PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

One-step Isolation and Characterization of Nuclear Membranes

Ariane Monneron

Phil. Trans. R. Soc. Lond. B 1974 **268**, 101-108
doi: 10.1098/rstb.1974.0018

Phil. Trans. R. Soc. Lond. B. **268**, 101–108 (1974) [101]
Printed in Great Britain

One-step isolation and characterization of nuclear membranes

By Ariane Monneron
Institut Pasteur, Paris, France

[Plates 36–37]

Purified fractions of nuclei from rat liver and isolated calf thymocytes are treated with 0.5 M $MgCl_2$ or with an alkaline buffer, pH 8.5. Such procedures dissociate nuclear membranes from the chromatin. The membranes are simply floated in a gradient. The recovery in the membrane band obtained by the Mg^{2+} technique is 55 to 60 %. Freeze-etching studies of the membrane pellets provide a good way to check their purity, pores being very conspicuous. A 5′-nucleotidase and an ATPase are detected on the nuclear membranes of thymocytes, as well as a still undescribed 3′-nucleotidase. However, no marker enzymes for the nuclear membrane have been found. The topology of sugar residues on the nuclear membranes is studied by means of lectins. Concanavalin A binds in large amounts to nuclear envelopes, both on the inner and outer leaflets, but not to pores.

It is of interest to try to isolate and purify nuclear membranes, because such structures are endowed with a set of functions not shared with other cytoplasmic or plasma membranes. They indeed represent the defensive wall for the cell's genetic material that has to be kept sheltered from the fluctuations of everyday life. Chromatin itself is firmly anchored on its inner surface. However, such a stronghold would be meaningless without highly selective 'doormen' sorting out and forwarding messages to be sent in and out of the nucleus. The holes in the nuclear envelope, the pores, are thus just as important to study as the membrane itself, although their function appears to be exactly opposite. When pore complexes have been successfully isolated, they will probably display highly selective enzymic equipment. However, the fascination of pores should not polarize us to the point of forgetting that the membrane itself, after all, may well bear receptors for hormones or other signals, the study of which has yet to be developed.

Another property peculiar to the nuclear membrane is its intriguing disappearance at each mitosis – not common behaviour for other membranes. Since nuclear membranes are reconstituted at each telophase, this process should be rewarding to study as a special model of membrane synthesis.

Nuclear membranes may have properties that vary with the cell cycle, or condition. Plasma membranes are widely studied now in this respect. The properties of both types of membranes should be compared, more especially as nuclear membranes often contaminate plasma membrane fractions, where they are overlooked due to their lack of known specific marker enzymes.

Finally, pathological processes affect nuclear membranes, e.g. when they are crossed by viruses leaving the nucleus on maturation.

These and many other problems warrant attempts to isolate nuclear membranes in simple, quick and quantitative ways.

We present here two such methods, based on the peeling off of the chromatin from the nuclear membranes by simple chemical treatment of the nuclei, which allows at the same time the one-step flotation of the membranes in a gradient. In one case, a high concentration of divalent cations is used, in the other, an alkaline pH.

Purification of nuclei

Purification of nuclear membranes is made relatively simple, as nuclei may be prepared in fractions almost devoid of other membranes. Indeed, the degree of purity of nuclear membranes depends on that of the nuclei. Ideally, such a situation avoids the need for marker enzymes.

A good principle for the isolation of nuclei is to spin them down from a homogenate through layers of sucrose (Blobel & Potter 1966). Since the nuclei are usually the densest structures, the contaminating cytoplasmic components do not reach the dense sucrose layer in which nuclei collect. Retention of cytoplasmic material and incomplete disruption of cells should of course be avoided. Such a technique yielding clean liver nuclei has been used for further preparation of liver nuclear membranes (Kashnig & Kasper 1969; Monneron, Blobel & Palade 1972). Procedures for isolating liver nuclei based on other principles (Chauveau, Moulé & Rouiller 1956; Widnell & Tata 1964) have also been used for the preparation of nuclear membranes, usually in combination with techniques derived from Blobel & Potter's work (Berezney, Funk & Crane 1970; Franke *et al.* 1970; Agutter 1972; Berezney, Macaulay & Crane 1972; Kay, Fraser & Johnston 1972). Some of these are very lengthy. Still other techniques have been devised for cultured cells (Penman 1966).

The case of isolated lymphocytes is critical, as these very small cells are difficult to disrupt without damaging their comparatively huge nuclei. We homogenized the cells in a dense sucrose solution (70%, w/v, in TKM buffer: 0.05 M tris-HCl, pH 7.5, 0.025 M KCl, 0.005 M $MgCl_2$) by use of a Potter Teflon glass homogenizer (size C), with eight up and down strokes at high speed. As judged by e.m. control sections, the shearing forces are strong enough to break the cells without damaging the nuclei. The technique is then derived from Blobel & Potter's: the homogenate is adjusted to a sucrose concentration of 10 to 20%, layered over 1.65 M and 2.1 M sucrose, and centrifuged for 30 min at 135000 *g* in a SW 27 rotor. The nuclear pellet is washed twice in TKM, mixed with glycerol and stored at −20 °C. Figure 3, plate 36, shows a section through such a pellet. Nuclear pellets should be routinely checked for contamination by enzymic determinations. Small numbers of mitochondria are sometimes present in Blobel & Potter's derived techniques. By comparison plasma membranes and other structures usually contaminate nuclear pellets obtained by other techniques, especially differential centrifugation. Great care has to be taken in the choice of plasma membrane-marker enzymes (De Pierre & Karnovsky 1973).

One-step isolation of nuclear membranes

Once the purity of the nuclear preparation is considered satisfactory, the membranes have to be prepared. Published techniques include: the disruption of nuclei by sonication (Zbarsky *et al.* 1969; Agutter 1972), or by a combination of sonication and high concentration of salt (Kashnig & Kasper 1969; Franke *et al.* 1970), or by incubation with DNAse, with or without subsequent treatment with high concentrations of salt (Ueda, Matsuura & Date 1969; Berezney *et al.* 1970; Chardonnet & Dales 1972; Kay *et al.* 1972). Many of these techniques require several steps and consequently are lengthy; the yield of membranes is often not stated, or does not appear to be high; and the use of enzymes or sonication is not always easy to control.

