THE PERMEABILITY OF THE AMPHIBIAN OOCYTE NUCLEUS, *IN SITU*

SAMUEL B. HOROWITZ

From the Department of Biology, Michigan Cancer Foundation, Detroit, Michigan 48201

ABSTRACT

Ultralow temperature radioautography, suitable for the quantitative localization of diffusible solutes, was used to study the permeability of the nuclear envelope in the intact amphibian oocyte Sucrose-^{3}H solutions were injected into mature oocytes, in volumes of 0 016–0 14% of that of the cell, and the subsequent movement of the solute was recorded. The resultant radioautographs show diffusion gradients in the cytoplasm and nucleus, and concentration gradients across the nuclear envelope Analysis of these gradients discloses that the nuclear envelope is as permeable as a comparable structure composed of cytoplasm, and is about 10^8 times more permeable than the oocyte plasma membrane The diffusion coefficient of sucrose in cytoplasm is 2×10^{-6} cm^2/sec, or about one-third its diffusivity in pure water. This reduction can probably be accounted for by an effective lengthening of the diffusional path because of obstruction by cytoplasmic inclusions. The nuclear : cytoplasmic sucrose concentration ratio at diffusional equilibrium is about 3 05, or 1.6 times as great as expected from the water content of the two compartments This asymmetry is attributed to an unavailability of 36% of the cytoplasmic water as solvent Finally, sucrose entry into oocytes from a bathing solution was monitored by whole cell analysis and radioautography. These and the microinjection results are consistent with a model in which sucrose entry into the cell is entirely limited by the permeability of the plasma membrane. The results are inconsistent with cell models that hypothesize a short-circuit transport route from the extracellular compartment to the nucleus, and with models in which cytoplasmic diffusion is viewed as limiting the rate of solute permeation.

INTRODUCTION

Differences in the nuclear and cytoplasmic concentrations of a variety of solutes have been observed repeatedly (1–4). However, we have no concrete idea of the role that these solute asymmetries play in cell function, nor of the identity of either the cellular structures or mechanisms responsible for their maintenance.

In part our ignorance is due to the preeminent role of the plasma membrane in cellular material transactions This surface transport barrier controls solute movement into the entire cell and makes it difficult to study solute movements into the nucleus. The surface-to-volume ratio of the nucleus is greater than that of the cell itself, hence rate constants for filling the nucleus are intrinsically greater than for filling the cell. As a consequence, when solute movement into the nucleus involves passage through the cell membrane a measure of nuclear membrane permeability is possible only if nuclear permeability is appreciably less than plasma membrane permeability This usually is not the case

To overcome this problem many studies of nuclear permeability have relied on isolated nuclei in artificial media (e g., references 5–9), preparations which are heterogeneous and un-

stable. Nuclear transport studies *in situ*, on the other hand, involve bypassing the cortical membrane by microinjection. These have been limited by the difficulty of determining the regional intracellular concentration of all but a few types of material. Consequently, *in situ* studies have relied either on osmotic methods (10) with the ambiguity of interpretation inherent in this approach (10, 11, see below), or on the use of relatively large electron-opaque particles (12–14) and dyes (15, 16).

The advent of radioautographic techniques suitable for the quantitative localization of diffusible solutes (17, 18) makes it possible to determine the local concentration of any solute that can be radioactively labeled. As a consequence new approaches to the problem of nuclear permeability are available.

In the present experiments a solution of sucrose-^{3}H is injected into the cytoplasm of the mature amphibian oocyte, and time is permitted for the evolution of a transcellular sucrose gradient. Diffusion is then effectively terminated by quenching the oocyte at −190°C. The oocyte is sectioned at low temperatures, and the local cytoplasmic and nuclear solute concentrations are determined by ultralow temperature radioautography.

The place of maximal solute concentration in the cytoplasm is at the injection site, and concentration decreases with distance from this point. The nucleus is astride this gradient, and the manner in which it influences the gradient is determined by its permeability. Consequently, comparison of solute distribution within nucleus and cytoplasm at different times permits an assessment of the permeability of the nuclear membrane.

Figure 1 shows schematically the design of an experiment and some of the results to be expected a priori. Injection (Fig. 1 *a*) is made into the vegetal hemisphere of the oocyte to minimize the possibility of damage to the nucleus. The injectate and a small volume of silicone oil (the hydraulic fluid of the micropipette apparatus) enter the cytoplasm. After a period of time (*t*), the oocyte is frozen and sectioned, ideally in a plane passing through both the nucleus and injection site (Fig. 1 *b*).[1] A grain density profile taken along the dotted line in Fig. 1 *b* is expected to exhibit the features

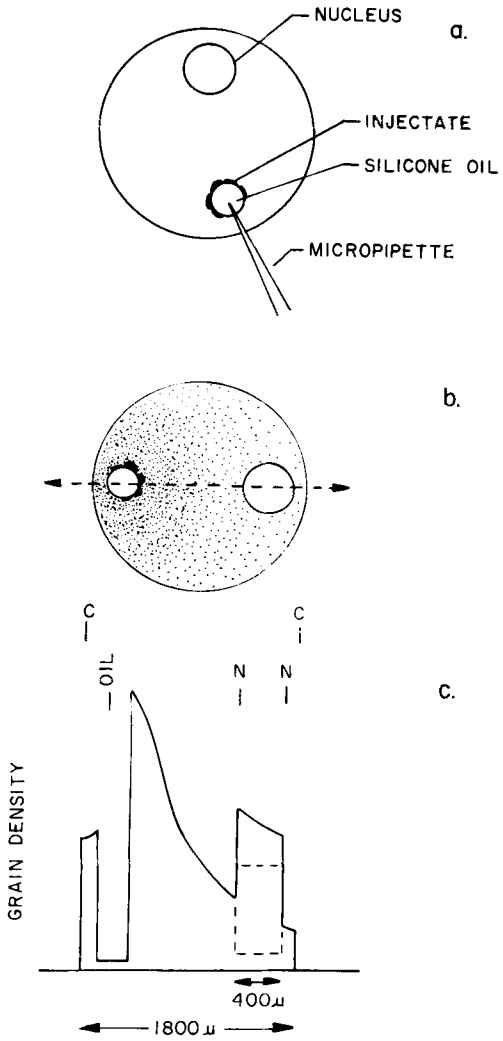

FIGURE 1 Schematic representation of the microinjection experiments. See text for explanation. *C* and *N*, cell and nuclear boundaries.

shown in Fig. 1 *c*. A point of high grain density is at the site of sucrose injection. Adjacent to it, on the left in this case, is the silicone oil, an area of low grain density (because of the insolubility of the sucrose). To the right, a diffusion gradient is shown falling away from the injection site. Sharp discontinuities are expected at the cell membranes (C).

Before continuing, an important point should be noted. Sucrose is a highly polar solute whose distribution in biological material reflects the distribution of solvent water. Because of a high concentration of yolk platelets and other inclusions,

[1] This orientation is rarely achieved; in practice, one must generally study the injection site and the nuclear area in different sections.

the cytoplasm of the mature *Rana pipiens* oocyte is only 45% water, while the nucleus, which is devoid of these inclusions, is about 85% water (4) Hence, if the nucleus and cytoplasm have the same concentration of sucrose-^{3}H on the basis of water content, the concentration on a volume basis is higher in the nucleus.[2] Since radioautographic grain density is proportional to the concentration of the radioactive source on a volume basis when the sucrose-^{3}H concentrations in nucleus and cytoplasm are similar on a water basis, the grain density is higher over the nucleus than over the cytoplasm.

