tion. Les résultats ne se trouvent pas sensiblement modifiés en faisant varier la longueur de l'apex entre 2 et 10 mm.

Discussion et conclusion. Le rhizomorphe du *Sphaerostilbe repens* bien que se développant dans un milieu compact exige l'oxygène pour sa croissance et ne peut donc pas s'affranchir d'un métabolisme exclusivement fermentaire. Cependant, il est important que la pression d'oxygène soit appliquée à l'entrée du canal central et non sur la face externe de l'apex. Il apparaît donc que la concentration en oxygène doit être plus faible sur la face externe apicale du rhizomorphe qu'à l'entrée du canal central. Smith et Griffin[10] mentionnent qu'une concentration supérieure à 4% en contact avec l'apex des rhizomorphes d'*Armillariella elegans* est de nature à inhiber leur croissance. En même temps, chez ce champignon, l'apex brunit et dédifférencie des articles lâchement accolés les uns aux autres, formant ainsi de véritables «pores» qui mettent en communication, par endroit, le canal central avec le milieu externe. Chez le *Sphaerostilbe repens,* les corémies régénérées par l'apex seraient l'équivalent de ces «pores respiratoires».

On peut penser que le brunissement de l'apex correspond à la formation de produits apparentés aux mélanines. Selon Smith et Griffin[10], ces produits seraient de nature, entre autre, à empêcher la diffusion des éléments nutritifs, inhibant ainsi la croissance de l'organe.

Comme chez l'Armillaire, le canal central du rhizomorphe du *Sphaerostilbe repens* semble capable de laisser diffuser l'air jusqu'à l'apex. Ceci semble appuyé par le fait que les articles de la médulla à proximité du canal central sont extrêmement riches en mitochondries, notamment à l'extrémité du rhizomorphe[3].

1 J.J. Guillaumin, Cah. Orstom. Sér. Biol. *12*, 51 (1970).
2 J. Dexheimer et B. Botton, C.r. Acad. Sci. Paris *281D*, 1093 (1975).
3 B. Botton et J. Dexheimer, Z. Pflanzenphysiol. *85*, 429 (1977).
4 R.D. Goos, Am. J. Bot. *49*, 19 (1962).
5 P.R. Ly, DEA Biologie végétale. Université de Nancy I, 1974.
6 B. Botton et P.R. Ly, Biologia Pl. *18*, 436 (1976).
7 J. Reitsma, Phytopath. Z. *5*, 461 (1932).
8 P.J. Snider, Mycologia *51*, 693 (1959).
9 M. Jacques-Félix, Bull. Soc. mycol. Fr. *84*, 161 (1968).
10 A.M. Smith et D.M. Griffin, Aust. J. biol. Sci. *24*, 231 (1971).
11 D.M. Griffin, dans: Ecology of Soil Fungi, p. 113. Chapman and Hall, London 1972.
12 C. Casalis-Gougerot, DEA Biologie végétale. Université de Nancy I, 1976.

Nuclear pores. Can they expand and contract to regulate nucleocytoplasmic exchange?

N.J. Severs[1] and E.G. Jordan

Cell Pathology Unit, The School of Pathology, The Middlesex Hospital Medical School, Riding House Street, London WIP 7LD, and Biology Department, Queen Elizabeth College, Campden Hill, London W8 7AH (England), 20 January 1978

Summary. Recent evidence has revived the idea that translocation of macromolecules between nucleus and cytoplasm may be regulated via expansion and contraction of the entire diameter of the nuclear pore complex. The present investigation does not support this hypothesis.

Nuclear pore complexes (NPCs) are thought to play a key role in communication between nucleus and cytoplasm, thus allowing integration of the specialized activities of these 2 cellular compartments. Precisely how NPCs mediate selective nucleocytoplasmic exchange of macromolecules is a problem which has challenged cell biologists for over 2 decades, and the progress made in this field has been given detailed treatment in several recent reviews[3-7].

Quantitatively, macromolecule translocation could conceivably be controlled by modulation of the proportion of nuclear envelope occupied by NPC area - operating via changes in NPC number or size. Values for overall NPC diameter reported in the earlier literature range from 30 nm[8] to 200 nm[9]. Thus, regulation by expansion and contraction of NPCs might originally have seemed a plausible hypothesis[10]. However, various methodological considerations later revealed that such differences do not reflect the in vivo state and it became generally agreed that true NPC diameter in fact shows little variation[3,5,7,11].

