PROTEIN MIGRATION INTO NUCLEI

I. Frog Oocyte Nuclei In Vivo Accumulate Microinjected Histones, Allow Entry to Small Proteins, and Exclude Large Proteins

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ABSTRACT

A technique is presented which enables one to measure the extent to which a protein enters and accumulates in the nucleus of the frog oocyte. In this method, the protein, labeled with ^{125}I, is microinjected into the oocyte. After incubation, the oocyte is manually enucleated and the radioactivity in the nucleus and cytoplasm is determined.

Using this technique, proteins lighter than 20,000 daltons were found to enter the nucleus and completely equilibrate between the nucleus and cytoplasm within 24 h. The entry of proteins heavier than 69,000 daltons was severely hindered. Histones and histone fractions entered as quickly as other small proteins, but, in contrast to these proteins, they accumulated in the nucleus to different extents, depending on the total amount of histone injected into the oocyte and the identity of the histone. Evidence is presented that histone fractions compete with each other for accumulation in the nucleus.

Two important aspects of the relationship between the nucleus and the cytoplasm of a cell are the distribution of proteins between these two compartments and the ability of certain cellular components to pass through the nuclear membrane.

That in vivo ^{3}H-labeled cytoplasmic proteins of *Xenopus* eggs and oocytes can enter transplanted nuclei was shown by Arms (1) and Merriam (2). Gurdon (3), in the first study using purified labeled proteins, labeled bovine serum albumin (BSA), and calf thymus histones with ^{125}I and injected these into eggs and oocytes. Autoradiography showed that although [^{125}I]BSA could enter oocyte nuclei and brain nuclei transplanted into eggs, it always remained more concentrated in the cytoplasm. [^{125}I]Histones, on the other hand, were concentrated 115-fold in 20 h by oocyte nuclei and at least 2.5-fold in 50 min by nuclei transplanted into unfertilized eggs.

In this paper, it is shown that the technique of manual enucleation of frog oocytes yields nuclear-cytoplasmic concentration ratios comparable to those obtained by autoradiography and, in addition, allows further analysis of the material in the nuclear and cytoplasmic fractions. Using this technique, a detailed study is presented of the

nuclear-cytoplasmic partitioning of various proteins microinjected into frog oocytes.

MATERIALS AND METHODS

Beef pancreas trypsin inhibitor (BPTI), lysozyme, myoglobin, ovalbumin, BSA, γ-globulin, and calf thymus histone were purchased from Sigma Chemical Co., St. Louis, Mo. Pure histone fractions were prepared and donated by E. W. Johns.

To prepare *Xenopus* erythrocyte histone, blood was obtained from adult frogs by heart puncture using a heparinized syringe. The blood was diluted into 0.15 M NaCl, 0.015 M trisodium citrate (SSC) at 4°C, and centrifuged for 5 min at 1,000 *g*. The pelleted erythrocytes were washed twice with SSC, then twice with 0.25 M sucrose containing 3 mM $MgCl_2$ and 7.5 mM Tris-HCl pH 7.5 (SMT). After the erythrocytes were lyzed with 0.5% NP40 (BDH Chemicals Ltd., Poole, England) in SMT, the nuclei were pelleted by centrifugation for 5 min at 3,000 *g*, washed twice with SMT, then extracted according to the method of Bonner et al. (4) with 0.4 N H_2SO_4 for 30 min at 4°C. After the extract was centrifuged, the supernate was added to 4 vol of ethanol and stored at −20°C over night. The resulting precipitate was collected and washed twice with ethanol, dried, and stored in a desiccator.

The proteins were labeled with ^{125}I (Amersham/Searle Corp., Arlington Heights, Ill., $Na^{125}I$) using the method of Greenwood et al. (5) or Marchalonis (6). Proteins labeled by both methods were found to yield similar results. BPTI was precipitated with 10 vol of acetone several times to remove unreacted ^{125}I and finally dissolved in a modified Barth saline solution (7). All other proteins were dialyzed against this medium to remove free ^{125}I.

The specific activity of each protein was determined by analyzing an aliquot of solution for protein using the Lowry et al. (8) method, and for radioactivity using a Wallac Decem-GTL 300–500 gamma counter. Because the protein solutions still contained some free ^{125}I (10–30%) an aliquot was also assayed for the fraction of protein-bound ^{125}I by precipitation in 20% TCA-10 mM NaI. The specific activities noted in Table III are in terms of TCA precipitable counts per minute per nanogram of protein.

Aliquots of the [^{125}I]protein solutions (3–10 mg protein/ml) were microinjected into the vegetal hemisphere of large *Xenopus* oocytes (7). Since the oocyte nucleus is in the animal hemisphere, injection into the other insures that the nuclear membrane is not punctured and that material is not injected directly into the nucleus. After injection, the oocytes were incubated in modified Barth solution at 19°C for various periods of time.

