Cytoplasmic Vacuoles of Rous Virus Transformed Cells are Organelles Involved in Cation Uptake

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SUMMARY

Cytoplasmic vacuoles induced during transformation of cells by Bryan strain Rous sarcoma virus (RSV-BH) have been studied using the cationic dye, neutral red (NR). Both the rate of uptake and the accumulation of NR are greater in RSV-BH transformed cells than non-transformed cells. However, uptake was greater in vacuolated than in non-vacuolated cells, whether or not they were transformed. The NR was incorporated into pre-existing vacuoles in the absence of cytoplasmic staining, suggesting the existence of direct channels from the cell surface to the vacuoles. Other low mol. wt. cationic dyes could also be incorporated into vacuoles, although those with branched structures or cationic weights greater than 330 were excluded. No anionic dyes were incorporated.

Infection of cells with a virus mutant, RSV-BH-Ta, induces temperature-dependent vacuolization. After a shift to the vacuole-permissive temperature, vacuoles developed at different rates and with morphological variations with different cations. Vacuoles which had formed in the presence of several cations, (K⁺, Rb⁺, tris⁺, choline⁺) failed to disappear when cells were incubated at a temperature sufficient to revert vacuoles formed in Na⁺-containing medium. No short-term effects of Cl⁻ replacements (Br⁻, I⁻, or SO₄²⁻) on vacuolization or reversal were observed.

The results suggest that these vacuoles are organelles involved in cation uptake. A possible function for these organelles in RSV-BH induced malignancy is discussed.

INTRODUCTION

Cytoplasmic vacuoles are observed in a variety of cells in culture under a variety of conditions. Vacuolization is usually attributed to degenerative processes leading to the death of the cells. However, cells transformed by the Bryan strain of Rous sarcoma virus (RSV-BH) contain numerous vacuoles (DiStefano & Dougherty, 1965; Bader, Ray & Brown, 1974), yet grow and divide through many generations.

An earlier study showed that RSV-BH-induced vacuoles are in fact cytoplasmic organelles, bounded by membrane, and are composed largely of water (Bader & Bader, 1976). No large molecules were detectable in the vacuoles. A number of isolated observations suggested to us that the organelles forming the vacuoles may have a function in maintaining cellular cation levels: (a) vacuolated RSV-BH transformed cells accumulate more water than non-transformed cells (Bader et al. 1974), and monovalent cations are commonly associated with water in physiological processes; (b) of all the components in defined cellular growth medium, only Na⁺ was found essential to the development of vacuoles (Bader & Bader,
1974; 1976); and (c) exposure of vacuolated cells to NR, a cationic dye (Fig. 1), results in the selective incorporation of the dye into vacuoles (Bader & Bader, 1976). This latter observation was examined more carefully and the results are presented here. These studies indicate that the organelles forming the vacuoles have a specific function in cation uptake.

METHODS

**Virus.** The Bryan 'high titre' strain of Rous sarcoma virus (RSV-BH), a transforming virus, is mixed with Rous-associated virus (RAV1), a subgroup A non-transforming avian leukemia virus. The mutant RSV-BH-Ta, also called tdBE1BH, transforms infected cells at 37 °C, but when shifted to 41 °C, these infected cells revert to normal phenotype (Bader, 1972). RSV-BH-Ta stocks also contain RAV1, but the presence of this virus has no effect on the transformation process. Another transforming virus, the Schmidt-Ruppin strain of Rous sarcoma virus (RSV-SR), transforms chick embryo cells to a morphologically different phenotype than ESV-BH or RSV-BH-Ta.

**Cells and media.** Chick embryo cells were prepared from 10-day-old embryos, and replated at 2- to 3-day intervals. Cultures were grown in Eagle's minimum essential medium (MEM) supplemented with dextrose (2 g/1 final concentration), sodium pyruvate (5 mM), 10% tryptose phosphate broth (Difco), 5% foetal bovine serum, penicillin (50 units/ml), streptomycin (50 µg/ml), tylosine (50 µg/ml), and gentamycin (20 µg/ml). Cultures were maintained in humidified, CO2-atmosphere incubators at 41, 39 or 37 °C, as dictated by individual experiments.

