ULTRASTRUCTURE AND PERMEABILITY OF NUCLEAR MEMBRANES

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ABSTRACT

The fine structures of nuclear envelopes known to have different permeability properties were compared. Membranes of salivary gland cell nuclei of *Drosophila* (third instar) and *Chironomus* (prepupae), which are strong barriers to ion diffusion, and membranes of oocyte nuclei (germinal vesicle) of *Xenopus* and *Triturus*, which are much more ion-permeable, show no essential difference in size, frequency, and distribution of their membrane gaps ("pores") which could account for the marked disparities in membrane permeability. The gaps are occupied by diffuse electron-opaque material with occasional central regions of strong opacity. This material may possibly account for the high diffusion resistance of *Drosophila* and *Chironomus* nuclear envelopes, where the resistance is far too great to allow free diffusion through the gaps. But material of this kind is also present in the more permeable nuclear envelopes of *Xenopus* and *Triturus* oocytes, and there are no convincing structural differences discernible with the techniques employed.

INTRODUCTION

The permeability properties of a variety of nuclear envelopes have recently been examined with electrical techniques. The envelopes were found to fall into two broad categories (16): one category, which includes the nuclear envelopes of oocytes of *Xenopus* and *Triturus*, are highly permeable structures which offer no appreciable resistance to ion flow beyond that of their nucleoplasm (11 and 12); and another, to which belong the nuclear membranes of gland cells of *Drosophila* and *Chironomus* larvae, are strong barriers to ion flow (10 and 15). The present paper deals with the fine structure of these nuclear envelopes, and in particular with their gaps in surface structure. It is an attempt at correlating surface structure with electrical measurements.

MATERIALS AND METHODS

Nuclei of salivary gland cells of third instar larvae of *Drosophila flavorepleta* and of prepupae of *Chironomus thummi*, and nuclei of transparent oocytes (up to 350 μ in diameter) of *Xenopus laevis* and *Triturus viridescens* were studied. The salivary glands were either fixed *in situ* or isolated from the animals, placed in cold buffered (veronal-acetate) 2 per cent osmium tetroxide with sucrose at pH 7.4 for 4 hours, dehydrated in acetone, and embedded in Araldite. Part of the material was fixed first in cold buffered 6.25 per cent glutaraldehyde (pH 7.6) (23). The oocytes were isolated in groups from the animals and processed for electron microscopy in an identical manner. All observations were made on nuclei *in situ*.

Two-micron thick sections of the Araldite-embedded tissue were used for phase contrast observation. 800- to 900-A-thick sections were stained with lead hydroxide (13) and examined under a calibrated Siemens Elmiskop I.

