Determination of the Intracellular State of Soluble Macromolecules by Gel Filtration In Vivo in the Cytoplasm of Amphibian Oocytes

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Abstract. A method to examine the diffusible state and the sizes of major cytoplasmic proteins in a living cell is described. Small (40–300 μm) commercially available gel filtration beads of a broad range of M_r exclusion limits were microsurgically implanted into the cytoplasm of oocytes of the frog, *Xenopus laevis*, usually after metabolic labeling of oocyte proteins with [^{35}S]methionine. After equilibration in vivo for several hours, the appearance of the implanted cells, notably the bead–cytoplasm boundary, was examined by light and electron microscopy of sections and found to be unaffected. After incubation the beads were isolated, briefly rinsed, and their protein contents examined by one- or two-dimensional gel electrophoresis. We show that diffusible proteins can be identified by their inclusion in the pores of the gel filtration beads used and that their approximate sizes can be estimated from the size exclusion values of the specific materials used. The application of this method to important cell biological questions is demonstrated by showing that several "karyophobic proteins," i.e., proteins of the cytosolic fraction which accumulate in the cytoplasm in vivo, are indeed diffusible in the living oocyte and appear with sizes similar to those determined in vitro. This indicates that the nucleo-cytoplasmic distribution of certain diffusible proteins is governed, in addition to size exclusion at nuclear pore complexes and karyophilic "signals," by other, as yet unknown forces. Some possible applications of this method of gel filtration in vivo are discussed.

Most biochemical work is done on soluble molecules present in the so-called "cytosolic" fraction obtained by cell homogenization and defined as material not pelleted under certain centrifugation conditions (in most cases, ~10^5 *g*; for definition see references 4 and 33). It is often tacitly assumed that the properties of such molecules, determined in this way, are identical to their properties in the living cell. However, this assumption may well be unjustified, in view of the very high particle and protein concentration and the high densities of solid structures present in both the cytoplasmic and the nucleoplasmic compartments (for discussion and literature see references 4, 17, and 18). Consequently, and somewhat paradoxically, at present there is more information about the intracellular state of structure-bound molecules, specifically proteins, despite the fact that these are more problematic for biochemical analyses due to their lower solubility. In fact, we know very little about the actual in vivo state of the numerous proteins that are readily extractable.

To obtain information about the diffusibility, the size, and the shape of a given molecule, say protein, in the living cell two major lines of methodology have been developed. The first approach is based on the introduction of the specifically labeled compound into the cell, usually by microinjection or membrane fusion techniques, followed by the recording of the form and the rate of distribution of this labeled material within the cell (e.g., 1–3, 7–11, 14–16, 19–25, 28–30, 37, 42, 44–46, 48–51, 53–57). In this connection, however, it should be noted that in many of these experiments artificial probes have been used, including extracellular and heterologous proteins which do not occur in the cytoplasm of the normal host cell. Determinations of the rates of diffusion of such injected proteins, for example by autoradiography (for reviews see references 42 and 44) or fluorescence techniques (e.g., 28, 45, 51) theoretically would allow estimations of the specific Stokes radii and of the viscosity of the specific intracellular compartment. However, such calculations have, to our knowledge, not been published so far. There are, however, several fundamental problems associated with the technique of protein microinjection. One is the possibility of selective intracellular sequestration and the altered life-time of the modified protein used in the host cytoplasm (e.g., 48, 54). Another problem is the possibility that the injected molecules may interact with the regulatory systems of the cell, resulting in "regulatory artifacts" such as down-regulation of synthesis (e.g., reference 5) or the disintegration of cellular structures.

An alternative approach to the identification of diffusible components of the cytoplasm has been described by Horowitz, Paine, and collaborators (22–25, 36, 39, 40, 42). This method is based on the microinjection of a droplet of warm gelatine solution into the ooplasm of the frog, *Xenopus laevis*, followed

by short incubation (1–2 min) of the oocyte in ice-cold buffer solution, which results in gelling of the gelatine protein and the formation of a roundish "reference phase." Upon equilibration at 13°C the oocyte is snap-frozen in liquid nitrogen, the gelled reference phase spheroid removed by cryomicrodissection, and its contents analyzed biochemically (39, 40, 42). While this procedure is useful in deciding whether a molecule is truly in a diffusible form or not it does not allow the determination of its intracellular size or shape.

In the present study we describe a new method to determine both the ability of endogenous cytoplasmic proteins to diffuse as well as their Stokes radii by implanting micro-beads of suitable gel filtration materials into the cytoplasm of oocytes of *Xenopus laevis*. Using this method we have been able to fractionate in vivo truly soluble cytoplasmic proteins according to their actual molecular sizes. In particular we show that certain soluble proteins restricted to the cytoplasm ("karyophobic proteins"; 8) are indeed diffusible and occur, in the living cell, with sizes similar to those determined in vitro.

