NEGATIVE STAINING AND ADENOSINE TRIPHOSPHATASE ACTIVITY OF ANNULATE LAMELLAE OF NEWT OOCYTES

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ABSTRACT

Semi-isolated annulate lamellae were prepared from single newt oocytes (*Triturus alpestris*) by a modified Callan-Tomlin technique. Such preparations were examined with the electron microscope, and the negative staining appearance of the annulate lamellae is described. The annulate lamellae can be detected either adhering to the nuclear envelope or being detached from it. Sometimes they are observed to be connected with slender tubular-like structures interpreted as parts of the endoplasmic reticulum. The results obtained from negative staining are combined with those from sections. Especially, the structural data on the annulate lamellae and the nuclear envelope of the very same cell were compared. Evidence is presented that in the oocytes studied the two kinds of porous cisternae, namely annulate lamellae and nuclear envelope, are markedly distinguished in that the annulate lamellae exhibit a much higher pore frequency (generally about twice that found for the corresponding nuclear envelope) and have also a relative pore area occupying as much as 32% to 55% of the cisternal surface (compared with 13% to 22% in the nuclear envelopes). The pore diameter and all other ultrastructural details of the pore complexes, however, are equivalent in both kinds of porous cisternae. Like the annuli of the nuclear pore complexes of various animal and plant cells, the annuli of the annulate lamellae pores reveal also an eightfold symmetry of their subunits in negatively stained as well as in sectioned material. Furthermore, the annulate lamellae are shown to be a site of activity of the Mg-Na-K-stimulated ATPase.

INTRODUCTION

Nearly all the information on annulate lamellae accumulated till today (more than 70 publications; most recently evaluated in an excellent review by Kessel, reference 36) is based entirely on electron microscopic observations of ultrathin sections through intact cells. It is the purpose of the present study to report on some attempts to gain more information on annulate lamellae by applying the techniques of negative staining and of enzyme localization on semi-isolated annulate lamellae of oocytes of the Alpine newt. Furthermore, since the annulate lamellae are generally thought of as originating directly from the nuclear envelope, (e.g. 2, 3, 10, 11, 15, 27–29, 32–34, 48, 57, 63, 65) special importance has been laid, in this investigation, on collecting structural data for comparing the nuclear envelope and the annulate lamellae of the very same material.

MATERIALS AND METHODS

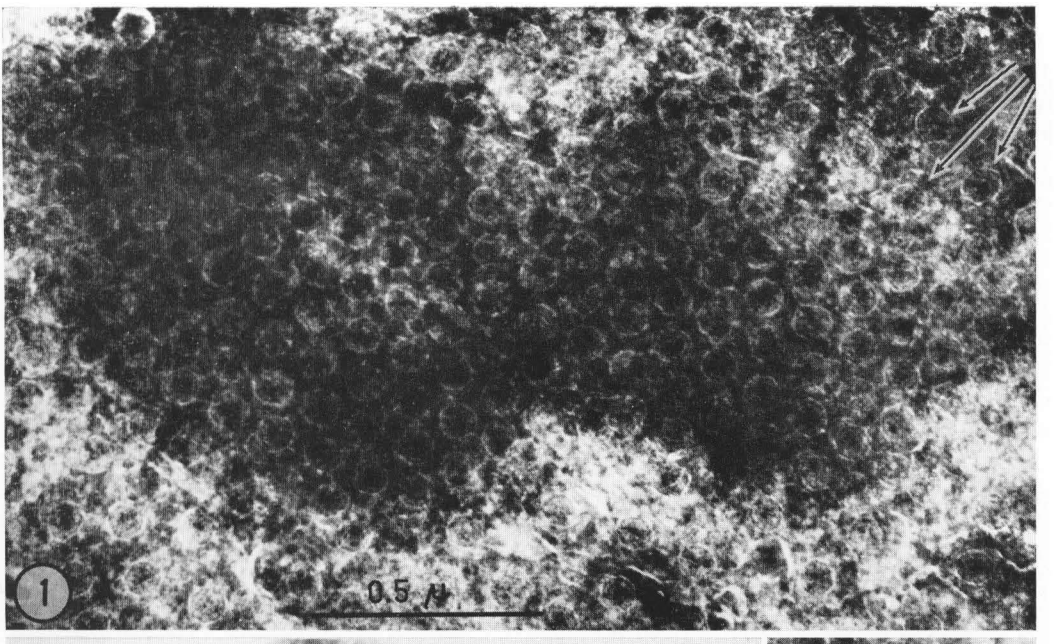
Ovaries were removed from freshly decapitated *Triturus alpestris* and immediately brought into medium A consisting of 0.1 M KCl and 0.1 M NaCl in a ratio of

5:1 (7, 21) or to medium B of the same composition but with added 2 mM or 10 mM $MgCl_2$. The nucleus of a nearly mature oocyte was isolated according to the method of Callan and Tomlin (8) and quickly transferred onto a Formvar-coated grid. Then the nuclear membrane and the associated annulate lamellae were prepared by a technique slightly modified from that employed by other authors (21, 49). In order to preserve the structural integrity of the annulate lamellae as well as of the nuclear envelope, we refrained from any washing, because the cisternae of the annulate lamellae adhering to the nuclear envelope are easily detached. After most of the solution was drawn off, the flattened nuclear envelope-annulate lamellae complexes were fixed for 15 min at 4°C by addition of a drop of 1% OsO_4, buffered to pH 7.2 with 0.1 M cacodylate. Then they were washed twice in double distilled water and negatively stained with 4% phosphotungstic acid (PTA) which had been adjusted to pH 7.2. Whereas medium B gave much better preservation of the annulus and the internal material of the pore complexes of both nuclear envelopes and annulate lamellae, medium A had the advantage of giving a more distinct appearance of the pores, i.e. clearly recognizable pore margins, probably because of a removal of pore complex material as a result of the lack of any bivalent cations. The influence of different cations on the structures of the pore complex will be described in a more detailed paper (19). In order to demonstrate the Mg-Na-K-stimulated ATPase activity the method of Wachstein and Meisel (66) was used as modified by Marchesi and Palade (43). Nuclei of mature oocytes were collected in medium B and transferred to the incubation medium which contained 40 mM Tris-maleate buffer, pH 7.0, 4 mM ATP, 4 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl, and 0.5 mM $Pb(NO_3)_2$. The reactions were performed at 37°C for 30 min either with unfixed nuclei or with nuclei prefixed in 1% glutaraldehyde (0.05 M cacodylate buffered to pH 7.2) for 10–20 min at room temperature. Control experiments to test the specificity of the reaction were carried out in the following media: 1.) lacking ATP; 2.) containing 4 mM glucose-6-phosphate instead of ATP; 3.) containing 4 mM Na_2HPO_4 instead of ATP, so that the distribution of lead phosphate precipitates adhering to the structures in question could be examined (compare reference 22); 4.) containing ATP, but with added 1 mM Ouabain. The nuclei were then postfixed for 3 hr in 4% glutaraldehyde, cacodylate buffered to pH 7.2, washed thoroughly with buffer, and osmicated with 1% OsO_4 buffered in the same way for 2 hr, dehydrated by Sitte's acetone vapor method (61), embedded in Araldite, and sectioned on a Reichert ultramicrotome with diamond knives. Sections were stained with uranyl acetate and lead citrate. The micrographs were made with a Siemens Elmiskop Ia. Instrument magnification reading was controlled by use of a grating replica. Furthermore, nuclei isolated by the technique described above were directly fixed in cacodylate-buffered 4% glutaraldehyde in the cold, washed, post-osmicated, and prepared for ultrathin sectioning work as mentioned above.