The behavior of the sucrose gradient at the nuclear boundary (N) will depend on the permeability of the nuclear envelope and the diffusional properties of nucleoplasm (Fig. 1 *c*). As a limiting case, if the nuclear envelope were totally impermeable, nuclear grain density would be at background levels at all values of *t*. At the other limit, in which neither the nuclear envelope nor the nucleoplasm constitutes an appreciable barrier to solute movement, the nuclear sucrose concentration at all values of *t* merely reflects the concentration in the adjacent cytoplasm. This is depicted by the solid line in Fig. 1 *c* in which the diffusion gradient in the water of the cytoplasm is continued, albeit at a higher concentration on a volume basis, through the water of the nucleus

The dashed lines through the nucleus in Fig. 1 *c* represent cases in which the nuclear envelope constitutes a substantial but not impermeable transport barrier In this circumstance the nuclear profile would be flat, reflecting the rate-limiting influence of the envelope. At shorter times than are required to achieve diffusional equilibrium, the ratio of nuclear to cytoplasmic grain densities would be less than that which prevails at equilibrium.

Other possibilities exist. For example, the nuclear envelope could constitute a negligible barrier to transport while diffusion in nucleoplasm was appreciably slower than in cytoplasm. In this case an intranuclear diffusion gradient would be seen with its peak at a point on the nuclear surface and its trough close to the nuclear center. A variety of other inhomogeneous distributions could be imagined, but in point of fact, these will not be necessary for the interpretation of the experimental results.

[2] Factors which play a secondary role, such as the ability of the water in each of the compartments to serve as a solvent, will be considered in the Discussion.

MATERIALS AND METHODS

Oocytes

Rana pipiens were obtained within a week of use from a New Jersey dealer. Until use, they were kept at 4°C in spring water. Only mature ovarian oocytes, stage Y_5 of Kemp (19), were used. The isolation technique, and the physical characteristics of these cells have been described elsewhere (18).

The microinjection experiments and most of the incubation experiments were done from January through March, 1971. A small number of incubation experiments on Wisconsin frogs, generously provided by Dr. M. DiBerardino, were performed in late March.

Solutions

Ringer's solution contained 104.7 mM NaCl, 2.5 mM KCl, 1 0 mM $CaCl_2$, 1 2 mM $MgCl_2$, 17.3 mM $NaHCO_3$, 2.0 mM NaH_2PO_4, 1 2 mM Na_2HPO_4 The pH was 7.2–7.3 The Ringer's used in incubation experiments contained 1.0 mM sucrose.

Sucrose-^{3}H (D-fructose-1-^{3}H) was obtained from New England Nuclear Corp., Boston, Mass. Radiochemical purity was claimed by the manufacturer to be >99.4%. This, and the identity of the compound, were confirmed by radiochromatography of the compound and its acid hydrolysate in the two systems described below.

Sucrose-^{3}H is supplied in a 7:3 ethanol-water solution. For microinjection, this was concentrated by drying 10-μl portions with a gentle stream of nitrogen in the bottom of a 0 5 ml polyethylene BEEM capsule. Final dryness was achieved at 10^{-2} torr at room temperature for 1 hr. The dried sucrose was redissolved in 5 μl of water, and this is the injectate. Injection solutions were prepared on two occasions and had activities of 179 μCi/μl and 100 μCi/μl as determined with calibrated micropipettes (see below) and liquid scintillation counting. The nominal concentrations of sucrose, using the specific activities supplied by the manufacturer, were 32 mM and 18 mM, respectively.

For incubation experiments the original sucrose-^{3}H solution was lyophilized and resuspended in 0.5 ml of Ringer's adjusted so that the final sucrose concentration was 1.0 mM.

Incubation

After isolation in sucrose-Ringer's, oocytes were transferred to a vial and allowed to come to the incubation temperature of 20.0°C. The supernatant was

carefully removed and 0.5 ml of 1.0 mM sucrose-Ringer's containing sucrose-^{3}H was added. The activities of the bathing solutions were either 33 or 330 μCi/ml The vial was agitated continuously for the first 50 min and intermittently thereafter. At preselected times an oocyte was removed with a copper wire loop and fine-tipped forceps, blotted, and rinsed for 15 sec in ice-cold sucrose-Ringer's and blotted again. Further treatment depended on the purpose of the experiment.

Oocytes used for radioautography were frozen in O.C.T., a commercial mounting media (Lab-Tek Inc , Westmont, Ill.), as described below.

Oocytes used in determining the time course of sucrose entry into the cell were weighed on a Cahn electrobalance (Cahn Instrument Co., Paramount, Calif.), and placed in 1.2 ml of distilled water in an ampul which was then heat sealed The oocytes were then extracted in boiling water for 20 min. Other oocytes were taken at various times to determine dry weight.

The radioactivity of the incubation fluid did not change after the original dilution.

Radioactivity was determined in 1.0 ml aliquots of water dissolved in 20 ml of Bray's solution (20) and counted in a Packard 3214 liquid scintillation spectrometer at 2°C.

Microinjection

Microinjection was accomplished using a Chamber's type unit filled with silicone oil (100 centistoke dimethylpolysiloxane; Dow-Corning 200 fluid). The micropipette holder (E. Leitz, Inc., Rockleigh, N. J) was manipulated by a micromanipulator.

Micropipettes were drawn from clean 0.8 mm OD capillary tubing with a micropipette puller (Industrial Science Associates M1 [Industrial Science Associates, Inc , Ridgewood, N. Y.]). The micropipettes were filled with 5 centistoke silicone oil before use. The tips of the pipettes as drawn were generally too fine (1–2 μ) for use, and were enlarged by being passed fleetingly over a drop of concentrated hydrofluoric acid and rinsed with water. When used, the tip width was 6–12 μ.

Micropipettes were calibrated after use with tritiated water (100 mCi/g). In early experiments injectate volumes were in the range 3.1 to 3.5 $\times$ 10^{-6} cm^3. In later experiments, we used smaller volumes, in the range of 3.8 to 4 8 $\times$ 10^{-7} cm^3. These are, respectively, about 0.14% and 0.016% of oocyte volumes. The radioactivity injected was 0.57–0.60 μCi/oocyte in the early experiments, and 0.038–0.048 μCi/oocyte in the later experiments.

The injection of oocytes was done in a moist chamber which allowed their orientation. Injection was made into the vegetal hemisphere to avoid damaging the nucleus. After microinjection, the oocyte was transferred to another moist chamber, in which it sat in a drop of Ringer's solution on a small pad of filter paper until frozen.

Radioautographic Technique

The radioautographic technique has been detailed elsewhere (18, 21). Briefly, it involves the following steps.

(*a*) Individual oocytes are frozen in O.C.T. by quenching in dichlorodifluoromethane cooled to −160°C and are then placed directly into liquid nitrogen. The elapsed time from immersion into O.C.T. until freezing is about 10 sec.

(*b*) Sectioning and tissue-to-emulsion contact is done at −50°C in a modified Wedeen type cryostat. Sections are cut 4–10 μ in thickness (depending on the cutting qualities of the oocyte) and transferred to a Teflon sheet backed by a microscope slide. A radioautographic plate is placed, emulsion downward, on the sections, to form a sandwich—slide, Teflon, sections, emulsion, slide—which is tightly clamped together. An effort was made to make serial sections of the entire oocyte. However, low temperature sectioning is extremely idiosyncratic, and we were never completely successful in this

(*c*) The sandwiches are incubated at −72°C for 18 hr to 43 days Exposure is terminated by separating the sandwich and developing. The sections are bleached for 48 hr as described in reference 18.

(*d*) Grain densities are determined by grain counting at magnifications of either 1563 or 2500 in dark field illumination, using a Whipple-Hauser type eyepiece micrometer. To assure that the grain density is proportional to the tritium content of the underlying section, two corrections are applied to the raw counts: (1) background densities are subtracted, (2) multiple-hit corrections are made with the aid of standard curves derived from radioautographs of discs of poly(butyl-^{3}H) methacrylate (18, 22). Grain densities, G, are expressed, unless otherwise noted, as grains per 1000 μ^2 per hours of radioautographic exposure.

