Phagolysosome Formation, Cyclic Adenosine 3':5'-Monophosphate and the Fate of *Salmonella typhimurium* within Mouse Peritoneal Macrophages

By M. E. W. CARROL, P. S. JACKETT, V. R. ABER AND D. B. LOWRIE

MRC Unit for Laboratory Studies of Tuberculosis,
Royal Postgraduate Medical School, Du Cane Road, London W12 0HS

(Received 19 April 1978; revised 22 August 1978)

Salmonella typhimurium did not inhibit fusion of lysosomes with the phagocytic vacuoles in infected macrophages and caused no increase in cyclic adenosine 3':5'-monophosphate. Glutaraldehyde-killed bacteria showed rapid ultrastructural degeneration within the phagolysosomes. In contrast, untreated bacteria were resistant to digestion by lysosomal enzymes. Intracellular survival of this species appears to depend on resistance to, and not evasion of, lysosomal enzymes.

INTRODUCTION

Normally when micro-organisms are ingested by mammalian cells, lysosomes fuse with phagosomes and the intraphagosomal micro-organisms are digested by lysosomal enzymes within the resulting phagolysosomes. Three ways in which intracellular parasites escape this fate have been described. First, *Mycobacterium lepraemurium*, *Leishmania mexicana* and *L. donovani* resist lysosomal enzyme attack and proliferate within phagolysosomes (Hart *et al.*, 1972; Alexander & Vickerman, 1975; Chang & Dwyer, 1976). Second, *Mycobacterium leprae* and *Trypanosoma cruzi* escape from phagosomes and multiply within the cytoplasm (Evans & Levy, 1972; Kress *et al.*, 1975; Noguiera & Cohn, 1976). Third, failure of lysosomes to fuse with phagosomes is apparent in cells infected with *Mycobacterium tuberculosis* (Armstrong & Hart, 1971), *Mycobacterium microti* (Hart *et al.*, 1972; Lowrie *et al.*, 1975*a*), *Toxoplasma gondii* (Jones & Hirsch, 1972), *Chlamydia psittaci* (Friis, 1972; Todd & Storz, 1975) and *Encephalitozoon cuniculi* (Weidner, 1975).

Evidence has been presented suggesting that *M. microti* inhibits phagolysosome formation in macrophages by increasing the cyclic adenosine 3':5'-monophosphate (cyclic AMP) content of the infected cells (Lowrie *et al.*, 1975*a*). We have therefore tested the hypothesis that cyclic AMP could be the inhibitor common to other instances of failure of phagosome/lysosome fusion.

Salmonella typhimurium is not killed but multiplies slowly within macrophages (Lowrie *et al.*, 1979). Studies of phagolysosome formation and of the cyclic AMP content of macrophages infected with this organism are reported here.

METHODS

Macrophages and bacteria. Mouse peritoneal macrophage monolayers were obtained and *Salmonella typhimurium* LT2 was prepared for phagocytosis as described by Lowrie *et al.* (1979), except that in some experiments, where stated, Dulbecco's salt solution (Oxoid) replaced Hanks' salt solution (HSS) in phagocytosis medium. This variation was introduced to avoid inhibition, by the glucose component of HSS, of bacterial cyclic AMP synthesis during phagocytosis.

Exposure of macrophages to ferritin and bacteria. Macrophages were exposed to bacteria at various bacterium:phagocyte ratios. Between 7 and 14 d after establishment of the monolayers, macrophages were exposed to ferritin and then to bacteria, essentially as described by Armstrong & Hart (1971). Ferritin served as an electron-opaque marker of secondary lysosomes in electron microscopy. Maintenance medium (MM; Lowrie *et al.*, 1979) was withdrawn from the monolayers and temporarily stored, the monolayers were rinsed with HSS containing 0.007% (w/v) sodium bicarbonate at 37 °C and then overlaid with 2.5 ml phagocytosis medium [HSS containing 2.5% (v/v) newborn calf serum (Gibco-Biocult, Paisley, Scotland)] containing 1% (w/v) ferritin (Cd-free; Miles Labs, Slough). The monolayers were incubated with ferritin for 3.5 h at 37 °C then rinsed twice with HSS containing 0.035% sodium bicarbonate. The original MM was then replaced and the dishes were incubated for a further 3 h. The dishes were again rinsed with warm HSS containing 0.007% bicarbonate and inoculated with either live or glutaraldehyde-killed bacteria in phagocytosis medium, or with phagocytosis medium alone (control), and incubated at 37 °C for 2 h to allow phagocytosis. Some of the monolayers were then vigorously rinsed twice with warm HSS containing 0.035% bicarbonate and incubated for a further 1 h with fresh MM. Immediately after the phagocytosis period, or after the subsequent 1 h incubation, replicate monolayers were either assayed for cyclic AMP content and the number of stainable bacteria per macrophage or processed for electron microscopy.

