Phil. Trans. R. Soc. Lond. B. **268**, 67–93 (1974)
Printed in Great Britain

NUCLEAR ENVELOPES

Structure and biochemistry of the nuclear envelope

By W. W. Franke

Division of Membrane Biology and Biochemistry, Institute of Experimental Pathology, German Cancer Research Centre, Heidelberg, and Department of Cell Biology, Institute of Biology II, University of Freiburg, Freiburg i. Br., Germany

[Plates 23–33]

The ultrastructure of the nuclear evelope is described in various cell types with special emphasis on its pore complexes (p.c.). The architecture of the p.c. is defined against the properties of other membranous pore formations. Evidence is presented that the non-membranous p.c. components contain ribonucleoproteins but do not represent the attachment sites of nuclear chromatin. The possible dynamic nature of the p.c. material is discussed in relation to nucleocytoplasmic translocation processes. DNA of the nuclear genome is firmly attached to interporous sections of the inner nuclear membrane. The stability of this attachment is demonstrated, and chemical and conformational characteristics as well as periods and kinetics of replication are given for both isolated membrane DNA and the corresponding chromatin *in situ*. The membrane-associated chromatin is dominated by a heterochromatinous character; it does not represent a transitory membrane interaction of replicating DNA. It is hypothesized that membrane-attachment of specific regions of the chromosomes are a means to their ordered arrangement during interphase and prophase. Structure, lipid, protein and enzyme pattern of the nuclear membranes, as well as the incorporation kinetics, underline the relationship to the endoplasmic reticulum.

Introduction

Except for a few isolated remarks as to the significance of a 'membrane' around the cell nucleus and its physicochemical nature in studies of the earlier light microscopists, including some with polarized light (Hertwig 1893, 1906; Schmidt 1929, 1937; Chinn 1938) there was little experimental work on the nuclear envelope until 1950. Recent progress and the interest of cell biologists and biochemists in this membranous structure has come from three chief sources: (i) the advancement of electron-microscopic preparation techniques for biological material; (ii) the development of methods for isolating nuclear envelope material by either micromanipulatory techniques, mostly from giant nuclei, in rather good structural integrity (Callan & Tomlin 1950; Gall 1954, 1956, 1964, 1967; Merriam 1961, 1962; Franke & Scheer 1970*a*; Scheer 1972; Fabergé 1973) or, in a more or less fragmented form, by disrupting isolated nuclei (with mechanical shearing, sonication, chromatin swelling, the use of chelating agents) and/or removing the bulk nuclear nucleoproteins by limited DNAase digestion or high salt concentrations and then separating and collecting the envelope fragments by flotation or sedimentation in solutions or gradients of appropriate densities (Franke 1966, 1967; Bornens 1968; Kashnig & Kasper 1969; Zbarsky, Perevoshikova, Delektorskaya & Delektorsky 1969; Berezney, Funk & Crane 1970; Franke *et al.* 1970; for further references see the articles by Feldherr 1972; Matsuura & Ueda 1972; Monneron, Blobel & Palade 1972; Kay & Johnston 1973; Kessel 1973; Zbarsky 1972*b*; Franke 1974; Franke & Scheer 1974). (iii) Further, apart from the general cytological interest in this structure, which is quantitatively only a minor membrane component in most cell types, special interest has recently arisen from the widely discussed hypotheses that this envelope is not only a means for establishing the nucleo-cytoplasmic compartmentalization but

also plays a role in regulating such fundamental process as the functional ordering of chromosomes or distinct chromosomal regions (see, for example, Comings 1968; Hollande & Valentin 1968; Kubai & Ris 1969; Comings & Okada 1970; Engelhardt & Pusa 1972; Rae & Franke 1972; Rimpau & Lelly 1972; Franke & Scheer 1974), the replication (Comings 1968; Alfert & Das 1969; for recent critical discussions see: Blondel 1968; Williams & Ockey 1970; Deumling & Franke 1972; Fakan, Turner, Pagano & Hancock 1972; Barrieux, Long & Garren 1973; Comings & Okada 1973; Franke *et al.* 1973; Hubermann, Tsai & Deich 1973; Infante *et al.* 1973; Kay & Johnston 1973; Oppenheim & Wahrman 1973; Wise & Prescott 1973; Yamada & Hanaoka 1973; Franke & Scheer 1974), and the nucleocytoplasmic exchange of macromolecules, in particular of RNA and proteins (literature discussed in: Feldherr & Harding 1964; Gall 1964; Goldstein 1964, 1970; Verhey & Moyer 1967; Merriam 1969; Stevens & André 1969; Franke & Scheer 1970*b*; Bouteille 1972; Feldherr 1972; Paine & Feldherr 1972; Kay & Johnston 1973; Kessel 1973; Watts 1973; Franke & Scheer 1974).

The nuclear envelope as a part of the endoplasmic reticulum (e.r.)

The nuclear envelope is a cisterna (the 'perinuclear cisterna') consisting of the inner and outer nuclear membrane and the enclosed perinuclear space of variable (though for any given cell type somewhat characteristic) luminal width. The outer nuclear membrane exhibits, in many cell types, direct continuity with e.r. cisternae (figure 1), periplastidal cisternae (figure 2; see, for example, Gibbs 1962, 1970; Falk & Kleinig 1968) and annulate lamellae (AL, see below) and can have polyribosomes associated with its cytoplasmic fact. The inner membrane may show occasional continuities with cisternae located in the nuclear interior (these include the intranuclear annulate lamellae, INAL) and can be attached to chromatin and nucleolar structures.

Description of plate 23

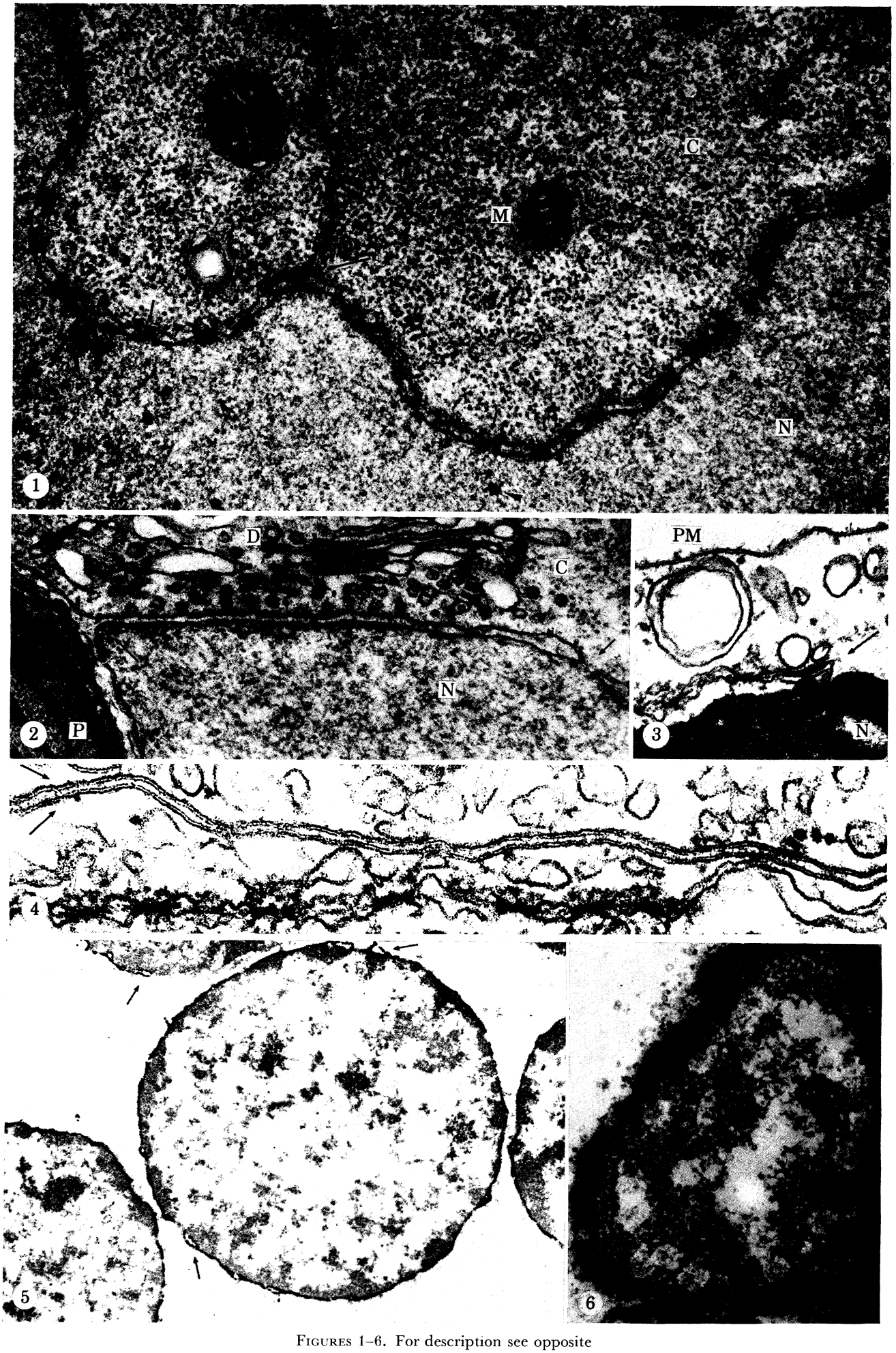
Figure 1. The nuclear envelope (here in an lampbrush stage oocyte of the clawed toad *Xenopus laevis*) defines the boundary between the nucleoplasm (N) and the cytoplasm (C). The cytoplasm is distinguished by its particles and organelles such as the mitochondria (M), ribosomes, and the membranes of the endoplasmic reticulum (e.r.) system which frequently are continuous with the outer nuclear membrane (a site of continuity is indicated, e.g., by the long arrow). The small arrows denote some nuclear pore complexes; the arrowheads point to some of the ribonucleoprotein aggregates. (Magn. × 54000.)

Figure 2. Continuity of nuclear envelope with the periplastidal cisterna in the Xanthophycean alga *Botrydium granulatum* (long arrow at the left). Note also the blebbing activity (vesicle formation) in the smooth surface section adjacent to the dictyosome (D). N, nucleoplasm, C, cytoplasm, P, plastid. The small arrow in the right denotes a nuclear pore complex. (Magn. × 49000.)

Figure 3. Local extension and folding back (indicated by the arrow) of the nuclear envelope during spermiogenesis of the newt, *Triturus alpestris*, surrounding the condensed pericentriolar nuclear pocket (N). PM, plasma membrane. (Magn. × 74000.)

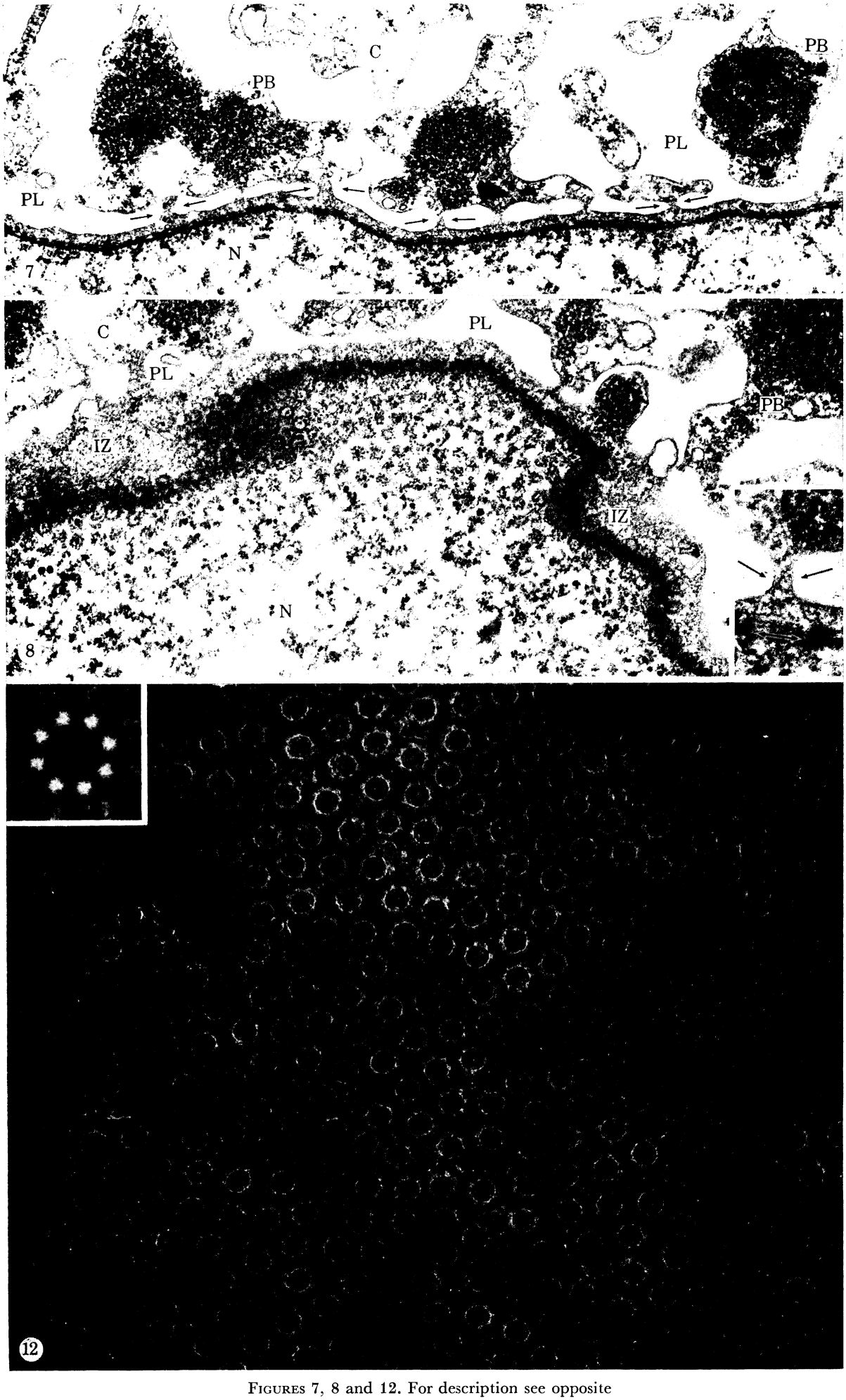
Figure 4. Membranes of the peripheries of two adjacent differentiating sperm cells (some as in figure 3). Note that the plasma membranes (denoted by the arrows) are broader and reveal a much clearer trilaminar 'dark–light–dark' (unit membrane) pattern than the internal membranes of the e.r. system and the nuclear envelope (a typical perinuclear cisterna with many pore complexes is shown in the bottom part of the figure). (Magn. × 113000.)

Figure 5, 6. Electron-microscopic localization of glucose-6-phosphatase activity in hepatocyte nuclei (for details of method see Kartenbeck *et al.* (1973) as seen in cross (figure 5) and tangential (figure 6) sections. Note that the activity is associated with the entire nuclear envelope but is excluded from the pore complexes (e.g. figure 6). The arrows denote sites in which inner and outer nuclear membrane are somewhat separated, thus demonstrating that this enzyme activity is associated with both membranes (Magns: figure 5, × 40000; figure 6, × 57000.)



FIGURES 1–6. For description see opposite

(*Facing p.* 68)



FIGURES 7, 8 and 12. For description see opposite

Commonly the nuclear envelope is thus envisaged as a locally differentiated cisterna of the whole e.r. which excludes the characteristic cytoplasmic membranous organelles and components (such as mitochondria, plastids, dictyosomes, lysosomes, vacuoles and vesicles, peroxisomes, endosymbionts) but also non-membranous components (such as ribosomes, glycogen and other particulate storage polysaccharides, lipid droplets, centrioles) from the compartment in which the nuclear genome (the chromosomes) and its associated structures, including the primary transcription products, are located (figure 1). This separation of nucleoplasm and cytoplasm, however, is not found in all interphase cells, in particular not in many cell types with RNA-synthetically inactive nuclei such as various spermiogenetic stages (see, for example, Robison 1966; Moses & Wilson 1970; Langreth 1969; for further references see Franke 1974, and Franke & Scheer 1974), and consequently is not ultimately critical for cellular viability.

In addition, this strict barrier function of the nuclear envelope is not without exceptions. In a great many cell types and/or physiological changes one finds not infrequently nuclei which do contain lipid droplets, or glycogen, or membranes of the e.r. (or Golgi) type, most characteristically again in unbalanced cell types or stages such as during tumour growth (e.g. figure 17 to 19; for references see Franke & Scheer 1974). Even the existence of intranuclear (probably symbiotic) bacteria has been reported (for literature see the work on euglenoids: Leedale 1969). There is also an increasing number of references describing partial or preferential breakdown of the nuclear envelope in various cytopathological situations (David 1964; Blackburn 1971; Zentgraf & Franke 1974).

Apart from its obvious functioning, in most cells, as a 'semipenetrable' nucleo-cytoplasmic barrier the quantitative contribution of the nuclear envelope to the functions of the whole e.r. is apparently negligibly low in most cell types. The close morphological relationship of the nuclear envelope and the e.r. has recently been substantiated by findings of far-reaching homologies in structure and chemical composition. Like the rough e.r. membranes both nuclear membranes appear, in many cells, to be somewhat thinner after the conventional electron-microscopical preparation techniques (e.g. 6 to 8 nm in thin sections, *ca.* 10 nm in freeze-etch; Kartenbeck, Zentgraf, Scheer & Franke 1971; Morré, Mollenhauer & Bracker 1971) than, for example, dictyosomal and plasma membranes and usually reveal less clearly the classic tri-

---

Description of plate 24

Figures 7, 8. During its growth the primary nucleus of the green alga, *Acetabularia mediterranea*, develops a special perinuclear lacuna (PL) which is seen in cross-section in figure 7 and in grazing section in figure 8. Here the sequence of nucleo-cytoplasmic zones is: (i) nucleoplasm (N); (ii) nuclear envelope (indicated, for example, by pores in figure 8 and the two-sided arrows in the inset in figure 8); (iii) intermediate zone (IZ) which is sandwiched between the primary ('true') nuclear envelope and the perinuclear lacuna ('secondary nuclear envelope'); (iv) the perinuclear lacuna (PL) which is also interrupted by pores (indicated by the pairs of arrows in the figures); (v) a juxtanuclear cytoplasmic (C) zone which is characterized by the frequency of aggregates of dense particles and fibrils (perinuclear bodies, PB). Note that the typical cytoplasmic structures and organelles (including the ribosomes) are excluded not only from the nucleoplasm but also from the intermediate sandwich zone. (Magns: figure 7, × 21 600; figure 8, × 28 000; inset in figure 8, × 54 000.)

Figure 12. Negatively stained (PTA) part of a large nuclear envelope piece manually isolated from a *Xenopus laevis* oocyte (lampbrush stage; for details of preparation see Franke & Scheer 1970*a*). Under the conditions used here (absence of divalent cations) some of the non-membranous pore complex material has been lost, thus leaving the pore outline especially clear. In preparations in which the annular granules are preserved one usually finds them arranged in an eightfold symmetry (the insert shows a Markham rotation analysis of a negatively stained nuclear envelope isolated from onion root tip). (Magn. 57 500.)

lamina 'unit membrane' substructure (figure 4; Grove, Bracker & Morré *et al.* 1968). In the terminology of Morré and co-workers (1971), the nuclear membranes represent the least differentiated (or 'least matured') state in the prototypical sequence of membrane transformation: nuclear envelope (rough e.r.)–smooth e.r.-Golgi apparatus–secretory vesicles–plasma membrane. Both the relationship to the e.r., in particular to the rough e.r., and the difference from plasma membrane and, to lesser degree, from dictyosomal membrane (and, of course, from other organelle membranes like inner mitochondrial and plastidal membrane) is reflected by the lipid composition of the nuclear envelope (e.g. low cholesterol and sphingomyelin; little, if any, cardiolipin; little, if any, glycolipid; high phosphatidylcholine; e.g. Gurr, Finean & Hawthorne 1963; Lemarchal & Bornens 1969; Keenan, Berezney, Funk & Crane 1970; Franke *et al.* 1970; Kleinig 1970; Khandawala & Kasper 1971; Kleinig, Zentgraf, Comes & Stadler 1971; Stadler & Kleinig 1971; Zentgraf, Deumling, Jarasch & Franke 1971; Keenan, Berezney & Crane 1972; Sato, Fujii, Matsuura & Ueda 1972; Jarasch *et al.* 1973; Franke 1974; Kasper 1974), and by its protein and enzyme activity pattern (table 1, figures 5 and 6; Kashnig & Kasper 1969; Zbarsky *et al.* 1969; Berezney *et al.* 1970; Berezney, Macaulay & Crane, 1972; Franke *et al.* 1970; Kasper 1971, 1974; Zentgraf *et al.* 1971; Kay, Fraser & Johnston 1972; Monneron *et al.* 1972; Zbarsky 1972*a*, *b*; Jarasch *et al.* 1973; Bornens & Kasper 1973; Kartenbeck, Jarasch & Franke 1973; Jarasch & Franke 1974).

The relationship between the nuclear envelope and the e.r. system is so marked that hitherto no enzyme activity is known which allows distinction of the nuclear membranes from microsomal membranes (for some controversial discussions see Kashnig & Kasper 1969; Zbarsky *et al.* 1969; Franke *et al.* 1970; Kasper 1974; Franke 1974; Berezney *et al.* 1972; Moore & Wilson 1972; Ichikawa & Mason 1973; Zbarsky 1972; Jarasch *et al.* 1973; Kartenbeck *et al.* 1973; Jarasch & Franke 1974).

The few reports of an enrichment of certain enzyme activities in nuclear membrane preparations from special cells and tissues (table 1) must be confirmed before these activities can be considered as 'nuclear membrane marker enzymes' in the specific material. There have also been reports that DNA polymerase-like activities might be associated with the nuclear membranes. These enzymes would be suitable candidates for identification of nuclear membranes among other cell membranes (see, for example, Yoshida, Modak & Yagi 1971; O'Brien, Sanyal & Stanton 1972). However, these observations have been refuted by other authors (e.g. Deumling & Franke 1972; Kay, Fraser & Johnston 1973). So far only the occurrence of membrane-associated nuclear DNA and, perhaps, of a special nuclear envelope RNA (Franke *et al.* 1973; Franke & Scheer 1974; Kasper 1974) might serve as a chemical component distinguishing the nuclear envelope from other cellular membranes but even here the literature is controversial (compare, for example, Kay, Haines & Johnston 1971; Fakan *et al.* 1972; Mizuno, Stoops & Sinha 1971; Mizuno, Stoops & Pfeiffer 1971*b*; Franke *et al.* 1973) and caution is recommended, especially in view of experiments of Kubinski, Gibbs & Kasper (1972), who demonstrated that some endomembranes are capable of binding free nucleic acids in a firm (and not yet understood) manner.

There are, however, some proteins present in isolated and high-salt-treated nuclear membranes which do not occur in microsomal and plasma membranes prepared in parallel as has demonstrated by gel electrophoreses of the membrane proteins (Franke *et al.* 1970; Monneron *et al.* 1972). However, these have not yet been correlated with any function. It is possible that some of these specific nuclear membrane proteins are associated with the nuclear pore com-

TABLE 1. SPECIFIC ENZYME ACTIVITIES AND CYTOCHROME CONTENTS OF NUCLEAR MEMBRANES, NUCLEI AND MICROSOMES[a]

enzyme and cell material	nuclear membranes	nuclei	micro-somes	reference
glucose-6-phosphatase				
rat liver	2.8 (h)[b]	22.5	198	Zbarsky *et al.* (1969)
	0.0 (l)[b]			
	355 (h)	35	520	Kashnig & Kasper (1969)
	255 (l)			
	1.7	32	122	Franke *et al.* (1970)
	160	22	176	Kay *et al.* (1972)
	0	—	—	Agutter (1972)
	90	22	140	Kartenbeck *et al.* (1973)
	83 (h)	—	130 (h)	
	98 (l)	—	162 (l)	
rat liver, 1 to 2 days before birth	20	8.3	33	
pig liver	1.7	15	392	Franke *et al.* (1970)
bovine liver	120	29	245	Berezney *et al.* (1972)
rat thymus	1.5	1.0	2.2	Jarasch *et al.* (1973)
enzyme activities associated with glucose-6-phosphatase in rat liver				
mannose-6-phosphatase	75	21	112	Kartenbeck *et al.* (1973)
pyrophosphatase	77	35	123	
PP_i-glucose phosphotransferase	62	19	125	
mannose-6-phosphate-glucose phosphotransferase	71	19	102	
ATP-glucose phosphotransferase	12	2.8	13	
GTP-glucose phosphotransferase	10	3.2	15	
CTP-glucose phosphotransferase	13	4.2	23	
ADP-glucose phosphotransferase	14.5	3.0	17	
GDP-glucose phosphotransferase	5.8	2.3	19	
CDP-glucose phosphotransferase	17	6.0	23	
Mg^{2+}-ATPase				
rat liver	233 (h)	37	73	Zbarsky *et al.* (1969)
	393 (l)			
	213 (h)	57	—	Delektorskaya & Perevoshchikova (1969)
	360 (l)			
	39 (h)	18	178	Kashnig & Kasper (1969)
	29 (l)			
	93	22	122	Franke *et al.* (1970)
	87	22	128	Kartenbeck *et al.* (1973)
pig liver	115	60	223	Franke *et al.* (1970)
bovine liver	117	29	171	Berezney *et al.* (1972)
Zajdela hepatoma	87 (h)	40	—	Zbarsky *et al.* (1969)
	127 (l)			
Ehrlich ascites tumour	160 (h)	106	—	Zbarsky *et al.* (1969)
	490 (l)			
calf thymus	65	—	78	Reilly (1971)
rat thymus	52	17.5	208	Jarasch *et al.* (1973)
hen erythrocytes	1.7	0.8	—	Zentgraf *et al.* (1971)
erythropoetic cells from anaemic hens	3.1	1.3	—	Jarasch (1973)
$(Na^+ + K^+)$-stimulated ATPase				
rat liver	0.0	0.0	—	Delektorskaya & Perevoshchikova (1969)
	5.0	5.0	65	Franke *et al.* (1970)
	3.3	1.7	37	Kartenbeck *et al.* (1973)

TABLE 1 (*cont.*)

enzyme and cell material	nuclear membranes	nuclei	microsomes	reference
calf thymus	0.0	—	19	Reilly (1971)
rat thymus	2.2	2.0	180	Jarasch *et al.* (1973)
hen erythrocytes	0.5	0.5	—	Zentgraf *et al.* (1971)
	ca. 0.2	*ca.* 0.2	—	Jarasch (1973)
2,4-dinitrophenol-stimulated ATPase				
rat liver	40 (h) 167 (l)	23	—	Delekotorskaya & Perevoshchikova (1969)
	0	1	11	Jarasch (1973)
rat thymus	6	0.5	10	Jarasch (1973)
5′-nucleotidase				
rat liver	0	—	—	Agutter (1972)
	3.3	10	48	Jarasch (1973)
hen erythrocytes	0.5	0.5	—	Zentgraf *et al.* (1971)
p-nitrophenylphosphatase				
rat liver				
pH 4.8	53	183	685	Franke *et al.* (1970)
pH 4.5	12.5	89	50	Kartenbeck *et al.* (1973)
pig liver (pH 4.8)	167	405	852	Franke *et al.* (1970)
rat liver				
pH 10.5	28	40	153	Franke *et al.* (1970)
pH 9.0	2.5	18.3	4.0	Kartenbeck *et al.* (1973)
pig liver	35	52	118	Franke *et al.* (1970)
β-glycerolphosphatase				
rat liver (pH 4.5)	4.1	42	15.6	Kartenbeck *et al.* (1973)
rat liver (pH 9.0)	1.6	15	3.0	Kartenbeck *et al.* (1973)
other phosphohydrolase activities in rat liver				
GTPase	18	13	75	Kartenbeck *et al.* (1973)
CTPase	3.3	8.6	11.1	
ADPase	1.6	8.6	10.3	
GDPase	2.5	20	37	
CDPase	1.6	5.8	6.8	
Glucose-1-phosphatase	1.6	1.6	4.5	
NADPH-cytochrome *c* reductase				
rat liver	6.5 (h) 7.7 (l)	2.5	18	Zbarsky *et al.* (1969)
	18	—	49	Franke *et al.* (1970)
	104	—	332	Kasper (1971)
	51	93	34	Kay *et al.* (1972)
	29	11	57	Jarasch (1973)
liver of phenobarbital-treated rats	92	—	636	Kasper (1971)
liver of 3-methylcholanthrene-treated rats	120	—	358	Kasper (1971)
pig liver	21	—	44	Franke *et al.* (1970)
bovine liver	5.2	0.89	83	Berezney *et al.* (1970)
rabbit liver	60	40	220 (s)[c] 150 (r)[c]	Ichikawa & Mason (1973)
liver of phenobarbital-treated rabbits	100	60	290 (s) 250 (r)	Ichikawa & Mason (1973)
liver of Triton-WR 1339-treated rabbits	80	50	230 (s) 200 (r)	Ichikawa & Mason (1973)
rat thymus	0	trace	—	Jarasch *et al.* (1973)
hen erythrocytes	0	0	—	Jarasch (1973)

TABLE 1 (*cont.*)

enzyme and cell material	nuclear membranes	nuclei	micro-somes	reference
NADPH-Δ^4-3-ketosteroid 5α-reductase				
rat ventral prostate	3.7×10^{-3}	0.6×10^{-3}	0.065×10^{-3}	Moore & Wilson (1972)
L-gulono-γ-lactone dehydrogenase				
rabbit liver	790	560	1290 (s) 1020 (r)	Ichikawa & Mason (1973)
liver of phenobarbital-treated rabbits	1050	650	1620 (s) 1450 (r)	Ichikawa & Mason (1973)
liver of Triton-WR 1339-treated rabbits	700	530	2000 (s) 1580 (r)	Ichikawa & Mason (1973)
benzpyrene hydroxylase				
rat liver	8.6	—	121	Kasper (1971)
Liver of phenobarbital-treated rats	6.8	—	395	Kasper (1971)
liver of 3-methylcholanthrene-treated rats	140	—	1115	Kasper (1971)
o-chloro-3,4-benzaniline pyrene hydroxylase				
rat liver	0.27	0.25	1.25 (s) 1.02 (r)	Ichikawa & Mason (1973)
liver of phenobarbital-treated rabbits	0.74	0.43	1.47 (s) 1.15 (r)	Ichikawa & Mason (1973)
liver of Triton-WR 1339-treated rabbits	0.32	0.25	1.42 (s) 1.02 (r)	Ichikawa & Mason (1973)
N-demethylase				
rat liver	0.087	—	0.80	Kasper (1971)
liver of phenobarbital-treated rats	0.10	—	1.7	Kasper (1971)
NADH-cytochrome *c*-reductase				
rat liver	60 (h) 16 (l)	21	283	Zbarsky *et al.* (1969)
	379 (h) 381 (l)	50	902	Kashnig & Kasper (1969)
	100	—	350	Franke *et al.* (1970)
	267	108	759	Kay *et al.* (1972)
	552	—	981	Kasper (1971)
liver of phenobarbital-treated rats	401	—	653	Kasper (1971)
liver of 3-methylcholanthrene-treated rats	437	—	698	Kasper (1971)
pig liver	45	—	150	Franke *et al.* (1970)
bovine liver	2100	242	4150	Berezney *et al.* (1970)
	840	—	2000	Berezney *et al.* (1972)
hen erythrocytes	170	330	—	Zentgraf *et al.* (1970)
rotenone-insensitive NADH-cytochrome *c* reductase				
rat liver	109	27	386	Jarasch (1973)
rat thymus	17	5	31	Jarasch *et al.* (1973)
hen erythrocytes	170	310	—	Jarasch (1973)
erythropoetic cells from anaemic hens	159	287	—	Jarasch (1973)
rotenone-inhibited NADH-cytochrome *c* reductase				
rat liver	58	12	—	Jarasch (1973)

