J. Hyg., Camb. (1973), **71**, 515
With 4 plates
Printed in Great Britain

Interactions of TRIC agents with macrophages and BHK-21 cells observed by electron microscopy

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(*Received* 18 *January* 1973)

SUMMARY

TRIC agents do not multiply in mouse peritoneal macrophages in culture but have a toxic effect on them, whereas they multiply readily in BHK-21 cells. Sections of macrophages and of BHK-21 cells fixed during the first 90 min after inoculation were examined by electron microscopy. Macrophages ingested all forms of the organism, which were eventually degraded in lysosomes. However, elementary bodies were distinguished from other TRIC particles by the delay in their transfer to lysosomes. BHK-21 cells ingested elementary bodies selectively, and in these cells the organisms were neither found in lysosomes nor degraded. Instead they showed morphological changes that probably represented an early stage of development.

INTRODUCTION

The agents of trachoma and inclusion conjunctivitis (TRIC agents) multiply in various kinds of cell in culture but generally cause little or no cytopathic effect. However, if mouse peritoneal macrophages *in vitro* ingest even a small number of infective organisms per cell, the macrophages are killed (Taverne & Blyth, 1971). Such destruction of macrophages may contribute not only to the fatal effects of large doses of TRIC organisms given intravenously to mice but perhaps also to the cell damage that is seen in the conjunctiva in the natural disease, trachoma. It is likely that the toxic effect of TRIC organisms on macrophages is mediated through the lysosomal system, since it is accompanied by changes in the distribution of the lysosomal enzyme acid phosphatase(Taverne, Blyth & Ballard in preparation).

This report describes the events observed by electron microscopy in macrophages that have ingested TRIC organisms. The study was undertaken to determine the relationship of ingested organisms to the systems of cytoplasmic vacuoles of macrophages. A comparison is made with the early events observed after infection of BHK-21 cells, which support the multiplication of the organisms.

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METHODS

Cell culture

Methods for BHK-21 cells and for macrophages, together with infectivity titrations in BHK-21 cells, have been described (Taverne & Blyth, 1971).

Macrophages

These were obtained from the peritoneal cavity of mice of strain CS-1 without artificially stimulating the production of exudate.

TRIC agents

Although preliminary observations were made on cell cultures inoculated with material from infected yolk sacs, the results reported here were obtained from experiments with suspensions made from infected BHK-21 cells. Macrophage cultures were inoculated with strain HAR-2*f* (Taverne, Blyth & Reeve, 1964). For the inoculum BHK-21 monolayers infected 44 hr. earlier with a yolk-sac pool were disrupted, incubated with 0·5% trypsin for 30 min. at 37° C. and centrifuged at 8000 ***g*** for 20 min. The pellet was resuspended in phosphate buffered saline (pH 7·4) containing 0·25 M sucrose and 10 % calf serum and was stored at −70° C. The suspension contained $2{\cdot}4 \times 10^9$ inclusion-forming units/ml. For the experiment with BHK-21 cells, a suspension of strain MRC-4*f* (Taverne *et al.* 1964) was kindly supplied by Miss M. Harrison of the Trachoma Unit. It contained $6{\cdot}0 \times 10^8$ inclusion-forming units/ml. and $2{\cdot}0 \times 10^9$ total TRIC organisms/ml. (counted by the dark-ground technique of Reeve & Taverne, 1962).

Latex balls

These were obtained from Serva Entwicklungslabor, Heidelberg, and were 0·357 μm. in diameter.

Inoculation of cultures

Overnight cultures of BHK-21 cells or macrophages were inoculated with 1 ml. of TRIC suspension containing about 200 infective organisms per cell and centrifuged at 1000 ***g*** for 10 min. at 35° C. The medium was then changed and the cultures were returned to an atmosphere of 5% CO_2 at 35° C.

Electron microscopy

After removal of the medium, groups of 6 cultures were fixed for 30 min. at 0° C. with cacodylate buffer containing 0·3% OsO_4 and 1·7% glutaraldehyde (Hirsch & Fedorko, 1968). The cultures were rinsed with two changes of ice-cold 0·1 M sodium acetate at 5 min. intervals, and were then stained with 0·25% uranyl acetate for 30 min. at 0° C. After two further rinses the cells were scraped from the cover-slips and collected by filtration on 0·8 μm. membrane filters (Sartorius no. 11304). The cells were overlaid with a drop of agar solution and cooled on ice.

The filter disks carrying the cells were dehydrated in isopropanol which was replaced with xylene and then infiltrated with Araldite in which they were embedded. Thin sections were stained with lead citrate (Venable & Coggeshall, 1965).

Demonstration of acid phosphatase activity

The cultures were fixed in buffered glutaraldehyde, incubated in Gomori's lead nitrate-glycerophosphate medium and post-fixed with osmium tetroxide. After the cells had been scraped from the coverslips, dehydration and embedding were carried out as above.

RESULTS

Morphological composition of infective suspensions

Samples of suspensions used to inoculate cell cultures were deposited by centrifugation and sections of the deposits were examined in the electron microscope. In addition to TRIC organisms the suspensions contained cellular debris, which was more abundant in specimens from yolk-sac material than in those from BHK-21 cells. All the developmental forms of TRIC organisms, ranging from large reticulate bodies (RB) to small, easily recognized elementary bodies (EB) were present in both kinds of suspension.

Classification of TRIC particles

Preliminary experiments suggested that the intracellular fate of ingested particles depended, among other things, on their nature. This suggestion was investigated by localizing, identifying and counting the various types of particle within cells.

Although the population of TRIC organisms was made up of a continuous range of morphological forms, it was arbitrarily divided into three groups, namely EBs, RBs and condensed bodies (CBs) (Plate 1). This differentiation between the classes was necessarily subjective; it depended both on size (in relation to the estimated level of section through the particles) and on structure. Small intermediate forms that contained many ribosomes and no dark centre (nucleoid) were grouped with RBs, whereas those of a similar size whose contents showed definite condensation or few ribosomes were grouped with EBs. Condensed bodies were recognized to be a type of TRIC particle after it was found that there was a continuous series of forms from slightly distorted EBs that were a little denser than normal to severely shrunken and extremely dense particles (Plate 1*d*, *e*).

