SEMIPERMEABILITY OF THE NUCLEAR MEMBRANE IN THE INTACT CELL*

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ABSTRACT

The osmotic properties of nuclei in intact cells were studied by injecting solutions into the cytoplasm of amphibian oocytes. Subsequent changes in nuclear volume were recorded photographically. The injection of solutions containing polyvinylpyrrolidone or bovine serum albumin caused changes in nuclear volume which were related to the colloid osmotic pressure of the solution injected. The concentration in which no significant nuclear volume change occurred (the isotonic range) was 1.0 to 1.5 per cent polyvinylpyrrolidone (2.0 to 3.75 $\times$ 10^{-4} M). 2 per cent bovine serum albumin had no significant effect on nuclear volume, whereas 4 per cent caused a significant decrease. The significance of these findings is discussed in terms of the permeability characteristics of the nuclear membrane.

INTRODUCTION

It has been known for some years that the cell nucleus behaves osmotically (Churney, 1942). When whole cells are placed in solutions of different salt concentrations, both the cell and nucleus are found to undergo changes in volume. If the nucleus is indeed an osmometer, and is thus surrounded by a semipermeable membrane, we must assume that some soluble component(s) of the cell does not freely penetrate the nuclear membrane (*i.e.* there is a physical or physiological impermeability to some soluble component(s)). One possible way to determine the nature of this soluble component(s) is to vary the concentration of specific components in the nuclear environment, and then note any changes in nuclear volume. This has been done in the case of amphibian oocytes by isolation of the large germinal vesicle nuclei in solutions of different composition (Callan, 1949; Goldstein and Harding, 1950). These studies indicated that ions or molecules of low molecular weight readily penetrate the nuclear membrane. Of the components tested, only protein and certain other substances of high molecular weight were found to exert an osmotic effect in this isolated system.

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The process of isolation may have marked effects on the properties of the nucleus and its membrane. In the present studies, osmotic properties of the nucleus were studied by microinjection of certain solutions into the cytoplasm. Any subsequent changes in nuclear volume were thus observed in the intact cell.

Materials and Methods

The relatively small (200 to 300 μ in diameter), transparent oocytes of *Rana pipiens* were used. A small piece of ovary was removed from a freshly pithed female. The ovarian tissue was rinsed in calcium-free Ringer solution and the subsequent dissections were carried out in calcium-free Ringer. Calcium was avoided since it has been described as deleterious to isolated germinal vesicles (Duryee, 1948) and is known to cause colloidal changes in the cytoplasm (Heilbrunn, 1952) which might indirectly affect the nucleus. The large heavily pigmented oocytes were removed with a pair of watchmaker's forceps under a dissecting microscope. The smaller, transparent oocytes could then be easily observed. Single oocytes, or small groups of 2 to 4 oocytes were then prepared. Cutting the ovarian tissue by means of moving a fine bent needle over the connective tissue proved to be a satisfactory way to accomplish this final step. At the end of about 10 minutes one to several oocytes were placed in a hanging drop in a moist chamber. The preparation was then examined more carefully. Any oocyte which appeared injured (for example, oocyte surface pulling away from the follicular layer) was not used. Also, any preparation which had been out of the animal for more than an hour was not used.

Preliminary attempts to inject these oocytes were not always successful. The follicle cells plus intercellular fibers provided a tough layer for the micropipette to penetrate. Satisfactory results were obtained by placing the micropipette up against the oocyte surface. Penetration was then accomplished by a single vibration of the micropipette, which was brought about by a light tap on the micromanipulator base (Tobias and Bryant, 1955). Care was taken to avoid contact of the micropipette with the nucleus. However, if such contact did occur the experiment was not used. Injection caused an increase in cross-sectional area of the *oocyte*, which, on the average, was close to 18 per cent. The micropipette itself had an opening at the tip of about 1.5 micra.

In all experiments, photographic records on 16 mm. movie film were obtained. Motion pictures were taken of the injection, and short scenes were taken at minute intervals for at least 5 minutes following injection. In some experiments, observations were made at 30 minutes after injection. In every case, the cell was checked for changes in appearance for a period at least twice as long as that required for the experiment. The oocyte was turned over in order to check its appearance from several angles. Measurements of nuclear size were obtained from the film. A film viewer was adapted so that the image from a single frame could be focussed on a piece of tracing paper. An outline of the nucleus could thus be accurately and rapidly traced. Each tracing was cut out and weighed. This procedure was carried out three times for each measurement. The weighings were taken as an index of the maximum cross-sectional area of the nucleus.