TABLE 1 (*cont.*)

enzyme and cell material	nuclear membranes	nuclei	micro-somes	reference
rat thymus	0.0	2	—	Jarasch (1973)
hen erythrocytes	15	20	—	
erythropoetic cells from anaemic hens	38	74	—	
NADH-ferricyanide reductase				
rat liver	642	205	—	Jarasch (1973)
bovine liver	2590	—	6030	Berezney *et al.* (1972)
NADH-menadione reductase				
rat liver	12	5	—	Jarasch (1973)
NADH oxidase in the prescense of cytochrome *c*				
rat liver	108	18	—	Kuzmina *et al.* (1969)
	118	27	—	Jarasch (1973)
bovine liver	522	90	—	Berezney *et al.* (1970)
	502	56	—	Berezney *et al.* (1972)
rat thymus	28	14	—	Jarasch (1973)
NADH-oxidation in the absence of added electron acceptor				
rat liver	22	25	—	Jarasch (1973)
succinate oxidase in the presence of cytochrome *c*				
rat liver	29	6	—	Kuzmina *et al.* (1969)
	12	4	—	Jarasch (1973)
bovine liver	8	4	—	Berezney *et al.* (1970)
rat thymus	trace	4	—	Jarasch (1973)
succinate PMS-reductase				
rat liver	16	4.3	—	Jarasch (1973)
Bovine liver	3.7	—	1.4	Berezney & Crane (1971)
rat thymus	11	8	—	Jarasch (1973)
succinate-ferricyanide reductase				
rat liver	11	—	—	Jarasch (1973)
succinate-cytochrome *c* reductase				
rat liver	15	3	—	Jarasch (1973)
bovine liver	4	3	—	Berezney *et al.* (1970)
succinate-INT-reductase				
rat liver	10	3.3	—	Jarasch (1973)
glutamate dehydrogenase				
rat liver	48 (h)	15	4.0	Zbarsky *et al.* (1969)
	27 (l)			
	32	28	40	Franke *et al.* (1970)
p-nitrophenylethylamine oxidase				
rat liver	16.5	1.9	0.5[d]	Gorkin (1971)
tyramine oxidase				
rat liver	0.3	—	—	Jarasch (1973)
benzylamine oxidase				
rat liver	0.6	—	—	Jarasch (1973)
cytochrome *c* oxidase				
rat liver	19 (h)	3.0	3.1	Zbarsky *et al.* (1969)
	15.7 (l)			
	266	84	132	Jarasch (1973)
bovine liver	548	98	24	Berezney & Crane (1971)
	426	48	—	Berezney *et al.* (1972)
rat thymus	163[e]	18	—	Conover (1970)
	75	40	77	Jarasch (1973)

TABLE 1 (*cont.*)

enzyme and cell material	nuclear membranes	nuclei	micro-somes	reference
hen erythrocytes	0	0	—	Zentgraf *et al.* (1971)
	39	17	—	Jarasch (1973)
erythropoetic cells from anaemic hens	45	17	—	Jarasch (1973)
tetrachloroquinol oxidase				
rat liver[f]	11	4	—	Jarasch (1973)
bovine liver[g]	17	—	—	Berezney & Crane (1972)
rat thymus	6	2	—	Jarasch (1973)
oxidative phosphorylation				
rat liver	11–40	1.4	—	Zbarsky (1972*b*)
	0	0	—	Jarasch (1973)
endogenous respiration				
rat liver	8	1.5	—	Kuzmina *et al.* (1969)
	0	*ca.* 3	—	Jarasch (1973)
rat thymus	*ca.* 2	5	—	Jarasch (1973)
hen erythrocytes	4	7	—	Jarasch (1973)
arylsulphatase A+B				
rat liver	9.3 (h) 1.6 (l)	2.2	1.6	Zbarsky *et al.* (1969)
proteinase				
rat liver	0.0	2.1	2.1	Zbarsky *et al* (1969)
acetylesterase				
rat liver	113 (h) 19 (l)	58	—	Pokrovsky *et al.* (1970)
carboxyesterase				
rat liver	115 (h) 0 (l)	64	—	Pokrovsky *et al.* (1970)
NAD pyrophosphorylase				
rat liver	0.03	1.4	trace	Jarasch (1973)
hen erythrocytes	0.17	16	—	Zentgraf *et al.* (1971)
b-type cytochromes				
rat liver	0.123	—	—	Jarasch (1973)
rat thymus	0.080	—	—	Jarasch (1973)
calf thymus	0.043 to 0.075 (h) 0.096 to 0.285 (l)	—	—	Ueda *et al.* (1969)
	0.033 (H1)[h] 0.094 (H2)[h] 0.173 (l)	—	—	Matsuura & Ueda (1972)
cytochrome b_5				
rat liver	0.034	—	0.130	Franke *et al.* (1970)
	0.183	—	0.492	Kasper (1971)
	0.062	—	0.172	Jarasch (1973)
liver of phenobarbital-treated rats	0.210	—	0.498	Kasper (1971)
liver of 3-methylcholanthrene-treated rats	0.174	—	0.546	Kasper (1971)
pig liver	0.026	—	0.250	Franke *et al.* (1970)
bovine liver	0.398	—	1.020	Berezney & Crane (1971)
rabbit liver	0.45	0.08	0.85 (s) 0.75 (r)	Ichikawa & Mason (1973)
liver of phenobarbital-treated rabbits	0.55	0.140	1.95 (s) 1.61 (r)	Ichikawa & Mason (1973)
liver of Triton WR 1339-treated rabbits	0.32	0.03	1.00 (s) 0.87 (r)	Ichikawa & Mason (1973)

TABLE 1 (*cont.*)

enzyme and cell material	nuclear membranes	nuclei	micro-somes	references
cytochrome *b*				
rat liver	0.0165	—	—	Jarasch (1973)
cytochrome P-450				
rat liver	0.025	—	0.180	Franke *et al.* (1970)
	0–0.22	—	0.620	Kasper (1971)
	0.094	—	—	Jarasch (1973)
liver of phenobarbital-treated rats	0	—	1.57	Kasper (1971)
pig liver	0.032	—	0.300	Franke *et al.* (1970)
bovine liver	0.055	—	1.24	Berezney & Crane (1971)
rabbit liver	0.53	0.07	1.69 (s) 1.45 (r)	Ichikawa & Mason (1973)
liver of phenobarbital-treated rabbits	0.55	0.14	1.95 (s) 1.61 (r)	
liver of Triton-WR 1339-treated rabbits	0.61	0.05	2.05 (s) 1.83 (r)	
cytochrome c_1 (+c)				
rat liver	0.010	—	—	Jarasch (1973)
rat thymus	0.010	—	—	Jarasch (1973)
calf thymus	*ca.* 0.0085	—	—	Matsuura & Ueda (1972)
cytochrome aa_3				
rat liver	0.034	—	—	Jarasch (1973)
	0	—	—	Khandwala & Kasper (1971)
bovine liver	0.051	—	0.0	Berezney & Crane (1971)
rat thymus	0.020	—	—	Jarasch (1973)
calf thymus	0.057 to 0.075 (h) 0.077 to 0.170 (l)	—	—	Ueda *et al.* (1969)
	0.021 (H1) 0.058 (H2) 0 to 0.063 (l)	—	—	Matsuura & Ueda (1972)
cytochrome *a*				
rat liver	0.018	—	—	Jarasch (1973)
cytochrome a_3				
rat liver	0.015	—	—	Jarasch (1973)

[a] Specific activities of the enzymes are expressed as nmoles substrate metabolized per min per mg protein; in the cases of the oxidases two electron equivalents are considered as substrate. Content of cytochromes is expressed as nmol/mg protein.
[b] Heavy (h) and light (l) membranes.
[c] Smooth (s) and rough (r) ER.
[d] Ergastoplasm.
[e] Membrane-enriched fraction from isolated thymus nuclei.
[f] In the presence of 55 μM protamine.
[g] In the presence of 120 μM protamine.
[h] Matsuura & Ueda (1972) obtained two heavy membrane fractions from thymus nuclei, H1 and H2.

plexes and/or are responsible for the colchicine binding activity which is enriched in isolated nuclear membranes relative to other membrane fractions (Stadler & Franke 1972; Franke, Stadler & Krien 1972; Stadler & Franke 1974). There have also been reports as to an occurrence of various glycoproteins in the nuclear membranes (Kawasaki & Yamashina 1972) but it is not yet clear whether these are specific proteins, i.e. different from those found in other endomembranes and in plasma membranes.

While in most cell types the major part of the nuclear envelope is rough, i.e. set with polyribosomes (figures 9, 10, 24, 46), and apparently is engaged in the synthesis of intracisternal (including secretory) and membrane proteins just like other sections of the e.r. system (Avrameas & Bouteille 1968; Leduc, Avrameas & Bouteille 1968; Avrameas 1970; Franke *et al.* 1971) there are also morphological indications that some areas of the nuclear envelope can have a smooth character and contribute to vesicle formation and to subsequent flow of intracisternal and membrane material into adjacent (juxtanuclear) dictyosomes (figure 2; see also Zeigel & Dalton 1962; Moore & McAlear 1963; Bouck 1965; Weston, Greider, Ackermann & Nikolewski 1965; Whaley 1966; Kessel 1971; Morré *et al.* 1971; Weston, Ackermann, Greider & Nikolewski 1972). At least in some algae and lower fungi such blebbing areas seem to be fixed with respect to other cell organelles and/or other parts of the nucleus. Thus, the nuclear envelope may be composed of functionally different membrane areas and constitute a mosaic of variable amounts and pattern of rough and smooth e.r. character.

In addition, that the nuclear envelope is not only engaged in protein synthesis and translocation but in some cellular situations is a site of localized storage of intracisternal proteins as has become clear from the findings of various enzymes and proteinaceous crystals within the perinuclear cisterna (see Behnke & Moe 1964; Cassier & Fain-Maurel 1968; Poux 1969; Fahimi 1970; Heath, Greenwood & Griffiths 1970; Herzog & Miller 1970, 1972; Leedale, Leadbeater & Massalski 1970; Perrin 1970; Wergin, Gruber & Newcomb 1970; Blackburn 1971; Strum, Wicken, Stanbury & Karnovsky 1971).

Furthermore, it is generally believed that the nuclear membranes, like e.r. membranes, are capable of synthesizing lipids, in particular phospholipids. The localization of an enrichment of fatty acid-activating (with coenzyme A) and phospholipid-incorporating activity, in nuclear membranes, relative to plasma membranes, has recently been shown for the hen erythrocyte (Stadler & Franke 1973).

Although the continuity of the nuclear envelope with the e.r.-system is frequent, there are some cell types in which no such continuity exists. Examples of this are those cells in which the e.r.-system has been greatly, or even totally, reduced such as in the nucleated mature erythrocytes of amphibia, reptilia, and birds but also other cell systems as, for instance in the primary nucleus of *Acetabularia* and the tetraspore mother cell nuclei of the red alga, *Corralina*. While in the latter post-meiotic stages there exists a special perinuclear arrangement of radially oriented e.r.-cisternae not connected with, but rather distinctly separated from, the nuclear surface (Peel, Lucas, Duckett & Greenwood 1973) the primary nucleus of *Acetabularia* develops during its dramatic growth a special 'perinuclear lacuna' which encloses the whole nucleus and is separated by an about 60 nm thick fibrillarly textured 'intermediate plasmatic zone' from the nuclear envelope (figures 7 and 8; Werz 1964; Boloukhère 1970; Woodcock & Miller 1973; Franke & Scheer 1974). It is interesting to note that this perinuclear lacuna is also interrupted by pores of a fairly uniform diameter which, however, are different from true pore complexes by not possessing the granular and fibrillar structures attached (figure 8). There have been no membrane continuities shown between the true nuclear envelope and the perinuclear lacuna. This additional perinuclear lacuna, which actually constitutes a fenestrated 'secondary nuclear envelope', then disappears in later stages before the onset of formation of secondary nuclei. Considering the similarity and continuity of nuclear envelope and e.r. and the dissimilarity of these membranes to the plasma membrane, together with the lateral mobility of membrane components (Singer & Nicholson 1972), it is hard to accept the view which has been put

forward by some authors (and which has become widely popularized in textbooks) that the nuclear envelope and the e.r. are continuous with the plasma membrane, thus allowing direct communication of the lumina of these endomembrane cisternae with the extracellular medium (McAlear & Edwards 1959; Robertson 1959, 1964; Buvat 1963; Aldrich & Vasil 1970; Carothers 1972*a*, *b*). However, none of the morphological demonstrations of such a continuity presented are really unequivocal, in particular since some of the micrographs shown suggest artificial membrane vesiculation and myelinization in the regions discussed, indicating possible membrane breakdown and refusion processes. At least the experiments in which the intracellular distribution of electron-dense marker particles added to the extracellular liquid was followed argue against a true permanent communication of the extracellular space with the endomembrane lumina (see, Behnke 1968; Ritch & Philpott 1969; Sedar 1969; however, also the recent article of Hoenigsmann & Wolf 1973). In the author's opinion, if there is such continuity it could be only short-lived and predominantly vectorial, e.g. transitory fusion of endomembrane with plasma membrane in an exocytosis-like manner. There exist indications that the nuclear envelope membranes are not homogeneous in character but may contain smaller or larger areas with a different structure and composition. For example, the membrane around the pore complexes might be somewhat different from the interporous parts. In some nuclei regions of the inner nuclear membrane, or of both membranes, appear thickened or richer in contrast (compare the review by Stevens & André 1969), usually coincident with a narrowing of the perinuclear space in this region, sometimes down to luminal widths of 8 nm or even below (in the postnuclear cap region of bull-sperm nuclei even cisternal collapse and fusion of inner and outer nuclear membrane has been reported, Wooding & O'Donnell 1971). Such localized nuclear envelope modifications, which frequently are associated with webs of fine fibrillar material or with microtubules, have been described during spermiogenesis of a variety of organisms for the nuclear surface regions adjacent to the acrosomal vesicle and/or the centriolar basis (e.g. figures 49 and 51), during intranuclear mitoses for the polar regions or the regions of chromosomal attachment to the envelope (see below; also figures 47), and, during meiotic prophase, for the attachment zone of the synaptonemal complexes (Moses 1960, 1968). A comprehensive review of the various forms of localized nuclear membrane differentiations has recently appeared (Franke & Scheer 1974). The existence of such localized differentiations shows, at least, that the nuclear envelope is not uniform in function but rather that special sites are contained within it which are functionally different.

Description of plate 25

Figure 9. The nuclear envelope of a hen (early) erythroblast (from bone marrow). Note the granulo-fibrillar appearance of the material associated with the nuclear pore membranes (indicated by the arrows; shown at higher magnification in the insert). Note also the trilaminar substructure of the nuclear membranes in some areas. CH, chromatin; PC, perinuclear cisterna; M, mitochondrion. Note also ribosomes on the outer nuclear membrane and juxtanuclear microtubules (in the top part). (Magn. × 125 000.)

Figure 10. A cross-section through a meristematic cell of an onion root tip. Here the non-membranous constituents of the nuclear pore complexes (denoted by the arrows) exhibit a predominantly granular aspect. Note that the pore complexes correspond with the relatively electron translucent 'channels' through the condensed chromatin. (Magn. × 195 000.)

Figure 11. Appearance of a nuclear pore complex after isolation of the nuclear envelope (from a maturing oocyte of *Xenopus laevis*; for details see Scheer 1972). N, nucleoplasmic face; C, cytoplasmic face. The annular granula are denoted by the arrows. Note also the dense material in the equatorial plane of the pore and the tangles of fibrils attached to the inner annulus. (Magn. × 174 000.)

FIGURES 9–11. For description see opposite

(*Facing p.* 78)

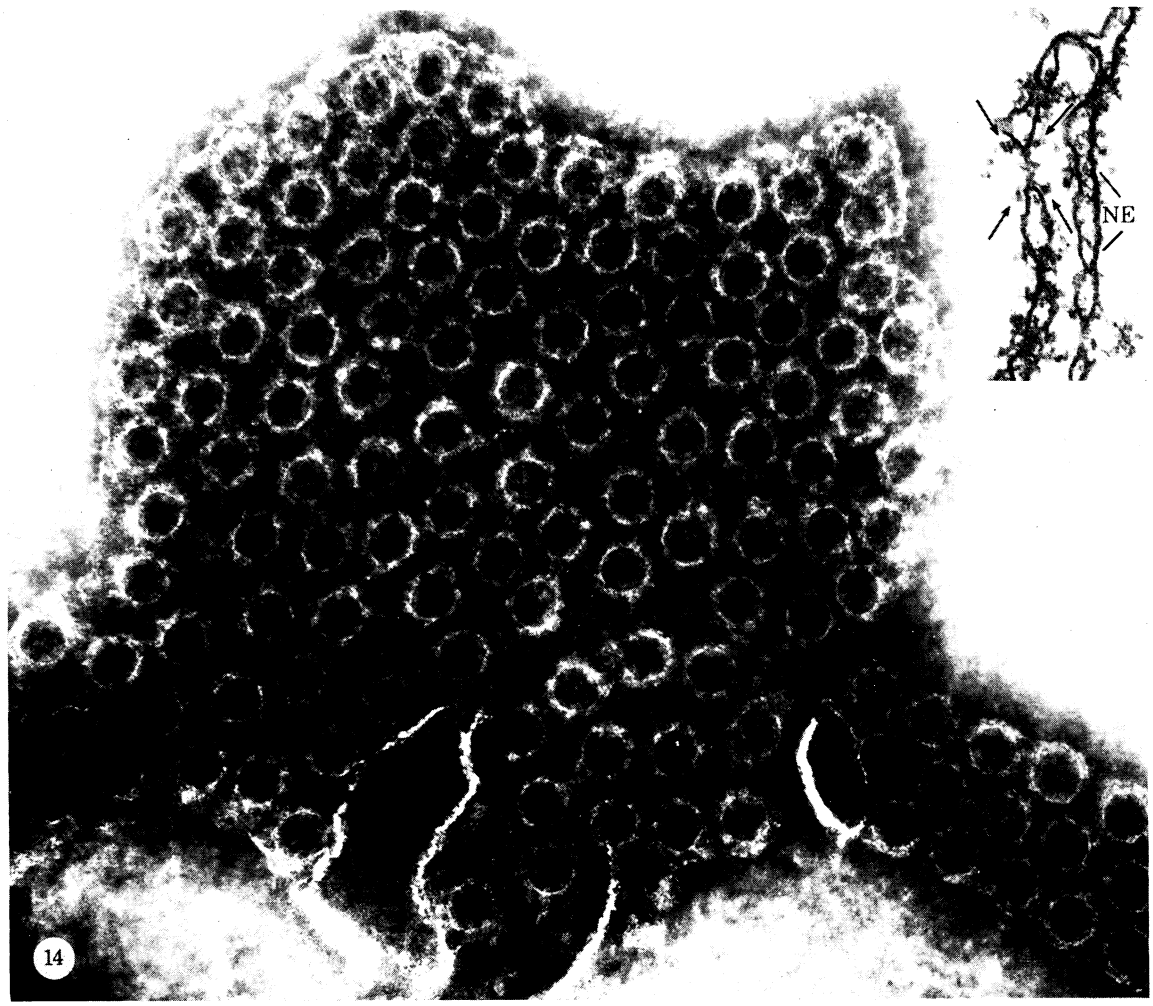


FIGURE 14. Pore complex structures in annulate lamellae (AL) isolated from lampbrush stage amphibian oocytes as revealed by negative staining (figure 14, from *Triturus alpestris*) and ultrathin section (insert, from *Xenopus laevis*). Note that such AL can be in continuity with the nuclear envelope (NE in the insert), and also the identical pore complex composition (e.g. at the arrows in the inset), and the very dense packing of such AL pore complexes. (Magn. ×80000; inset ×55000; compare Scheer & Franke 1969.)

The close relationship of the nuclear envelope to the e.r. is further indicated by observations that in the 'open mitosis' immediately upon disintegration of the nuclear envelope into cisternal fragments ribosomes appear also on the former inner nuclear membrane (see, for example, Esau & Gill 1969; Pickett-Heaps 1970). Correspondingly, there exist observations suggesting that e.r. cisternae contribute to the reformation of the nuclear envelope in anaphase-to-telophase; the first pieces of new nuclear envelope identifiable on the chromosomal surfaces, sometimes already containing pore complexes (figure 29), to be derived from, and sometimes are still continuous with, ribosome-bearing cisternae of e.r. Occasionally such e.r. cisternae may then be entrapped in the forming envelope and give rise to the formation of intranuclear cisternae including annulate lamella (see, for example, Maul 1970; for discussion see Franke & Scheer 1971; Franke, Scheer & Fritsch 1972).

The ultrastructure of the nuclear pore complex

The structure which is characteristic of, but not exclusively confined to, the nuclear envelope is the pore complex. The fact that pore complexes of identical subarchitecture appear also in cytoplasmic and intranuclear annulate lamellae shows that this structure is not concerned with functions unique to the nucleocytoplasmic boundary. The substructure of the (nuclear) pore complex has been studied intensely in the past decade and, after some early controversy in the literature (for discussion see, for examples, the articles of Wischnitzer 1958; Watson 1959; Merriam 1961; Gall 1964, 1967; Franke 1966, 1967, 1970*a*, 1974; Sichel 1966; Vivier 1967; Stevens & André 1969; Abelson & Smith 1970; Roberts & Northcote 1970; Feldherr 1972; Wunderlich & Speth 1972; Kessel 1973), there is now general agreement among most workers in this field with the following model (figure 13; Watson 1959; Merriam 1961; Gall 1964; Franke 1966, 1967, 1970*a*; Franke & Scheer 1970*a*; Roberts & Northcote 1970, 1971; LaCour & Wells 1972; Hanzely & Olah 1973). The pores are constituted by fusions of inner and outer nuclear membrane. This fusion leaves fenestrations of a rather uniform distribution diameter, with mean values between 60 and 80 nm (inner pore diameter), the specific mean value being constant for a given nuclear type (figure 12; see, for example, Gall 1964, 1967; Franke & Scheer 1970*a*, *b*). Frequently, with the freeze fracture technique, somewhat larger pore diameters are found than in ultrathin sections or in negatively stained, isolated nuclear envelope fragments. This may indicate that some shrinkage occurs during dehydration and isolation (compare, for example, Branton & Moor 1964; Franke 1966, 1970*a*; Franke *et al.* 1970; Speth & Wunderlich 1970; Kartenbeck *et al.* 1971). This is not observed, however, in some nuclei such as in the amphibian oocyte (Kartenbeck *et al.* 1971; Scheer 1973). There have been, and still are, discussions as to whether the true perimeter of the pore is circular or polygonal (pro circularity: Franke 1966, 1967, 1970; Franke & Scheer 1970*a*; Roberts & Northcote 1970, 1971; pro polygonality: Gall 1967; Kessel 1969; Abelson & Smith 1970; Maul 1971). Associated with the membrane surfaces in this pore region are granular and fibrillar structures which in the aggregate constitute the pore complex (Watson 1959). On either margin of the pore cylinder lie eight granular components (10–20 nm in diameter) which are symmetrically spaced and represent the structured components within the ring of the annulus (Callan & Tomlin 1950; Afzelius 1955; Watson 1959; Merriam 1961, 1962; Gall 1954, 1956, 1964; Rebhun 1956; Bajer & Molé-Bajer 1969; Daniels, McNiff & Ekberg 1969; Franke 1966, 1967, 1970*a*; Fisher & Cooper 1967; Franke & Scheer 1970*a*; Roberts & Northcote 1970, 1971; LaCour & Wells 1972; Fabergé 1973). These annular granules can appear either

compact and dense (e.g. in figures 10, 11) or as more loosely aggregated coils of fibrils (figure 9), the specific aspect depending perhaps on both the preparation method and the cell type studied (Franke 1970*a*). In addition, eight dense particles lie within the pore and are tightly attached to the pore wall. These particles can appear as distinct globules (see, for example, Roberts & Northcote 1970) or as cones projecting toward the centre (Franke 1970*a*; Franke & Scheer 1970*a*) and sometimes can constitute a whole massive pore plug in the equator plane. These 'peripheral granules' or 'projecting tips' are also arranged in an eightfold radial symmetry which corresponds to that of the granules in the inner and outer annulus. In a great many preparations these eight centripetally protruding structures appear to taper into fibrils indicating a spoke-like pattern (Watson 1959; Merriam 1961; Vivier 1967; Yoo & Bayley 1967; Daniels *et al.* 1969; Franke 1970*a*). In the pore interior some other fibril arrangements such as an 'inner ring' and a variety of pore-traversing filaments have also been noted (e.g. Yoo & Bayley 1967; Wunderlich & Franke 1968; Kessel 1969; Franke 1970*a*; Franke & Scheer 1970*a*). The very centre of the pore can be occupied by another electron-opaque granule or rod, the 'central granule' (Pollister, Gettner & Ward 1954; for reviews see Gall 1964, Franke 1970*a*; Feldherr 1972; Kessel 1973). Another category of fibrillar structures is those fibrils which terminate at the annular or central granules and which are usually much more conspicuous on the nuclear side of the pore complexes (see, for example, Franke 1970*a*; Franke & Scheer 1970*a*). These inner annulus-attached fibrils can appear integrated into a cylinder which extends from the pore complex deep into the nucleoplasm, this cylinder usually being contained in the channels cutting through the (when present) peripheral condensed chromatin.

This architecture of the pore complex (figure 13) is universal to all eukaryotes (Franke 1970*a*). Minor modifications such as a pronounced fibrillar aspect in some cells, an almost total absence of annular and distinct internal substructures in the nuclear pores of mature nucleated

---

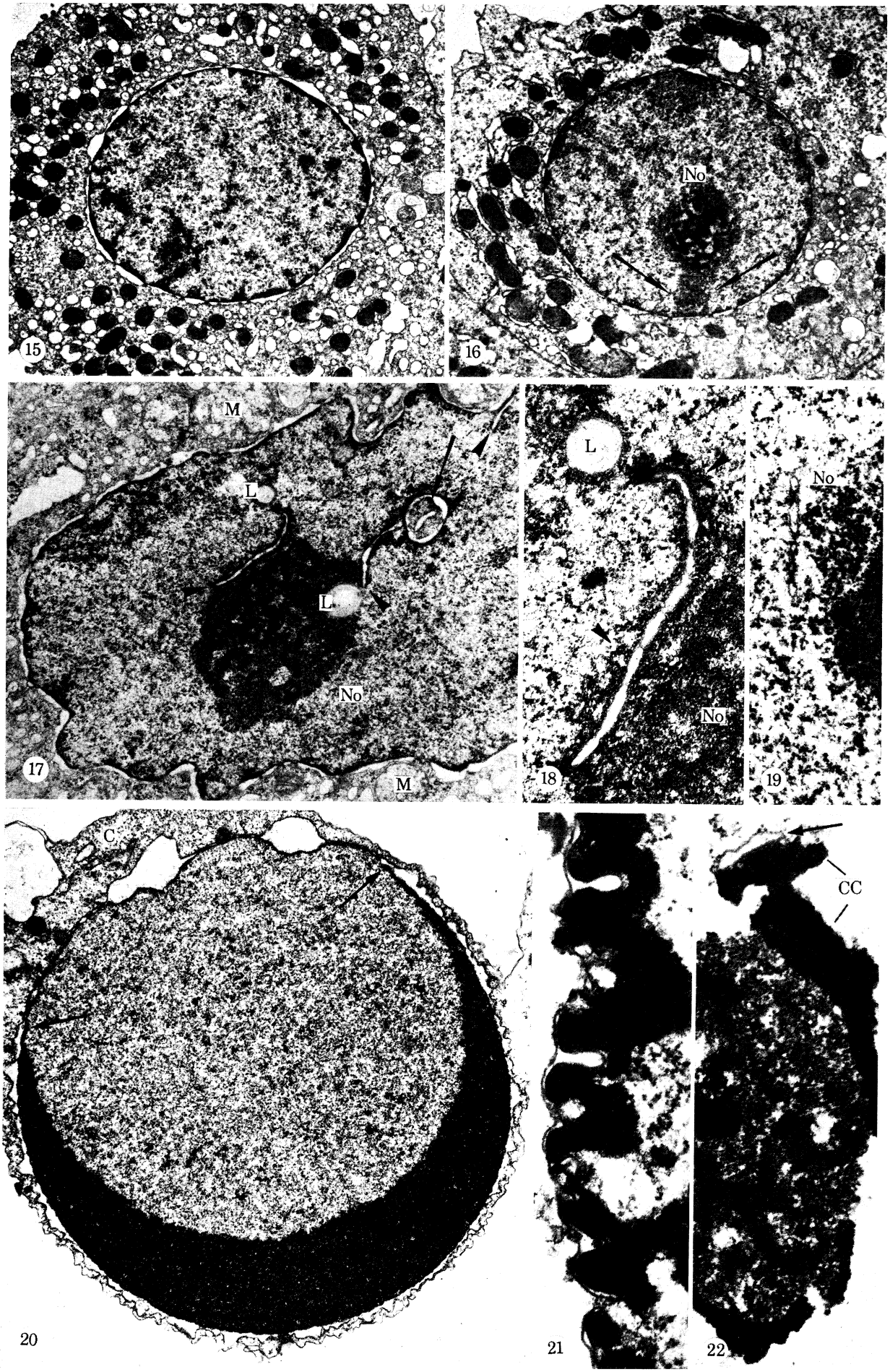
Description of plate 27

Figures 15–19. Associations of condensed chromatin with the nuclear envelope or intranuclear cisternae. Condensed chromatin is, in a great many cell types, characteristically associated with the interporous regions of the nuclear envelope (demonstrated in figure 15 in a Morris hepatoma cell). One of the most prominent blocks of such peripheral condensed chromatin is the nucleolar pedicle (arrows in figure 16) which anchors the perinucleolar heterochromatin at the nuclear envelope, thus positioning the nucleolus (No.). In addition to such nuclear membrane attachment, blocks of condensed chromatin are also often seen in association with cisternae of the nuclear interior (arrowheads in figure 17 and 18), either isolated cisternae or proliferations of the inner nuclear membrane (figures 17 and 18 show a Morris hepatoma nucleus with deep cytoplasmic indentations, e.g. at the arrow, and intranuclear lipid droplets L). M, mitochondria, No, nucleolus. Figure 19 shows, in a cultured plant cell (*Haplopappus gracilis*), a typical association of an intranuclear annulate lamella (INAL) with nucleolus (No)-associated condensed chromatin. (Magns: figure 15, ×5800; figure 16, ×4500; figure 17, ×12800; figure 18 (magnification of part of figure 17) ×39400; figure 19, 34200.)

Figure 20. Chromatin condensation (here revealed during nuclear pyknosis in a hepatoma tumour) takes place in the nuclear periphery and often (though not generally) is accompanied by the disappearance of pore complexes in this region (the margins of the pyknotic chromatin crescent are denoted by the arrows). (Magn. ×12000.)

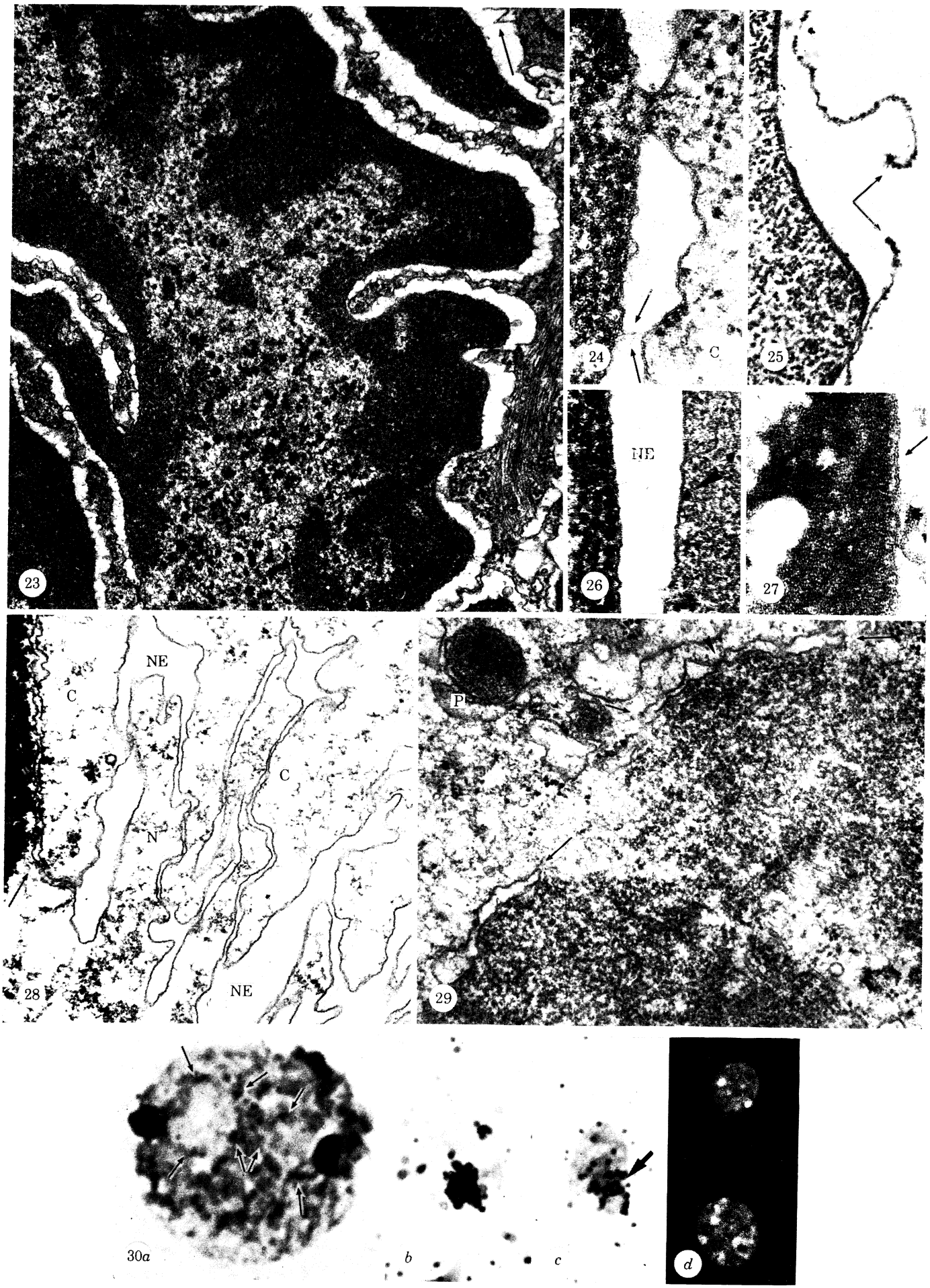
Figure 21. In some cases of nuclear envelope inflation, in particular in isolated nuclei (this figure shows an isolated nucleus from rat femur muscle), the inner nuclear membrane remains closely associated with the profile of the peripheral dense chromatin whereas the outer membrane does not. (Magn. ×39400.)