Resolution

The resolution of radioautographs can be assessed by assuming that the concentration of sucrose-^{3}H at the surface of an oocyte, previously injected and allowed to come to diffusional equilibrium, changes as a step function. The grain density profile is then determined through plane of the surface. An example is given in Fig 2. Resolution, defined as the distance over which grain density falls by 50%, is about 3 μ. This compares favorably with our earlier result, with tritium, of 14 μ, using glycerol-^{3}H (18) and reflects the improvements in technique since the earlier study.

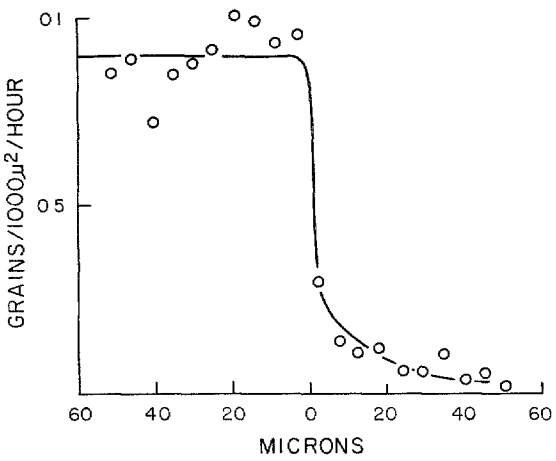

FIGURE 2 Grain density profile taken at the border of an 8 μ section of an oocyte microinjected with sucrose-^{3}H, and permitted to come to diffusional equilibrium Distance is measured from edge of the cell. Each point is the average of six counts.

Paper Chromatography

Descending chromatography, utilizing untreated strips of Whatman No 1 paper, was employed to monitor the radiopurity of the injectate, and the possibility of enzymatic conversions of the sucrose-^{3}H in the cell Two solvent systems were employed: (*a*) *n*-butanol-pyridine-water (10:3:3) and (*b*) *n*-propanol-ethyl acetate-water (7:1:2).

The paper strips were run overnight at room temperature. The strips were dried, and scanned for radioactivity with an automatic strip scanner and Geiger-Muller flow counter Urea-phosphate spray (23) was used to locate carrier sucrose and fructose

RESULTS

Fig 3 *a* is a photomicrograph in dark-field illumination of a section that passes through both injection site and nucleus, in an oocyte frozen 982 sec (*t*) after injection The cytoplasm is refractile because of a high concentration of particulates, especially yolk platelets and pigment granules The dark sphere is a pool of silicone oil, which is devoid of particulate material and radioactivity. The bright area on its right edge is due to the high silver grain density that underlies the injected sucrose-^{3}H solution The cell nucleus, n, is almost devoid of particulate matter but appears bright because of the very high grain density in the underlying emulsion.

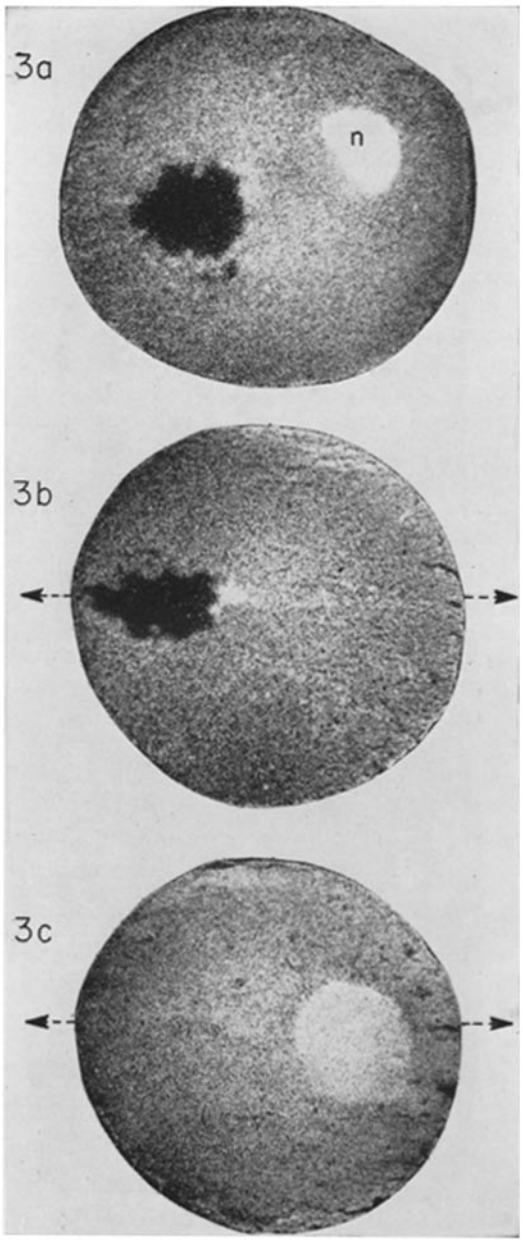

FIGURE 3 Micrographs in dark field illumination of sections of microinjected oocytes. (*a*) Section through the injection site and nucleus (*n*) of an oocyte at t = 982 sec (*b*) Section through the injection site and cytoplasm of an oocyte at t = 1646 sec. (*c*) Section through the nucleus of the same oocyte. See text for additional explanation × 30.

Injection Site and the Cytoplasmic Gradient

Fig. 3 *b* is a photomicrograph of an oocyte at $t = 1646$ sec in a plane that includes the injection site but not the nucleus. The silicone oil appears as a dark subellipsoid on the left side of the section The bright area immediately to its right is the area of high grain density that marks the location of the injected pool. The quantitative distribution of sucrose-^{3}H in this section is given in Fig. 4, in which the corrected grain densities, G, over the entire section are displayed. The regions of highest grain density are adjacent to the silicone oil. Grain density decreases radially in all directions from the injection site in a transcytoplasmic gradient.

A more detailed picture of the variation in G across the section is provided in Fig. 5 This is a profile along the axis indicated by the arrow in Fig. 3 *b*. It is similar to that seen in oocytes of t from 900 to 2100 sec. There is an eightfold difference in G from the site of injection to the most distant edge of the cell. The profile has two components: a peak at the site of injection, and a less steep cytoplasmic gradient The cytoplasmic gradient is due to the diffusion of sucrose and accounts for about 28% of the total gradient. The peak marks the volume occupied by the injected water which does not completely disperse immediately Two observations make this clear. First, the peak is not seen when injection volumes are less than 0 5 mμl. Second, the ratio of G at the peak to G in adjacent cytoplasm is about 3, the ratio expected from the relative water contents of injectate and cytoplasm (see Discussion). The delayed disappearance of injected water demonstrates, as one might expect, that the interdiffusion of water with cellular protein and particulate matter is slower than the diffusion of the labeled solute in water and cytoplasm.