No harmful effects were observed within the cells following injection. In a few cases, however, as a result of injection, the oocyte surface pulled away from the layer

of follicle cells, leaving a space between the surface and the follicle cell layer. Such experiments were not used.

In some of the experiments a high molecular weight synthetic polymer, polyvinylpyrrolidone (PVP) was used (kindly supplied by Antara Chemicals, General Aniline and Film Corporation, Philadelphia). This substance, which has been used as a blood substitute, has the advantage of being non-toxic, relatively non-reactive, and of known average molecular weight (molecular weight, 40,000; with a range of from about 30,000 to 50,000 in the sample used). In other experiments, a preparation of crystalline bovine serum albumin (BSA) was used. The molecular weight of BSA has been reported to be 65,360 (Creeth, 1952). For experiments in which KCl and NaCl alone were used, the solution consisted of nine parts 0.126 M KCl and one part 0.123 M NaCl (Bialaszewicz, 1929), hereafter referred to as the standard salt solution. The PVP or BSA was dissolved in this standard salt solution which was adjusted slightly in each case so that all solutions had exactly the same tonicity *with respect to the cell membrane* (isosmotic with amphibian Ringer). Actually, it should be noted that with regard to the cell membrane, the PVP would be expected to have little osmotic effect. For example, the highest concentration of PVP used, 4 per cent, represents a molar concentration of only 0.001 M. This value could conceivably be higher if an appreciable fraction of components with molecular weights less than 40,000 were present in the PVP preparations used. The pH of each solution was adjusted to approximately 6.5 by the addition of NaOH.

RESULTS

The measurements of change in cross-sectional area of the nucleus indicate clearly that the PVP has an effect on nuclear volume, the extent of which depends on the PVP concentration. Figs. 1 through 4 show photographic prints made from the 16 mm. motion picture records of two experiments. Figs. 1 and 2 show an oocyte before and 3 minutes after the injection of standard salt solution. An increase in nuclear size has taken place. Figs. 3 and 4 show an oocyte before and 3 minutes after the injection of 4 per cent PVP. This solution has brought about a decrease in size of the nucleus. This size change occurred very rapidly, and was essentially complete within 3 minutes. For example, in Table I, line 8, the mean per cent area change at 3 minutes after injection of 4 per cent PVP was −13.59. The change between 3 minutes and 5 minutes, however, was insignificant (line 9). Unless otherwise stated, all the data reported represent measurements made at 3 minutes after injection. For each solution a series of about ten experiments was performed. The data are shown in Table I.

Control experiments indicated that penetration and withdrawal of the micropipette in itself had no significant effect on nuclear size (Table I, line 1). Only in those cases in which solution was injected into the oocyte were significant changes in nuclear size found. In other control experiments, it was demonstrated that the viscosity of the solutions injected was not responsible for any mechanical effects on nuclear size. The viscosity of the 4 per cent

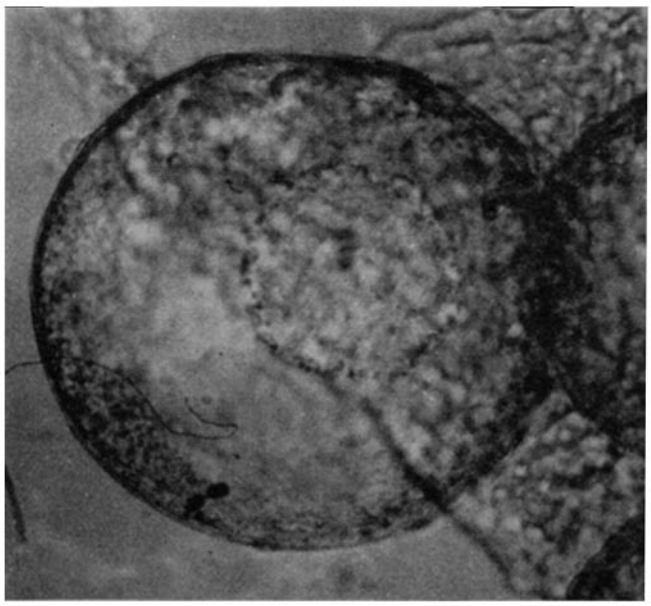

FIG. 1. An oocyte before injection.