RESULTS AND DISCUSSION

In the negatively stained preparations annulate lamellae can be detected either lying upon the nuclear envelope (Fig. 1) or separated from it (Fig. 2). In the former association the annulate lamellae, as a consequence of the accumulative height of the resulting stack of cisternae, show much more electron contrast than the underlying nuclear envelope alone, resembling the "dense patches" described by Merriam (49) for air-dried preparations of *Rana pipiens* oocyte nuclei. That the association of the annulate lamellae with the nuclear envelope survives the Callan-Tomlin procedures might be due to the cisternal continuity of both membrane systems (e.g. insert, Fig. 15) or to the fibrillar material which seemingly connects them, (e.g. Fig. 19). The isolated annulate lamellae are frequently seen connected with slender tubular-like PTA-repellent profiles (Figs. 3 and 4) presumed to be fragments of the endoplasmic reticulum (ER) which is also well known to be in cisternal continuity with the annulate lamellae (3, 28, 31, 38–40, 46, 47, 60, for further references see 36). The ER origin of the membranous tubules might also be indicated by their finely granular surface (diameter of the granules at about 40–50 A) in areas where they seem to be more flattened (e.g. insert, Fig. 3). This finely grained appearance has repeatedly been reported as a feature common to negatively stained ER membranes (9, 23, 30, 55).

At the borders of the annulate lamellae, sometimes sites of membrane rupture are prominent in the staining (Figs. 2 and 3). The rupture lines (or the native borders) of the annulate lamellar sheets preferentially circumscribe the pore complexes (e.g. Fig. 6), thus indicating that the pore complexes are sites of enhanced structural stability (compare also the isolated nuclear envelope pieces presented in references 75 and 51). Occasionally annulate lamellae sheets lie close together, separated only by a small isthmus of PTA (Fig. 5). It cannot be decided whether such annulate lamellae pieces are fissure remnants of one cisterna or whether they represent two annulate



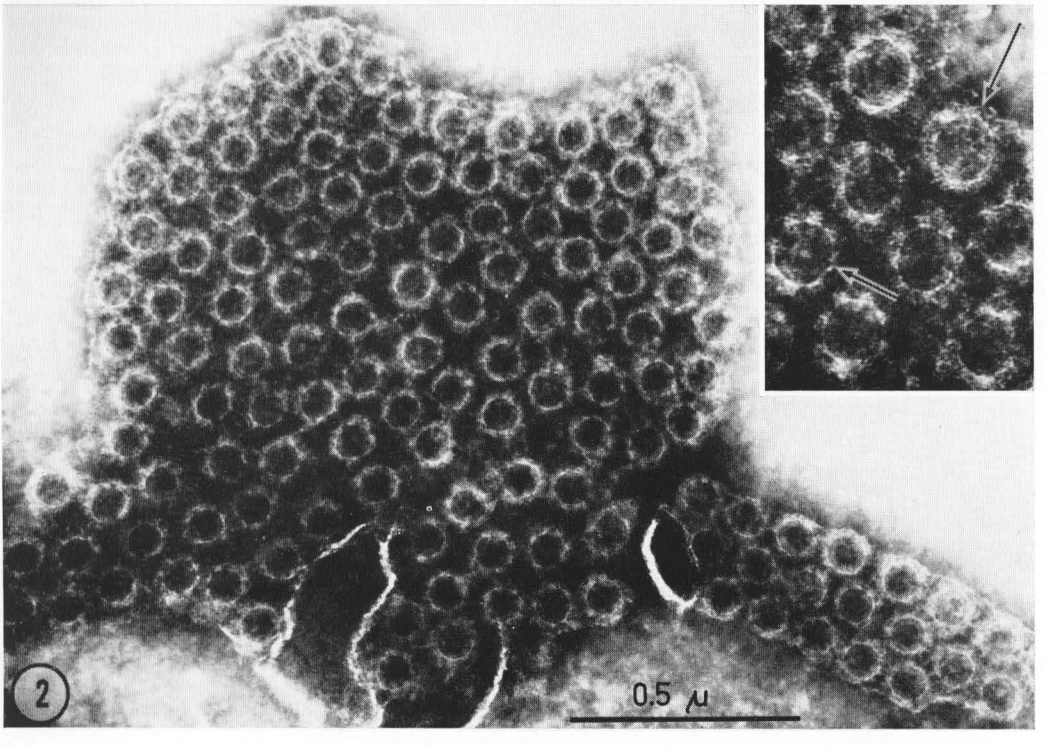


FIGURE 1 Negatively stained preparation from an oocyte of *Triturus alpestris* showing an annulate lamella (area with high contrast) lying upon the isolated nuclear envelope (area with less contrast). Nuclear pores (arrows) reveal a markedly greater spacing than the annulate lamellae pores. × 60,000.

FIGURE 2 Negatively stained sheet of an annulate lamella of a newt oocyte detached from the nuclear envelope. The dense packing of the pores is obvious. × 60,000. *Insert* shows some ultrastructural details of the annulate lamellae pore complex, e.g., the central dot, the annular subunits, the tip-like projections from the annular subunits into the pore lumen (left arrow), and the thin strands within the pore complex (right arrow). × 105,000.