Materials and Methods

Animals and Radiolabeling of Oocyte Proteins

African clawed toads of the species *Xenopus laevis* were purchased from the South African Snake Farm (Fish Hoek, Cape Province, South Africa) and kept in water tanks at 20 °C. Manually defolliculated oocytes of stage VI (13) were incubated for 24 h at 19°C in modified Barth's medium (6) containing [^{35}S]-methionine (final concentration 0.5 mCi/ml; New England Nuclear, Boston, MA). After incubation the oocytes were washed three times in the same medium without radioactive methionine.

Implantation of Beads

For all preparations a stereomicroscope (M8, Wild, Heerbrugg, Switzerland) was used. A small puncture was made in the oocyte plasma membrane with a fine needle, aiming at the vegetal hemisphere of oocytes that had previously been radioactively labeled with [^{35}S]methionine. Individual gel filtration beads that had been swollen in modified Barth's medium for 24 h were taken with fine forceps, and one to three beads were introduced per oocyte by gentle pushing into the vegetal ooplasm. These manipulations did not take more than 40 s. Usually after ~10 min the oocyte surface membrane had sealed again and re-assumed normal cortical morphology.

Diameters and properties of the beads used in the present study are listed in Table I.

Isolation of Beads after Implantation

After 1–6 h of incubation the oocytes were placed in buffer A (75 mM KCl, 25 mM NaCl, 10 mM Tris-HCl, pH 7.2), opened with forceps, and the beads were individually picked out. The isolated beads were then cleaned from adherent cytoplasmic material by repeated sucking up and down through a fine-bored pipette in ice-cold buffer. The whole procedure did not take more than 30 s. The proteins retained in the beads were then extracted by 3 min boiling in approximately the same volume of SDS-containing sample buffer (31), followed by cooling to room temperature and precipitation of the protein with 9 vol cold acetone (−20°C). The precipitates were allowed to settle during 24 h incubation at −20°C.

Isolation of Cytoplasmic Fractions

Nuclei and cytoplasms were quickly isolated from oocytes of the same stage, separately collected in buffer A, and processed as described previously (8).

Gel Electrophoresis

For one-dimensional SDS PAGE in 10% acrylamide gels, the system of Thomas and Kornberg (52) was used. Samples were solubilized by boiling in SDS sample buffer (31). For two-dimensional gel electrophoresis the non-equilibrium pH gradient gel electrophoresis (NEPHGE) system of O'Farrell et al. (38) was applied for separation in the first dimension, followed by second dimension electrophoresis by SDS PAGE in 18% acrylamide slab gels according to Thomas and Kornberg (52). Gels were stained with Coomassie Blue and then processed for fluorography as described by Laskey and Mills (34). Reference proteins used for estimation of M_r values were as previously described (Fig. 2 of reference 8). For co-electrophoresis in NEPHGE separations the fraction of soluble ooplasmic proteins from unlabeled oocytes as previously shown (Fig. 5*c* of reference 8) was added to facilitate identification of radioactive components seen only by fluorography.

Light and Electron Microscopy

The oocytes were conventionally fixed for 45 min in cold, buffered 2.5% glutaraldehyde, washed in sodium cacodylate buffer, and postfixed in 2% osmium tetroxide for 2 h. After rinsing in distilled water, specimens were dehydrated in graded ethanol solutions and embedded in Epon 812 (47). Sections of ~1 μm were used for light microscopy. Ultrathin sections were prepared and stained according to standard procedures (cf. 47).

Results

Morphology of Oocytes after Implantation of Beads

The appearance of an oocyte of *Xenopus laevis* with an implanted Sephadex G-150 bead is shown in the phase-contrast photograph of an ~1-μm thick section in the survey montage of Fig. 1. Similar pictures have been obtained for the other kinds of gel filtration beads. The implanted bead is located deep in the cell and is surrounded by cytoplasm of normal appearance, amidst the numerous yolk platelets and mitochondria. The nucleus ("germinal vesicle"), which is sometimes difficult to distinguish from the implanted beads when manipulating under the stereomicroscope (the beads are usually somewhat smaller, relatively hard, and opaque), is easily recognized in such sections by the numerous nucleoli. Characteristically, the cytoplasmic components are not randomly distributed in the immediate vicinity of the implanted beads. Rather a small cytoplasmic zone of 3–8 μm is formed which is devoid of yolk platelets but contains mitochondria,