Another point to be noticed in Fig. 5 is that the grain density profile drops off sharply at the cell surface, indicating that the membrane constitutes a substantial barrier to sucrose transport This interpretation is confirmed by studies of the kinetics of sucrose influx into oocytes from Ringer's solution, described below

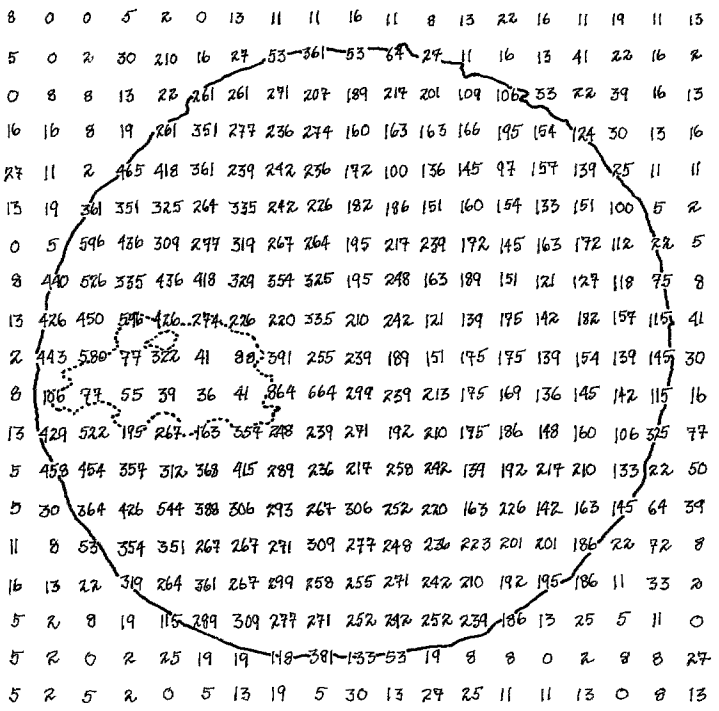

FIGURE 4 Local grain densities in grains per 1000 μ^2 per 100 hr of radioautographic exposure, over a section that passes through the injection site of an oocyte injected with sucrose-^{3}H. $t = 1646$ sec. The section is that shown in Fig. 3 *b* though inverted. × 52.

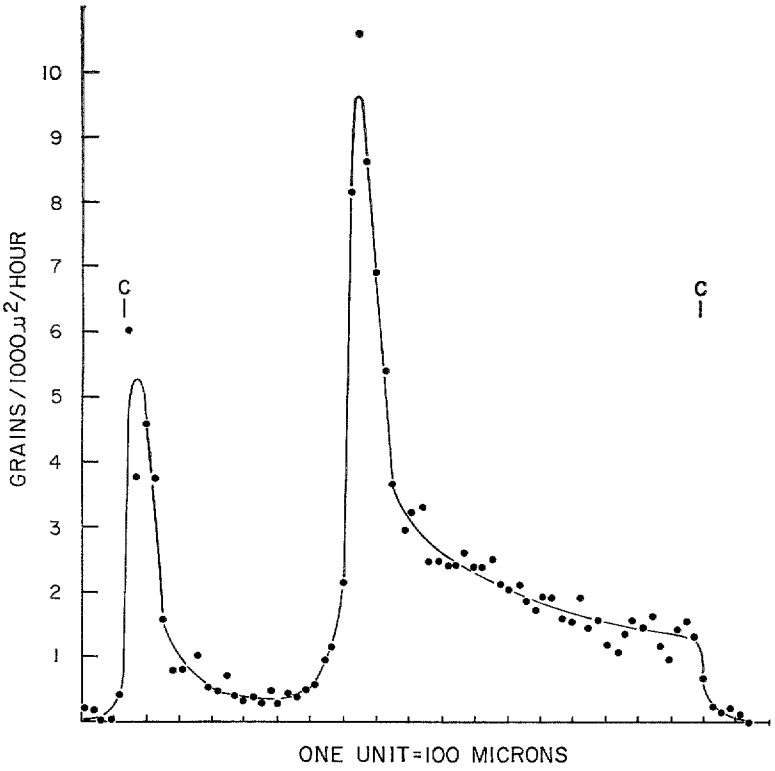

FIGURE 5 Grain density profile through the injection site and cytoplasm of an oocyte injected with sucrose-^{3}H. t = 1646 sec. The vertical bars (C) mark the cell boundaries. See text for additional explanation.

Fig. 6 provides another example of a profile through an injection site. In this case the oocyte was frozen at t = 2058 sec It shows the same features as the profile in Fig. 5; a cytoplasmic diffusion gradient, peak concentration at the site of injection, low concentration in the silicone oil, and discontinuity at the cell surface

Concentration gradients in the cytoplasm are steepest at short times after microinjection A profile from the shortest experimental time available (t = 250 sec) is given in Fig. 7. This passes tangentially to the edge of the silicone oil pool and through or close to the injection site. There is a 75-fold decrease in grain density from the high point of the gradient to the most distal edge of the cell, where G approaches zero.

It is possible to estimate the cytoplasmic diffusion coefficient, D_c, for sucrose, comparing the right leg of the profile in Fig 7 to theoretical diffusion profiles. We will assume that the injection volume is sufficiently small to constitute an instantaneous point source and that solute reflectance from the cell boundary is negligible The equation (24) describing the concentration of sucrose-^{3}H, C, in the cytoplasm is then

$$C(r) = \frac{M}{8(\pi D_c t)} e^{-r^2/4D_c t} \quad \text{Equation 1}$$

where r is radial distance from the injection site and M is the total amount of sucrose diffusing This can be normalized to

$$C(r)/C(0) = G(r)/G(0) = \exp(-r^2/4 D_c t) \quad \text{Equation 2}$$

where $C(0)$ is the concentration and $G(0)$ is the grain density at r = 0.

A comparison of the normalized, experimentally derived profile at t = 250 sec with theoretical profiles for three values of D is made in Fig 8. The distribution of experimental points agrees rather

well with the form of the diffusion curves and is best approximated by the line for $D = 2 \times 10^{-6}$ cm²/sec.

Efforts to estimate D_c from longer-time experiments are greatly complicated by the reflection of the diffusing sucrose as it increases in concentration at the cell boundary. Such determinations have not been attempted. The value of D_c of 2×10^{-6} cm²/sec appears consistent with the gradients we have studied, and with the disappearance of a discernible gradient between 2000 and 3000 sec (see below).

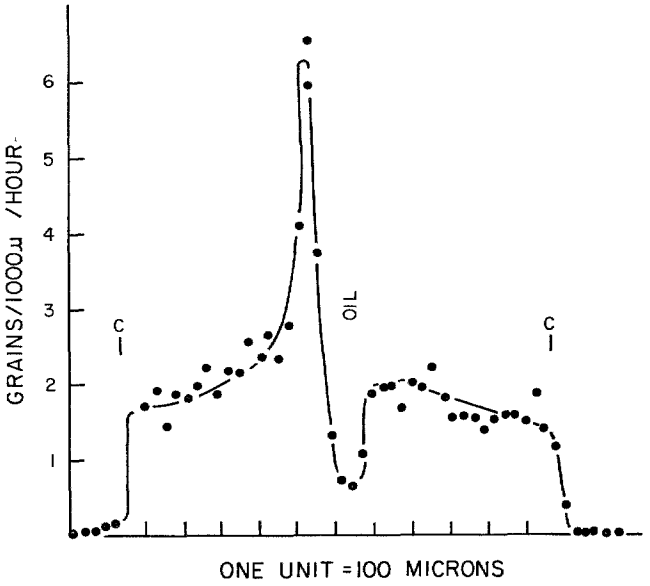

FIGURE 6 Grain density profile through the injection site and cytoplasm of an oocyte injected with sucrose-^{3}H. $t = 2058$ sec. The vertical bars (C) mark the cell boundaries