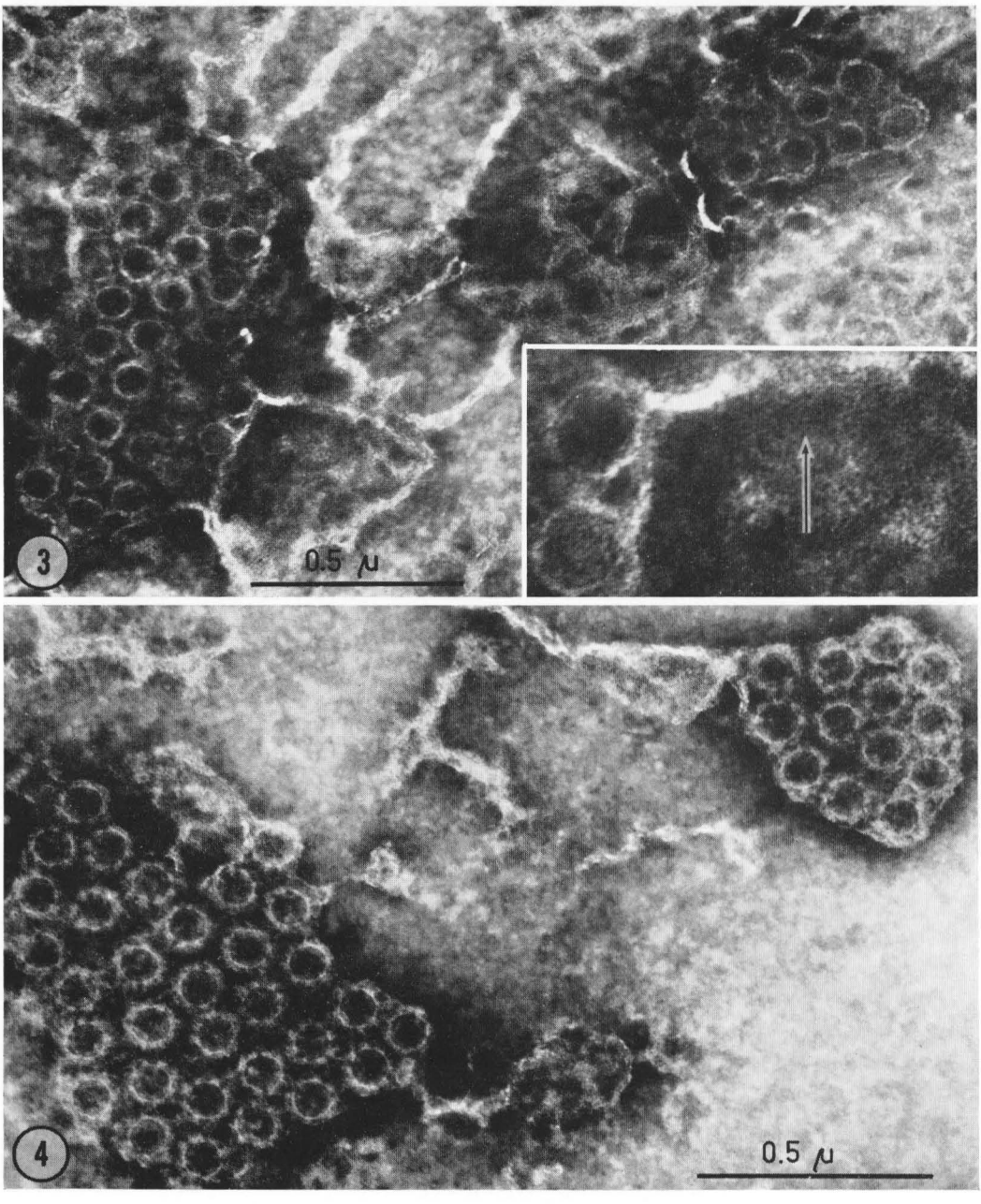


FIGURE 3 Negatively stained annulate lamellae connected to slender tubule-like strands interpreted as parts of the endoplasmic reticulum system. × 60,000. *Insert* shows the granular surface (arrow) of such tubules in areas where they seem to be flattened. × 160,000.

FIGURE 4 An annulate lamellae-tubule association similar to that shown in Fig. 3, but from a preparation in which $MgCl_2$ was added in a concentration of 2 mM. The annular subunits at the margins of the majority of the pores are better preserved. × 66,000.

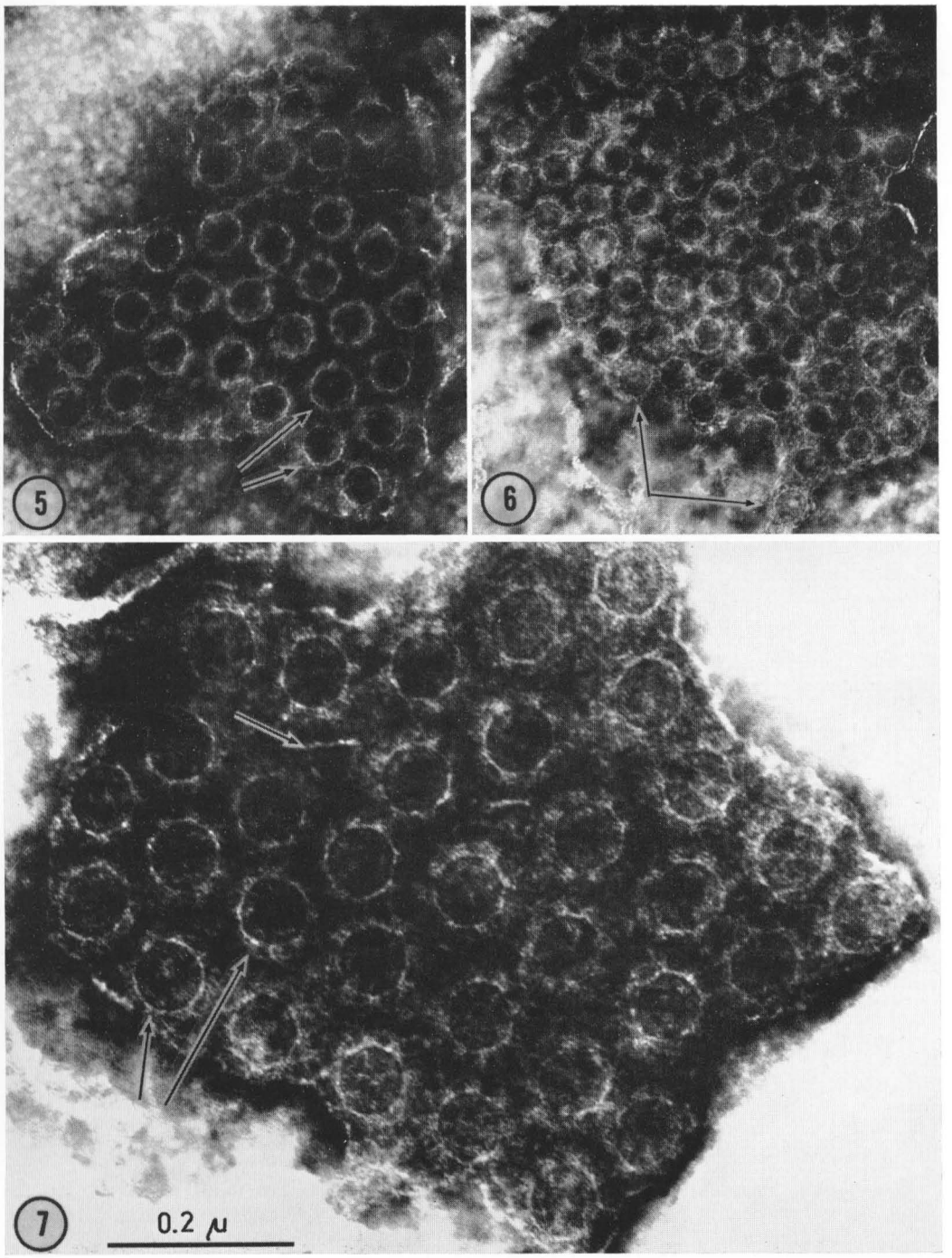


FIGURE 5 Two closely adjacent annulate lamellae pieces. Note remarkable differences in pore diameters in the same annulate lamella (arrows). × 72,000.