Cells were infected (2 to 10 focus-forming units per cell) as secondary cultures within 24 h after transfer, and redistributed at 2- to 3-day intervals thereafter. Transformation was evident in RSV-BH or RSV-BH-Ta infected cells within a day after infection, and practically all cells in infected cultures were transformed within 6 days after infection. Infected cultures were used for experiments on the second day after replating before cells became confluent.

**Neutral red.** Neutral red (NR) was diluted from a stock 1% solution into Earle's balanced salt solution (BSS) or Dulbecco's phosphate-buffered saline (PBS), depending on the nature of the experiment. The dye-containing solution was passed through a 0.22 µm filter before use. The usual concentration of NR used in uptake studies was 1 mM. The dye was assayed by its absorption at 530 µm at low pH (< 5.0). After exposure of cells to NR, the cells were rinsed with cold PBS, and 1 ml of NaOH (0.1 N) was added. When cells had disintegrated, samples were removed for protein analysis (Lowry et al. 1951) and 0.1 N-HCl was added to the remainder to dissolve NR aggregates, and to produce the intense red colour analysed by absorption.

**Chemical treatments.** Glutaraldehyde was diluted to 2% (v/v) in cacodylate buffer (pH 6.5). Triton X-100 was diluted to 0.1% (v/v) in PBS.
RESULTS

Incorporation of neutral red into vacuoles

Chick embryo (CE) cells transformed by the virus, RSV-BH, contain many cytoplasmic vacuoles, while few vacuoles are apparent in non-transformed cells (Bader et al. 1974). When NR is added to the cell cultures, the dye is incorporated within a few minutes into the vacuoles of the transformed cells (Fig. 2a and b). Lesser amounts of stain are found in small perinuclear areas of the non-transformed CE cells (Fig. 2c and d). In both cell types, the NR is restricted to the vacuoles and the small perinuclear areas. No stain is found in nuclei, which are clearly outlined in transformed cells. Cytoplasm, except for the vacuoles and small areas, is devoid of stain.
Fig. 3. Incorporation of neutral red into pre-existing vacuoles. (a) Phase contrast micrograph of vacuolated cells before addition of NR. (b) Non-phase micrograph of same field. (c) Non-phase micrograph 10 min after addition of NR. (d) Non-phase micrograph of another region of vacuolated cells before addition of NR. (e) 1 min after addition of NR; (f) 2 min later; (g) 10 min after addition of NR.
### Vacuoles of Rous transformed cells

#### Table 1. Concentration of neutral red by vacuolated cells*

<table>
<thead>
<tr>
<th>Extracellular volume</th>
<th>Extracellular nmol</th>
<th>Intracellular nmol</th>
<th>Approximate concentration factor†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>11</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>12</td>
<td>5.9</td>
</tr>
<tr>
<td>3</td>
<td>200</td>
<td>12</td>
<td>9.2</td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>200</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>12</td>
<td>200</td>
<td>16</td>
<td>25</td>
</tr>
</tbody>
</table>

* Vacuolated, RSV-BH transformed cells were exposed (at $t_0$) to 200 nmol of NR in the indicated volumes of PBS. Cultures were agitated at room temperature (21 °C) for 6 h. Extracellular fluids were collected, the cells were rinsed with cold PBS before lysing, and NR was assayed (at $t_0$).

† The approximate aqueous volume of cellular vacuoles per culture was calculated: assuming $5.1 \times 10^{-9}$ ml/cell, about 90% of which is water (Bader et al. 1974) = $4.6 \times 10^{-9}$ ml water/cell, of which approx. 50% is in vacuoles = $2.3 \times 10^{-9}$ ml water/cell in vacuoles, × $2.2 \times 10^6$ cells per culture = $5.1 \times 10^{-2}$ ml in intravacuolar water per culture. The concentration factor is the concentration of NR in vacuolar water divided by the concentration of extracellular NR remaining after 6 h.

