatographed on thin layer chromatography[22] (Table III). For electron microscopy, pellets of membranes were double fixed in glutaraldehyde and osmium tetroxide with and without ruthenium red [23] dehydrated and embedded in epoxy resins. Grids were stained with uranyl acetate and lead citrate.

Results and discussion. Data on total homogenates of transitional epithelium have already been reported[4]. The transitional epithelium plasma membrane fraction herein reported, seemed relatively pure as indicated by electron microscopy and by absence of enzymes which are markers of other cell fractions. As for the enzymes which are currently considered to be markers of plasma membranes[24], only adenosinetriphosphatase (Mg-dependent) and β-D-glucosidase were found in significant amounts (Table I). The low levels of 5-nucleotidase might be a characteristic of transitional epithelium.

The present study indicated that the plasma membrane fraction of transitional epithelium contained 3 times more sialic acid and 5 times more hexosamines than the homogenates of whole transitional epithelium[4]. The plasmalemma fraction contained twice as much sialic acid than the other cell fractions. No significant enrichment in neutral carbohydrates was observed in the plasma membrane fraction with respect to the total homogenates. Yet, total homogenates of this epithelium revealed high leveles of neutral carbohydrates[4]. This is in agreement with

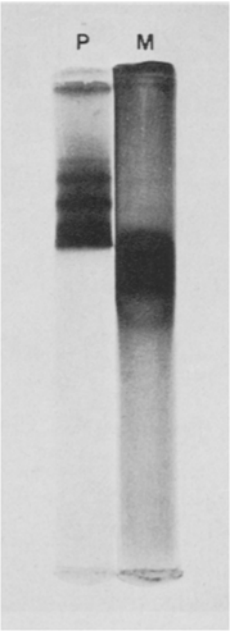

Fig. 2. Disk electrophoresis. Gels were run for 60 min at room temperature. 5% SDS was added to homogenates of plasma membrane fractions. 40 µl of sample were placed in 0.25 ml of thick pore gel or spacing gel. P, gel stained with amidoblack B; M, gel stained with mucicarmine. Several distinct bands stained both for proteins and sugars. The fastest moving band in M has no corresponding band in P.

Table I. Enzymes of a plasma membrane fraction

5 Nucleotidase (µmoles of P per h/mg protein)	[a]5.3 ± 0.24[b]
Adenosine-triphosphatase (mg- dependent) (µmoles of P per h/mg protein)	10.02 ± 0.72
Glucose-6-phosphatase (µmoles of P per h/mg protein	1.06 ± 0.03
β-D-glucosidase (µmoles of substrate/mg protein/min)	13.86 ± 2.01
α-D-glucosidase	n.a.
β-glucuronidase	n.a.
Succinic dehydrogenase	n.a.

[a] Values are average of 3 assays of membrane fraction preparation. [b] Standard error. n.a., not assayable.

Table II. Carbohydrates composition of a plasma membrane fraction (nmoles/mg protein)

Neutral carbohydrates	Hexosamines	Sialic acid
1,065.33 ± 45.5	299.00 ± 11.8	73.5 ± 1.8

Data are the mean of 3 determinations from the exfoliated transitional epithelium of 5 bladders ± Standard error.

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histochemical data indicating large amounts of glycogen in transitional epithelium (A. EYNARD, R. ROVASIO and B. MONIS, unpublished observations).

Polyacrylamid gels revealed several protein bands, some of which stained with mucicarmine, an empirical procedure for carbohydrates (Figure 2). Chromatograms of this plasma membrane fraction indicated the presence of glucose, glucosamine and traces of galactose and mannose (Figure 3).

Table III. Total and lipid-bound sialic acid in transitional epithelium

Fraction	Sialic acid (nmoles/mg protein)		
	A) Total	B) Lipid-bound[a]	B/A (%)
Total homogenates	24	3.3	13.7
Plasma membrane	70	16.1	23.0

[a] Total homogenate of 20 bladders. The plasma membrane fraction was obtained from 50 bladders.

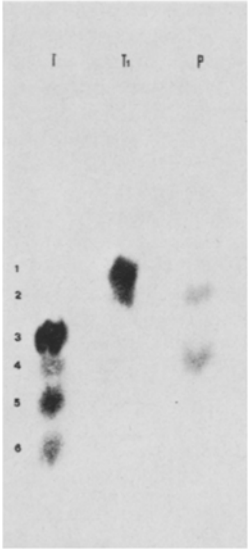

Fig. 3. Hydrolyzed plasma membrane fractions was chromatographed for 16 h in *n*-butanol-pyridine- 0.1 *N* HCl (5/3/2 v.v). Neutral sugars T, D-galactose (3), D-glucose (4), D-mannose (5), 1-fucose (6). Aminosugars T_1, galactosamine (1) and D-glucosamine (2).

The present data confirmed and expanded previous observations indicating that transitional epithelium of urinary tract of sheep is notably rich in carbohydrates, some of which are largely contained in the plasma membrane. This seemed in correspondence with light and electron microscopic observations indicating the presence in this epithelium of a carbohydrate-containing fluffy coat, a differentiation of the plasma membrane[2].