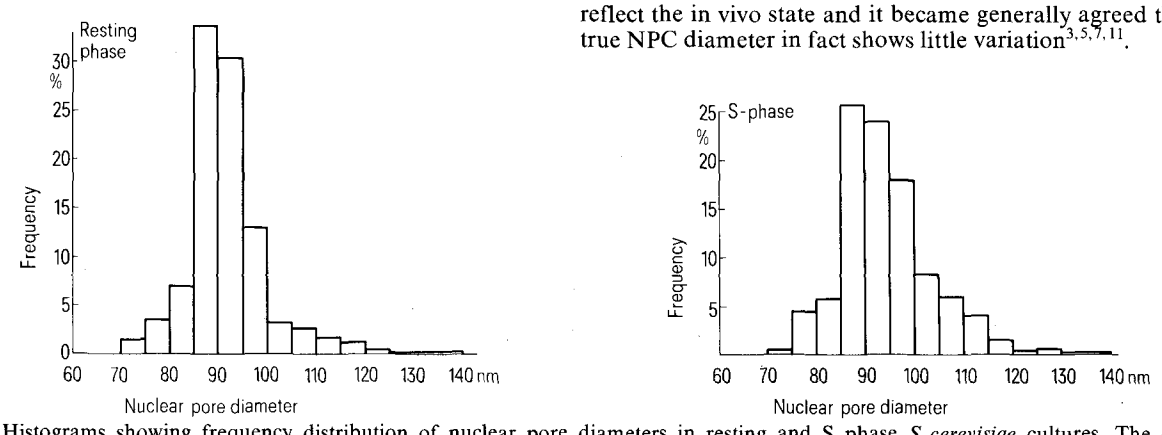


Fig. 1. Histograms showing frequency distribution of nuclear pore diameters in resting and S phase *S. cerevisiae* cultures. The cells were synchronized, sampled at 0 min and 90 min after inoculation, and freeze-fractured without pretreatment as described previously[13,14]. Approximately 500 nuclear pores from 50 nuclear envelopes were measured for each sample. Pores on steeply inclined areas of nuclear membrane fracture face were excluded. A single peak between 85 and 95 nm is shown in both resting and S phase cells, and no 2nd peak between 120 and 175 nm is present. A small number of pores in the range 115-140 nm is encountered but inspection of these indicates that they arise when the fracture path departs from the membrane before reaching the true pore periphery. A similar frequency distribution was obtained in unfixed *Chlorella* cells (results not shown).

Willison and Rajaraman[2] have recently cast serious doubt on this conclusion. They propose that the narrow central channel through the annulus (i.e. the non-membranous electron-dense NPC component) can be opened and closed by relaxation and contraction of the entire NPC. Their evidence is based on the demonstration of large (120–175 nm diameter) NPCs in unfixed (directly frozen and pre-glycerinated) freeze-etched human lymphocyte nuclei. The large NPCs were absent in glutaraldehyde-fixed samples where only the usual (~ 100 nm diameter) NPCs were observed. Thus, Willison and Rajaraman[2] conclude that glutaraldehyde fixation induces NPC closure.

Confirmation of these results is imperative if NPC expansion/contraction is to be accepted as a general cellular regulatory mechanism. Accordingly, we have re-investigated the possible significance of NPC diameter in a range of plant and animal cell types. Specimens were fixed in buffered 1–2.5% glutaraldehyde and cryoprotected in buffered 20–30% glycerol, treated with glycerol without prefixation, or untreated prior to freezing. Freeze-fracture replicas were prepared by the Bullivant[12] technique. Diameters of NPCs were measured on electron micrographs across the region of fracture departure from the membrane around the pore. In 1 cell type, *Saccharomyces cerevisiae*, comparison of NPC diameter in resting (starved) cells (inactive in macromolecular synthesis) and S phase cells (active in DNA, RNA and protein synthesis) was made.