RESULTS

In the following account, certain aspects of nuclear

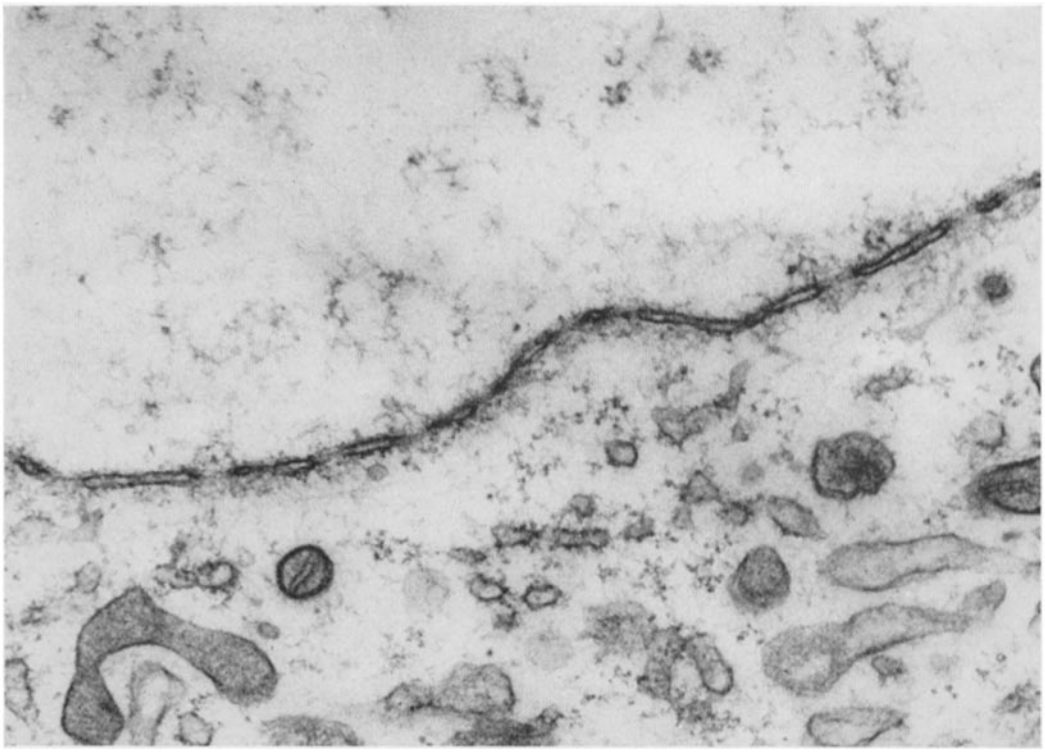


FIGURE 1 Electron micrograph of the nuclear envelope of a salivary gland cell of *Drosophila flavorepleta* in transverse sections. Several gaps (annuli) in membrane structure appear in the field. The gaps contain electron-opaque material which extends into the nucleoplasm and cytoplasm. × 60,000.

surface structure are described which may be expected to relate to nuclear surface permeability. The chief objects of description are the annular gaps in membrane structure (hereafter referred to as annuli). The aim was to examine the question of the role of these annuli in membrane permeability, and to compare their structure, frequency, and distribution in nuclear envelopes which electrical measurement has shown to be of distinctly different permeability characteristics. Descriptions of the fine structure of nuclear envelopes of some of the genera used in the present study were already available, and some in excellent detail (2, 3, 5–7, 9, 21, and 27). However, for present purposes it was essential that the membrane material used for electron microscopy and electrical measurements be immediately comparable. The material employed here for electron microscopy was, therefore, obtained from the same species and developmental stages as that used in the earlier electrophysiological work. This was particularly important in view of the recent finding that nuclear membrane permeability undergoes changes during development under the influence of a growth hormone (10 *a*). The material was isolated and handled as in the electrophysiological work, and was fixed in a state similar to that in which the electrical

TABLE I

Annulus Dimensions and Spacings

Material	Gap diameter	Gap depth	Gap spacing
	A	*A*	*A*
Drosophila flavo-repleta	700 ± 37	215 ± 11	1350 ± 28
Chironomus thummi	525 ± 11	196 ± 8	1000 ± 17
Triturus viridescens	475 ± 6	290 ± 9	1150 ± 19
Xenopus laevis	450 ± 19	290 ± 12	950 ± 61

Mean values with standard errors.