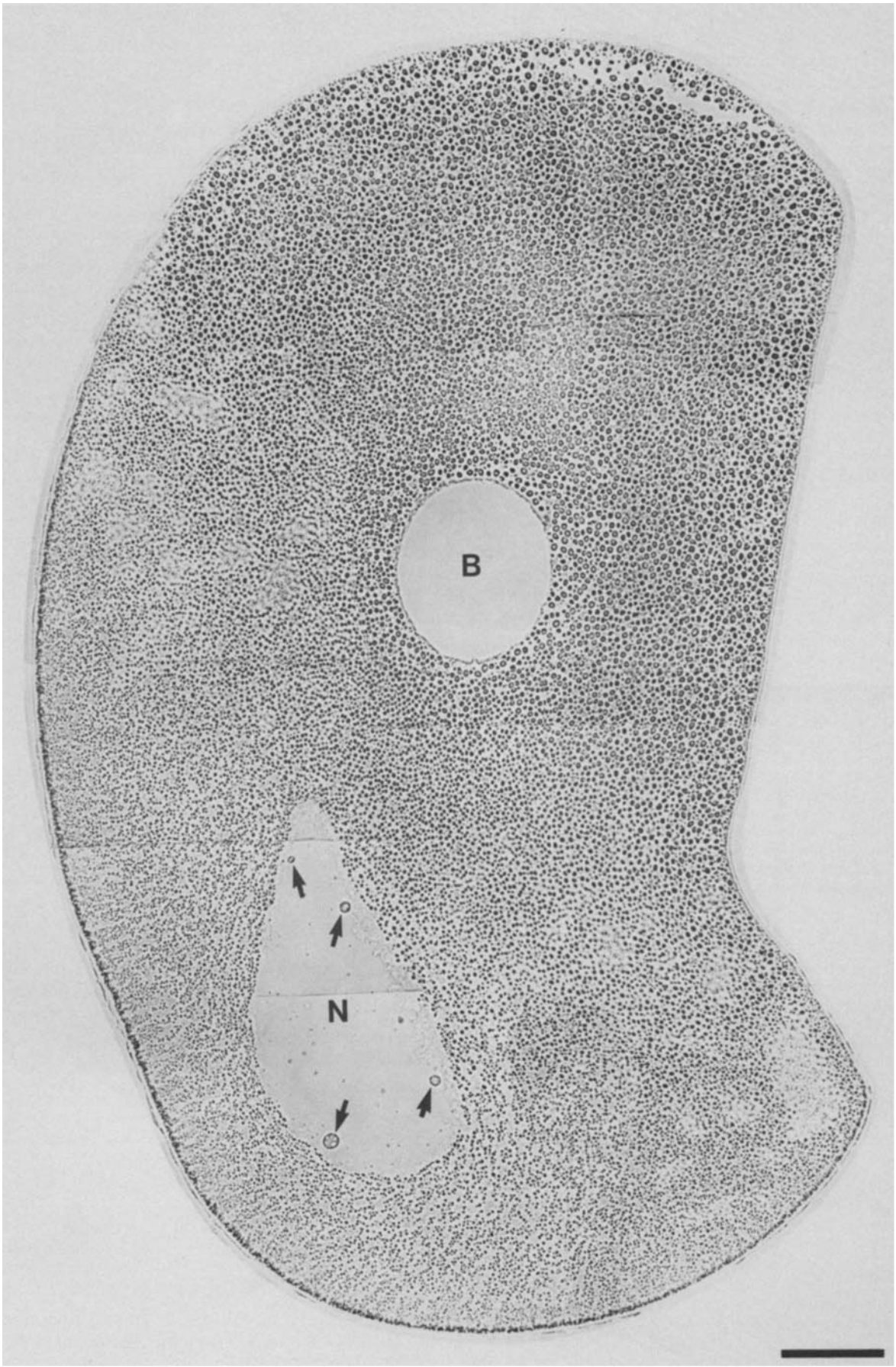
*Table I. Technical Data of Beads Used for Implantation**

Type of beads	Mean exclusion limit‡	Fractionation range of proteins	Bead diameter§
	$M_r \times 10^{-3}$	$M_r \times 10^{-3}$	μm
Bio-Gel P-150 coarse	150	15–150	150–300
Bio-Gel P-60 coarse	60	3–60	150–300
Bio-Gel P-6 coarse	6	1–6	150–300
Sephadex G-150	150	5–300	40–120 (60–150)
Sephadex G-25 medium	5	1–5	50–150 (60–180)

* As indicated by the manufacturer.

‡ As determined by globular proteins.