Figure 22. In preparations of nuclear substructures (here is shown a fraction of nucleoli isolated from rat liver) one frequently recognizes the intimate attachment of nucleoli (the central body), condensed chromatin (CC), and the nuclear envelope membranes (arrow). (Magn. ×30800.)



FIGURES 15–22. For description see opposite

(*Facing p.* 80)



FIGURES 23–30. For description see opposite

erthyrocytes (Kartenbeck *et al.* 1971), and some intracisternal electron-opaque appendages at the pore walls in erythropoietic and spermiogenetic stages, i.e. stages of decrease in nuclear RNA-synthetic activities (Fawcett 1966; Picheral 1970), have been noted. The components described are associated rather firmly with the membrane as demonstrated by the fact that during proper nuclear envelope isolation they remain fixed to the pores (figure 11; Franke 1966, 1967; Franke & Scheer 1970*a*; Scheer 1972; Price, Harris & Baldwin 1972; Fabergé 1973) and are partially removed only after washing with low or high salt concentrations, particularly when bivalent cations are absent (see also Mentré 1969; Franke *et al.* 1970; Agutter 1972).

In most nuclei one has, at the first view, the impression of a random distribution of the nuclear pores. Detailed analysis, however, has shown that even in such non-ordered pore arrangements there is deviation from randomness, probably a simple consequence of the existence of a minimal possible interpore distance (Maul, Price & Lieberman 1971). In some nuclear types, however, various forms of highly regular pore arrays have been observed, linear arrangements as well as hexagonal or square packing patterns (Drawert & Mix 1961; Northcote & Lewis 1968; Wunderlich & Franke 1968; Flickinger 1970; Wecke & Giesbrecht 1970; Folliot & Picheral 1971; Kartenbeck *et al.* 1971; Neushul & Walker 1971; Roberts & Northcote 1971; Thair & Wardrop 1971; Lott, Larsen & Whittington 1972; LaFountain & LaFountain 1973). An especially striking example of an ordered nuclear pore array has been shown by Teigler & Baerwald (1972) in haemocytes of the cockroach as well as in Malpighian tubule cells of a leafhopper in which hexagonal dense-packed clusters of nuclear pores are separated by relatively large nuclear surface areas without any pores at all. In addition, there are many examples showing that pore density is higher in some nuclear surface regions than in others (see, for example, Meyer 1963; Aldrich & Vasil 1970; LaCour & Wells 1972; Scheer & Franke 1972), and this is particularly clear in sperm cells, where pores are usually totally lacking in the regions associated with the

---

Description of plate 28

Figures 23–29. Further examples of the significance and importance of the association of peripheral condensed chromatin with the nuclear envelope. Condensed chromatin usually underlies the deep cytoplasmic invaginations (seen in figure 23 in an epithelial cell of the Wolffian duct of the newt, *Triturus alpestris*). Frequently this peripheral chromatin reveals a regular substructural organization in the form of *ca.* 20 nm large particles of knobs lining the inner nuclear membrane (figure 24, 26, in young erythroblasts of hen bone marrow.) In figures 23 and 24, note also the intracisternal membrane-to-membrane threads (indicated by the arrows). Figures 25 and 27 show in rat liver nuclei, isolated and washed in low salt concentrations, the relative stability of the association of the inner nuclear membrane with the peripheral chromatin (e.g. arrow in figure 27) at sites where the outer nuclear membrane has been inflated and disrupted (arrows in figure 25) or totally lost (figure 27). Figure 28 illustrates the bizarre folding and inflation of the nuclear envelope in pollen mother cells of *Canna generalis* in regions where it is obviously not connected to the nucleolar or chromatinous structures (transition shown at the arrow). Figure 29 presents the situation of anaphase-to-telophase stages of mitosis (here in a Morris hepatoma cell) in which the individual chromosomes become associated with cisternal pieces (arrows), some of them attached with ribosomes indicating their rough e.r. character, in which pore complexes can occasionally already be detected (arrowhead in the top part). NE, nuclear envelope; N, nucleoplasm; C, cytoplasm; P, peroxisome. (Magns: figure 23, × 50000; figure 24, × 87000; figure 25, 33000; figure 26, × 89000; figure 27, × 105000; figure 28, × 44000; figure 29, × 46000.)

Figure 30. Peripheral chromatin blocks, in particular the nucleolar pedicles, are rich in constitutive heterochromatin as demonstrable by the so-called Giemsa-technique (in (*a*) Sertoli cell of mouse; the nucleolar circumferences are denoted by the small arrows), *in situ* hybridization with RNA complementary to defined repetitive DNA fractions (e.g. cRNA to mouse satellite DNA in thin section autoradiographs in mouse Sertoli cell, (*b*) and hepatocyte, (*c*)), and staining with the fluorochrome quinacrine-HCl (mouse hepatocytes in (*d*)).

acrosome and the centriolar basis and are often confined to the basal part, the so-called 'redundant nuclear envelope' (for detailed discussion see Franke & Scheer 1974). An exceptional case of ordered nuclear pore distribution is the marine dinoflagellate *Noctiluca*, where pore complexes are confined to special invaginations of the nuclear envelope, the 'ampullae' (Afzelius 1963; Soyer 1969). Pore complexes of the same subarchitecture as in the nuclear

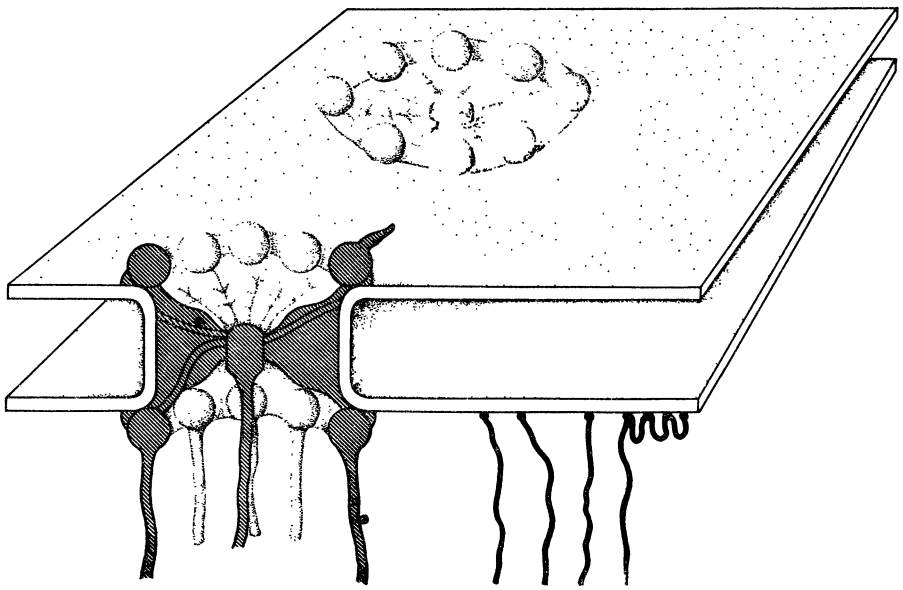
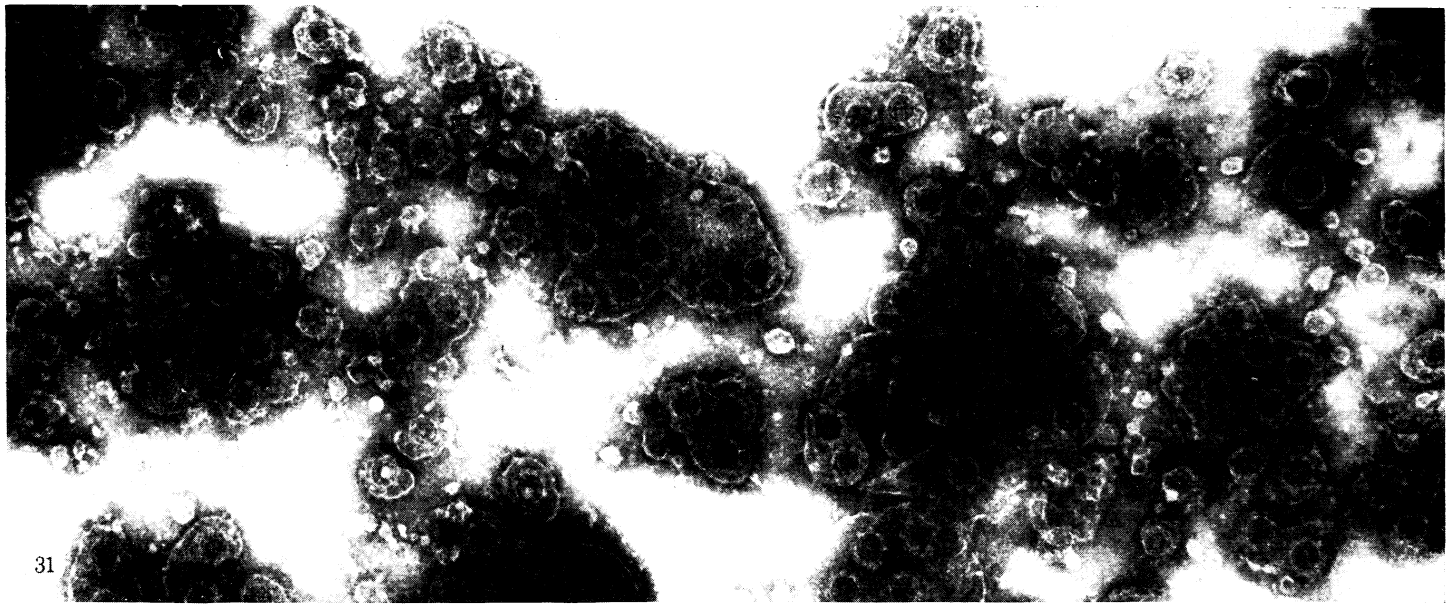
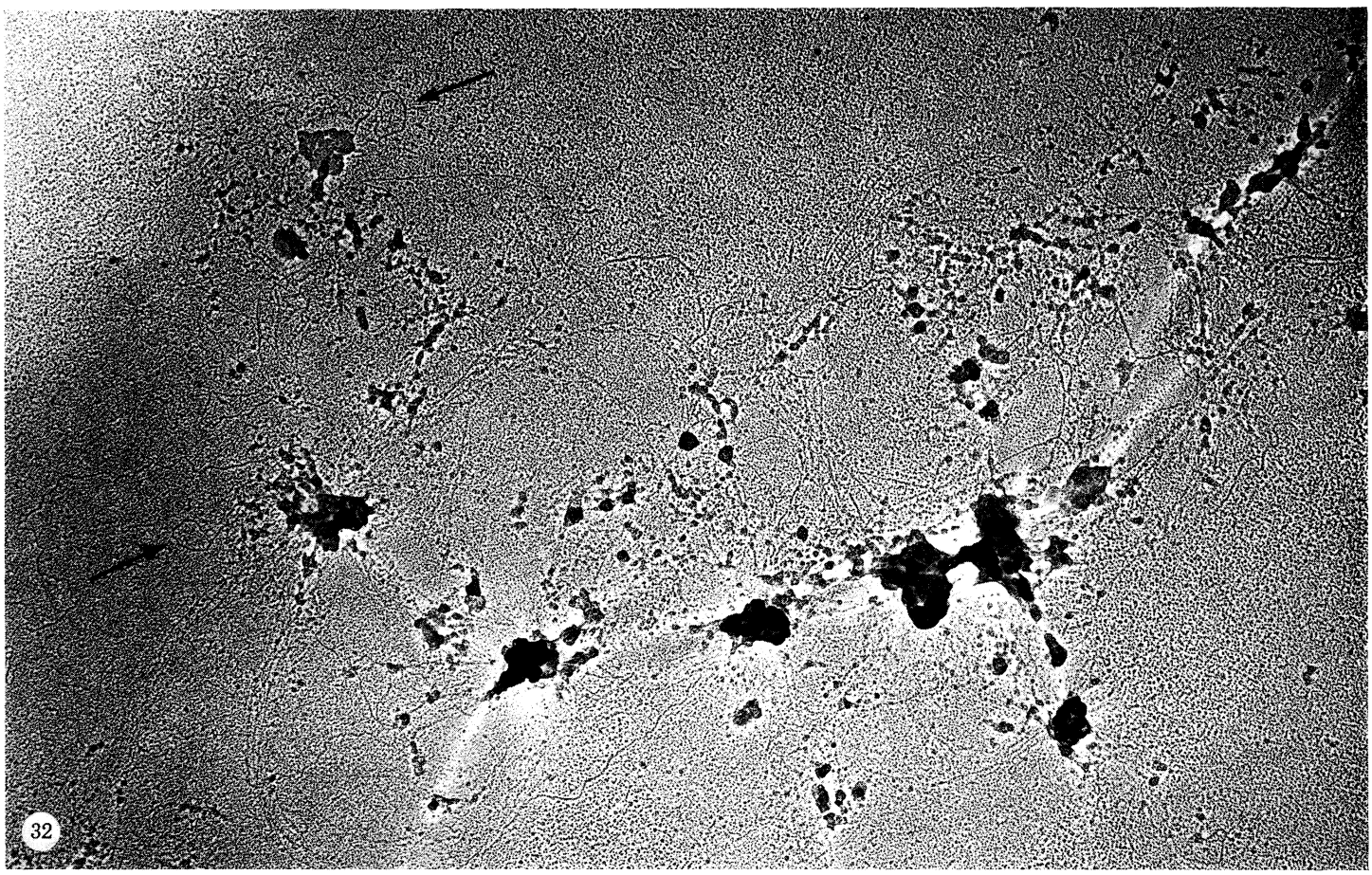


FIGURE 13. Schematic drawing of the nuclear pore complex architecture and the association of chromatin strands with the inner nuclear membrane. The pore complex consists of an inner (nucleoplasmic) and an outer (cytoplasmic) annulus, each composed of eight subunit granules symmetrically distributed on either pore margin, eight conical tips centripetally projecting from the pore wall and also arranged in an eightfold symmetry, (frequently) a central granule or central rod, and fibrils attached to all these granular components, especially to the inner annular granules. Specific preparation techniques yield results suggesting that all these components contain, or are made up by, fibrillogranular substructures. The strands shown in the interporous region (in the right) represent two extreme alternatives of the attachment of chromatin strands with the inner nuclear membrane, namely terminal attachment (left) and garland-like loops (in the right).

envelope occur in the cytoplasmic (figure 14) as well as in the intranuclear (figure 19) annulate lamellae of animal (for reviews, see Kessel 1968; Wischnitzer 1970) and plant (Franke *et al.* 1972; Scheer & Franke 1972) cells, although often at a higher frequency than in the nuclear envelope (see also Scheer & Franke 1969).

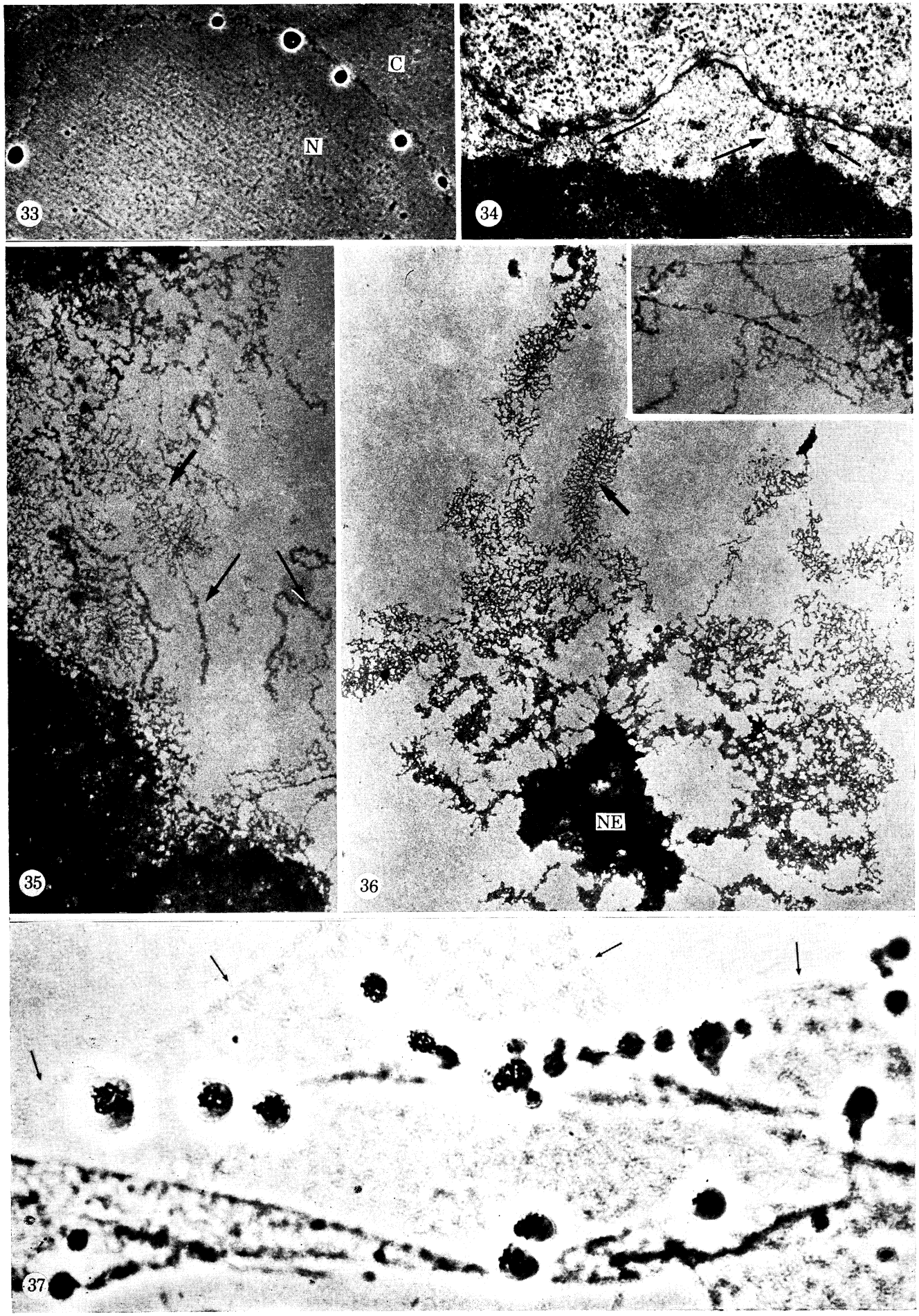
The frequency of pores, i.e. the number of pores per square micrometre of nuclear surface, can vary in different areas on the same nucleus but the average pore frequency is also different in different nuclear types. In some nuclei pore complexes have been claimed to be totally absent (e.g. Jenkins 1967; Longo & Anderson 1968), in other nuclei, mostly those which have rather low RNA synthesis ability, there are very few pores (for example, in mature erythrocytes 3–4/μm^2) whereas in more active nuclei the pore frequency can reach high values (for instance, about 60 in amphibian oocytes). In comparing pore frequency data it is important to keep in mind that different numbers are obtained with the three chief electron microscopic procedures: freeze-etch preparation reveal lower pore frequencies than thin sections, and in negatively stained preparations of isolated nuclear envelope fragments one obtains even higher figures





FIGURES 31, 32. When nuclear membranes isolated from rat liver (typical fraction shown in negative staining in figure 31) are purified and spread with the Kleinschmidt technique the membrane fragments reveal associations with DNA molecule strands which frequently appear in loop formations (arrows in figure 32; for details see Franke *et al.* 1973*a*). (Magns: figure 31, ×71 000; figure 32, ×32 000.)

(*Facing p.* 82)



FIGURES 33–37. For description see opposite

(Franke 1970*a*; Speth & Wunderlich 1970; Kartenbeck *et al.* 1971). This methodological difference, however, again varies from cell type to cell type and is not observed at all in amphibian oocytes (Kartenbeck *et al.* 1971). The common view is that nuclear pore frequency might be correlated with RNA synthetic activity (Merriam 1962; Franke & Scheer 1970*b*; LaFountain & LaFountain 1973; Maul *et al.* 1972) but this simple correlation does not generally hold. For example, mature oocytes in amphibia do maintain relatively high pore frequencies, in contrast to mouse oocytes where they seem to disappear before germinal vesicle breakdown (cf. Scheer 1973; Szollosi, Calarco & Donahue 1972); pore frequencies are also not reduced when RNA synthesis is blocked by inhibitors (for detailed discussion see Eckert, Franke & Scheer 1972).

The rates and mechanisms of pore complex formation are generally unknown. Pore formation can be rather rapid, as can be deduced from the work of Scheer (1973), who determined a net increase of pore complexes per *Xenopus laevis* oocyte nucleus during mid-lampbrush phase of about 500 pores/min. Increases in pore number during the cell cycle and in stimulated lymphocytes have also been shown (Maul *et al.* 1971, 1972; Scott, Carter & Kidwell 1971). The lifetime of nuclear pore complexes and their individual components, respectively, have not been determined to date. As to the mechanism of how cisternal fenestrations and pore complexes, in particular, may be formed, the present author has proposed that pore formations are results of localized membrane disintegration and healing processes, perhaps induced by definite particles (to explain their size uniformity), during which a part of the former membrane material is excluded from the refused membrane and might be contained in the fibrillogranular structures associated with the pores (Franke, Eckert & Krien 1971; Franke 1974).

Association of the nuclear envelope with chromosomes and extrachromosomal DNA

In most nuclear types the periphery is enriched in condensed chromatin. This peripheral dense chromatin (figure 15) is associated with the interporous areas of the inner nuclear membrane and leaves the channels which run into the pore complexes (e.g. figures 9 and 10). The peripheral sheath of condensed chromatin usually contains distinct chromosomal markers such as the sex chromatin, the nucleolar pedicle (figure 16), and, in meiosis, the termini of the synaptonemal complexes. Nuclear evaginations, invaginations, sheets, and bridge-connexions between nuclear lobi are also characterized by such a tight membrane-association of condensed

---

Description of plate 30

Figures 33–36. Associations of the nucleolar envelope with the extrachromosomal nucleoli of amphibian oocytes (lampbrush stage of *Triturus alpestris*). In these nuclei the majority of the nucleoli are accumulated in the periphery (figure 33) and appear to be linked to the nuclear membranes by aggregates of fibrils spanning between the nucleolar cortex and the adjacent nuclear pore complexes (figure 34, arrows), i.e. by structures presumed to represent ribonucleoproteins. In spreadings of isolated nuclear envelopes using the technique of Miller & Beatty (1969*a*), however, one can also identify relatively thick fibrils (*ca.* 25 nm) which appear in continuity with typical rDNA containing axes (thin arrows in figure 35 and inset figure 36; the thick arrows denote some typical matrix units, i.e. rDPN covered with gradients of ribonucleoprotein fibrils). (Magns: figure 33, × 460; figure 34, × 83 000; figure 35, × 23 000; figure 36, × 19 500; insert in figure 36, × 26 000.)

Figure 37. While the relatively small amount of rDNA present in the amplified nucleoli of *Triturus alpestris* oocytes can readily be detected with the [^{3}H]actinomycin D binding technique (see the grains) the nuclear envelope (upper margin marked by arrows) appears to be free of radioactivity, thus indicating the absence of DNA in the pore complexes (for details see Scheer 1972). (Light micrograph, × 925.)

chromatin (for references see Haynes & Davies 1973; Franke & Scheer 1974). One gains the impression that it is this condensed peripheral chromatin which stabilizes the envelope in deviations from spherical structure and is mostly responsible for the special nuclear morphology (e.g. figures 23 and 28; see in particular the example of the isolated 'contracted' muscle nucleus: figure 21; Franke & Schinko 1969). Similarly, intranuclear cisternae, whether or not they contain pore complexes, are frequently unterlaid by such layers of condensed chromatin (figures 17–19), and these are especially frequent in association with perinucleolar heterochromatin (figures 17–19). It is not clear whether these interactions reflect the association of a special (mostly condensed) chromatin fraction with the envelope or a 'condensing influence' from the membrane, or both. It is also important the recall in this connexion that condensation during mitotic prophase usually begins at the envelope and proceeds centripetally (the same has been reported for induced prophase-like chromatin condensation as well: Robbins, Pederson & Klein 1970). Chromatin condensation during nuclear pyknosis also tends to begin from the nuclear envelope (figure 20) and, interestingly, nuclear pore complexes disappear from the region which is occupied by the condensed chromatin crescent. Figure 28 shows, in a pollen mother cell of *Canna generalis*, another demonstration of the determination jo nuclear morphology by interaction of the envelope with dense intranuclear structures; in this case the nuclear envelope regions not tightly bound to chromatin or nucleoli have bizarre foldings and also tend to inflate.† The intimate interaction of the condensed peripheral chromatin with the inner nuclear membrane apparently is also the reason for the finding that (i) nuclear subfractions containing pieces of dense chromatin usually carry nuclear envelope fragments along with them (e.g. the nucleoli shown in figure 22), and (ii) that the inner nuclear membrane appears to be so much more resistant against mechanical stress and is preserved in situations where the outer membrane has been disrupted or lost (figures 25 and 27). Moreover the intimate membrane interaction of the surface of the condensed chromosomes in mitotic anaphase–telophase stages (figure 29) seems also to be a principle of nuclear envelope formation. The situation suggests that chromosomes contain some components which have a preference to tightly interact with membrane material. As was first shown by Davies and co-workers (Davies 1967, 1968; Davies & Small 1968) the outermost layer(s) of this envelope-associated chromatin consist(s) of rod- or granule-like substructures (diameters from 18–23 nm) which are regularly spaced (figures 24 and 26).

Studies on the peripheral chromatin with cytological techniques (Giemsa technique; *in situ* hybridization with [^{3}H]cRNA to fractions of repetitive DNA sequences followed by thin sectioning and autoradiography; staining with quinacrine fluorochromes) have indicated that some of these peripheral blocks of condensed chromatin contain, or are enriched in, heterochromatin. In the mouse, for example, the pericentromeric heterochromatin demonstrable by its content of light satellite DNA (figure 30; Bianchi, Sweet & Ayres 1971; Franke & Krien 1972; Rae & Franke 1972; Franke 1974; see also Franke *et al.* 1973*a*) is enriched in the nuclear periphery. This view of a preferential localization of aggregates of 'true' heterochromation (for definition see Lima-de-Faria 1969) in the very periphery has also been supported by studies of the replication rate and time course as well as of the chemical characteristics (composition, renaturation kinetics, etc.) of the small fraction of nuclear DNA which is recovered in firm

† There seem to be at least three factors contributing to the maintenance of luminal width of the perinuclear cisterna. The association with dense nuclear or cytoplasmic structures, the pore frequency, and the occurrence of intracisternal membrane-to-membrane threads (figure 24; Franke, Zentgraf, Kartenbeck & Scheer 1973).

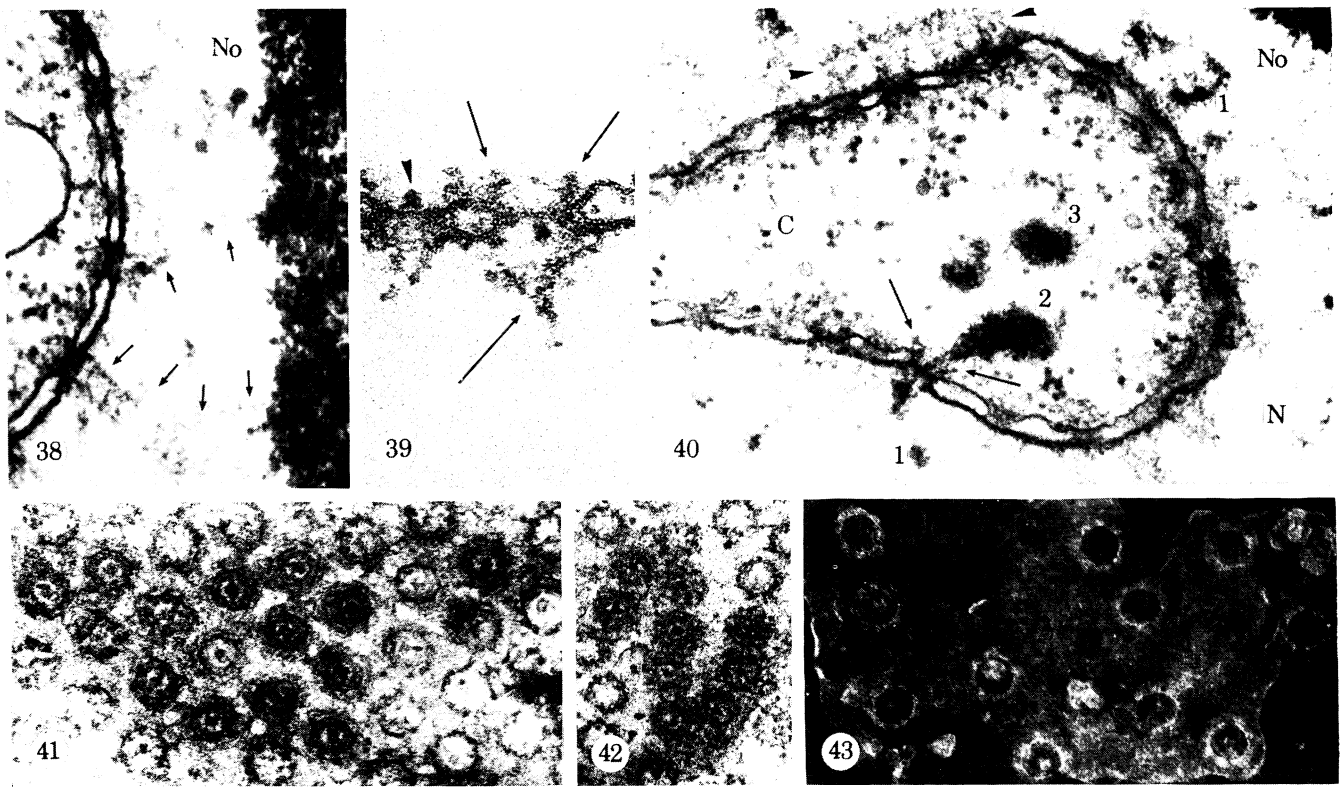
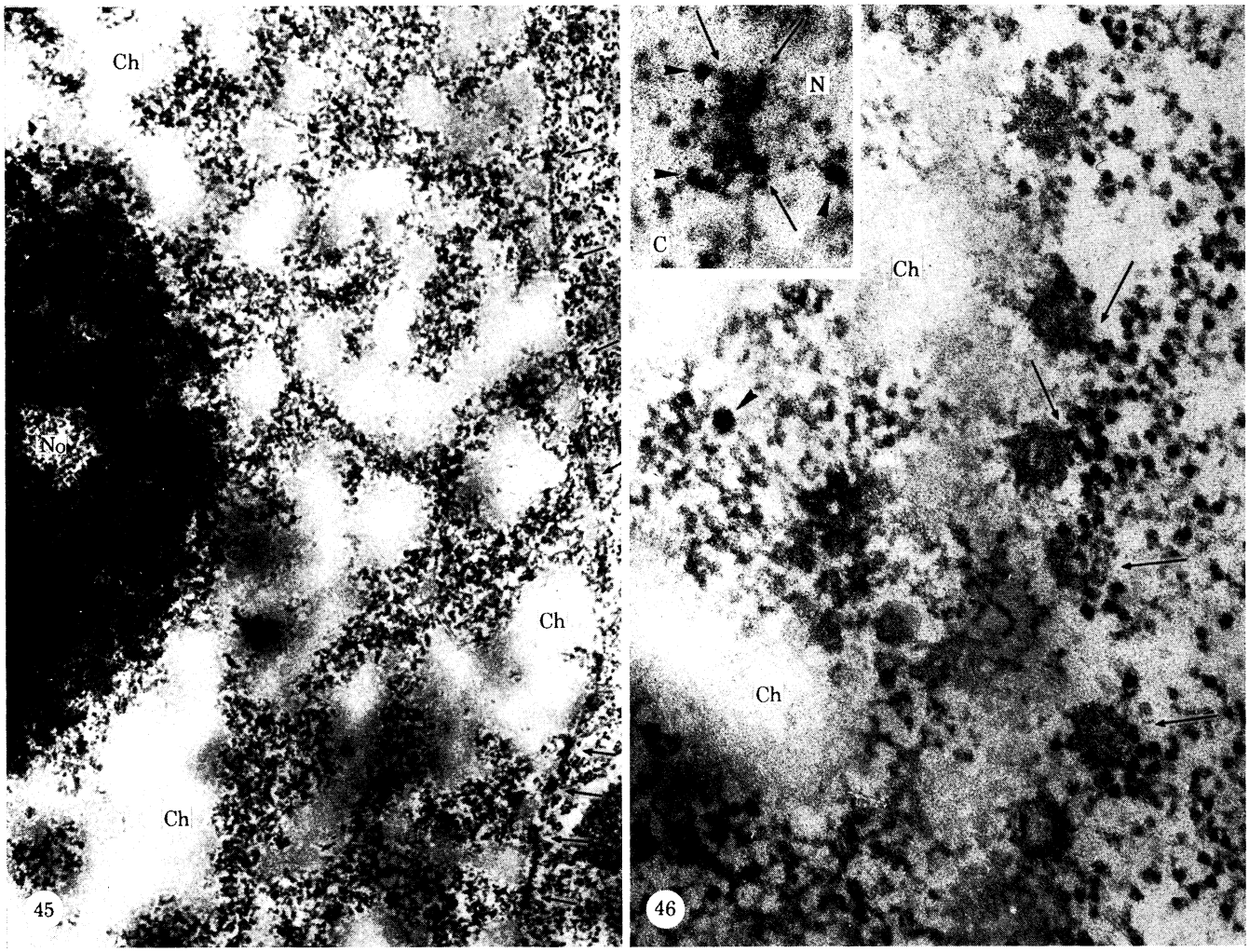


FIGURE 38–40. Details of (presumably nucleolus-derived) structures associated with the nuclear pore complexes in lampbrush stage amphibian oocytes. Direct morphological continuity of groups of fibrils attached to the inner annulus with the nucleolar (No) periphery (denoted by arrows in figure 38) is frequent. Figure 39 shows a pore complex in an isolated nuclear envelope (from *Xenopus laevis*) with the inner annulus attached fibrils (long arrow), annular granules (the outer annulus is denoted in the right pore by the smaller arrows), and one centrally located dense particle which may be either on the nucleoplasmic side, off the pore centre (pore in the right), within the pore, or in contrast (the central granule in the left pore is denoted by the arrowhead) 'already' on the cytoplasmic side of the pore. Note also the difference in electron opacity between the annular granules and the central particles. Figure 40 illustrates the typical form of transport of larger dense aggregates into the cytoplasm. These aggregates assume a 'dumb-bell-shape' when passing the pore complex, indicating that only the very centre (*ca.* 10–15 nm diameter) of the pore is accessible for their passage. The numbers suggest a sequence of such nucleocytoplasmic translocation. (The arrows denote again the outer annulus, the arrowheads in the upper part point to pore complex associated fibrils.) N, nucleoplasm; C, cytoplasm; No, nucleolus. (Magns: figure 38, × 60 000; figure 39, × 121 000; figure 40, × 53 800.)