FIGURE 6 In some of the negatively stained preparations the newt oocyte annulate lamellae pore complexes seem to be associated with fuzzy, finely-stranded material. Note that the borders or the rupture lines can often be seen as running around the pore complexes (arrows), which indicates that the pore complexes are sites of enhanced structural stability. × 57,000.

FIGURE 7 Besides circular outlines of the pore circumferences (lower arrows), many types of polygonality can be seen in the annulate lamellae pores. Occasionally holes, presumably artifacts, can also be detected (upper arrow). × 135,000.

cisternae closely associated in situ, possibly in the same stack (e.g., compare Figs. 17 and 19).

The annulate lamellae can easily be distinguished from parts of the nuclear envelope by their markedly increased pore frequency. Table I presents a comparison of corresponding structural data on the nuclear envelope and on annulate lamellae from the very same oocyte, i.e., on the very same grid. The pore diameter and the pore complex ultrastructure of the annulate lamellae agree with those of the nuclear envelope (Table I; compare also references, 1, 5, 25, 27, 45, 58, 65), even though a difference in the pore frequencies in both kinds of porous cisternae is apparent. The pore frequency of the annulate lamellae of the newt oocytes in general is twice that of the nuclear envelope in the same cell and can be found to amount to values of about 130 pores per μ^2 (Fig. 8). Evidence for this significantly increased pore frequency of the annulate lamellae comes also from ultrathin sectioning work (e.g. Figs. 15 and 18) where pore frequencies up to 175 pores per μ^2 could be counted in tangential sections of annulate lamellae (Table I; e.g., Fig. 16). The increased pore frequency in the annulate lamellae as compared with the nuclear envelope was also observed by Merriam (49), who mentioned an increase of 188% in *Rana* eggs, and by Hertig and Adams (28) for human oocytes. Except in the case of the macronuclear envelope of *Tetrahymena pyriformis* (70, 71), porous cisternae with pore frequencies of more than 100 pores per μ^2 so far have not been reported although a great many studies have presented data referring to this subject (e.g. 2, 6, 14, 16–18, 24, 41, 50, 52, 68, 75).

Of special interest is the comparison of the nuclear envelope and the annulate lamellae of the same material. Since it could be established from measurements of large nuclear envelope areas (30 to 260 μ^2) that the nuclear pore frequencies of newt oocytes in different stages of maturation never exceed 70 pores per μ^2 (Scheer, unpublished data), the increased pore frequency of the annulate lamellae seems to be one of their characteristic properties. This dense packing of the pores in the annulate lamellae often must

TABLE I

Comparison of Structural Data of Annulate Lamellae and Nuclear Envelope of Single Triturus Alpestris Oocytes

	Pore frequency		Pore diameter		Surface occupied by pore area	
Oocyte No.	nuclear envelope	annulate lamellae	nuclear envelope	annulate lamellae	nuclear envelope	annulate lamellae
	Pores/μ^2		A	A	%	%
1	54	126	708 ± 23	694 ± 28	21.3	47.7
2	48	130	692 ± 21	686 ± 22	18.1	48.0
3	42	136	652 ± 19	689 ± 24	14.0	50.7
4	49	86	760 ± 47	726 ± 26	22.2	35.6
5	36	84	810 ± 26	770 ± 34	18.6	39.1
6	50	90	675 ± 22	670 ± 20	17.9	31.7
7	38	90	658 ± 30	690 ± 22	12.9	33.7
8	53	126	721 ± 28	733 ± 28	21.6	53.2
9	42	124	652 ± 19	695 ± 25	14.0	47.0
10	47	97	756 ± 47	697 ± 36	21.1	37.0
11	35	82	795 ± 25	780 ± 24	17.4	39.2
12	51	90	712 ± 21	680 ± 27	20.3	32.7
13	38	80	790 ± 22	760 ± 22	18.6	36.3
14	32	80	770 ± 29	735 ± 23	14.9	33.9
15	38	90	664 ± 30	675 ± 13	13.2	32.2
16	63	136	ca. 660	ca. 650	22	45
17	64	175	ca. 650	ca. 630	21	55
18	51	150	ca. 660	ca. 620	17	45
19	63	130	ca. 640	ca. 650	20	43

Values of nos. 1–15 were taken from negatively stained preparations. Nos. 16–19 refer to calculations made from tangential (pore frequencies) and transverse (pore diameters) sections.