§ For Bio-Gel beads (purchased from Bio-Rad Laboratories, München, FRG) the diameter range of hydrated beads is given. For Sephadex beads (purchased from Pharmacia, Uppsala, Sweden) the range of diameters of the dry beads is indicated; the size ranges determined after swelling in Barth's medium are given in brackets.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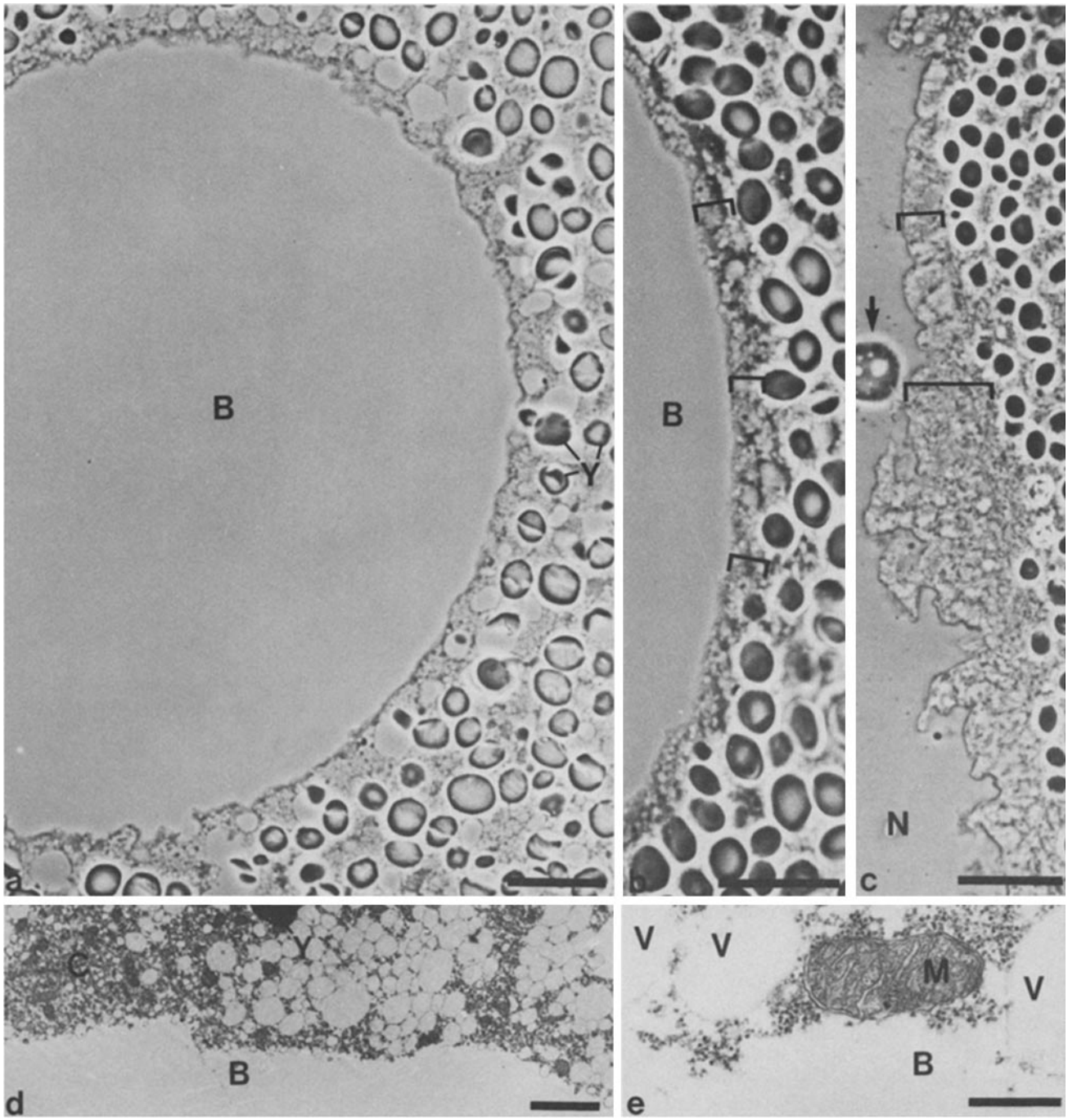

Figure 2. Light (*a–c*, phase-contrast optics) and electron (*d* and *e*) micrographs showing 1 μm (*a–c*) and ultrathin (*d* and *e*) sections through the cytoplasm of *Xenopus* oocytes after implantation of beads of Sephadex G-150. 6 h after implantation the oocytes were conventionally fixed, and sections were analyzed by microscopy. (*a* and *b*) Sections showing the implanted bead (*B*) and the appearance of the adjacent cytoplasm. The brackets in *b* denote the special zone of cytoplasm surrounding the bead from which yolk platelets (some are denoted *Y*) are largely excluded. (*c*) Section showing the zone surrounding the nucleus (*N*; nucleolus is indicated by arrow). The special perinuclear cytoplasmic zone devoid of yolk platelets is denoted by brackets. (*d* and *e*) Sections showing the electron microscopic appearance of the bead (*B*) and the adjacent cytoplasm (*C*). The yolk platelets (*Y*) are excluded from the zone directly bordering on the bead surface, whereas mitochondria (*M*), translucent vesicles (*V* in *e*), and polyribosomes are not. Bars represent 20 μm (*a–c*), 2.5 μm (*d*), and 0.5 μm (*e*).