Estimation of cyclic AMP and stainable bacteria in macrophage monolayers. Monolayers were chilled on ice, rinsed twice with ice-cold HSS and the majority of cells were scraped free into 2 ml HSS using a silicone rubber 'policeman'. Each cell suspension was added to 1 ml 12% (w/v) perchloric acid, dispersed by ultrasonication (Biosonik microprobe, Bronwill Scientific Co., Rochester, N.Y., U.S.A.; 60 W output for 20 s) and duplicate samples were assayed for DNA (Giles & Myers, 1965) and cyclic AMP. Samples for cyclic AMP assay were partially purified on 7 × 0.5 cm columns of AG 50W-X8 H⁺ resin (BioRad) by washing with 3 ml 1 mM-potassium phosphate buffer, pH 7.0 (Kuo & Greengard, 1972). Cyclic AMP was eluted with 6 ml water, freeze-dried and assayed using a competitive protein binding assay kit (The Radiochemical Centre, Amersham). Typically, 92% of cyclic [³H]AMP incorporated in samples as an internal standard was recovered. Cyclic AMP:macrophage ratios were calculated using the DNA estimates. The mean number of cell-associated bacteria per macrophage was estimated by light microscopy of residual monolayer cells (Lowrie *et al.*, 1979).

Assay of bacterial cyclic AMP. It was anticipated that bacterial cyclic AMP might significantly contribute to the total cyclic AMP in infected macrophages (Lowrie *et al.*, 1975*a*). Accordingly, in each experiment, samples of the bacterial suspension in phagocytosis medium were assayed for intra- and extra-bacterial cyclic AMP both before and after 2 h incubation in glass tubes at 37 °C. Bacteria were pelleted by centrifugation and disrupted by 10 min ultrasonication in ice-cold 12% perchloric acid. Supernatant phagocytosis medium, sterilized by membrane filtration (0.22 µm pore size; Millipore), and bacterial extracts were purified and assayed for cyclic AMP as described above.

Electron microscopy. Monolayers were processed in duplicate for electron microscopy essentially as described by Hirsch & Fedorko (1968). Medium was removed from the dishes and the monolayers were immediately flooded with freshly mixed ice-cold fixative solution (3 ml). This contained 0.67% (w/v) osmic acid and 0.83% (v/v) glutaraldehyde buffered at pH 7.2 with 0.1 M-sodium cacodylate. The dishes were placed on ice and after 2 to 3 min the fixative was removed and replaced with fresh fixative (3 ml). After 1 h the monolayers were rinsed twice with Ringer's solution and flooded with 0.25% (w/v) uranyl acetate in 0.1 M-veronal acetate buffer, pH 6.3. After 15 min the monolayers were again rinsed in Ringer's solution and dehydrated through a graded series of ethanol concentrations. All solutions were ice-cold and monolayers were allowed to warm to room temperature during three final rinses in absolute ethanol. The dishes were flooded with propylene oxide and gently agitated while the solvent dissolved the surface plastic and the monolayers came free as fragmented sheets. This method of harvesting the cells avoided the extensive cell damage which otherwise occurred when fixed cells were scraped free into aqueous suspension. The monolayer sheets were transferred to glass tubes, allowed to settle through propylene oxide (3 × 10 ml) and then infiltrated with a mixture of equal volumes of propylene oxide and complete Araldite resin mixture for 18 h at room temperature. The cell sheets were finally centrifuged through resin and blocks were cured at 60 °C for 7 d.