FIGURES 41–43. Central granules can vary in size and form, (e.g. in the negatively stained HeLa nuclear envelope piece shown in figure 43, for details see Comes & Franke 1970), in frequency and electron opacity (see the thin sections of figures 41, 42). They are not confined to nuclei actively engaged in RNA synthesis such as the lampbrush stage (*Xenopus laevis*) nucleus studied in figure 42 but can also be frequent in (inactive') nuclei such as that of the *Triturus alpestris* maturing sperm cell shown in figure 41. (Magns: figure 41, × 70 000; figure 42, × 58 000; figure 43, × 70 000.)

(*Facing p.* 84)



FIGURES 45, 46. In many cytochemical tests the materials contained in the chromatin channels react as typical RNA-containing structures (here shown after the EDTA differentiation of uranyl stain as described by Bernhard (1969); onion root tip cells, for details see Franke & Falk, (1970)). The pore complex components also react in this manner (seen in cross-section in figure 45 and in the inset of figure 46 and in grazing section in figure 46). The arrows in figure 45 and in the inset denote pore complexes, those in figure 46 point to insertions of cytoplasmic polyribosomes at the pore complex annuli. Ch, chromatin (Magns: figure 45, ×27000; figure 46, ×102000; inset, ×98000.)

association with isolated nuclear membranes (Kay *et al.* 1971; Deumling & Franke 1972; Fakan *et al.* 1972; Franke *et al.* 1973*a*). The intimate association of some DNA with the nuclear envelope can also be demonstrated directly in nuclear envelope fragments (figure 31) which have been purified by flotation in 4 M CsCl and in sucrose gradients (for details see Franke *et al.* 1973*a*) and then have been spread using the Kleinschmidt technique (figure 32). The results suggest that at least some of the peripheral, envelope-associated DNA molecules are arranged in a garland-like fashion (figure 32; cf. Comings 1968; Ormerod & Lehmann 1971). The function of this membrane-attachment of DNA (and chromosomes) is far from clarified. Earlier suggestions that it represents replicating regions or initiator sites of replication, ideas which have created a boom of work on this membrane-DNA, could not be experimentally substantiated (see the references quoted in the Introduction; particularly detailed critical discussions of these problems are contained in the articles by Fakan *et al.* 1972; Kay & Johnston 1973; Huberman *et al.* 1973; Franke *et al.* 1973*a*). The amounts of DNA which are obtained with the isolated nuclear membranes are generally very small but surprisingly variable with different preparation methods (ranging from 0 to 8% of the dry mass of the membrane fraction; Kashnig & Kasper 1969; Zbarsky *et al.* 1969; Berezney *et al.* 1970; Franke *et al.* 1970; Zentgraf *et al.* 1971; Agutter 1972; Kay *et al.* 1972; Monneron *et al.* 1972; Jarasch *et al.* 1973; Franke *et al.* 1973*a*).

Another question is whether extrachromosomal (e.g. the amplified nucleolar) DNA molecules could also be associated with the nuclear envelope. We have studied this in the amphibian oocyte lampbrush stage in which the majority of the many amplified nucleoli lie close to the nuclear envelope (figure 33), obviously connected to it by thin filaments, most of them being pore complex-attached (figure 34). When the nuclear envelope is isolated from such a nucleus, nucleolar material tends to stick to the nuclear envelope ghost until it is washed off (for details of preparation see Scheer 1972, 1973). When we prepared such isolated nuclear envelopes according to the spreading and positive staining technique of Miller (Miller & Beatty 1969; for the specific preparation details see Scheer, Trendelenburg & Franke 1973) we noted that (*a*) transcriptionally active rRNA cistrons were dragged with the envelope (figures 35 and 36) and (*b*) that the strands closest to the remnants of the nuclear envelope (NE in figures 35 and 36) were thickened extensions of the rDNA–protein axes (M. Trendelenburg, U. Scheer & W. W. Franke, unpublished observations). This might serve as an indication that amplified DNA not contained in chromosomes may also be directly associated with, and perhaps anchored at, the nuclear envelope. Furthermore, the observation that such 'amplified nucleoli' then can detach from the envelope and are translocated into the nuclear interior later during oocyte maturation suggests that such a dynamic, transitory attachment to the envelope might play a physiological role in optimally positioning the rRNA cistrons.

Several authors have favoured the notion that the nuclear pore complexes (i.e. the inner annuli) represent sites of attachment of chromosomal DNA and chromatin strands, respectively (Claude 1964; DuPraw 1965; Comings & Okada 1970; Lampert 1971; Maul 1971; Engelhardt & Pusa 1972; Sorsa 1972). Recent evidence, including stratification experiments (Beams & Mueller 1970), chemical analysis of nuclear envelopes containing millions of pore complexes but neither chromosomes nor nucleoli attached (from maturing amphibian oocytes; Scheer 1972), binding experiments using [^{3}H]actinomycin D (figure 37; Scheer 1972), cytochemical digestion and differential staining techniques (see, for example, Mentré 1969; Franke & Falk 1970; and figures 45 and 46), and the observation that pore complex structures occur in AL

and nuclear envelope sacs, which have been detached from the chromatin-associated moiety, as well (for references see above and Franke & Scheer 1974) strongly indicate that the fibrils attached to the inner annulus do not represent chromatin but rather ribonucleoprotein strands and that the interaction with the chromatin is mainly in the interporous regions.

Association of the nuclear envelope with structures containing ribonucleoproteins

All analyses of isolated nuclear membranes (see the references quoted in the previous chapters) have found a variable amount of RNA which is tightly bound to these membranes and cannot be removed from them with mechanical shearing, sonication, low salt, high salt, chelating agents (for bivalent cations), and agents which weaken hydrogen bounds. Although some of this membrane-bound RNA might be related to a similar RNA fraction found in e.r. membranes (for review see Shapot & Davidova 1971) there is some RNA present in nuclear membranes which is different from e.r. RNA (Kasper 1974).

In addition, it is clear from a series of studies that *in situ* the nuclear envelope can be, apparently somewhat variably, associated with ribonucleoproteinaceous structures.

(i) The outer nuclear membrane bears in most cells actively engaged in protein synthesis polyribosomes (figures 9, 10, 46; an obvious exception is, for example, the primary nucleus of *Acetabularia*, figures 7 and 8). Some of these nuclear envelope polyribosomes can be very close to the outer annulus, sometimes with the individual ribosomes being in register with

(ii) The annular granules (figures 44 to 46), which in size and cytochemical behaviour resemble ribosomes but are not identical with them (Gall 1956; Mentré 1969; Mepham & Lane 1969; Franke 1970*a*; Franke & Falk 1970; Franke & Scheer 1970*a*; Jacob & Danieli 1972; Franke 1974). This holds for the granules of inner and outer annulus. The inner annulus reveals another relationship with a ribonucleoprotein structure, namely fibrillar connexions with the material of the nucleolar periphery which have the same cytochemical differential staining appearance (e.g. figures 38, 45, 46; Franke & Scheer 1970*a*, *b*, 1974; Franke & Falk 1970;

Description of plate 33

Figures 47–51. Associations of the nuclear envelope with microtubules and various (micro)filamentous structures are observed in many cell types. Attachment of intranuclear microtubules at the inner nuclear membrane can occur, e.g., by terminal insertion in a dense polar plaque within the perinuclear cisterna or apposed to the inner nuclear membrane (the latter is demonstrated here by the spindle apparatus of the lower fungus, *Phycomyces blakesleeanus*, figure 47; insertion of the microtubule bundle denoted by arrows; P_1 and P_2 are the two polar plaques). It can, however, also be brought about by lateral microtubule-membrane cross-linkages such as with the microtubule (MT, crossbridges indicated by arrowheads; in a macronucleus of the ciliate, *Tetrahymena pyriformis*, strain GL, figure 48, arrow denotes ribosomes on the outer nuclear membrane). Close associations of microtubules with the outer nuclear membrane are even more common and are particularly conspicuous during spermiogenesis in which they have been proposed to function in (sperm head) nuclear shaping processes (figure 49 shows a rat sperm cell in which the whole post-acrosomal region of the nucleus is manchetted by rows of microtubules; the transition is indicated by arrows). These tubules sometimes seem to be bridged to the outer nuclear membrane by dense threads (inset; arrows). Various forms of perinuclear filaments are known, some of them are indicative of cytopathological changes, and again their connexions to the nuclear envelope can be very stable (figure 50 shows, in a grazing section, that an isolated HeLa cell nucleus has still attached filaments and, sometimes, microtubules). Figure 51 illustrates the dense brush of regularly spaced filaments (between the arrows) connecting the centriolar basis with the outer nuclear envelope in a rat sperm cell.

N, nucleoplasm; C, cytoplasm; NE, nuclear envelope. (Magns: figure 47, × 65000; figure 48, × 170000; figure 49, × 21000; inset in figure 49, × 55000; figure 50, × 46000; figure 51, × 110000.)

FIGURES 47–51. For description see opposite

(*Facing p.* 86)

Kessel 1973). It is these pore-complex-associated fibrillar masses which fill the 'chromatin channels' mentioned earlier (figure 45). In addition, finely filamentous connexions are sometimes revealed between the annulus and perichromatin granules, again a structure known to contain RNA (Monneron & Bernhard 1969).

(iii) The pore complex structure discussed most with respect to a possible RNP-content is the central dense element. Like the annular granules, this particle can reveal spatial relationships

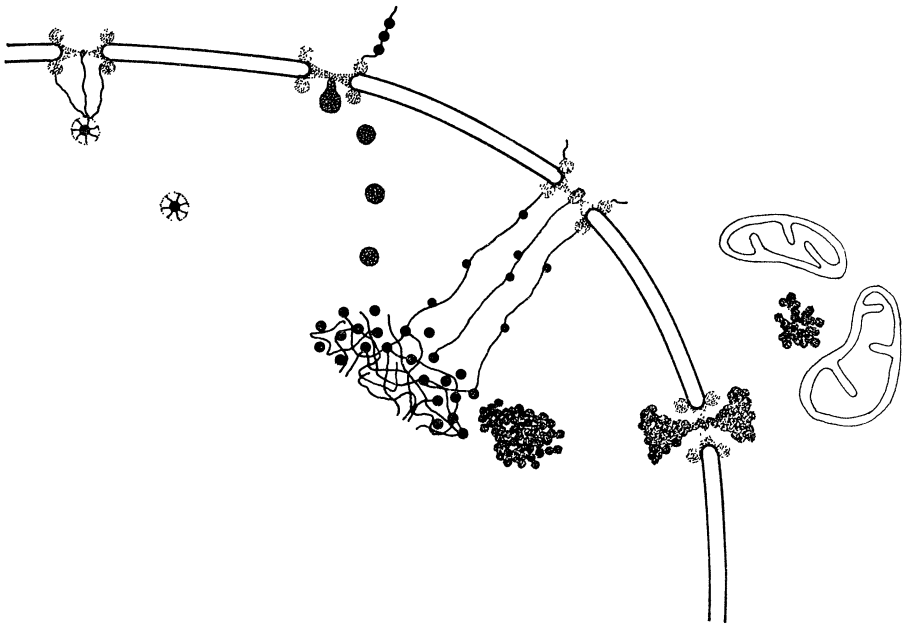


FIGURE 44. Illustration depicting the various forms of associations of structures supposed to contain ribonucleoproteins with the nuclear pore complexes: (i) perichromatin granules approach the pores but apparently do not penetrate them; (ii) small (35–55 nm diameter) granules approach the pores and might penetrate them; (iii) direct fibril continuity between nucleolar cortex and inner annulus and/or central granule; and (iv) transport of larger, dense aggregates through the pore complexes in their typical dumbbell-shape state: these aggregates then accumulate in the perinuclear cytoplasm and then frequently show associations with mitochondria.

and connexions with the RNP structures aforementioned but, in contrast to the annular granules, it exhibits a diversity of sizes and forms. It can appear right in the centre of the pore, with diameters ranging from 3.5 to 30 nm (see, for example, figures 41 to 43), it can be located off the pore plane either on the nucleoplasmic or the cytoplasmic side (e.g. figure 39), it can appear as a fibrillar thread or as part of some characteristic structures apparently being in the process of nucleocytoplasmic translocation such as the Balbiani-ring-derived granules in *Chironomus* salivary glands (Beermann 1964; Stevens & Swift 1966), the RNP-helicles in some amoebae (Stevens 1967), and the massive particles described in amphibian oocytes (figure 40; Clerot 1968; Franke & Scheer 1970*b*). This morphology of the central elements has led many authors to the concept that this particle might just represent material in passage through the so-called central channel of the pore complex, i.e. the innermost part of approximately 15 nm diameter, which seems to be accessible for the movement of large molecules and particles through the pore (review: Feldherr 1972). As indicated by the numbers in figure 40 the idea is that such dense intranuclear particles or aggregates approach the pore, then elongate and slip through the pore centre, thereby transitorily assuming a dumb-bell shape, and finally come to lie in the perninuclear cytoplasm where they again round off to more spherical forms. However, a central

granule is not *per se* an indicator of nucleocytoplasmic transport of RNP as demonstrated by the central granule frequency in cytoplasmic AL, in some nuclei not synthesizing RNA such as in maturing sperm and oocyte cells and in cells treated with inhibitors of RNA synthesis (figure 41; for detailed discussion see Eckert *et al.* 1971, and Franke & Scheer 1974). A summary of all the various forms of structural relationships of the pore complex with RNP structures is given in figure 44.

From both the cytochemical reactions and the analyses of isolated purified nuclear envelopes of maturing *Xenopus* oocytes (Scheer 1972) one can conclude that the nuclear pore complex components do contain RNA (0.4×10^{-4} pg per average pore complex) but very little, if any, DNA. The amount of nuclear RNA bound to the nuclear envelope (i.e. predominantly to its pores, at least in the amphibian oocyte) can represent as much as 8% (in *Xenopus* oocytes). It might be that a considerable part of this is RNP which is in the process of nucleocytoplasmic translocation in an intermediary binding to lipoproteinaceous material of the membrane (or associated with the membrane) with a specific turnover constant characteristic for the pore flow rate (Franke 1970*b*) of RNA in the specific cell system. Gel electrophoretic analyses of the nuclear envelope associated RNA in the amphibian oocytes (lampbrush and maturing stages) have indicated (*a*) a high percentage of ribosomal type RNA, and (*b*) an enrichment of later stages of processing in this fraction (Franke & Scheer 1974). This might serve as an indication that the final rRNA processing (and perhaps also the final assembly with proteins) takes place at the pore complex (Franke & Scheer 1970*b*).

Association of the nuclear envelope with microtubules and microfilaments

Proteinaceous structures like the microtubules and the various (micro)filaments can be tightly associated with plasma membranes and endomembranes, frequently mediated by special lateral linkage structures (Franke 1971*a–d*). Such associations are particularly frequent, and in some cases very regular and conspicuous, at the nuclear envelope. The cytological literature contains many examples of this.

(i) Terminal insertions of cytoplasmic and intranuclear microtubules at certain sites of the nuclear envelope, which often are modified into nuclear envelope associated dense plaques, or convergence of microtubules on to membrane-associated indistinct filamentous aggregates or osmiophilic knobs (e.g. figure 47; Zickler 1970; Franke 1971*a*, 1974; Franke & Reau 1973). Such situations suggest that the nuclear envelope membranes contain localized sites (in nuclear divisions, for example, at the poles) which can serve as 'microtubule organizing centres' (m.t.o.c., Pickett-Heaps 1969; for axonemes see Ockleford & Tucker 1973).

(ii) Lateral associations of microtubules with either the inner (e.g. figure 48; Wilson 1968; Franke 1971) or the outer (figure 49; Kessel 1966; references in Franke & Scheer 1974) nuclear membrane. Here, the association frequently seems to be stabilized by 3.5 to 7 nm thick lateral microtubule membrane cross-bridges (figure 48, 49). Formations of microtubules parallel to the nuclear surface are especially frequent in some spermiogenetic stages (McIntosh & Porter 1967; Moses & Wilson 1970; Fawcett, Anderson & Phillips 1971).

(iii) Filamentous connexions between the outer nuclear membrane and other endomembranes, plasma membrane, outer membranes of mitochondria or plastids, or non-membranous, densely staining aggregates such as those associated with centriolar bases (e.g. figure 51) and

other filamentous aggregate structures such as rhizoplasts, connecting pieces and myofilaments (references collected in Fawcett & Phillips 1970; Franke 1970*c*, *d*, 1971*d*; Franke & Scheer 1974).

(iv) Irregular formations of bundles of filaments and/or microtubules in the nuclear periphery (e.g. figure 50) as they are especially frequent in cytopathological situations (review Franke 1971*d*).

The tight interaction of these proteinaceous structures with the nuclear membranes is perhaps best demonstrable by their attachment to isolated nuclei (e.g. figure 50; Holmes & Choppin 1968; Franke 1971*a*). The functions of all these nuclear membrane associated microtubules and filaments are not clear. They have been implicated with hypotheses that the microtubular associations play a role in nuclear shaping (see, however, Fawcett *et al.* 1971), in nuclear division, especially in intranuclear mitoses (the aforementioned review articles), in nuclear migrations (Holmes & Choppin 1968; Girbardt 1968) and in providing a perinuclear rigid skeleton. It has also been noted that microtubules often appear when and where nuclear membranes disintegrate (e.g. by Moses & Wilson 1970; Franke 1971*d*). Both microtubular and filamentous associations have also been hypothesized to provide a means to orientating the nucleus with respect to other cell structures.

I am indebted to my colleagues and students for many valuable discussions and for contributing illustrations to this article; in particular I thank Drs U. Scheer, J. Stadler, E. D. Jarasch, H. Falk and Mr J. Kartenbeck, M. Trendelenburg and H. W. Zentgraf. I am also indebted to Professor T. W. Keenan (Purdue University, Lafayette, Ind., U.S.A.) for reading and correcting the manuscript.

References

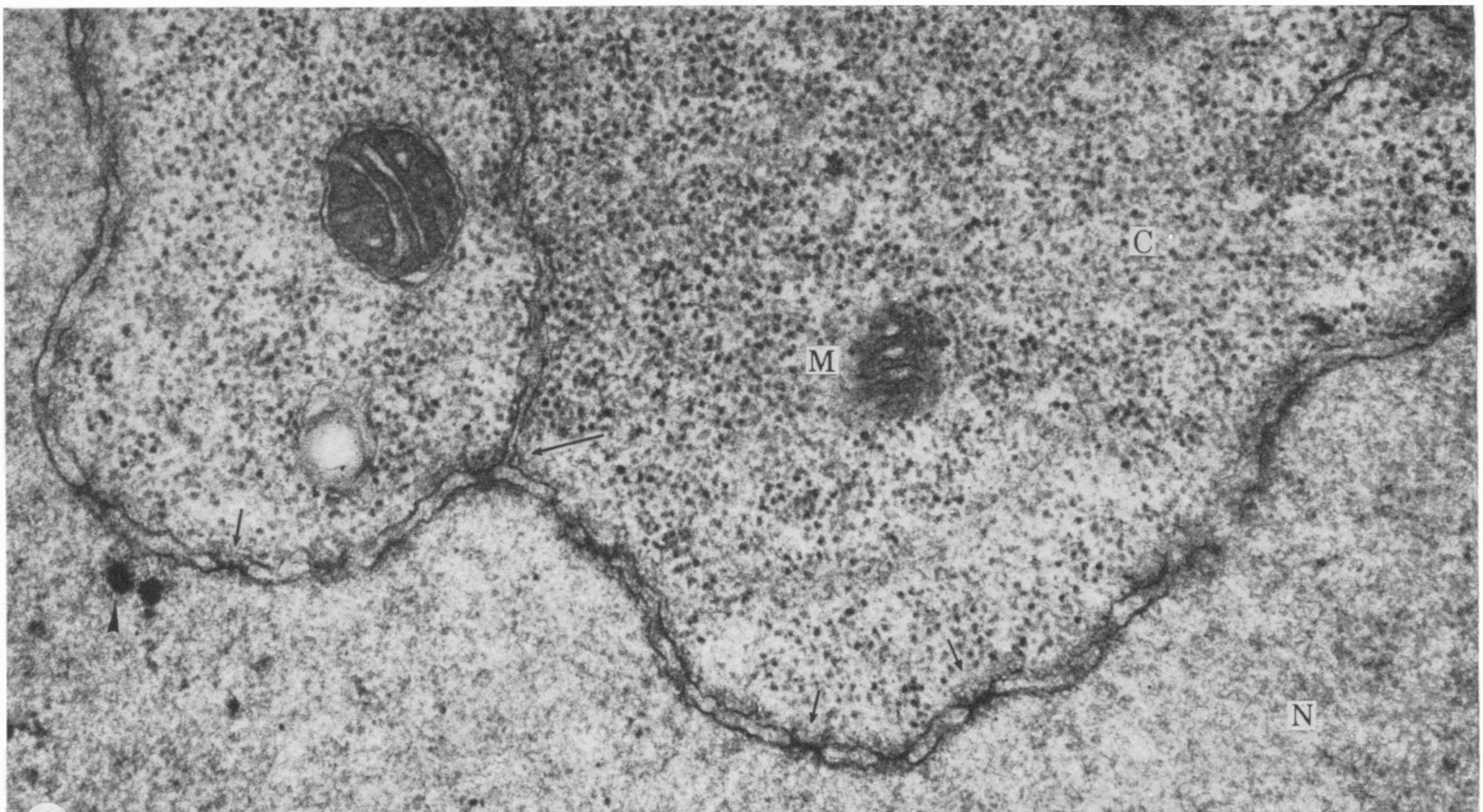
Abelson, T. & Smith, G. H. 1970 *J. ultrastruct. Res.* **30**, 558–588.
Afzelius, B. A. 1955 *Expl Cell Res.* **8**, 147–158.
Afzelius, B. A. 1963 *J. Cell Biol.* **19**, 229–238.
Agutter, P. S. 1972 *Biochim. biophys. Acta* **255**, 397–401.
Aldrich, H. C. & Vasil, J. K. 1970 *J. ultrastruct. Res.* **32**, 307–315.
Alfert, M. & Das, N. K. 1969 *Proc. natn. Acad. Sci. U.S.A.* **63**, 123–128.
Avrameas, S. 1970 *Int. Rev. Cytol.* **27**, 349–385.
Avrameas, S. & Bouteille, M. 1968 *Expl Cell Res.* **53**, 166–176.
Bajer, A. & Molé-Bajer, J. 1969 *Chromosoma* **27**, 448–484.
Barrieux, A., Long, G. L. & Garren, L. D. 1973 *Biochim. biophys. Acta* **312**, 228–242.
Beams, H. W. & Mueller, S. 1970 *Z. Zellforsch mikrosk. Anat.* **108**, 297–308.
Beermann, W. 1964 *J. expl. Zool.* **157**, 49–61.
Behnke, O. 1968 *J. ultrastruct. Res.* **24**, 412–433.
Behnke, O. & Moe, H. J. 1964 *J. Cell Biol.* **22**, 633–640.
Berezney, R. 1973 *Meth. Cell Physiol.* **8** (in the Press).
Berezney, R. & Crane, F. L. 1971 *Biochem. Biophys. Res. Commun.* **43**, 1017–23.
Berezney, R. & Crane, F. L. 1972 *J. biol. Chem.* **247**, 5562–5568.
Berezney, R., Funk, L. K. & Crane, F. L. 1970 *Biochim. biophys. Acta* **223**, 61–70.
Berezney, R., Macaulay, L. K. & Crane, F. L. 1972 *J. biol. Chem.* **247**, 5549–5561.
Bernhard, W. 1969 *J. ultrastruct. Res.* **27**, 250–265.
Bianchi, N. O., Sweet, B. H. & Ayres, J. 1971 *Expl Cell Res.* **69**, 236–239.
Blackburn, W. R. 1971 *Pathobiol. Ann.* pp. 1–31.
Blondel, B. 1968 *Expl Cell Res.* **53**, 384–356.
Bornens, M. 1968 *C. r. hebd. Séanc. Acad. Sci., Paris* **266**, 596–599.
Bornens, M. & Kasper, C. B. 1973 *J. biol. Chem.* (in the Press.)
Boloukhère, M. 1970 In *Biology of Acetabularia* (ed. J. Brachet and S. Bonotto), pp. 145–175. New York: Academic Press.
Bouck, G. B. 1965 *J. Cell Biol.* **26**, 523–537.
Bouteille, M. 1972 *Expl Cell Res.* **74**, 343–354.
Buvat, R. 1963 *Int. Rev. Cytol.* **14**, 41–155.
Branton, D. & Moor, H. 1964 *J. Ultrastruct. Res.* **11**, 401–411.

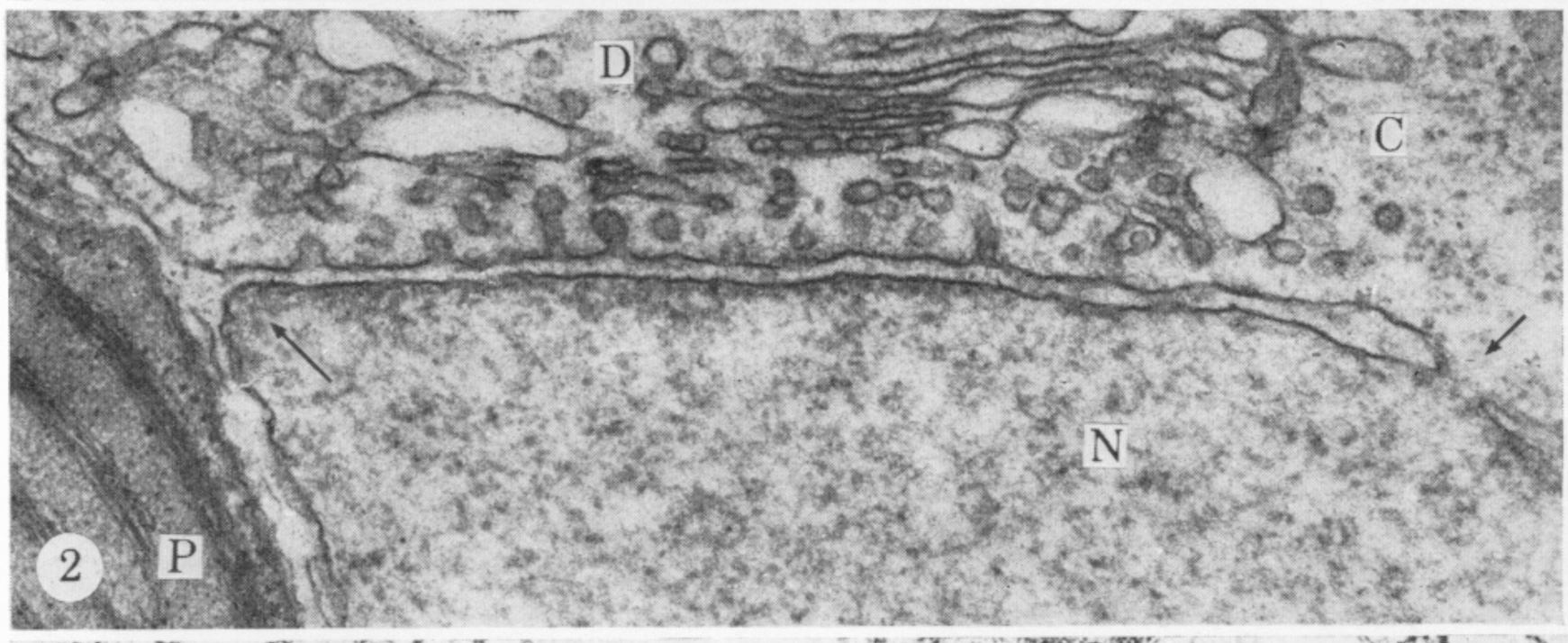
Callan, H. G. & Tomlin, S. G. 1950 *Proc. R. Soc. Lond.* B **137**, 367–378.
Carothers, Z. B 1972*a* *Science, N.Y.* **175**, 652–653.
Carothers, Z. B. 1972*b* *J. Cell Biol.* **52**, 273–282.
Cassier, M. P. & Fain-Maurel, M. A. 1968 *C. r. hebd. Séanc. Acad. Sci., Paris* **266**, 686–689.
Chinn, P. 1938 *J. Cell comp. Physiol.* **12**, 1–11.
Claude, A. 1964 In *Cellular control mechanisms and cancer* (ed. P. Emmelot and O. Mühlbock), pp. 41–48. Amsterdam: Elsevier.
Clerot, J. C. 1968 *J. Microscopie* **7**, 240–248.
Comes, P. & Franke, W. W. 1970 *Z. Zellforsch. mikrosk. Anat.* **107**, 240–248.
Comings, D. E. 1968 *Am. J. Human Gen.* **20**, 440–461.
Comings, D. E. & Okada, T. A. 1970 *Expl Cell Res.* **62**, 293–298.
Comings, D. E. & Okada, T. A. 1972 *Adv. Cell & molec. Biol.* **2**, 309–384.
Comings, D. E. & Okada, T. A. 1973 *J. molec. Biol.* **75**, 609–618.
Conover, T. E. 1970 *Archs Biochem. Biophys.* **136**, 541–562.
Daniels, E. W., McNiff, J. M. & Ekberg, D. R. 1969 *Z. Zellforsch. mikrosk. Anat.* **98**, 357–363.
David, H. 1964 *Z. mikroskop. anat. Forsch.* **71**, 526–550.
Davies, H. G. 1967 *Nature, Lond.* **214**, 208–210.
Davies, H. G. 1968 *J. Cell Sci.* **3**, 129–150.
Davies, H. G. & Small, J. V. 1968 *Nature, Lond.* **217**, 1122–1125.
Delektorskaya, L. N. & Perevoshchikova, K. A. 1969 *Biokhimiya* **34**, 199–203.
Deumling, B. & Franke, W. W. 1972 *Hoppe-Seylers Z. Physiol. Chem.* **253**, 287–297.
DeZoeten, G. A. & Gaard, G. 1969 *J. Cell Biol.* **40**, 814–823.
Drawert, H. & Mix, M. 1961 *Z. Naturforsch. mikrosk. Anat.* **16***b*, 545–551.
DuPraw, E. J. 1965 *Proc. natn. Acad. Sci. U.S.A.* **53**, 161–168.
Eckert, W. A., Franke, W. W. & Scheer, U. 1972 *Z. Zellforsch. mikrosk. Anat.* **127**, 230–239.
Engelhardt, P. & Pusa, K. 1972 *Nature, New Biol.* **240**, 163–166.
Esau, K. & Gill, R. H. 1969 *Can. J. Bot.* **47**, 581–591.
Fabergé, A. C. 1973 *Z. Zellforsch.* **136**, 183–190.
Fahimi, H. D. 1970 *J. Cell Biol.* **47**, 247–262.
Fakan, S., Turner, G. N., Pagano, J. S. & Hancock, R. 1972 *Proc. natn. Acad. Sci. U.S.A.* **69**, 2300–2305.
Falk, H. & Kleinig, H. 1968 *Arch. Mikrobiol.* **61**, 347–362.
Fawcett, D. 1966 *An atlas of fine structure.* Philadelphia, London: W. B. Saunders.
Fawcett, D. W., Anderson, W. A. & Phillips, D. M. 1971 *Devl Biol.* **26**, 220–251.
Fawcett, D. W. & Phillips, D. M. 1970 In *Comparative spermatology* (ed. B. Bacetti), pp. 13–28. New York: Academic Press.
Feldherr, C. M. 1972 *Adv. Cell & molec. Biol.* **2**, 273–307.
Feldherr, C. M. & Harding. C. V. 1964 *Protoplasmatologia, Vienna* **5**, 35–50.
Fisher, H. W. & Cooper, T. W. 1967 *Expl Cell Res.* **48**, 620–622.
Flickinger, C. J. 1970 *Expl Cell Res.* **60**, 225–236.
Folliot, R. & Picheral, B. 1971 *J. Microscopie* **10**, 219–224.
Franke, W. W. 1966 *J. Cell Biol.* **31**, 619–623.
Franke, W. W. 1967 *Z. Zellforsch. mikrosk. Anat.* **80**, 585–593.
Franke, W. W. 1970*a* *Z. Zellforsch. mikrosk. Anat.* **105**, 405–429.
Franke, W. W. 1970*b* *Naturwissenschaften* **57**, 44–45.
Franke, W. W. 1970*c* *Naturwissenschaften* **57**, 503.
Franke, W. W. 1970*d* *Z. Zellforsch. mikrosk. Anat.* **111**, 143–148.
Franke, W. W. 1971*a* *Protoplasma, Vienna* **73**, 263–285.
Franke, W. W. 1971*b* *Expl Cell Res.* **66**, 486–489.
Franke, W. W. 1971*c* *Cytobiologie, Stuttgart* **4**, 150–159.
Franke, W. W. 1971*d* *Z. Naturforsch.* **26***b*, 626–627.
Franke, W. W 1974 *Int. Rev. Cytol.* (in the Press).
Franke, W. W., Deumling, B., Ermen, B., Jarasch, E. D. & Kleinig, H. 1970 *J. Cell Biol.* **46**, 379–395.
Franke, W. W., Deumling, B., Zentgraf, H. W., Falk, H. & Rae, P. M. M. 1973*a* *Expl Cell Res.* **81**, 365–392.
Franke, W. W. Eckert, W. & Krien, S. 1971*b* *Z. Zellforsch. mikrosk. Anat.* **119**, 577–604.
Franke, W. W. & Falk, H. 1970 *Histochemie* **24**, 266–278.
Franke, W. W. & Kartenbeck, J. 1969 *Experientia, Basel*, **25**, 396–398.
Franke, W. W. & Krien, S. 1972 *Naturwissenschaften* **59**, 37–38.
Franke, W. W., Morré, D. J., Deumling, B., Cheetham, R. D., Kartenbeck, J., Jarasch, E. D. & Zentgraf, H. W. 1971*a* *Z. Naturforsch.* **26***b*, 1031–1039.
Franke, W. W. & Reau, P. 1973 *Arch. Mikrobiol.* **90**, 121–129.
Franke, W. W. & Scheer, U. 1970*a* *J. ultrastruct. Res.* **30**, 288–316.
Franke, W. W. & Scheer, U. 1970*b* *J. ultrastruct. Res.* **30**, 317–327.
Franke, W. W. & Scheer, U. 1971 *Cytobiologie, Stuttgart* **4**, 317–329.