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**Fig. 4.** Uptake of NR into vacuolated RSV-EH transformed (▲, △) and non-vacuolated (●, ○) CE cells. Cells were exposed to 1 mM-NR in Eagle’s MEM at 39 °C. At the indicated time periods, culture fluids were removed, the cells were washed, and incorporated NR was determined (▲, ●). After 60 min several cultures were rinsed in cold PBS, Eagle's MEM was added, and the cultures were returned to the incubator. At 20 min intervals thereafter, intracellular NR was determined as a measure of the efflux of NR from the cells (△, ○).
Formation of new vacuoles in RSV-BH transformed cells occurs slowly, and is incompatible with the formation of new vacuoles during incorporation of stain. Furthermore, the NR appeared to be incorporated into pre-existing vacuoles. To confirm this, individual cells were photographed before and after addition of NR. Many pre-existing vacuoles became stained (Fig. 3), and no newly arising stained vacuoles were evident. Nonetheless, after a short incubation (10 min) with NR, many cells still contained unstained vacuoles, suggesting that some vacuoles were less readily accessible than others to the dye.

Incorporation of NR into pre-existing vacuoles in the absence of cytoplasmic staining suggested that either the vacuoles had a high binding affinity for NR, or the stain entered directly into the vacuoles without being exposed to cytoplasmic constituents. Cytoplasm contains macromolecules, particularly RNA and some proteins, which might be expected to bind NR, while in earlier studies a variety of staining procedures failed to detect macromolecules in vacuoles (Bader & Bader, 1976). To examine this point further, cells were exposed to NR for a few minutes to allow staining of the vacuoles. The dye then was removed and the cells were treated with glutaraldehyde to increase membrane permeability. The NR diffused out of the vacuoles, and both cytoplasm and nuclei became stained. To varying extents, similar effects were observed after treating stained cells with hydrochloric acid (0.1 N), trichloracetic acid (0.1 N), Triton X-100 (0.1 %, v/v), or water. In all cases, previously unstained cytoplasm and nuclei acquired a red hue after the release of NR from the vacuoles. These results suggested that NR in exogenous medium entered vacuoles through channels without being exposed to the cytoplasm.

Concentration of neutral red in vacuoles

The notion of a direct channel from the extracellular milieu to vacuoles further suggested that vacuolar contents could be in equilibrium with the medium. This was tested by adding a constant amount of NR (200 nmol) in differing volumes of PBS and incubating for several hours. The dye remaining in the medium, and that incorporated into the cells, was assayed (Table 1). Practically all of the NR was removed from the medium and was found to be concentrated intracellularly in the vacuoles. By estimating the approximate water content of the cells (Bader et al. 1974), it could be calculated that the NR was concentrated nearly 25000-fold in the culture containing the largest solvent volume (12 ml).

Increased rate of uptake in vacuolated cells

The staining of vacuoles could be attributed to either an increased rate of uptake of NR in vacuolated compared to non-vacuolated cells, or to an increased accumulation in vacuolated cells of NR taken up at the same rates by vacuolated and non-vacuolated cells. Comparison of non-vacuolated chick embryo cells and the same cells transformed by RSV-BH showed that both the rate of uptake of NR and its accumulation were greater in the vacuolated cells (Fig. 4). The exogenous NR was removed from some cultures after 1 h, and the loss of dye from the cells during the next hour was determined (Fig. 4). The total decrease was similar in vacuolated and non-vacuolated cells, indicating that both cell types contained a low-affinity, reversible binder for NR. However, most of the NR incorporated into vacuolated cells was retained, and microscopic examination revealed no loss of stain from the vacuoles.

Although these observations were made in cells transformed by RSV-BH, vacuolization, not virus transformation per se, is responsible for the increased rate of NR uptake. Cells transformed by another strain of RSV, RSV-SR, are usually non-vacuolated, and take up NR in a manner similar to non-transformed chick embryo cells (Fig. 5). These cells can be
Vacuoles of Rous transformed cells

Fig. 5. Uptake of NR into vacuolated (△—△) and non-vacuolated (●—●) CE cells transformed by RSV-SR. Cells were exposed to 1 mM-NR in Eagle’s MEM at 39 °C. At the indicated time periods, culture fluids were removed, the cells were washed, and incorporated NR was determined.