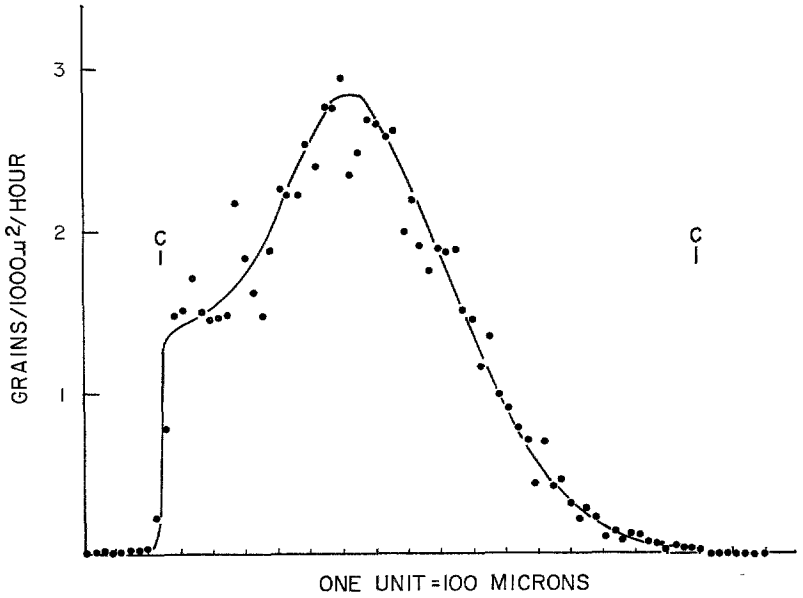

FIGURE 7 Grain density profile through the injection site and cytoplasm of an oocyte injected with sucrose-^{3}H. $t = 250$ sec. The vertical bars (C) mark the cell boundaries.

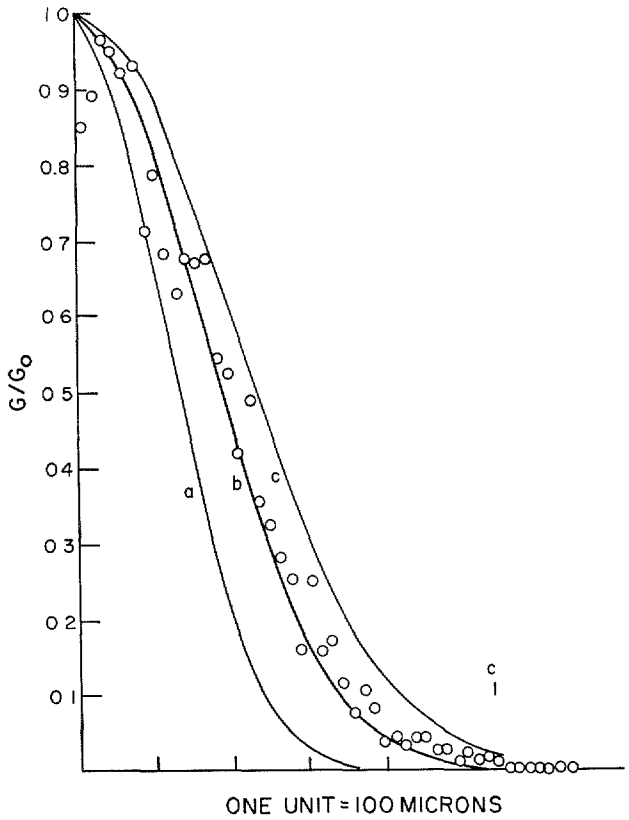

FIGURE 8 Normalized grain density profile. The points are experimental and are those of the right leg of the profile in Fig. 7 The lines are theoretical for three values of D at 250 sec. (*a*) 1.0×10^{-6} cm^2/sec, (*b*) $2\,0 \times 10^{-6}$ cm^2/sec, (*c*) 3.0×10^{-6} cm^2/sec. See text for additional explanation.

Nucleus

Fig 3 *c* is a photomicrograph of a section in a plane parallel to that of Fig. 3 *b* (which passes through the injection site) but displaced approximately 400 μ so that this section passes through the nucleus Grain densities over the entire section are given in Fig. 9. They are highest in the vegetal hemisphere, at the area closest to the injection site, and decrease from there. Grain density in the nucleus is sharply higher than in the surrounding cytoplasm.

The ratio of G in nucleus and cytoplasm is proportional to the ratio of the sucrose-^{3}H concentrations on a volume basis in these compartments.[3] Since there is a cytoplasmic gradient, it is most satisfactory to compare the concentration in adjacent positions in the nucleus and cytoplasm. This can be done by determining G at positions (a, b, c, $\cdots$) inside, but along the edge of the nucleus (G_n^a, G_n^b, $\cdots$) and in the immediately adjacent cytoplasm (G_c^a, G_c^b, $\cdots$). The mean of the ratios of adjacent nuclear and cytoplasmic sites

$$\bar{X}_{n/c} = [G_n^a/G_c^a + G_n^b/G_c^b + \cdots]/N \quad \text{Equation 3}$$

in Fig 9 is $3\,2 \pm 0\,6$.

A more detailed picture of the variation of sucrose-^{3}H concentration along the axis indicated

[3] The ratio of grain densities is actually very close to being *equal* to the ratio of the solute concentrations on a volume basis in the two compartments Two small corrections would be necessary to make the two ratios equal (*a*) a correction for differences in the electron opacity of nucleus and cytoplasm, and (*b*) a slight increase in the multihit correction for G over the cytoplasm as a consequence of the fact that the solute is not uniformly distributed locally but tends to be restricted to the region between the yolk platelets (for details see reference 18). These corrections are probably in opposite directions and are unlikely to exceed 5%.

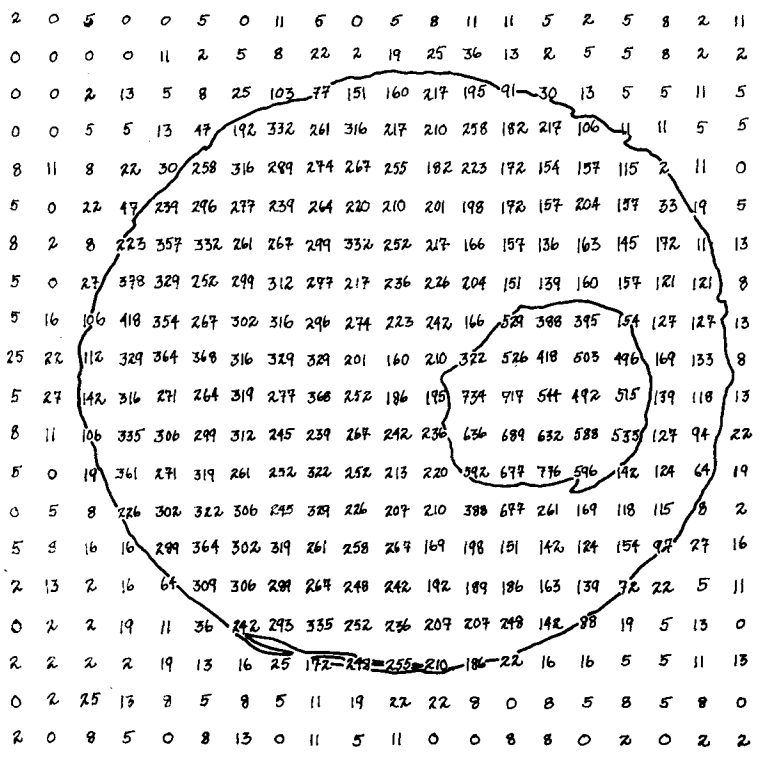

FIGURE 9 Local grain densities in grains/1000 μ^2 per 100 hr of radioautographic exposure, over a section that passes through the nucleus of an oocyte injected with sucrose-^{3}H. t = 1646 sec. The section is that shown in Fig. 3 *c*. × 52.

by arrows in Fig. 3 *c* is provided in Fig. 10. There are two regular features of nuclei astride a cytoplasmic concentration gradient: (*a*) a higher concentration of solute than in the cytoplasm, and (*b*) a transnuclear gradient that parallels that of the cytoplasm. Within the experimental error, the slopes of nuclear and cytoplasmic gradients are the same.