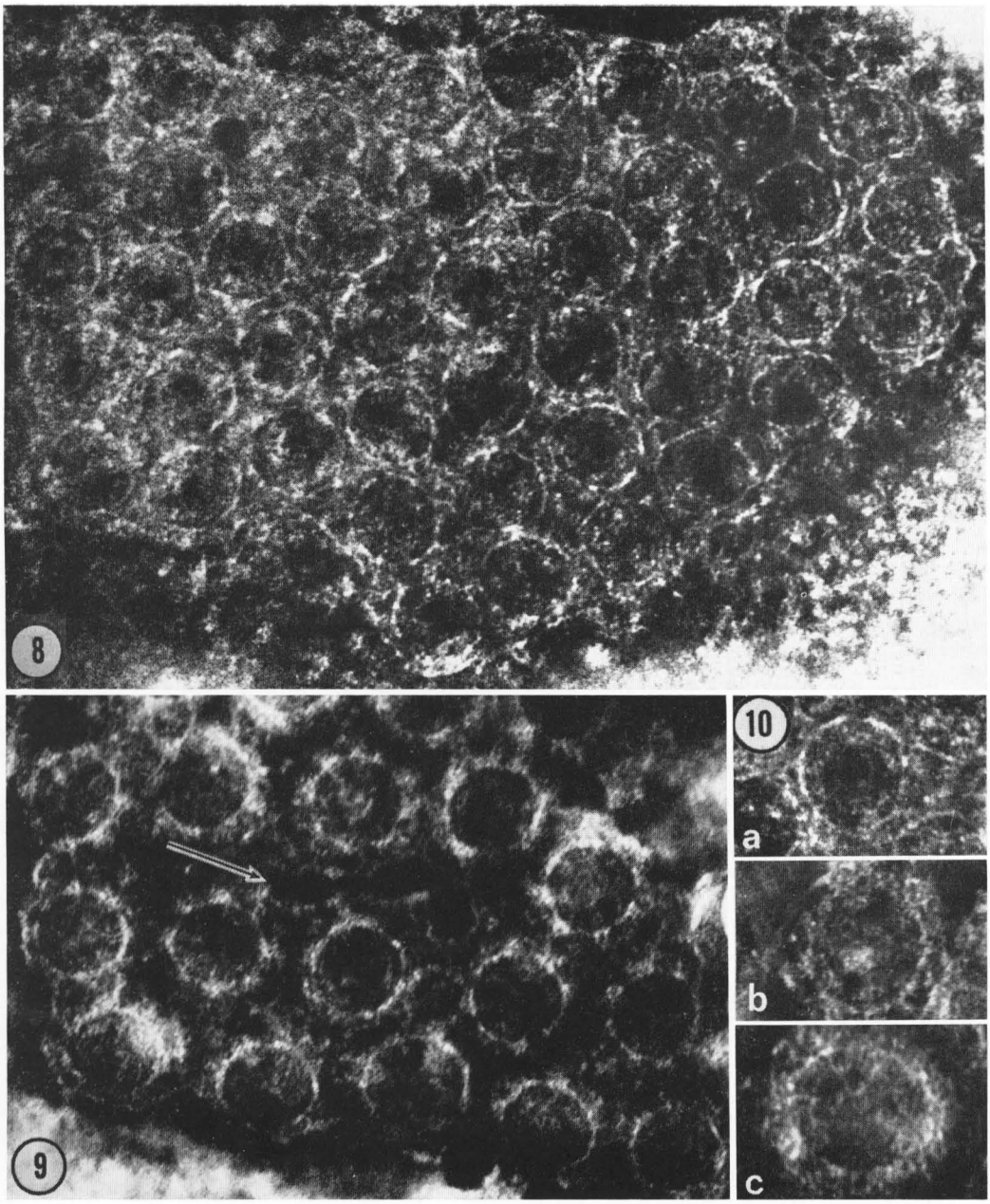


FIGURE 8 Extremely dense packing of the pores in annulate lamellae frequently results in a hexagonal array. × 160,000.

FIGURE 9 Negatively stained annulate lamella from newt oocyte revealing an irregularly shaped hole or indentation (arrow) and pore complexes which reveal strand-like material and radial projections from the annular subunits. × 160,000.

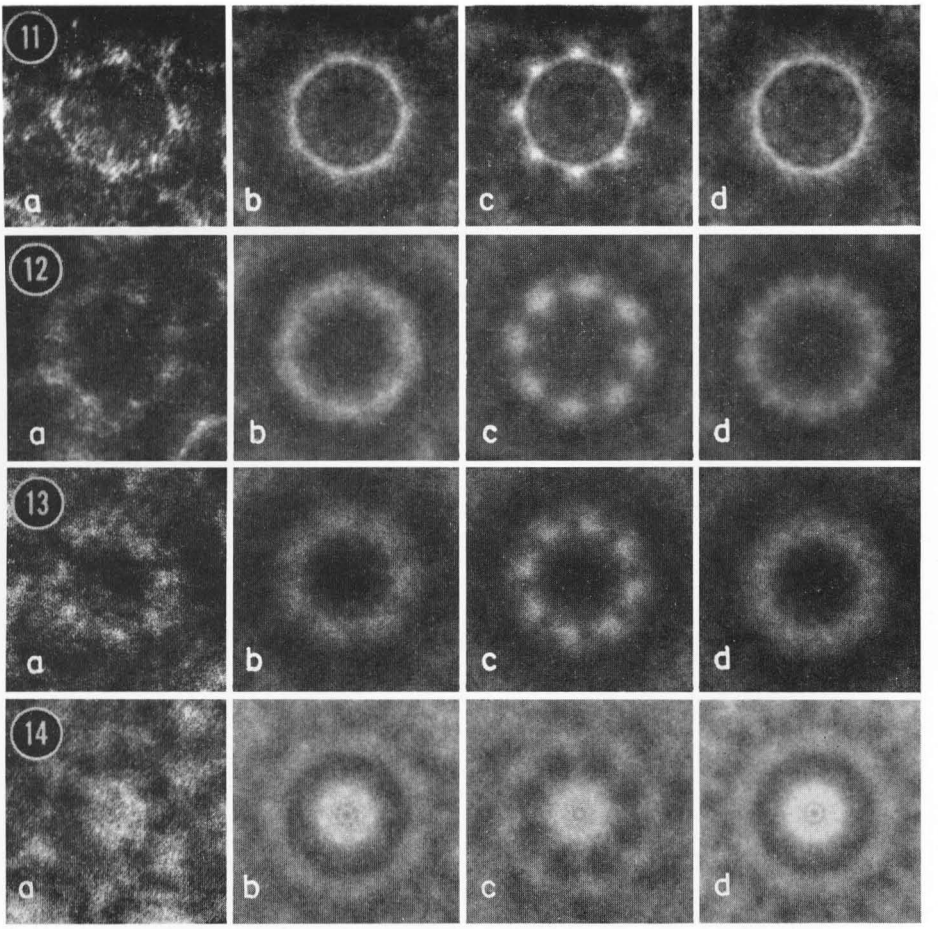
FIGURES 10 *a–c* Comparison of three main structural features of the inner part of the annulate lamellae pore complex as seen in negatively stained preparations. *a*, shows the diffuse material within the pore lumen leaving a small "central channel"; *b*, the pore center is occupied by the "central dot" or "central granule"; *c*, thin, 25 A-wide strands extend into the lumen of the pore. *a* and *b*, × 200,000; *c*, × 240,000.

result in a regular array (Figs. 7 and 8). Such hexagonal patterns have been repeatedly reported for annulate lamellae by other authors (4, 5, 26, 39, 42, 45, 46, 48, 49, 56–60, 63).

The negative staining appearance of pore complexes in Callan-Tomlin preparations is dependent, to a certain degree, on the kind and strength of ions present in the preparation medium and on the length of time the isolation procedure has taken (19). In those preparations of the *Triturus alpestris* eggs, in which solely alkaline cations were used, the true pore diameter (membrane to membrane) of the annulate lamellae was especially clear and was determined to be an average 706 ± 38 A (mean value from more than 250 measurements). This value is in the range of the corresponding values for the nuclear envelope pores of the same material (Table I; compare also reference 21). That remarkable differences between the pore diameters, however, can occur in the same annulate lamellae piece can be seen, for example, from Fig. 5. The agreement in pore widths of both the annulate lamellae and nuclear envelopes could also be confirmed by measuring the transversely sectioned pores in ultrathin sections (Figs. 17–19). Thus, as a result of the increased pore frequency of the annulate lamellae, their relative pore area, i.e. the percentage of surface unit occupied by pore area, is strikingly high. All values so far calculated for the annulate lamellae are greater than 31%, whereas the relative pore area in the corresponding nuclear envelopes never exceeds 23% (Table I). The data for nearly mature *Triturus alpestris* eggs are twice those reported by Merriam (49) for *Rana pipiens* oocytes in the comparable stage of development. In a great number of annulate lamellae the extremely dense spacing of the pore complexes brings about values for the relative pore area that are even greater than 50% (Fig. 8). Such values are, again, by far higher than those that have been reported for any nuclear envelopes (for comparison, see references 20, 70). Faced with the increased pore frequency and relative pore area, one is tempted to conclude that, whatever the special function of the pore complexes in both kinds of cisternae might be, the structural potential of this function is remarkably amplified in the annulate lamellae.