Table 2. Uptake of neutral red into non-transformed CE cells*

<table>
<thead>
<tr>
<th>Temperature of culture maintenance (°C)</th>
<th>Vacuoles</th>
<th>Dye incorporated (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>41</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>37</td>
<td>+</td>
<td>96</td>
</tr>
</tbody>
</table>

* Non-transformed CE cells were maintained for 4 days without serum at 41 or 37 °C. Neutral red was added and cultures were incubated at 39 °C for 5 min.

induced to vacuolate by removal of serum from the growth medium, and such treatment resulted in an increased capacity for uptake of the dye (Fig. 5).

Also, non-transformed chick embryo cells can be induced to vacuolate by exposing the cells to serum-free medium at 37 °C for several days. Similar cells maintained at 41 °C remain non-vacuolated. These non-transformed vacuolated cells took up NR at a greater rate than non-vacuolated cells (Table 2).

Dependence of uptake on dye concentration

Neutral red at various concentrations was added to vacuolated RSV-BH cells and incorporation was measured. Above 4 mM no increase in the rate of dye uptake was observed indicating that the channels for uptake were saturated at these levels. However, even at
Table 3. Incorporation of cationic stains into vacuoles*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ionic mol. wt</th>
<th>Ionic charge</th>
<th>Vacuolar staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysoidin Y</td>
<td>214</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Thionin</td>
<td>229</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Neutral red</td>
<td>254</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acridine orange</td>
<td>267</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methyl red</td>
<td>268</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Auramine O</td>
<td>269</td>
<td>+</td>
<td>±†</td>
</tr>
<tr>
<td>Azure B</td>
<td>271</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>285</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Basic fuchsin</td>
<td>303</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Safranin</td>
<td>315</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Alizarin red S</td>
<td>319</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Bismarck brown Y</td>
<td>320</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Methylene green</td>
<td>330</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>Malachite green</td>
<td>330</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Metanil yellow</td>
<td>352</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>373</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Methyl green</td>
<td>387</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>444</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Phloxin B</td>
<td>784</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Trypan blue</td>
<td>869</td>
<td>–</td>
<td>0</td>
</tr>
</tbody>
</table>

* All compounds were dissolved at 1 mM in 0.15 M-NaCl, 0.01 M-tris-HCl, and the solutions adjusted to pH 7.4. The solutions were added to vacuolated RSV-BH transformed cells, which then were maintained at room temperature or at 39 °C. The cultures were examined periodically up to 1 h while the stain was present, and again after removal of the stain.

† ±, Weak but definite selective staining of vacuoles.

saturating concentrations for the rate of uptake, incorporation of dye increased with extended exposure, demonstrating that the capacity of the vacuoles for dye had not been saturated.

**Temperature, energy and pH**

Vacuolated transformed cells were equilibrated at various temperatures, then exposed to NR. Uptake rates during 30 min at 37 and 41 °C were essentially identical. Uptake at 5 °C proceeded at a rate approximately two-thirds that at 41 or 37 °C, and at 21 °C uptake was about three-quarters that of the higher temperatures. The substantial rate of uptake at 5 °C suggests that NR enters the vacuoles by diffusion, and argues against a pinocytotic mechanism.

A requirement for energy was examined by depleting cultures of glucose and other metabolites for 24 h before addition of the dye. This treatment was insufficient to prevent uptake of dye. Pre-treatment of cells for 1 h with dinitrophenol (1 mM) had no effect on NR uptake.

Uptake of NR increased slightly with increasing pH over the physiological range (pH 6.8 to 7.8), a consistent observation in several experiments. This effect of pH can probably be attributed to a general effect of pH on cell surfaces as indicated in other types of experiments (Bader & Bader, 1976; Bader, Lew & Brown, 1976).

**Other dyes**

A number of other dyes were examined for their ability to stain vacuoles under physiological conditions. In general, only cationic, basic molecules of ionic mol. wt. less than 330 were incorporated into vacuoles (Table 3). Azure B (mol. wt. 271) and acridine orange (mol. wt. 267) stained as avidly as neutral red (mol. wt. 254), and auramine O (mol. wt. 269)
Vacuoles of Rous transformed cells

at an equivalent concentration has a less intense colour, but also stained vacuoles. Methylene blue (mol. wt. 285) and methylene green (mol. wt. 330) have general structures similar to the above dyes, but were incorporated to a lesser extent than the smaller molecules. Bismarck brown Y (mol. wt. 320) is dibasic and stains vacuoles weakly. Neither chrysoidin Y (mol. wt. 214) nor thionin (mol. wt. 229) were incorporated into vacuoles. Other cationic dyes of mol. wt. higher than 330, or with branched structures, failed to stain the vacuoles, and none of the anionic dyes tested were incorporated into vacuoles.

