The Nuclear Envelope after $KMnO_4$ Fixation.* By Joseph G. Gall. (*From the Department of Zoology, University of Minnesota, Minneapolis.*)‡

Studies with the electron microscope have revealed a structural plan common to the nuclear envelopes of many types of cells (*e.g.* 1–6, 8, 11, 13). Nearly all envelopes examined to date consist of two membranes each roughly 50 to 80 A in thickness separated by a perinuclear space of somewhat greater, but variable dimensions. In addition there exist discontinuities in the envelope which generally appear as "pores" in transverse sections or as "annuli" in tangential and surface views. There is, however, considerable uncertainty regarding the patency of the pores and the fine structure of the annuli. Nearly all investigators have studied the nuclear envelope in sections of OsO_4-fixed cells embedded in methacrylate. An important exception is the original and still invaluable study of Callan and Tomlin (4), who devised a remarkably simple technique for isolating and spreading nuclear envelopes free of other cell components (*cf.* also 3, 6). The nuclear envelope has also been seen in sections of permanganate-fixed tissue, where it appears to be well preserved (9). It seemed, therefore, that new information might be gained from a study of isolated envelopes fixed in permanganate.

Materials and Methods

Oocytes of the newt, *Triturus viridescens*, were removed from the body cavity of freshly killed animals and placed in 0.1 M KCl. The giant nucleus is easily

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removed by breaking open the oocyte with two pairs of jewelers' forceps. The nucleus is freed of adherent yolk by sucking it in and out of a pipette several times. Calcium ions are avoided in the isolation medium to prevent clotting of the yolk; calcium is also known to cause drastic structural changes in the chromosomes. The nucleus is transferred to a drop of 0.1 M KCl on a formvar-coated glass slide. The solution is drawn off with a sliver of filter paper until the nucleus is well flattened. Another drop of 0.1 M KCl is then added to break open the nucleus and wash out its contents. The piece of envelope originally on the bottom of the nucleus adheres tightly to the formvar film through this and subsequent manipulations. Fixatives or various test solutions may next be added to the preparation. In the present case fixation was carried out for 5 to 15 minutes with 1 or 2 per cent $KMnO_4$, unbuffered or adjusted to pH 7.3 with 0.007 M phosphate. At the end of fixation the membrane was rinsed thoroughly in distilled water and allowed to dry in air. The formvar film was floated off and mounted on a grid in the conventional fashion. Observations were made with an RCA EMU 3D electron microscope at electronic magnifications up to ~30,000.

RESULTS AND DISCUSSION

The general appearance of a nuclear envelope fixed in $KMnO_4$ is shown in Fig. 1. Scattered rather uniformly over most of the surface are circular areas of low density which appear at first glance to be holes or pores in an otherwise continuous sheet. I shall use the customary designation "pores" for simplicity, although it is quite clear that these areas are not patent under the present conditions of isolation. Pores of similar appearance were described by Luft (9) in his original paper on $KMnO_4$ fixation for electron microscopy. In the isolated newt envelopes their diameter is somewhat variable, ranging from about 1,000 to 1,400 A, while in the mouse liver nuclei pictured by Luft they are somewhat smaller, about 850 A in diameter. One fact stands out immediately: these pores have approximately twice the diameter customarily described for the pores in OsO_4-fixed nuclear envelopes, *i.e.* twice the diameter of the central clear area within the annulus (6, 13). Furthermore, the annulus itself, whatever may be its fine structure in OsO_4-fixed material, is missing.

One can reconcile the differences between OsO_4- and $KMnO_4$-fixed material in basically two ways: (1) One can assume that an annulus is a short cylindrical tube (1, 14) or a circlet of granules (7, 10, 13) fitted into or surrounding a pore. $KMnO_4$ fixation preserves the membranous parts of the envelope, but permits complete dissolution of the annuli, whereas OsO_4 preserves both membranes and annuli. (2) $KMnO_4$ preserves the envelope as in life, and the annuli seen after OsO_4 fixation are artifacts produced by shrinkage of material around the pores (4). The first interpretation is compatible with the fact that $KMnO_4$, generally speaking, preserves membranes better than other structures. The small granules associated with the endoplasmic reticulum are not present after $KMnO_4$ (9), and this is a fact of particular interest since a relationship between these granules and the annuli has been suggested by several authors (7, 10, 12, 13). However, thickened rims around the pores are occasionally seen after $KMnO_4$, especially in regions which appear to have been stretched during preparation. The second interpretation remains, therefore, a possibility.