RBs and EBs were subdivided according to their condition; those considered to be imperfect are referred to as 'sick', for the sake of brevity. This separation was based on an assessment of such characters as shape, continuity of membranes and their relationship to the cytoplasm, structure of the cytoplasm and the amount of apparently empty space within the organism (Plate 1).

Entry into macrophages

In macrophages fixed immediately after the inoculation period many organisms were already enclosed within cytoplasmic vacuoles. Because inoculation involved centrifugation for 10 min., some of these organisms may have been inside the cell for most of this time. Other organisms were in the process of entering the cell, and many were found in a layer of inoculum deposited on the cell surface.

Particles entered the cell by phagocytosis. In many instances single organisms

were enfolded by small cytoplasmic processes so that they were transferred into the cell within a closely fitting membrane, accompanied by a minimum quantity of fluid (Plate 2*a*, *b*, *c*). All types of TRIC particle could enter singly and even a mitochondrion from the inoculum was found closely invested within a cytoplasmic vacuole, apparently after entering the cell in this way. In other instances larger cytoplasmic processes surrounded a portion of the deposited inoculum, enclosing a group of organisms of various types together with fluid and cell debris or yolk granules (Plate 2*d*). As a result, vacuoles containing a mixture of different types of material were carried into the cytoplasm.

Fate of organisms within macrophages

Two types of cytoplasmic vacuoles contained TRIC organisms. First, there were large irregularly shaped vacuoles that contained many particles of different sorts (Plate 3*a*). Although some of these vacuoles were the result of the phagocytosis of varied material in a single mass, some contained, in addition to TRIC particles, amorphous material identical with that found in vacuoles in uninoculated cells (Plate 3*c*). Its presence suggested that fusion had occurred between vacuoles containing TRIC particles and pre-existing lysosomes. That some of these vacuoles were lysosomes was confirmed by the Gomori procedure for demonstrating acid phosphatase activity (Plate 3*b*).

Other vacuoles contained one, or occasionally two, TRIC particles within a tightly investing membrane (Plate 4). The isolated particles were assumed to have entered singly. By analogy with the classification used for vacuoles enclosing ingested *Histoplasma capsulatum* (Dumont & Robert, 1970) we refer to the close-fitting vacuoles as 'tight' (T) vacuoles. Although there appeared to be a clear distinction between the two types of vacuole, a few were found that did not fit precisely into either category. These were round and larger than T vacuoles; they contained a single EB, or a single sick EB, surrounded by fluid and were most common in specimens fixed 40 or 80 min. after inoculation (Plate 3*d*). Since they contained only one organism they were classified as T vacuoles. As entry into BHK-21 cells appears to be entirely into T vacuoles (see below) special attention was directed to T vacuoles in macrophages and a quantitative survey was made of the fate of the TRIC particles within them. In the experiment selected for analysis, latex balls equal in number to infective organisms were added to the inoculum so that the reaction of the macrophage to TRIC particles and to a more inert particle could be compared. At various times after inoculation the particles were classified according to the system already described, and those within T vacuoles were distinguished from those in other vacuoles. For the sake of precision in classification the rare vacuoles containing more than one particle but otherwise identical with T vacuoles were not classified with them but were included with the other vacuoles.

All types of TRIC particles and latex balls were found within the cells at all times (Table 1). An indication of the reliability of the counts is given by the fact that throughout the period of observation the ratio of small TRIC particles (EBs, sick EBs and CBs taken together) to large particles (RBs and sick RBs) remained

Table 1. *TRIC particles counted in macrophages at intervals after inoculation**

Type of particle	TRIC pellet†	0 min.		20 min.		40 min.		80 min.	
		L	T	L	T	L	T	L	T
EB	28	16	10	37	14	18	15	3	4
CB	20	27	18	10	5	31	6	27	3
Sick EB	26	22	10	70	8	59	4	66	6
RB	67	0	10	5	1	1	2	1	0
Sick RB	14	52	7	51	3	66	2	56	0
Total TRIC	155	117	55	173	31	175	29	153	13
Latex balls	—	12	4	27	1	12	1	22	2

L: in vacuoles that resembled lysosomes. T: in 'tight' (T) vacuoles.

* Although approximately the same number of cell sections were examined at each time the volume of cytoplasm surveyed was not constant. Direct comparison between different times is therefore not valid.

† Numbers of particles in a representative inoculum (before addition of latex balls).

relatively constant. (No conversion of EBs into RBs should have occurred within 80 min. since this process requires at least 4 hr., even in a cell which supports the multiplication of TRIC organisms.) Immediately after inoculation about a third of all organisms were present in numerous T vacuoles; this proportion and the absolute number of T vacuoles decreased with time.

Direct comparison of the numbers of each class of TRIC particle in samples of macrophages taken at different times is not valid since, although about the same number of cell sections were examined in each case, their thickness inevitably varied. This difficulty cannot immediately be overcome by converting the numbers to proportions. For instance, suppose that the number of EBs in T vacuoles is expressed as the proportion of all EBs counted in that sample. The changes observed in this proportion with time would not simply reflect an altered relationship between EBs and T vacuoles, but could be affected by changes in the number of EBs resulting from, say, their conversion to sick EBs, if this occurred predominantly in one type of vacuole.

If all particles had an equal chance of being found in T vacuoles, then the number of particles of a particular class in T vacuoles, expressed as the proportion of all particles found in these vacuoles (the 'observed' proportion) would approximate to the total number in that class expressed as a proportion of all intracellular particles, the latter being an estimate of the 'expected' proportion. The ratio of the observed proportion of each class found in T vacuoles to its expected proportion gives a measure by which the behaviour of the different classes can be compared. This ratio allows valid comparisons to be made both within one sample and between different samples. A ratio greater than 1 indicates that more particles of that class were found in T vacuoles than would be expected if the relationship of all types of particles to T vacuoles was the same.