In Fig. 11 we have plotted two profiles through an oocyte at t = 1004 sec. The upper profile passes through cytoplasm and nucleus, and shows the same features as Fig. 10. The lower figure is a parallel profile through the same section taken just outside the nucleus, and shows the continuity of the cytoplasmic gradient.

The presence of a diffusional gradient in the nucleus parallel to that in the cytoplasm was one of the possible experimental results considered in the Introduction. This result indicates that the nuclear envelope is a negligible barrier, having no more slowing effect on sucrose transport than a comparable membrane were it composed of cytoplasm.

In the absence of a significant transport barrier at the nuclear surface, the ratio of nuclear: cytoplasmic sucrose-^{3}H should be the same at all values of t. That this is the case is shown in Table I, which is the tabulation of $\bar{X}_{n/c}$ for the eight oocytes in which the nuclear and cytoplasmic raw grain densities permitted reliable determination of G_n and G_c. No significant variation from the mean of 3.05 occurred during the period (16 min to 3 hr) represented in these experiments.

Injected oocytes did not exhibit a cytoplasmic or nuclear gradient after about 3000 sec, by which time diffusional equilibrium had been achieved. The grain density profile in Fig. 12, which passes through the nucleus and cytoplasm, makes this point. Profiles through oocytes at t = 11,040 and 12,750 sec are essentially identical. The uniform distribution of sucrose supports a conclusion reached in other studies (13, 21), that in the mature oocyte the solvent water is uniformly distributed within the cytoplasm and the nucleus. This is not the case in immature oocytes (21).

Metabolism

The possibility that sucrose-^{3}H might undergo appreciable enzymatic conversion within the cell was ruled out by radiochromatography of the cell contents after microinjection. Oocytes were injected with sucrose-^{3}H solution and frozen 10^4 sec later. In preparation for chromatography they were thawed, mashed in 10 μl of 10 mM cold sucrose, and heated in boiling water for 5 min. The

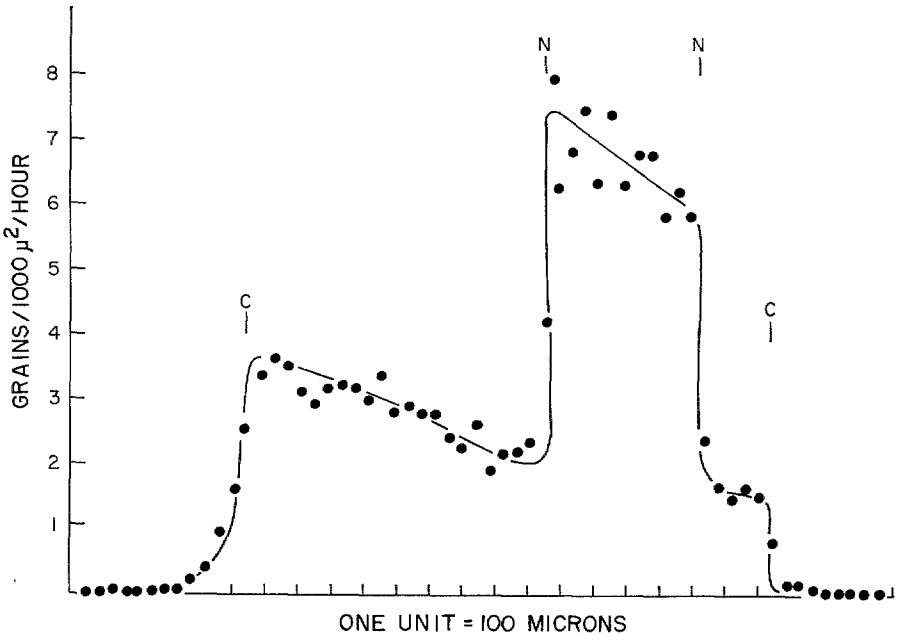

FIGURE 10 Grain density profile through the cytoplasm and nucleus of an oocyte injected with sucrose-^{3}H. t = 1646 sec. The vertical bars mark the cell (C) and nuclear (N) boundaries. See text for additional explanation.

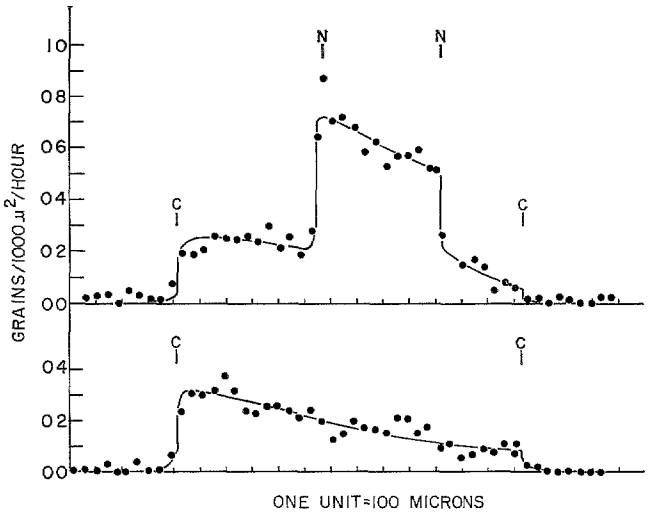

FIGURE 11 Parallel grain density profiles through the cytoplasm (lower) and cytoplasm and nucleus (upper) of an oocyte injected with sucrose-^{3}H. t = 1004 sec. The vertical bars mark the cell (C) and nuclear (N) boundaries.

resultant cell suspensions were then run in the two solvent systems described under Methods In each case the activity moved as a single peak coinciding with the sucrose standard. In solvent I, the R_T relative to fructose was 0.37, in solvent II, it was 0 60. Insoluble cell components remained at the origin of the chromatogram, and no radioactivity was detected there We conclude that, within the resolution of the method, all of the intracellular radioactive material was chemically unmodified sucrose.

Entry of Sucrose into the Intact Oocyte

Oocytes were incubated in Ringer's-sucrose-^{3}H for two purposes (*a*) To determine the permeability of the cortical membrane to sucrose for comparison with nuclear permeability values (*b*) To test whether short circuit routes exist from the extracellular compartment to the nucleus or to other intracellular compartments, routes that do not require the solute to move through the cortical membrane

TABLE I

The Ratios of the Concentrations of Sucrose in the Nucleus and Cytoplasm after Injection

Time after injection (seconds)	Nuclear-cytoplasmic grain density ratio ($\bar{X}_{n/c}$)
982	3.05 ± 0.45
1,004	3.24 ± 0.76
1,646	3.00 ± 0.49
1,701	3.28 ± 0.56
2,090	3.16 ± 0.32
3,015	2.53 ± 0.48
3,026	3 38 ± 0.61
11,040	2.76 ± 1.04
	Mean = 3.05 ± 0 28

The time course of sucrose-^{3}H uptake by the whole oocyte is shown in Fig 13. At 22 hr the activity in the oocyte water had reached only 3 8% of the activity in the bathing solution Of this, about 25% was taken up in the first 5 min and is extraoocytic, as can be seen from radioautographs (below) This is referred to as the fast fraction The remaining activity enters the cell extraordinarily slowly. The permeability coefficient, P, is 7.4×10^{-9} cm/sec from

$$P = \frac{ka}{3} \qquad \text{Equation 4}$$

where the constant, k, derived from the final two points of Fig 13, is $3\ 06 \times 10^{-7}$ sec^{-1}, and a is the oocyte radius Because of the large errors intrinsic to determinations of slow influx rates in the presence of appreciable extracellular material, this value of P should be viewed as providing only an order of magnitude

A number of oocytes were incubated for extended periods (up to 25 hr) at high activities, in an attempt to get sufficient sucrose-^{3}H into the