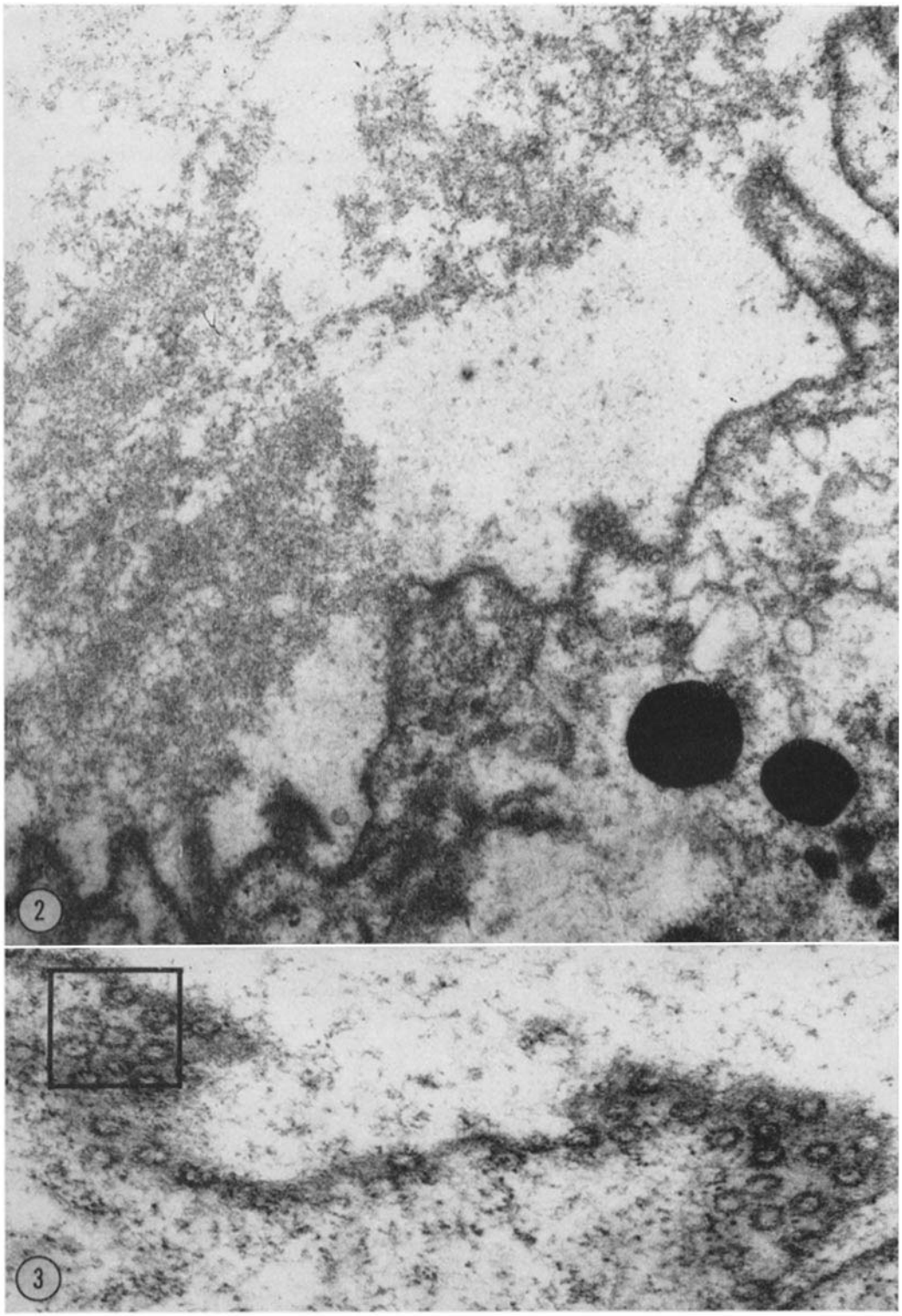


FIGURE 2 Tangential views of the nuclear envelope of *Drosophila flavoreχleta* showing distributions of annuli. × 21,500.

FIGURE 3 Tangential view of a hexagonal array of annuli (inside the square). Central dots of strong opacity are evident in some annuli. × 72,000.

measurements had been done (11 and 15). Moreover, the procedures followed in processing and examining the material of the various kinds of nuclear envelopes were kept as constant as possible.

Gland Cell Nuclei

The nuclei of salivary gland cells of *Drosophila* and *Chironomus* present the double membrane structure characteristic of nuclear envelopes of a wide variety of cells (see 8, 25, and 26 for reviews). The two membranes appear to merge at frequent intervals and form the well known annuli which, in sections normal to the plane of the nuclear envelope, appear as gaps in membrane structure (Figs. 1 and 4). The annuli are seen in all regions of the nuclear envelope and appear to be distributed in roughly hexagonal arrays in sections which are parallel to the plane of the nuclear envelope (Figs. 2, 3, and 5). Table I summarizes the dimensions and spacings of the annuli. Measures of inner diameter, of center-to-center spacing, and of distribution of annuli were obtained from tangential views; and the depth of annuli, which includes the thickness of the two membranes, was obtained from cross-sections.

Sections through the annuli which are perpendicular to the plane of the nuclear envelope reveal the presence of strands of electron-opaque material filling the gaps in membrane structure and often projecting beyond the gaps into the cytoplasm and nucleoplasm (Figs. 1 and 4). A dot of stronger electron opacity is seen in some tangential sections at the center of the annulus (Figs. 3 to 5). Similar formations have also been seen in nuclei of other cells (1, 4, 7, 14, 17 *a*-20, 22, 24–27).

Oocyte Nuclei

Electron micrographs of the oocyte nuclei show a membrane structure and gap arrays similar to those of the gland cell nuclei (Figs. 6 to 9). There are differences in gap dimensions and gap frequency; but these differences are small (Table I). The envelopes and their gaps appear essentially similar in structure to those of the gland cell nuclei in both glutaraldehyde- and osmium tetroxide–fixed materials. Again, one finds strands of electron-opaque material in the gaps (Figs. 6 to 8) which is somewhat less prominent than that observed in the gland cell nuclei, and occasional central dots of greater opacity (Figs. 7 to 9).