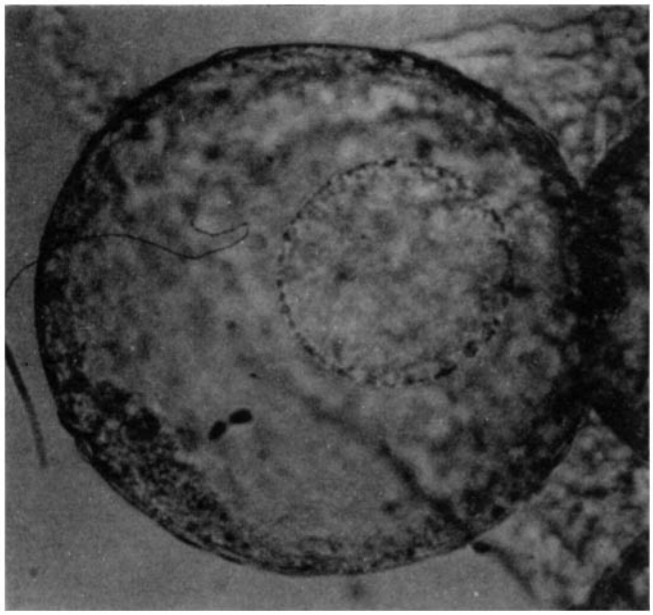

FIG. 2. The same oocyte shown in Fig. 1, 3 minutes after the injection of standard salt solution. A small increase in nuclear volume has taken place.

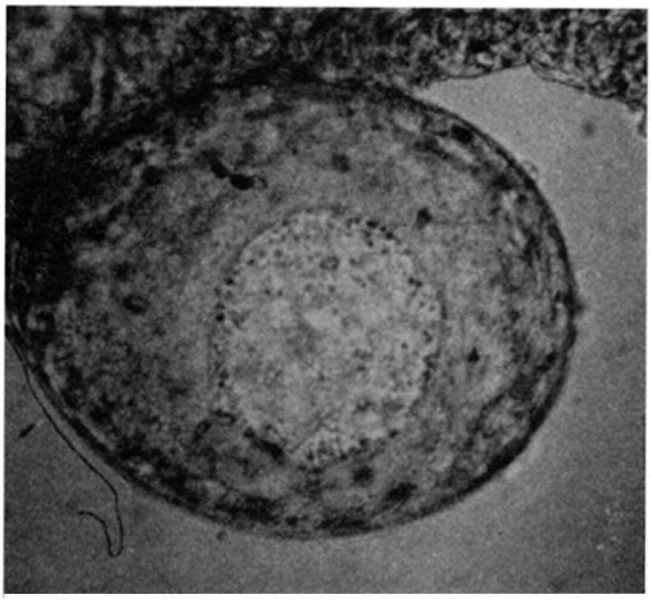

FIG. 3. Another oocyte before injection.

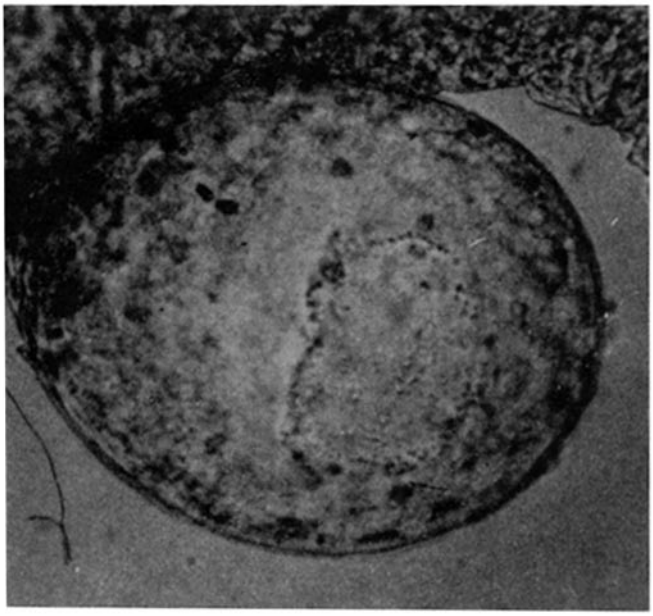

FIG. 4. The same oocyte shown in Fig. 3, 3 minutes after the injection of 4 per cent PVP. There has been a decrease in nuclear volume.