Figure 1. Survey light micrograph montage of several pictures (phase-contrast optics) of a *Xenopus oocyte* with an implanted bead of Sephadex G-150. 6 h after implantation of the beads into the cytoplasm, the oocyte was conventionally fixed, dehydrated and embedded, and sections of 1 μm were examined by light microscopy. *N*, nucleus; the nucleoli are well visible (*arrows*). *B*, implanted bead. Bar, 100 μm.

small vesicles, ribosomes, and other small components (Fig. 2, *a*, *b*, *d*, and *e*). In its general appearance this "zone of exclusion" of yolk platelets around the implanted beads is reminiscent of the somewhat broader perinuclear zone (Fig. 2*c*). This oocyte morphology was preserved for at least 6–8 h after implantation. We have not followed the further fate of oocytes with beads implanted. However, we have noted some altered oocyte morphology such as a white-spotty appearance of the oocytes after overnight incubation.

Analyses of Proteins Recovered from Implanted Beads

Proteins specifically retained in the porous cavities of the gel filtration beads exposed to the cytoplasm of the living oocyte for 1–6 h were eluted in buffers containing SDS and the polypeptides were extracted and separated by SDS PAGE (Fig. 3) or two-dimensional gel electrophoresis (Fig. 4). Polypeptides larger than M_r 10,000 were recognized only among the proteins that had entered beads with relatively large pore sizes such as Bio-Gel P-60 and P-150 or Sephadex G-150 (Fig. 3*a*), whereas no protein larger than M_r 10,000 was recovered from small pore size beads such as Bio-Gel P-6 and Sephadex G-25 (Fig. 3*b*, lanes *6* and *7*). Furthermore, the absence of such proteins from P-6 and G-25 beads, as well as the fact that the abundant yolk proteins were not recognized in Coomassie Blue–stained analyses of materials recovered from such beads, also demonstrates that nonspecific adsorption of cytoplasmic proteins is not a serious artifact of this method.

Different proteins were recovered from the different types of implanted gel filtration beads. Typical abundant cytoplasmic proteins such as actin were found in similar concentrations in all three materials of relatively large pore sizes, i.e., Sephadex G-150, Bio-Gel P-150 and P-60 (Figs. 3 and 4), indicating that a large proportion of the diffusible actin of the cytoplasm (cf. 10, 40) is in the monomeric state. We also detected radioactivity corresponding to coelectrophoresed "high mobility group protein" HMG-A in extracts from all three types of beads (not shown), confirming the soluble state of cytoplasmic HMG-A previously reported (27). However, while major large polypeptides of M_r 100,000–230,000 were retained in Sephadex G-150 and, though to a lesser degree, Bio-Gel P-150 beads (Fig. 3, lanes *3* and *4*; Fig. 4, *b* and *c*), polypeptides larger than M_r 95,000 were absent from Bio-Gel P-60 beads (Fig. 3, lane *5*, and Fig. 4*d*). However, some polypeptides in the M_r range of 60,000–95,000 were seen among the contents of P-60 beads (Fig. 3*a*), i.e., exceeding somewhat the exclusion limit given for this material by the manufacturer (Table I). Whether the inclusion of some polypeptides of M_r 60,000–95,000 in Bio-Gel P-60 beads in vivo is due to anomalous properties of the specific proteins or simply reflects some inaccuracy of the assumed exclusion limits cannot be decided at present. The size fractionation of proteins recovered from the implanted beads can also be seen from the patterns of polypeptides obtained on two-dimensional gel electrophoresis. Some polypeptides such as the relatively large cytoplasmic components C1 (polypeptide M_r ca. 230,000), C3 (M_r 69,000), C4 (M_r 64,000), and C5 (M_r 61,000) did enter the P-150 and G-150 beads (Fig. 4, *b* and *c*) but not the other types of beads examined (Fig. 4*d*). In addition, some polypeptides with M_r values below M_r 150,000 were also seen only in analyses of contents of the larger pore size beads (Fig. 4*b*) but not in beads with smaller pores (e.g., Fig. 4*d*), indicating that these small polypeptides are contained in large complexes.