After incubation, oocytes to be autoradiographed were fixed overnight in Perenyi's solution (9), embedded in Paraplast paraffin wax (Raven Ltd.), sectioned at 7 μm, dipped in Ilford K2 emulsion, and exposed for 3–7 days. After the autoradiographs were developed, silver grains were counted over randomly chosen areas of the nucleus or cytoplasm. In both these compartments the grains were evenly distributed throughout with no local accumulations. No grains were found over the cell wall or follicle cells.

Manual enucleation of fixed oocytes was performed on oocytes fixed for 1 h in either Perenyi's solution or 20% TCA. In these fixatives the nucleus becomes a small white ball surrounded by a crumbly yellow cytoplasm so that once the oocyte is torn open the nucleus can easily be identified under a low power binocular microscope and removed with a pair of forceps. Each nucleus and the remainder of the oocyte after the nucleus was removed was assayed for radioactivity. All the extranuclear radioactivity is assumed to be cytoplasmic because no significant amount was found in the follicle cells which surround the oocyte.

When free $Na^{125}I$ was injected into oocytes which were then incubated for 24 h and fixed for 1 h in Perenyi's solution or 20% TCA, less than 1% of the injected radioactivity remained in the oocyte. Therefore, the amount of protein corresponding to the amount of radioactivity in the nucleus or cytoplasm could be determined directly from the specific activities as previously described.

Manual enucleation of live oocytes was performed in half-strength modified Barth saline solution under a low power binocular microscope. The oocyte was slit at the animal pole and gently squeezed with forceps to extrude the nucleus. The nucleus and cytoplasm were quickly transferred to separate containers in preparation for sodium dodecyl sulfate (SDS) gel electrophoresis. Results from gamma counting of single oocytes injected with [^{125}I]myoglobin or [^{125}I]BSA followed by gamma counting of the isolated nucleus and cytoplasm from the same oocytes show that the recovery of each of these two proteins during the manual enucleation of live oocytes is consistently over 90%.

SDS gels were run according to Laemmli (10) except that a 15 or 18% acrylamide gel with an acrylamide-bisacrylamide ratio of 200:1 was used. Cytoplasmic samples were limited to 0.2 oocyte to prevent overloading of the gel by yolk protein. Acid urea gels of histone fractions were run according to Panyim and Chalkley (11).

Each band in calf thymus total histone was identified using purified calf thymus histone fractions. Electrophoresis in an 18% SDS gel resolved the five calf thymus histone fractions, but the *Xenopus* erythrocyte F2a2 and F2b were not well resolved.

After electrophoresis the gels were attached to paper, dried by vacuum, and autoradiographed with Kodirex AP 54 film. Before drying, some gels were stained in a solution containing 0.1% Coomassie Brilliant Blue (Schwarz Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.), 45% methanol, and 10% acetic acid, and destained in the above solution lacking dye.

RESULTS

Determination of Relative Protein Concentration in Nucleus and Cytoplasm

In order to more easily measure the relative concentration of an injected radioactive protein in the oocyte nucleus and cytoplasm, two methods based on the manual enucleation of live or fixed oocytes were compared with autoradiography of fixed oocyte sections. All three methods produced similar results when tested on oocytes injected with [^{125}I]myoglobin or [^{125}I]BSA.

Autoradiographs of sections of oocytes injected with [^{125}I]myoglobin and [^{125}I]BSA such as those shown in Figs. 1 *a* and *b* were used to determine the density of silver grains over the nucleus and cytoplasm and thereby to calculate a nuclear-cytoplasmic concentration ratio for these two proteins