PVP solution was about 4 times that of water. Although this is not a very high viscosity, it was thought of some importance to employ a "viscosity control" (*i.e.*, injection of a solution with the same viscosity, but with considerably less colloid osmotic pressure). This was done by the use of a different preparation of PVP, with a molecular weight of 360,000. Solutions of this preparation with the same viscosity as 4 per cent PVP (molecular weight, 40,000), have a considerably lower colloid osmotic pressure. Since these viscosity controls

TABLE I

Change in Size of the Nucleus 3 Minutes Following Injection

Solution used	No. of experiments	Mean per cent area change	Significance of difference of mean from zero (t-test)	
			Significance	Probability of this result due to chance
Injection* control	10	−1.17	Not significant	$0.10 < P < 0.15$
K—Na (9:1)	12	+7.64	Significant	$P < 0.005$
1.2 × K—Na (9:1)	11	+7.17	Significant	$P < 0.005$
0.5 per cent PVP	10	+6.74	Significant	$P < 0.005$
1.0 per cent PVP	9	+1.43	Not significant	$0.10 < P < 0.150$
1.5 per cent PVP	10	−1.58	Not significant	$0.40 < P < 0.450$
2.0 per cent PVP	9	−5.24	Significant	$P < 0.005$
4.0 per cent PVP	10	−13.59	Significant	$P < 0.005$
4.0 per cent PVP	10	+1.19‡	Not significant	$0.15 < P < 0.200$
1.96 per cent BSA	10	+0.94	Not significant	$0.25 < P < 0.300$
4.00 per cent BSA	9	−6.22	Significant	$P < 0.005$

* Micropipette inserted into oocyte and withdrawn, but no solution injected.

‡ Mean per cent area change between 3 minutes and 5 minutes after injection.

showed relatively little effect on nuclear size, compared with 4 per cent PVP of 40,000 molecular weight, it was assumed that the viscosity of the solution was relatively unimportant, and was not responsible in itself for the observed changes in nuclear size.

Data on the effects of different concentrations of PVP are listed in Table I. Every injection of 0.5 per cent PVP caused an increase in nuclear size. The average of 10 experiments was 6.74 per cent, which is a significant change (see columns 4 and 5). 1 per cent PVP showed a small increase in nuclear size, which, however, proved to be statistically insignificant. 1½ per cent PVP caused a *decrease* of 1.58 per cent, but here again, the change was not significant. 2 per cent PVP, on the other hand, brought about a significant decrease of 5.24

per cent in nuclear size, and the decrease was −13.59 per cent with 4 per cent PVP. In a few experiments, which are not recorded in Table I, higher concentrations were used. 10 per cent PVP caused a marked shrinkage of the nucleus with a shrivelling of the nuclear membrane. Measurements of these changes in nuclear size with the injection of higher concentrations were not made. Emphasis was rather placed on measurements following the injection of concentrations closer to the "isotonic" range.

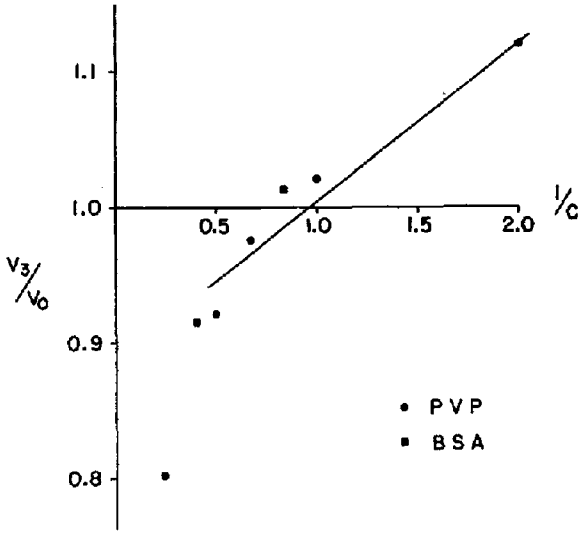

FIG. 5. The ratio of nuclear volume at 3 minutes after injection, over the volume before injection, is plotted as a function of the inverse of the per cent concentration of PVP. The circles represent PVP and the squares, BSA. The per cent concentrations of BSA are expressed in terms of the per cent concentrations of PVP which have equivalent molarities. The straight line is a regression calculated on the basis of all PVP experiments except those in which 4 per cent PVP was used.

In Fig. 5, nuclear volume (V) is plotted as a function of the reciprocal of the PVP concentration ($1/C$). A certain variability is evident. This is undoubtedly due in part to variations from one experiment to the next in the amount of solution injected. The data, nevertheless, do appear to fall along a straight line for the small volume changes, which is some indication that nuclear volume as a function of the colloid osmotic pressure of the cytoplasm behaves according to Boyle's law.