A question of considerable importance is whether the pores provide an unobstructed pathway between cytoplasm and nucleus. A first approach to this question can be made by determining, if the pores in isolated, fixed membranes are open. Certainly pores like those in Fig. 1 are *thinner* areas of the envelope, since metal shadowing shows them as depressions, at least when the shadowing is done from the nuclear side of the envelope. On the other hand, a true hole cannot be demonstrated unequivocally so long as the envelope is spread on a supporting film of plastic. To avoid this problem nuclear envelopes were spread on thick carbon films which contained numerous small holes about 0.5 to 2 micra diameter. The nuclear envelope is stable over most of these holes. The nuclear pores are not patent under these conditions of isolation. Rather they are covered by a fine granulation (~30 to 40 A) which also extends over the remainder of the envelope (Fig. 2). Unfortunately the interpretation of this experiment in terms of the normal nuclear envelope is equivocal. Examination of holes in the carbon film in regions *outside* the limits of the nuclear envelope shows that most of these are likewise covered by a finely granular film (Fig. 3). In fact, the entire grid is coated by what is perhaps a thin layer of denatured protein derived from the nucleoplasm during the isolation procedure. This layer, although probably only a few hundred A thick, is remarkably strong, forming stable sheets over holes several micra in diameter. Thus, even if the pores in the nuclear envelope are originally open, they are certainly covered by this additional material after fixation

and air drying. As yet no method of removing the unwanted layer has been discovered.

In summary, isolated nuclear envelopes fixed in $KMnO_4$ differ somewhat from those fixed in OsO_4. Circular areas of low density are seen in place of the customary annuli. These circular areas are not true holes in the *isolated* envelope, but the material covering them is at least in part an extraneous precipitate and not a portion of the envelope itself.

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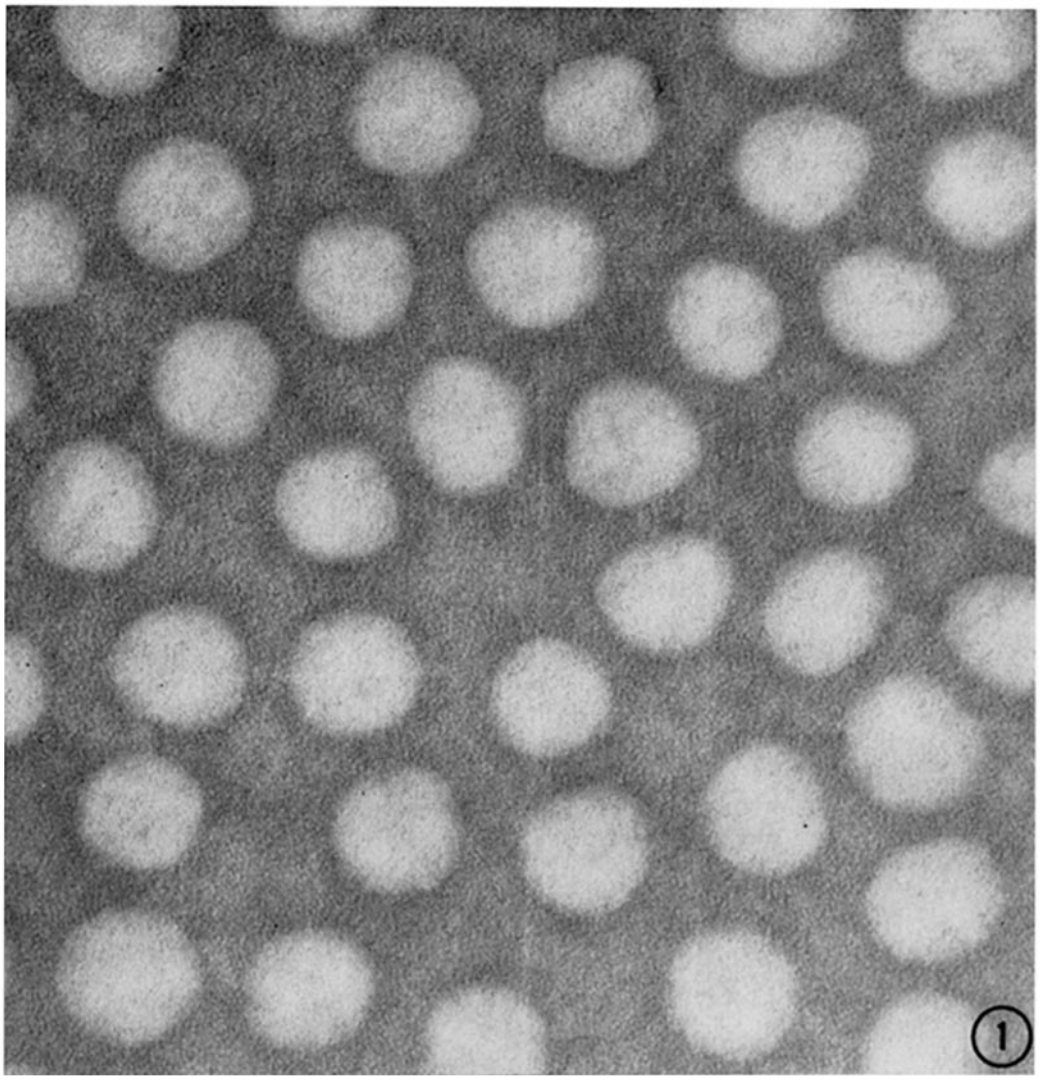
Explanation of Plate