On the basis of Willison and Rajaraman's[2] hypothesis, large diameter NPCs would be expected in unfixed material but not in fixed specimens. Furthermore, in directly frozen (unfixed) *S. cerevisiae*, more large diameter NPC might be

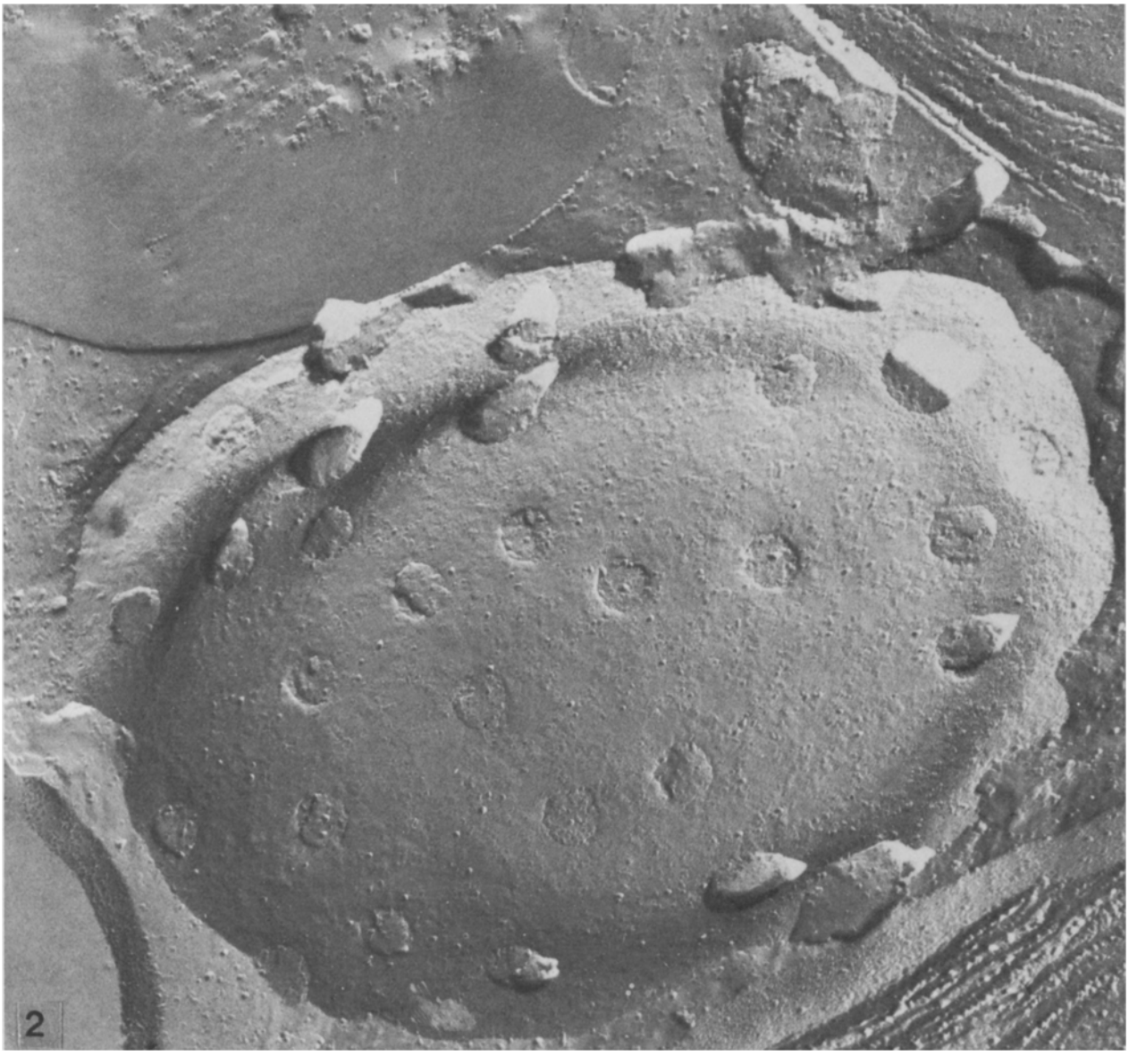


Fig. 2. Freeze-fracture replica of a directly frozen (unfixed, unglycerinated) *Chlorella pyrenoidosa* cell, showing the E-face of the outer nuclear membrane of a convexly fractured nucleus. On the flatter areas of membrane, the nuclear pores are relatively uniform in shape and diameter, and sometimes suggest a polygonal outline. As the membrane slopes towards the periphery of the nuclei, the shape, size and appearance of the pores is less regular; an observation best explained in terms of fracturing behaviour[16]. No *bona fide* large diameter nuclear pores were observed in more than 40 *Chlorella* nuclear envelopes examined. × 69,400.