Quantitative ultrastructural surveys of bacterial morphology and phagolysosome formation were done essentially as described by Armstrong & Hart (1971). Widely interrupted series of thin sections were cut from replicate blocks prepared from each monolayer so that many different cells could be examined. Sections were stained with uranyl acetate, randomly coded to avoid bias during microscopy and examined using a Philips EM 300 electron microscope. Each cell profile encountered which contained intracellular bacteria was examined to assess whether the bacteria were free within the cytoplasm or confined within a membrane lined vesicle. Each vesicle which contained one or more bacteria was counted as either a phago-

Table 1. *Change in amount of cyclic AMP in macrophage monolayers exposed to living or glutaraldehyde-killed Salmonella typhimurium*

Bacterial suspensions were incubated with monolayers for 2 h at 37 °C at different bacterium:phagocyte ratios. Monolayers were then rinsed and examined either immediately or after a further 1 h incubation in maintenance medium. Cell-associated bacteria were counted by light microscopy of Giemsa-stained monolayers. The average cyclic AMP content of replicate control (bacterium-free) monolayers was subtracted from the average content of the macrophages exposed to bacteria and the difference is shown. In experiments 3 and 4, bacteria were suspended in Dulbecco's salt solution instead of Hanks' salt solution during phagocytosis.

State of inoculum	Expt no.	Cyclic AMP content of control monolayers $\pm$ s.d. [pmol (10^7 macrophages) $^{-1}$]	Bacterium: phagocyte ratio during phagocytosis	Immediately after phagocytosis			After 1 h subsequent incubation		
				No. of replicate monolayers	Average no. of bacteria per macrophage	Average change in cyclic AMP [pmol (10^7 macrophages) $^{-1}$]	No. of replicate test monolayers	Average no. of bacteria per macrophage	Average change in cyclic AMP [pmol (10^7 macrophages) $^{-1}$]
Live	1	14.2 $\pm$ 3.6	750:1	2	85	-3.3	2	NC	-9.6*
	2	4.1 $\pm$ 0.6	120:1	3	19	+0.3	3	21	+0.2
	3	15.7 $\pm$ 2.3	37:1	3	21	+0.6	4	26	-0.5
	4	13.1 $\pm$ 2.9	25:1	3	12.5	-3.2	4	15.8	+0.5
	5	4.1 $\pm$ 1.0	18:1	3	2.3	+0.8	3	2.5	+1.3
Dead	1	14.2 $\pm$ 3.6	150:1	2	20	-4.2	2	15	-2.3
	2	4.1 $\pm$ 0.6	120:1	3	16	-1.0	3	11	-0.9
	3	15.7 $\pm$ 2.3	37:1	3	5.5	+1.1	3	2.8	+3.1
	4	13.1 $\pm$ 2.9	25:1	3	6.5	+0.5	3	4.4	-2.9
	5	4.1 $\pm$ 1.0	19:1	3	1.7	-1.0	3	1.1	-1.1

* Significant change ($P < 0.01$). NC, Too many bacteria to count.

Fig. 1. Electron micrograph of an ultrathin section of a macrophage which had ingested glutaraldehyde-killed *S. typhimurium*. Numerous bacteria can be seen in various stages of degradation within vesicles which are identified as phagolysosomes by their ferritin content. Examples are indicated by double arrows. Some of the ferritin-labelled lysosomes not yet fused with the phagolysosomes are indicated by single arrows. Bar marker represents 1 μ m.

lysosome or a phagosome, according to the presence or absence of ferritin, the marker for lysosomal contents, within the same vesicle. Each bacterium was examined for signs of morphological damage and each phagosome or phagolysosome was accordingly recorded as containing either intact bacteria only or at least one damaged bacterium. Morphological integrity was not used as a criterion of bacterial viability (c.f. Armstrong & Hart, 1971).

RESULTS

Change in cyclic AMP content of macrophage monolayers

The results of counts of bacteria and assays of cyclic AMP in monolayers of five representative experiments are summarized in Table 1. No significant increase in monolayer cyclic AMP content was seen after phagocytosis of either living or dead bacteria, even when phagocytosis took place in the absence of exogenous glucose (experiments 3 and 4), that is in conditions which favour bacterial cyclic AMP synthesis (Rickenberg, 1974). Analyses of pooled data revealed no correlation between change in cyclic AMP and the number of monolayer-associated bacteria. In experiment 1, in which overwhelming bacterial infection was established, a significant decrease in macrophage cyclic AMP content occurred. This accompanied damage to the monolayer, manifested 1 h after phagocytosis as detachment of rounded-up cells.