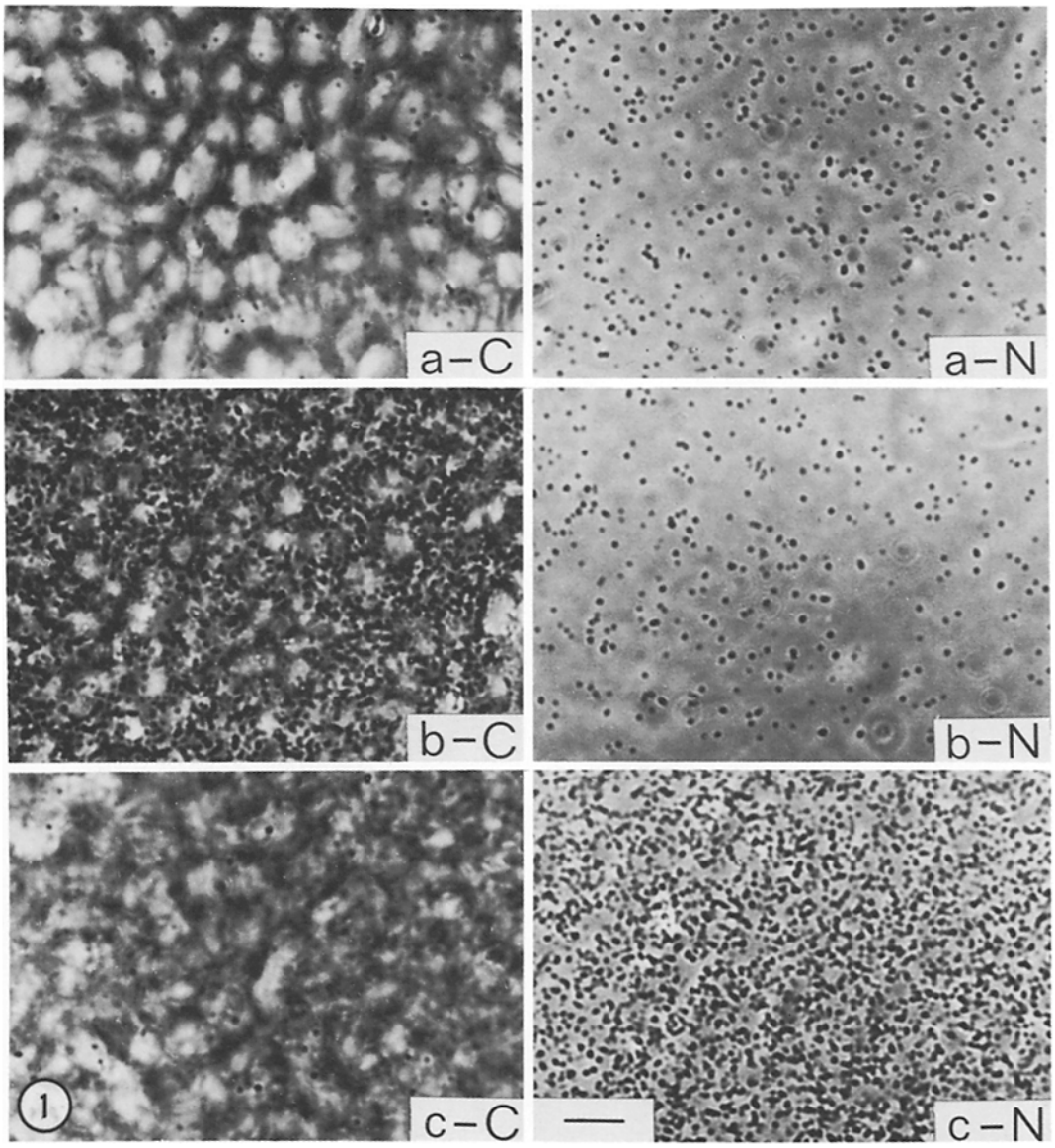

FIGURE 1 Autoradiographs of the nucleus (*N*) and cytoplasm (*C*) of oocytes injected with ^{125}I-labeled proteins. (*a*) [^{125}I]myoglobin. (*b*) [^{125}I]BSA. (*c*) [^{125}I]calf thymus histone F2a1. Figs. 1 *a* and *b* were injected 24 h before, and Fig. 1 *c* 18 h before fixation and preparation for autoradiography as described in Materials and Methods. The bar is 1 μm long.

(Table I). From the nuclear-cytoplasmic concentration ratios, and a knowledge of the relative volumes of the nucleus and cytoplasm, one can calculate for these two proteins the percentage of the total oocyte radioactivity that is in the nucleus. These values are shown in Table II, column a.

Values for the same parameter, but determined by manual enucleation of either live oocytes or fixed oocytes, are presented in Table II, columns b and c, respectively. The close agreement of the results from these three methods indicates that the methods based on manual enucleation of oocytes are as valid as autoradiography for determining nuclear-cytoplasmic concentration ratios and the percentage of total oocyte radioactivity in the nucleus.

Partitioning of Various Proteins between Nucleus and Cytoplasm

Figs. 1 *a–c* show that [^{125}I]myoglobin, [^{125}I]-BSA, and [^{125}I]histone all partition very differently between the nucleus and cytoplasm of oocytes. To help discover the factors behind these differences, the partitioning of several proteins of various molecular weights and charges was studied.

The relevant properties of these proteins are listed in Table III. Four are neutral or slightly acidic proteins of various molecular weights but several small basic proteins, lysozyme, BPTI, and various histones are included. All but γ-globulin are monomeric so that dissociation of subunits cannot be a factor interfering with the interpretation of the results.

Fig. 2 presents the results obtained when various amounts of these proteins were injected into oocytes and incubated for 24 h (18 h for histones). For all the proteins except histones the points lie on straight lines, the slopes of which are equal to the fractions of each protein found in the nucleus.