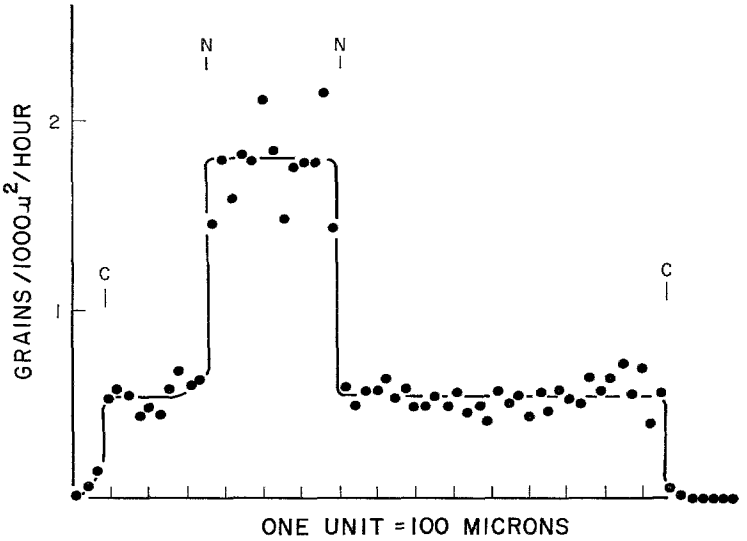

FIGURE 12 Grain density profile through the cytoplasm and nucleus of an oocyte injected with sucrose-^{3}H and permitted to go to diffusional equilibrium before freezing. t = 3026 sec. The vertical bars mark the cell (*C*) and nuclear (*N*) boundaries.

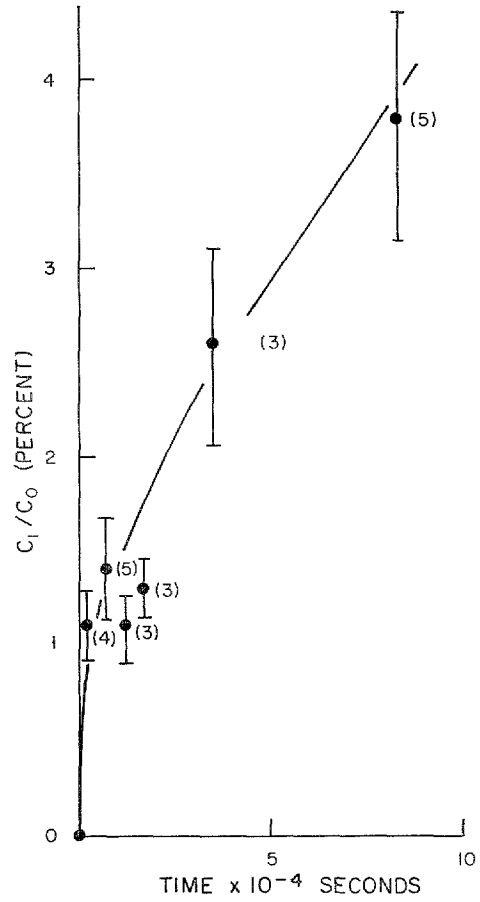

FIGURE 13 Uptake of sucrose-^{3}H by oocytes of *Rana pipiens* expressed as the ratio of the mean concentration of sucrose-^{3}H in cell water (C_i) to that in the incubation medium (C_o) Indicated are the number of determinations, means and standard deviations of the mean

cell to permit reliable grain counting This was successful in only a single case which we believe had a damaged cortical membrane $\bar{X}_{n/c}$ in this oocyte after 25 hr of incubation was 3 98 ± 0 54, somewhat higher than the values obtained by microinjection.

To determine the distribution of the sucrose-^{3}H of the fast fraction, oocytes were incubated for short periods of time (t_I) at very high activities (330 μCi/ml), rinsed in the same manner as used to obtain the data in Fig 13, and frozen for radioautography. Radioautographic exposure was carried out for 43 days to allow for the low activities involved. Fig. 14 is a grain density profile through such a cell (t_I = 1360 sec) and adherent extracellular material. This profile shows that no fast fraction sucrose is detectable in either the cytoplasm or nucleus, while a *relatively* large quantity remains extracellular This extracellular activity is largely associated with adherent follicular epithelium, at locations where the epithelium is torn away from the oocyte, the grains are found over the epithelium

No diffusional gradient is seen from the surface of the cell into the cytoplasm Assuming D_c to be 2 0 × 10^{-6} cm^2/sec, such a gradient would fall to one-half of the surface concentration in 870 μ and would be easily detected. The actual decline in grain density observed can be accounted for by the radioautographic resolution of 3 μ

DISCUSSION

Nuclear Envelope Transport

The most striking facts to emerge from this study are (*a*) that the rate of sucrose movement through the oocyte nuclear envelope is indistinguishable from its rate through cytoplasm and (*b*) that the diffusion of sucrose in cytoplasm is itself rapid.

The nuclear envelope is a heterogeneous structure, consisting of an inner and outer membrane, the enclosed cisterna, and a system of complex pores, but for present purposes it may be treated as a homogeneous sheet Its permeability coefficient, P_n , is then given by

$$P_n = \frac{D_m K_d}{a} \qquad \text{Equation 5}$$

where a is the envelope thickness, about 300 A (25), D_m is the diffusion coefficient in the envelope, and K_d is the envelope/cytoplasm distribution coefficient

$D_m K_d$ cannot be appreciably less than 2 0 × 10^{-6} cm^2/sec, the value for a membrane composed of cytoplasm, nor greater than 1 5 × 10^{-5} cm^2/sec. the value were the nuclear envelope made of water (taking the K_d for water/cytoplasm as 3). P_n for sucrose, then, is in the range of 0.7–5 0 cm/sec, or 10^8 times greater than the permeability of the oocyte plasma membrane

Sucrose was chosen for study because its large radius, about 4 4 A (26), places it near the upper limit of porosity of cell membranes Hence, nuclear permeability to sucrose provides a significant clue to the ability of the nuclear membrane to quantitatively distinguish small solutes on the basis of size or lipid solubility We conclude that in the oocyte this ability is greatly diminished or lacking.

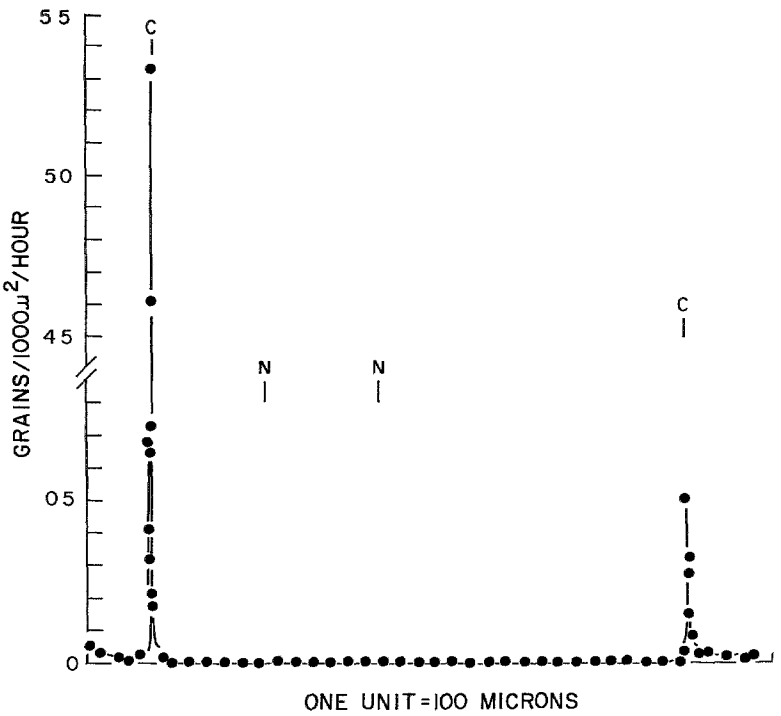

FIGURE 14 Grain density profile through the cytoplasm and nucleus of an oocyte incubated in sucrose-^{3}H for 1360 sec. The vertical bars mark the cell (*C*) and nuclear (*N*) boundaries. See text for additional explanation.