The proportions of various types of particle in T vacuoles at various times are compared with the proportions in both types of vacuole taken together in Fig. 1. Each pair of columns represents the counts of intracellular particles at a particular time after inoculation. The left-hand column represents the proportions of each

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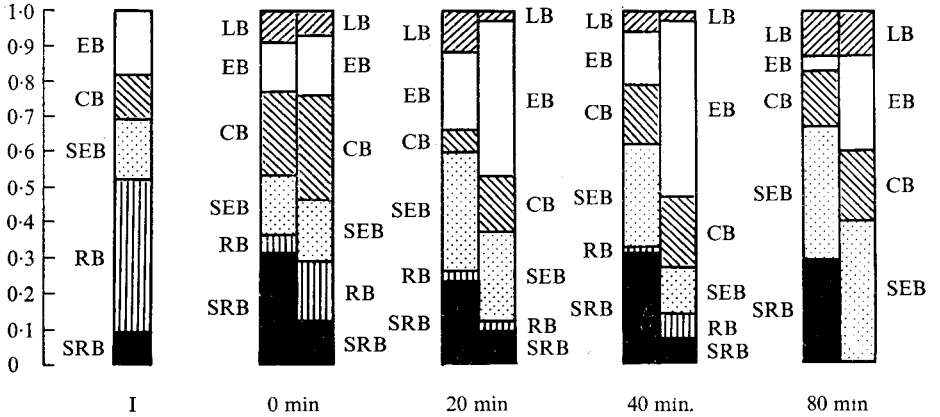

Fig. 1. The proportions of different types of TRIC particles in macrophages at intervals after inoculation. The single column marked I shows the proportions in a representative inoculum before the addition of latex balls. The left-hand column of each pair represents the proportions in all vacuoles at each time; the right-hand column shows those in 'tight' (T) vacuoles. The proportions are derived from the numbers in Table 1. EB, Elementary body; CB, condensed body; SEB, sick elementary body; RB, reticulate body; SRB, sick reticulate body; LB, latex balls.

Table 2. *The ratio of the observed number of particles in T vacuoles to the expected number, assuming random distribution between all vacuoles*

	0 min.	20 min.	40 min.	80 min.
EB	1·15	1·41*	1·68*	1·88*
CB	1·19	1·51	1·09	1·13
Sick EB	0·99	0·83	0·59	1·03
RB	∞ *	1·12	1·86	0·00
Sick RB	0·46*	0·54	0·32*	0·00*
Latex balls	0·84	0·37	0·67	1·00

* These ratios differ significantly from 1·00 ($P < 0·05$) using the χ^2 test with Yates's correction.

type of particle in all vacuoles and the single column on the extreme left shows the morphological composition of a representative suspension of organisms without latex balls. Thus the composition of the inoculum can be compared with that of the intracellular particles at different times after inoculation. The right-hand column of each pair shows the proportions in T vacuoles only. If all classes of particle had the same chance of being in T vacuoles the proportions shown in the left-hand column should provide an estimate of those to be expected in the right-hand column, and the columns should match.

The ratio of the height of a segment in the right-hand column to the height of the corresponding segment in its left-hand neighbour is the measure of behaviour referred to above. To aid comparison between different classes of particles at different times this ratio is tabulated (Table 2). When the numbers of intracellular particles in each class and the proportion in T vacuoles were analysed the following points emerged:

There were always more normal EBs in T vacuoles than expected and this

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Table 3. *TRIC particles counted in BHK-21 cells immediately after inoculation and 80 min. later**

Type of particle	TRIC pellet†	0 min.	80 min.
EB	30	36	51
CB	19	31	13
Sick EB	16	5	7
RB	28	1	3
Sick RB	40	0	1
Total TRIC	133	73	75
Latex balls	—	0	0

* All particles were in T vacuoles. Direct comparison between counts at different times is not valid (see note to Table 1).

† The numbers of particles in the inoculum (before the addition of latex balls).

discrepancy increased with time. Condensed bodies were the only other particles that were always found in T vacuoles more frequently than expected. Immediately after inoculation the number of EBs and of sick EBs expressed as a proportion of the total particles found in the cells compared well with that in the inoculum, but by 80 min. the proportion of normal EBs was noticeably decreased, whereas that of sick EBs was increased (Fig. 1). The ratio of EBs to sick EBs decreased continuously with time. Thus normal EBs were apparently being replaced by 'sick' ones.

By far the greater proportion of RBs in the cells were classified as 'sick' and were found in lysosomes. Normal RBs were seldom found intracellularly except immediately after inoculation when they were all in T vacuoles.

Somewhat fewer latex balls than normal EBs were seen within cells, although the number added had been calculated to equal the number of infective EBs. However, it is probable that some EBs classified as 'normal' were not infective, so that this result does not imply that EBs and latex balls were ingested with differing efficiency. The great majority of the latex balls were in lysosomes.

Entry into BHK-21 cells

In BHK-21 cells fixed immediately after the inoculation period small TRIC particles, both EBs and CBs, were readily found either in the process of entering the cell or within cytoplasmic vacuoles. Although reticulate bodies were abundant in the inoculum (Plate 1*a*) and were observed outside infected cells, few were found in cytoplasmic vacuoles (Table 3; Fig. 2) and most of these contained an early nucleoid, indicating that they were probably forms intermediate between EBs and RBs. Debris from the inoculum was seldom phagocytosed by BHK-21 cells, and although the inoculum contained latex particles equal in number to infective particles, none were found intracellularly.

The surface of BHK-21 cells was smooth in comparison with that of macrophages and all particles apparently entered singly; phagocytosis of groups of particles by means of large cell processes was not observed.

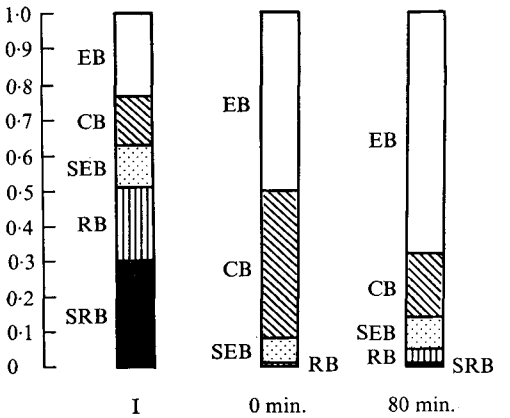

Fig. 2. The proportions of different types of TRIC particles in BHK-21 cells at intervals after inoculation. The column marked I shows the proportions in the inoculum before the addition of latex balls. The remaining columns represent the proportions in the cells, at each time; all the particles were in 'tight' (T) vacuoles. No latex balls were found within BHK-21 cells in this experiment. The proportions are derived from the numbers in Table 3. For abbreviations see Fig. 1.