Bacterial cyclic AMP production

During 2 h incubation at 37 °C the number of bacteria suspended in HSS containing 2.5% serum (phagocytosis medium) increased approximately fourfold. Although the total cyclic AMP in the suspension remained unchanged, the cyclic AMP content of the bacteria

Fig. 2. Electron micrograph of an undamaged bacterium in an ultrathin section of a macrophage which had ingested living *S. typhimurium*. The organism shows intact cell membranes (CM) and an even finely-granular cytoplasm. An electron-dense zone (EDZ) is apparent in the macrophage cytoplasm adjacent to the phagosomal membrane (PM). Several small lysosomes in the process of fusing with the phagosome are indicated by arrows. Bar marker represents 0.5 μm .

decreased about fourfold from 1 to 2 down to 0.2 to 0.5 pmol (10^8 bacteria) $^{-1}$. In contrast the bacteria did not multiply when incubated for 2 h in Dulbecco's salt solution containing 2.5% serum and the total cyclic AMP present increased approximately fivefold to 5 to 8 pmol (10^8 bacteria) $^{-1}$. The additional nucleotide was essentially all extracellular.

Ultrastructural changes in macrophage monolayers

Representative macrophage monolayers were processed 1 h after phagocytosis in experiments 1 to 4 (see Table 1). Degenerative ultrastructural changes in macrophage morphology were seen only in the heavily infected monolayers of experiment 1, consisting of loss of cytoplasmic fine structure in rounded cells. A toxic effect of the bacteria was inferred. All other monolayers showed a well-preserved ultrastructure with abundant ferritin-labelled secondary lysosomes and a variable proportion of unlabelled small vesicles, some of which were apparently primary lysosomes arising from the Golgi apparatus.

Salmonellae were always found within phagosomes or phagolysosomes in healthy macrophages. Degenerating macrophages in experiment 1 occasionally contained intact bacteria which appeared to be free in the cytoplasm.

Some damaged intracellular bacteria were always observed regardless of whether the macrophages had ingested untreated or glutaraldehyde-killed organisms; damaged bacteria showed swelling, cytoplasmic patchiness and wall disruption. The most severe bacterial damage was observed in macrophages which contained pre-killed bacteria (Fig. 1). Bacteria phagocytosed from living inocula were minimally damaged and manifested swelling only. The macrophage cytoplasm adjacent to vacuoles which contained intact bacteria from live inocula frequently showed an increased electron density (Fig. 2). The same phenomenon

Table 2. *Phagolysosome formation and bacterial digestion in macrophages containing either untreated or glutaraldehyde-killed Salmonella typhimurium*

The numbers of bacterium-containing phagosomes observed during electron microscopy were subdivided according to whether the intraphagosomal bacteria were damaged or intact and whether the phagosomes had or had not fused with ferritin-labelled lysosomes.

State of inoculum	Expt no.*	No. of bacterium-containing cell profiles examined	Structural appearance of intraphagosomal bacteria†	No. of phagosomes which were in the following states of fusion with lysosomes:		
				Fused	Not fused	Doubtful
Live	1	34	Intact	84	18	10
			Damaged	36	2	2
			Doubtful	7	3	2
	2	33	Intact	53	2	2
			Damaged	6	0	0
			Doubtful	2	0	0
	3	38	Intact	32	11	11
			Damaged	15	0	4
			Doubtful	2	5	0
	4	36	Intact	54	8	3
			Damaged	14	0	0
			Doubtful	8	0	0
Dead	1	19	Intact	0	0	0
			Damaged	36	0	0
			Doubtful	0	0	0
	2	42	Intact	7	0	0
			Damaged	136	0	0
			Doubtful	3	0	0
	3	18	Intact	3	0	0
			Damaged	17	0	1
			Doubtful	1	0	0
	4	49	Intact	6	2	2
			Damaged	45	2	6
			Doubtful	0	1	0

* Experiment numbers correspond to those in Table 1.

† Occasional phagosomes which contained both damaged and intact bacteria were scored as containing damaged ones.

Table 3. *Subdivision of pooled data from Table 2 (excluding the doubtful categories) according to the proportion of phagosomes which contained damaged bacteria and the proportion which had fused with lysosomes*

State of inoculum	Total no. of bacterium-containing phagosomes found (A)	Structural appearance of bacteria in phagosomes	Phagosomes containing bacteria		Phagosomes which had fused with lysosomes	
			No. (B)	% of A	No.	% of B
Live	335	Intact	262	78	223	85†
		Damaged	73	22*	71	97‡
Dead	254	Intact	18	7	16	89†
		Damaged	236	93*	234	99‡

* Significant difference between these two percentages ($P < 0.001$).

†, ‡ Significant difference between these pairs of percentages ($P < 0.001$).

in rabbit macrophages infected with *Listeria monocytogenes*, *Staphylococcus aureus* or *Salmonella typhi* was illustrated by Leake *et al.* (1971), but its significance is not known.