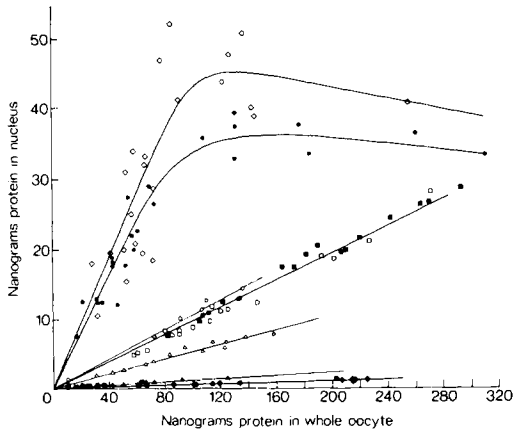

FIGURE 2 Partitioning of various proteins between the nucleus and cytoplasm. Specific activities for the [^{125}I]-proteins are listed in Table III. ●, calf thymus histone; ◇, *X. laevis* erythrocyte histones; ○, lysozyme; □, myoglobin; ■, BPTI, △, ovalbumin; ▲, BSA; ◆, γ-globulin. (The abscissa for BPTI is compressed fourfold.) All techniques are described in Materials and Methods. The oocytes were incubated for 24 h after microinjection. Each point represents the data from one oocyte.

TABLE I

Nuclear-Cytoplasmic Concentration Ratios for [^{125}I]Myoglobin and [^{125}I]BSA Injected Oocytes

Protein	Oocyte	Nucleus (N)		Cytoplasm (C)		Background (B)		N − B	C − B	$\frac{N-B}{C-B}$
		Number of unit areas	Grains/area	Number of unit areas	Grains/area	Number of unit areas	Grains/area	Grains/area	Grains/area	
BSA	1	10	15.0 ± 3.5	4	66.8 ± 6.9	40	5.4 ± 0.5	9.6 ± 4.0	61.4 ± 10.9	0.16
	2	10	10.1 ± 2.0	4	68.5 ± 6.8	40	1.2 ± 0.4	8.9 ± 2.4	67.3 ± 7.2	0.13
	3	10	13.3 ± 3.8	4	71.8 ± 7.9	40	0.4 ± 0.3	12.9 ± 4.1	71.4 ± 8.2	0.18
Myoglobin	4	5	31.2 ± 3.7	14	13.3 ± 4.7	40	2.7 ± 0.4	28.5 ± 4.1	10.6 ± 5.1	2.7
	5	5	37.8 ± 5.9	14	15.0 ± 4.1	40	3.0 ± 0.7	34.8 ± 9.6	12.0 ± 4.4	2.9
	6	10	33.1 ± 3.1	10	13.2 ± 1.7	40	2.4 ± 0.3	30.7 ± 3.4	10.8 ± 2.0	2.8

Oocytes were injected with [^{125}I]myoglobin or [^{125}I]BSA, incubated for 24 h in modified Barth saline, fixed, and prepared for autoradiography as described in Materials and Methods. Grains were counted in randomly chosen nuclear and cytoplasmic areas of oocyte autoradiographs like those in Figs. 1 *a* and *b*. The results are presented as the mean ± standard error.

TABLE II

Percentage of Total Radioactivity in Nucleus

Protein	Method (a) From grain counting	(b) Live dissection	(c) From fixed and dissected oocytes
BSA	0.7 (1)	0.5	
	0.5 (2)	0.6	
	0.7 (3)	0.5	0.6
		0.8	
Myoglobin	10.1 (4)	9.1	9.7
	10.8 (5)	9.0	
	10.6 (6)	11.0	

Nuclear radioactivity as percent of total radioactivity in the oocyte, measured by three methods. (a) These values were calculated from the nuclear cytoplasmic concentration ratios in Table I by the formula

$$\%N = 100\% \frac{4R}{96 + 4R}$$

where R is the nuclear cytoplasmic concentration ratio. The formula is derived by rearranging this formula for R.

$$R = \frac{\%N}{\%V_n} \div \frac{\%C}{\%V_c}$$

where $\%N$ and $\%C$ are the percentage of the total radioactivity in the nucleus and cytoplasm, respectively, and where $\%V_n$ and $\%V_c$ are the percentages of the total oocyte volume in the nucleus and cytoplasm. $\%V_n$ was found to be 4% by measuring the diameters of the nucleus and the whole oocyte in autoradiograph sections. (b) Each value is from one oocyte dissected as described in Materials and Methods. (c) These values were calculated from the graphs for [^{125}I]myoglobin and [^{125}I]BSA in Fig. 2.

How the curves of Fig. 2 vary with increasing times of incubation is shown in Fig. 3. The partitioning of the smaller proteins, such as histones and myoglobin, did not change after 18 and 24 h, respectively. The amounts of both BSA and ovalbumin in the nucleus increased by about 50% between 24 and 48 h, although the absolute values were very different. Ovalbumin continued to enter the nucleus, until at 72 h it had entered to the same extent as myoglobin.