Fate of organisms within BHK-21 cells

At the two times when samples were taken, TRIC particles or their residues were not found in lysosomes. However, lysosome-like vacuoles were not common in our BHK-21 cells, whether or not the cells were infected. Cytoplasmic vacuoles containing TRIC particles were of only one type; they contained one (or in rare instances, two) TRIC particles within a tightly investing membrane (T vacuoles) (Plate 4*a*, *b*). Sometimes the vacuoles also contained a few small irregular vesicles. Immediately after inoculation the vacuoles were situated at the periphery of the cell, but 80 min. later groups were found concentrated to one side of the nucleus. They lay in or near a zone relatively free from organelles and containing intracellular fibrils and polyribosomes, adjacent to the Golgi apparatus. Nearly all the EBs were classified as healthy, but in many of them the cell wall had separated from the cytoplasmic membrane leaving an electron-transparent zone surrounding the cytoplasm, which itself appeared normal (Plate 4*b*). Thus the overall diameter of the organism increased while that of its cytoplasm remained unaltered. Quantitative analysis demonstrated that immediately after inoculation a higher proportion of the intracellular particles were CBs than 80 min. later (Table 3; Fig. 2). At the earlier time a few vacuoles containing EBs or CBs were surrounded by small vesicles about 50 nm. in diameter, some of which were fusing with (or budding from) the vacuolar membrane. These vesicles resembled the small Golgi vesicles implicated in the synthesis and concentration of secretion products. After 80 min. more of the vacuoles that contained TRIC particles were associated with small vesicles, and more vesicles surrounded each vacuole (Plate 4*b*).

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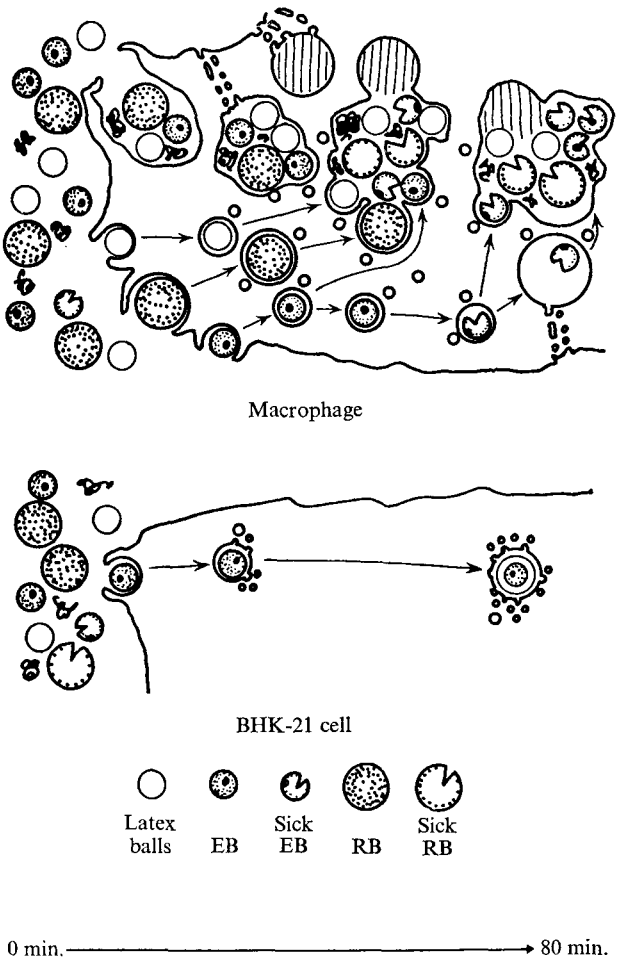

Fig. 3. Diagram of the sequence of events in macrophages and BHK-21 cells following inoculation with TRIC and other particles.

DISCUSSION

A diagram showing our interpretation of the sequence of events during and after entry of TRIC organisms into macrophages and BHK-21 cells is presented (Fig. 3); the main features which distinguish the reactions of macrophages to these organisms from the reactions of BHK-21 cells are listed (Table 4). One of the most striking contrasts is that macrophages rapidly ingested all kinds of particles whereas BHK-21 cells ingested EBs and CBs selectively to a remarkable degree. Conditions of culture can of course materially alter the efficiency with which cells ingest particles, but it is unlikely that the different experimental conditions used for handling macrophages and BHK-21 cells would account for the differences in phagocytic ability observed here.

Both kinds of cell take in particles singly. For macrophages this is the commonest form of entry, and their lower selectivity results primarily from their ability to

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Table 4. *Differences between macrophages and BHK-21 cells in their interactions with TRIC organisms*

Macrophages	BHK-21 cells
All types of particle enter by phagocytosis	EBs (and CBs) enter by selective phagocytosis
Organisms and other particles enter singly and in groups	Organisms enter singly
TRIC organisms found singly in close-fitting vacuoles, or with other material in phagolysosomes	TRIC organisms found singly in close-fitting vacuoles only
EBs degraded in lysosomes	EBs neither transferred to lysosomes nor degraded
Small vesicles do not accumulate around close-fitting vacuoles containing single TRIC organisms	Small vesicles accumulate around and fuse with, vacuoles containing TRIC organisms
Organism does not show early signs of development	Wall of TRIC organism separates from its plasma membrane (early development?)
Organisms do not multiply	Organisms multiply
Organisms are toxic to the cells	Organisms are not toxic to the cells

ingest a wider range of particles in this way. In addition macrophages, unlike BHK-21 cells, ingest particles in groups, a type of entry that is obviously not selective. BHK-21 cells ingested latex balls much less efficiently than EBs and CBs (although 131 EBs and CBs were counted in the cells, no latex balls were found) whereas macrophages ingested EBs and latex balls equally well. However, latex balls can enter BHK-21 cells in some circumstances (unpublished experiments). It is clear that both EBs and CBs have a specific stimulating effect on phagocytosis which, at least in BHK-21 cells, is superior to that possessed by either RBs or latex balls equal in size to EBs. Since this property cannot be due solely to the size of the particles, it must reside in some physicochemical property of their external surface.