The relative numbers of intact or damaged bacteria and the relationship between bacterial damage and exposure to lysosomal contents are shown in Table 2 and illustrate two main features. First, bacteria from killed inocula were damaged much more often than those from the live inocula. Second, lysosomes had usually fused with phagosomes regardless of whether the bacteria were from a living or a killed inoculum.

Statistical analyses of the results shown in Table 2 indicated that significantly more of the intracellular bacteria were damaged in the macrophages supplied with dead bacteria than in those given living bacteria ($P < 0.001$) (Table 3). Although fusion tended to be more frequent with the pre-killed bacteria, empirically weighted logit analysis, in a $2 \times 2 \times 2$ contingency table of the proportions of phagosomes fused with lysosomes, showed that the nature of the inoculum, whether of live or dead bacteria, did not significantly affect the extent of fusion of lysosomes with phagosomes and no significant interactions were revealed ($P > 0.05$). In contrast, damaged bacteria were significantly more often found in phagolysosomes than were intact bacteria ($P < 0.001$).

DISCUSSION

Studies of the interaction of *S. typhimurium* strains of low virulence with macrophages had shown that rapid and extensive phagolysosome formation followed phagocytosis by mouse peritoneal macrophages both *in vitro* and *in vivo* (Fauve & Delaunay, 1966; Blanden, 1968). It was possible, however, that events with virulent bacteria would be different. Takeuchi (1967) observed that virulent bacteria which had been engulfed by macrophages during their passage through the wall of the ileum in infected mice were often in phagolysosomes but the viability of these organisms was uncertain. Our experiments showed that phagolysosome formation is extensive even with living virulent bacteria and we could observe no substantial inhibition of the fusion process or escape from the phagolysosome. Intracellular proliferation (Lowrie *et al.*, 1979) evidently occurs within these vesicles. In this respect the organism resembles *M. lepraemurium* and *Leishmania* species (Hart *et al.*, 1972; Alexander & Vickerman, 1975; Chang & Dwyer, 1976).

The bacteria were resistant to lysosomal enzymes when alive, as were the 20% of 'dead' organisms in the 'live' inoculum. Only minimal damage was seen when they were intracellular. Similar resistance was observed in the studies of Takeuchi (1967) and Blanden (1968). This contrasts strikingly with the rapid destruction of the glutaraldehyde-killed bacteria. The general appearance and stages of bacterial disintegration, even with glutaraldehyde-killed organisms, closely resembled those illustrated by Blanden (1968) and Leake *et al.* (1971) from studies, respectively, of *S. typhimurium* in peritoneal macrophages from BCG-vaccinated mice and of *S. typhi* in pulmonary alveolar macrophages from BCG-vaccinated rabbits. Resistance of *S. typhimurium* to attack by lysosomal enzymes is probably conferred by the structure of the wall lipopolysaccharide (Friedberg & Shilo, 1970; Tageson & Stendahl, 1973), just as resistance in *M. lepraemurium* seems to reside in that organism's thick lipid envelope (Draper & Rees, 1970).

Salmonella typhimurium further resembles *M. lepraemurium* in not causing an increase in cyclic AMP in infected macrophages. Although *M. lepraemurium* as isolated seemed to have very little cyclic AMP content or synthetic capacity (Lowrie *et al.*, 1975*b*), *S. typhimurium* was able to synthesize and release up to 8 pmol (10^8 bacteria) $^{-1}$ when incubated for 2 h in glucose-free phagocytosis medium. Hence, if the bacteria continued cyclic AMP synthesis at a similar rate in the intracellular environment, the large numbers of intracellular bacteria attained in this study might have been expected to cause detectable increases in the nucleotide in the monolayers. That no increase was seen might be attributable to any or all of the following factors: decreased synthesis by intracellular bacteria, destruction of

released bacterial cyclic AMP by lysosomal enzymes or depression of the level of macrophage-synthesized cyclic AMP by toxic bacterial products. The latter phenomenon was apparent in the significant decrease in the nucleotide in overwhelmingly infected monolayers.

Considered in isolation, the observation that *S. typhimurium* neither raises the cyclic AMP content of infected macrophages nor inhibits phagolysosome formation neither supports nor detracts from the hypothesis that fusion inhibition in other infections is cyclic AMP-mediated. Nevertheless, the correlation between fusion inhibition and cyclic AMP enhancement has been sustained: live *M. microti* and *M. bovis* BCG possess substantial capacity to elevate cyclic AMP levels and inhibit fusion; dead bacteria, inert particles and live *M. lepraemurium* and *S. typhimurium* possess neither (Lowrie, 1978).