The results presented in Figs. 2 and 3 are valid only if the [^{125}I]material in the nucleus and cytoplasm of the injected oocyte is the same protein as that injected. To prove this, oocytes that had been injected with [^{125}I]proteins 24 h previously were manually enucleated and the separated nuclear and cytoplasmic fractions were compared by SDS gel electrophoresis. When [^{125}I]myoglobin, ovalbumin, BSA, and γ-globulin were injected (Fig. 4), the [^{125}I]material recovered from

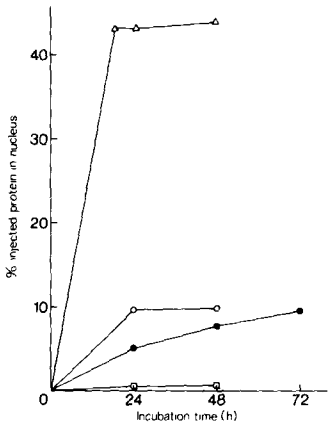

FIGURE 3 Kinetics of protein partitioning between the nucleus and cytoplasm. 10-20 oocytes injected with each [^{125}I]protein were incubated for the times incubated, then fixed, and analyzed as described in Materials and Methods. The percentage of each protein in the nucleus at each time point was determined from a graph like Fig. 2. The value for [^{125}I]histone was taken at subsaturating concentrations. Δ, calf thymus histone; O, lysozyme; ●, ovalbumin; □, BSA.

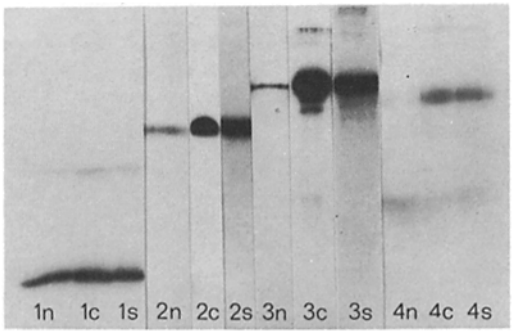

FIGURE 4 Autoradiographs of SDS gels of [^{125}I]-proteins from oocyte fractions. Oocytes injected with various [^{125}I]proteins were manually enucleated. The nuclear and cytoplasmic fractions were electrophoresed beside the pure [^{125}I]protein on 18% SDS gels which were then autoradiographed. The gels are myoglobin (*1*); ovalbumin (*2*); BSA (*3*); and γ-globulin (*4*). The fractions are nuclear (*n*) and cytoplasmic (*c*) and the [^{125}I]protein before injection (*s*).

TABLE III

Properties of the Proteins Used in This Study

	Native mol wt	Isoelectric point	Sp. act.	Molecular dimensions
	daltons		*cpm ^{125}I/ng*	*Å*
γ-globulin (bovine)	160,000 (17)	7 (18)	2,700	235 × 44 (17)
				142 × 85 × 45* (19)
BSA	67,000 (17)	4.6 (17	1,300	150 × 38 (17)
Ovalbumin (chicken)	44,000 (18)	4.6-4.9 (17)	200	63 × 23 (17)
Myoglobin (whale)	17,816 (18)	6.99 (17)	420	70 × 24 (17)
				43 × 34 × 23* (20)
Lysozyme (chicken)	14,600 (18)	11.0-11.2 (17)	1,200	60 × 24 (17)
				45 × 30 × 30* (21)
BPTI	6,513 (18)	10.0-10.5 (22)	530	29 × 19* (23)
Calf thymus histones	11,000-20,000 (18)	11 + (17)	170	
Frog erythrocyte histone	11,000-20,000 (18)	11 + (17)	1,250	
Calf thymus F1	20,000 (24)		480	
F2a1	11,282 (25)		1,050	
F2a2	15,000 (24)		1,850	
F2b	13,774 (26)		810	

The numbers in parentheses indicate the reference for each piece of data.

* Determined by X-ray crystallography. (Otherwise by hydrodynamic methods.)

both the nuclear and cytoplasmic fractions had the same molecular weight as the [^{125}I]protein originally injected. These results indicate that the injected proteins are stable in oocytes.

Since BSA and γ-globulin entered the nucleus only to a small extent in 24 h, it is possible that the radioactivity in the nucleus in these cases was not intact BSA or γ-globulin, but breakdown products. A small molecular weight impurity or breakdown product comprising 5-10% of the injected material could account for the results seen in Fig. 2. Fig. 4 shows that for [^{125}I]BSA, the nuclear [^{125}I] material is BSA; therefore BSA does enter these nuclei although very slowly.

However, Fig. 4 also shows that for [^{125}I]γ-globulin the nuclear [^{125}I]material is enriched in the smaller subunit. When the [^{125}I]γ-globulin bands were cut out from the gel and their radioactivity was determined in a gamma counter, it was found that the ratios of ^{125}I in the small and large subunits were 0.34 in the starting material, 0.40 in the cytoplasmic material, and 4.1 in the nuclear material; the nucleus is enriched more than 10-fold with the smaller subunit. Presumably a fraction of the γ-globulin has dissociated in the cell and the small chains have preferentially entered the nucleus. These results mean that intact γ-globulin enters oocyte nuclei much less than indicated in Fig. 2 and perhaps not at all.