We present here two techniques allowing a one-step isolation of nuclear membranes, one based on the use of relatively high concentrations of divalent ions, the other, on the use of an

alkaline pH. The first one, already published (Monneron *et al.* 1972), relies on the fact that by use of electrolytes only, one can dissociate the nuclear envelope from its bound chromatin. Rat liver nuclei were thoroughly mixed in centrifuge tubes with a small volume of 1.8 M sucrose, 0.5 M in $MgCl_2$, 0.05 M in tris-HCl, pH 7.5. The suspension immediately became homogeneous and very viscous. E.m. studies showed that well-preserved nuclear envelopes and smaller

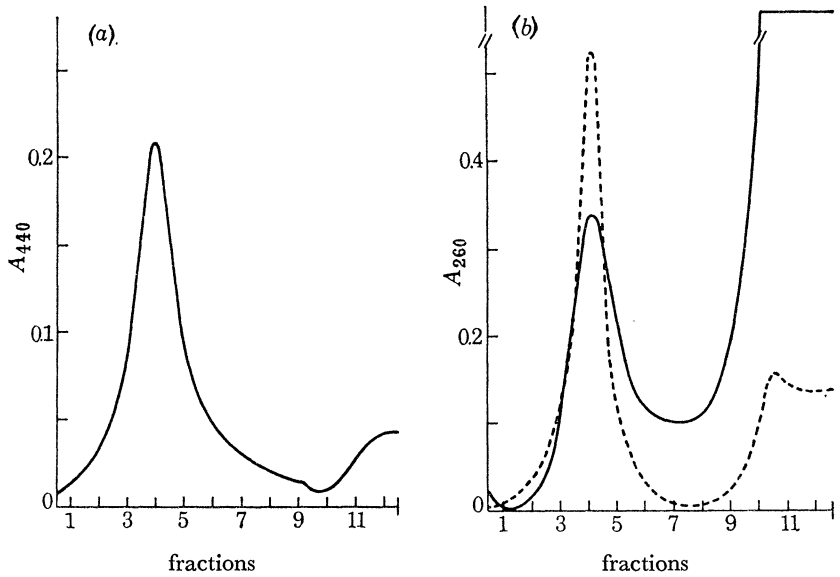


FIGURE 1. Rat liver nuclear membranes prepared by the high Mg^{2+} technique. Continuous gradient in 0.5 M Mg^{2+} Cl_2 sucrose. Recording at, left, 440 nm, right, 260 nm. Only the sharp membranous peak is recorded at 440 nm, whereas, at 260 nm, the bottom of the tube, containing the DNA, shows a strong absorption. The dotted line corresponds to the recording of the isolated membrane fraction recentrifuged in a continuous gradient (20 to 55%).

membrane vesicles were freed from their previous association with chromatin. The nuclear suspension was overlayered by a linear sucrose gradient having the same ionic composition and pH as the load. After a short centrifugation (190000*g* average, 2 h), a milky band of membranes was found suspended in the gradient at a relative density of 1.17 to 1.18 (figure 1). Assuming that practically all the nuclear phospholipids are located in the membranes, the recovery of membranes, in the gradient was 74%, and 55–60% in the sharp membranous band. The composition of this band was 23 ± 2% phospholipids, 73 ± 4% proteins (phospholipid: protein, 0.31), 3 ± 1% RNA and 0.6 ± 0.2% DNA (carbohydrates were not studied).

Other divalents ions, such as calcium, could be used as effectively. Replacement of 0.5 M $MgCl_2$ by 1M KCl also yielded very clean membranes. However, the flotation of the membranes took a longer time, and the recovery was not as high as with $MgCl_2$.

Such a technique, based on the sole use of electrolytes, can be applied to a wide range of cells. However, we encountered difficulties when working with calf thymocyte nuclei. The concentration of DNA in such nuclei is indeed very high, due to their small size (about 4 to 5 μm in diameter, as compared to 7 to 10 μm in rat liver cells). The viscosity of a thymocyte nuclear preparation treated with a high Mg^{2+} concentration is such that it entraps nuclear envelopes to a great extent, reducing the recovery of membranes in the gradient. Osmotic shock was ineffective in breaking such nuclei. Different pH values were tested, and it was found that a pH of 8.5 leads to the dissociation of chromatin from nuclear envelopes, without any increase in

the viscosity of the suspension. Nuclei were mixed with 1.8 M sucrose in TKM buffer, pH 8.5, and submitted to eight strokes in a Potter homogenizer at full speed. The suspension was overlayered by a linear, or by a discontinuous gradient, in sucrose solutions of pH 8.5, and centrifuged for 2 h at 135000*g*. A milky band was found at a sucrose density of 1.19 to 1.20 (figure 2). The recovery of membranes in this band, based on the recovery of phospholipids was 35 to

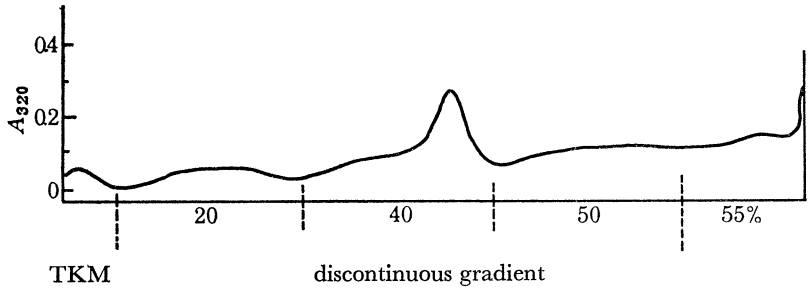


FIGURE 2. Calf thymocyte nuclear membranes prepared by the pH 8.5 method. Recording at 320 nm. Discontinuous gradient in TKM-sucrose pH 8.5.

40%; the composition of the membranes was $65 \pm 4\%$ proteins, $21 \pm 2\%$ phospholipids (phospholipid: protein, 0.32), $14 \pm 2\%$ RNA and 0.0% DNA (carbohydrates not studied). We thus obtained thymocyte nuclear membranes freed from nuclear content by means of an alkaline pH only, without treating nuclei with DNAse, as in Kay *et al.*'s work (1972).

CHARACTERIZATION OF NUCLEAR MEMBRANES

Published techniques of nuclear membrane isolation usually include ultrastructural and biochemical characterizations. We will discuss here some points of ultrastructure, of cyto- and immuno-cytochemistry, and a few data concerning the binding of lectins.