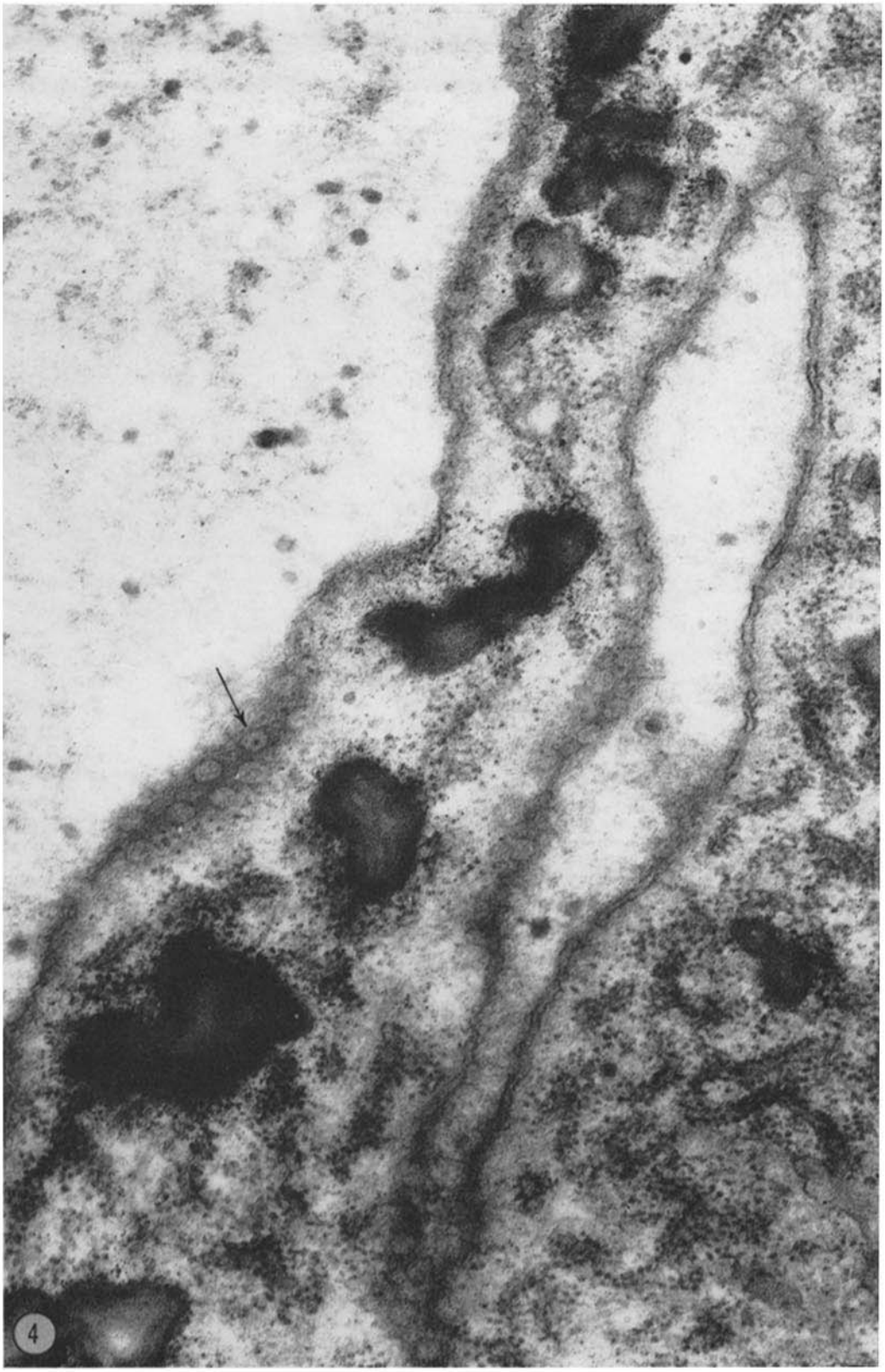
DISCUSSION

The Question of the Membrane Pores

The prominent feature of the surface structure in the nuclei of *Drosophila* and *Chironomus* gland cells is the presence of annular gaps in the nuclear envelopes, which, as in nuclei of a wide variety of other cells (*cf.* 8 and 26), are distributed rather regularly over the envelope. The question to be examined first is whether these gaps constitute free communications between nucleoplasm and cytoplasm. By free communication, we mean a gap filled with material of a resistivity of an order of magnitude similar to that of cytoplasm or nucleoplasm; that is, a resistivity of the order of 100 Ω cm (15). Even a cursory consideration makes this seem unlikely, since the total gap area amounts to so large a fraction of the total envelope area. In *Drosophila*, the gap area, which is readily calculated from the electron micrographs, amounts to 25 per cent and, in *Chironomus*, to 26 per cent of the total nuclear envelope area. Thus, if the gaps were free communications, they would shunt the total transverse resistance of the envelope to a magnitude approaching that presented by an equivalent membrane made entirely of nucleoplasm or cytoplasm (10^{-3} – 10^{-4} Ω cm^2) (Fig. 10). But the actual envelope resistance of these nuclei, as given by electrical measurements, is several orders of magnitude greater than that of such a hypothetical perforated membrane (15 and 17).

We may now examine this question more rigorously in the light of the data provided by the electron micrographs and electrical measurements. The resistance of a gap of the dimensions as in the *Drosophila* nuclear envelopes treated as a cylindrical volume conductor filled with cytoplasm or nucleoplasm, or as a thin disc submerged in a volume conductor of the resistivity of cytoplasm or nucleoplasm is 10^7 Ω (details of calculation of this