In some cases, following the injection of 4 per cent PVP, the medium surrounding the oocyte was made hypotonic by the addition of glass-distilled water to the calcium-free Ringer's. This resulted in a return of the shrunken nuclei to a larger size, indicating that the PVP-induced shrinkage is reversible.

If these volume changes are indeed an indication of the osmotic properties of the nucleus, then the isotonic concentration of PVP would lie in the range of 1.0 to 1.5 per cent. Since the molecular weight of this particular preparation of PVP is about 40,000, the isotonic range would be 2.5 to 3.75 $\times$ 10^{-4} M. It is quite possible that this does not reflect the total concentration of soluble high molecular weight components in the cytoplasm (see Discussion). Also, as has been pointed out above, the fact that the figure of 40,000 represents an average molecular weight must be taken into consideration. If smaller molecules comprise an appreciable fraction of the total, the isotonic range as stated might be too low.

Experiments similar to those with PVP were carried out with a preparation of crystalline bovine serum albumin (BSA, molecular weight, 65,360). A solution of 1.96 per cent BSA made up in standard salt solution was injected into a series of 10 oocytes. This concentration of BSA, which is equivalent on a molar basis to 1.2 per cent PVP, caused no significant change in nuclear volume. 4 per cent BSA, on the other hand, brought about a significant decrease in nuclear size.

DISCUSSION

In the above experiments, all solutions had the same total osmotic pressure. The colloid osmotic pressure, on the other hand, varied with the concentration of PVP or BSA. If we assume that the volume changes we are dealing with are osmotic in nature, we can conclude from the data in Table I that nuclear volume is dependent upon the colloid osmotic pressure of the cell. If we increase the colloid osmotic pressure of the cytoplasm by the injection of high concentrations of PVP or BSA, the nucleus shrinks. Lower concentrations cause an increase or very little change. The increase brought about by injecting standard salt solution, as well as a solution 20 per cent more concentrated than this (Table I, line 3) is interpreted as a result of the dilution of normal cytoplasmic colloids.

It would also follow from these results that the nuclear membrane of the intact oocyte does not allow the *free* penetration of molecules of 40,000 molecular weight and larger. The osmotic properties would suggest either a physical or physiological impermeability to large molecules. There is no information in the reported experiments to distinguish between these two possibilities.

Coons *et al.* (1951) have attempted to follow the distribution of protein antigens at the cellular level, at various periods of time after intravenous injection in the rat. Localization of the protein in tissue sections was accomplished by the fluorescent antibody staining technique. In these experiments, the fluorescein–antibody complex diffuses through a histological section of tissue and reacts with the protein antigen. The presence of fluorescence in the section is interpreted as an indication of the presence of the protein antigen at that site. Fluorescence appeared in various cells shortly after injection. It also appeared

in the nuclei, and, moreover, in some cases the nucleus was more fluorescent than the cytoplasm.

Schiller, Schayer, and Hess (1952) have employed a different technique. They injected fluorescein-labelled protein, and then sought the appearance of fluorescence directly in tissue sections. They found fluorescence in the cytoplasm, but it did not appear in the nuclei. We thus have an apparent discrepancy which may possibly be the result of the specific technique involved; and which has not, to our knowledge, been resolved as yet.

As indicated above, the osmotic experiments with germinal vesicles indicate a physical or physiological impermeability to large molecules. Comparison of these results with those on somatic cells referred to above should perhaps be made with great caution. In addition to the fact that quite different methods were employed, there may be significant differences between the properties of germinal vesicles and those of the nuclei of somatic cells.

It is conceivable that penetration of large molecules does indeed occur under conditions in which the nucleus would still maintain itself as an osmometer. Just for example, if a process comparable to pinocytosis should occur at the nuclear surface, cytoplasmic proteins could be taken into the nucleus, while the nuclear membrane itself retained its low permeability to these molecules at all times. The colloid osmotic pressure could subsequently be regulated by incorporation of the newly acquired protein into the solid structures of the nucleus (rendering them osmotically inactive), or, conceivably, by a mechanism for the active removal of protein from the nucleus. These thoughts, which perhaps border on wild speculation, are presented here merely to indicate that it is conceivable to have a substance which is osmotically effective on the nucleus, and yet which may under certain circumstances get into the nucleus. This point has been made very well before by Stern and Mirsky (1953), who state: "It is questionable, however, whether 'semipermeability' of a membrane and the passage of macromolecules across it are incompatible. If a parallel is drawn from the many studies of 'active transport' across cell membranes, it would appear quite probable for the nuclear membrane to act structurally as a barrier to free diffusion between nucleus and cytoplasm while functioning by energy-requiring mechanisms in the transportation of substances to and from the nucleus." A great deal more investigation is obviously required.