Besides the pores, irregularly shaped holes or fissures, presumed to be artificially induced, can also be found (e.g., Figs. 7 and 9).

The annulus material which is a dominant structural feature of tangentially sectioned pore complexes of annulate lamellae as well as of nuclear envelopes seems to disappear during the course of the preparation if bivalent cations or other stabilizing substances are omitted (19). In the negatively stained annulate lamellae the globular subunits of the annulus showed up as prominent areas of less contrast revealing either distinct particles (e.g. Figs. 4, 13) or spots with less distinct contours (e.g., Figs. 2, 5, 7, 9, 12). These more particulate constituents of the annulus, when analyzed with Markham's rotational technique (44), generally were found to be arranged in the precise pattern of an eightfold symmetry at the margin of the pore (Figs. 11–13). Such a symmetrical arrangement of eight globular subunits can also be encountered in tangentially sectioned annulate lamellae (Figs. 14 and 16). The annular globules which are 130–170 A in diameter (compare the data summarized in reference 36) are also seen in transverse sections through pore complexes as being located on both sides and upon the margin of the pore (Figs. 17 and 18). From the combined findings from sections and negative staining work, it now seems to be established that the annulus of the annulate lamellae pore complexes has the same substructural composition as has been reported previously for the annulus of the nuclear envelope pores in quite different kinds of cells. Eight (or, in some instances, nine, reference 17) globular subunits are distributed around the pore margin in a precise symmetry (e.g., 1, 12, 14, 16, 18, 45, 51, 53, 54, 64, 67, 69, 75). Another structural element of the annulate lamellae pore complexes that sometimes can be observed both in negatively stained preparations and in sections is the so-called "central dot" or "central granule" (e.g., Figs. 2, 10 *b*, 16; diameters of 100 to 200 A). The occurrence of this central granule, however, seems to be less frequent in annulate lamellae pores than in nuclear envelope pores (ref. 36, p. 16). It should be emphasized, however, that, in negatively stained annulate lamellae, central structures of smaller sizes (diameters about 25 to 50 A) can sometimes be discerned which appear either granular or strandlike and in some instances seem to be connected to the pore margin (e.g. Figs. 2, 6, 9, 10 *c*), an association comparable to that observed in pores of isolated envelopes of pea



FIGURES 11–14 Markham analyses of annulate lamellae pore complexes demonstrate that an enhancement of the radial symmetry of the annular subunits occurs only for n = 8 (*c*), but not for n = 7 (*b*), n = 9 (*d*) and any other value of n up to 11. × 200,000.

FIGURE 11 Negatively stained pore complex after isolation in a medium consisting solely of alkaline chlorides.

FIGURE 12 Negatively stained pore complex after isolation in a medium to which 2 mM $MgCl_2$ was added.

FIGURE 13 Pore complex as isolated after addition of 10 mM $MgCl_2$ to the alkaline chloride medium.

FIGURE 14 Pore complex of an annulate lamella as seen in a section cut tangentially to an annulate lamella.

seedling nuclei (75) and *Tetrahymena* macronuclei (70, 71). These filament-connected granular structures are apparently identical to the granule-filament complexes which have been reported by Kessel in sections through stacks of annulate lamellae in oocytes of *Rana pipiens* and of the ophiurid *Ophioderma panamensis*, and in spermatocytes of the crayfish *Orconectes virilis* (35). Another interesting observation concerning the internal structures of the pore complex is that the lumen of the pore seems to be partially filled with some sort of loosely textured "fluffy" material either forming conical projections from the pore margin (e.g. Figs. 2 and 9) or, being more evenly distributed along the margin, leaving an innermost "central channel" recognizable as a more in-