FIGURE 4 Electron micrograph of the nuclear envelope of a salivary gland cell of *Chironomus thummi*. The irregular nuclear surface offers views in the transverse plane (to the right) showing membrane gaps similar to those of Fig. 1, and in the tangential plane (to the left) a central dot in one annulus (arrow). × 60,000.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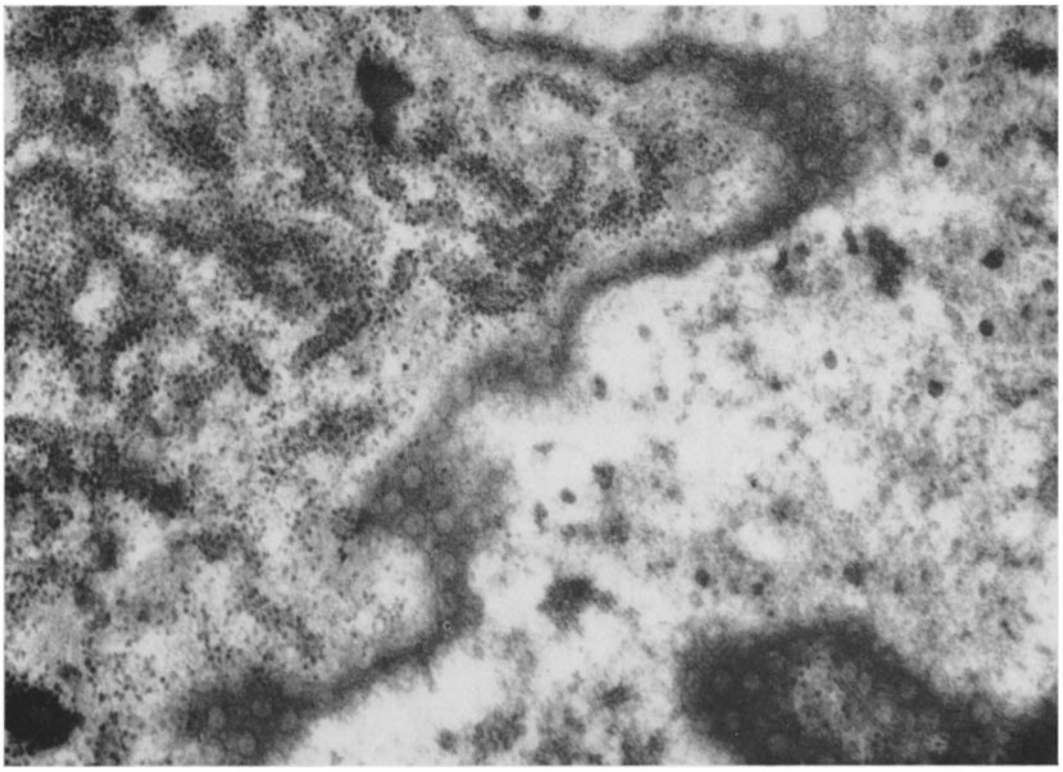
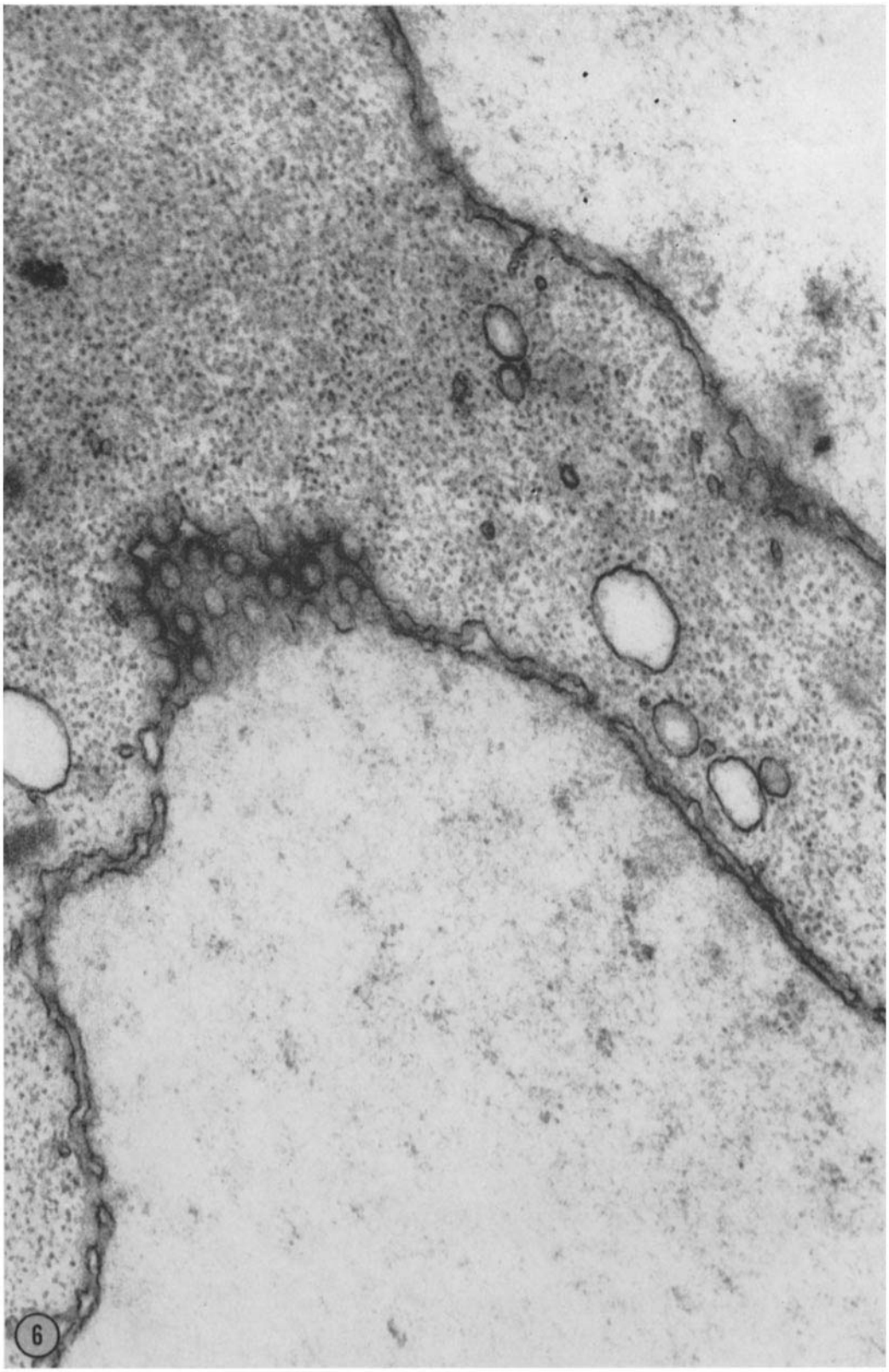