Ultrastructural data

Sections through pellets of nuclear membranes are shown in figure 4, plate 36 (method using a high $MgCl_2$ concentration), and figures 5 and 6, plates 36 and 37 (pH 8.5 method). The first technique yields clean membranes, without any adherent material; vesicles of different sizes are swollen and contain tubular structures. This aspect is due to the hypotonicity of fixatives (Monneron *et al.* 1972). The second technique yields extremely contorted or tubular membranous structures, and ribosomes, still bound to the vesicles derived from the outer nuclear membrane, are numerous. Such membranes can be washed by centrifugation in appropriate salt solutions. Pores are the natural markers of nuclear envelopes, no other membranous system, with the exception of annullate lamellae, displaying them. In thin sections of nuclear membranes, they may be difficult to recognize, due to the profound structural changes occurring during homogenization and osmotic, or physicochemical treatments. Nuclear envelopes are usually fragmented into much smaller vesicles that seal themselves, and pores may be torn or extremely distended (figure 6, plate 37). However, when large sheets of membranes are tangentially sectioned, pores may be clearly seen (figure 4, plate 36) (Monneron *et al.* 1972). Apart from pores, no other features differentiate the two sheets of the nuclear envelope; rough endoplasmic reticulum is comparable to the outer leaflet, and smooth endoplasmic reticulum to the inner leaflet. Pores are somewhat easier to recognize in negatively stained preparations, as

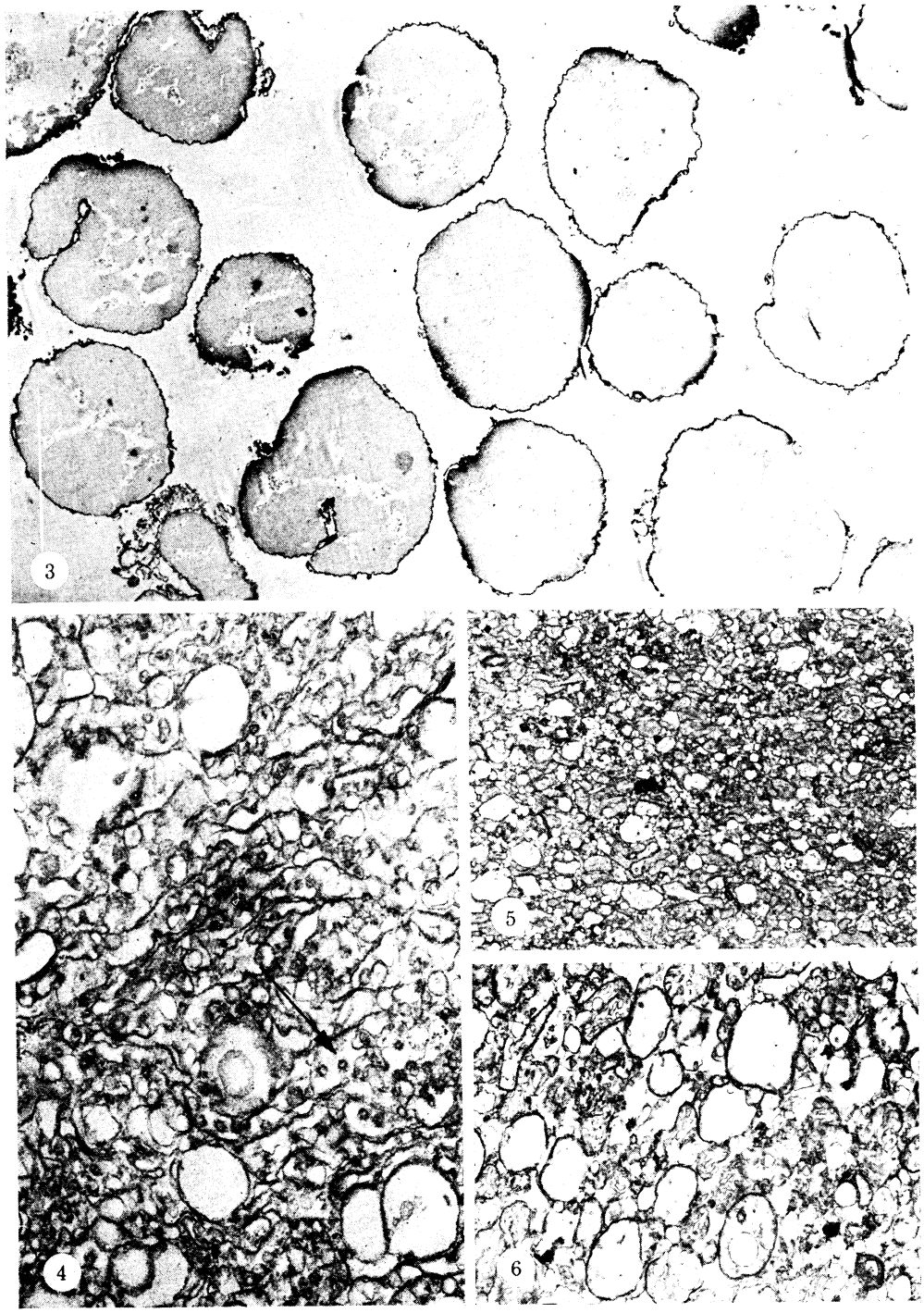


FIGURE 3. Calf thymocyte nuclei, prepared from isolated cells, as described in the paper. Unstained section. The nuclear envelope is dense, due to its binding of concanavalin A. (Nuclei treated with the lectin before e.m. processing). (Magn. $\times 6700$.)

FIGURE 4. Nuclear membrane pellet obtained through the high Mg^{2+} method. Fixation: glutaraldehyde and OsO_4. The arrow points to pores seen in front view. (Magn. $\times 22\,000$.)

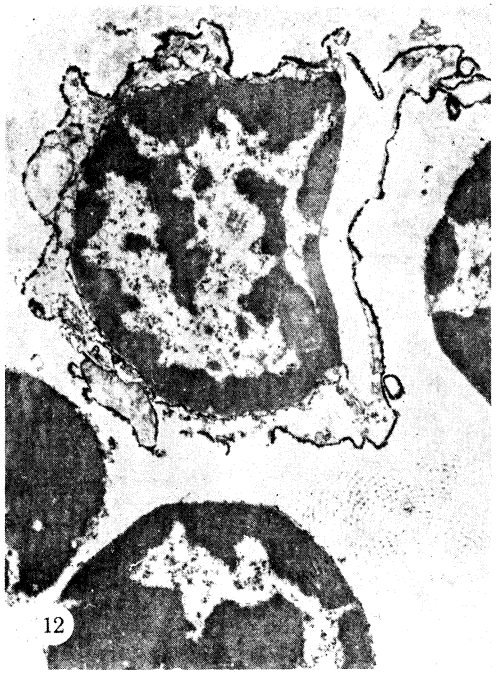
FIGURE 5. Nuclear membrane pellet obtained by the pH 8.5 method. Same fixation as in figure 4. Membranes are very contorted and ribosomes are numerous. (Magn. $\times 4600$.)