That both EBs and CBs are ingested by BHK-21 cells in preference to other types of particle and that once inside the cell both are treated in the same way – both by macrophages and by BHK-21 cells – supports our recognition on morphological grounds that they are closely related. It is possible that CBs are damaged EBs that are not viable, but it seems more likely that they represent a proportion of EBs that react differently to fixation so that osmotic changes cause more distortion and shrinkage. If, in addition, EBs become more permeable to solutes used in preparation for electron microscopy during the early stages of development so that fewer shrink, then the observation that the ratio of CBs to EBs in BHK-21 cells decreases with time (Table 3) supports the hypothesis that some CBs are viable EBs. A similar phenomenon can be seen during the developmental cycle of *Rickettsiella melolonthae* where some of the mature organisms, when observed by electron microscopy, appeared as denser and smaller forms than the multiplying

organism (Devauchelle, Meynadier & Vago, 1972). Again, when Gram-negative bacteria are prepared for electron microscopy, some organisms appear dense with convoluted membranes while others from the same culture appear larger with a smooth profile.

Once within the cells, the main difference between the response of macrophages and that of BHK-21 cells to TRIC organisms is that EBs and CBs are transferred into lysosomes in the former but not in the latter. This is demonstrated by the presence within macrophages of degraded EBs in vacuoles, some of which were shown to contain a lysosomal enzyme. In these cells, the ratio of EBs to sick EBs decreases with time but in BHK-21 cells it remains constant over the same time interval and no degraded EBs are found in lysosome-like vacuoles.

It is usually assumed that the fate of a particle after phagocytosis is to enter a digestive vacuole, but there are exceptions to this rule. Intracellular parasites must escape digestion within their host cell either because they are resistant to the action of lysosomal enzymes or because they avoid contact with them. Both these mechanisms of resistance exist among the *Mycobacteria*. For instance, *M. lepraemurium* is clearly resistant to the action of the enzymes since it multiplies within lysosomes (Brown & Draper, 1970); indeed its multiplication in rat fibroblasts was enhanced when the conditions of culture increased lysosomal activity (Brown, Draper & D'Arcy Hart, 1969). By contrast, living organisms of *M. tuberculosis* strain H37Rv multiplied in macrophages within vacuoles that did not fuse with lysosomes, although damaged organisms entered lysosomes and were digested (Armstrong & D'Arcy Hart, 1971). Rickettsiae avoid contact with lysosomal enzymes in one of two ways: either, like *Rickettsia sennetsu* and *Rickettsiella melolonthae*, they multiply in vacuoles that remain separate from lysosomes (Anderson, Hopps, Barile & Berheim, 1965; Devauchelle *et al.* 1972), or, like *Rickettsia prowazeki* and *Rickettsia rickettsii*, they escape into the cytoplasm of the host cell (Anderson *et al.* 1965). In macrophages parasitized by the protozoon *Toxoplasma gondii*, two populations of organisms were seen: about half the organisms degenerated in typical phagocytic vacuoles containing acid phosphatase, whereas the rest were enclosed in vacuoles that contained no acid phosphatase, remained morphologically normal and eventually divided (Jones & Hirsch, 1972).

TRIC organisms escape digestion in BHK-21 cells because the vacuole in which they multiply is not a lysosome. Other Chlamydia also appear to prevent the transfer of lysosomal materials to phagocytic vacuoles as was shown by Friis (1972) for the meningopneumonitis agent in L cells. Either the TRIC agent actively inhibits fusion of its vacuole with lysosomes or it lacks a factor that stimulates fusion. This failure of fusion in BHK-21 cells is unlikely to result solely from the lower concentration of lysosomes in the host cell cytoplasm (thus decreasing the probability of collision) because vacuoles containing TRIC particles accumulate near the nucleus where they are close to lysosomes and to the Golgi apparatus, which is the source of vesicles containing the acid hydrolases for the lysosomal system.

Close examination of our results reveals that, in macrophages, EBs persist longer in T vacuoles than other types of particle. For instance, RBs were trans-

ferred to lysosomes and degraded so rapidly that normal RBs were rarely found intracellularly. Thus, as in BHK-21 cells, EBs are treated differently from other particles, since their entry into lysosomes is delayed; even so the majority of EBs are transferred to lysosomes and degraded within 80 min. of entry.

The absence of multiplication of TRIC organisms in macrophages may be associated with their destruction in lysosomes, but why they enter lysosomes in macrophages but not in BHK-21 cells is obscure. The simplest hypothesis, that vacuoles containing the organism escape fusion in BHK-21 cells because lysosomes are rare, is unlikely for the reasons given above. Another possibility is that the organism begins to develop in T vacuoles within macrophages as it does in BHK-21 cells but that in macrophages the development is abortive and as a result the vacuoles fuse with lysosomes. The enlarged T vacuoles containing a single EB may result from fusion of pinocytotic vesicles that contain ingested culture medium with the T vacuole, and would thus represent the first stage of the recruitment of the vacuole into the lysosomal system. Two changes were observed in infected BHK-21 cells 80 min. after inoculation that were never seen in macrophages at any time; one concerned the morphology of EBs, the other the interaction of the host cell cytoplasm with T vacuoles containing EBs. The separation of the cell wall from the cell membrane of developing EBs was seen only rarely at the end of the inoculation period, affected most particles by 80 min., and was no longer evident 6 hr. after inoculation (unpublished observations). The electron-transparent zone seems likely to result from the expansion of the cell wall of the organism and its appearance may be associated with the alteration in permeability of EBs that occurs (at least in the case of the meningopneumonitis agent growing in L cells) within one hour after the end of the infection period (Tamura, 1971).

The small vesicles clustered around T vacuoles containing EBs also appear to be associated with the development of the organism; they do not contain acid phosphatase (unpublished observations). In macrophages T vacuoles were never surrounded by these small vesicles although larger vesicles were common throughout the cytoplasm.