FIGURE 5 Tangential section of the nuclear envelope of *Chironomus thummi* showing arrays of annuli. × 45,000.

and following points in this paragraph are given in reference 15). This is the resistance of an isolated gap. An additional resistance arises due to the close proximity of gaps, by interaction of potential fields between neighboring gaps. This increases the effective gap resistance by a factor whose upper limit is approximately 2. The distribution of the gaps, as seen in electron micrographs, is roughly hexagonal (with six equidistant neighboring gaps) and a center-to-center distance (b) in between gaps of 1350 A. In such an array, there are $2/\sqrt{3}b^2$ gaps, 6.5×10^9 gaps per cm^2 of envelope; and the upper limit of transverse membrane resistance of unit area, as given by analog computations which take into account interaction between neighboring gaps, is 1.7×10^{-3} Ω cm^2. Nearly the same value of resistance is obtained for a membrane such as in *Chironomus*. The possibility of membrane distortion arising from the technical procedures in electron microscopy introduces some uncertainty as to the actual gap distribution which, of course, may not be strictly hexagonal. But variations in distribution, even coarse ones, introduce relatively small changes in resistance. For instance, two distributions as different as a hexagonal array, as above, and a square array (with four equidistant neighboring gaps) differ in resistance by less than 20 per cent. The order of magnitude of the resistance of such a hypothetical porous membrane remains the same, namely, 10^{-3} Ω cm^2. The actual