Effect of Cytoplasmic Yolk on the Partitioning of Proteins

Myoglobin, lysozyme, BPTI (after 24 h), and ovalbumin (after 72 h) all entered the oocyte nucleus to the extent of 9.7-11% (Fig. 2), even though the nucleus is only 4% of the oocyte volume. With myoglobin, the same value of 10-11% was obtained by each of the three methods used (Table II). That the oocyte nucleus should concentrate such widely differing proteins, all of which are foreign to this type of cell, would be a surprising finding.

An alternative explanation is that not all the cytoplasm is accessible to the labeled proteins. In fact, much of the cytoplasm of these oocytes consists of yolk platelets. In Fig. 1 (BSA especially) it can be seen that most of the grains lie over the interplatelet cytoplasm and that relatively few lie over the yolk platelets themselves. The fraction of accessible volume can be estimated by centrifugation because under low salt conditions the yolk is insoluble. Table IV shows that after centrifugation the aqueous layer accounted for 32-34% of the oocytes by volume, and contained 73-77% of injected [^{125}I]myoglobin. If this layer is considered to consist mainly of nucleoplasm and cytoplasm accessible to an injected protein, the nucleus is then 12% of this volume, a value that agrees closely with

TABLE IV

Determination of the Relative Nuclear and "Accessible" Cytoplasmic Volumes

Volume of fraction				Ratios			% of total [^{125}I]myoglobin in each component		
Pellet	Aqueous	Lipid	Total	Aqueous/ total	Nucleus/ total	Nucleus/ aqueous	Pellet	Aqueous	Lipid
μl	μl	μl	μl	%	%	%	%	%	%
36.3	18.2	12.1	66.6	32.4	(4)	12.3	14	73	13
39.8	27.7	13.8	81.3	34.1	(4)	11.7	12	77	11

Clumps of ovary consisting almost wholly of large oocytes were dried on paper towels and homogenized in a loose fitting homogenizer. Ovarian connective tissue was removed from the homogenate and 0.3 mg of myoglobin was added per ml of homogenate (calculated to be 0.3 μg/oocyte). An oocyte which had been injected with [^{125}I]myoglobin (0.3 μg) and incubated for 24 h was added to the above mixture, which was rehomogenized. Aliquots were taken up in 100-μl capillaries which were sealed at one end in a flame and centrifuged at 4,000 *g* for 15 min. The height of each fraction in the capillaries was measured and converted into volume (the 11.55-cm capillaries held 100 μl each). The tubes were then frozen and cut into three segments corresponding to the three oocyte fractions for determination of the [^{125}I]myoglobin content.

In the calculations, the aqueous layer is assumed to consist of the nucleoplasm and extra-yolk ("accessible") cytoplasm. The nuclear volume percentage (4%) is derived as in Table II.

the results from the experiments on the partitioning of small proteins. The most reasonable conclusion from these data is that myoglobin, lysozyme, BPTI, and ovalbumin are not concentrated, but merely partition between the nucleus and the "accessible" cytoplasm. Other authors (12, 13) also report the concentration of microinjected macromolecules in oocyte nuclei and attribute this concentration to exclusion from the cytoplasm. These results are of considerable importance in work dealing with the partitioning of materials between a nucleus and a yolk-filled cytoplasm. Results showing a two- to threefold nuclear concentration of materials in oocytes should be interpreted in the light of this finding.

Accumulation of Histones in Oocyte Nuclei

Even after correcting for the excluded cytoplasmic volume of the oocyte, total histones from calf thymus and frog erythrocyte are found to accumulate in the oocyte nucleus. Because total histone is a mixture of proteins, it is necessary to clarify these results by determining to what extent each histone fraction accumulated in the nucleus. To this end, the histone fractions were separated on SDS gels. Fig. 5 shows first that while four fractions of calf thymus and frog erythrocyte histone are clearly present in similar mass amounts (Fig. 5, *1m* and *2m*), only two, F2a1 and F2b, are appreciably labeled with ^{125}I (Fig. 5, *1s* and *2s*). By cutting out the bands and determining the amount of radioactivity in each, F2a1 plus F2b were found to account for more than 90% of the total radioactivity in both histone preparations. In Fig. 5, it is also quite noticeable that F2a1 accounts for a larger proportion of the radioactivity in the nucleus than in the cytoplasm (compare gel *1n* with *1c* and *2n* with *2c*). Gamma counting of the sliced gel showed that for calf thymus histone, the radioactivity in the cytoplasm is 28% F2a1 and 59% F2b while that in the nucleus is 56% F2a1 and 35% F2b. Similar results were found for frog erythrocyte histone