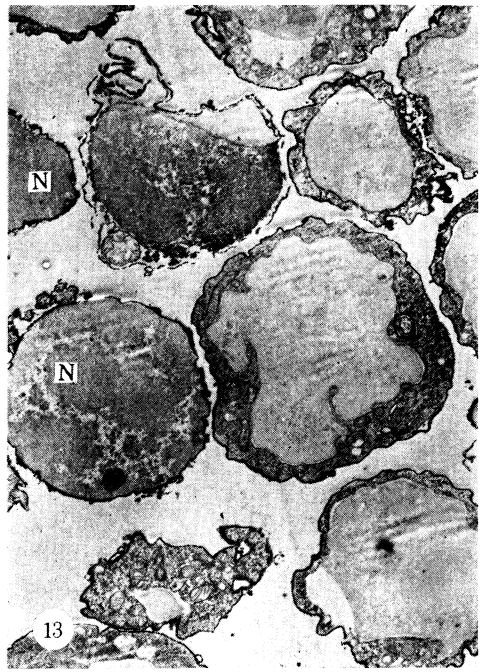
FIGURE 6. Higher magnification of the same preparation, showing budding of the vesicles which are filled with tubules or smaller vesicles. (Magn. $\times 10\,000$.)

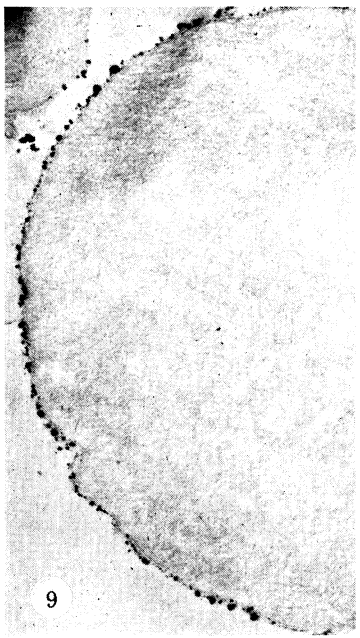
(*Facing p.* 104)

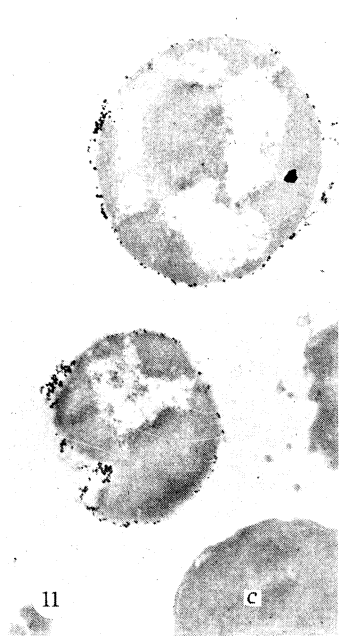
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES









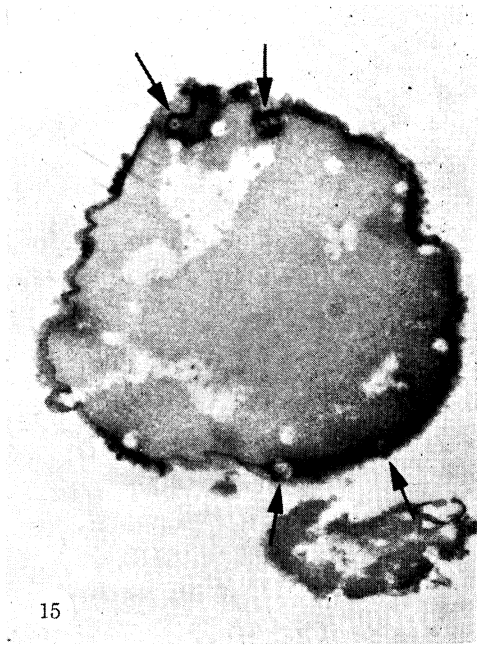


FIGURE 7–15. For description see opposite

shown by several beautiful pictures (Franke 1966; Franke & Scheer 1970; Harris & Agutter 1970; Price, Harris & Baldwin 1972; Kay *et al.* 1972). However, this technique does not permit contamination to be evaluated. A very useful technique that may contribute to a better understanding of membrane fractions is freeze-etching. As the fracture plane follows that of the membrane, many more pores are seen than in sections, and they are easy to recognize, as pits or mounds, on the fractured planes as well as on etched surfaces (figures 7 and 8, plate 37). Swollen vesicles are seen, where long channels originating from distended pores are prominent. A study of the different views from the fractured surfaces has been published (Monneron *et al.* 1972). Freeze-etching should be used more often to check the purity of such membrane fractions, as it is able to characterize quickly and in great detail a large number of the isolated structures.

Enzymes associated with nuclear membranes

No specific marker-enzyme has yet been assigned to nuclear membranes. In this respect, the inner and the outer leaflet of the envelope are probably very different, and pores represent still another area. Several enzymes existing in the endoplasmic reticulum have been found in nuclear membrane fractions, with, sometimes, a lower yield than in microsomes (Kashnig & Kasper 1969; Ueda *et al.* 1969; Franke *et al.* 1970; Kasper 1971; Zentgraf, Deumling, Jarasch & Franke 1971; Agutter 1972; Berezney & Crane 1972; Berezney, Macaulay & Crane 1972; Kay *et al.* 1972). Some autoradiographical and biochemical data have lead to the hypothesis that DNA polymerase could be associated, at least temporarily, with the nuclear membrane. (Comings & Kakefuda 1968; Hanaoka & Yamada 1971; Mizuno, Stoops & Peiffer 1971; Yoshida, Modak & Yagi 1971; Hatfield 1972; O'Brien, Sanyal & Stanton 1972; Infante *et al.* 1973). These data are very controversial (Williams & Ockey 1970; Kay, Haines & Johnston 1971; Deumling & Franke 1972; Fakan, Turner, Pagano & Hancock 1972; Freienstein, Freitag & Süss 1973). We have tested rat liver nuclei from partially hepatectomized rats in *in vitro* systems allowing the incorporation

Description of plate 37

Figures 7, 8. Views of a freeze-etched pellet of nuclear membranes obtained by the high Mg^{2+} method. In figure 7, only small areas of vesicles are seen, but pores may be recognized in many of them. In figure 8, the fracture plane and the etching reveals a larger sheet of nuclear envelope. (Magn.: figure 7, ×25000; figure 8, ×29000.)

Figure 9. Thymocyte nucleus incubated in a Wachstein–Meisel type of medium containing 5′-TMP. The nuclear envelope is covered with lead phosphate deposits. (Unstained section; magn. ×12000.)

Figure 10. Thymocyte nucleus incubated with 3′-TMP. (Unstained section; magn. ×13000.)

Figure 11. Suspension of thymocytes and nuclei incubated with ATP (in the presence of 10 mM Mg^{2+}, and without K^{+}). The nuclear envelopes are labelled (c, cell). (Unstained section; magn. ×6000.)