FIGURE 6 The nuclear envelope of an oocyte of *Xenopus laevis*. Views in the transverse plane showing small numbers of membrane gaps containing electron-opaque material, and in the tangential plane showing hexagonal arrays of annuli. × 60,000.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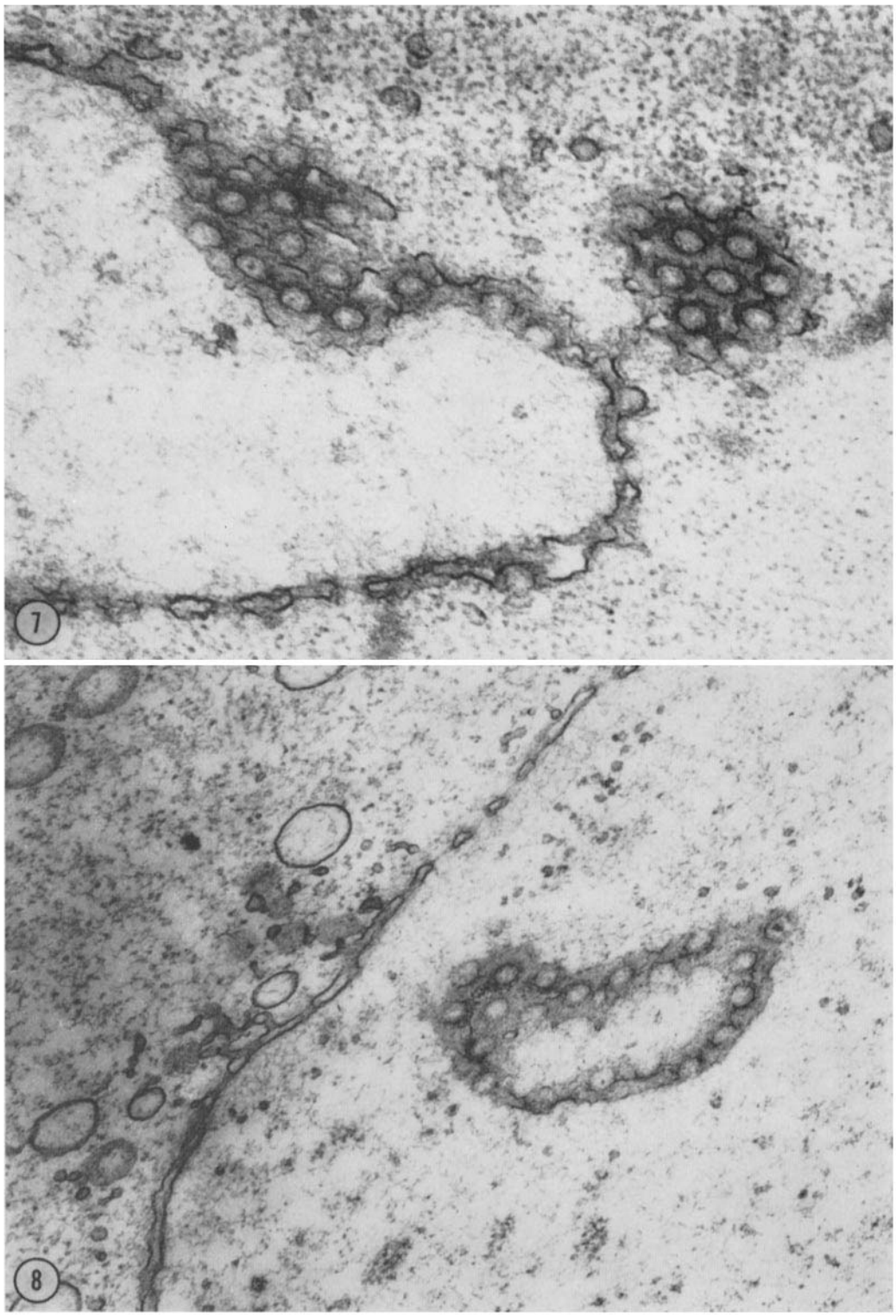

FIGURE 7 The hexagonal distribution of annuli and the central dots are seen in this electron micrograph of the nuclear membrane of a *Xenopus* oocyte. × 68,000.

FIGURE 8 Nuclear membrane of oocyte of *Triturus viridescens* with features similar to those seen in Fig. 6. × 58,000.

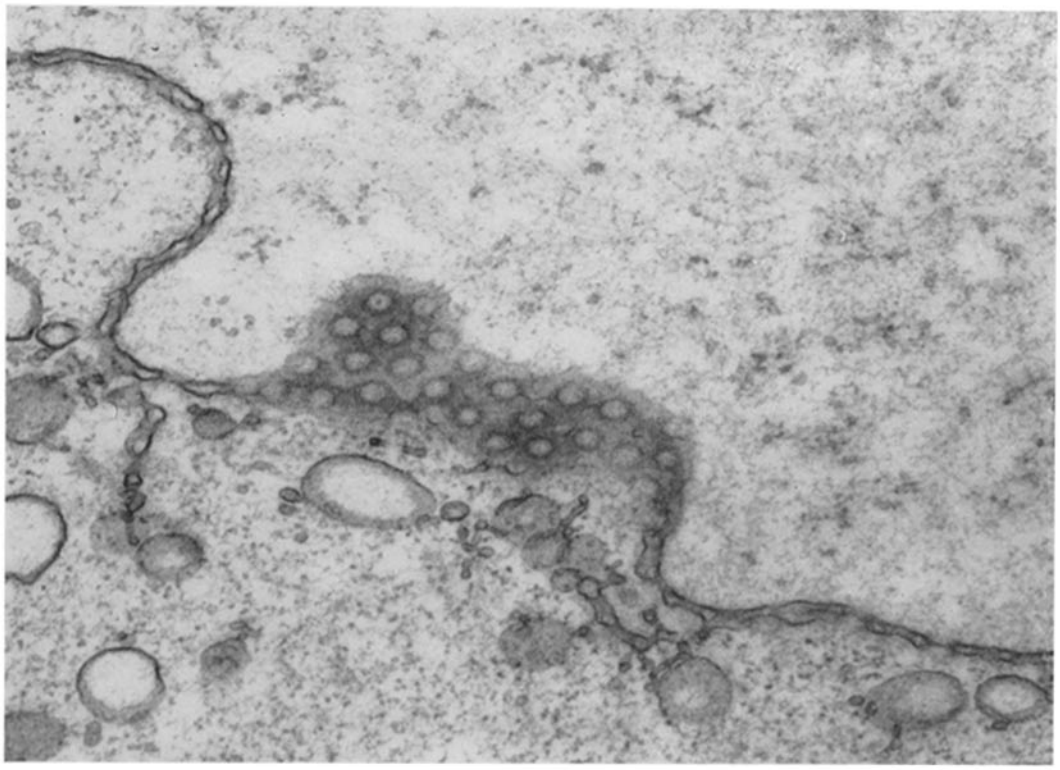

FIGURE 9 Arrays of annuli and central dot structures of the nuclear membrane of *Triturus* oocytes. × 54,000.