There has been in this study no systematic attempt to determine a "threshold" range of molecular weight, below which the molecules would be osmotically ineffectual. The value of 2.5 to 3.75 $\times$ 10^{-4} M reflects only the concentration of cytoplasmic molecules which do not readily penetrate the nuclear membrane. It may be that molecules of fairly high molecular weight, but below 40,000, can readily penetrate. The determined value of 2.5 to 3.75 $\times$ 10^{-4} M, of course, would not reflect the concentration of these penetrating molecules. Previous work with the large oocytes of *Rana pipiens* indicated that sucrose, with a molecular weight of 342, readily penetrates the isolated germinal vesicle nucleus,

whereas egg albumin, with a molecular weight of approximately 34,500, does not (Goldstein, and Harding, 1950). Further studies with a series of molecular weights below 40,000 might be of value in characterizing the nature of the nuclear membrane. Information on the relation between molecular dimension and osmotic effectiveness for a series of compounds of graded molecular weights might be important to the question of the patency of the "pores" which have been described in the nuclear membranes of various cells (for example, Watson, 1955; Afzelius, 1955; Pappas, 1956).

Very preliminary attempts have been made to extend this study to somatic cell nuclei. These were complicated by the fact that these nuclei are smaller and suitable preparations for micromanipulation are more difficult to obtain. A few observations, however, have been made on the nuclei of frog striated muscle fibers and nucleated red blood cells. No quantitative measurements of size change were made. However, the injection of 4 per cent PVP into the striated muscle fibers appeared to cause a shrinkage of the nuclei. Injection was made in close proximity to the nuclei. The injection of standard salt solution caused an increase. The red blood cells were studied in quite a different way. The cells were first immersed in glass-distilled water. After 2 minutes, which was sufficient to cause hemolysis and an increase in nuclear size, they were transferred to solutions of PVP. Here again, quantitative measurements were not made. A very obvious shrinkage of the nucleus was obtained after immersion in 20 per cent PVP. Although the preliminary results with red blood cells indicate that nuclear shrinkage is obtained only with relatively high concentrations of PVP further extension of these observations is required before any definite conclusions can be drawn. The method of treatment in this case is quite different from that used with the oocytes and this may have some effect on the results. It is possible, however, that the colloid osmotic pressure of the cell interior varies with the cell type. Red cells with their high concentrations of hemoglobin may possibly have a higher colloid osmotic pressure than oocytes.

In the further extension of these osmotic and permeability experiments, particularly with regard to somatic cells, it would be of some interest to compare the properties of: (1) nuclei isolated by some of the standard methods for mass isolation, (2) nuclei immediately after isolation by the mildest possible treatment, and (3) nuclei within the intact cell.

A more complete knowledge of the osmotic properties of nuclei may be of value in studies on isolated nuclei as well as in experiments on nuclear transplantation. Those who have succeeded in transplanting cell nuclei (Commandon and de Fonbrune, 1939; Lorch and Danielli, 1953) stress the fact that a brief contact of the nucleus with the external medium inactivates the nucleus, rendering it incapable of taking part in further development. The lack of protein (or some other high molecular weight component) in suitable concentration may be partly responsible for this.

Molecules below the colloidal range, however, may also be important osmotically to the isolated nucleus. Mirsky *et al.* (1956) and Allfrey *et al.* (1957) have noted that the capacity of isolated thymus lymphocyte nuclei to synthesize protein and RNA varies with the concentration of sucrose in the isolation medium. A peak in synthetic activity of the nuclei is reached at a concentration of sucrose close to 0.2 M. This correlation between the synthetic activity of the isolated nuclei and the osmotic pressure of the medium has led Mirsky and Allfrey to suggest that the nucleus, or at least some components within the nucleus, behave osmotically. Because of the high permeability of the nuclear envelope in the isolated thymus nucleus to many high molecular weight substances (ribonuclease, deoxyribonuclease, histones, protamines, and basic dyes) they assume that the semipermeable membrane of importance in their experiments must lie within the nucleus: "The marked effects of sucrose concentration on nuclear activity suggest that the fine structure necessary for nuclear protein synthesis is in osmotic balance with its environment, and that nuclear function can vary with that balance. This is reminiscent of experiments on the extended and contracted states of interphase chromosomes." There is, therefore, the possibility of a complex osmotic system within the nucleus.

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