Figure 12. A suspension of calf thymocytes and nuclei treated first with rabbit anti-calf thymocyte serum, then with sheep anti-rabbit IgG coupled with peroxidase. The plasma membrane is labelled, whereas the nuclear envelope is unlabelled. Section stained with uranyl acetate and lead. (Magn. ×12000.)

Figure 13. Suspension of calf thymocytes treated with concanavalin A. The lectin is revealed by the peroxidase reaction, using diaminobenzidine. The two nuclei seen in this field have their envelope stained at least as much as the plasma membrane of neighbouring cells. (Fixation: glutaraldehyde and osmic acid; unstained section; magn. ×5100.)

Figure 14. Calf thymocyte nuclear preparation treated with concanavalin A. The arrow points to a pore. The labelling is interrupted at this level. (Unstained section; magn. ×18000.)

Figure 15. Same preparation as figure 14. Tangential sections of pores (arrows) show that no concanavalin A is revealed inside the pore (except for the central granule which has not yet been studied in this respect). Section lightly stained with lead. (Magn. ×18000.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

of [^{3}H]dTTP in acid-insoluble material, according to Bollum's technique (1968). Nuclei were very active. Nuclear membranes prepared from such nuclei did not contain any radioactive constituent, and isolated nuclear membranes incubated in a similar medium did not incorporate any radioactivity, either with or without added native liver primer DNA. Indeed, autoradiography of the labelled nuclei did not show any predominent labelling of the nuclear envelope region. However, one cannot conclude from these experiments that DNA polymerase is not a membrane-bound enzyme. It may be there transiently or it may be denatured by the fractionation procedure, or else some other essential enzymes or ligands may have been removed or damaged.

In the course of a cytochemical study of thymocyte plasma membranes (Monneron 1974), we observed several enzymes to be active on the nuclear envelopes. Enzymic hydrolysis of 5′-TMP was shown (figure 9, plate 37), lead precipitates being much more abundant on nuclei than on whole cells. This 5′-nucleotidase activity was also demonstrated with 5′-AMP, although to a lesser extent. 3′-TMP was hydrolysed at the level of the nuclear membrane, (figure 10, plate 37). However, we have also demonstrated this activity at the plasma membrane of some thymocytes. Finally, an ATPase activity, not dependent upon K^+ or Na^+, was regularly found (figure 11, plate 37). This result should be compared with Chardonnet & Dales (1972), who found an APTase activity on the nuclear envelope of Hela cells, mainly at the pore level. Such observations do not afford specific enzymic markers for nuclear membranes, but underline the fact that contamination of plasma membranes by nuclear membranes and vice versa is not easily assessed on the basis of so-called enzymic markers.

Immunochemistry and immunocytochemistry should help in such studies. In an attempt to purify thymocyte plasma membranes, we have isolated rabbit antibodies against the surface of whole calf thymocytes. These antibodies were thoroughly adsorbed with calf liver homogenate. The resulting antiserum reacted strongly with plasma membranes, but to a lesser extent with nuclear membranes, as shown both by biochemistry and by immunocytochemistry, using sheep anti-rabbit IgG coupled with peroxidase (figure 12, plate 37) (Monneron 1973). Antibodies directed against purified nuclear membranes are being prepared.

Binding of lectins to nuclear membranes

Lectins such as concanavalin A and wheat-germ agglutinin have been shown to induce the aggregation of nuclei (Nicolson, Lacorbière & Delmonte 1972). Specific carbohydrate residues have thus been demonstrated on nuclear envelopes, which is in agreement with other chemical data (Kashnig & Kasper 1969; Keshgegian & Glick 1973). However, to our knowledge, no quantitative data are available, nor has the precise location of the lectin-binding sites on the nuclear envelope been shown. We have treated thymocytes and thymocyte nuclei with concanavalin A, which was then titrated, or demonstrated at the e.m. level by means of the Avraméas (1970) peroxidase technique. Isolated nuclei that contaminated whole cell preparations were rather more labelled by concanavalin A than were plasma membranes (figure 13, plate 37). Pure preparations of thymocyte nuclei bound as much or more ($1\frac{1}{2}$ times as much) concanavalin A than an equivalent number of whole thymocytes. All nuclei bound concanavalin A (figure 3), and both leaflets of the nuclear envelope were labelled, the outer one apparently more heavily. The concanavalin labelling was usually interrupted at the pore level (figures 14 and 15, plate 37). The nuclear surface of the inner leaflet of the membrane was never labelled. Treatment of nuclei with detergents, such as Triton X-100 and DOC (as

described by Penman (1966) but at a much lower concentration) prevented the binding of concanavalin A to nuclear membranes. Hence, large amounts of specific sugar residues can be detected in nuclei, and are localized mainly, if not exclusively, on nuclear membranes. We submitted isolated nuclei to concanavalin A at 37 °C for 1 h. While control thymocytes showed obvious cap formation, the distribution of concanavalin A binding-sites on the nuclear membranes did not seem to be modified. However, the complexity of the nuclear envelope made images difficult to interpret. It is not surprising, however, that displacement of carbohydrate-containing structures should be minimal in such a membrane, interrupted as it is by pores and with its chromatin tightly bound to one of its leaflets, ribosomes to the other. Other lectins should be used in combination with freeze-etching studies.

The nuclear envelope obviously plays a very important role in the control mechanisms of cell metabolism, by itself and by its pores. Very little is known yet about its structure, composition and enzymic equipment, nor about how it changes with the cell cycle, cellular development and in pathological conditions. Study of the plasma membrane is, in these respects, far ahead. It becomes increasingly more necessary to compare carefully both types of membranes, and therefore simple and rapid techniques yielding well-controlled purified fractions are essential. In order that the fractions be representative, the yield of membranes should be known, and be as high as possible, and several criteria should be used to ensure its purity. The one-step isolation procedures that we propose seems to provide nuclear membranes in an acceptable yield and purity.

I wish to thank J. C. Bénichou and D. Segrétain for technical assistance and support, and Dr A. Ryter for interest in this work. Supported in part by Centre National de la Recherche-Scientifique (Laboratoire Associé No. 88) and by Delegation Générale á la Recherche Scientifique et Technique (Contrat No. 71-7-3082).

References (Monneron)

Agutter, P. S. 1972 The isolation of the envelopes of rat liver nuclei. *Biochim. biophys. Acta* **255**, 397–401.

Avraméas, S. 1970 Immunoenzyme techniques: enzymes as markers for the localization of antigens and antibodies. *Int. Rev. Cytol.* **27**, 349–385.

Berezney, R. & Crane, F. L. 1972 Characterization of electron transport activity in bovine liver nuclear membranes. *J. biol. Chem.* **247**, 5562–5568.