present in the synthetically active S phase than in the inactive resting phase. However, neither of these predictions was met in the present study. Only 1 size class of NPC diameter corresponding to the 'closed' NPC of Willison and Rajaraman[2], was observed in unfixed, unglycerinated *Chlorella* and in similarly prepared resting and S phase *S. cerevisiae* (figures 1–3). Variation around the mean diameter reflects the different ways in which NPCs may be fractured[11,15,16]; the fracture plane often leaving the membrane before reaching the equatorial margin of the pore. No difference in NPC diameter was observed between fixed and unfixed *S. cerevisiae, Schizosaccharomyces pombe* or

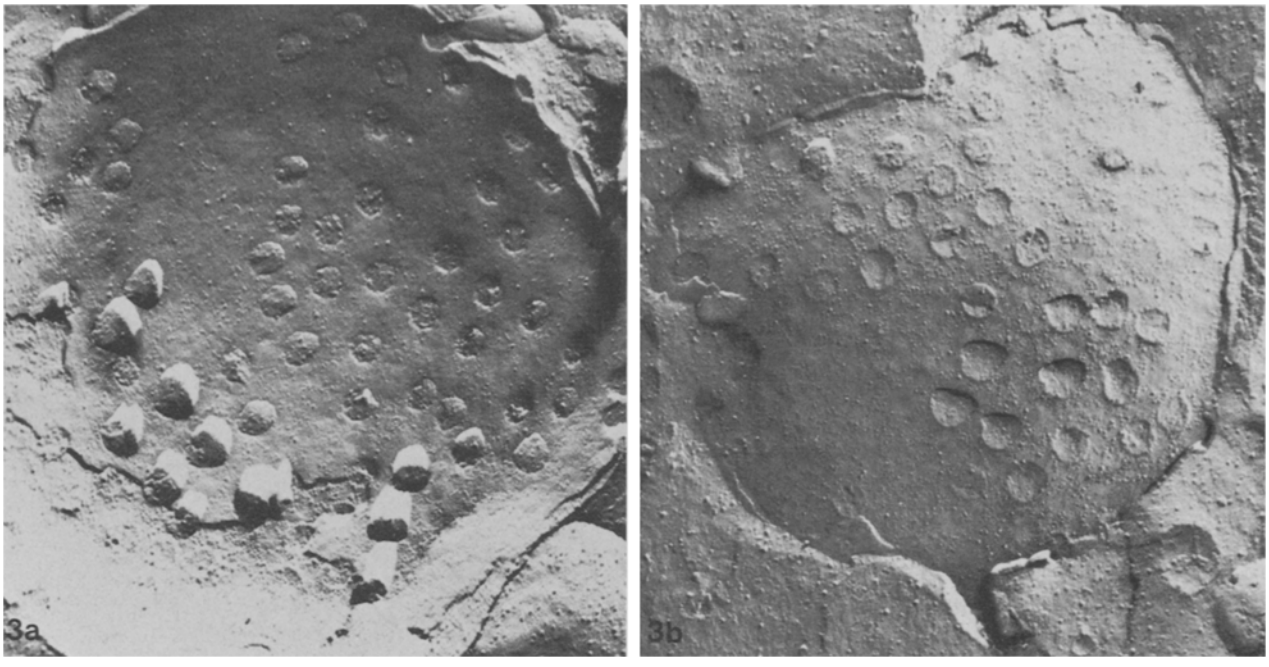


Fig. 3. Freeze-fractured nuclei from directly frozen (unfixed, unglycerinated) S phase *S. cerevisiae cells. a* concavely fractured; *b* convexly fractured. The main portion of *a* illustrates the E-face of the inner nuclear membrane. The P face is shown in *b*. As with *Chlorella*, pores that are fractured evenly around their periphery present uniform diameters. Some pores differ by being uneven, larger and by having long shadows (*a*, lower left). This happens in inclined areas of membrane where the fracture plane veers to the horizontal in the non-membranous pore interior. A complementary view is seen on the lower right of *b*. No difference in the size and appearance of nuclear pores in resting compared with S phase *S. cerevisiae* was observed. × 40,000.