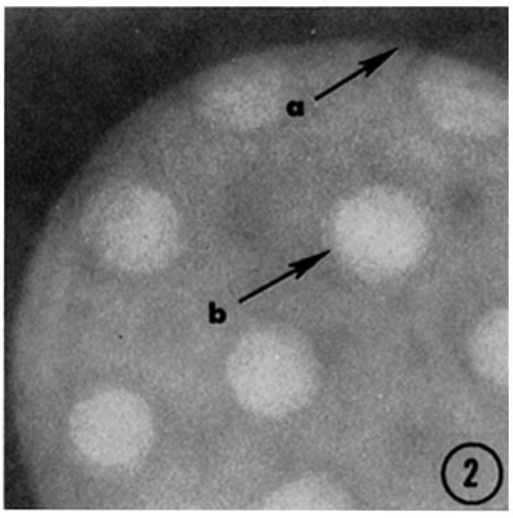
Plate 75

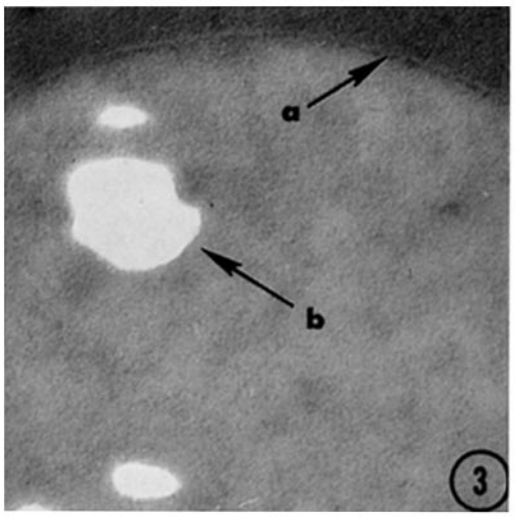
Fig. 1. Surface view of a small portion of the nuclear envelope from a newt oocyte. Isolated in 0.1 M KCl, spread on a formvar film, and fixed in 2 per cent $KMnO_4$. The roughly circular areas of low density ("pores") have approximately the same diameter as the annuli customarily seen after OsO_4 fixation (1,000 to 1,400 A). Their diameter is thus about twice that of the pores described from OsO_4-fixed material, *i.e.* twice the diameter of the central clear area of the annulus. The fine granulation ($\backsim$30 to 40 A) is part of the specimen and not photographic grain, as shown by the fact that identical patterns are found in successive micrographs of a through-focus series. Furthermore, the granulation is brought into relief by metal shadowing. Approximately × 140,000.

Fig. 2. A similar preparation spread on a carbon film. The arrow *a* indicates the perimeter of a hole in the support, the arrow *b* the perimeter of a pore in the nuclear envelope. It is evident that the pore is covered by a fine granulation similar to that seen over the remainder of the envelope. The pores here are smaller, on the average, than those in Fig. 1. Approximately × 140,000.

Fig. 3. Micrograph from the same grid as Fig. 2, but in an area outside the limits of the isolated nuclear envelope. The arrow *a* indicates the perimeter of a hole in the carbon supporting film. Note that the hole is covered by a finely granular film torn in a few regions (*e.g.* arrow *b*). The presence of this film (denatured protein?) over the entire specimen grid precludes a decision as to the patency of the nuclear pores. The lighter line around the hole in the carbon film looks like an overfocus fringe, but is in reality a less dense part of the film itself. This micrograph, like Figs. 1 and 2, is slightly underfocused. Approximately × 140,000.







(Gall: Nuclear envelope after $KMnO_4$ fixation)