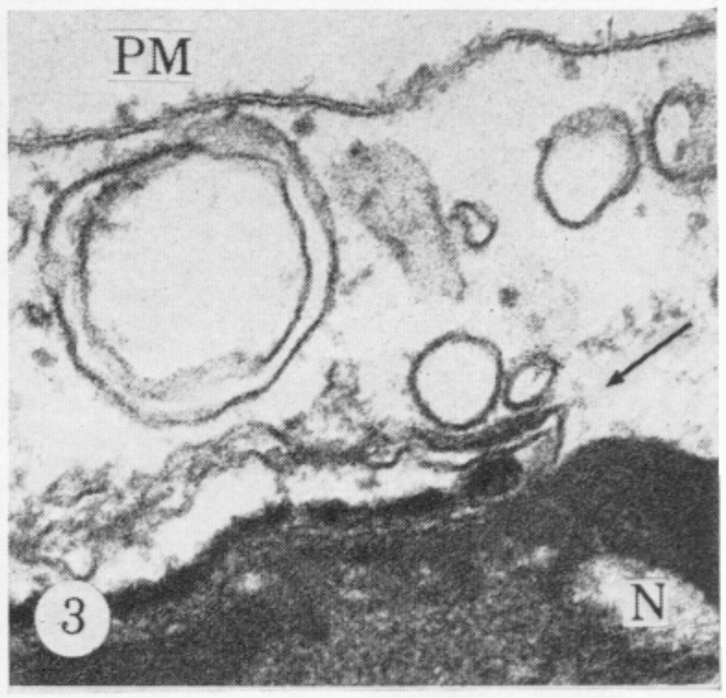
Franke, W. W. & Scheer, U. 1974 In *The cell nucleus* (ed. H. Busch). New York: Academic Press (in the Press).
Franke, W. W., Scheer, U. & Fritsch, H. 1972 *J. Cell Biol.* **53**, 823–827.
Franke, W. W. & Schinko, W. 1969 *J. Cell Biol.* **42**, 326–331.
Franke, W. W., Stadler, J. & Krien, S. 1972 *Beitr. Pathol.* **146**, 289–291.
Franke, W. W., Zentgraf, H. W., Scheer, U. & Kartenbeck, J. 1973 *Cytobiologie, Stuttgart* **7**, 89–100.
Gall, J. G. 1954 *Expl Cell Res.* **7**, 197–200.
Gall, J. G. 1956 *J. biophys. biochem. Cytol.* **4**, suppl. 393–395.
Gall, J. G. 1964 *Protoplasmatologia, Vienna* **5**, 4–25.
Gall, J. G. 1967 *J. Cell Biol.* **32**, 391–399.
Gibbs, S. P. 1962 *J. Cell Biol* **14**, 433–444.
Gibbs, S. P. 1970 *Ann. N.Y. Acad. Sci.* **175**, 454–473.
Girbardt, M. 1968 In *Aspects of cell mobility*. 22nd Symp. *Soc. exp. Biol.*, pp. 249–259. Oxford University Press.
Goldstein, L. 1964 *Protoplasmatologia, Vienna* **5**, 51–71.
Goldstein, L. 1970 *Adv. Cell Biol.* **1**, 187–210.
Gorkin, V. Z. 1971 *Experientia* **27**, 30.
Grove, S. N., Bracker, C. E. & Morré, D. J. 1968 *Science, N.Y.* **161**, 171–173.
Gurr, N. J., Finean, J. B. & Hawthorne, J. N. 1963 *Biochim. biophys. Acta* **70**, 406–411.
Hanaoka, F. & Yamada, M. 1971 *Biochem. biophys. Res. Commun.* **42**, 647–653.
Hanzely, L. & Olah, L. V. 1973 *Trans. Am. microsc. Soc.* **92**, 35–44.
Haynes, M. E. & Davies, H. G. 1973 *J. Cell Sci.* **13**, 139–171.
Heath, I. B., Greenwood, A. D. & Griffith, H. B. 1970 *J. Cell Sci.* **7**, 445–461.
Hertwig, O. 1893 *Die Zelle und die Gewebe.* Jena: Fischer Verlag.
Hertwig, R. 1906 In *Handbuch der vergleichenden und experimentellen Entwicklungslehre der Wirbeltiere* (ed. O. Hertwig), **1**, 474–568. Jena: Fischer Verlag.
Herzog, V. & Miller, F. 1970 *Z. Zellforsch. mikrosk. Anat.* **107**, 403–420.
Herzog, V. & Miller, F. 1972 *J. Cell Biol.* **53**, 662–680.
Hoenigsmann, H. & Wolff, K. 1973 *Expl Cell Res.* **80**, 191–209.
Hollande, A. & Valentin, J. 1968 *C. r. heb. Séanc. Acad. Sci., Paris* **266**, 367–370.
Holmes, K. V. & Choppin, P. W. 1968 *J. Cell Biol.* **39**, 526–542.
Huberman, J. A., Tsai, A. & Deich, R. A. 1973 *Nature, Lond.* **241**, 32–36.
Ichikawa, Y. & Mason, H. S. 1973 *Proc. 2nd Int. Symp. Oxidases and Related Redox Systems* (ed. T. King, H. S. Mason & M. Morrison). Baltimore: University Park Press. (In the Press.)
Infante, A. A., Nauta, R., Gilbert, S., Hobart, P. & Firshein, W. 1973 *Nature, New Biol.* **242**, 5–8.
Jacob, J. & Danieli, G. A. 1972 *Cell Diff.* **1**, 119–125.
Jarasch, E. D. 1973 Thesis, University of Freiburg, Germany.
Jarasch, E. D. & Franke, W. W. 1974 *J. biol. Chem.* (in the Press).
Jarasch, E. D., Reilly, C. E., Comes, P., Kartenbeck, J. & Franke, W. W. 1973 *Hoppe-Seyler's Z. Physiol. Chem.* **354**, 974–986.
Jenkins, R. A. 1967 *J. Cell Biol.* **34**, 463–481.
Kartenbeck, J., Zentgraf, H. W., Scheer, U. & Franke, W. W. 1971 *Adv. Anatomy, Embryology & Cell Biology* **45**, 1–55. Heidelberg: Springer Verlag.
Kartenbeck, J., Jarasch, E. D. & Franke, W. W. 1973 *Expl Cell Res.* **81**, 175–194.
Kashnig, D. M. & Kasper, C. B. 1969 *J. biol. Chem.* **244**, 3786–3792.
Kasper, C. B. 1971 *J. biol. Chem.* **246**, 577–582.
Kasper, C. B. 1974 In *The cell nucleus* (ed. H. Busch). New York: Academic Press. (In the Press.)
Kawasaki, T., & Yamashina, I. 1972 *J. Biochem.* **72**, 1517–1525.
Kay, R. R., Fraser, D. & Johnston, I. R. 1972 *Europ. J. Biochem.* **30**, 145–154.
Kay, R. R., Haines, M. E. & Johnston, I. R. 1971 *FEBS Lett.* **16**, 233–236.
Kay, R. R. & Johnston, I. R. 1973 *Subcell. Biochem.* **2**, 127–166.
Keenan, T. W., Berezney, R. & Crane, F. L. 1972 *Lipids* **7**, 212–215.
Keenan, T. W., Berezney, R., Funk, L. K. & Crane, F. L. 1970 *Biochim. biophys. Acta* **203**, 547–558.
Kessel, R. G. 1966 *J. ultrastruct. Res.* **16**, 293–304.
Kessel, R. G. 1968 *J. ultrastruct. Res.* **10**, suppl., 1–82.
Kessel, R. G. 1969 *Z. Zellforsch. mikrosk. Anat.* **94**, 454–461.
Kessel, R. G. 1971 *J. ultrastruct. Res.* **34**, 260–275.
Kessel, R. G. 1973 *Progr. Surface Membrane Sci.* **6**, 243–329.
Khandwala, A. S. & Kasper, C. B. 1971 *Biochim. biophys. Acta* **233**, 348–357.
Kleinig, H. 1970 *J. Cell Biol.* **46**, 396–402.
Kleinig, H., Zentgraf, H. W., Comes, P. & Stadler, J. 1971 *J. biol. Chem.* **246**, 2996–3000.
Kubai, D. F. & Ris, H. 1969 *J. Cell Biol.* **40**, 508–528.
Kubinski, H., Gibbs, P. & Kasper, C. B. 1972 *Biochim. biophys. Acta* **281**, 244–252.
Kuzmina, S. N., Zbarsky, I. B., Monakhov, N. K., Gaitzkhoki, V. S. & Neifakh, S. A. 1969 *FEBS Lett* **5**, 34–36

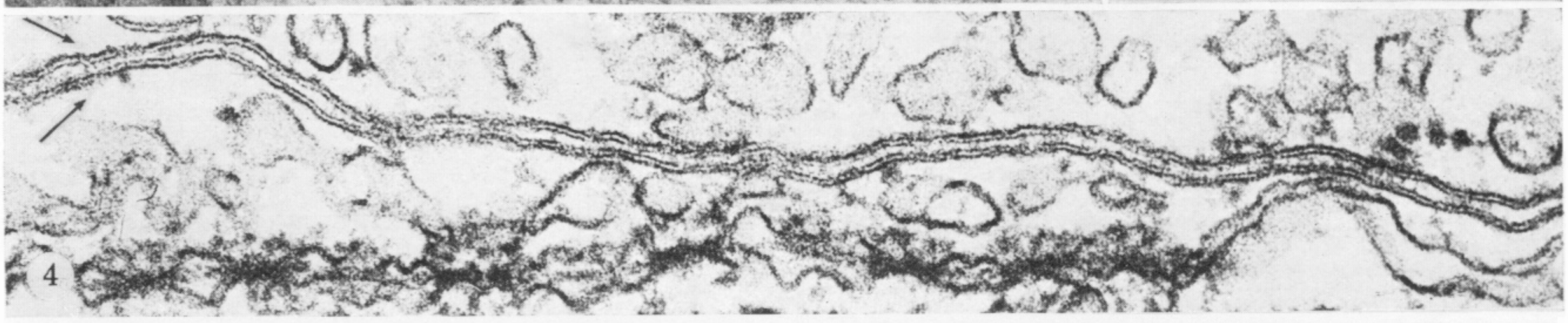
LaCour, L. F. & Wells, B. 1972 *Z. Zellforsch. mikrosk. Anat.* **123**, 178–194.
LaFountain, J. R. & LaFountain, K. L. 1973 *Expl Cell Res.* **78**, 472–475.
Lampert, F. 1971 *Humangenetik* **13**, 285–292.
Langreth, S. G. 1969 *J. Cell Biol.* **43**, 575–603.
Leduc, E. H., Avrameas, S. & Bouteille, M. 1968 *J. exp. Med.* **127**, 109–122.
Leedale, G. F. 1969 *Österr. Bot. Z.* **116**, 279–294.
Leedale, G. F., Leadbeater, B. S. C. & Massalski, A. 1970 *J. Cell Sci.* **6**, 701–709.
Lemarchal, P. & Bornens, P. 1969 *Bull. Soc. Chim. Biol.* **51**, 1021–1046.
Lima-de-Faria, A. 1969 In *Handbook of molecular cytology* (ed. A. Lima-de-Faria), pp. 277–325. Amsterdam: North Holland.
Longo, F. J. & Anderson, E. 1968 *J. Cell Biol.* **39**, 339–368.
Lott, J. N. A., Larsen, P. L. & Whittington, C. M. 1972 *Can. J. Bot.* **50**, 1785–1790.
Markham, R. S., Frey, S. & Hills, G. J. 1963 *Virology* **20**, 88–102.
Matsuura, T. & Ueda, K. 1972 *Archs Biochem. Biophys.* **150**, 440–450.
Maul, G. G. 1970 *J. ultrastruct. Res.* **31**, 375–380.
Maul, G. G. 1971 *J. Cell Biol.* **51**, 558–562.
Maul, G. G., Maul, H. M., Scogna, J. E., Lieberman, M. W., Stein, G. S., Hsu, B. Y. & Borun, T. W. 1972 *J. Cell Biol.* **55**, 433–445.
Maul, G. G., Price, J. W. & Lieberman, M. W. 1971 *J. Cell Biol.* **51**, 405–414.
McAlear, J. H. & Edwards, G. A. 1959 *Expl Cell Res.* **16**, 689–692.
McIntosh, J. R. & Porter, K. R. 1967 *J. Cell Biol.* **35**, 153–173.
Mepham, R. H. & Lane, G. R. 1969 *Nature, Lond.* **221**, 288–289.
Mentré, P. 1969 *J. Microscopie* **8**, 51–62.
Merriam, R. W. 1961 *J. Biophys. Biochem. Cytol.* **11**, 559–570.
Merriam, R. W. 1962 *J. Cell Biol.* **12**, 79–90.
Merriam, R. W. 1969 *J. Cell Sci.* **5**, 333–349.
Meyer, G. F. 1963 *Chromosoma* **14**, 207–255.
Miller, O. L. & Beatty, B. R. 1969 *Science, U.S.A.* **164**, 955–957.
Mizuno, N. S., Stoops, C. E. & Pfeiffer, R. L. 1971*a* *J. molec. Biol.* **59**, 517–525.
Mizuno, N. S., Stoops, C. E. & Sinha, A. A. 1971*b* *Nature, Lond.* **229**, 22–24.
Monneron, A. & Bernhard, W. 1969 *J. ultrastruct. Res.* **27**, 266–288.
Monneron, A., Blobel, G. & Palade, G. 1972 *J. Cell Biol.* **55**, 104–125.
Moore, R. T. & MaAlear, J. H. 1963 *J. Cell Biol.* **16**, 131–140.
Moore, R. J. & Wilson, J. D. 1972 *J. biol. Chem.* **247**, 958–967.
Morré, D. J., Mollenhauer, H. H. & Bracker, C. E. 1971 In *Origin and continuity of cell organelles* (ed. J. Reinert & H. Ursprung), pp. 82–126. Berlin: Springer Verlag.
Moses, M. J. 1960 In *Proc. IVth Int. Conf. Electron Microsc.*, 1958, pp. 230–233. Berlin: Springer Verlag.
Moses, M. J. 1968 *A. Rev. Gen.* **2**, 363–412.
Moses, M. J. & Wilson, M. H. 1970 *Chromosoma* **30**, 373–429.
Neushul, M. & Walker, D. C. 1971 *J. Phycol.* **7**, suppl. 3–13.
Northcote, D. H. & Lewis, D. R. 1968 *J. Cell Sci.* **3**, 199–206.
O'Brien, R. L., Sanyal, A. B. & Stanton, R. H. 1972 *Expl Cell Res.* **70**, 106–155.
Ockleford, C. D. & Tucker, J. B. 1973 *J. ultrastruct. Res.* **44**, 369–387.
Oppenheim, A. & Wahrman, J. 1973 *Expl Cell Res.* **79**, 287–294.
Ormerod, M. G. & Lehmann, A. R. 1971 *Biochim. biophys. Acta* **228**, 331–343.
Paine, P. L. & Feldherr, C. M. 1972 *Expl Cell Res.* **74**, 81–102.
Peel, M. C., Lucas, I. A. N., Duckett, J. G. & Greenwood, A. D. 1973 *Z. Zellforsch. mikrosk. Anat.* **147**, 59–74.
Perrin, A. 1970 *Protoplasma, Vienna* **70**, 131–134.
Picheral, B. 1970 *J. Microscopie*, 979–982.
Pickett-Heaps, J. D. 1969 *Cytobios* **3**, 257–280.
Pickett-Heaps, J. D. 1970 *Protoplasma, Vienna* **70**, 325–347.
Pokrovsky, A. A., Zbarsky, J. B., Ponomareva, L. G., Gapparov, M. M., Tutelayan, V. A., Lashneva, N. V., Perevoshchikova, K. A. & Delektorskaya, L. N. 1970 *Biokhimiya* **35**, 346–347.
Pollister, A. W., Gettner, M. & Ward, R. 1954 *Science, U.S.A.* **120**, 789.
Poux, N. 1969 *J. Microscopie* **8**, 855–866.
Price, M. R., Harris, J. R. & Baldwin, R. W. 1972 *J. ultrastruct. Res.* **40**, 178–196.
Rae, P. M. M. & Franke, W. W. 1972 *Chromosoma* **39**, 443–456.
Rebhun, L. Y. 1956 *J. biophys. biochem. Cytol.* **2**, 93–104.
Reilly, C. E. 1971 Diploma-Thesis, University of Freiburg, Germany, pp. 1–41.
Rimpau, J. & Lelley, T. 1972 *Z. PflZücht.* **67**, 197–201.
Ritch, R. & Philpott, C. W. 1969 *Expl Cell Res.* **55**, 17–24.
Robbins, E., Pederson, T. & Klein, P. 1970 *J. Cell Biol.* **44**, 400–416.
Roberts, K. & Northcote, D. H. 1970 *Nature, Lond.* **228**, 385–386.

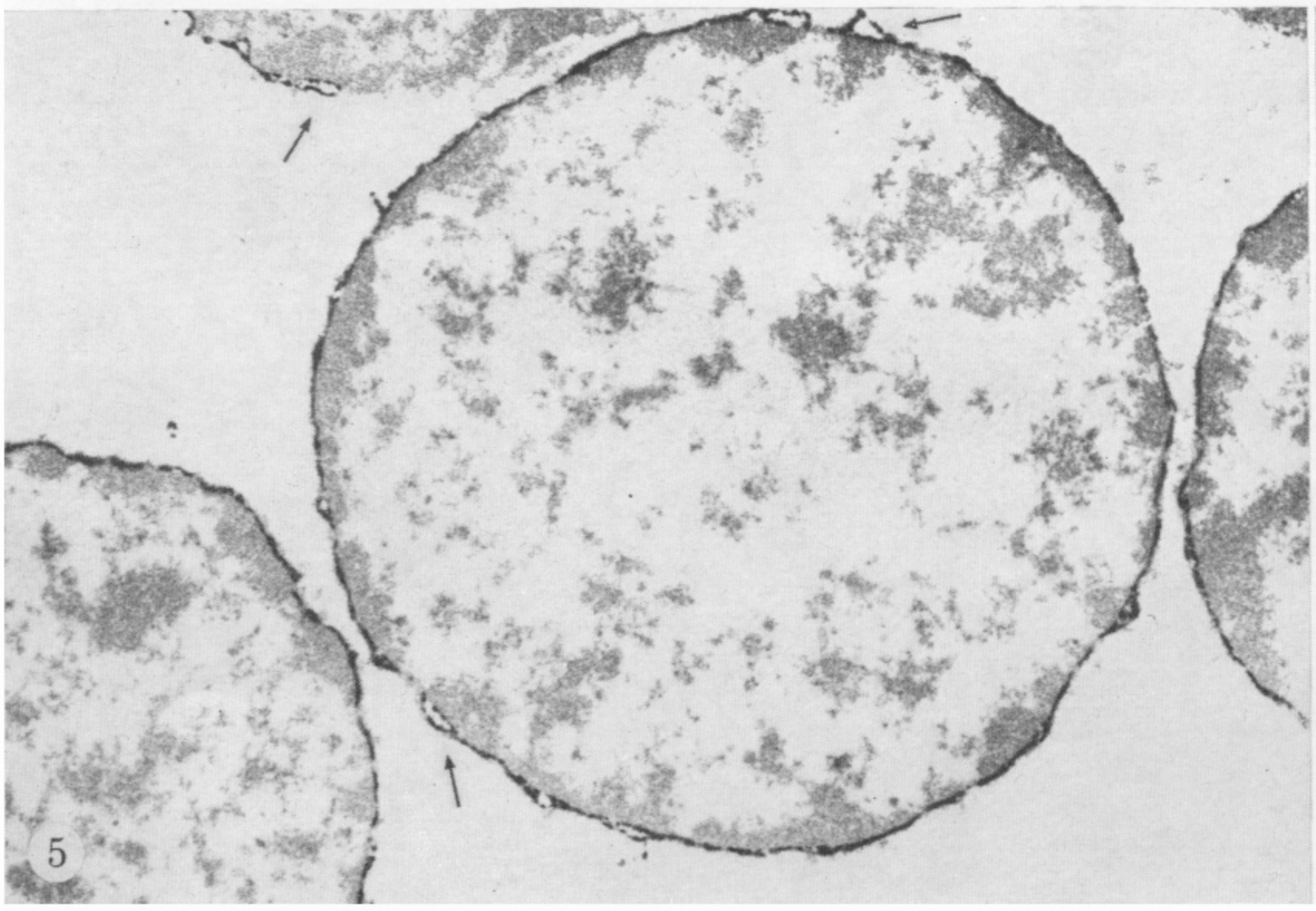
Roberts, K. & Northcote, D. H. 1971 *Microscopica Acta* **71**, 102–122.
Robertson, J. D. 1959 In *The structure and function of subcellular components* (ed. E. M. Cook). Cambridge University Press.
Robertson, J. D. 1964 In *Cellular membranes in development* (ed. M. Locke), pp. 1–82. New York: Academic Press.
Robison, W. G. 1966 *J. Cell Biol.* **29**, 251–266.
Sato, T., Fujii, T., Matsuura, T. & Ueda, K. 1972 *J. Biochem.* **72**, 1049–1051.
Scheer, U. 1972 *Z. Zellforsch. mikrosk. Anat.* **127**, 127–148.
Scheer, U. 1973 *Devl Biol.* **30**, 13–28.
Scheer, U. & Franke, W. W. 1969 *J. Cell Biol.* **42**, 519–533.
Scheer, U. & Franke, W. W. 1972 *Planta, Berlin* **107**, 145–159.
Scheer, U., Trendelenburg, M. & Franke, W. W. 1973 *Expl Cell Res.* **80**, 175–190.
Schmidt, W. J. 1929 *Protoplasma, Vienna* **7**, 353–394.
Schmidt, W. J. 1937 *Protoplasma- Monographien* **11**, 1–388. Berlin: Gebrüder Bornträger Verlag.
Scott, R. E., Carter, R. L. & Kidwell, W. R. 1971 *Nature, Lond.* **233**, 219–220.
Sedar, H. W. 1969 *J. Cell Biol.* **43**, 179–184.
Shapot, V. S. & Davidova, S. Y. 1971 In *Progr. Nucleic Acid Res. Molec. Biol.* **11**, 81–101.
Sichel, G. 1966 *La Cellule* **66**, 97–102.
Singer, S. J. & Nicholson, G. L. 1972 *Science, N.Y.* **175**, 720–731.
Sorsa, V. 1972 *Hereditas* **72**, 215–222.
Soyer, M. O. 1969 *J. Microscopie* **8**, 569–588.
Speth, V. & Wunderlich, F. 1970 *J. Cell Biol.* **47**, 772–777.
Stadler, J. & Franke, W. W. 1972 *Nature, New Biol.* **237**, 237–238.
Stadler, J. & Franke, W. W. 1973 *Biochim. biophys. Acta* **311**, 205–213.
Stadler, J. & Franke, W. W. 1974 *J. Cell Biol.* **60**, 297–303.
Stadler, J. & Kleinig, H. 1971 *Biochim. biophys. Acta* **233**, 315–319.
Stevens, A. R. 1967 *The control of nuclear activity* (ed. L. Goldstein), pp. 189–271. Englewood Cliffs, N. J.: Prentice Hall.
Stevens, B. J. & André, J. 1969 In *Handbook of molecular cytology* (ed. A. Lima-de-Faria), pp. 837–871. Amsterdam: North-Holland.
Stevens, B. J. & Swift, H. 1966 *J. Cell Biol.* **31**, 55–77.
Strum, J. M., Wicken, J. R., Stanbury, J. R. & Karnovsky, M. J. 1971 *J. Cell Biol.* **51**, 162–175.
Szollosi, D., Calarco, P. G. & Donahue, R. P. 1972 *Anat. Rec.* **174**, 325–340.
Teigler, D. J. & Baerwald, R. J. 1972 *Tissue and Cell* **4**, 447–456.
Thair, B. W. & Wardrop, A. B. 1971 *Planta, Berlin* **100**, 1–17.
Ueda, K., Matsuura, T., Date, N. & Kawai, K. 1969 *Biochem. biophys. Res. Comm.* **34**, 322–327.
Verhey, C. A. & Moyer, F. H. 1967 *J. exp. Zool.* **164**, 195–226.
Vivier, E. 1967 *J. Microscopie* **6**, 371–390.
Watson, M. L. 1959 *J. biophys. biochem. Cytol.* **6**, 147–156.
Watts, J. W. 1973 In *Cell biology and medicine* (ed. E. E. Bittar), pp. 265–288. New York, London: Wiley.
Wecke, J. & Giesbrecht, P. 1970 In *Proc. Sept. Congr. Int. Microscope Electronique*, Grenoble, pp. 233–234.
Wergin, W. P., Gruber, P. J. & Newcomb, E. H. 1970 *J. ultrastruct. Res.* **30**, 533–557.
Werz, G. 1964 *Planta, Berlin* **62**, 255–271.
Weston, J., Ackerman, G. A., Greider, M. H. & Nikolewski, R. F. 1972 *Z. Zellforsch. mikrosk. Anat.* **123**, 153–160.
Weston, J. C., Greider, M. H., Ackerman, G. A. & Nikolewski, R. F. 1965 *J. appl. Phys.* **36**, 2627.
Whaley, W. G. 1966 In *Organisation der Zelle.* III. *Probleme der biologischen Reduplikation* (ed. P. Sitte), pp. 340–371. Berlin: Springer Verlag.
Williams, C. A. & Ockey, C. H. 1970 *Expl Cell Res.* **63**, 365–372.
Wilson, H. J. 1968 *J. Cell Biol.* **40**, 854–859.
Wischnitzer, S. 1958 *J. Ultrastruct. Res.* **1**, 201–222.
Wischnitzer, S. 1970 *Int. Rev. Cytol.* **27**, 65–100.
Wise, G. E. & Prescott, D. M. 1973 *Proc. natn. Acad. Sci. U.S.A.* **70**, 714–717.
Woodcock, C. L. F. & Miller, G. J. 1973 *Protoplasma, Vienna* **77**, 331–341.
Wooding, F. B. P. & O'Donnell, J. M. 1971 *J. ultrastruct. Res.* **35**, 71–85.
Wunderlich, F. & Franke, W. W. 1968 *J. Cell Biol.* **38**, 458–462.
Wunderlich, F. & Speth, V. 1972 *J. Microsc.* **13**, 361–381.
Yamada, M. & Hanaoka, F. 1973 *Nature, New Biol.* **243**, 227–230.
Yoo, B. Y. & Bayley, S. T. 1967 *J. ultrastruct. Res.* **18**, 651–659.
Yoshida, S., Modak, M. J. & Yagi, K. 1971 *Biochem. biophys. Res. Commun.* **43**, 1408–1414.
Zbarsky, I. B. 1972*a* *Prog. Modern Biol. Acad. Sci. USSR* **73**, 1–25.
Zbarsky, I. B. 1972*b* In *Methods in cell physiol.* **5**, 167–198.
Zbarsky, I. B., Perevoshchikova, K. A., Delektorskaya, L. N. & Delektorsky, V. V. 1969 *Nature, Lond.* **221**, 257–259.
Zeigel, R. F. & Dalton, H. J. 1962 *J. Cell Biol.* **15**, 45–54.
Zentgraf, H. W., Deumling, B., Jarasch, E. D. & Franke, W. W. 1971 *J. biol. Chem.* **246**, 2986–2995.
Zentgraf, H. W. & Franke, W. W. 1974 *Beitr. path. Anat.* (in the Press).
Zickler, D. 1970 *Chromosoma* **30**, 287–304.