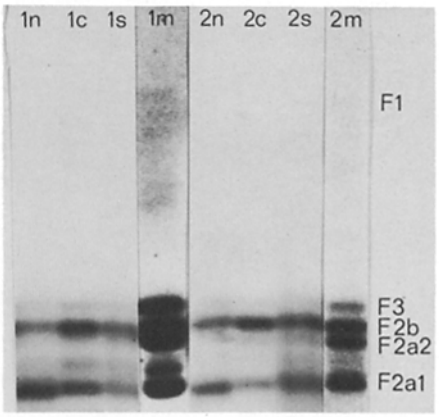

FIGURE 5 Autoradiographs and mass staining of [^{125}I]-histones from oocyte fractions on SDS gels. Oocyte fractions were prepared and electrophoresed as in Fig. 4. The proteins are *Xenopus* erythrocyte histone (*1*) and calf thymus histone (*2*). The fractions are as in Fig. 4 except that gels (*m*) show the mass stain of the histone.

fractions. Clearly F2a1 has concentrated to a greater extent in the nucleus than has F2b. Because the other fractions were poorly labeled, it is difficult to determine to what extent they accumulated in the nucleus.

To remedy this difficulty, pure histone fractions were labeled with ^{125}I and tested for accumulation. On acid urea gels, the labeled fractions showed less than 5% contamination by other fractions. No results were obtained with F3 because, for some reason as yet unexplained, it was found to be toxic to oocytes.

To what extent each of these four fractions accumulated in oocyte nuclei is shown in Fig. 6. Parameters for these curves and for those of total histones in Fig. 2 are given in Table V. All the histones tested accumulated in the nucleus, but at low concentrations F2a1 has a noticeably higher affinity than the other histones for the nucleus. The maximum amount that a nucleus will accumulate also differs for different histones. From these data it is clear that the four histone fractions accumulate in oocyte nuclei, although quantitatively they differ in their behavior.

The finding (Table V) that calf thymus F1 and F2b accumulate in the oocyte nucleus to the same extent as total calf thymus histone indicates that at least some of the histones may accumulate by the same mechanism and therefore may compete with each other. A more direct test for competition between histone species was peformed as follows. A solution of [^{125}I]F2a2 at 2 mg/ml was divided into two aliquots and unlabeled histone F2a1 was dissolved in one aliquot to a final concentration of 10 mg/ml. Each solution was injected into a series of oocytes so that the amount of [^{125}I]F2a2 injected would saturate the nucleus. After a 24-h incubation, the nuclei of oocytes injected with [^{125}I]F2a2 alone contained 16.0 ± 1.0 ng of [^{125}I]F2a2 per nucleus (average of eight determinations ± SE), while nuclei of oocytes injected with [^{125}I]F2a2 and unlabeled F2a1 contained 1.8 ± 0.3 ng of [^{125}I]F2a2 per nucleus (average of 12 determinations ± SE). A fivefold excess of unlabeled F2a1 caused a ninefold decrease in the saturation level of [^{125}I]F2a2. The finding that these two histones compete indicate that possibly all the histones compete in some way.

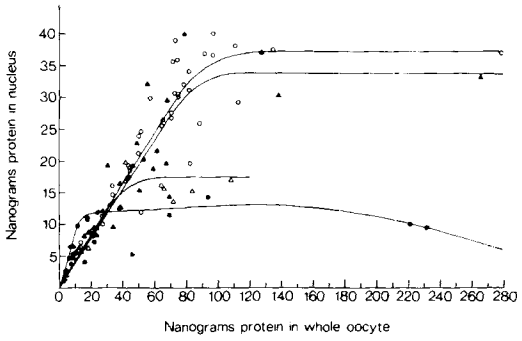

FIGURE 6 Partitioning of various histone fractions between the nucleus and cytoplasm. All procedures are described in Materials and Methods. ●, F2a1; △, F2a2; O, F1; ▲, F2b.

TABLE V

Parameters of Histone Accumulation in Oocyte Nuclei

Histone	Concentration ratio (nuclear/cytoplasmic)*	"Saturation" (nanogram/nucleus)
Frog erythrocyte total	6.8	45
Calf thymus total	5.3	35
Calf thymus F1	5.9	37
F2a1	40.0	12
F2a2	5.7	17
F2b	5.3	33

* Calculated from data in Figs. 2 and 6 using the formula developed in Table II, but divided by 2.8 to correct for excluded cytoplasmic volume.

DISCUSSION

Effect of Size of Protein Molecules on Their Rate of Entry into the Oocyte Nucleus

The data in Figs. 2 and 3 indicate that the size of a protein is the predominant factor affecting its ability to enter the oocyte nucleus. The smaller proteins studied (BPTI, lysozyme, and myoglobin), whether basic or neutral, equilibrate between the nuclear and cytoplasmic compartments in 24 h or less, while the larger proteins, BSA and γ-globulin, enter extremely slowly. Ovalbumin (63 × 23 Å) is somewhat hindered in its entry into the nucleus but still equilibrates in 72 h. From these data, one can estimate that particles up to 45 Å (lysozyme and myoglobin, Table III) in the longest dimension can easily pass through the nuclear membrane while particles larger than 60 Å (ovalbumin, Table III) enter only slowly.