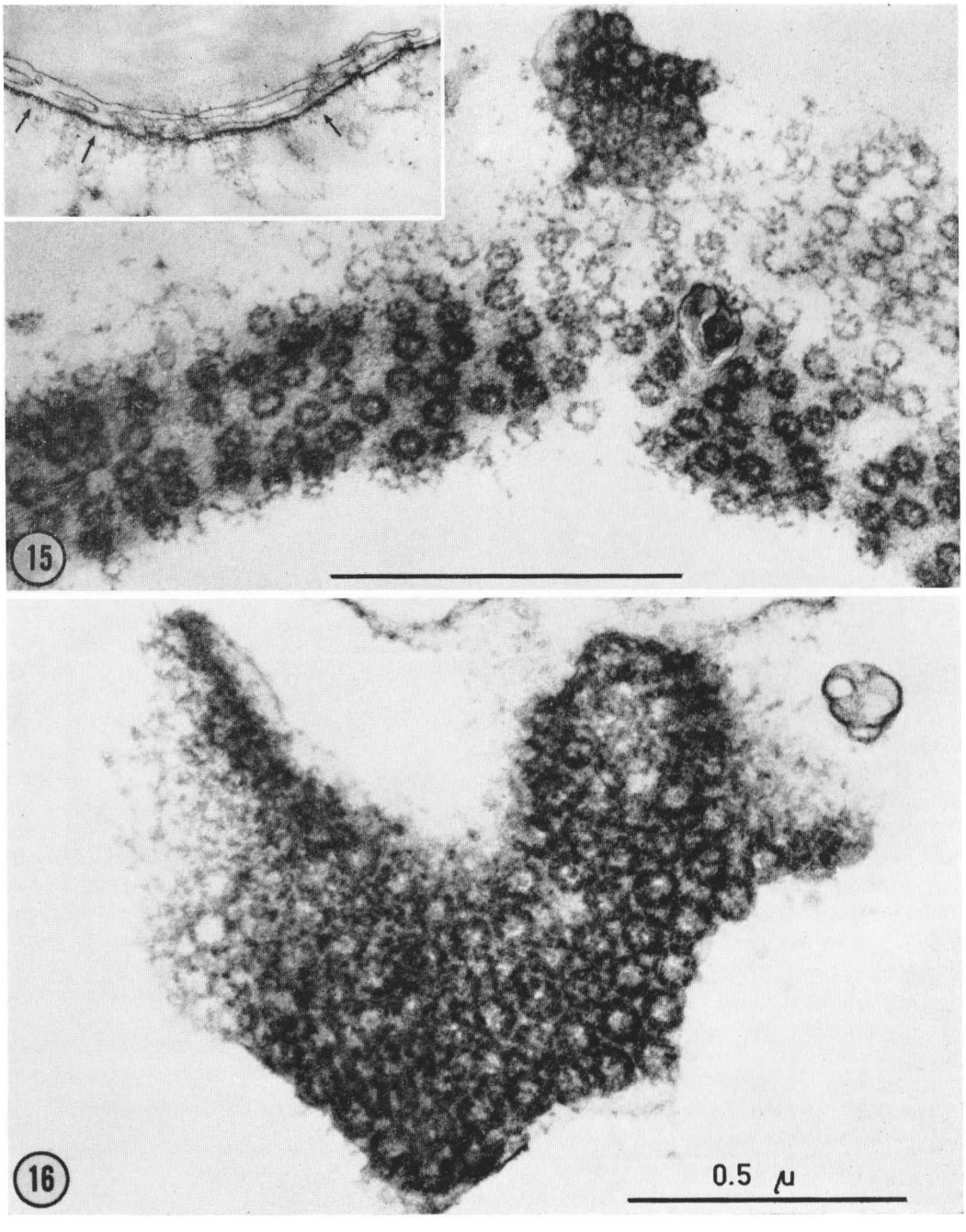


FIGURE 15 Section grazing the envelope of a nucleus isolated from a *Triturus alpestris* oocyte (lower part) as well as an annulate lamella located on the outer side of the nuclear envelope (upper part). The higher pore frequency and the more regular array of the pores in the annulate lamella is striking. Scale indicates 1 μ. × 50,000. *Insert* shows the cisternal continuity of the nuclear envelope and the annulate lamellae (arrows) as frequently observed in the preparations described. × 31,000.

FIGURE 16 Section cut tangential to an annulate lamella connected to the isolated nuclear envelope of a newt oocyte in which the densely packed pore complexes reveal central dots and the subunits of the annuli. × 80,000.

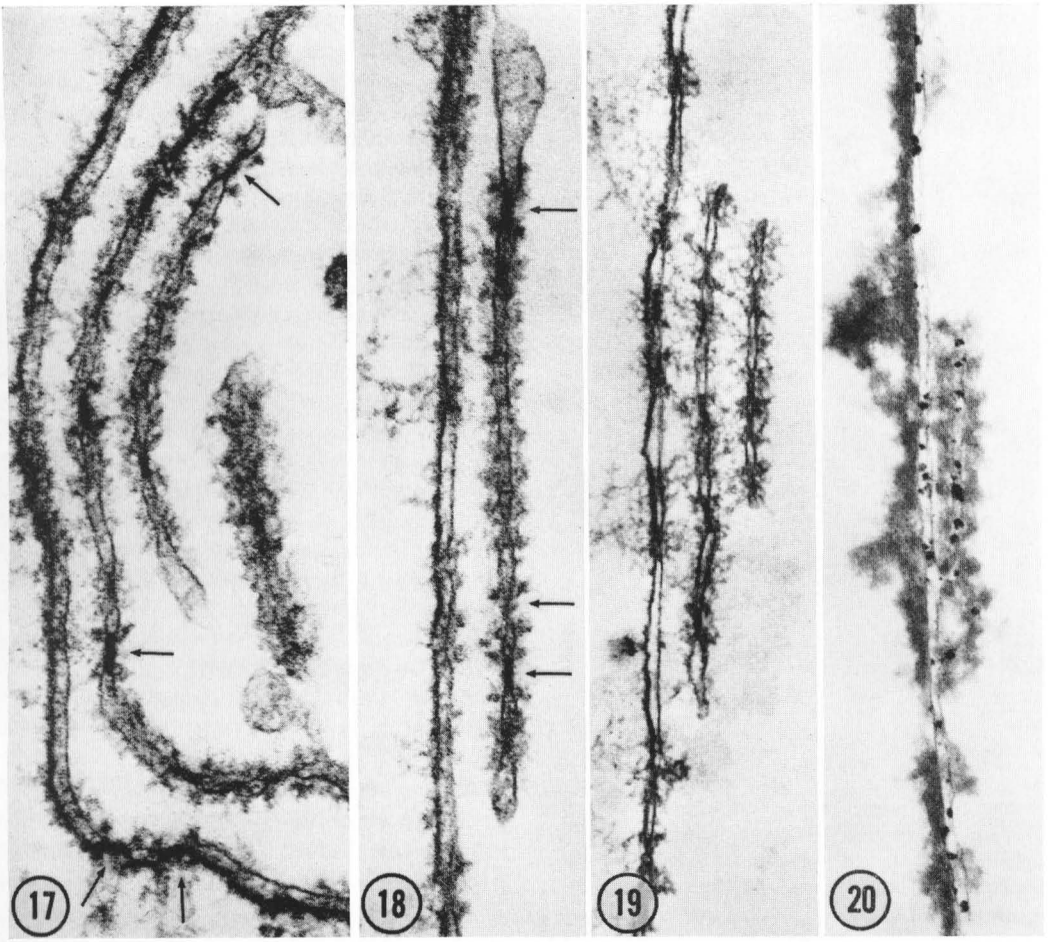


FIGURE 17 Transverse section through a stack of annulate lamellae adhering to a nuclear envelope (left) isolated by a Callan-Tomlin preparation. Arrows point to pore complexes in which the globular subunits of the annulus can be recognized as lying upon the pore margin in both the nuclear envelope and in the annulate lamellae. × 85,000.

FIGURE 18 Transversely sectioned nuclear envelope (left) and annulate lamella. Ultrastructural details of the annulate lamellae pore complexes can be seen (arrows). A comparison of these two porous membranes indicates also the higher pore frequency of the annulate lamellae. × 83,000.

FIGURE 19 A stack of two annulate lamellae which adhered to the nuclear envelope (left) during the preparation. Many fine strands of electron-dense material seem to connect all three cisternae. This indicates that not only cisternal continuity but also such connecting strands could be responsible for the survival of the annulate lamellae-nuclear envelope association through the procedures of Callan-Tomlin isolations. × 65,000.

FIGURE 20 Transversely sectioned nuclear envelope plus annulate lamella after the ATPase reaction. Precipitated lead phosphate can be seen along the nuclear membranes and the annulate lamella. Non-membrane material, e.g. the peripheral chromatin at the nucleoplasmic side of the nuclear envelope, appears to have swollen during the incubation in the enzyme reaction assay. × 58,000.