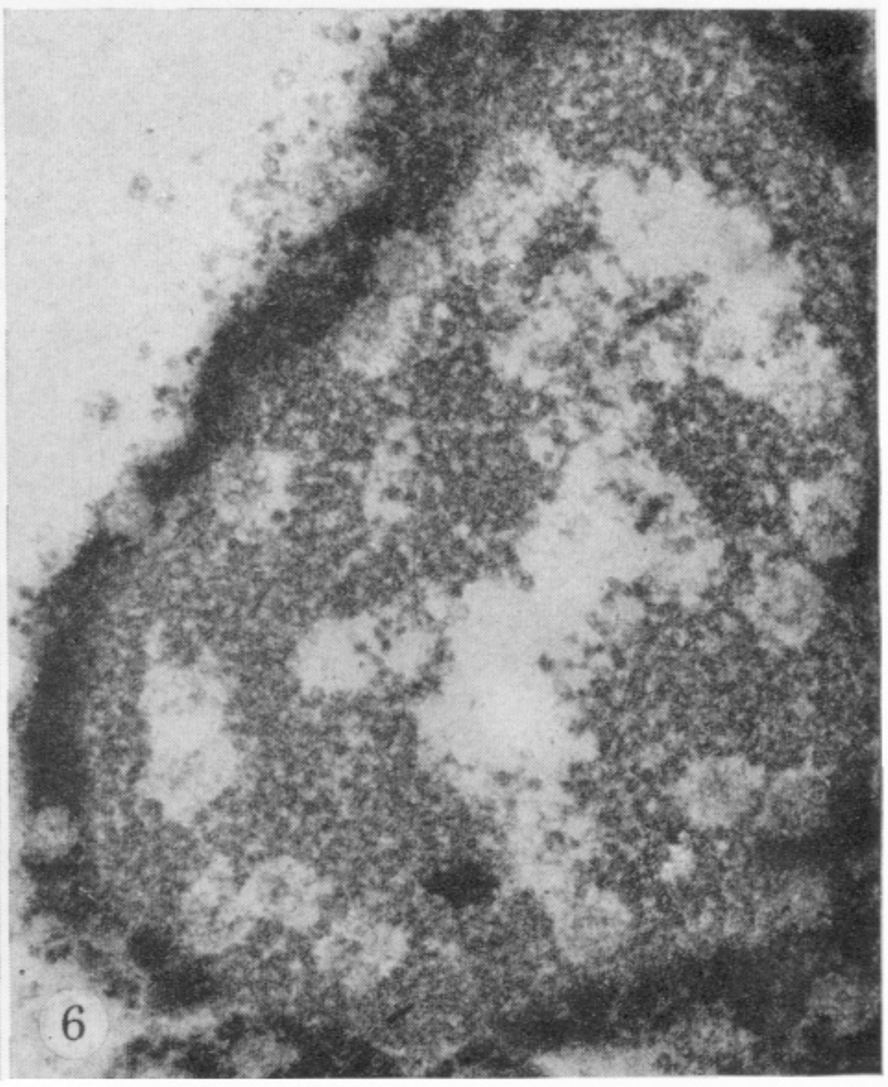




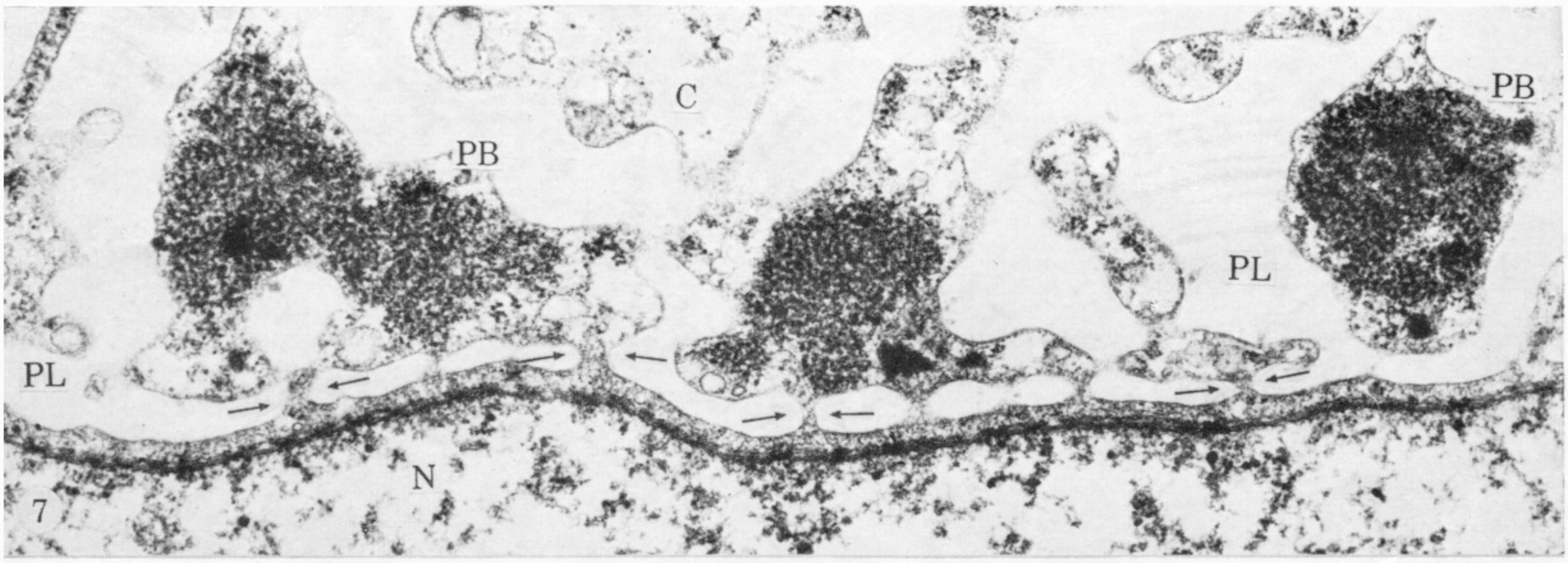


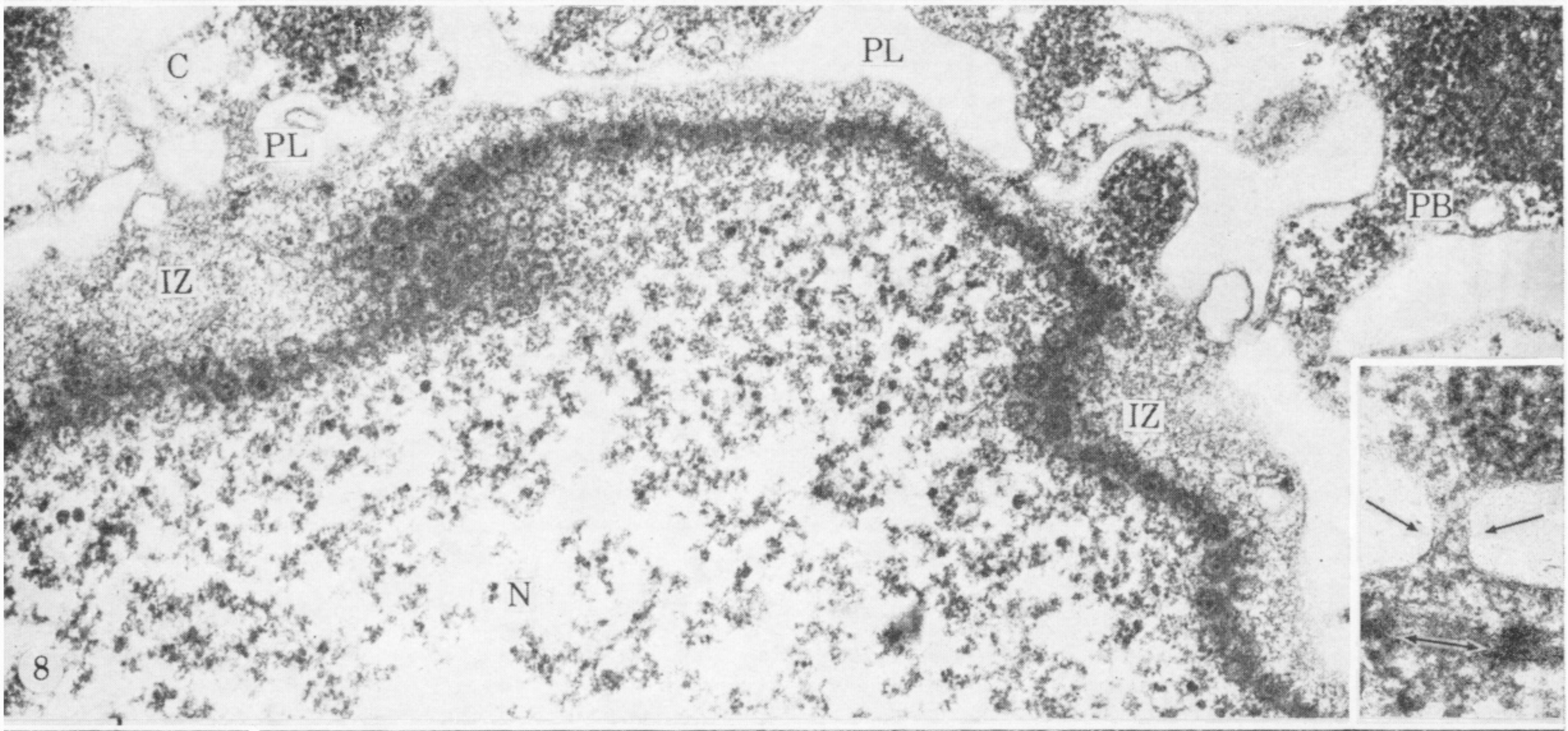


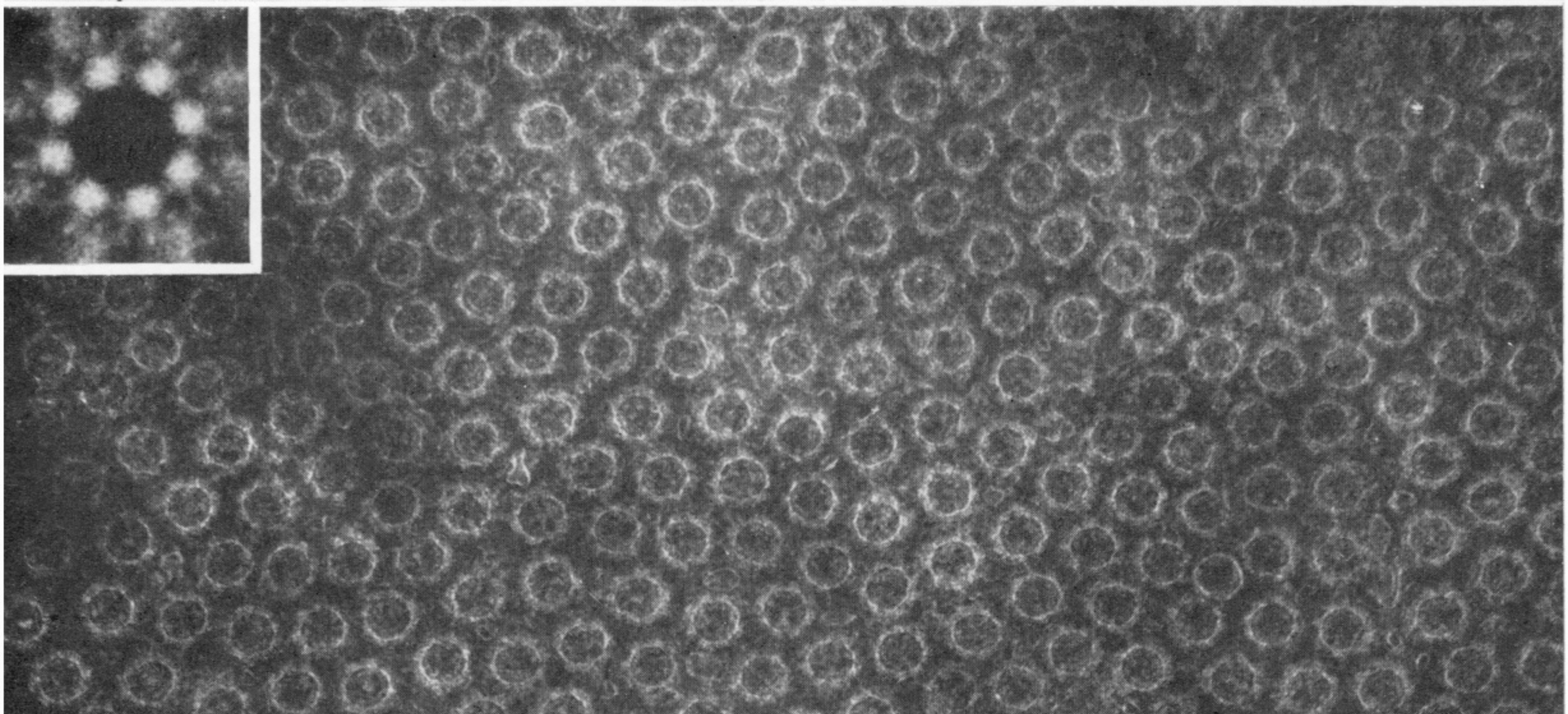




FIGURES 1–6. For description see opposite







FIGURES 7, 8 and 12. For description see opposite

PC

CH

M

9

10

N

C

11

FIGURES 9–11. For description see opposite

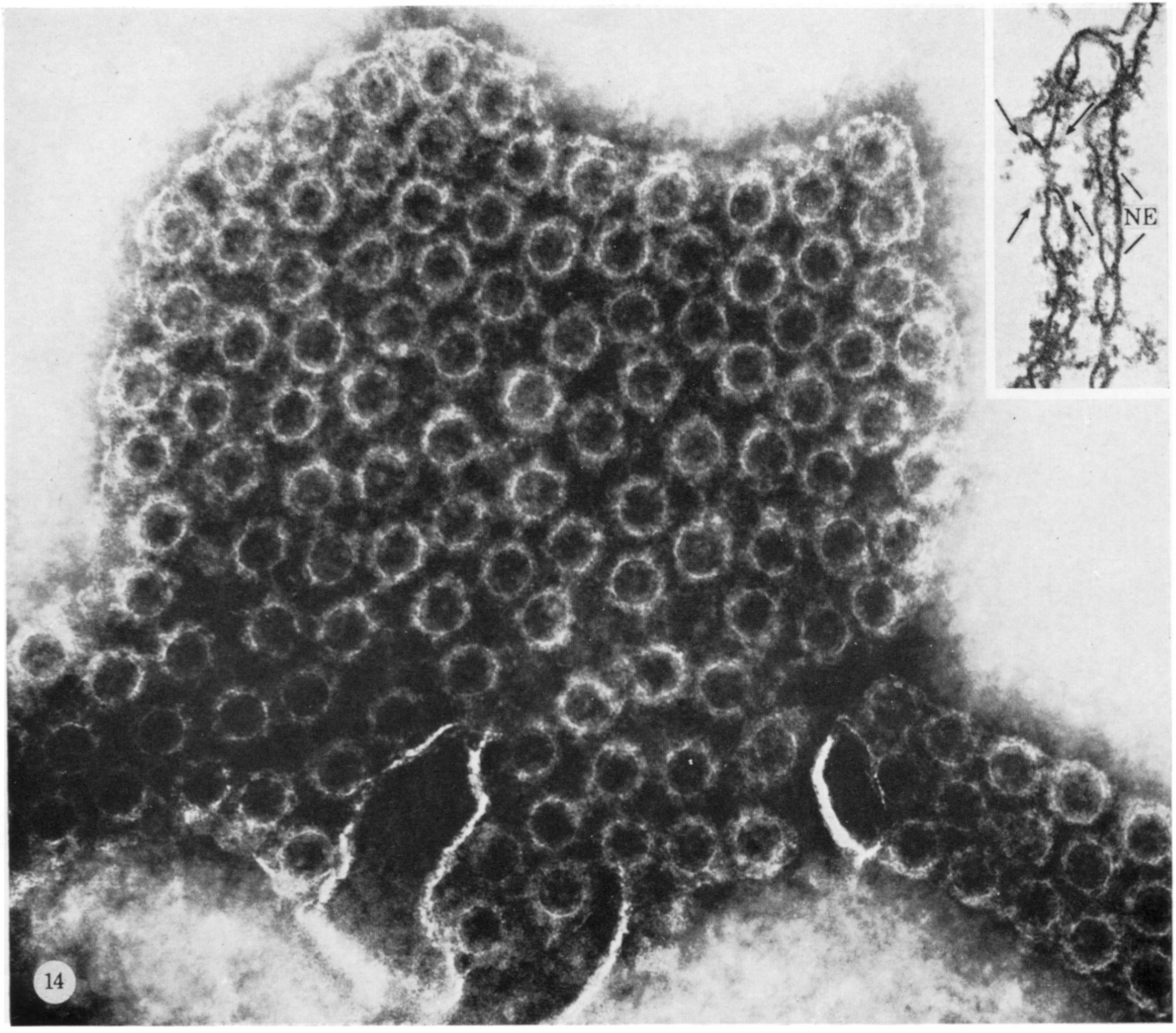
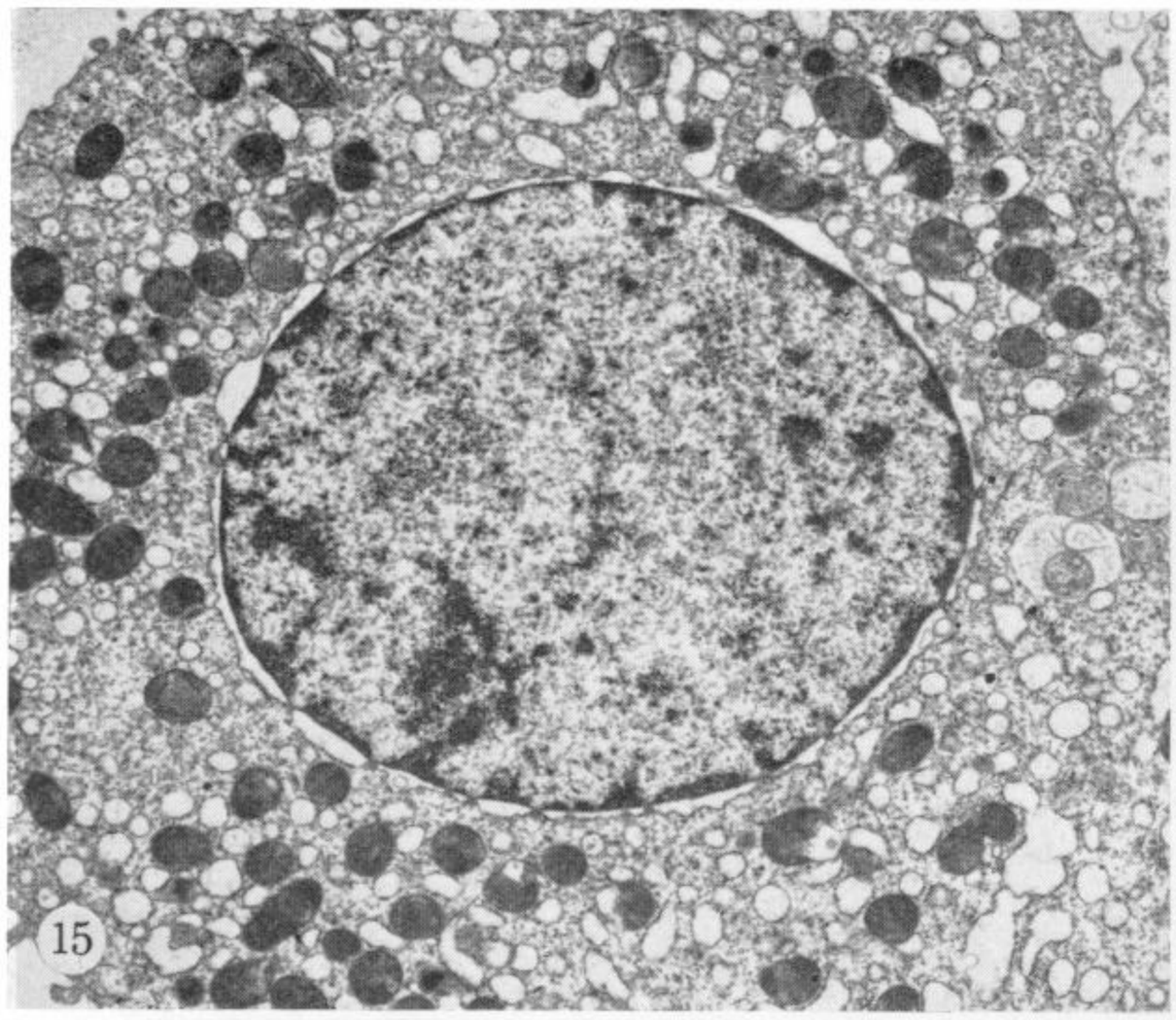
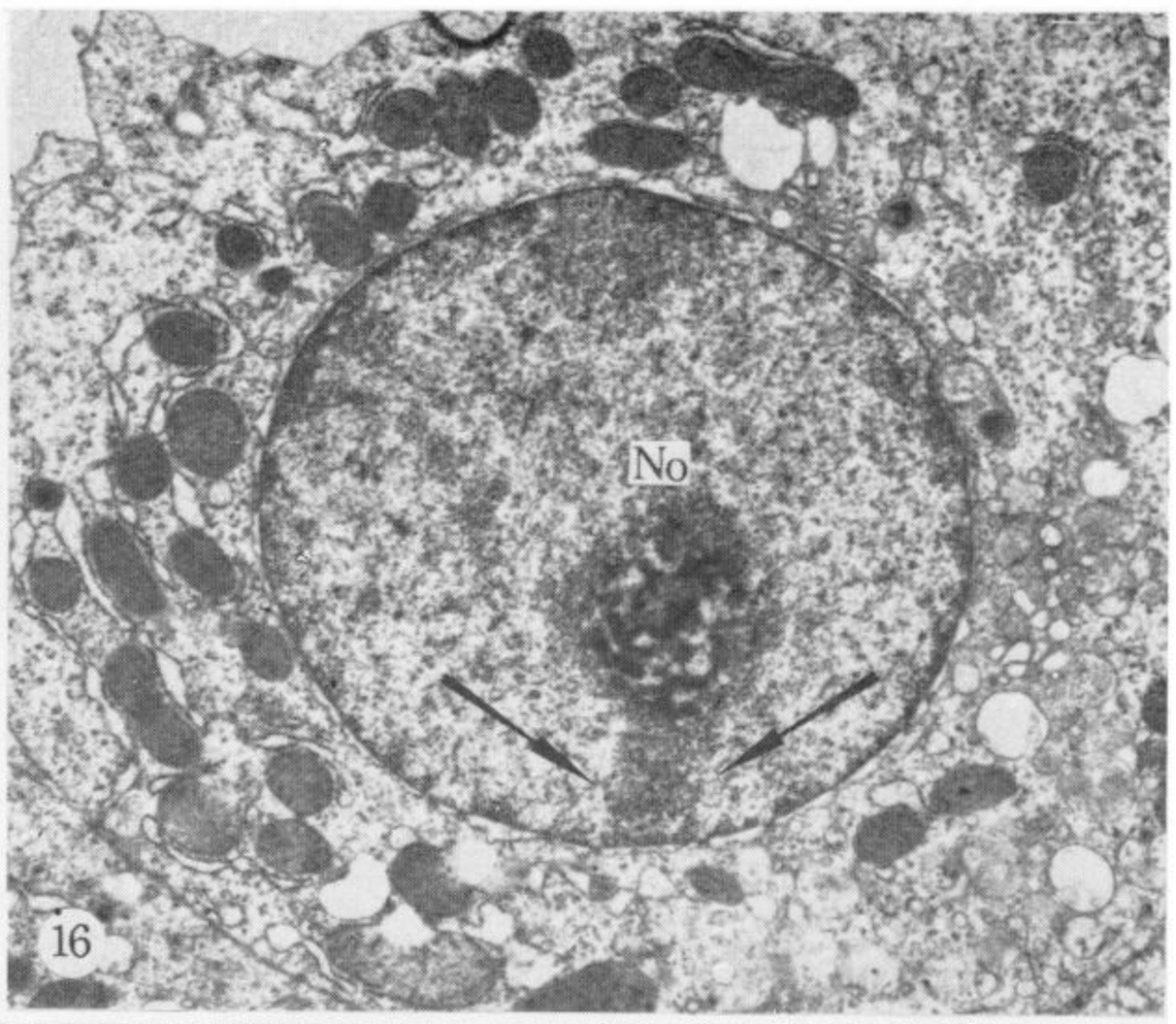
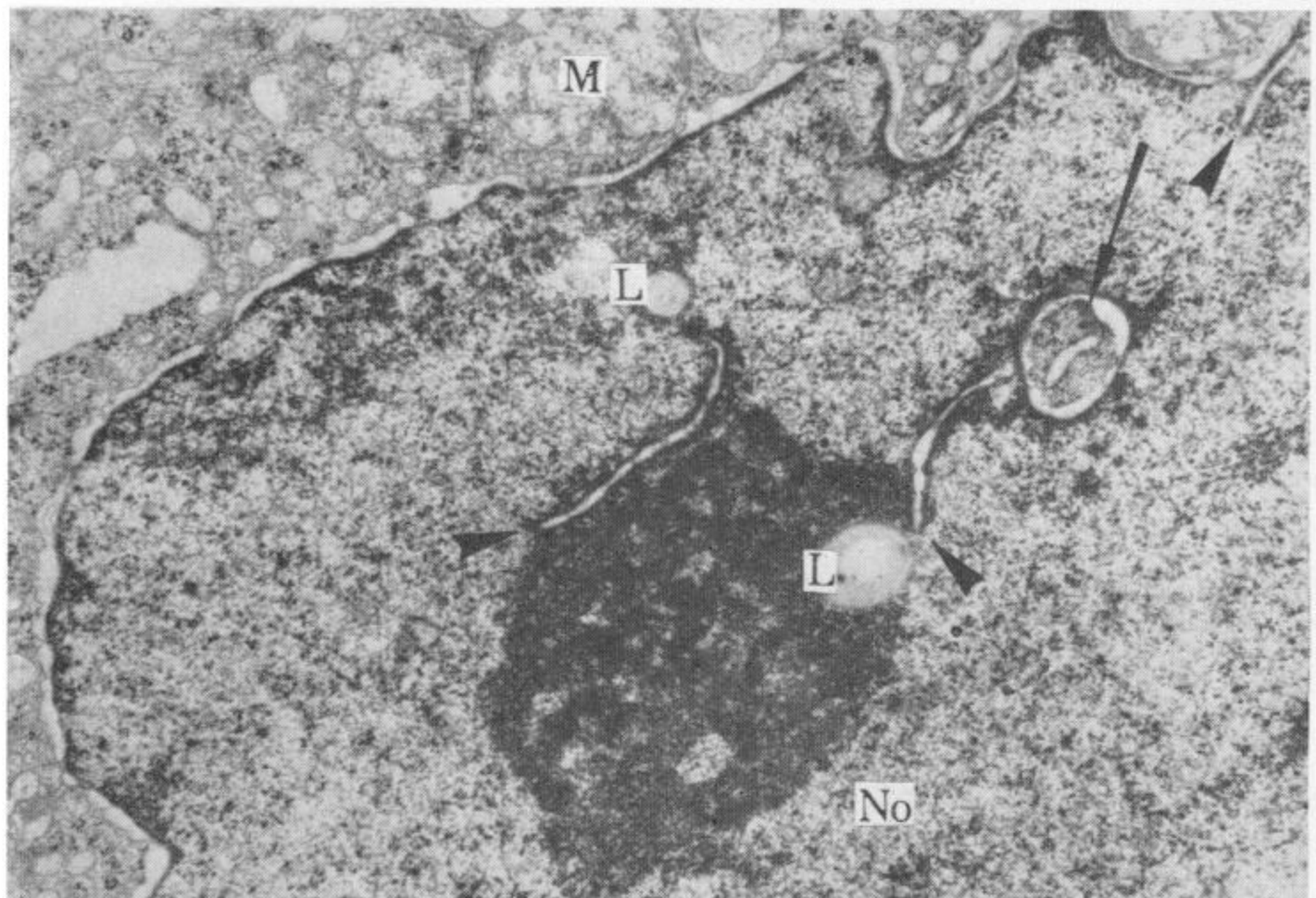
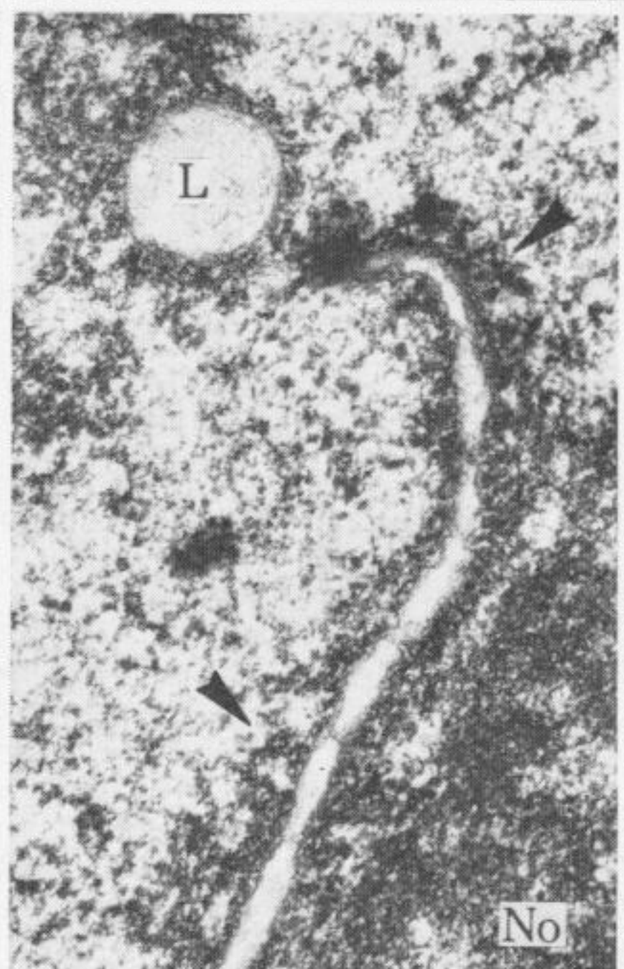


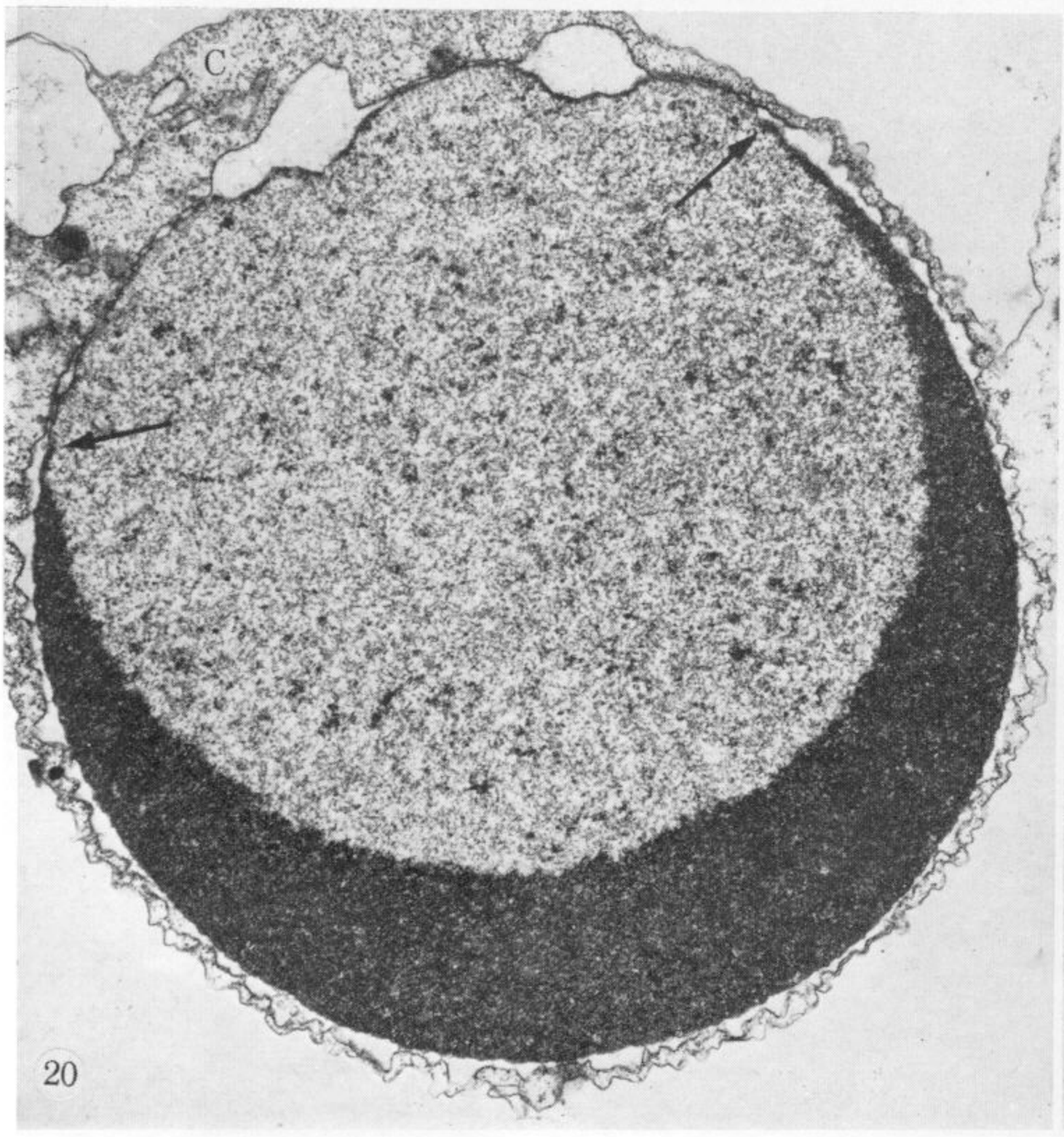
FIGURE 14. Pore complex structures in annulate lamellae (AL) isolated from lampbrush stage amphibian oocytes as revealed by negative staining (figure 14, from *Triturus alpestris*) and ultrathin section (insert, from *Xenopus laevis*). Note that such AL can be in continuity with the nuclear envelope (NE in the insert), and also the identical pore complex composition (e.g. at the arrows in the inset), and the very dense packing of such AL pore complexes. (Magn. ×80000; inset ×55000; compare Scheer & Franke 1969.)