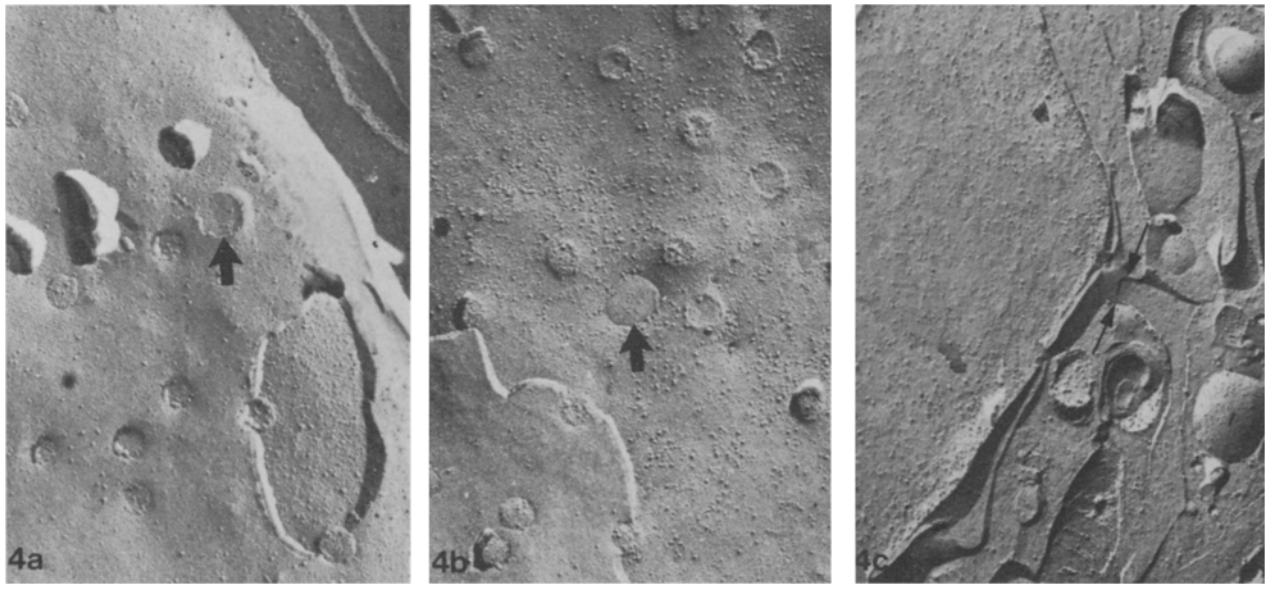


Fig. 4. Freeze-fracture replicas of glutaraldehyde-fixed and glycerinated human diploid (MRC-5) fibroblasts. Breaks (arrows) larger than nuclear pores can be seen in the outer nuclear membrane E-face (*a*) and P-face (*b*). Unlike nuclear pores, the membrane at their periphery shows a curvature towards the cytoplasm. Inspection of cross-fractured nuclei (*c*) reveals that these breaks arise at sites where the outer nuclear membrane is thrown into folds or where it joins with the endoplasmic reticulum (arrows). *a* and *b*, × 46,000, *c* × 33,000.