The morphological events reported here suggest that entry of EBs into lysosomes is the key to their toxic effect on macrophages, which probably results from a reaction between infective EBs and the lysosomes in which they lie. Again, entry into lysosomes is likely to be the essential step required for inactivation of the organism. By contrast, EBs never enter lysosomes in BHK-21 cells so that no toxic effect occurs and the organism remains free to multiply.

We would like to thank Mrs Anne Martin, Mrs Anne Fitzpatrick and Mr Ronald Ballard for their excellent technical assistance.

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EXPLANATION OF PLATES

Trachoma particles and vacuoles are classified according to the scheme described in the text. EB, Elementary body; CB, condensed body; RB, reticulate body; T vacuole, 'tight' vacuole.

Plate 1

(*a*) A representative micrograph of a pellet of an inoculum prepared from BHK-21 cells infected with TRIC organisms. Arrows indicate forms intermediate between RBs and EBs.

(*b*)–(*g*) Individual particles from an inoculum, all at the same magnification. (*b*) EB, (*c*) sick EB, (*d*) and (*e*) CB, (*f*) RB, (*g*) sick RB.

Plate 2

Ingestion of particles by macrophages.

(*a*) TRIC particles are apparently adsorbed to the cell membrane, which in one instance is indented.

(*b*), (*c*) Two stages in the phagocytosis of single particles.

(*d*) A cytoplasmic process engulfs a mixed group of particles.

Plate 3

Particles within lysosomes in macrophages.

(*a*) Different types of particle are within a single lysosome with membranous debris which may have originated from TRIC organisms.

(*b*) This lysosome is in a cell treated by the Gomori technique. It contains damaged TRIC particles (arrows) together with a specific precipitate indicating localized acid phosphatase activity.

(*c*) This lysosome contains two CBs and a damaged RB as well as amorphous material which is probably condensed serum protein from the culture medium.

(*d*) This EB lies in an enlarged T vacuole. Several coated vesicles (possibly primary lysosomes; Novikoff, Novikoff, Quintana & Hauw, 1971) lie near the vacuole, but this association was rare and not restricted to this type of vacuole.

Plate 4

Particles within T vacuoles in BHK-21 cells and macrophages, all at the same magnification.
(*a*) An EB immediately after entry into a BHK-21 cell.

(*b*) Three organisms in BHK-21 cells 80 min. after inoculation. The EB on the right shows separation between its wall and its cell membrane. The two bodies marked with arrows are assumed to be tangentially sectioned T vacuoles containing TRIC particles. All three vacuoles are surrounded by small cytoplasmic vesicles.

(*c*)–(*g*) Particles in macrophages.

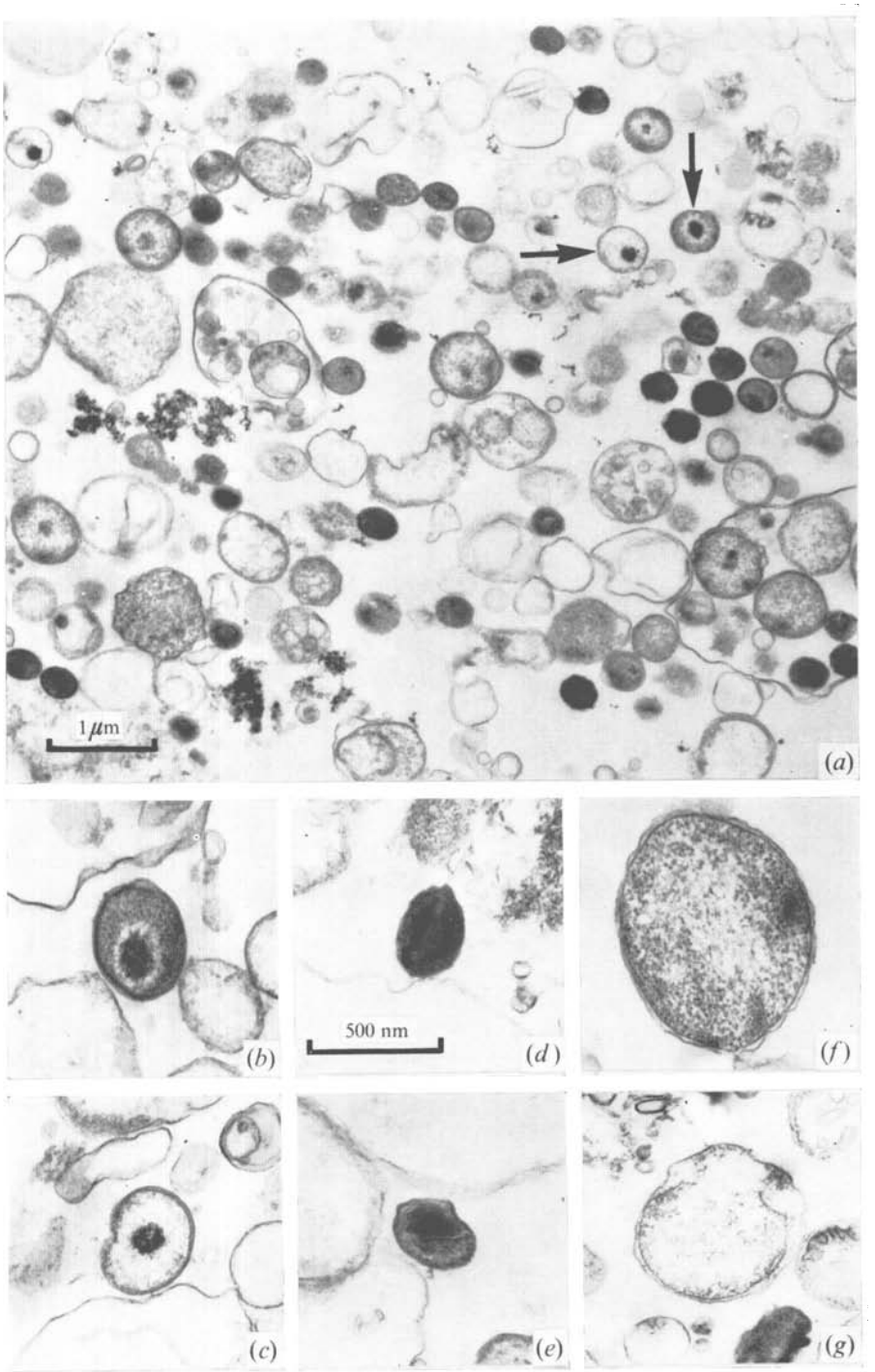
(*c*) An EB 20 min. after inoculation.