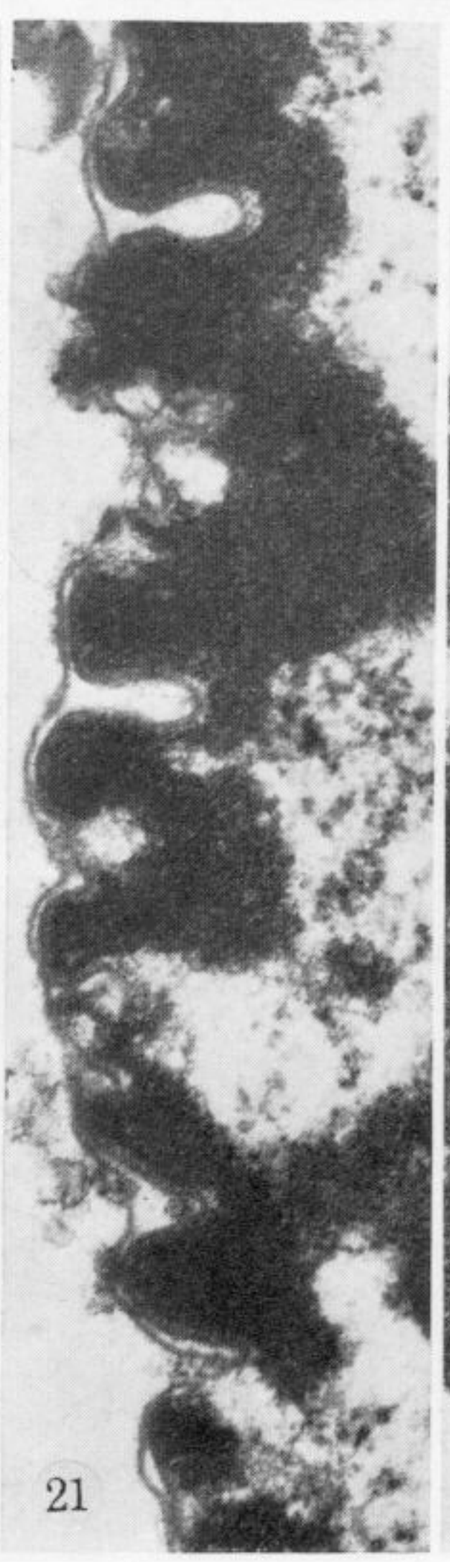


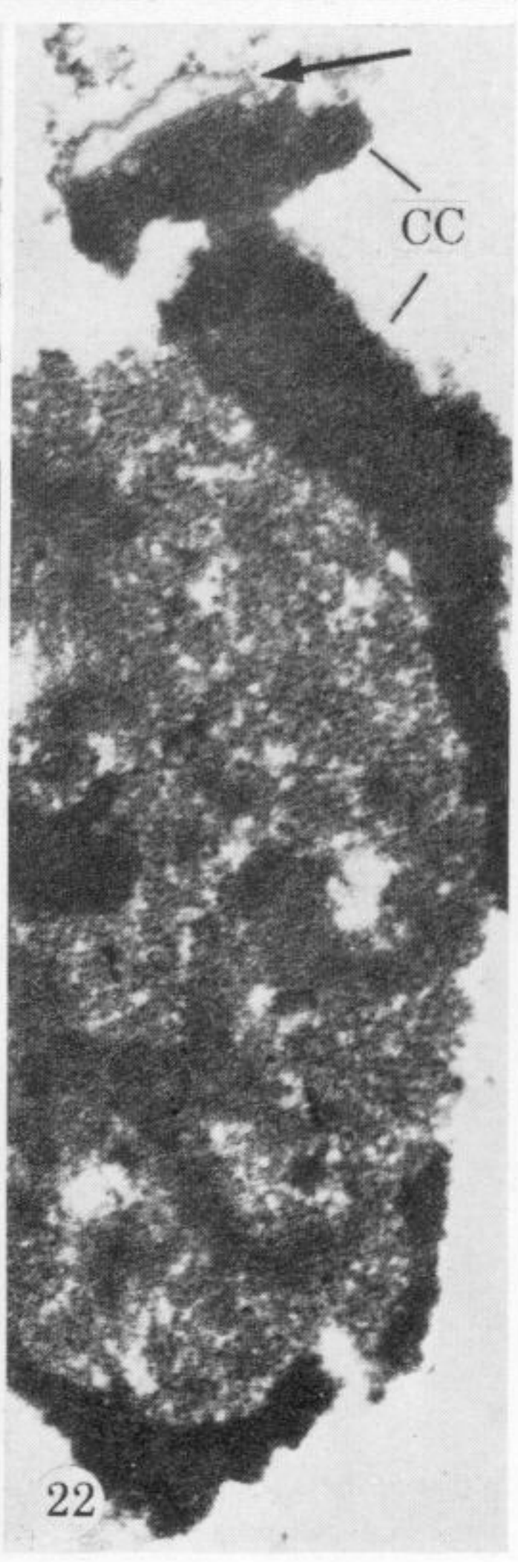






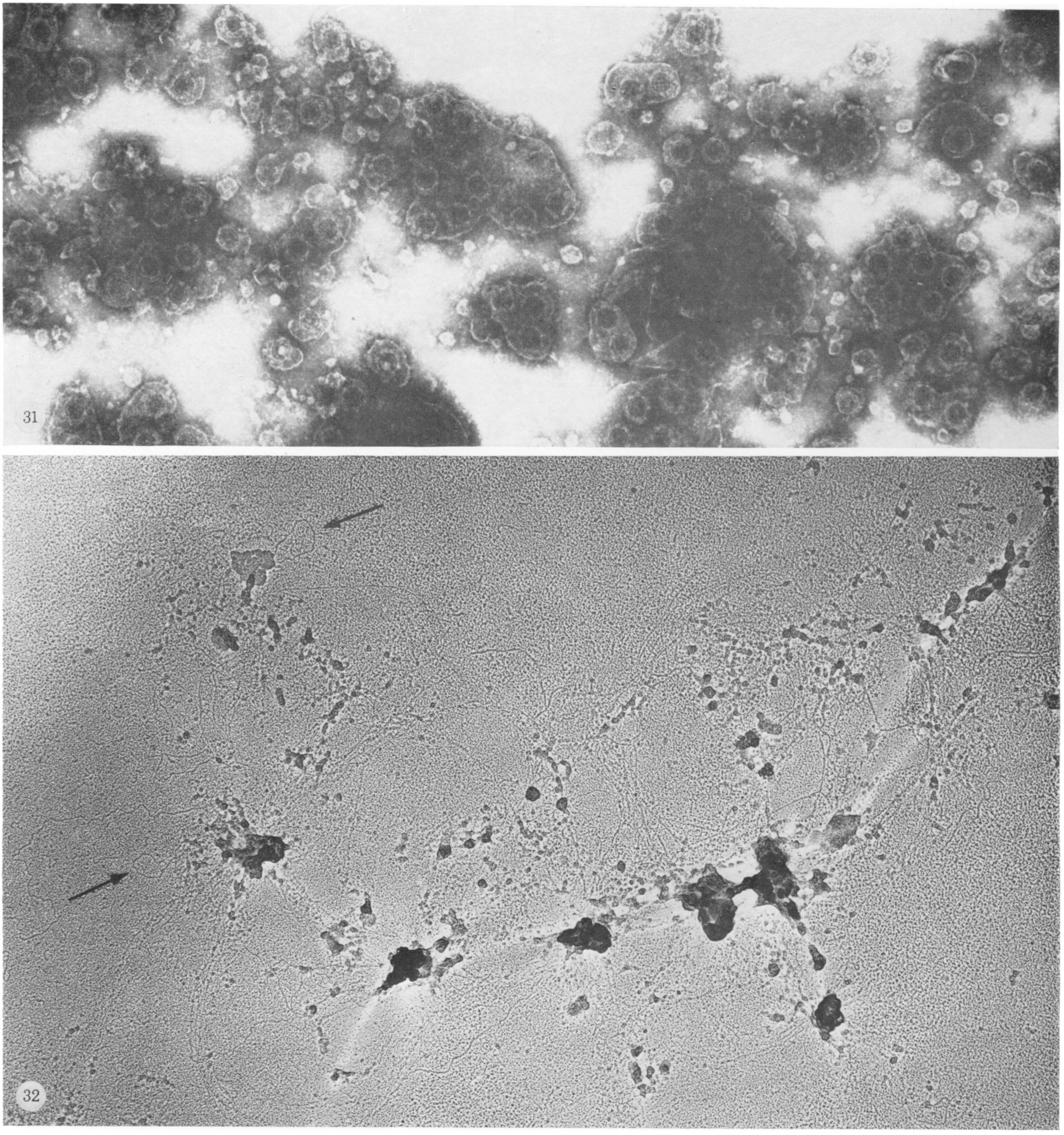




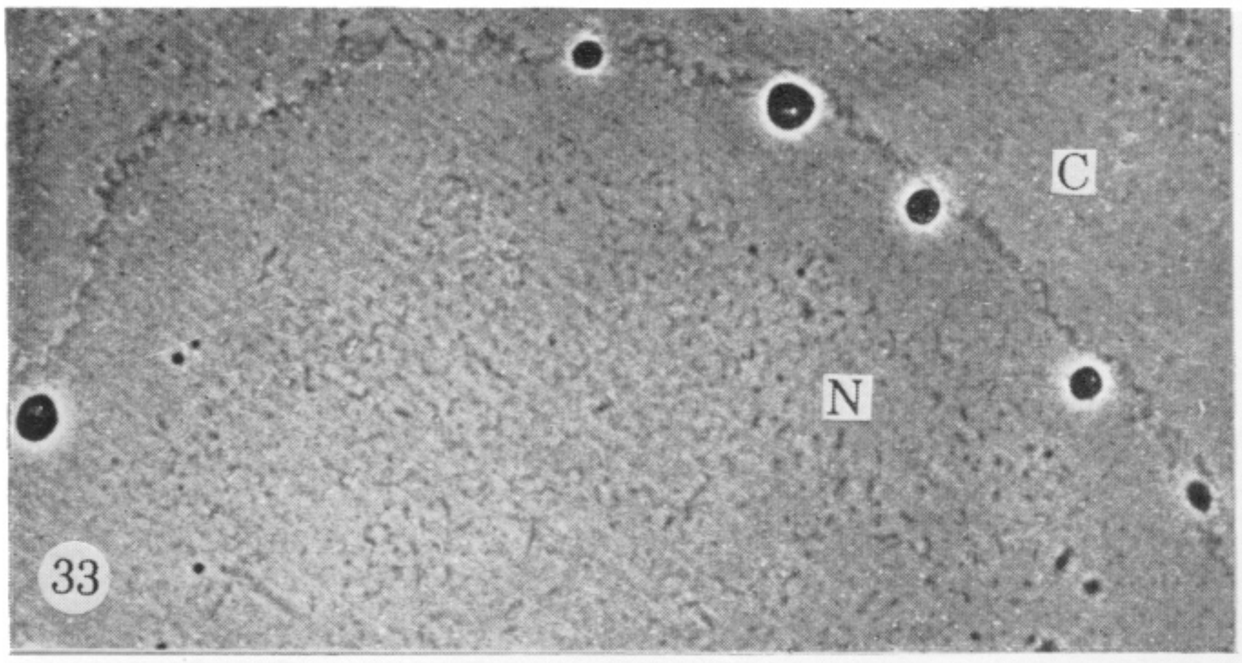


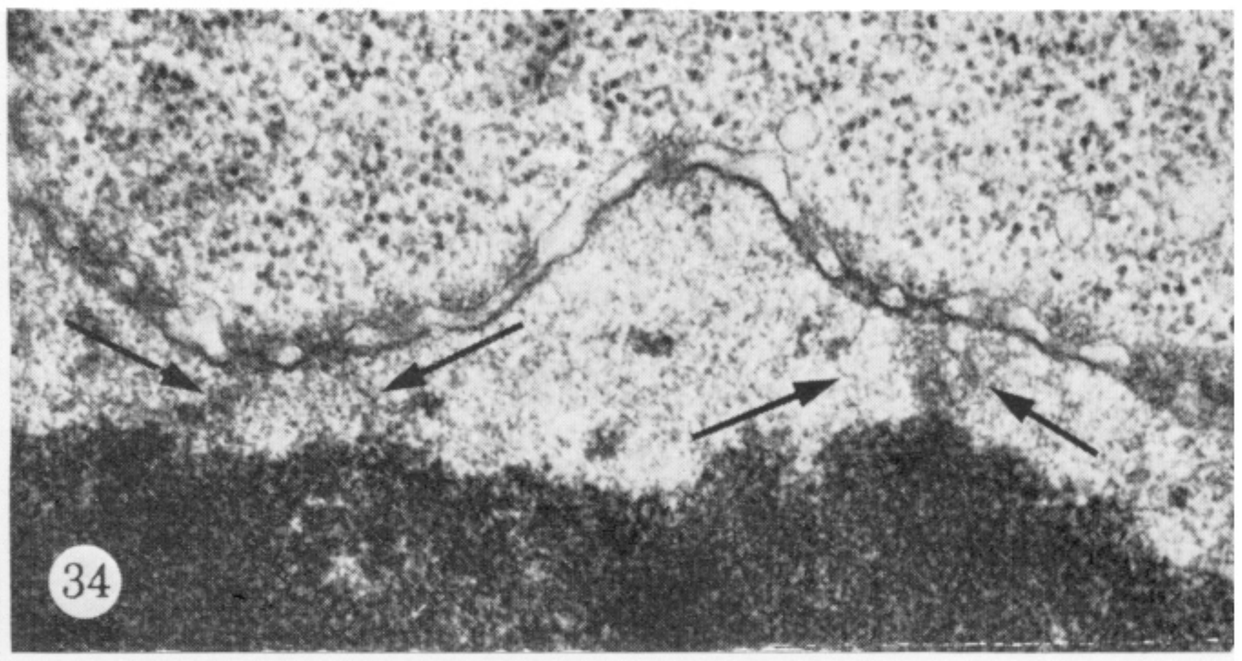
FIGURES 15–22. For description see opposite

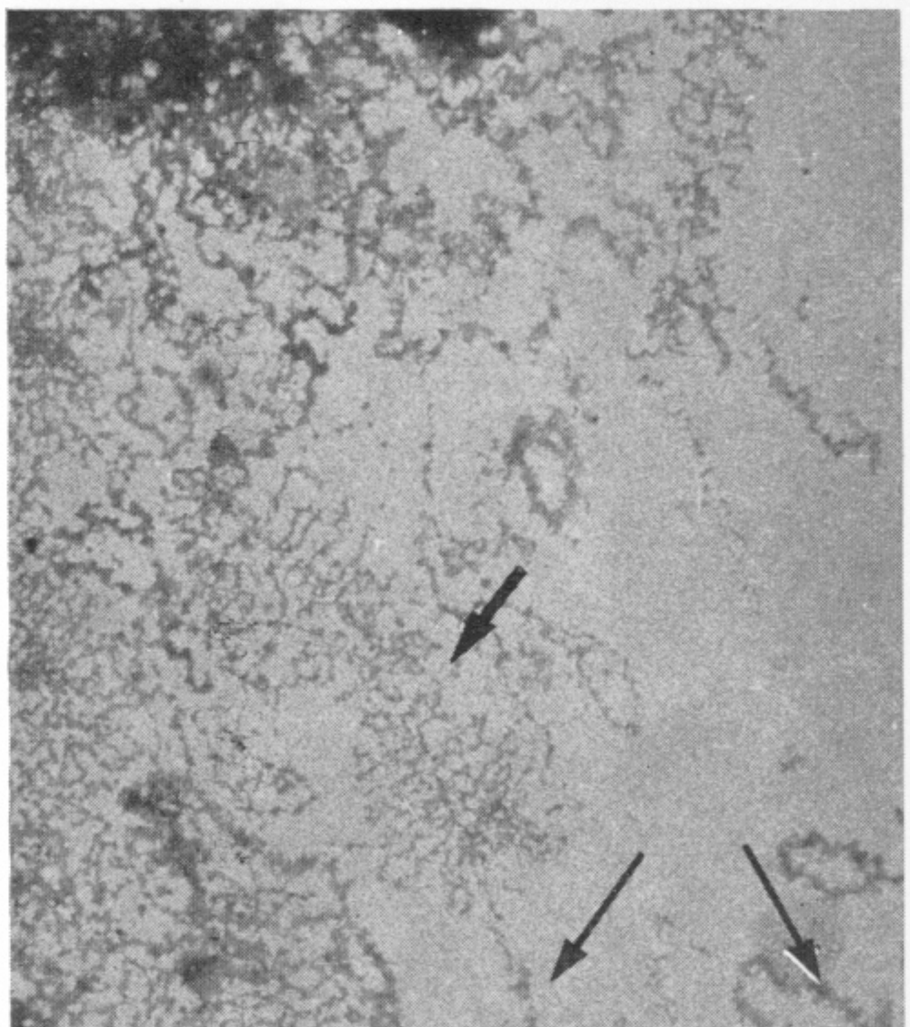
FIGURES 23–30. For description see opposite

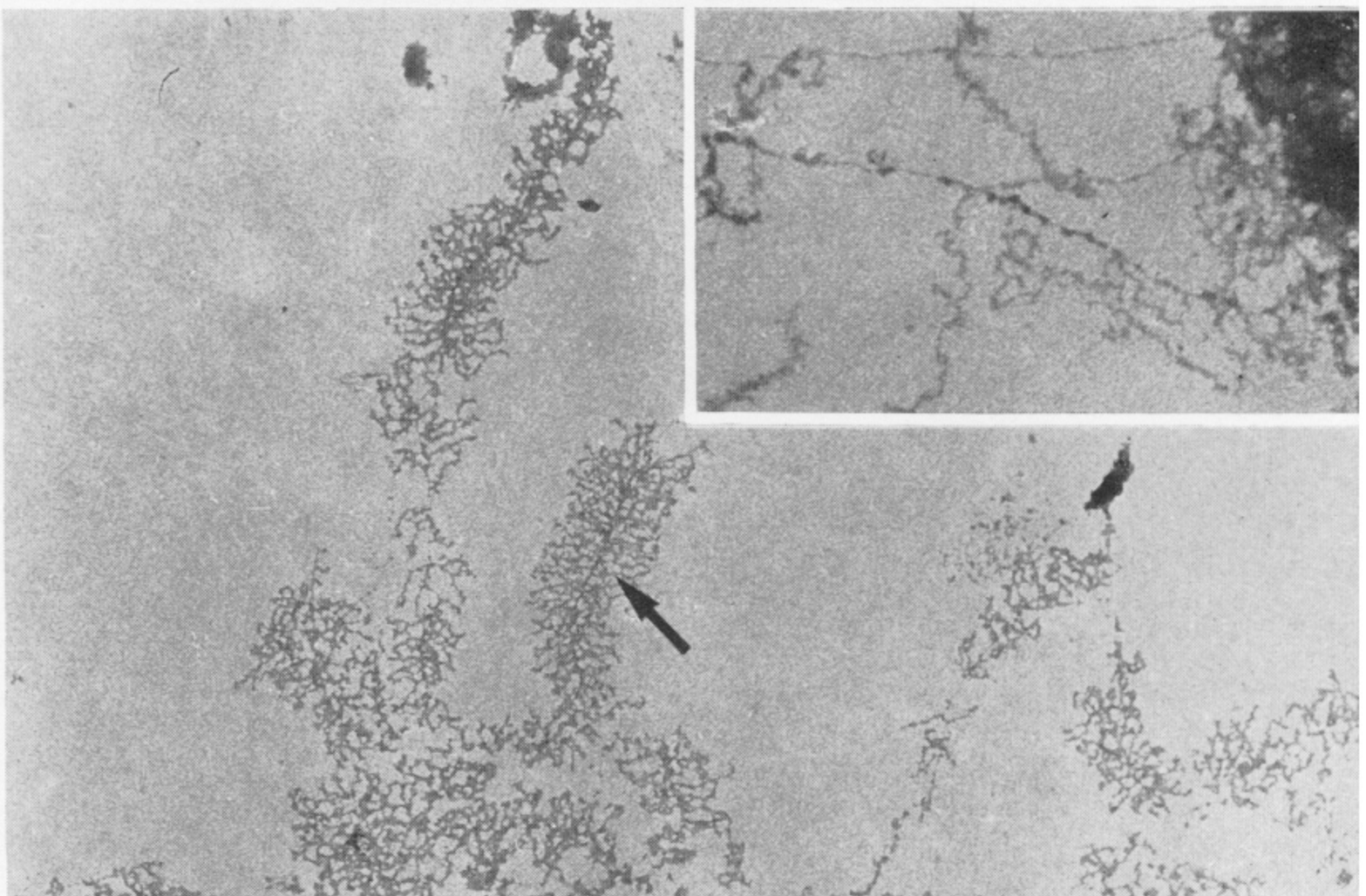


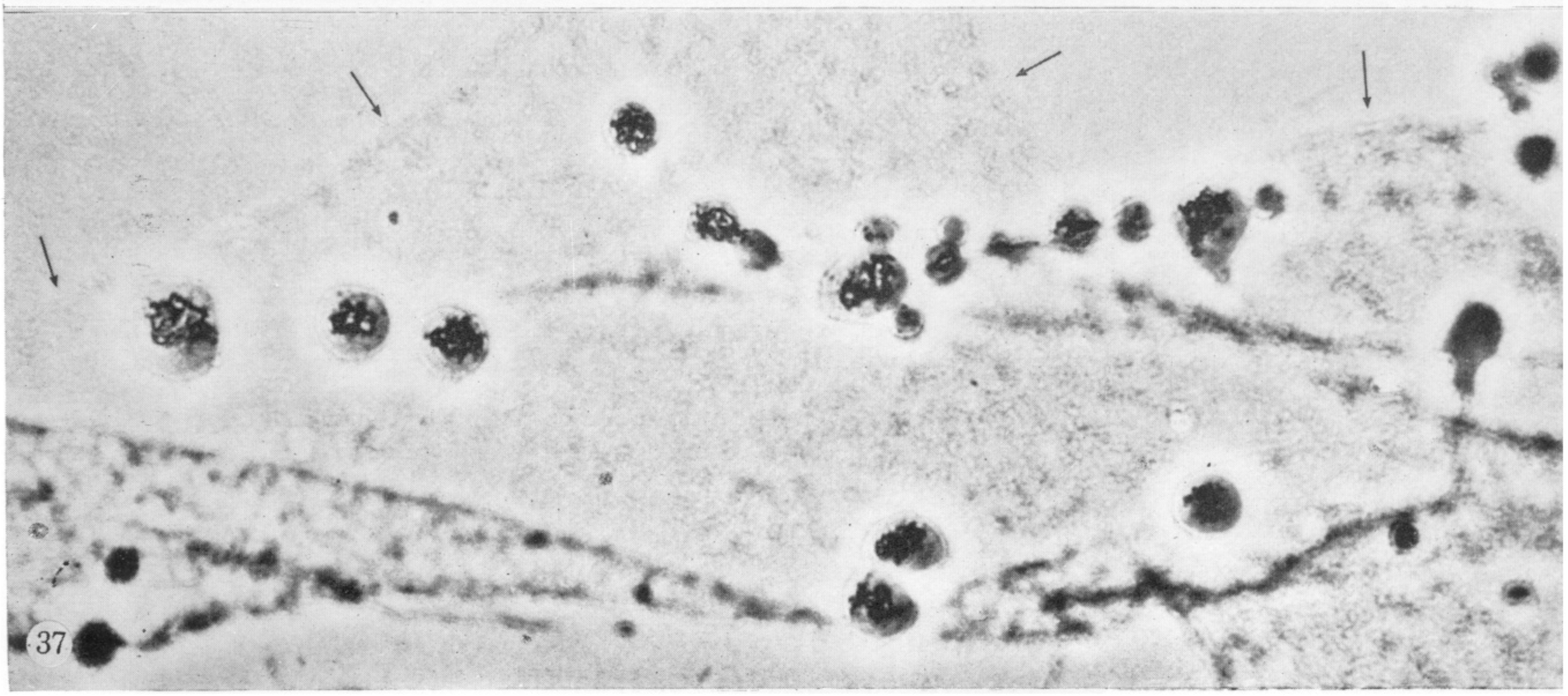
FIGURES 31, 32. When nuclear membranes isolated from rat liver (typical fraction shown in negative staining in figure 31) are purified and spread with the Kleinschmidt technique the membrane fragments reveal associations with DNA molecule strands which frequently appear in loop formations (arrows in figure 32; for details see Franke *et al.* 1973*a*). (Magns: figure 31, ×71 000; figure 32, ×32 000.)