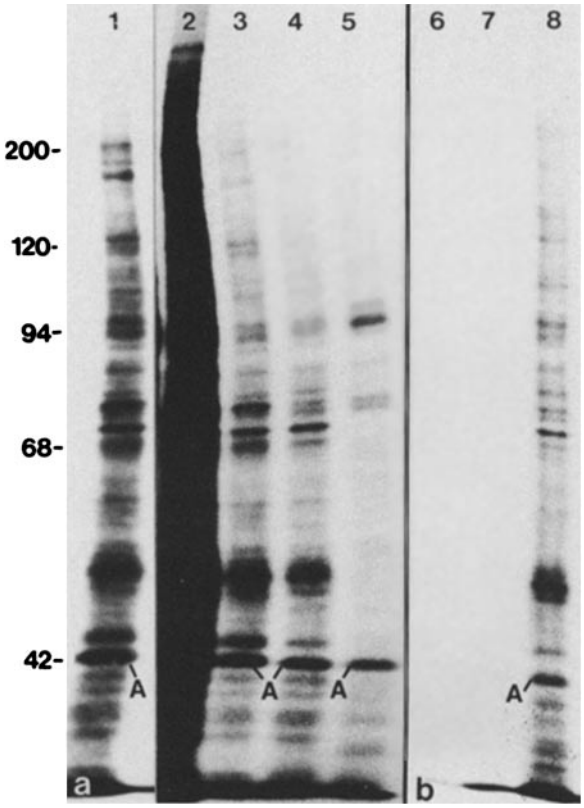

Figure 3. SDS PAGE of soluble cytoplasmic proteins from *Xenopus* oocytes analyzed by gel filtration in vivo using implantation of beads. Proteins were labeled by incubation of oocytes for 24 h with [^{35}S]-methionine. Then a total of 100 beads were implanted into the cytoplasm of a total of 30 oocytes. 6 h after implantation the beads were manually isolated, briefly rinsed, and the protein contents eluted in SDS sample buffer and analyzed by SDS PAGE. Proteins were visualized by autoradiography (M_r values $\times 10^{-3}$ of reference proteins visualized by Coomassie Blue staining are indicated on the left margin; the position of actin is denoted by *A*). (*a*) Polypeptides eluted from large pore size beads: lane *1*, total soluble proteins, i.e., not pelletable by centrifugation at 100,000 *g* for 1 h, from a simple enucleated cytoplasm, short exposure time 24 h; lane *2*, same as lane *1*, exposure time 6 d, individual polypeptide bands are not resolved after the exposure time applied here; lane *3*, protein from 100 Sephadex G-150 beads (same exposure time as in lane *2*); lane *4*, proteins from 100 Bio-Gel P-150 beads; lane *5*, protein from 100 Bio-Gel P-60 beads. (*b*) Results with small pore size beads (different set of experiments); lane 6, eluate from 100 Sephadex G-25 beads; lane *7*, eluate from 100 Bio-Gel P-6 beads; lane *8*, eluate from 100 Sephadex G-150 beads shown for comparison (similar experiments as shown in lane 3). Note absence of any protein label in lanes *6* and *7*.

In this study we specifically examined whether certain proteins accumulating in the cytoplasm, i.e., the "karyophobic" polypeptides termed C1–C9 (8), which have been identified as soluble proteins in cell homogenates, are indeed "soluble" proteins in the living cell. As shown in Fig. 4 most proteins of this category did indeed migrate into gel filtration beads, including the large polypeptide C1 (Fig. 4*b*) as well as the much smaller proteins C3–C9 (Fig. 4, *b–d*). The sizes of the proteins containing these polypeptides could be estimated