Berezney, R., Funk, L. K. & Crane, F. L. 1970 The isolation of nuclear membrane from a large scale preparation of bovine liver nuclei. *Biochim. biophys. Acta* **203**, 531–546.

Berezney, R., Macaulay, L. K. & Crane, F. L. 1972 The purification and biochemical characterization of bovine liver nuclear membranes. *J. biol. Chem.* **247**, 5549–5561.

Blobel, G. & Potter, V. R. 1966 Nuclei from rat liver: isolation method that combines purity with high yield. *Science, N.Y.* **154**, 1662–1665.

Bollum, F. J. 1968 Deoxyribonucleotide polymerizing enzymes from calf thymus gland. *Methods in enzymology* (ed. S. P. Colowick & N. O. Kaplan), vol. 12, part B, p. 169. New York: Academic Press.

Chardonnet, Y. & Dales, S. 1972 Early events in the interaction of adenoviruses with Hela cells. III. Relationship between an ATPase activity in nuclear envelopes and transfer of core material: a hypothesis. *Virology* **48**, 342–359.

Chauveau, J., Moulé, Y. & Rouiller, C. L. 1956 Isolation of pure and unaltered liver nuclei. Morphology and biochemical composition. *Expl Cell Res.* **11**, 317–321.

Comings, D. E. & Kakefuda, T. 1968 Initiation of DNA replication at the nuclear membrane in human cells. *J. molec. Biol.* **33**, 225–229.

De Pierre, J. W. & Karnovsky, M. L. 1973 Plasma membranes of mammalian cells. A review of methods for their characterization and isolation. *J. Cell Biol.* **56**, 275–302.

Deumling, B. & Franke, W. W. 1972 Nuclear membranes from mammalian liver, V. *Hoppe-Seyler's Z. physiol. Chem.* **353**, 287–297.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Fakan, S., Turner, G. N., Pagano, J. S. & Hancock, R. 1972 Sites of replication of chromosomal DNA in a eukaryotic cell. *Proc. Natn. Acad. Sci., U.S.A.* **69**, 2300–2305.
Franke, W. W. 1966 Isolated nuclear membranes. *J. Cell Biol.* **31**, 619–623.
Franke, W. W., Deumling, B., Ermen, B., Jarasch, E. & Kleinig, H. 1970 Nuclear membranes from mammalian liver. I. Isolation procedure and general characterization. *J. Cell Biol.* **46**, 379–395.
Franke, W. W. & Scheer, U. 1970 The ultrastructure of the nuclear envelope of amphibian oocytes: a reinvestigation. *J. ultrastruct. Res.* **30**, 288–316.
Freienstein, C. M., Freitag, H. & Süss, R. 1973 Membrane-associated DNA ('M-band') in growing and confluent 3T3 cells. *F.E.B.S. Lett.* **30**, 170–172.
Hanaoka, F. & Yamada, M. 1971 Localization of the replication point of mammalian cell DNA at the membrane. *Biochem. biophys. Res. Commun.* **42**, 647–653.
Harris, J. R. & Agutter, P. 1970 A negative staining study of human erythrocyte ghosts and rat liver nuclear membranes. *J. ultrastruct. Res.* **33**, 219–232.
Hatfield, J. M. R. 1972 Investigation of a complex associated with replicating DNA in L cell nuclei. *Expl Cell Res.* **72**, 591–595.
Infante, A. A., Nauta, R., Gilbert, S., Hobart, P. & Firshein, W. 1973 DNA synthesis in developing sea-urchins: role of a DNA-nuclear membrane complex. *Nature New Biol.* **242**, 5–8.
Kashnig, D. M. & Kasper, C. B. 1969 Isolation, morphology and composition of the nuclear membrane from rat liver. *J. biol. Chem.* **244**, 3786–3792.
Kasper, C. B. 1971 Biochemical distinctions between the nuclear and microsomal membranes from rat hepatocytes. The effect of phenobarbital administration. *J. biol. Chem.* **246**, 577–581.
Kay, R. R., Haines, M. E. & Johnston, I. R. 1971 Late replication of the DNA associated with the nuclear membrane. *F.E.B.S. Lett.* **16**, 233–236.
Kay, R. R., Fraser, D. & Johnston, I. R. 1972 A method for the rapid isolation of nuclear membranes from rat liver. Characterization of the membrane preparation and its associated DNA polymerase. *Eur. J. Biochem.* **30**, 145–154.
Keshgegian, A. A. & Glick, M. C. 1973 Glycoproteins associated with nuclei of cells before and after transformation by a RNA virus. *Biochemistry* **12**, 1221–1226.
Mizuno, N. S., Stoops, C. E. & Peiffer, R. L. 1971 Nature of the DNA associated with the nuclear envelope of regenerating liver. *J. molec. Biol.* **59**, 517–525.
Monneron, A. 1973 A one-step isolation procedure for plasma membranes of cultured thymocytes. *Ninth International Congress for Biochemistry*, Stockholm.
Monneron, A. 1974 Ultrastructural cytochemistry of membrane-localized enzymes in thymocytes: elusive marker enzymes. *J. Histo. cytochem.* (submitted).
Monneron, A., Blobel, G. & Palade, G. E. 1972 Fractionation of the nucleus by divalent cations. Isolation of nuclear membranes. *J. Cell Biol.* **55**, 104–125.
Nicolson, G., Lacorbière, M. & Delmonte, P. 1972 Outer membrane terminal saccharides of bovine liver nuclei and mitochondria. *Expl Cell Res.* **71**, 468–473.
O'Brien, R. L., Sanyal, A. B. & Stanton, R. H. 1972 Association of DNA replication with the nuclear membrane of Hela cells. *Expl Cell Res.* **70**, 106–112.
Penman, S. 1966 RNA metabolism in the Hela cell nucleus. *J. molec. Biol.* **17**, 117–130.
Price, M. R., Harris, J. R. & Baldwin, R. W. 1972 A method for the isolation and purification of normal rat liver and hepatom a nuclear 'ghosts' by zonal centrifugation. *J. ultrastruct. Res.* **40**, 178–196.
Ueda, K., Matsuura, T. & Date, N. 1969 The occurrence of cytochromes in the membranous structures of calf thymus nuclei. *Biochem. biophys. Res. Commun.* **34**, 322–327.
Widnell, C. C. & Tata, J. R. 1964 A procedure for the isolation of enzymatically active rat-liver nuclei. *Biochem. J.* **92**, 313–317.
Williams, C. A. & Ockey, C. H. 1970 Distribution of DNA replicator sites in mammalian nuclei after different methods of cell synchronization. *Expl Cell Res.* **63**, 365–372.
Yoshida, S., Modak, M. J. & Yagi, K. 1971 DNA polymerase associated with nuclear membrane of calf thymus. *Biochem. biophys. Res. Commun.* **43**, 1408–1414.
Zbarsky, I. B., Perevoshchikova, K. A., Delektorskaya, L. N. & Delektorsky, V. V. 1969 Isolation and biochemical characteristics of the nuclear envelope. *Nature, Lond.* **221**, 257–259.
Zentgraf, H., Deumling, B., Jarasch, E. D. & Franke, W. W. 1971 Nuclear membranes and plasma membranes from hen erythrocytes. *J. biol. Chem.* **246**, 2986–2995.