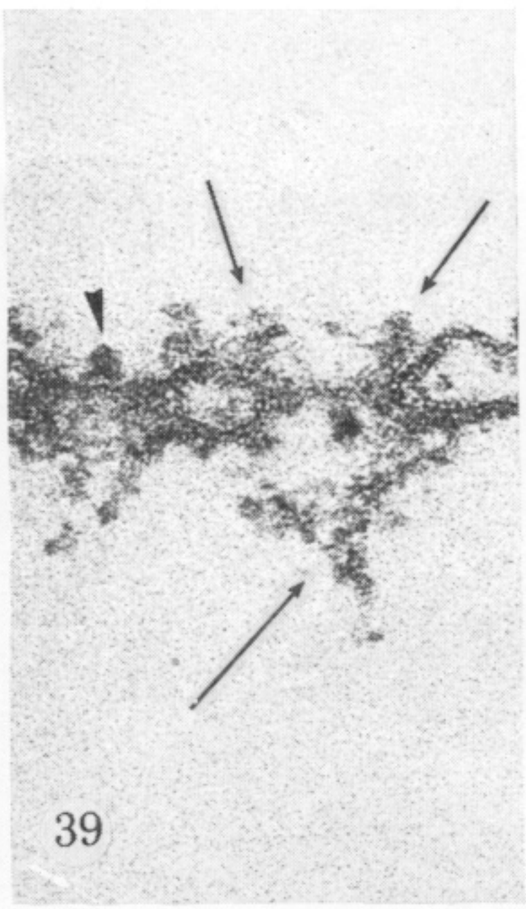


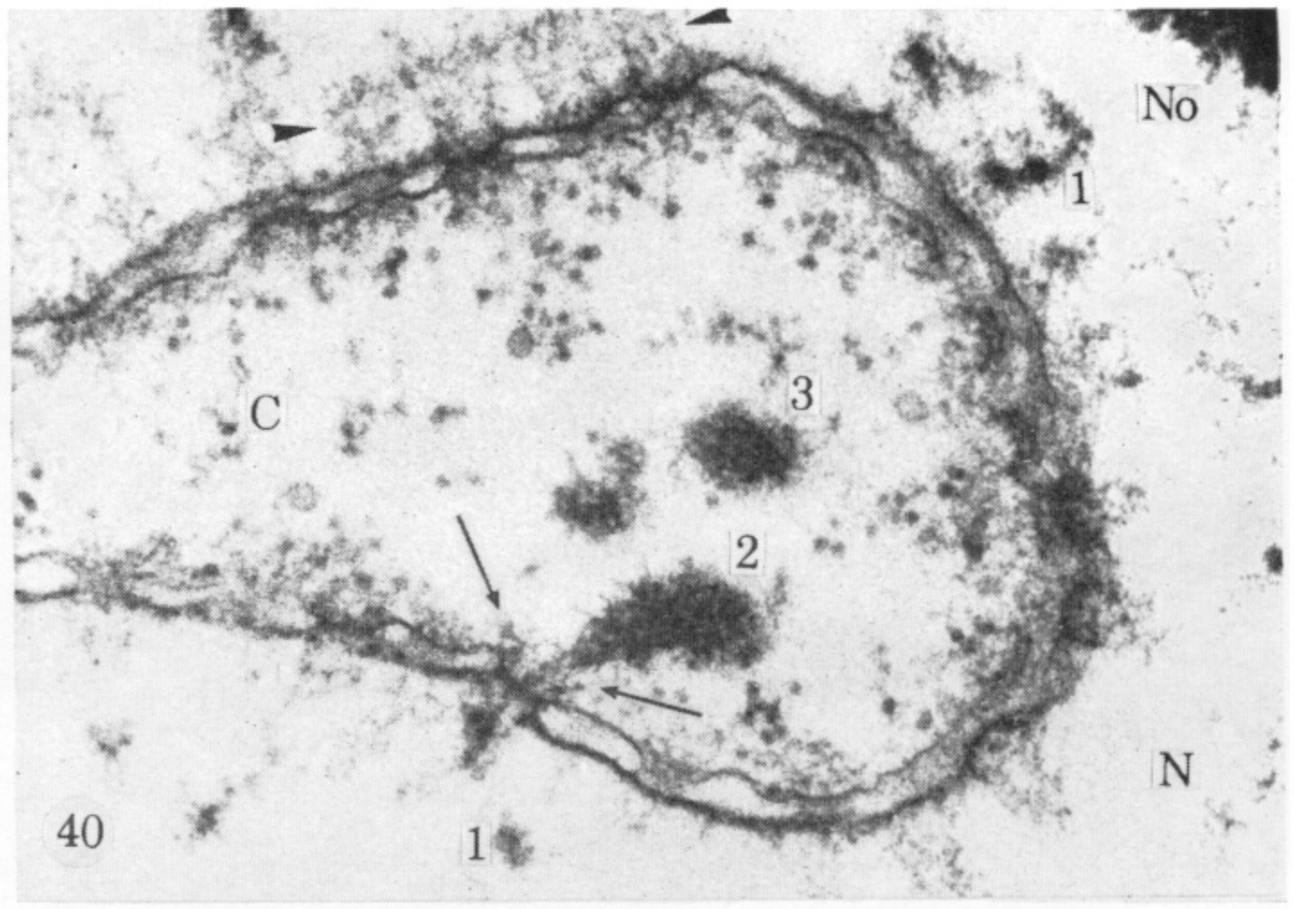


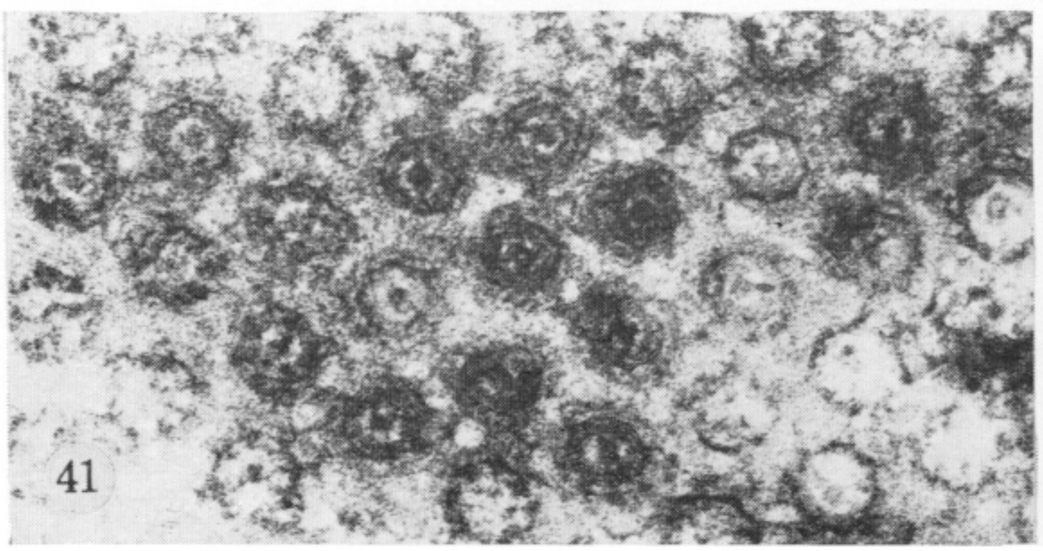


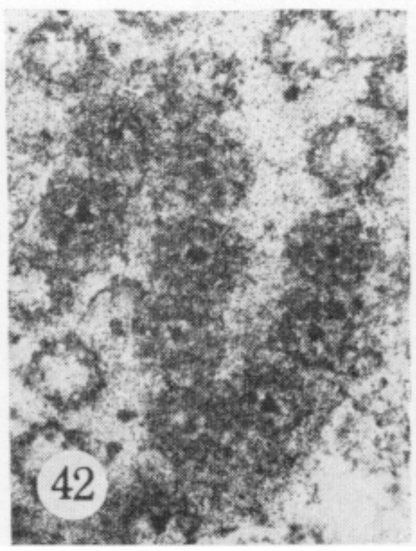


FIGURES 33–37. For description see opposite









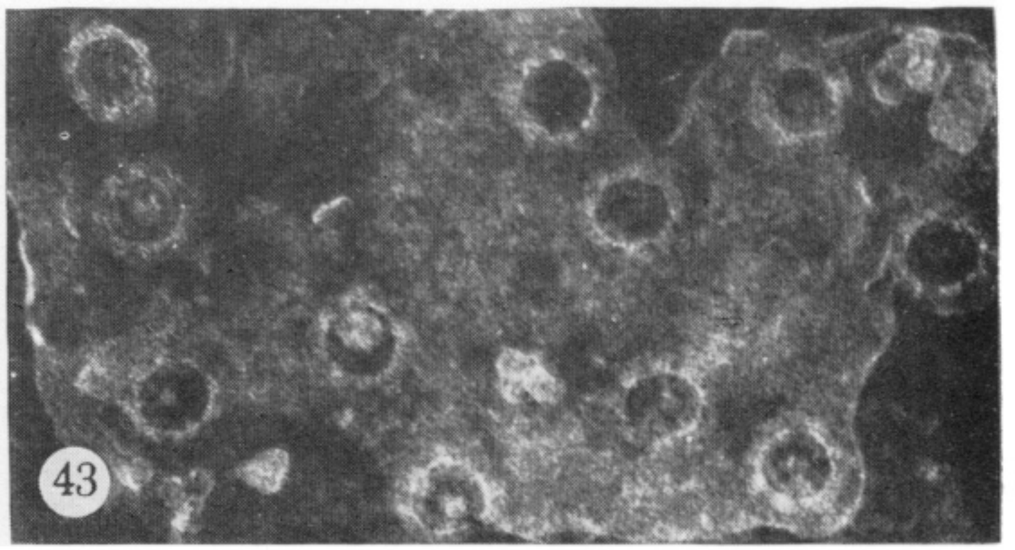
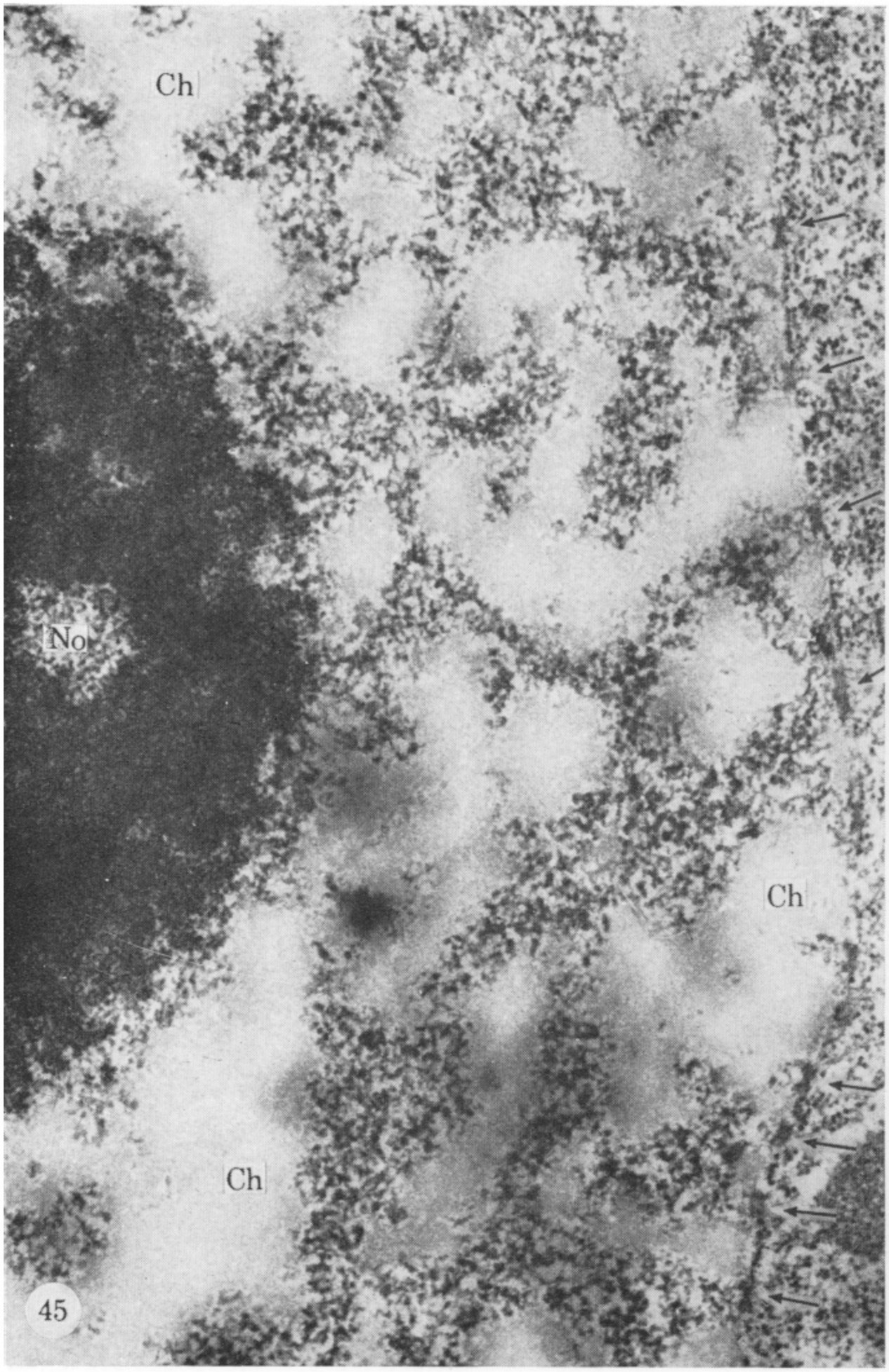
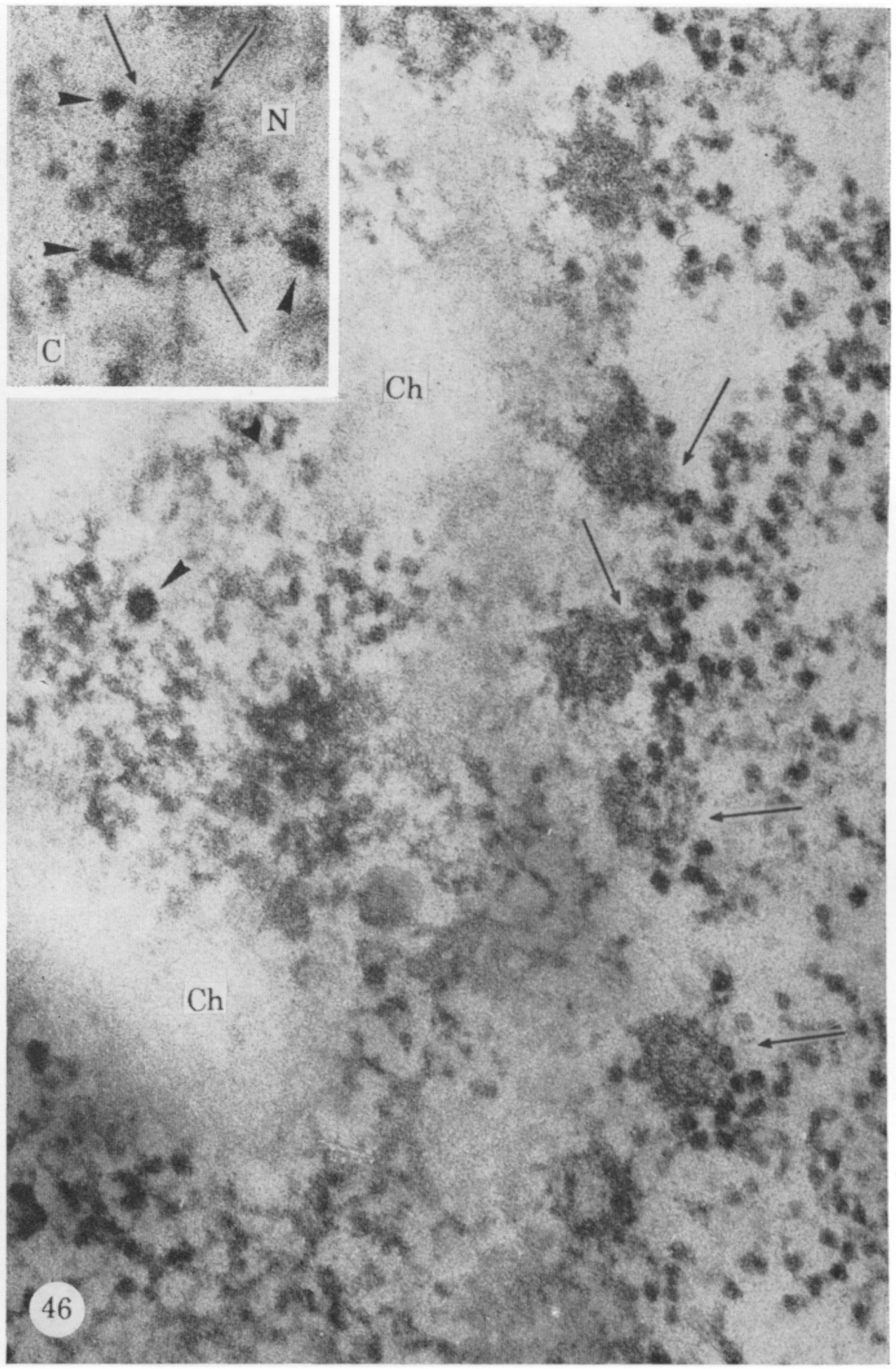


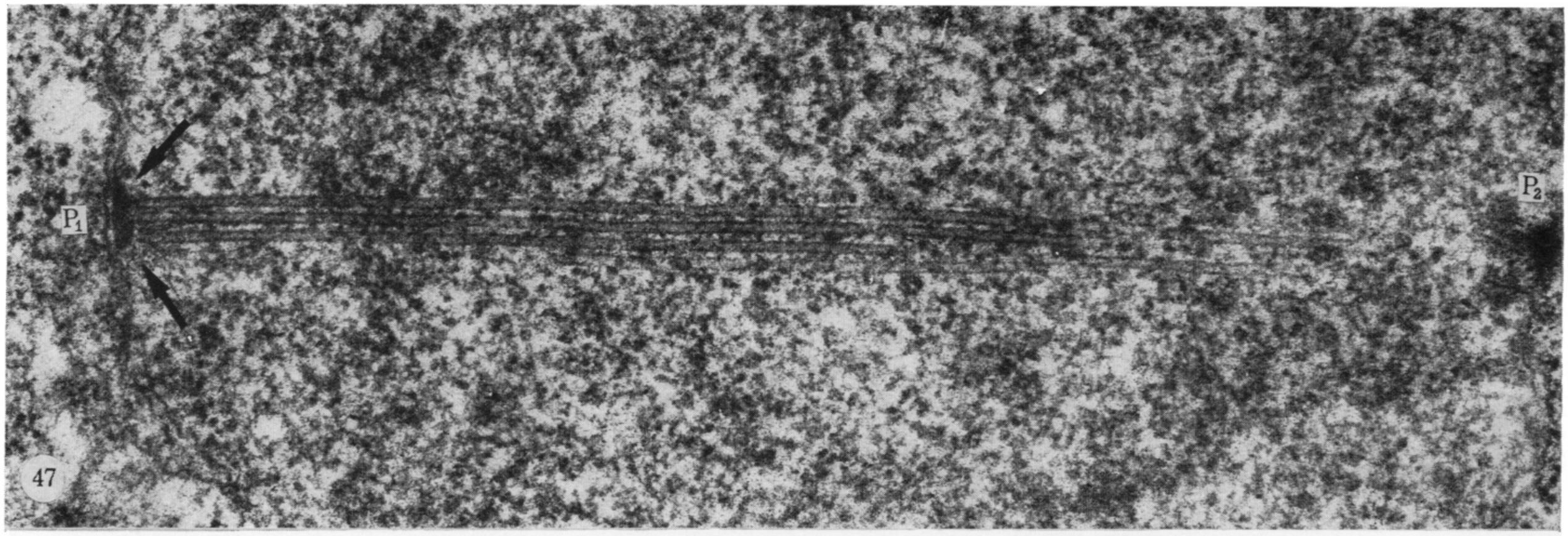
FIGURE 38–40. Details of (presumably nucleolus-derived) structures associated with the nuclear pore complexes in lampbrush stage amphibian oocytes. Direct morphological continuity of groups of fibrils attached to the inner annulus with the nucleolar (No) periphery (denoted by arrows in figure 38) is frequent. Figure 39 shows a pore complex in an isolated nuclear envelope (from *Xenopus laevis*) with the inner annulus attached fibrils (long arrow), annular granules (the outer annulus is denoted in the right pore by the smaller arrows), and one centrally located dense particle which may be either on the nucleoplasmic side, off the pore centre (pore in the right), within the pore, or in contrast (the central granule in the left pore is denoted by the arrowhead) 'already' on the cytoplasmic side of the pore. Note also the difference in electron opacity between the annular granules and the central particles. Figure 40 illustrates the typical form of transport of larger dense aggregates into the cytoplasm. These aggregates assume a 'dumb-bell-shape' when passing the pore complex, indicating that only the very centre (*ca.* 10–15 nm diameter) of the pore is accessible for their passage. The numbers suggest a sequence of such nucleocytoplasmic translocation. (The arrows denote again the outer annulus, the arrowheads in the upper part point to pore complex associated fibrils.) N, nucleoplasm; C, cytoplasm; No, nucleolus. (Magns: figure 38, × 60 000; figure 39, × 121 000; figure 40, × 53 800.)

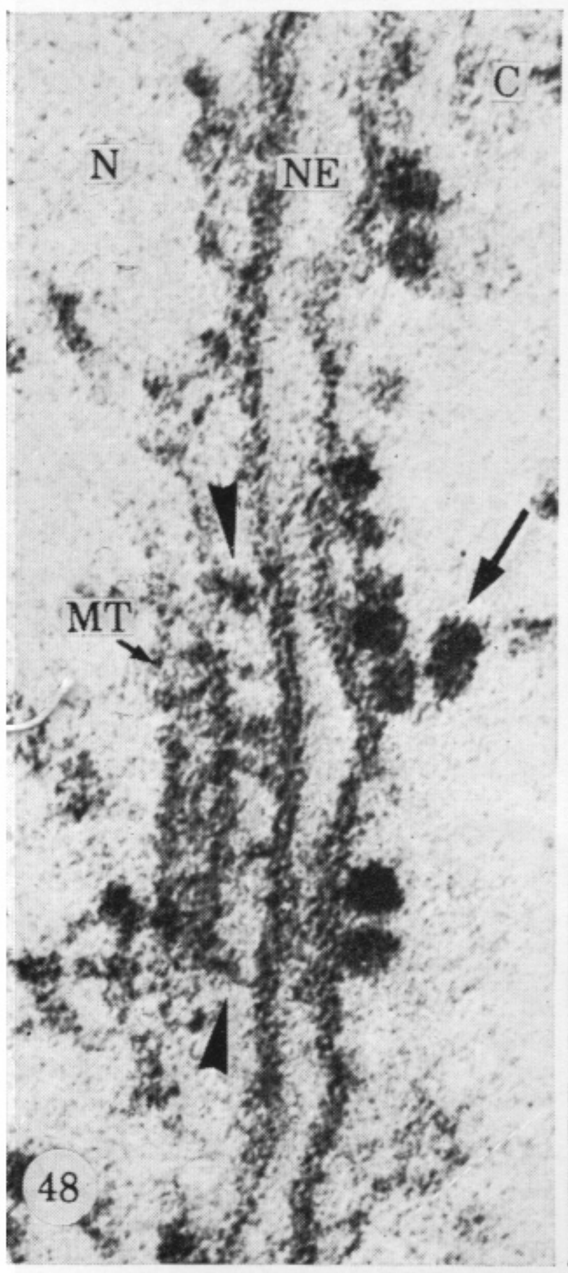
FIGURES 41–43. Central granules can vary in size and form, (e.g. in the negatively stained HeLa nuclear envelope piece shown in figure 43, for details see Comes & Franke 1970), in frequency and electron opacity (see the thin sections of figures 41, 42). They are not confined to nuclei actively engaged in RNA synthesis such as the lampbrush stage (*Xenopus laevis*) nucleus studied in figure 42 but can also be frequent in (inactive') nuclei such as that of the *Triturus alpestris* maturing sperm cell shown in figure 41. (Magns: figure 41, × 70 000; figure 42, × 58 000; figure 43, × 70 000.)

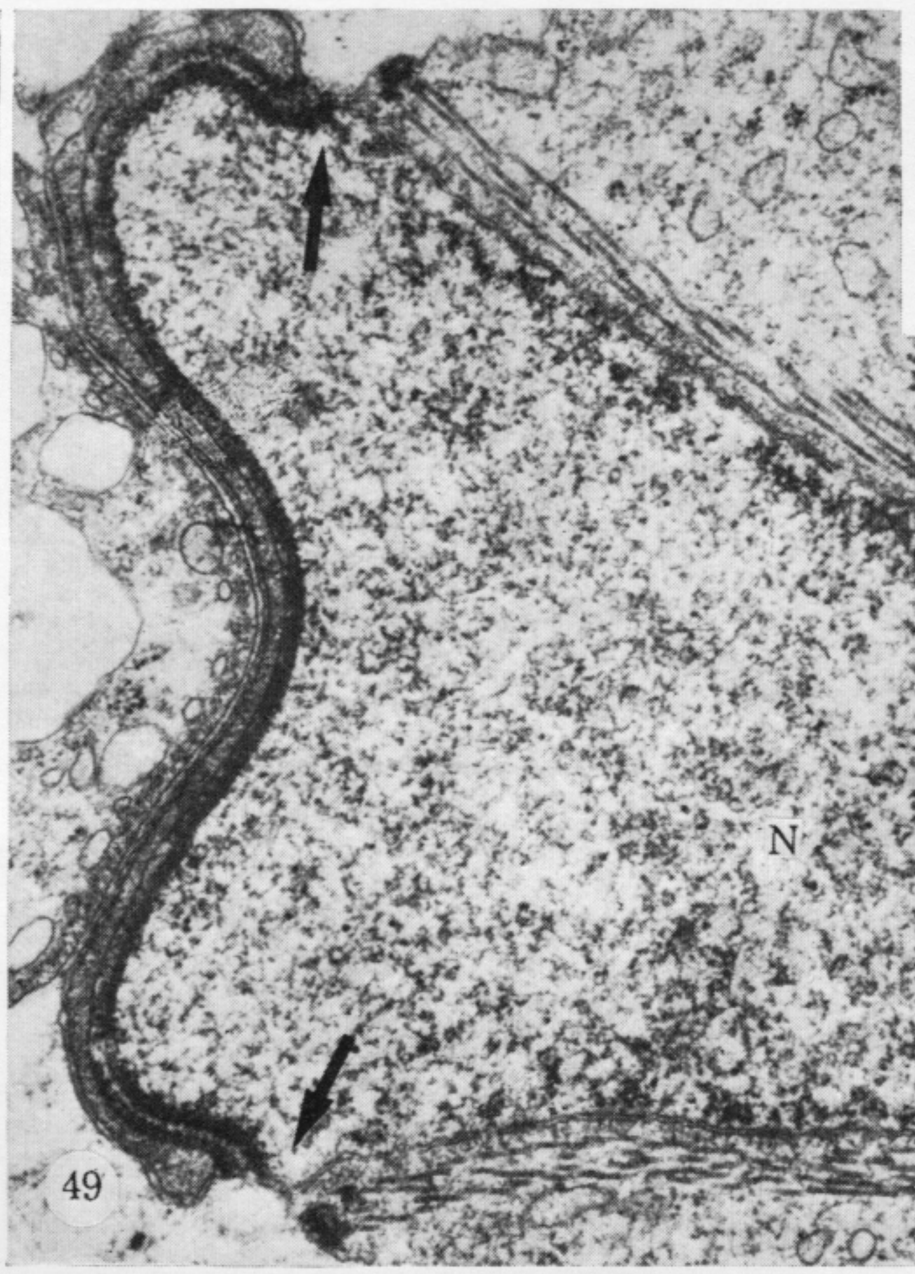


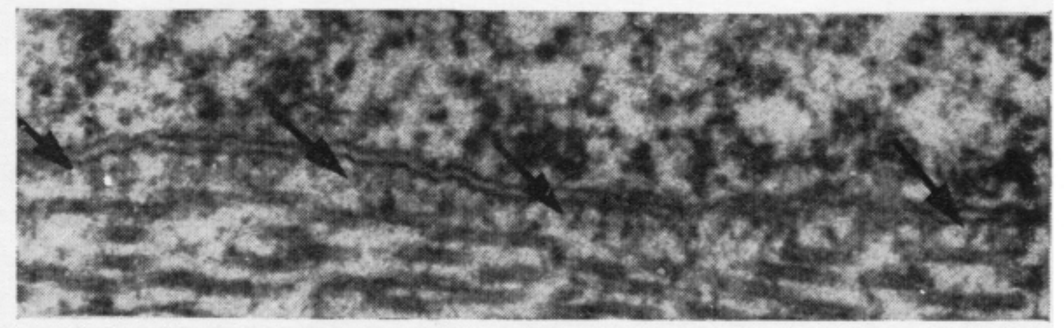


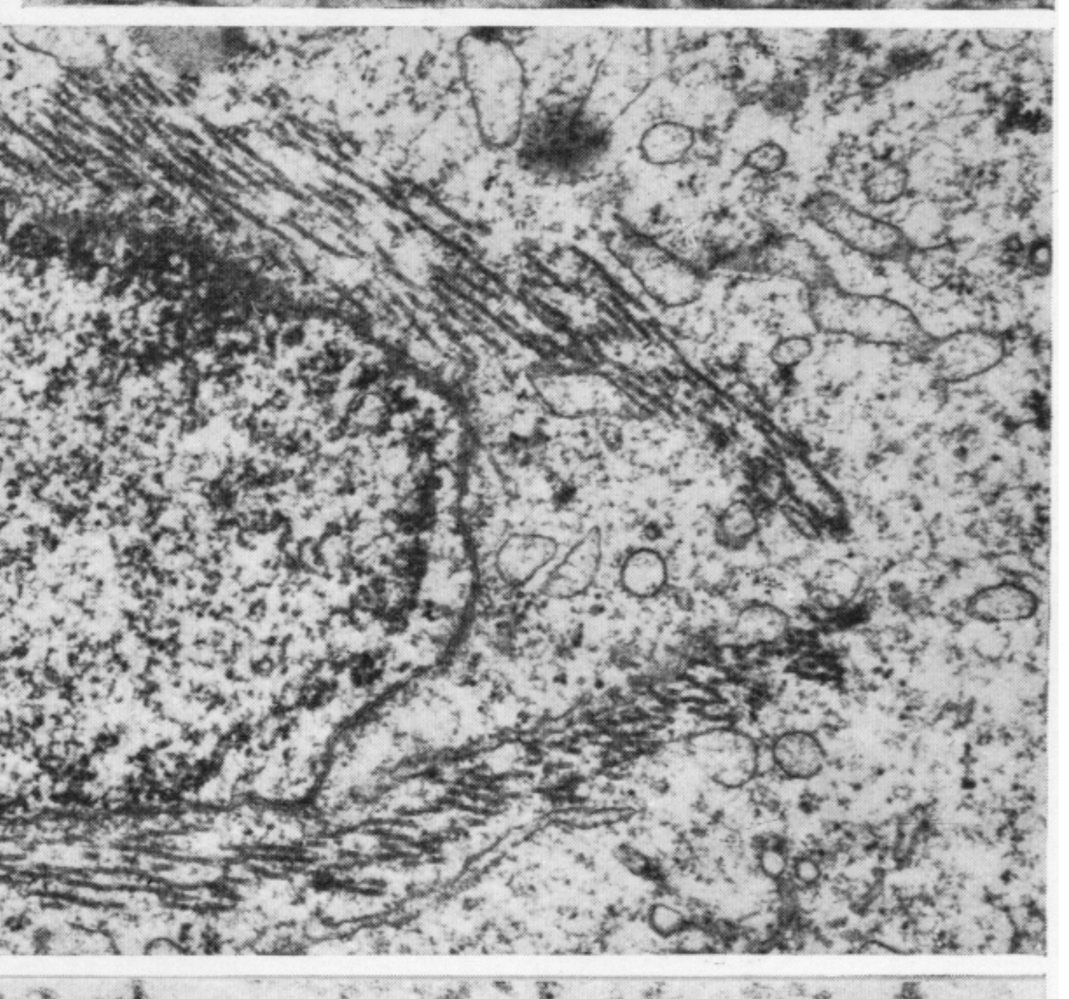
FIGURES 45, 46. In many cytochemical tests the materials contained in the chromatin channels react as typical RNA-containing structures (here shown after the EDTA differentiation of uranyl stain as described by Bernhard (1969); onion root tip cells, for details see Franke & Falk, (1970)). The pore complex components also react in this manner (seen in cross-section in figure 45 and in the inset of figure 46 and in grazing section in figure 46). The arrows in figure 45 and in the inset denote pore complexes, those in figure 46 point to insertions of cytoplasmic polyribosomes at the pore complex annuli. Ch, chromatin (Magns: figure 45, ×27 000; figure 46, ×102 000; inset, ×98 000.)

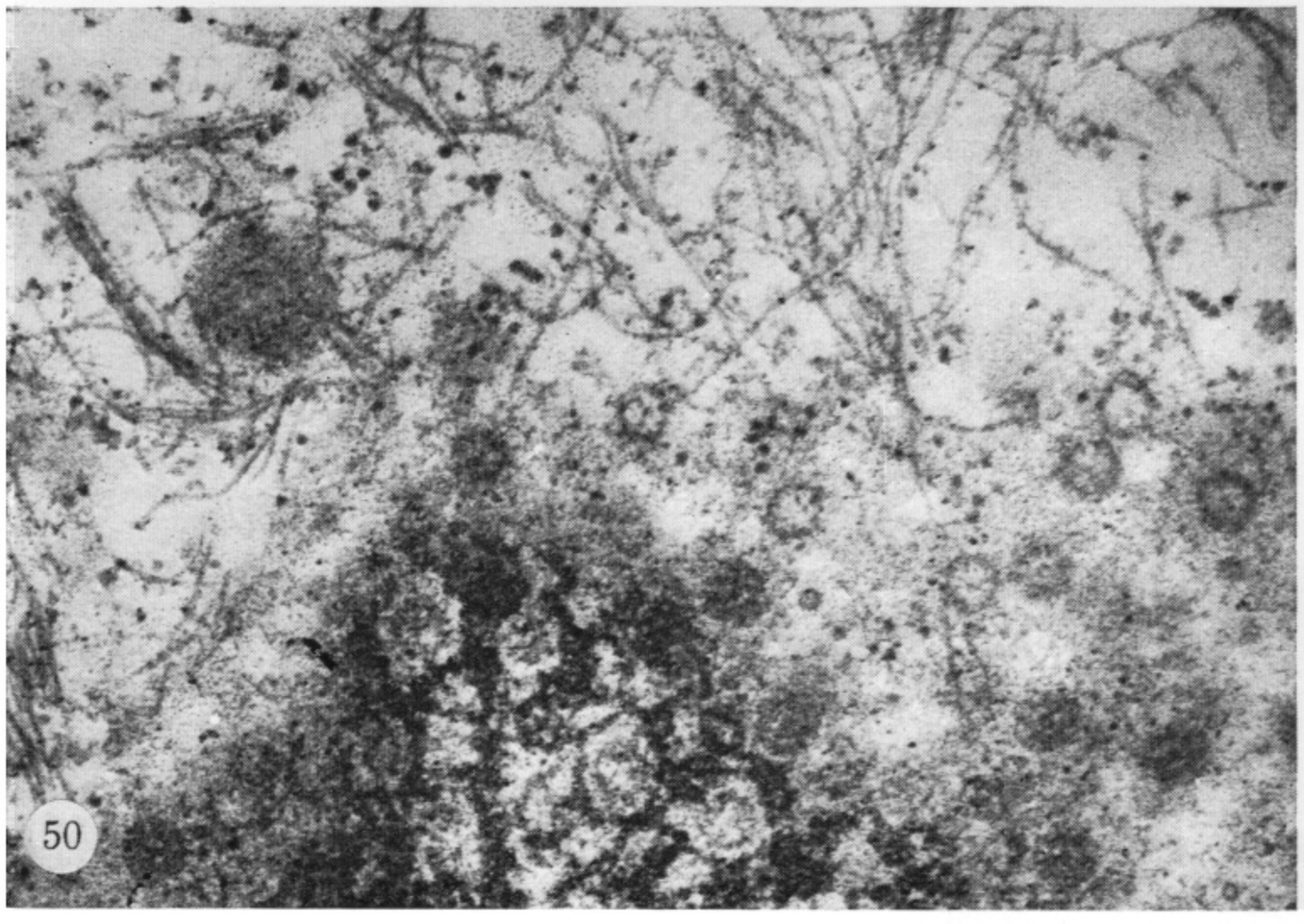


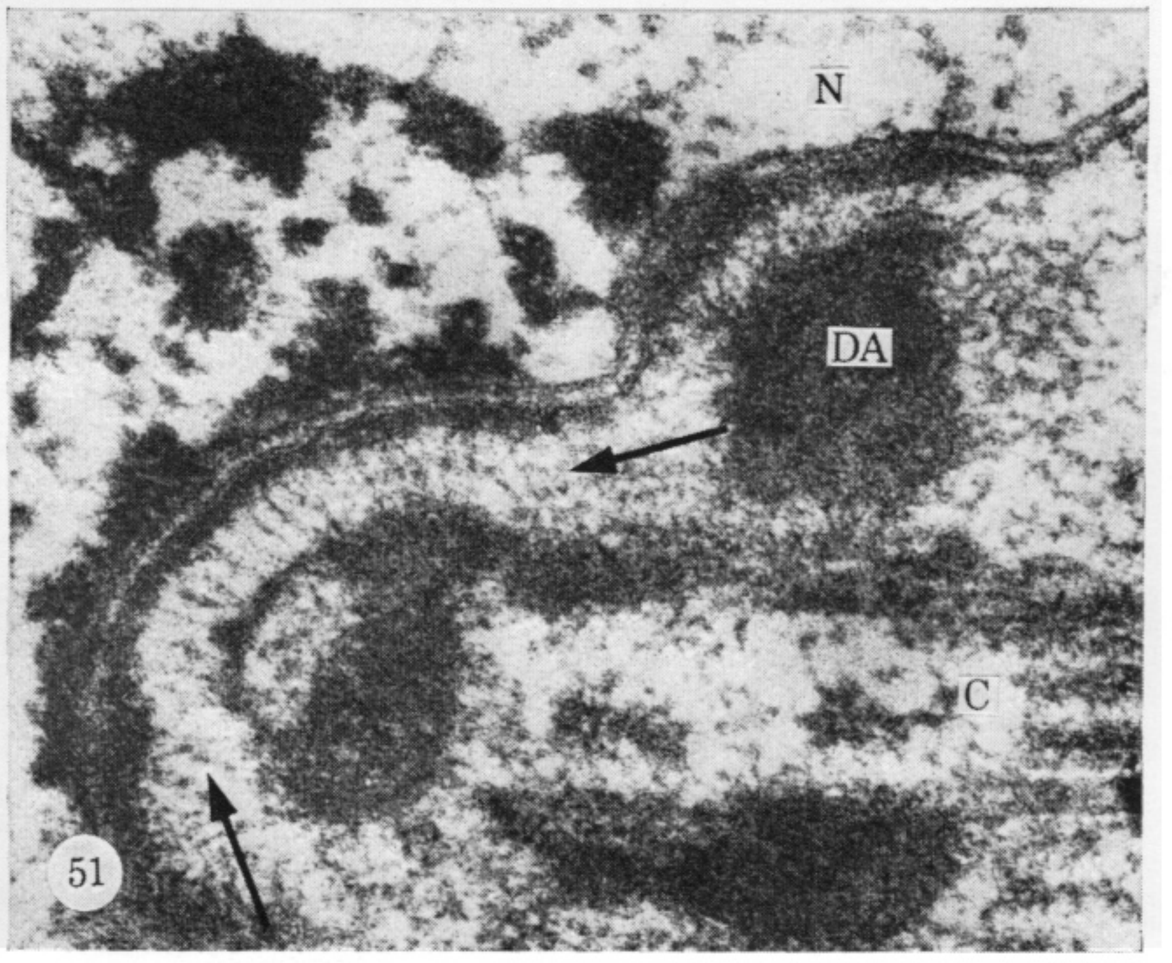












FIGURES 47–51. For description see opposite