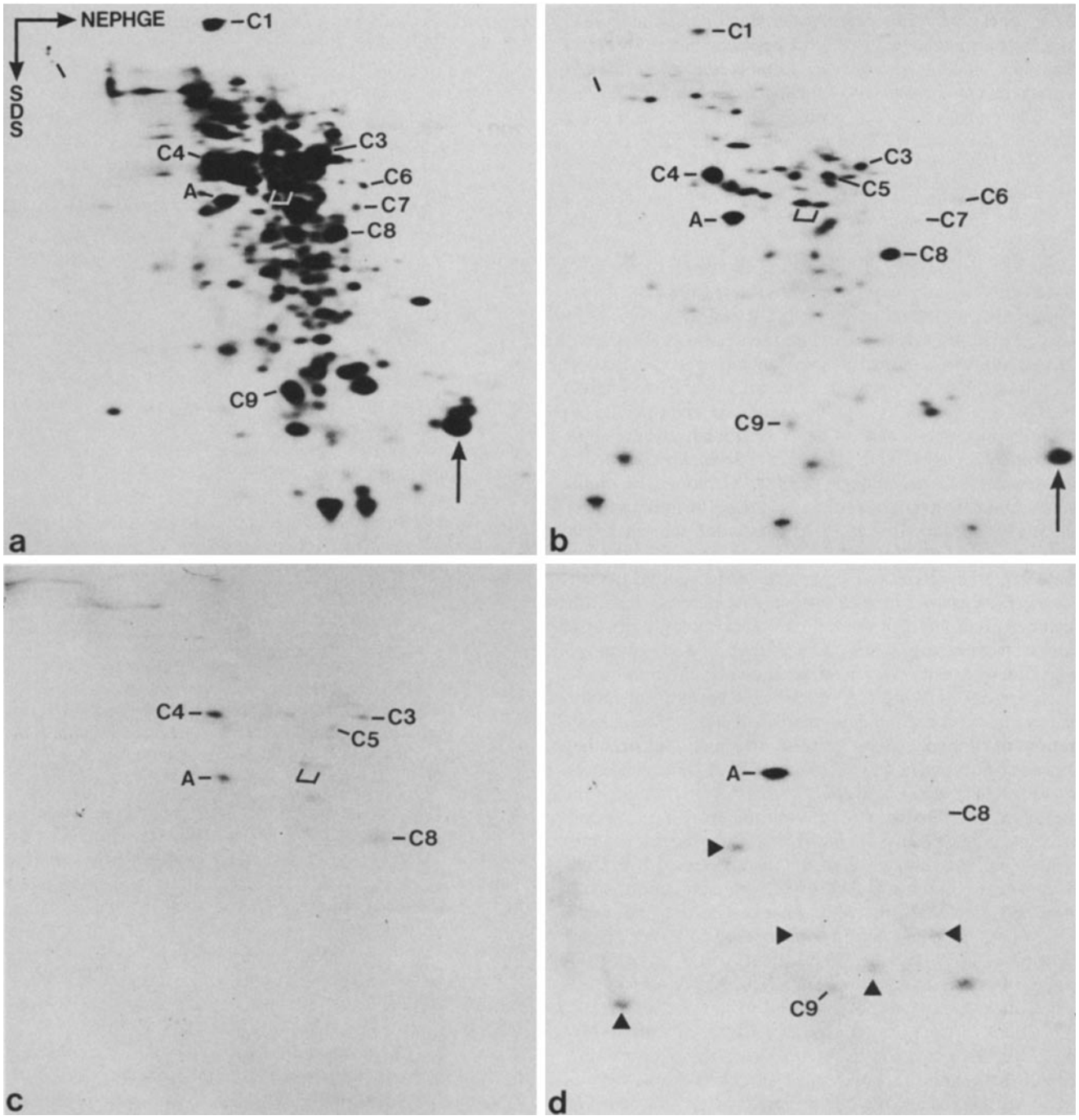

Figure 4. Two-dimensional gel electrophoretic analysis of the soluble proteins fractionated in vivo by implantation of gel filtration beads. A total of 300 beads was implanted into the cytoplasm of a total of 90 *Xenopus* oocytes previously labeled in vivo with [^{35}S]methionine. 6 h after implantation the beads were manually isolated, rinsed, and the proteins contained in the beads were analyzed by two-dimensional gel electrophoresis using non-equilibrium pH gradient electrophoresis (NEPHGE) in the first dimension and SDS PAGE (18% acrylamide) in the second dimension. (*a*) Proteins present in 100,000 *g* supernatant from five manually isolated and pooled ooplasms of *Xenopus*; (*b–d*) proteins present in Sephadex G-150 beads (*b*), Bio-Gel P-150 beads (*c*), and Bio-Gel P-60 beads (*d*). Proteins are visualized by autoradiofluorography. *C1–C9* denote the position of major karyophobic polypeptides as previously defined. The radioactivity incorporated in polypeptides *C6* and *C7* is characteristically low and the corresponding spots are only faintly seen in *a* and *b*. The bar in the upper left corners of *a* and *b* denotes the putative component *C2*; in these gels, however, the identity of this spot could not be fully ascertained. The positions of actin (*A*) and another major amphiphilic protein (bracket) are indicated. The arrows in *a* and *b* denote a major intrinsic reference protein, the arrowheads in *d* point to some lower molecular weight polypeptides which are included in the P-60 beads.

from their exclusion from certain gel filtration materials. For example, the abundant polypeptide C8 has been seen in the contents of Sephadex G-150 and Bio-Gel P-150 (Fig. 4, *b* and *c*) but was not detected in considerable amounts in Bio-Gel P-60 beads, in agreement with its apparent M_r of ~ 70,000 determined in vitro (8).

Similar results were obtained after 3, 6, and 10 h after bead implantation, indicating that equilibrium is reached within a few hours, apparently somewhat earlier than in the case of the usually larger gelatine reference phase structures (40).

Discussion

The bead implantation method described here, like the "gelatin reference phase-cryomicrodissection" method (22, 24, 25, 39, 40, 42), allows the decision whether a certain protein is structure-bound or exists in a diffusible state. In comparison to the "gelatine reference phase" procedure, however, the gel filtration bead method offers a number of technical advantages: (*a*) It uses well defined, commerically available materials; (*b*) different kinds of gel filtration beads can be used; (*c*) the implantation of the beads does not require temperature shifts such as transitory cooling of the recipient oocyte; (*d*) the implanted beads can be easily discerned; in fact, some manufacturers offer colored bead materials that would further facilitate such experiments, notably the re-isolation of the beads; (*e*) for analyses of proteins included in the cavities of the beads during incubation in the oocyte difficult cryodissection techniques are not required; (*e*) it does not introduce masses of a foreign protein and thus avoids possible artifacts of protein interactions and of peptides released from the gelatine. In addition to these technical advantages the bead implantation method offers the general possibility of molecular size (Stokes radius) fractionation gel filtration in the cytoplasm of a living cell. As in principle it is possible to also determine, by an independent method, the internal fluid space volume of the beads, quantitative studies should further allow the concentrations of specific proteins to be determined and thereby help in defining the dynamic equilibria between the soluble and the structure-bound states of the same molecule. Moreover, it may also be possible to identify the proteins included in the beads by sensitive immunological methods either by immunolocalization in situ (e.g., by immunofluorescence microscopy of sections through implanted beads) or, in vitro, by immunoassaying and immunoblotting of the proteins eluted from the beads upon re-isolation.

At present we cannot definitively assess the possible artifacts which the bead implantation method, like any other method, may entail. The microsurgical implantation described here may, like other invasive procedures such as microinjection, result in some transitory leakage of water and solubles as well as in disturbances of intracellular equilibria (cf. 36). However, this should not affect experiments in which relatively long incubation times are applied. Moreover, while the implanted beads are in a diffusional equilibrium with the solutes of the cytoplasmic phase its interior is not identical with the cytoplasmic solvent space but, like the gelatine mass of Horowitz and colleagues (22–25), represents an artificial "reference phase" in which proteins may behave differently. Finally, some quantitative losses of proteins from the beads may occur during the isolation and rinsing steps, as has been reported for manual isolations of nuclei from amphibian oocytes (43), although such losses should not be considerable in view of the short times involved (this is also indicated from some preliminary data on elution kinetics from the isolated beads).

In the present study we have specifically used the method of gel filtration in vivo to characterize the state of a certain group of cytoplasmic proteins, the "karyophobic proteins" (8). Such proteins accumulate in the cytoplasm of *Xenopus* oocytes as well as of other cells, e.g., human HeLa cells, but are not found in considerable amounts in the nucleus, although they do not seem to be stably bound to cytoplasmic structures but are recovered as soluble molecules in cytosolic fractions (8). The question of whether such proteins are truly soluble, i.e., diffusible, in the living cell or are structure-associated in vivo but solubilized during cell disruption and dilution in buffers appears to be answered for at least some of the more abundant proteins of this class by the results of our present study. The polypeptides designated C1 (M_r 230,000), C3 (M_r 69,000), C4 (M_r 64,000), C5 (M_r 61,000), C6 (M_r 47,000), C7 (M_r 40,000), C8 (M_r 35,000), and C9 (M_r ~ 15,000) enter the spaces of the implanted beads, and therefore we conclude that these proteins occur, exclusively or in parts, in diffusible forms in the *Xenopus* ooplasm. Consequently, the explanation that Goldstein and Ko (20) and DeRobertis (9) have discussed (see also the earlier results in references 2 and 3), namely, that some cytoplasm-restricted proteins may simply be excluded from translocation into the nucleus by being stably bound to, or integrated in, large cytoplasmic structures, does not seem to apply for these karyophobic proteins.

Our bead implantation method also allows estimations of the approximate sizes of the proteins containing these polypeptides. This is particularly relevant to the currently prevailing concept of particle size exclusion as the major principle of the accumulation of certain proteins in the cytoplasm (e.g., 1–3, 9, 41, 44, 45). For example, the large cytoplasmic polypeptide C1, which on SDS PAGE appears with a mobility corresponding to an M_r value of 230,000 (8), clearly enters Sephadex G-150 beads and, apparently to a lesser degree, also Bio-Gel P-150 beads, suggesting that protein C1 is present as a monomeric polypeptide in the ooplasm. Polypeptides C3–C5 of M_r 61,000–69,000 have been reported, from in vitro experiments, to be included in protein complexes of apparent M_r values between 100,000 and 150,000 (8). These values determined in vitro are in agreement with our finding in vivo that these proteins enter G-150 and P-150 beads but are excluded from Bio-Gel P-60 beads, indicating that these polypeptides occur in larger, perhaps dimeric complexes. In contrast, polypeptide C8 of M_r 35,000 enters the cavities of P-150 and G-150 beads but is greatly reduced among the proteins recovered from P-60 beads and totally excluded from P-6 and G-25 beads, a finding that is compatible with an overall protein M_r of ~70,000 determined in vitro (8). On the other hand, the small karyophobic polypeptide C9 with an SDS PAGE–derived M_r of 15,000 and an apparent total protein M_r of ~30,000 determined in vitro is able to enter the P-60 beads implanted into the living oocyte. The latter two proteins, C8 and C9, are also of special importance as their sizes are clearly below the protein size limits for passage across the nuclear pore complexes as reported by several authors (2, 3, 39, 41, 42, 44, 45). These examples of soluble karyophobic proteins, together with reports of cytoplasmic accumulations of some small RNA-binding proteins (9, 35, 58), a growing list of karyophilic proteins exceeding the size "limits" (3, 7–10, 14, 15, 29, 40, 49, 50), studies showing an amino acid sequence specificity of nuclear vs. cytoplasmic accumulation (26, 32), and the demonstration that the compartmentaliza-

tion of a given protein may depend on the state of cell differentiation (12), all point to the existence of more specific controls of the distribution of diffusible proteins between nucleoplasm and cytoplasm. Hopefully, methods to study the behavior of soluble proteins in vivo such as the gel filtration method described in this study will help in elucidating the forces involved in these forms of topological regulation.

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