

of artificial chromatin as a template for RNA synthesis^{10, 21}. But, it is most probable that these specific molecules are present in chromatin only in small quantities not detectable by analytical polyacrylamide gel electrophoresis and that the majority of NHC proteins has nonregulatory functions.

The difference between our findings and the findings reported in the literature could be explained by the following facts. Used procedure eliminated the possibility of contamination of our preparations with cytoplasmic proteins or nuclear sap proteins²² which could be tissue specific. In addition to working with purified nuclei, our analysis of the total chromosomal proteins by avoiding the different steps required for the subsequent isolation of NHC proteins eliminated additional possibilities for the formation of artefacts.

The most pronounced differences were reported between the electrophoretic patterns of NHC proteins isolated from nucleated erythroid cells and the electrophoretic patterns of NHC proteins isolated from other types of cells^{4, 7, 9}. The NHC proteins isolated from erythroid cells are less heterogeneous with some electrophoretic bands missing. In our opinion, these findings do not support the conclusion that the main electrophoretic bands of NHC proteins are involved in the regulation of gene expression. The nucleus of nucleated erythrocytes is completely inactive and also the reticulocytes are inactive with

respect to RNA synthesis²³. Therefore, it can be expected that the proteins (enzymes) required for DNA synthesis or RNA synthesis and processing are missing in the electrophoretic pattern of NHC proteins isolated from erythroid cells.

Zusammenfassung. Die Analyse mit Polyacrylamide-Gel-Elektrophorese von Chromatinproteinen an Hühner-Embryonen, Ratten Ascites Hepatoma, Rattenleber und verschiedenen Teilen des Rattengehirns hat ergeben, dass dieselben Hauptfraktionen der sauren Chromosomenproteine in allen untersuchten Geweben vorhanden sind.

H. FUJITANI and V. HOLOUBEK²⁴

Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston (Texas 77550, USA), 22 October 1973.

²¹ T. C. SPELBERG, L. S. HNILICA and A. T. ANSEVIN, *Biochim. biophys. Acta* 228, 550 (1971).

²² E. W. JOHNS and S. FORRESTER, *Eur. J. Biochem.* 8, 547 (1969).

²³ I. L. CAMERON and D. M. PRESCOTT, *Expl Cell Res.* 30, 609 (1963).

²⁴ This work was supported by a grant from the Robert A. Welch Foundation No. H-393. We thank Dr. C. W. KISCHER for his help with the collection of chicken embryos and for the determination of their developmental stage.

The Low Calcium Content of Cellular Systems Adapted to Flow

Recently EPSTEIN¹ stressed a few wellknown facts about the phloem and put forward a tentative explanation for their correlation with the observed very low calcium and boron content. In relation to these suggestions – which I would like to endorse – there seem to be a number of other rather interesting points and correlations which could be added to expand the view.

Phloem is not the only living plant tissue adapted to flow. If wounded, the latex system of many plants will show extensive flow. Especially the outflow of latex from *Hevea brasiliensis* has been extensively investigated. This latex is derived from a large drainage area and is extruded by means of osmotic attraction of water². The exuding latex can best be considered as a diluted cytoplasm³, as it contains both many proteins and plastids and an enormous array of enzymes. The low viscosity of latex is again linked with its very low calcium content, e.g. ca. 2% of the total cation content⁴.

This low calcium and high potassium content of both phloem and latex plasma would seem to be appropriate from the point of view of plasmatic viscosity. Monovalent ions cause swelling and lowered viscosity, while divalent ions favour an increased viscosity due to lower water content. The fluidity of latex can be related to a certain amount of swelling. In the ontogeny of the latex vessels in *Hevea* the cytoplasm increases in volume, while the original large vacuoles retract to a multi-disperse system of minute droplets^{3, 5}. The resulting immense number of luteoids⁶ ultimately only occupy a few percent of the volume of exuded latex. Also the electron microscope has produced evidence of the strong dilution of the phloem cytoplasm.

EPSTEIN¹ proposes an exclusion of calcium from the sieve tubes. The question arises as to where we must locate this process of restricting calcium activity. One could conceive of relating it to the process of vein-

loading. But there is also a possibility of a low supply towards the phloem from cells with a low calcium activity.

In the conception 'symplasm', the unity of cytoplasmic contents of living cells, connected by their plasmodesmata and including the phloem, is implied. From this point of view of cytoplasmic continuity there is reason to suspect a low calcium activity in the cytoplasm of surrounding cells as well. This would seem to be contrary to the generally moderate to high calcium content of most cells, but much of it is located in cell-walls or vacuoles. That much of the calcium in the plant could be non-essential has also been suggested by WALLACE, FROHLICH and LUNT⁷. Although cell-organelles, e.g. chloroplasts can accumulate calcium, some recent evidence from muscle physiology suggests very low calcium activities^{8–10}. For *Nitella translucens* a value of 8 mM in the flowing cytoplasm is mentioned¹¹, while for *Nitella flexilis* a value of 125 mM/l is given for potassium¹².

¹ E. EPSTEIN, *Experientia* 29, 133 (1973).

² W. H. ARISZ, *Arch. Rubbercult.* 12, 220 (1928).

³ L. K. WIERSUM, *Rev. gén. Caoutchouc* 35, 276 (1958).

⁴ C. F. FLINT and H. RAMAGE, *J. Soc. chem. Ind., Lond.* 54, 337 (1935).

⁵ F. R. MILANEZ, *Archos Serv. flor. Bras.* 2, 39 (1946).

⁶ J. RUINEN, *Ann. bogor.* 1, 27 (1950).

⁷ A. WALLACE, E. FROHLICH and R. O. LUNT, *Nature, Lond.* 209, 634 (1966).

⁸ L. PACKER and A. R. CROFTS, *Curr. Top. Bionerg.* 2, 24 (1967).

⁹ C. C. ASHLEY and E. B. RIDGWAY, in *A Symposium on Calcium and Cellular Function* (Ed. A. W. CUTHBERT; St. Martins Press, New York 1970), p. 42.

¹⁰ L. HURWITZ, D. F. FITZPATRICK, G. DEBBAS and E. J. LANDON, *Science* 179, 384 (1973).

¹¹ R. M. SPANSWICK and E. J. WILLIAMS, *J. exp. Bot.* 16, 463 (1965).

¹² U. KISHIMOTO and M. TAZAWA, *Plant Cell Physiol.* 6, 507 (1965).

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

| | | | |
|-----------------------------|-----|-------------------------------|-----|
| <i>Nitella</i> | 15 | <i>Ricinus</i> sprout | 3 |
| <i>Yucca</i> phloem exudate | 120 | <i>Ricinus</i> phloem exudate | 400 |
| <i>Hevea</i> 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 GOOR¹³, 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 meta-

bolically important phosphates and the large amount of organic anions. These organic anions – originating from nitrate reduction – and mainly coupled to the important potassium ion¹⁴, 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.

L. K. WIERSUM¹⁵

*Department of Plant Nutrition,
Institute for Soil Fertility, Haren-Gr. (The Netherlands),
15 November 1973.*

¹³ B. J. VAN GOOR and D. WIERSMA, in preparation.

¹⁴ W. DIJKSHOORN, Proc. 6th Int. Colloq. Plant Analysis Fert. Probl., Tel Aviv 1970, p. 447.

¹⁵ I thank E. EPSTEIN for reading the manuscript.

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 system¹. 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 glycocalyx². This was in accord with the observation of relatively high content of carbohydrates in the microsomal fraction of transitional epithelium³.

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 polyacrylamide gel electrophoresis.

Materials and methods. Transitional epithelium cells were obtained from sheep urinary bladder⁴. The cell

pellet was processed for the isolation of plasma membranes⁵. Cells were resuspended in 0.02 M *tris*-HCl, pH 8 and disrupted in a Dounce homogenizer 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 SW25 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 SW25 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).

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

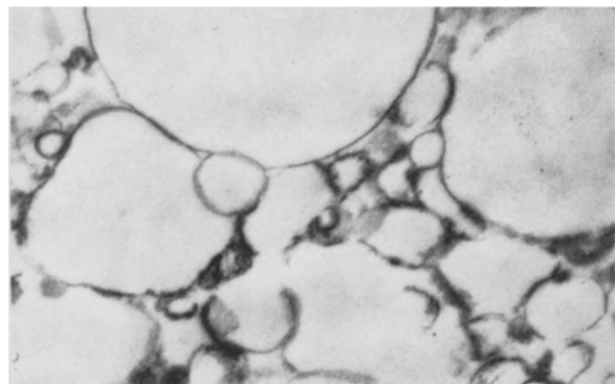


Fig. 1. Plasma membrane fraction. Profiles of membranous structures are seen. $\times 48,000$.

¹ B. MONIS, A. CANDIOTTI, A. GOMEZ and N. IBAÑEZ, Life Sci. Part II, 11, 699 (1972).

² B. MONIS and D. ZAMBRANO, Z. Zellforsch. 87, 101 (1968).

³ N. IBAÑEZ, A. CANDIOTTI and B. MONIS, Life Sci. Part II, 10, 989 (1971).

⁴ A. CANDIOTTI, N. IBAÑEZ and B. MONIS, Experientia 27, 551 (1971).

⁵ R. M. HICKS, J. Cell Biol. 45, 542 (1970).

⁶ L. A. HEPPEL and R. J. HILMOE, J. biol. Chem. 188, 665 (1951).

⁷ M. A. SWANSON, *Method of Enzymology*, (Academic Press, New York 1955), v. 2, p. 541.

⁸ T. K. RAY, Biochim. biophys. Acta 196, 1 (1970).

⁹ N. B. BOSMANN and J. BERNACKI, Expl. Cell Res. 67, 379 (1970).