Crystal violet (cationic, mol. wt. 373) was taken into cells, as was the anionic dye, phloxin B, but these stains were distributed throughout the cells, in contrast to those segregated in vacuoles.

Ionic requirement for vacuolization

Earlier studies had shown that Na⁺ was the only component of the cellular growth medium required for the development and disappearance of vacuoles (Bader & Bader, 1974, 1976). Cells infected with a mutant of RSV-BH, RSV-BH-Ta, are non-vacuolated at 41 °C, but become vacuolated after shifting to 37 °C (Bader, 1972). In order to examine specific ion requirements for vacuolization, solutions of various cation chlorides (buffered with 10 mM-tris-HCl to pH 7.4) were added to mutant-infected cells before shifting from 41 to 37 °C. Except for Li⁺, all these cations allowed the development of vacuoles, although in several cases vacuoles failed to disappear when cultures were shifted back to 41 °C. To summarize these results: (a) K⁺ – vacuolization was delayed compared to Na⁺, and vacuoles failed to disappear when cells later were shifted from 37 to 41 °C. (b) Rb⁺ – appearance of vacuoles was indistinguishable from cultures containing K⁺. (c) Cs⁺ – similar to K⁺ during first hour, but notably toxic thereafter. (d) Mg²⁺ – vacuolization was more extensive than with Na⁺, but the cells succumbed with extended exposure, and reversal could not be examined. (e) Ca²⁺ – vacuolization was slightly less than with Na⁺, and vacuoles disappeared with reversal of temperatures. Cells treated with Ca²⁺ acquired a long 'stretched out' morphology. (f) Tris⁺ – many obvious vacuoles appeared more rapidly than with Na⁺, and, failing to disappear, were very prominent after cells were shifted to 41 °C. (g) Choline⁺ – development of vacuoles was similar to cultures with tris⁺, although vacuoles were less refractile than tris⁺-exposed cells after shifting to 41 °C.

The role of anion in vacuolization also was examined, using Na⁺ as the only cation. Substitution of Br⁻, I⁻, or SO₄²⁻ for Cl⁻ had little effect on the morphology or rate of appearance of vacuoles in RSV-BH-Ta cells. Likewise, after shifting from 41 to 37 °C vacuoles which had developed in the presence of Br⁻, I⁻, or SO₄²⁻ disappeared when the cultures were returned to 41 °C. Thus, in contrast to the notable differences in the vacuolization process seen with different cations, differing anions had little effect. It should be noted, however, that extended exposure (longer than 6 h) to Cl⁻ substitutes resulted in gradual loss of cell viability, as observed from the subsequent growth of cells after replacement of the cation test medium with growth medium (cells exposed to 0.15 M-NaCl alone for 2 days recovered without obvious detriment).

Attempts to isolate vacuoles

Heavily vacuolated cells were homogenized in an attempt to release vacuoles for their eventual isolation and purification. No free vacuoles were detected after a variety of homogenization procedures using PBS, and other high Na⁺, low K⁺, buffers, with or without divalent cations. Increasing K⁺ (to 150 mM) and decreasing Na⁺ (to 10 mM) in the homogenization buffer to mimic cytoplasmic cation concentrations failed to dissociate free
vacuoles. When vacuoles were stained with NR prior to mild homogenization, some of the
dye was released during homogenization, but most was retained within amorphous membran-
ous pieces. These observations suggest that vacuoles, although appearing microscopically as
spherical membrane vesicles, develop as part of the membrane network.

DISCUSSION

Cytoplasmic vacuoles in cells transformed by RSV-BH, or in cells maintained without
serum, incorporate NR from the exogenous medium. Neutral red is selectively incorporated
into pre-existing vacuoles, in the absence of any significant staining of cytoplasm. The
rapidity of entry of NR into vacuoles, as well as the concentration of dye from the medium,
argue against a pinocytotic mechanism for incorporation of the NR, and we suggest that
NR enters the vacuoles through channels connecting the vacuoles with the cell surface.