(*d*) A sick EB lying in an enlarged T vacuole 80 min. after inoculation.

(*e*) An RB immediately after inoculation.

(*f*) A latex ball immediately after inoculation.

(*g*) A CB 20 min. after inoculation.



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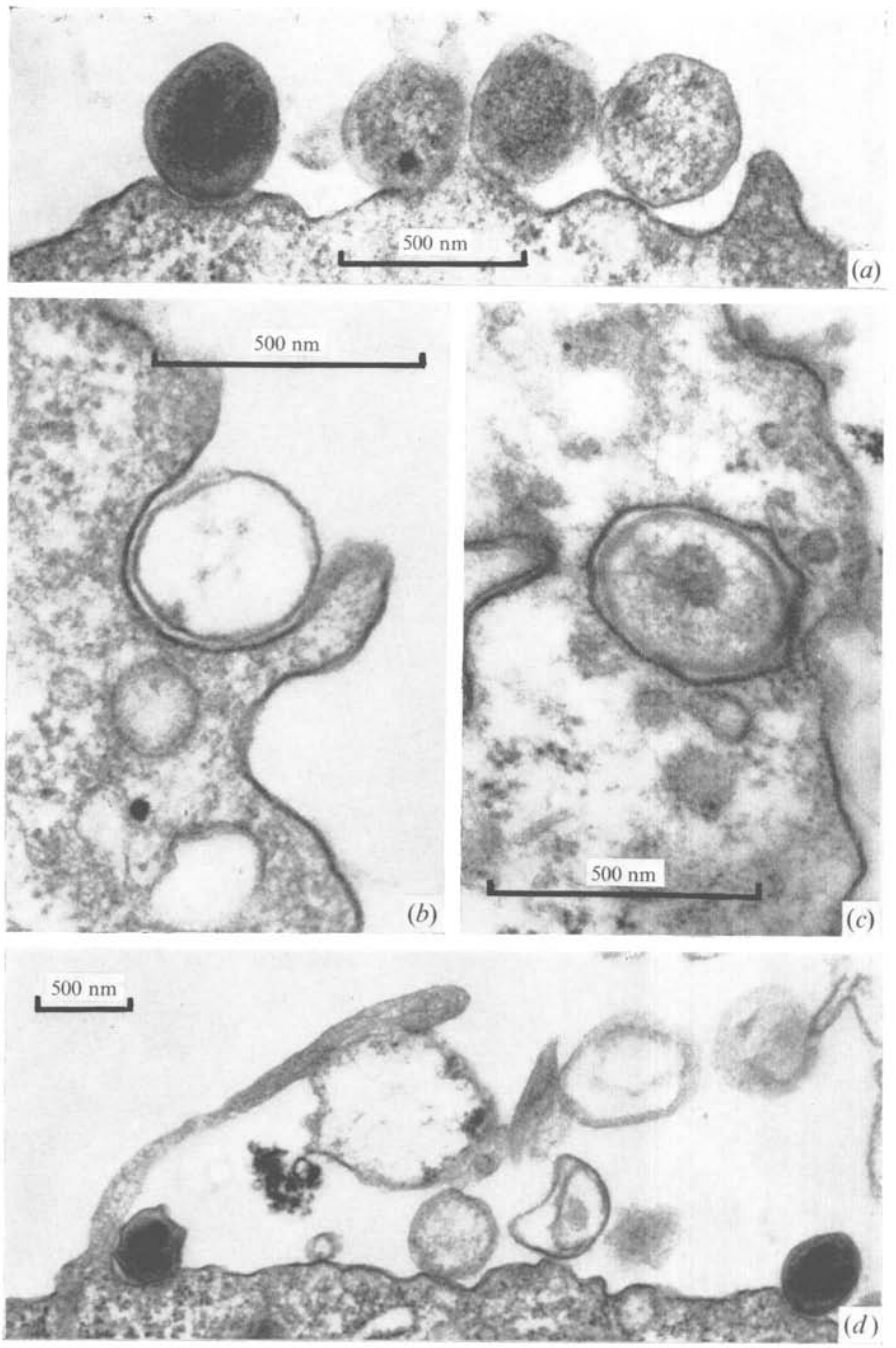