Values for normal and abnormal cells such as rat liver[24], Ehrlich ascitis cells[25], rat ascites hepatoma[26] and mouse fibroblast (L cells)[27] have been reported. Significant variations of various carbohydrates in cell surfaces were noted. These reports indicated that the highest concentration of sialic acid and hexosamines were found in the plasma membrane fraction of the cell types studied. In addition, our data indicated that roughly 23% of the sialic acid of plasma membrane of transitional epithelium is lipid bound. In fact, preliminary data of thin layer chromatography of sheep transitional epithelium indicated a preponderance of more polar gangliosides (polisialogangliosides), in agreement with observations that extraneural tissues contained polisialogangliosides with a pattern as complex as that of brain of mammals[28]. Values of lipid-bound sialic acid, such as 75 mg/100 dry weight in transitional epithelium, which is about 30% of values reported for brain, indicated that transitional epithelium content of lipid-bound sialic acid is even higher than that of the medulla of bovine adrenal[29]. PURO et al.[28] found that relatively low values of gangliosides in extraneural tissues, which varied from 2 to 10% of the content reported for brain.

BENEDETTI and EMMELOT[30,31] have reported that no less than 95% of sialic acid of plasmalemma of liver is bound to protein and that only traces appear to be gangliosides. Plasma membrane of sheep transitional epithelium would seem to contain significant levels of gangliosides, as was suggested by our findings that roughly one-fourth of the total sialic acid was lipid-bound.

The biological significance of carbohydrate components of cell surfaces is little known[32–34]. For cell surface components of transitional epithelium, it has been suggested that this epithelium is the source of the urinary Tamm-Horsfall (T-H) mucoprotein. This is supported by the observations that lipids which are characteristic of biological membranes are present in preparations of T-H mucoprotein[1,35,36]. The finding, under the electron microscope, of plasma membrane fragments in T-H mucoprotein, and the similar electrophoretic mobility of the urinary mucoprotein and transitional epithelium[1], would suggest that cell surface of transitional epithelium might be the origin of T-H mucoproteins. However more information is required in support of the view that

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complex carbohydrates of certain biological fluids might be released from the corresponding cell surfaces[1,37,38].

Resumen. Se estudió el contenido de hexosas, hexosaminas, metilpentosas y ácido siálico de una fracción de membrana plasmática del epitelio de transición de la vejiga de cordero. La concentración de ácido siálico y hexosaminas era 3 y 5 veces respectivamente más alta en membrana plasmática que en homogenato total. En cambio, los carbohidratos neutros no se enriquecieron significativamente. El 23% de ácido siálico se extrajo con los lípidos de esa fracción. Se presentan datos de cromatografía y electroforesis.

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Mode of Action of DDT Analogues: Molecular Orbital Studies

In earlier work a steric theory of DDT action has been used to design a large number of highly active DDT analogues[1,2]. The basis of this theory is that a molecule of an active compound has to form a 'molecular wedge' with two distinct features. Firstly, the apex of the 'wedge' (e.g. CCl_3 group of DDT) must have a particular size and shape, and secondly, the base comprises a multiple ring structure which must have electron donating substituents. The size limitation on the base part of the molecule is less restrictive than that on the apex.

It has been found that compounds synthesized to fit this model, while all possessing insecticidal activity, nevertheless exhibit considerable variations of this activity even in strictly standardized insect strains.

Recently, FUKUTO et al.[3] examined the structure/activity correlation for a series of DDT trichloroethane analogues by means of a multiple regression analysis using empirical substituent constants and mortality values in the housefly and *Culex* mosquito larvae. They obtained good correlations between log LD_{50} and a function of the steric substituent constant E_s.

In this preliminary note we report the first results of an alternative method of correlating the activity of DDT analogues of a similar chemical structure with other molecular properties. We assume that any differences in this activity can be attributed to differences in electronic structure as the molecules have all been selected according to rigorous steric criteria.

All our insect mortality data were obtained[1] on the susceptible strain (WHO/IN/1) of houseflies which were selected by sex, age and weight of pupae. The flies were kept at standard humidity and temperature and fed on cellulose free of traces of insecticides. Other controlled variables were the time of day of application of the insecticides and the time of mortality counting. The mortality calculations were carried out only for tests where control mortality was zero for the 48 h test period. The compounds were potentiated with a mixed function oxidase inhibitor (Sesoxane) to obtain the basic activity comparison without superimposed biochemical degradation of the insecticides.

To estimate the charge distribution on the molecules we have performed molecular orbital calculations at the CNDO/2 level. This method is suitable for the calculation of charge distributions of molecules[4]. The computer program we used was that published by POPLE and BEVERIDGE[5] extended to take molecules with 55 atoms and 125 valence electrons and modified slightly to run under the highest optimization available on the CD 6600 computer.

The equation relating activity to charge distribution is based on the method of CAMMARATA[6], and a similar equation has been successfully used by NEW and RICHARDS[7] in a study related to ours, the molecular orbital calculation of hapten-antibody interactions. Since we are assuming that all steric factors are constant, we have[6]

$$\log LD_{50} = k \sum_i \frac{q_{s_i}\, q_r}{D_{rs_i}} + C.$$

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Relationship between calculated charges on chemically unrelated DDT-type insecticides and mortality in a standardized strain of the housefly

Compound	LD_{50} (μg/♀ insect)	Charge on apex of molecules (10^{-4} × charge on an electron)
1,1-*Bis*-(*p*-ethoxyphenyl)-2,2-dimethyl propane	0.32	+ 210
1,1-*Bis*-(*p*-chlorophenyl)-2,2-dichloro cyclopropane	0.12	− 325
1,1-*Bis*-(*p*-ethoxyphenyl)-2-nitropropane	0.065	− 757
1,1-*Bis*-(*p*-ethoxyphenyl)-2-nitrobutane	0.061	− 679
2,2-*Bis*-(*p*-ethoxyphenyl)-3,3-dimethyl oxetane	0.01	−1776