Although appearing as refractile spheres in the cytoplasm, vacuoles could not be isolated
as such. The retention of NR within an amorphous membranous mass after cellular homo-
genization, suggests that many vacuoles are connected in an intracellular membranous net-
work. Whether each vacuole has its special channel to the surface, or whether some vacuoles
have no channels to the surface, but only channels to other vacuoles, remains to be resolved.
We favour the latter possibility.

Vacuolar contents are neither in equilibrium with the extracellular medium, nor freely
accessible to all extracellular solutes. Cells removed NR from the culture medium, and
concentrated it in vacuoles. The concentration cannot be attributed to ionic or steric binding
to large intravacuolar molecules, since no substance capable of binding NR, or any other
stain, could be detected in vacuoles (Bader & Bader, 1976).

Other cationic stains similar in structure to NR also were incorporated into vacuoles.
However, cationic molecules of mol. wt. higher than 330, or with branched structures, were
excluded, as well as all of the anionic dyes tested. This discrimination suggests that the
channel to the vacuoles is selective for cations below a certain steric size.

In cultures where Na+ was replaced by other cations, vacuolization developed after a
temperature shift of RSV-BH-Ta infected cells. The single exception occurred with Li+ as
the Na+ substitute. These results can be explained by assuming that all of the cations entered
the membrane-bound organelles which were to develop into vacuoles. However, only Li+
readily permeated the membrane, diffusing into the cytoplasm. Other cations, including Na+,
were restricted in their permeability, and, binding water, distended the organelles into ob-
servable vacuoles.

Vacuolization can be reversed in this system by placing cells at a higher temperature
Vacuoles disappeared when Na+ was the major cation, but vacuoles formed with most other
cations failed to diminish. It seems likely that the larger cations were unable to permeate
the vacuolar membrane, and remained trapped in the organelle.

If the increased incorporation of NR into vacuolated cells is an indication of a generalized
increase in capacity for cation uptake, one would expect uptake of cations from the growth
medium to increase as well. This, in fact, is the case. In other experiments, the rate of Na+
uptake was greater in vacuolated transformed cells than in non-vacuolated cells (Harris &
Bader, unpublished data).

Organelles incorporating acridine orange (Weissman & Gilgen, 1956; Robbins, Marcus &
Gonata, 1964), or NR (Ogawa, Mizunu & Okamoto, 1961; Allison & Mallucci, 1965), have
been studied, and shown to contain acid phosphatase, suggesting that the organelles were
lysosomes. Experiments examining the acid phosphatase activity of vacuoles and vacuolar
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membranes are in progress. Whatever the outcome, the presence or absence of acid phosphatase is insufficient information to describe a physiological role for vacuoles.

We suggest that the vacuoles observed in RSV-BH transformed cells, and in a variety of other cells under certain conditions, are organelles involved in the uptake of cations, especially Na\(^+\), from the extracellular medium. Channels direct the cations to the vacuoles, where the cations become concentrated, increasing their availability to the cell, or to a particular area of the cell. Cytoplasmic organelles in non-vacuolated cells also incorporate NR, albeit to a lesser degree than the prominent vacuoles. These organelles may serve a similar function to cation uptake, but by restriction of the channel, and/or decreased capacity, limit the amount of cation accessible to the cell. Vacuolar organelles are most likely to affect the uptake of Na\(^+\), since Na\(^+\) is the most abundant cation in physiological solutions. Other large cations may be less able to permeate the vacuolar membrane, and the flux of other cations may be more critically regulated by active transport processes.

The physiological role of vacuoles in the malignant potential induced by RSV-BH is a matter of special interest. Increased intracellular concentrations of Na\(^+\) could affect the extent or specificity of transcription, and be responsible for the failure of these transformed cells to respond to physiological regulatory pressures. The occurrence of vacuoles in non-transformed cells demonstrates that vacuolization per se is insufficient to make a cell malignant. These cases, however, are usually accompanied or preceded by growth-limiting treatments, and physiological changes related to vacuolization have not been studied.

REFERENCES


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