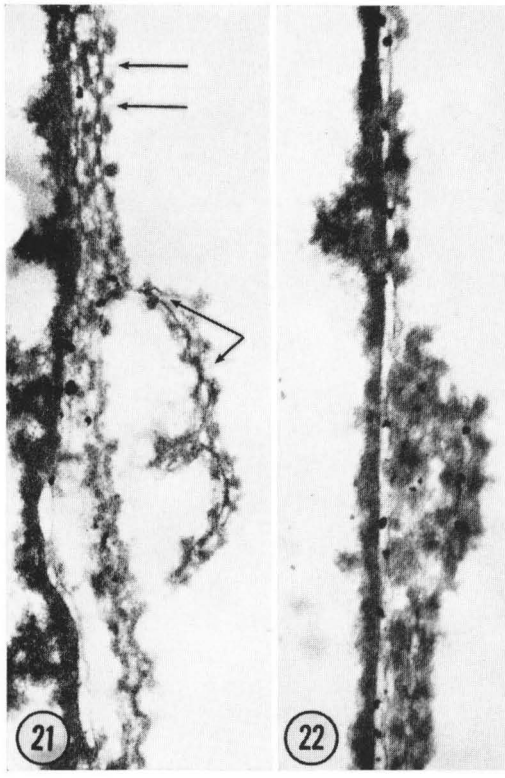


FIGURE 21 Nuclear envelope and annulate lamellae after an enzyme reaction assay in which ATP was replaced by glucose-6-phosphate. The number of deposits is markedly decreased. No deposit can be seen associated with the pore complexes of the annulate lamellae (arrows). × 58,000.

FIGURE 22 Ouabain-containing control of the ATPase reaction. Diminution of the number of deposits is not obvious. × 58,000.

tensely stained hole with a minimum diameter of about 200 to 250 A (e.g., Figs. 8 and 10 *a*). In general, such "filled" pore complexes seem to be particularly more frequent in preparations in which Mg^{2+} ions were added to the medium. Although the present lack of any knowledge on the chemical nature and functional meaning of the pore complex structure cannot be overlooked, it is noteworthy that, for instance, the "central dot" and the "central channel" are of the same order of magnitude that Stevens and Swift (62) and Feldherr (13) have reported as being a structural limitation for the passage of Balbiani ring granules and gold particles, respectively, through the nuclear envelope pore complex. The very same structural limitation can also be found in *Triturus* eggs for the passage of nucleolus-derived material through the nuclear pores (19). As far as the annulate lamellae-nuclear envelope relationship is concerned, the speculation of Bal et al. (3) that the internal structures of the pore complex could represent a form of "stored" informational RNA-protein material seems to be a valuable working hypothesis.

With reference to an interpretation of Gall (21) who explained the absence of a negatively stained (i.e. "white") annulus in amphibian and starfish oocyte nuclear envelopes as being caused by a penetration of the stain into the annulus material, we should mention that in the present study the annular material of both annulate lamellae and nuclear envelopes generally was detectable as negatively stained ("white"), provided that it was preserved by a proper type of isolation (for the annulate lamellae, see Figs. 4 and 10 *a–c*). This generally electron-translucent appearance of the negatively stained annulus has also been described for isolated nuclear envelopes of various materials by several other authors (16–18, 51, 71, 75).

Another statement made by Gall (20, 21), that nuclear pores were octagonal in outline rather than circular, has led Kessel to raise the question as to whether this eight-sided shape would also be true for annulate lamellae pores (ref. 36, particularly the remark on p. 75). As can be seen in Figs. 6–9 of the present article, circular outlines of the pores can be seen besides other outlines which are somewhat polygonal, preferentially octagonal, in shape. From our studies on nuclear envelopes isolated either by mass isolation (16, 17, 71) or by Callan-Tomlin techniques (19), we have the impression that such polygonality of pores reflects an artificial distortion during the preparation, especially at the very moment of spreading and drying, and does not reflect the in vivo state. That there exists an obvious preference for octagons may be due to the eight granular subunits lying equally spaced upon the pore margin, which thus possibly represent sites of greater stability during the distortion process. Moreover, it is interesting to note, in this connection, that such deviations from the circularity of the pore complex occur to a much lesser extent

in preparations to which stabilizing bivalent cations have been added.

In the course of experiments on the special enzyme activities of the nuclear envelope, we examined the question of whether annulate lamellae and nuclear envelopes have enzyme activities in common. The first inquiry concerns the activity of Mg-Na-K-ATPase. That nuclear envelopes, particularly their pore complexes, are sites of remarkable ATPase activity was reported first by Yasuzumi and his group (72) and by Klein and Afzelius (37). Both findings, however, cannot evade one of the objections generally raised against tissue blocks incubated in the Wachstein-Meisel assay, i.e., the question of whether the product of the capture reaction has remained at the very site of the reaction or has merely accumulated in a subsequent step. Experiments with isolated membranous material are by far more unequivocal with respect to this argument. Thus, the result of the enzyme localization experiment described in Methods which shows dense precipitates of lead phosphate along the nuclear envelope as well as the annulate lamellae (Fig. 20), provides strong evidence that annulate lamellae are a site of ATPase activity. In this connection one should bear in mind, however, that any cytochemistry on semi-isolated annulate lamellae by means of the conventional incubation assays are rendered difficult by a progressive detachment of the annulate lamellae cisternae from the nuclear envelope. This is especially the case when nonfixed material is employed. Thus also, for instance, the ER cisternae attached to the ends of the annulate lamellae were no longer seen after the incubation procedures. On the other hand, preparations made after the glutaraldehyde-prefixation described above were generally found to be nearly devoid of any lead phosphate deposit. An association of these deposits with the pore complexes, which has been reported for the nuclear envelopes of various kinds of cells (73, 74) and which could be repeatedly seen in the nuclear pores of the present material, was not observed in the annulate lamellae. Controls in which ATP was replaced by glucose-6-phosphate as the substrate showed generally many less deposits (Fig. 21). This might serve as an indication of the substrate specificity of the ATPase localization. Since glucose-6-phosphatase is widely regarded as an enzyme marker for ER elements, its obviously low activity in annulate lamellae directs one to the recent finding of Zbarsky and his group (76) that the nuclear envelope is a site of greatly increased ATPase activity but reveals an almost total absence of glucose-6-phosphatase. Precipitates of lead phosphate formed by Na_2HPO_4 simultaneously added to the incubation medium instead of ATP could be easily distinguished by their much greater size and by a quite different and nonspecific pattern of adhesion. Whereas the ouabain-containing controls so far have not revealed a noticeable diminuation of lead phosphate deposits (Fig. 22), a differentiation of the annulate lamellae ATPase into either Na-K-activated transport ATPase or the Mg-dependent ATPase requires further careful investigation. Our observation that the ATPase activity of the annulate lamellae as well as of the nuclear envelope is apparently not influenced by ouabain seems to be, again, in agreement with corresponding results of Zbarsky et al. (76).

The annulate lamellae obtained in the preparations described in this study are not necessarily representative for the entire population of annulate lamellae present in an oocyte, because only those which are adjacent or even connected to the nuclear envelope are accessible to a Callan-Tomlin preparation. Thus, from the outset it can not be excluded that the structural and enzymatic properties of annulate lamellae occurring at a greater distance from the nucleus are different from the structural and enzymatic properties of the juxtanuclear annulate lamellae. Since a great many investigators have accumulated information which suggests that the annulate lamellae originate from the nuclear envelope, migrate into the cytoplasm, and undergo breakdown (possibly into vesicles, see ref. 36), changes in such properties during the course of annulate lamellae development should not seem unexpected.

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