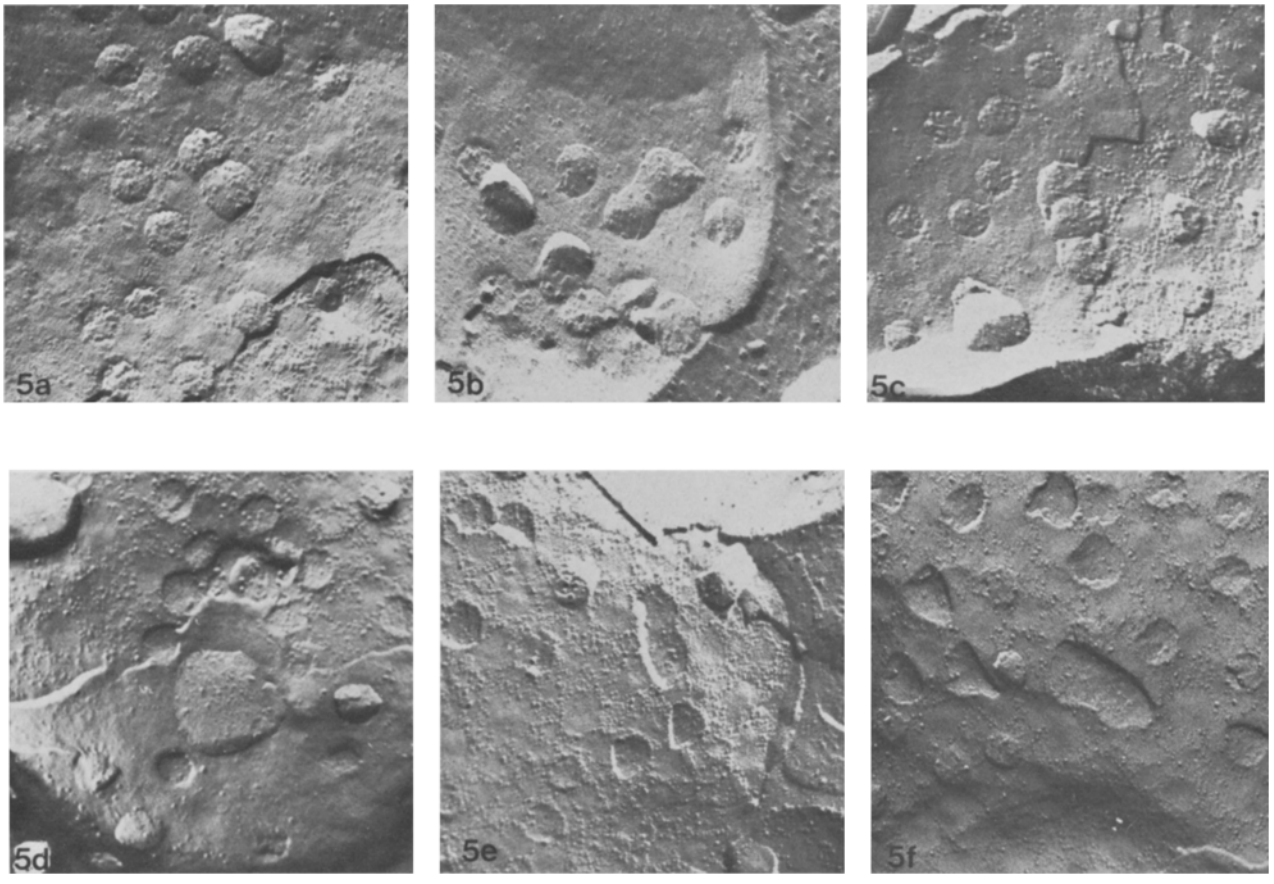


Fig. 5. Freeze-fracture replicas of nuclei of directly frozen *S. cerevisiae* cells illustrating the appearance of nuclear pores fractured out as a group. *a–c* are from concavely fractured nuclei and *d–f* from convexly fractured nuclei. *a* The fracture plane has departed from the membrane before reaching the margins of 2 adjacent pores, though they remain distinct. *b* as *a*, but the boundary between the 2 pores has not been exposed. *c* as *a*, but involving 3 adjacent pores. *d* a larger, roughly circular break, probably involving at least 4 pores. *e* and *f* breaks involving 2 pores – complementary to the appearance seen in *b*. × 50,000.

Chlorella. Although directly frozen higher plant and animal nuclei have not yet been examined, no difference between specimens treated with glycerol only and those fixed before glycerination has been detected. Larger somewhat distorted NPC were occasionally observed in specimens badly damaged by ice crystals and in those showing extensive cryoprotectant-induced artifacts but neither of these explanations can satisfactorily account for the results of Willison and Rajaraman[2] since their specimens appear to be well preserved.

'Breaks' resembling, though larger than, those formed at NPCs were often observed in trypsinized MRC-5 fibroblasts (figure 4) irrespective of whether or not they had been pre-fixed. These cells had irregularly shaped nuclei with frequent connections between the outer nuclear membrane (ONM) and endoplasmic reticulum (ER). Fractures across nuclear evaginations/invaginations and sites of ONM-ER continuity would be expected to produce larger 'breaks' than those created at NPCs. Other large 'breaks' in the nuclear envelope may arise when small clusters of NPCs are fractured out as a group (figure 5) which tends to occur when such NPCs are situated in a local depression or elevation of the nuclear surface. In our experience, the presence of large apparent 'pores' depends on morphological factors rather than pretreatment regime but again it is difficult to account for Willison and Rajaraman's[2] results in these terms since they observed NPCs of regular periphery and substructure.