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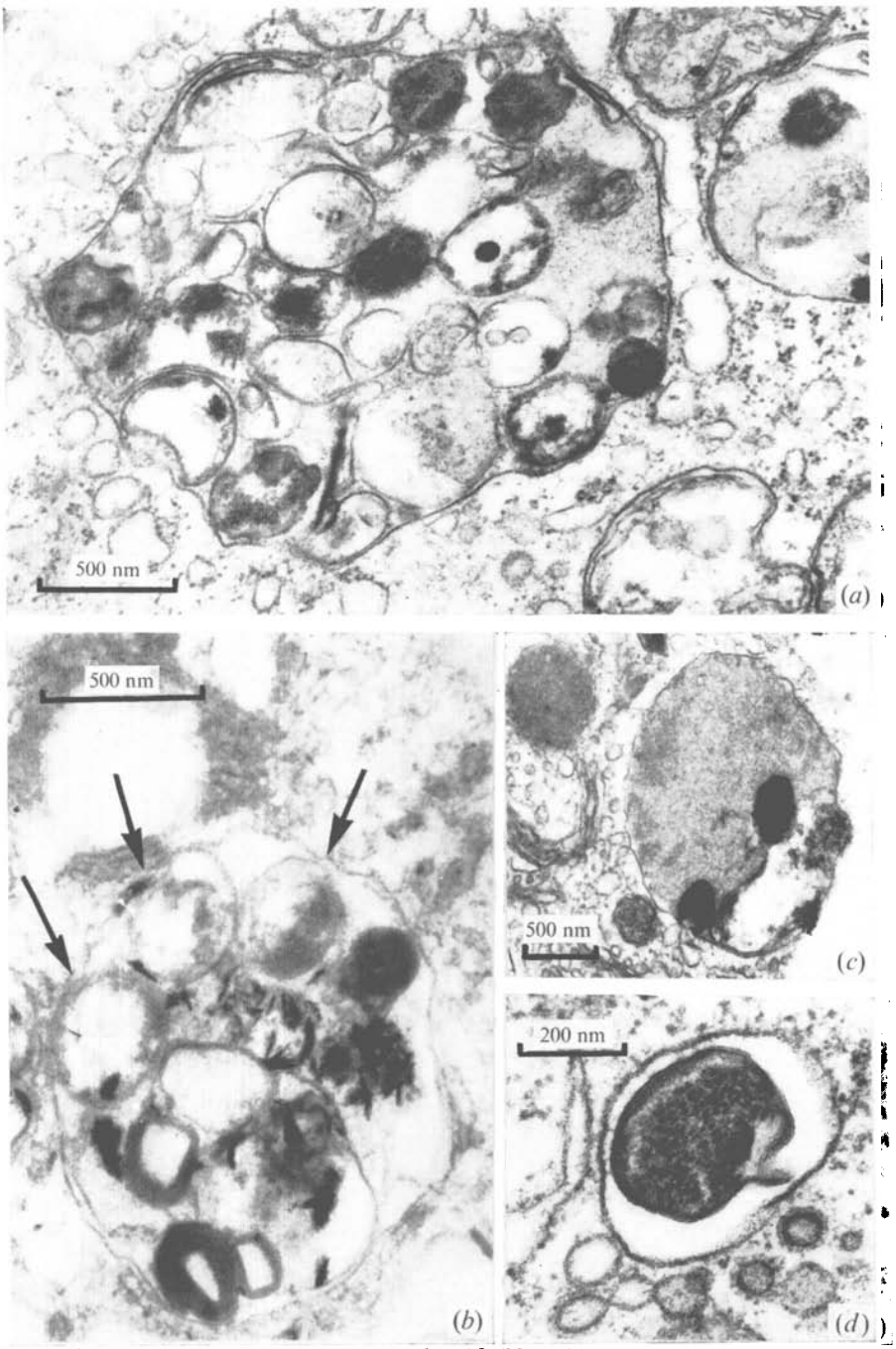

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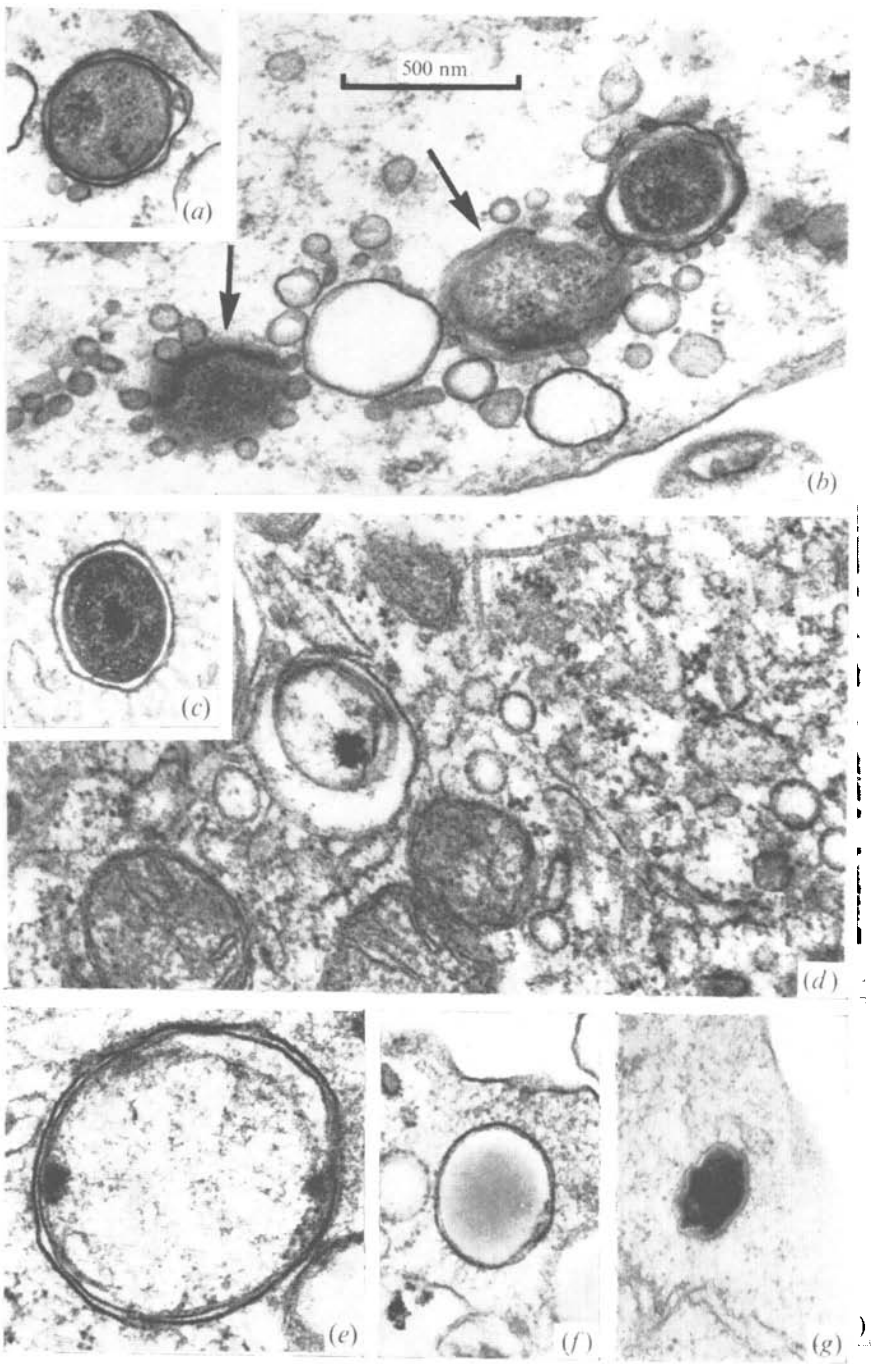

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