transverse resistance of the nuclear envelope in these cells, as obtained from direct conductance measurements in *in situ* nuclei, is of the order of 1 Ω cm². *Thus, clearly, the gaps in these nuclear envelopes cannot be free communications.*

The Question of the Diffusion Barrier

The resistance of the nuclear envelope is lower than that of many cell surface (plasma) membranes. But a surface structure with a resistance of 1 Ω cm² is still a strong barrier to ion diffusion. Its permeability is one-ten-thousandth of that of a porous structure of the kind pictured in Fig. 10. Permeabilities of such low order are generally associated with cellular surface structures that show continuous membranes. But here, the membranes are evidently discontinuous. What, then, are the structural elements that provide the nuclear surface with so high a resistance to ion flow? In electron micrographs of osmium tetroxide–fixed material, the discontinuities appear as gaps only as far as the highly ordered membrane structure is concerned, but they are not entirely structureless. As in other nuclear material (1, 4, 7, 14, 17 *a*-20, 22, 24–27), strands of electron-opaque material are visible in almost all gaps, and a small region of stronger opacity is seen in the center of many gaps. It is tempting to speculate that these materials are the additional diffusion barriers (in addition to the membranes) which confer upon the nuclear envelope its high diffusion resistance.

The question which then presents itself is, what accounts for the marked difference in permeability between envelopes of gland cell nuclei of *Drosophila* and *Chironomus*, on the one hand, and those of oocyte nuclei of various species, on the other. (The primary difference in ion mobility resides clearly at the nuclear surfaces; the resistivity of nucleoplasm is nearly the same in all cases, 100 Ω cm (12, 15, 16).) Electrical measurements in *in situ* nuclei of oocytes of species as diverse as *Xenopus*, *Triturus* (11), *Asterias*, *Nereis*, *Spisula*, and *Hydractinia* (12) show the nuclear envelope to be rather permeable to small ions. The upper limit of nuclear envelope

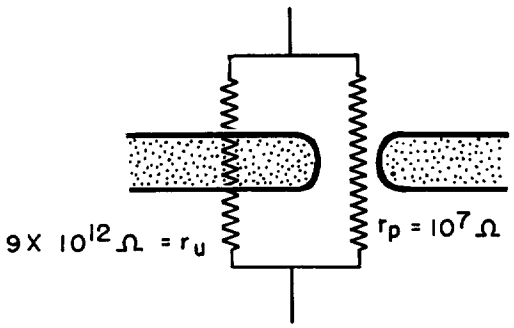

FIGURE 10 Resistance of a "porous" membrane. r_p is the resistance of one hypothetical pore in *Drosophila* if it were freely communicating and filled with a material like nucleoplasm or cytoplasm. r_u, the resistance of the continuous membrane portion associated with one hole, on the basis of a specific resistance of 10^3 Ω cm². The resulting membrane resistance is essentially equal to r_p. The *actual* resistance measured in the nuclear membrane is 1,000 to 10,000 times greater than r_p (15).

resistance is on the order of 10^{3} Ω cm² in all of these oocyte nuclei. Not all of these nuclear envelopes have as yet been studied as to their ultrastructure. But the envelopes which have now been examined under conditions comparable to those in which the electrical measurements were made, namely, those of *Xenopus* and *Triturus*, show no substantial differences in gap size, gap spacing, or gap distribution (Table I), and present no obvious differences in gap structure, compared with that of gland cell nuclei, which might account for the marked disparities in membrane resistance. This is rather disappointing. Part of the difficulties here probably reside in the relatively low degree of structural order in the gap material which makes difficult the recognition of possible structural differences. It would be fruitless at present and the results probably quite misleading, if one were to attempt a differentiation of the two envelope types, for example, on the basis of over-all electron opacity measurements of their gap material. The many variations inherent in the materials themselves are too great, even if variations in techniques for processing them are held at a minimum, as in the present study. A further difficulty resides in the general limitation of electron microscopy which provides bits of structural information selected rather arbitrarily by the limited range of techniques of fixation, staining, etc., available at present; such bits, of course, do not necessarily relate to surface permeability.

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