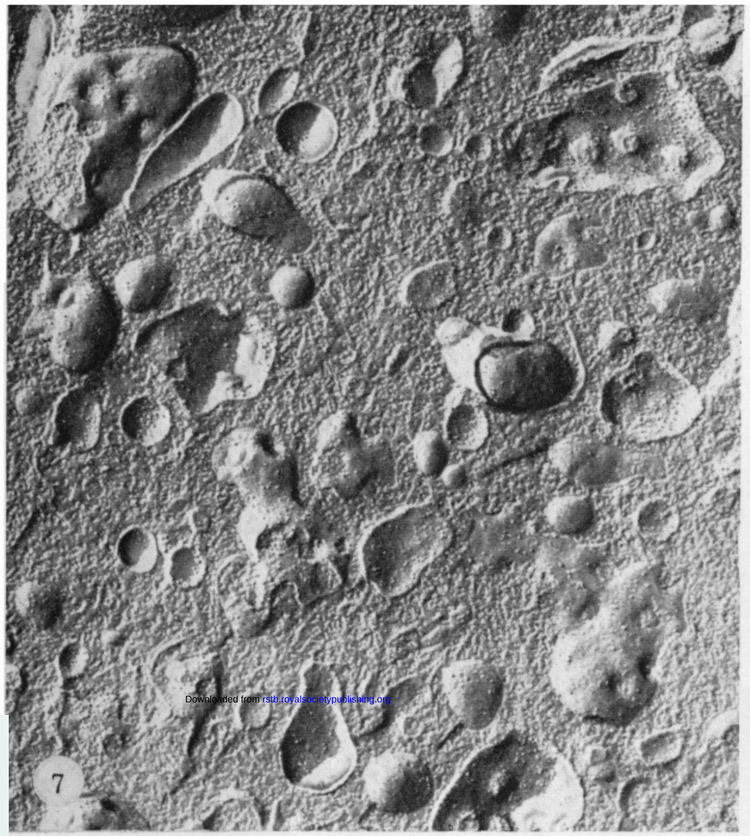
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

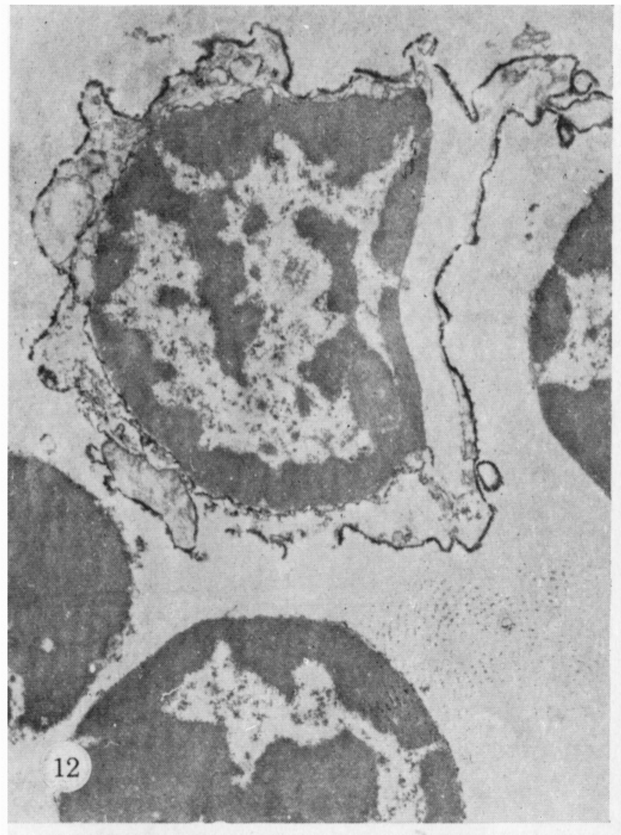
FIGURE 3. Calf thymocyte nuclei, prepared from isolated cells, as described in the paper. Unstained section. The nuclear envelope is dense, due to its binding of concanavalin A. (Nuclei treated with the lectin before e.m. processing). (Magn. $\times 6700$.)

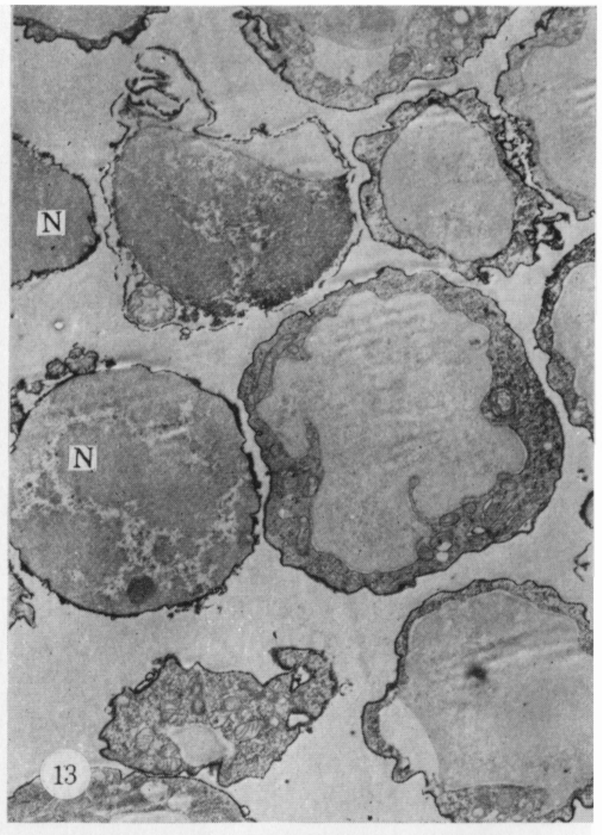
FIGURE 4. Nuclear membrane pellet obtained through the high Mg^{2+} method. Fixation: glutaraldehyde and OsO_4. The arrow points to pores seen in front view. (Magn. $\times 22\,000$.)