The ease with which sucrose passes from cytoplasm to nucleus raises the question as to whether the oocyte nuclear envelope constitutes a transport barrier for any solute One widely held belief is that larger solutes are excluded by sieving This view is largely based on the observation that concentrated solutions of macromolecules, in vitro (6, 27, 28) or in vivo (10), osmotically shrink the nucleus The interpretation of this type of experiment often is ambiguous While the observation may indeed mean that a semipermeable nuclear membrane is present, it equally may reflect the operation of other processes which can exclude macromolecules from the nuclear water during the period of observation Consequently, before osmotic shrinkage can be attributed to a membrane process it is necessary to demonstrate (*a*) that macromolecular exclusion is not a solubility phenomena and (*b*) that solute diffusion is complete at the time shrinking is observed. These points are difficult to demonstrate and, to our knowledge, have never been established in any nuclear study The only direct evidence bearing on the question appears to be the observation of Holtfreter (5) that hemoglobin diffuses into oocyte nuclei. This study was done on isolated nuclei and consequently must be interpreted with caution.

Our results imply that solutes moving from the extracellular compartment through the plasma membrane and cytoplasm to the nucleus of oocytes will be limited either by permeation of the plasma membrane or diffusion into cytoplasm, but not permeation of the nuclear envelope If there is an exception to this rule, it will depend upon some as yet unsuspected specific interaction of the nuclear envelope with a solute or class of solutes The radioautographic results, especially Fig 14, show that sucrose flux is limited by a cortical membrane. In previous studies, we have shown that the flux of another polyol, glycerol (18), and a monovalent cation, Na^+, is also limited by the cortical membrane (21) and the flux of a sulfhydryl compound, cysteamine, is limited by cytoplasmic diffusion (29). Thus, it is improbable that the nuclear membrane plays a significant role in the transport of these classes of substances

Siebert and his associates (30–32) have recently

suggested that solutes do not move from the extracellular compartment to the nucleus through the plasma membrane and cytoplasm. They postulate, instead, the existence of a short-circuit route through the cisterna of the endoplasmic reticulum to the perinuclear space and thence through the nuclear side of an asymmetric nuclear envelope into the nucleus proper Our previous studies on the oocyte have shown that such a model is unnecessary to explain the flux of glycerol (18), Na^+ (21), or cysteamine (29). The present microinjection study shows that no barrier exists to prevent a solute in the perinuclear space from spreading through both the nucleus and cytoplasm, and as a consequence the Siebert model cannot be correct for the oocyte.

Nuclear Pores

The nuclear pores of the amphibian oocyte are about 700 A in diameter and occupy 20% of the nuclear surface (33). As a consequence, it is possible to entertain a model in which nucleocytoplasmic solute exchange takes place entirely through the pores. If, for example, the pores contained material with the sucrose solubility and transport properties of water, the remainder of the nuclear envelope could be impermeable, and P_n for sucrose would still be about 1 0 cm/sec, quite sufficient to account for the observed properties. We know of no evidence to exclude such a model, except for the observation that nuclear pores are structurally similar and equally abundant in cells in which the nuclear envelope appears to be a barrier to small solute movement (34–35).

Solvent Water

The ratio of sucrose concentrations in nucleus and cytoplasm is 3 05 (Table I) We have shown previously that the ratio of the water contents is 1 88 (4) The apparent water space available to sucrose in the nucleus is therefore 1 6 times as great as in the cytoplasm The comparable figure for free glycerol (18) and Na^+ (21) is 1 3. We have explained these asymmetries by a model in which a significant portion of the cytoplasmic water is unavailable as solvent because of interactions with the macromolecular constituents of the cytoplasm The present results show that the quantity of water detected as unavailable is dependent upon the solute being studied

Nuclear water has been shown to be about 96% accessible as solvent for glycerol (18). Assuming that the same is true for sucrose, 36% of the cytoplasmic water is unavailable as solvent. This compares with 23% for glycerol and Na^+ A pattern such as this, in which larger molecules are denied access to solvent volumes available to smaller solutes, is, of course, the basis of exclusion chromatography (36, 37). Exclusion processes are likely to be significant in determining the distribution of a solute between any two phases in which water is held by a macromolecular matrix in one and is free or relatively free in the second. These processes may help account for known nucleocytoplasmic macromolecular and small solute asymmetries in other cells (38)

Another important class of intracellular solute asymmetries is known in the distribution of protein (39) and triglycerides (40) between cytoplasm and the cisternae of the endoplasmic reticulum in cells that synthesize these substances for "export" (see reference 11). Physical exclusion of the products of synthesis from the water of cytoplasm, but not from the water of the cisternae, could account for the concentrative ability of the latter Such a model implies that cisternal water is less restricted in its translational movements than cytoplasmic water This relative translational freedom could account for the role of the cisternae in facilitating and orientating intracellular solute movements

Diffusion in Cytoplasm

In previous studies, we concluded that the cytoplasmic diffusion coefficients for ^{22}Na (21), glycerol (18), and tritiated water (3) were equal to or greater than 0 2 times their diffusion coefficients in water Values of this magnitude are consistent with the notion that diffusion in cytoplasm is like diffusion in water, except for obstruction effects due to the presence of solids They exclude, for example, the possibility that the water of the oocyte cytoplasm forms a continuous, structured phase with "solid-like" diffusional properties (see reference 41) The results of the present study support this conclusion. The diffusion coefficient for sucrose in cytoplasm, D_c, of 2×10^{-6} cm^2/sec is 0 38 times D_w, its diffusion coefficient in aqueous solution If the decrease were entirely due to the effective lengthening of the diffusional path because of obstruction by macromolecules and cellular inclusions, then

$$D_c = D_w/\lambda^2 \qquad \text{Equation 6}$$

where λ is the factor by which the diffusional path is increased (42) The value of λ is only 1 6, suggesting that little if any increase in the intrinsic diffusional resistance of cytoplasm is required to account for its difference from water. This observation is in harmony with a number of estimates of D_c in other cells (43–46).

Nucleocytoplasmic Solute Asymmetries

In spite of our demonstration that the oocyte nuclear envelope *in situ* is highly permeable to sucrose, it would be premature to conclude that in seeking an explanation for nucleocytoplasmic solute asymmetries one can ignore the role of the nuclear membrane. The oocyte nucleus may be only one of a number of kinds of nuclei with respect to transport properties There is evidence for this in the comparative study of the electrical properties of immature oocyte and secretory gland nuclei reported by Loewenstein and associates (47) It is also conceivable that specialized control mechanisms capable of restricting and modulating the movement of specific classes of solutes are superimposed on a generally permeable membrane structure The existence of selective mechanisms, in the nuclear membranes of other cells, is suggested by the observations of McEwen et al on adenosine triphosphate (8), Allfrey et al. on amino acids (7), and Stirling and Kinter on galactose permeation (48). Nevertheless, the great permeability to sucrose shown in the present study leaves little room for doubt that nonmembrane processes are important determinants of nucleocytoplasmic solute asymmetries in the oocyte These determinants appear to be equilibrium processes such as binding to macromolecules and the related phenomenon of differential solubility in the water of nucleus and cytoplasm As such, these asymmetries are better thought of as reflecting the differences in the composition of nucleus and cytoplasm than reflecting the operation of mechanisms that control or determine these differences The evidence presented here argues against a cellular model in which nuclear processes are modulated by small solute concentrations established by a nuclear membrane control system

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