Our failure to demonstrate large NPCs in unfixed freeze-fractured specimens does not preclude the possibilities that NPCs can exist in 'open' and 'closed' configurations[7] and that changes in central channel or patent pore size are important in regulating transfer of macromolecules[17], but it does suggest that these changes are not dependent upon alterations in overall NPC diameter. Indeed, the primary regulatory properties of the NPC appear to be inherent in its structure and composition rather than in an ability to adjust NPC number or diameter. An alternative to the hypothesis of Willison and Rajaraman[2] is that the annulus serves an important function in maintaining a stable (and thus constant) opening (i.e. overall diameter) in the nuclear membranes. The symmetrical annulus architecture could provide an ordered structural framework into which functional components for processing macromolecules (e.g. RNA cleavage) and regulating their passage (e.g. ATPase) could be built. Thus, the annulus could be envisaged as consisting partly of structural elements, partly of functional components and partly of macromolecules actually in transit across the nuclear envelope. Although the evidence suggests in balance that the entire NPC cannot expand and contract, the fact that large diameter NPCs have occasionally been observed[2,18] makes further investigations in other systems essential before this question can be finally settled.

1 Acknowledgments. We are indebted to Dr J.H.M. Willison for discussion of his and Dr Rajaraman's results before their publication. We also wish to thank Dr C. Thurston, Dr D.H. Williamson and Dr K. Lambert for providing some of the specimens used in this investigation. N.J. Severs acknowledges the support of the Science Research Council and the Cancer Research Campaign.

2 J.H.M. Willison and R. Rajaraman, Proc. Fedn biol. Soc. *19*, 244a (1976); J. Cell Biol. *70*, 226a (1976); J. Microsc. *109*, 183 (1977).
3 W.W. Franke, Int. Rev. Cytol., suppl. *4*, 71 (1974).
4 W.W. Franke, Phil. Trans. R. Soc. Lond. B *268*, 67 (1974).
5 W.W. Franke and U. Scheer, in: The Cell Nucleus, vol. 1, p. 219. Ed. H. Busch. Academic Press, New York 1974.
6 W.W. Franke and U. Scheer, Symp. Soc. exp. Biol. *28*, 249 (1974).
7 F. Wunderlich, R. Berezney and H. Kleinig, in: Biological Membranes, p. 241. Ed. D. Chapman and D.F.H. Wallach. Academic Press, New York 1976.
8 H.G. Callan, J.R. Randall and S.G. Tomlin, Nature *163*, 280 (1949).
9 D. Branton and H. Moor, J. Ultrastruct. Res. *11*, 401 (1964).
10 E.J. DuPraw, in: DNA and Chromosomes, p. 205. Holt, Reinhart and Winston, New York 1970.
11 J. Kartenbeck, H. Zentgraf, U. Scheer and W.W. Franke, Adv. Anat. Embryol. Cell Biol. *45*, 1 (1971).
12 S. Bullivant, in: Advanced Techniques in Biological Electron Microscopy, p. 67. Ed. J.K. Koehler. Springer-Verlag, Heidelberg 1973.
13 E.G. Jordan, N.J. Severs and D.H. Williamson, Exp. Cell Res. *104*, 446 (1977).
14 N.J. Severs, Cytobios. *18*, 51 (1977).
15 A. Monneron, G. Blobel and G.E. Palade, J. Cell Biol. *55*, 104 (1972).
16 N.J. Severs and E.G. Jordan, J. Ultrastruct. Res. *52*, 85 (1975).
17 P.L. Paine, L.C. Moore and S. Horowitz, Nature *254*, 109 (1975).
18 T.P. Liu, Tissue Cell *4*, 493 (1972).