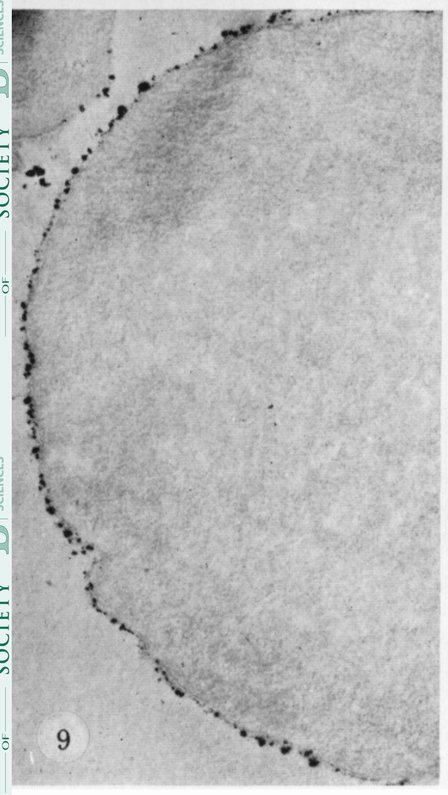
FIGURE 5. Nuclear membrane pellet obtained by the pH 8.5 method. Same fixation as in figure 4. Membranes are very contorted and ribosomes are numerous. (Magn. $\times 4600$.)

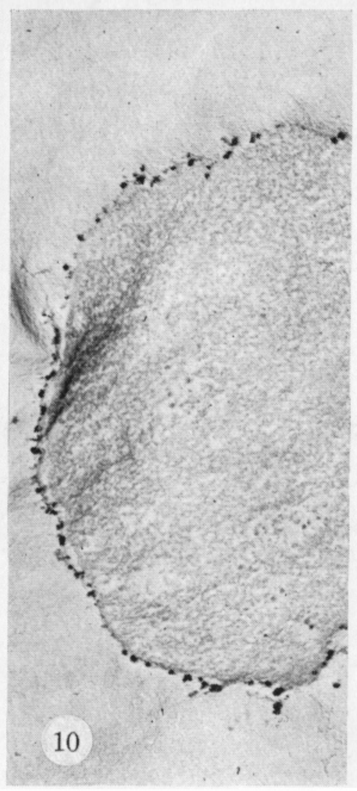
FIGURE 6. Higher magnification of the same preparation, showing budding of the vesicles which are filled with tubules or smaller vesicles. (Magn. $\times 10\,000$.)

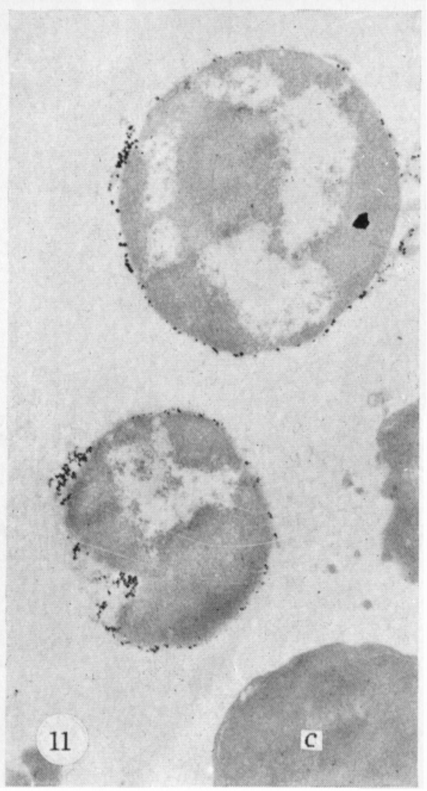


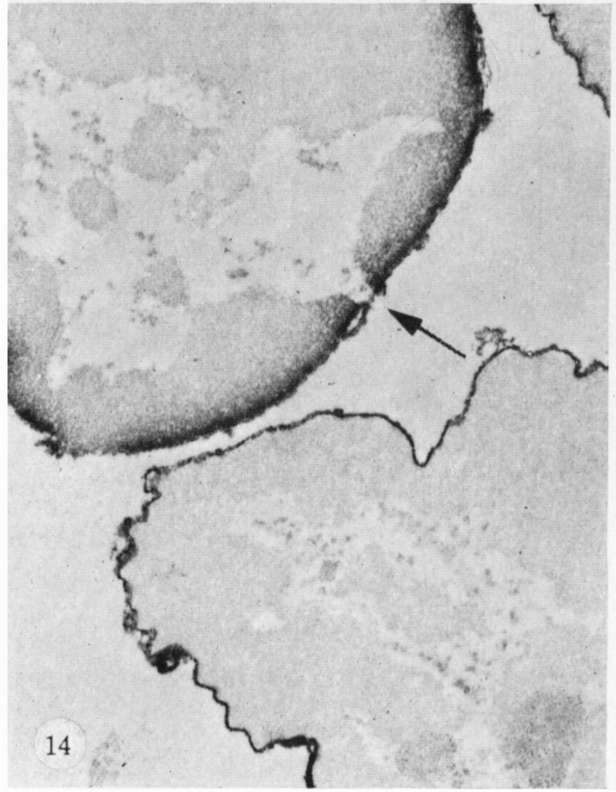












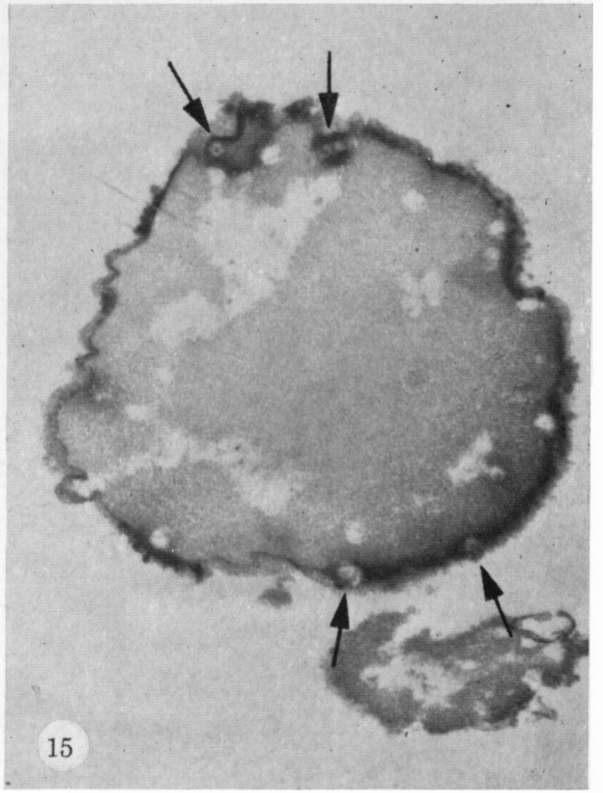


FIGURE 7–15. For description see opposite

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES