THE EFFECT OF POLY-L-LYSINE ON THE

UPTAKE OF REOVIRUS DOUBLE-STRANDED RNA

IN MACROPHAGES IN VITRO

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ABSTRACT

The effect of polycations on cultured mouse peitoneal macrophages has been examined.
Polycations, at concentrations greater than 5 μg/ml, are toxic for macrophages, as measured
by failure of the cells to exclude vital dyes. At toxic concentrations polycations bind in large
amounts to nuclei and endoplasmic reticulum, while at nontoxic levels polycations bind se-
lectively to the cell surface. Nontoxic concentrations of polycations stimulate binding of reo-
virus double-stranded (ds) RNA to the macrophages by forming polycation-dsRNA
complexes either in the medium or at the cell surface. These complexes enter the cell in
endocytic vacuoles and are concentrated in secondary lysosomes. Despite exposure to the
acid hydrolases within this cell compartment, the dsRNA and the polycation (poly-L-lysine)
are conserved in a macromolecular form within the vacuolar system. The mechanism(s) by
which the uptake of infectious nucleic acids and the induction of interferon by dsRNA are
stimulated by polycations are discussed.

INTRODUCTION

Polycations have been reported to enhance the in-
fectivity of single- and double-stranded viral RNA
(1), of double-stranded DNA (2), and of RNA
tumor viruses (3); they stimulate the cellular up-
take of a variety of macromolecules (1, 2, 4–6);
and they increase interferon production in cells
treated with double-stranded (ds) RNA (6–8).
These findings are of considerable general interest
in view of the slow rate at which large molecules
usually penetrate cells and the profound effects
that polynucleotides exert upon cellular metab-
olism once they gain access to the cytoplasmic
matrix.

The mechanism by which polycations stimulate
the uptake of polynucleotides has been examined
by several investigators (6, 7, 9–11). In general,
their results are consistent with the hypothesis that
polycations stimulate the binding of polynucleo-
tides to cells by forming cationic bridges between
the negative charges of the polynucleotide and the
cell surface. As a result, polynucleotide-polycation-
cell surface ternary complexes are presumably
formed. However, the events which follow the
polycation-stimulated binding of polynucleotides
to cell surfaces have not been examined in detail.
Comparatively little attention has been directed
toward the toxic effects of polycations upon living
cells and the relationship of polycation-induced
changes in cell permeability to the binding of a
variety of macromolecules to cells (6, 12–14). In
order to clarify some of these issues, we have ex-
amined the effects of several polycations on the

viability of mouse peritoneal macrophages in vitro. We have studied the uptake, intracellular fate, and localization of poly-L-lysine in these cells and the effect of this polycation upon the binding and uptake of reovirus dsRNA. A preliminary report of these studies has been presented (33).

MATERIALS AND METHODS

^3H-Labeled Reovirus dsRNA

[^{3}H]Uridine and [^{3}H]cytidine-labeled reovirus was grown and purified as described (15), dialyzed against phosphate-buffered saline (PBS) (16), and the RNA was extracted with an equal volume of water-saturated phenol at room temperature. The [^{3}H]dsRNA-containing aqueous layer was extracted with ethyl ether, to remove any remaining phenol, and placed on a 1.5 × 7 cm methylated albumin-silicic acid column (17).

The [^{3}H]dsRNA eluted in a single peak at 0.7 M NaCl. The peak fractions were combined, dialyzed exhaustively against PBS, passed through a 0.5 × 2 cm Sephadex G25 column which had been equilibrated with PBS, and the [^{3}H]dsRNA-containing fractions were combined. The final solution contained 37.5 µg dsRNA/ml (1 OD at 260 nm = 50 µg/ml dsRNA [18] of sp act 1.05 × 10^5 dpm/µg.

[^3H]Poly-L-lysine

[^{3}H]Poly-L-lysine, average mol wt 50,000, sp act 1.37 mCi/ml, was prepared by New England Nuclear (Boston, Mass.) using the Wilzbach procedure. Nonradioactive poly-L-lysine was purchased from Yeda (Rehovot, Israel), poly-L-ornithine from Sigma Chemical Co. (St. Louis, Mo.). DEAE-dextran (mol wt 2 × 10^6) was purchased from Pharmacia Fine Chemicals, Inc. (Uppsala, Sweden). Poly-inosinic-cytidylic acid (poly IC) was purchased from Microbiological Associates, Inc. (Bethesda, Md.).

Macrophage Cultures

Macrophages were obtained as described previously (19) and were cultivated in medium 199 with 30% newborn calf serum (NBCS) in 35 mm plastic Petri dishes (Falcon Plastics, Oxnard, Calif.) or in Leighton tubes for 24 h before experiments. The cell concentration was approximately 10^6 cells per dish, or 5 × 10^5 cells per Leighton tube.

The experimental incubations were performed using 1.5 ml medium 199 with 1% NBCS, and dsRNA and poly-L-lysine as stated. All incubations were performed at 37°C in 95% air, 5% CO_2. To determine cell viability, cover slip cultures were incubated with 1% trypan blue or 1% nigrosine in 0.9% NaCl for 2 min before microscopic examination.

Light Microscopy

Some of the morphologic studies were performed with cells grown on flying cover slips in Leighton tubes (19). After the appropriate incubation, the cells were fixed by immersion in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 15 min, rinsed, mounted in water, and examined under phase-contrast optics with a Zeiss Ultraphot II microscope (Carl Zeiss, Inc., New York). Light microscopic autoradiographs were prepared with an L4 Ilford suspension (Ilford, Ltd., Ilford, England). Exposure times varied from 1 to 12 wk, after which the micrographs were developed with Microdol.

Electron Microscopy

Cells on plastic Petri dishes were fixed with glutaraldehyde for 15 min, rinsed, fixed in 1% OsO_4 in 0.1 M phosphate buffer for 30 min, and dehydrated and embedded in Epon. Semithin and thin sections were made with a Porter-Blum Microtome equipped with a diamond knife. Autoradiographs were produced according to the method of Salpeter (20) or von Gaudecker (21) as described previously (22). Exposure time varied from 4 to 10 mo. Thin sections were stained with lead according to Reynolds and were examined in a Siemens Elmiskop I electron microscope (Siemens Corp., Medical Industrial Div., Iselin, N. J.).

Binding of Labeled Materials

After incubation of cells with the isotopically labeled compound to be tested, the medium was removed and the dishes were rinsed three times with 1 ml prewarmed 0.9% NaCl solution. The cells were scraped off the dish with a rubber policeman, suspended in 1 ml of saline, centrifuged for 5 min at 1,000 rpm, and the supernatant was discarded. The resulting cell pellet was washed once in 1 ml 0.9% saline and dissolved overnight at room temperature in 1.0 ml of 1 N NaOH. A sample of 0.2 ml was counted in 15 ml Bray's solution in a Nuclear-Chicago Mark II scintillation spectrometer (Nuclear-Chicago Corp., Des Plaines, Ill.). Quenching was corrected using an external standard. The results were expressed as counts per minute per dish. Preliminary experiments showed that the cell content of dishes showed little variation (~10%) as judged by protein assay (23).

Differential centrifugation was performed with a Spinco Ultracentrifuge equipped with an SW 39 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Fractions were collected dropwise
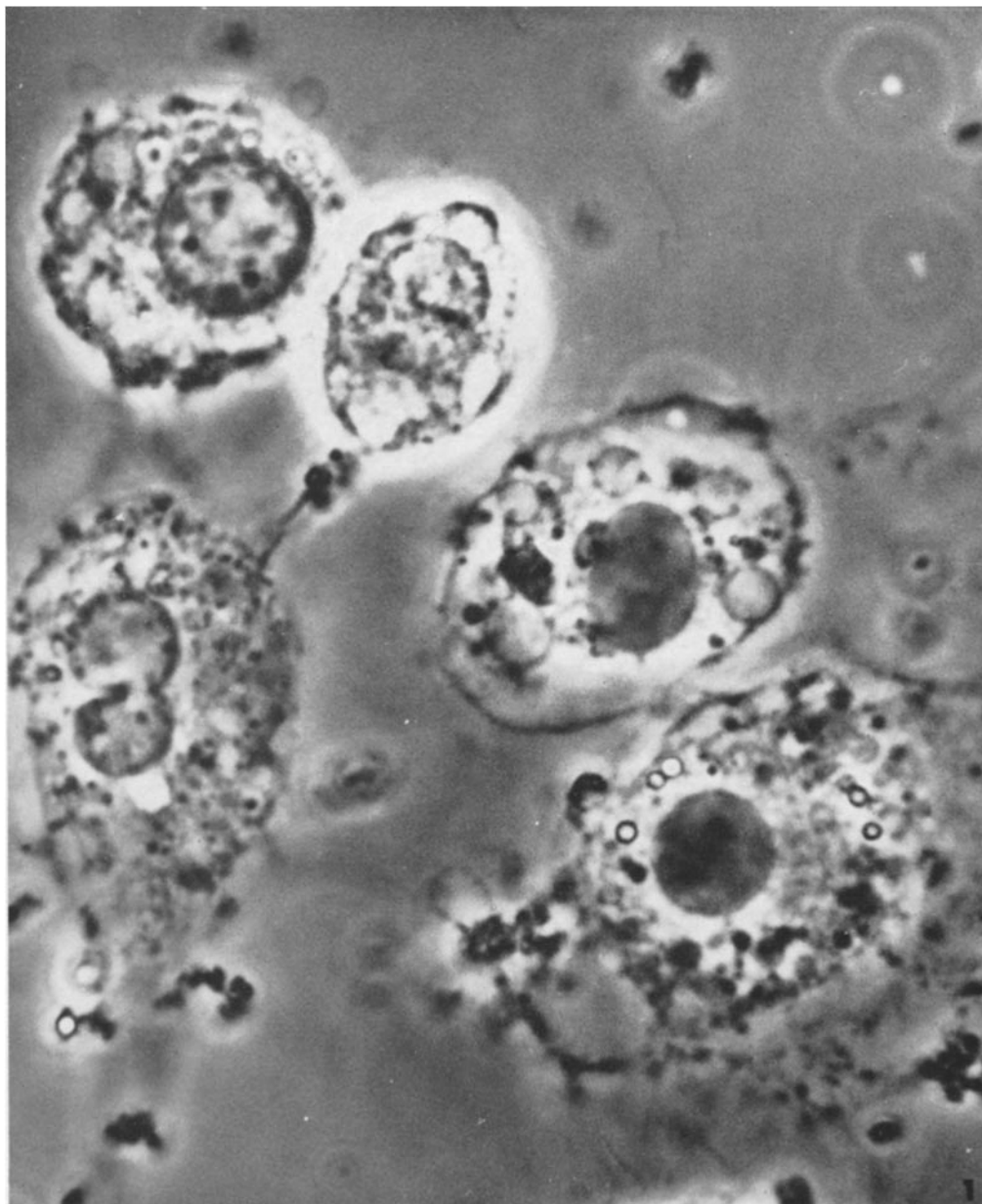
FIGURE 1 Phase-contrast micrograph of macrophages after exposure to 10 µg/ml poly-L-lysine in medium 199 with 1% NBCS for 1 h. All the cells are dead or damaged. × 3,000.

from the bottom of the tube and assayed for radio-label as described above.

RESULTS

Effect of Polycations on Cell Morphology and Viability

At all concentrations over 5 µg/ml there were clear indications of cell damage (Fig. 1). After 60 min of incubation with 10 µg/ml of the nonradio-active poly-L-lysine, more than 95% of the cells were dead as judged by phase-contrast microscopy or by the uptake of trypan blue or nigrosine (Fig. 2). The toxic effects as judged by phase-contrast microscopy consisted of rounding up of the cells, cytoplasmic swelling, retraction of organelles to the perinuclear area, and abnormal refractile proper-ties of the nuclei. The majority of the dead cells
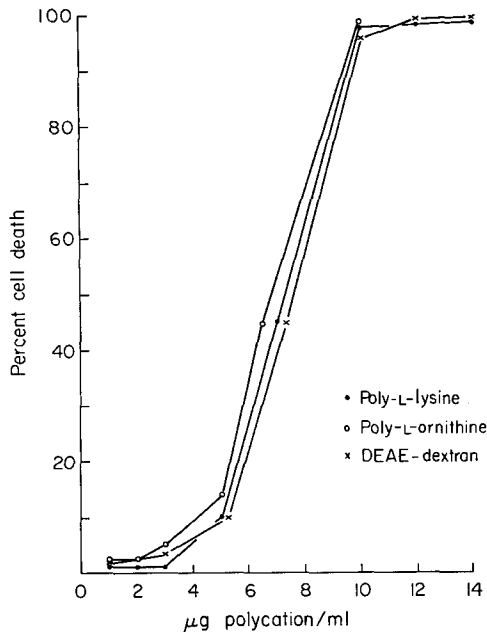
FIGURE 2 Toxic effect of polycations on macrophages. Macrophages were incubated with the indicated concentrations of polycations for 1 h at 37°C in medium 199 containing 1% NBCS. Cell viability was measured as described in Methods.

remained firmly attached to the glass surface. The ^{3}H-labeled poly-L-lysine was slightly less toxic per microgram than the nonradioactive compound. At concentrations below 5 μg/ml there were no clear-cut signs of cell damage using either the radioactive or the nonradioactive poly-L-lysine (Fig. 3); in either case less than 1% of the cells took up trypan blue.

Increasing the serum concentration to 10% resulted in a fivefold increase in the amount of poly-L-lysine required to kill 50% of the cells. The toxic effect of poly-L-lysine could be abolished by the simultaneous presence of poly IC. Thus, 20 μg of poly-L-lysine killed all the cells in 60 min. The addition of about 60 μg/ml of poly IC reduced the number of cells taking up trypan blue to that of the control. Intermediate concentrations of poly IC showed proportional protective effects.

There was no significant stimulatory effect of nontoxic doses of poly-L-lysine on pinocytosis in the macrophage as judged by counting pinocytic vesicles in the phase-contrast microscope (24). Poly-L-ornithine, DEAE-dextran, and poly-D-lysine gave similar dose-response results in terms of cytotoxicity (Fig. 2).

The Uptake of Poly-L-lysine

Experiments with 12 μg/ml of [^{3}H]poly-L-lysine (a toxic dose) showed a rapid temperature-independent binding of the material during the first hour of incubation; thereafter there was no significant additional binding of [^{3}H]polylysine by the dead cells (Fig. 4 a). Autoradiographs demonstrated that the radioactivity in this case was located mainly in the nucleus and in remnants of the endoplasmic reticulum (Fig. 5). In contrast, when nontoxic amounts of polylysine (2 μg/ml) were added, uptake continued for 5 h (Fig. 4 a) and accounted for 1–2% of the polylysine in the medium. Uptake at this concentration was temperature dependent and inhibited by incubation at 4°C. Autoradiographs of cells incubated under these conditions showed labeling mainly over the cell surface as well as occasionally over cytoplasmic vacuoles (Fig. 6).

It should be noted that although only twice as much radiolabeled polylysine was taken up after a 1 h exposure of macrophages to a toxic concentration (12 μg/ml), as compared with a nontoxic concentration (2 μg/ml) of this compound, there was a fivefold excess of unlabeled polylysine in the former instance (Fig. 4 a). Hence, macrophages exposed to the toxic concentration bound 10-fold as much polylysine as those incubated with the compound at nontoxic concentrations.

Exposure of cells to 2 μg/ml of [^{3}H]poly-L-lysine for 1 h with subsequent incubation in medium without the polycation for up to 6 h showed no significant loss of cell-bound radioactivity (Fig. 4 b). In contrast, washout studies with toxic concentrations (12 μg/ml) showed the loss into the medium of 50% of the labeled polycation by 6 h.

The Effect of Poly-L-lysine on the Uptake of dsRNA

In the absence of polylysine, there was insignificant binding of [^{3}H]dsRNA to macrophages. However, addition of 2 μg/ml cold poly-L-lysine increased the uptake of dsRNA dramatically (Fig. 7). Toxic doses of polylysine also increased the uptake of [^{3}H]dsRNA, but the extent of this effect showed marked variability in terms of time-course and total amount of [^{3}H]dsRNA bound.

To determine whether the order in which polylysine and dsRNA were added to the cells influenced the binding of [^{3}H]dsRNA, the experiments described in Table I were performed. Macrophages preincubated for 30 min with 2
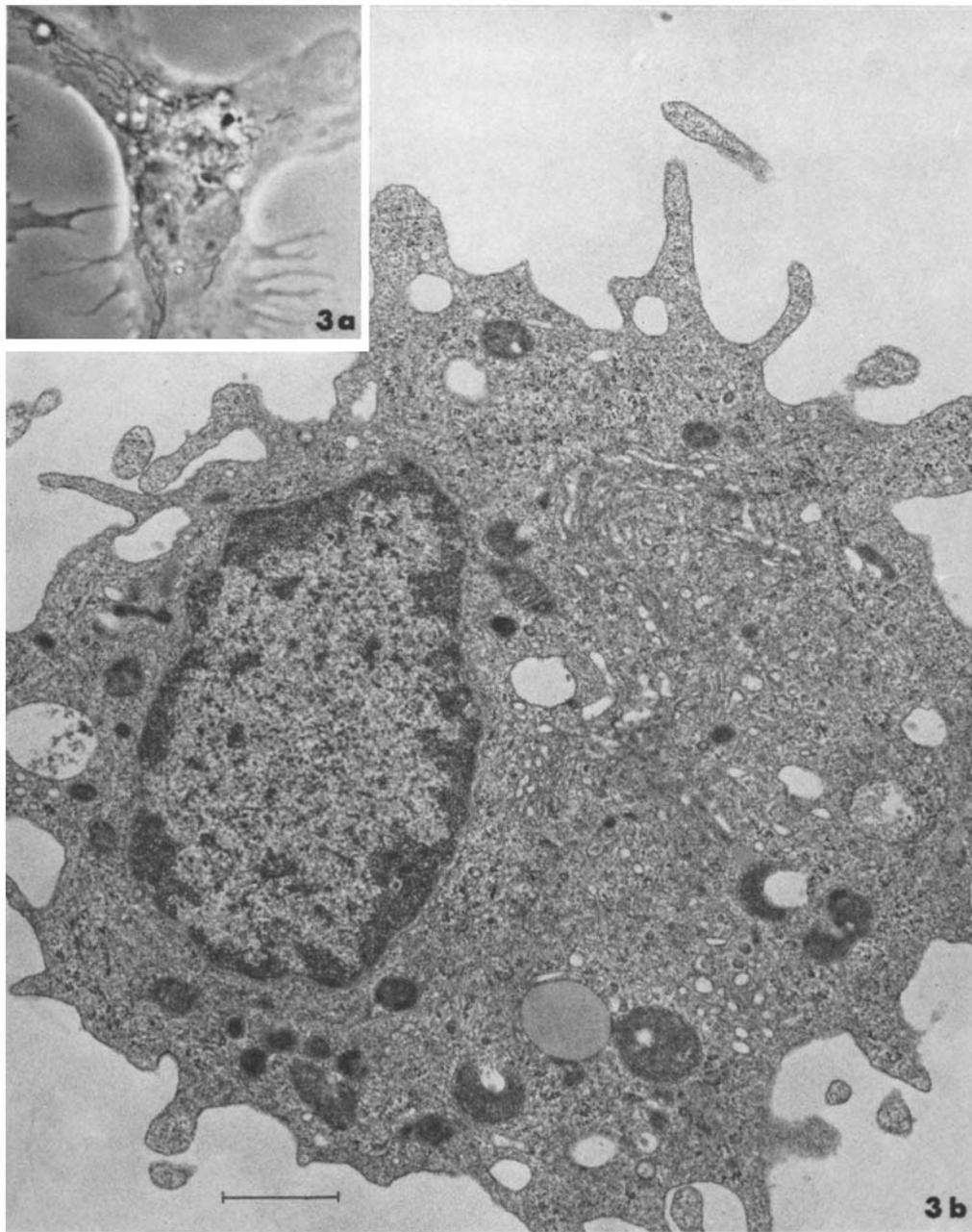
FIGURE 3 (a) Phase-contrast micrograph of macrophages after 6 h exposure to 2 μg/ml poly-L-lysine in medium 199 containing 1% NBCS. There are no signs of cell damage. × 1,500. (b) Electron micrograph from same experiment as Fig. 3 a. The macrophage is well preserved. × 16,250.

μg/ml polylysine bound as much [³H]dsRNA as cells incubated for a similar time period with both [³H]dsRNA and polylysine (Table I, lines a and c). Similar results were obtained with poly-L-arginine, poly-L-ornithine, and DEAE-dextran at concentrations of 2 μg/ml. However, the polylysine-stimulated binding of [³H]dsRNA was abolished by addition of poly IC before the addi-
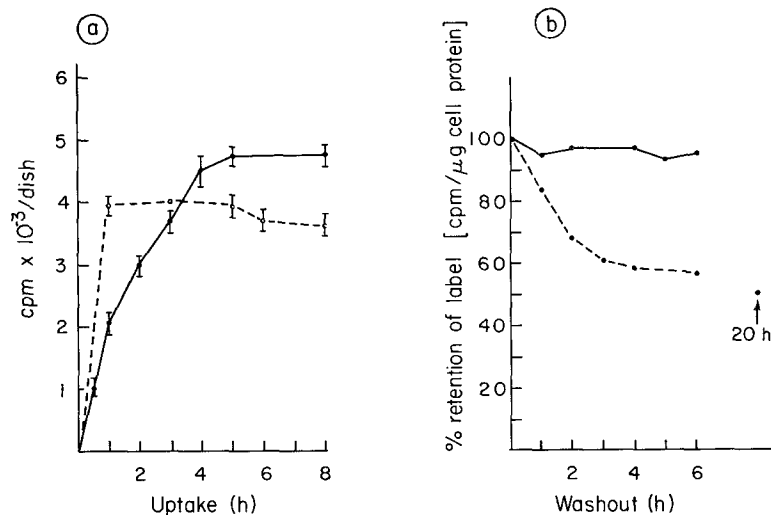
FIGURE 4 Uptake and stability of [³H]poly-L-lysine in macrophages. (a) Macrophage cultures were incubated with 2 μg/ml (nontoxic) or 12 μg/ml (toxic) poly-L-lysine for the indicated time periods and assayed for cell-bound radiolabel. ●——●, 2 μg/ml [³H]poly-L-lysine; O----O, 2 μg/ml [³H]poly-L-lysine + 10 μg/ml poly-L-lysine. (b) Macrophage cultures were incubated with 2 or 10 μg/ml of ³[H]poly-L-lysine for 1 h at 37°C. The plates were washed and incubated for the indicated time periods in medium 199 containing 1% NBCS. Cell-bound radiolabel was assayed as described in Methods. ●——●, 2 μg/ml [³H]poly-L-lysine; ●----●, 2 μg/ml [³H]poly-L-lysine + 10 μg/ml poly-L-lysine.

tion of [³H]dsRNA (Table I, line d). When the sequence of addition of the three compounds was reversed, i.e. preincubation with poly IC, followed by polylysine and then [³H]dsRNA, the results were similar to those obtained with polylysine alone (Table I, lines c and e). These data suggested that polylysine forms a stable complex with the cell surface and that it is the surface polylysine which complexes dsRNA and stimulates its uptake. To test this hypothesis macrophages were preincubated with unlabeled polylysine, rinsed, and incubated in the absence of polylysine. At suitable intervals the cells were exposed to [³H]dsRNA and the cell-bound radioactivity was measured. As indicated in Fig. 8, the enhanced capacity of macrophages pretreated with polylysine to bind [³H]dsRNA decayed rapidly and was completely abolished after a 4 h washout. To determine whether the decrease in the [³H]dsRNA-binding capacity of the cells was due to elution of polylysine into the medium or to ingestion of the polylysine by the cells, macrophages were assayed for their content of [³H]polylysine at intervals after a 30 min exposure to [³H]polylysine. Throughout the 6 h washout period there was no loss of cell-bound radioactivity. Evidence derived from other studies indicates that there was little or no hydrolysis of

poly-L-lysine after 4 h. Hence, it seems likely that the loss of [³H]dsRNA binding capacity reflects removal of polylysine from the cell surface by endocytosis.

The Uptake of Polycation-Polynucleotide Complexes

Our own experimental results, together with the data reported by others (25), suggested that dsRNA is taken up by macrophages as dsRNA-polycation complexes. We therefore formed such complexes in vitro by mixing polycations and polynucleotides. Some experiments were performed with poly-L-lysine-[³H]dsRNA complexes, but because of the relative scarcity of [³H]dsRNA most of the experiments were done with [³H]poly-L-lysine and poly IC. Complexes formed almost instantaneously at room temperature when solutions of poly-L-lysine and poly IC were mixed. These complexes could be sedimented by ultracentrifugation (Fig. 9). By increasing the ratio of poly IC to polylysine the formation of sedimentable complexes was enhanced (Fig. 9). When the pelleted complexes from these experiments were separated, resuspended, and added to macrophage cultures, their uptake was found to be proportional to the
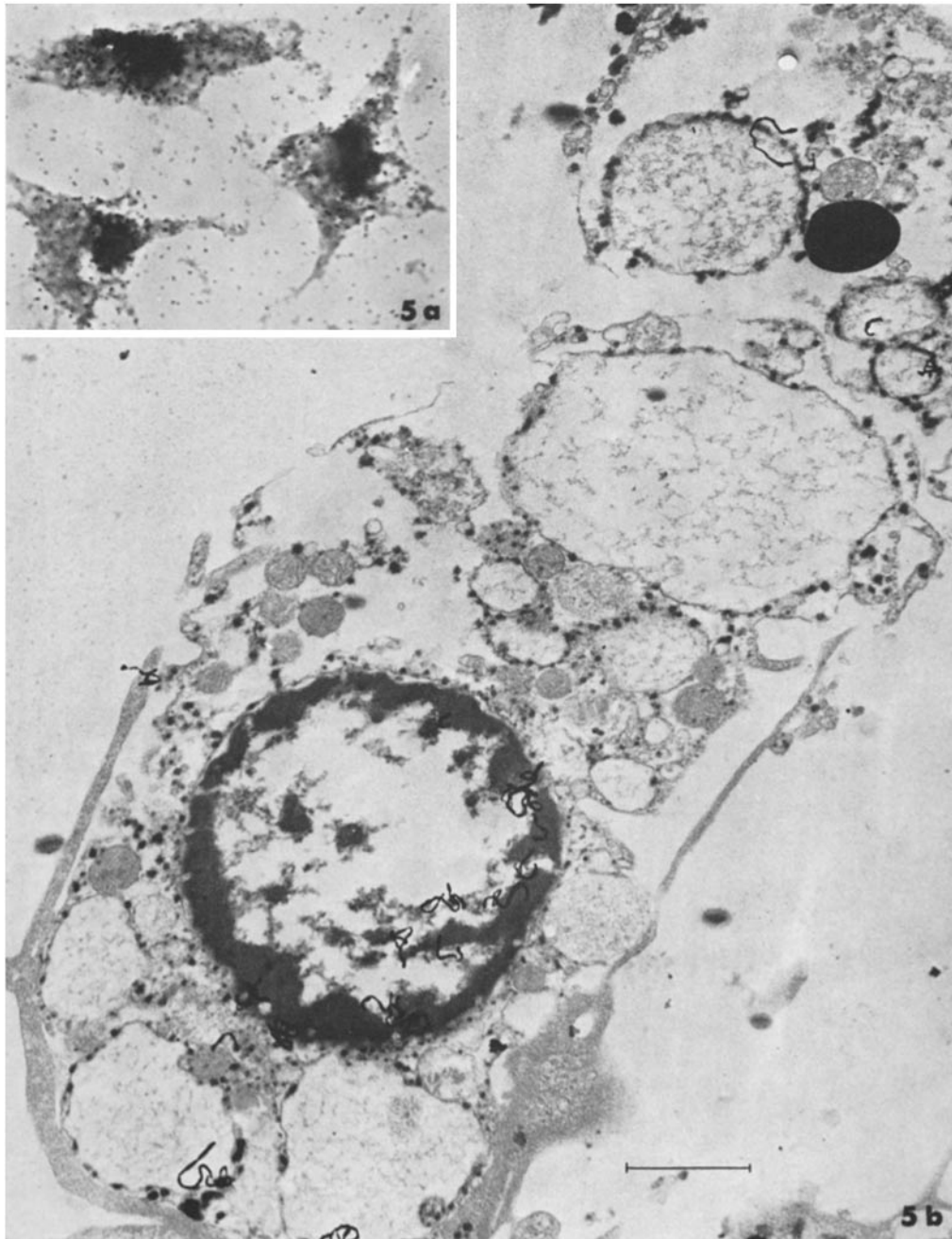
FIGURE 5   (a) Light microscopic autoradiograph of macrophages incubated with 10 μg of [³H]poly-ʟ-lysine for 1 h. Grains are seen over the cells as well as over the glass surface. × 1,500. (b) Electron microscopic autoradiograph from same experiment as Fig. 5 a. The cell is disintegrated. Grains are seen mainly over the nucleus as well as over remnants of endoplasmic reticulum. × 17,500.
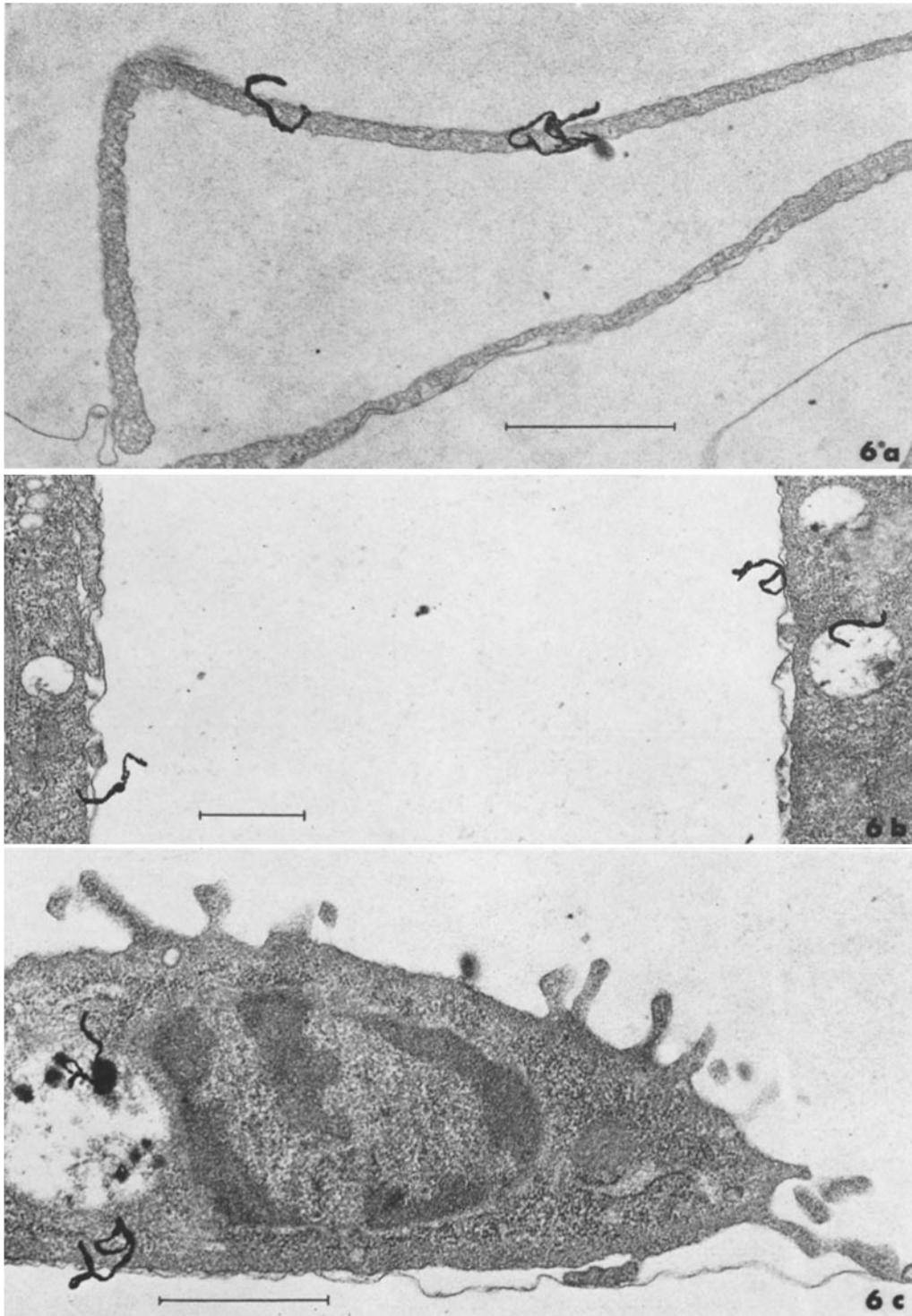
FIGURE 6 Electron microscopic autoradiographs after exposure of macrophages to nontoxic doses of radioactive poly-L-lysine (2 μg/ml). Grains are found mainly over or close to the cell surface (a), and less frequently over cytoplasmic vacuoles (b and c). (a) × 24,500; (b) × 15,750; (c) × 24,500.

Effect of Preincubation with Polycations and Polyanions on [³H]dsRNA Binding

	Order of addition*			Uptake [³H]dsRNA (% of control, line a)
	1st	2nd	3rd	
(a) None	None	Polylysine + [³H]dsRNA	100 ± 5
(b) None	None	[³H]dsRNA	10 ± 3
(c) None	Polylysine	[³H]dsRNA	100 ± 5
(d) Polylysine	Poly IC	[³H]dsRNA	15 ± 5
(e) Poly IC	Polylysine	[³H]dsRNA	110 ± 10

* Cells were incubated for 30 min at 37°C in medium 199 containing 1% NBCS and the compound to be tested. They were then washed in prewarmed medium and incubated for an additional 30 min with the second test compound, etc. Polylysine, 2 μg/ml; poly IC, 10 μg/ml; [³H]dsRNA, 0.37 μg/ml.
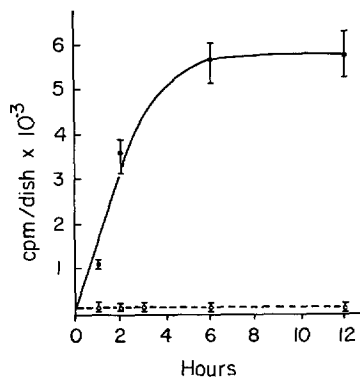

FIGURE 7 The uptake of [³H]dsRNA (0.37 μg/ml) in the presence (——) and absence (----) of nontoxic amounts of poly-L-lysine (2 μg/ml).
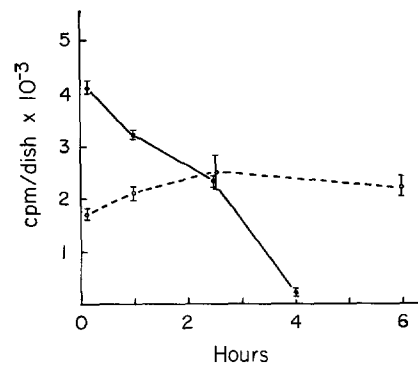

FIGURE 8 Change in [³H]dsRNA binding capacity of macrophage-polylysine complexes after removal of polylysine from the medium. Macrophage cultures were incubated with 2 μg/ml [³H]poly-L-lysine for 0.5 h. The cells were washed and assayed at the indicated time periods for cell-associated radiolabel (O----O). A parallel series of cultures was incubated with 2 μg/ml unlabeled poly-L-lysine for 0.5 h, washed, incubated with [³H]dsRNA (0.37 μg/ml) for 1 h at 37°C, and assayed for cell-associated radiolabel (●——●). Despite the complete retention of polylysine by the macrophages throughout the period examined, there is a rapid decrease in the capacity of the cells to bind [³H]dsRNA.

amount available to the macrophages (Fig. 10). The addition of poly-L-lysine or dsRNA to the preformed complexes did not change their uptake Phase-contrast micrographs of macrophages incubated in the presence of complexes showed the complexes as small refractile aggregates in the cytoplasm (Fig. 11 a). Autoradiography, confirmed that the radioactive complexes were present in membrane-bounded vacuoles in the macrophage cytoplasm (Fig. 11 b).

Fate of dsRNA

To determine the intracellular fate of the dsRNA, macrophages were incubated with [³H]dsRNA and polylysine and processed for light and electron microscopic autoradiography. Light microscopic autoradiography showed heavy labeling over the cytoplasm, especially over vacuoles in the perinuclear area (Fig. 12 a). This is the locus

of vacuoles derived from endocytic events. Electron microscopic autoradiography revealed that the label was located exclusively in vacuoles containing a granular material (Fig. 12 b). Extraction of the cells at this time confirmed that the label was still present in the form of [³H]dsRNA, as judged by its resistance to pancreatic ribonuclease digestion at high ionic strength and its susceptibility to digestion with this nuclease at low ionic strength (17, 18).
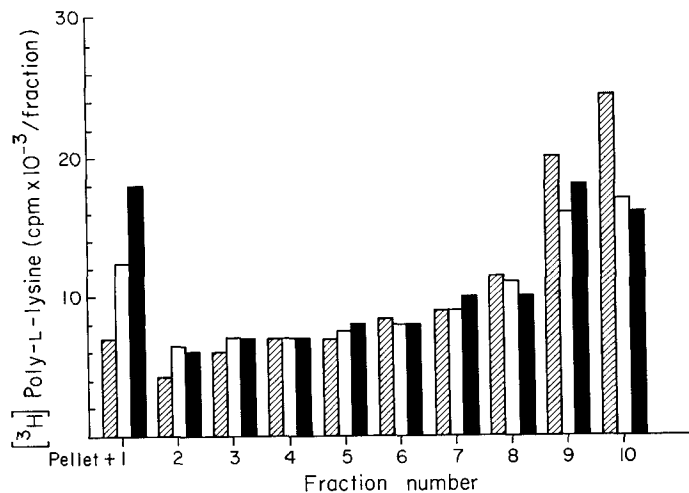
FIGURE 9 Sedimentation behavior of [³H]poly-L-lysine-dsRNA complexes. 40 μg of poly IC (hatched bars), 80 μg of poly IC (open bars), or 160 μg of poly IC (solid bars) were incubated with 4 μg [³H]poly-L-lysine for 12 h at 37°C. The samples were layered over 0.25 M sucrose in 0.1 M sodium phosphate, pH 7.4, and centrifuged for 4 h at 35,000 rpm in an SW 39 rotor. Increasing amounts of sedimentable-[³H]poly-L-lysine appear as the concentration of poly IC is increased.
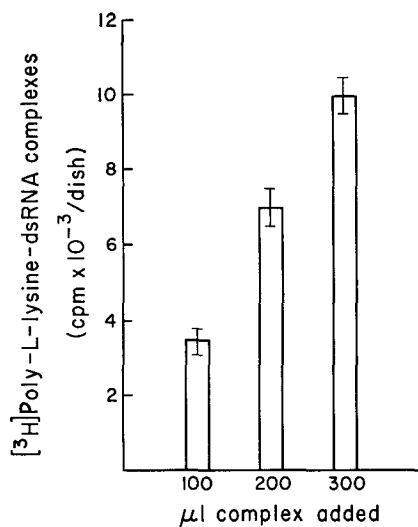

FIGURE 10 Uptake of [³H]poly-L-lysine-dsRNA complexes by macrophages. The uptake is proportional to the amounts of complexes available to the macrophages.

DISCUSSION

Polycations are markedly toxic for macrophages. In our experiments concentrations of poly-L-lysine (mol wt ~50,000), polyornithine, or DEAE-dextran (mol wt ~2 × 10⁶) in excess of 5 μg/ml caused loss of selective permeability, as judged by the inability of the cells to exclude vital dyes, lysis

of the cell membrane, and cell death. Dead cells bound about 10-fold more [³H]poly-L-lysine after a 1 h exposure than did viable ones (Fig. 4 a); in the former circumstance the radiolabeled polycation was bound to nuclei and endoplasmic reticulum (Fig. 5), while in the latter case it was localized exclusively on the plasma membrane and within endocytic vacuoles (Fig. 6). Thus the binding of polycations to cells, per se, cannot be used as a measure of biological activity and it is apparent that even a small proportion of damaged cells may give rise to spuriously increased uptake. These results cast doubt upon the biological significance of the reported entry of histones and other polycations (13, 14) into the nucleus of animal cells. Rather, it seems likely that polycations are merely reacting with nucleic acids and other anionic molecules in the nucleus and cytoplasm of damaged cells.

The absolute amount of polycation needed to alter cell viability is probably dependent upon several factors including the cell type, the degree of polymerization of the polycation, and the presence of other substances capable of reacting with the polycation, some of which may be present in serum. Cells can be protected from polycation-induced damage by poly IC or polyglutamic acid (5). This may explain why the high concentrations (25–1,000 μg/ml) of polycations used by some

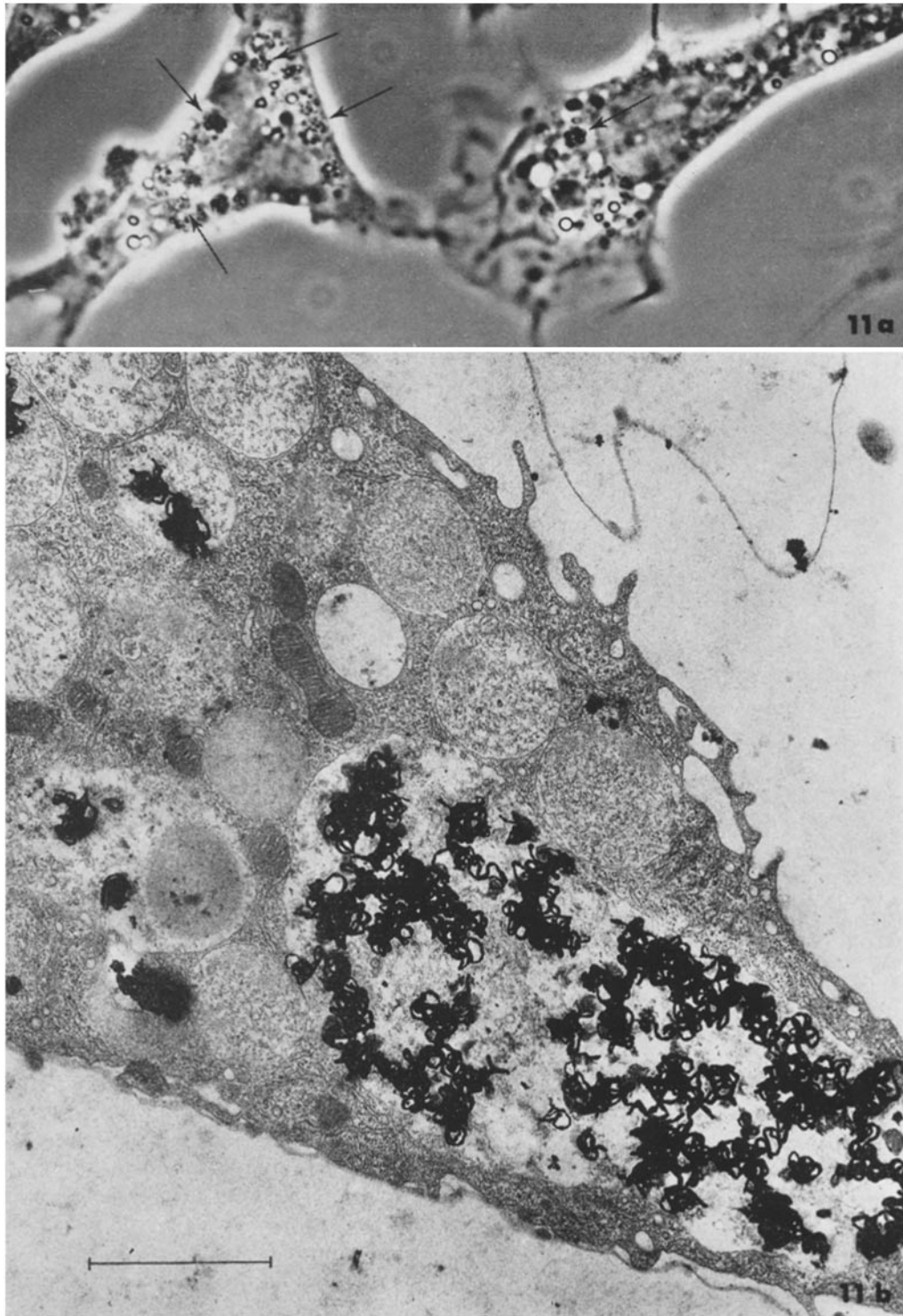FIGURE 11 (a) Phase-contrast micrograph displaying small aggregates of poly-L-lysine-dsRNA complexes taken up by macrophages after 6 h of exposure (arrows). × 1,500. (b) Electron microscopic autoradiograph demonstrating the localization of [³H]poly-L-lysine-dsRNA complexes after 6 h of incubation. The radioactivity is seen over several large vacuoles which contain granular material. × 27,000.

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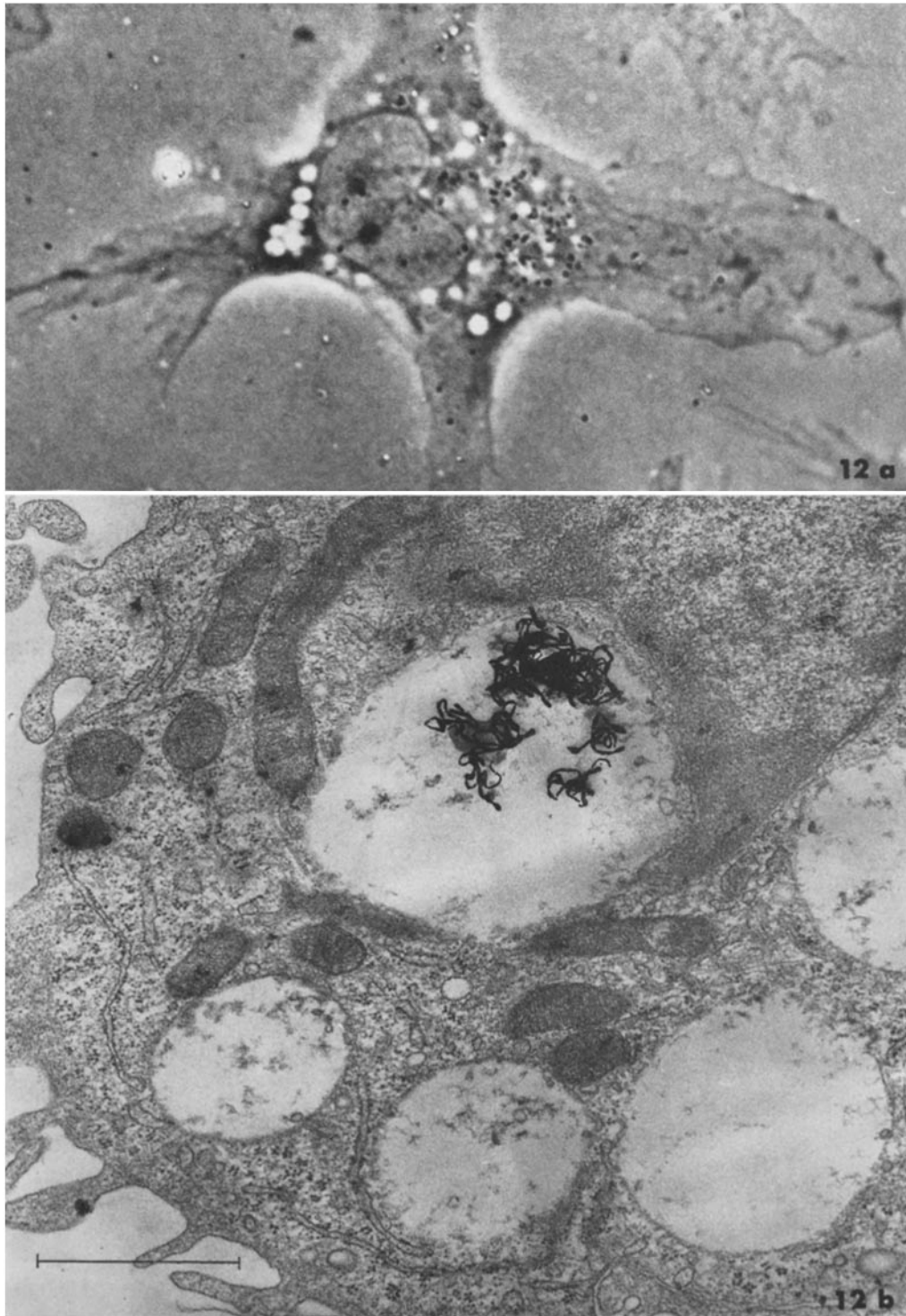
FIGURE 12 (a) Light microscopic autoradiograph of macrophages incubated for 6 h with 2 μg/ml poly-L-lysine and 0.75 μg/ml [³H]dsRNA. Many grains are seen over the cell, mainly in the peri-Golgi zone. There is no labeling above background over the nucleus or the remainder of the cell cytoplasm. × 3,750. (b) Electron microscopic autoradiograph from same experiment as Fig. 12 a. Grains are seen over a perinuclear vacuole containing granular material. Similar vacuoles located more peripherally in the cell are not labeled and do not contain the granular material seen in the labeled vacuole.

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investigators to stimulate nucleic acid uptake (2, 6, 11) and interferon production (8) have not destroyed the cells. Under some circumstances toxic levels of polycations may be responsible for the release of preformed interferon rather than for enhancing the capacity of dsRNA to induce interferon production.

The precise mechanisms by which polycations stimulate binding of nucleic acids and other polyanions to cells have not been rigorously defined. Polycations can serve as agglutinins, presumably by facilitating electrostatic interactions between the negatively charged surfaces of animal cells (26). It seems likely that polycations serve a similar function in binding polyanions to cell surfaces.

The particulate nature of the complex may also play a role in stimulating the uptake of dsRNA. In this regard, the complexing of soluble proteins with antibodies or their aggregation by heat results in a several thousand-fold increase in binding and uptake by macrophages (27). Soluble horseradish peroxidase, which does not bind to the macrophage plasma membrane, is pinocytized at a rate of 0.001 % of its extracellular *concentration* per hour. When complexed with IgG, it is interiorized 4,000-fold more rapidly and in proportion to the extracellular *load*. This is in keeping with the uptake of preformed polycation-polynucleotide complexes.

Cell surface-bound polycations are rapidly interiorized by endocytic mechanisms and become unavailable for subsequent binding of added polyanions (references 10, 11; Fig. 8). Competition experiments, such as those described in Table I, demonstrate that the polycation-stimulated uptake of [³H]dsRNA can be blocked by the addition of a competing polynucleotide, such as poly IC, before the addition of [³H]dsRNA. These data, taken together with morphological observations indicating that polycations do not alter the rate of pinocytosis in macrophages, clearly demonstrate that the polycation-stimulated uptake of dsRNA is not a reflection of increased membrane permeability or of a stimulated uptake of bulk fluids from the medium. Rather, they offer strong support for the hypothesis that polycations facilitate the binding of nucleic acids to the cell surface and that it is the polycation-polyanion complex which is removed from the surface by ongoing endocytic processes.

After endocytosis, dsRNA-polycation complexes were concentrated within the vacuolar system of the cell, presumably in secondary lysosomes.

Despite their exposure to the acid nucleases and proteases contained within this cellular compartment, both the [³H]dsRNA and the [³H]poly-L-lysine were conserved (Figs. 4, 8, 12) throughout the course of these experiments. Previous work (17) has demonstrated that the [³H]dsRNA used in these experiments is resistant to lysosomal nucleases, but the resistance of [³H]poly-L-lysine to hydrolysis by lysosomal proteases is surprising and remains unexplained.

Virtually all the endocytosed [³H]dsRNA was conserved within the vacuolar system, as judged by electron microscopic autoradiography. Thus these experiments offer no support for the hypothesis that nontoxic concentrations of polycations stimulate the transport of [³H]dsRNA into the cytoplasmic matrix. Rather, they argue strongly for the orthodox view which states that endocytosed macromolecules remain within the vacuolar system until they are degraded to nucleosides, oligosaccharides, or small peptides (28–30). Admittedly, we cannot rule out the possibility that even at nontoxic concentrations of polycations a small but biologically significant fraction of the [³H]dsRNA reaches the cytoplasmic matrix where it induces interferon production and cellular resistance to a variety of pathogens. Alternatively, it is possible that the biologically important event which triggers interferon production and/or release is the interaction of dsRNA with cellular membranes, and that polycations merely serve to facilitate this interaction; but it is also evident from studies with infectious viral nucleic acids that highly polymerized nucleic acids do enter the cytoplasmic matrix where they initiate the synthesis of progeny virions. In several cases (2, 11) the concentration of polycations required to maximally stimulate viral nucleic acid binding was substantially less than that required to stimulate maximal infectivity. Hence it seems likely that at low concentrations polycations enhance nucleic acid binding to the cell surface while at higher (perhaps minimally toxic) concentrations they facilitate the penetration of nucleic acids into the cytoplasmic matrix.

Whatever mechanism is involved, however, a note of caution seems in order. Polycations, single-stranded RNA (31), and dsRNA have been shown to cause cellular damage in vivo and in vitro, and recently interferon itself has been shown to sensitize cells to the toxic effects of dsRNA (32). Experiments designed to examine the biological effects of these macromolecules on cellular metab-

olism should rigorously exclude their possible cyto-
toxic effects, especially in the case of interferon,
when the parameter being measured is the failure
of cells to perform a given function such as support-
ing the replication of animal viruses.

REFERENCES

1. BISHOP, J. M., and G. KOCH. 1967. Purification
   and characterization of poliovirus-induced in-
   fectious double-stranded ribonucleic acid.
   J. Biol. Chem. **242:**1736.

2. WARDEN, D., and H. V. THORNE. 1969. Influ-
   ence of diethylaminoethyl-dextran on uptake
   and degradation of polyoma virus deoxyribo-
   nucleic acid by mouse embryo cells. *J. Virol.*
   4:380.

3. VOGT, P. V. 1967. DEAE-dextran: enhancement
   of cellular transformation induced by avian
   sarcoma viruses. *Virology.* **33:**175.

4. RYSER, H. J.-P., and R. HANCOCK. 1965. Histones
   and basic polyamino acids stimulate the
   uptake of albumin by tumor cells in culture.
   Science (Wash. D. C.). **150:**501.

5. AXLINE, S., and Z. A. COHN. 1970. In vitro
   induction of lysosomal enzymes by phago-
   cytosis. *J. Exp. Med.* **131:**1239.

6. PAGANO, J. S. 1970. Biologic activity of isolated
   viral nucleic acids. *Prog. Med. Virol.* **12:**1.

7. COLBY, C., and M. CHAMBERLIN. 1969. The
   specificity of interferon induction in chick
   embryo cells by helical RNA. *Proc. Natl.
   Acad. Sci. U.S.A.* **63:**160.

8. DIANZANI, F., P. CANTAGALLI, S. GAGNONI, and
   G. RITA. 1968. Effect of DEAE-dextran on
   production of interferon induced by synthetic
   double-stranded RNA in L-cell cultures. *Proc.
   Soc. Exp. Biol. Med.* **128:**708.

9. COLBY, C. 1970. The induction of interferon by
   natural and synthetic polynucleotides. *Prog.
   Nucleic Acid Res. Mol. Biol.* **11:**1.

10. WENTZKY, P., and G. KOCH. 1971. Influence of
    polycations on the interaction between polio-
    virus multistranded ribonucleic acid and
    HeLa cells. *J. Virol.* **8:**35.

11. WIEGERS, V. J., and G. KOCH. 1972. Interaction
    of poliovirus-induced double-stranded RNA
    with HeLa cells. *Arch. Biochem. Biophys.* **148:**
    89.

12. RYSER, H. J. P. 1967. Studies on protein uptake
    by isolated tumor cells. III. *J. Cell Biol.* **32:**737.

13. HANCOCK, R., and H. AMOS. 1968. Nuclear bind-
    ing of exogenous histones by L cells at low pH.
    J. Cell Biol. **36:**C1.

14. BECKER, F. F., and H. GREEN. 1969. The effects
    of protamines and histones on the nucleic acids
    of tumor cells. *Exp. Cell Res.* **19:**361.

15. SMITH, R. E., H. J. ZWEERINK, and W. K.
    JOKLIK. 1969. Polypeptide components of
    virions, top component and cores of reovirus
    type 3. *Virology.* **39:**791.

16. DULBECCO, R., and M. VOGT. 1954. Plaque
    formation and isolation of pure lines with
    poliomyelitis viruses. *J. Exp. Med.* **99:**167.

17. SILVERSTEIN, S. C., and S. DALES. 1968. The
    penetration of reovirus RNA and initiation of
    its genetic function in L-strain fibroblasts.
    J. Cell Biol. **36:**197.

18. SHATKIN, A. J. 1965. Inactivity of purified reo-
    virus RNA as a template for *E. coli* polymerases
    in vitro. Proc. Natl. Acad. Sci. U.S.A. **54:**1721.

19. COHN, Z. A., and B. BENSON. 1965. The dif-
    ferentiation of mononuclear phagocytes.
    Morphology, cytochemistry and biochemistry.
    J. Exp. Med. **121:**153.

20. SALPETER, M. M. 1966. *In* Methods in Cell
    Physiology. D. M. Prescott, editor. Academic
    Press, Inc., New York. 229.

21. VON GAUDECKER, B. 1967. RNA synthesis in the
    nucleolus of *Chironomus thummi* as studied by
    high resolution autoradiography. *Z. Zellforsch.
    Mikrosk. Anat.* **82:**536.

22. SELJELID, R., A. REITH, and V. F. NAKKER. 1970.
    The early phase of endocytosis in rat thyroid
    follicle cells. *Lab. Invest.* **23:**595.

23. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR,
    and R. J. RANDALL. 1951. Protein measure-
    ment with the Folin phenol method. *J. Biol.
    Chem.* **193:**265.

24. COHN, Z. A. 1966. The regulation of pinocytosis
    in mouse macrophages. I. Metabolic require-
    ments as defined by the use of inhibitors. *J.
    Exp. Med.* **124:**557.

25. HAVLIZA, D., and G. KOCH. 1971. Complex
    formation between poliovirus RNA and
    polycations. *Arch. Biochem. Biophys.* **147:**85.

26. KATCHALSKY, A., D. DANON, A. NEVO, and A.
    DE VRIES. 1959. Interactions of basic poly-
    electrolytes with the red blood cell. II. Agglu-
    tination of red blood cells by polymeric bases.
    Biochim. Biophys. Acta. **33:**120.

27. STEINMAN, R., and Z. A. COHN. 1972. The inter-
    action of particulate horseradish peroxidase
    (HRP)-anti HRP immune complexes with
    mouse peritoneal macrophages in vitro. *J.
    Cell Biol.* **55:**616.

28. DE DUVE, C., and R. WATTIAUX. 1966. Functions of lysosomes. *Annu. Rev. Physiol.* **28**:435.

29. COHN, Z. A., and B. A. EHRENREICH. 1969. The uptake, storage and intracellular hydrolysis of carbohydrates by macrophages. *J. Exp. Med.* **129**:201.

30. EHRENREICH, B. A., and Z. A. COHN. 1969. The fate of peptides pinocytosed by macrophages *in vitro*. *J. Exp. Med.* **129**:227.

31. KOCH, G., and J. M. BISHOP. 1968. The effect of polycations on the interaction of vival RNA with mammalian cells: studies on the infec-tivity of single- and double-stranded poliovirus RNA. *Virology.* **35**:9.

32. STEWART, W. E., II, E. DECLERCQ, A. BILLIAU, J. DESMYTER, and P. DESOMER. 1972. Increased susceptibility of cells treated with interferon to the toxicity of polyriboinosinic-polyribo-cytidylic acid. *Proc. Natl. Acad. Sci. U.S.A.* **69**:1851.

33. SELJELID, R., Z. A. COHN, and S. C. SILVERSTEIN. 1970. The influence of poly-L-lysine on the properties of cultivated macrophages. *J. Cell Biol.* **47**(2, Pt. 2):186a.