Cytochemical localization of surface carbohydrates on mycoplasma membranes[1]

H.-G. Schiefer, H. Krauss, U. Schummer, H. Brunner and U. Gerhardt

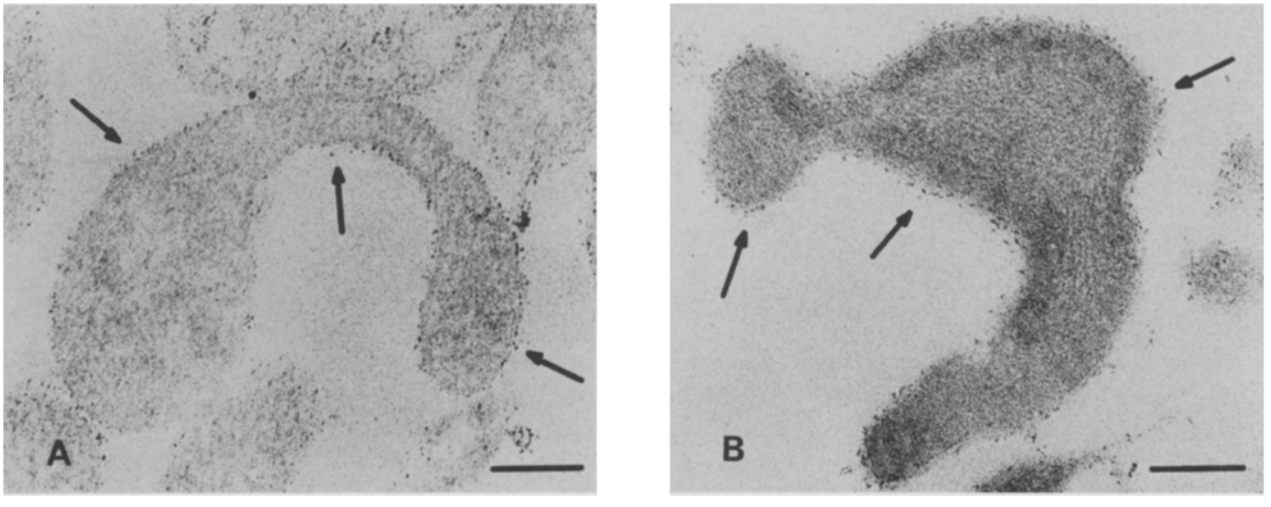
Institut für Medizinische Mikrobiologie, and Institut für Hygiene und Infektionskrankheiten der Tiere, Justus Liebig Universität, D-63 Giessen (Federal Republic of Germany), 6 February 1978

Summary. Surface carbohydrate structures, containing α-D-glucosyl or sterically closely related residues, were visualized on mycoplasma membranes by a cytochemical staining procedure with concanavalin A and iron-dextran complexes.

Mycoplasmas are the smallest microorganisms capable of autonomous growth. They possess no cell wall and intracytoplasmic membranes, but are endowed with a cytoplasmic membrane more stable than that of bacteria[2]. Many morphological and biochemical similarities exist between mycoplasma and mammalian plasma membranes[2]. For instance, in analogy to eukaryotic plasma mebranes, carbohydrates are exposed on the surface membranes of several mycoplasma species[2-5].

Surface carbohydrate structures on mycoplasma membranes were visualized in the electron microscope by applying a recently developed, sensitive staining procedure[6,7], which is based on the finding that concanavalin A (Con A) has at least 2 reactive sites: 1 binds to cellular carbohydrates containing α-D-glucosyl or sterically closely related residues[8], the 2nd acts as a receptor to bind glucose units of exogeneously added, electron-dense iron-dextran complexes.

Materials and methods. Mycoplasma neurolyticum type A (Sabin) Freundt and *Mycoplasma gallisepticum* Edward et Kanarek were kindly provided by J.G. Tully (Bethesda, USA) and E.A. Freundt (Aarhus, Denmark), respectively[3]. The mycoplasmas were grown and harvested, as described previously[5]. Intact mycoplasmas (2 mg of protein) were suspended in 2 ml of phosphate buffered saline (PBS), containing 0.002 M phosphate, pH 7.0, and 0.145 M NaCl, and allowed to react with 1 mg of Con A/ml for 60 min at 22 °C. After centrifugation, the mycoplasmas were washed



Mycoplasma neurolyticum (A) and *Mycoplasma gallisepticum* (B) labelled by the cytochemical staining procedure with Con A and iron-dextran particles. Cells were not contrasted by lead citrate and uranyl acetate. Marker of surface carbohydrate structures uniformly distributed over the entire membrane surface (arrows). Bar, 100 nm.