However, it is difficult from this study to determine unequivocally a maximum size of protein that can enter the nucleus because most spherical proteins in the relevant molecular weight range are usually composed of smaller subunits.

Since these polymeric proteins may dissociate in the cell (see Fig. 4, γ-globulin), it may be impossible to determine the size of the particles actually entering the nucleus. On the other hand, the monomeric proteins most suitable for this type of study are not spherical but oblong (i.e. ovalbumin and BSA, Table III). If the length of an oblong protein is longer than the pore diameter, its passage will be hindered because it can only enter end on (this may explain the slow but definite entry of BSA). Therefore, a spherical protein with a molecular weight the same as or even greater than that of an oblong one might enter relatively unhindered because of its greater compactness. In fact, oocyte proteins of at least 140,000 daltons (SDS molecular weight) can enter and accumulate in oocyte nuclei (14).

The results presented in this paper are consistent with other studies on the entry of biological macromolecules into nuclei. Paine and Feldherr (12) have found that lysozyme readily enters the nuclei of cockroach oocytes while larger proteins such as BSA enter these nuclei very slowly. Horowitz and Moore (13) have reported that inulin, a saccharide with a molecular weight of 5,500 daltons, entered the nuclei of frog oocytes in a matter of minutes, while Horowitz et al. (15) reported that dextrans with molecular weights exceeding 20,000 daltons enter these nuclei very slowly with half-times of days. On the basis of the Stokes-Einstein radii of these saccharides, these authors conclude that the sieving diameter of the nuclear membrane is between 30 and 64 Å.

Attempts to study the rate of entry of the smaller proteins at times less than 24 h were unsuccessful, because at any particular time the nuclear cytoplasmic concentration ratios varied greatly from one oocyte to the next. These results are probably due to the interaction of two factors. First, although it is possible to consistently inject into the cytoplasm rather than the nucleus of oocytes by injecting into the vegetal hemisphere, it is not possible to know how far from the nuclear membrane the solution was injected. Therefore, in some oocytes, the injected protein will take longer than in others to diffuse to the nuclear membrane. Secondly, if for these small proteins diffusion through the nuclear membrane is about as fast as diffusion through the cytoplasm, widely different nuclear-cytoplasmic concentration ratios will result between oocytes until the injected proteins have equilibrated between the two compartments. The rate at which these smaller proteins enter the nucleus could be studied using autoradiography of oocyte sections, as Horowitz and Moore (13) have done for inulin. They found that inulin (5,500 daltons) diffused across the nuclear membrane as fast as through the cytoplasm.

Accumulation of Histone

Histones from calf thymus or *Xenopus* erythrocytes not only enter oocyte nuclei but accumulate there. Since the oocyte nucleus contains 50 pg of DNA (16), and presumably 50 pg of histone in its chromosomes, the amount of injected histone accumulated by these nuclei (up to 37 ng for pure histone fractions, Table V), is in some cases about 700 times the amount already present in nucleohistone. It is quite likely, then, that these histones are not binding to DNA as nucleohistone. This interpretation is also supported by the following two findings. First, with both total calf thymus and frog erythrocyte histone, the nucleus seems to accumulate F2a1 to a greater extent than the other histone fractions (Fig. 5), even though in chromatin each histone fraction is similarly abundant. Second, the histone fractions seem to compete with each other for accumulation in the nucleus, so that the presence of one histone fraction can affect the amount of another in the nucleus, even though chromatin the amounts of the histone fractions are fixed relative to one another. However, the interpretations of the nuclear-cytoplasmic concentration ratios and saturation values of the various histone fractions and of the differences between fractions are complicated by the possibility that the oocyte may also contain in addition to the injected [^{125}I]histone endogenous histone in excess over that present in nucleohistone (E. D. Adamson and H. R. Woodland. Personal communication).

The finding that proteins larger than histones (myoglobin, lysozyme, and ovalbumin, Table III) probably passively diffuse into the oocyte nucleus indicates that histones may also enter by diffusion. Consequently, the accumulated histone is probably not actively transported across the nuclear membrane but is rather retained in the nucleus after entry, either by association with nuclear binding sites or by self-association to form particles too large to exit through the nuclear membrane.

The competition of the histone fractions for accumulation and the similarity of results obtained with either calf thymus or frog erythrocyte histone show that the mechanism of accumulation may involve characteristics common not only to the frog histone fractions but to histones of all species.

However, the most obvious characteristic common to all histones, namely their basicity, does not explain their behavior because other basic proteins (lysozyme and BPTI, Table III) do not accumulate in the oocyte nucleus while a class of microinjected acidic proteins do accumulate in the oocyte nucleus (14). Further studies should elucidate the histone characteristics necessary for their accumulation in the oocyte nucleus.

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