We thank Professor A. P. Waterson for the generous provision of facilities for electron microscopy.

REFERENCES

- ALEXANDER, J. & VICKERMAN, K. (1975). Fusion of host cell secondary lysosomes with the parasitophorous vacuoles of *Leishmania mexicana*-infected macrophages. *Journal of Protozoology* **22**, 502-508.
- ARMSTRONG, J. A. & HART, P. D'A. (1971). Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. *Journal of Experimental Medicine* **134**, 713-740.
- BLANDEN, R. V. (1968). Modification of macrophage function. *Journal of the Reticuloendothelial Society* **5**, 179-202.
- CHANG, K.-P. & DWYER, D. M. (1976). Multiplication of a human parasite (*Leishmania donovani*) in phagolysosomes of hamster macrophages in vitro. *Science* **193**, 678-680.
- DRAPER, P. & REES, R. J. W. (1970). Electron-transparent zone of mycobacteria may be a defence mechanism. *Nature, London* **228**, 860-861.
- EVANS, M. J. & LEVY, L. (1972). Ultrastructural changes in cells of the mouse footpad infected with *Mycobacterium leprae*. *Infection and Immunity* **5**, 238-247.
- FAUVE, R. M. & DELAUNAY, A. (1966). Résistance cellulaire à l'infection bactérienne. V. Modifications cytoplasmiques observées *in vitro*, dans des macrophages de souris, après injection de bactéries capables ou non de multiplication intracellulaire. *Annales de l'Institut Pasteur* **111**, Suppl. 5, 78-84.
- FRIEDBERG, D. & SHILO, M. (1970). Role of cell wall structure of *Salmonella* in the interaction with phagocytes. *Infection and Immunity* **2**, 279-285.
- FRIIS, R. R. (1972). Interaction of L cells and *Chlamydia psittaci*: entry of the parasite and host responses to its development. *Journal of Bacteriology* **110**, 706-721.
- GILES, K. W. & MYERS, A. (1965). An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature, London* **206**, 93.
- HART, P. D'A., ARMSTRONG, J. A., BROWN, C. A. & DRAPER, P. (1972). Ultrastructural study of the behavior of macrophages toward parasitic mycobacteria. *Infection and Immunity* **5**, 803-807.
- HIRSCH, J. G. & FEDORKO, M. E. (1968). Ultrastructure of human leucocytes after simultaneous fixation with glutaraldehyde and osmium tetroxide and 'post-fixation' in uranyl acetate. *Journal of Cell Biology* **38**, 615-627.
- JONES, T. C. & HIRSCH, J. G. (1972). The interaction between *Toxoplasma gondii* and mammalian cells. II. The absence of lysosomal fusion with phagocytic vacuoles containing living parasites. *Journal of Experimental Medicine* **136**, 1173-1194.
- KRESS, Y., BLOOM, B. R., WITTNER, M., ROWEN, A. & TANOWITZ, H. (1975). Resistance of *Trypanosoma cruzi* to killing by macrophages. *Nature, London* **257**, 394-396.
- KUO, J.-F. & GREENGARD, P. (1972). An assay method for cyclic AMP and cyclic GMP based upon their abilities to activate cyclic AMP-dependent and cyclic GMP-dependent protein kinases. *Advances in Cyclic Nucleotide Research* **2**, 41-50.
- LEAKE, E. S., EVANS, D. G. & MYRVIK, Q. N. (1971). Ultrastructural patterns of bacterial breakdown in normal and granulomatous rabbit alveolar macrophages. *Journal of the Reticuloendothelial Society* **9**, 174-199.
- LOWRIE, D. B. (1978). Tubercle bacilli in infected macrophages may inhibit phagosome-lysosome fusion by causing increased cAMP concentrations. In *Molecular Biology and Pharmacology of Cyclic Nucleotides*, pp. 311-314. Edited by G. Folco & R. Paoletti. Amsterdam: Elsevier/North Holland.
- LOWRIE, D. B., JACKETT, P. S. & RATCLIFFE, N. A. (1975a). *Mycobacterium microti* may protect itself from intracellular destruction by releasing cyclic AMP into phagosomes. *Nature, London* **254**, 600-602.
- LOWRIE, D. B., JACKETT, P. S. & RATCLIFFE, N. A. (1975b). Failure of phagosome/lysosome fusion in *Mycobacterium microti*-infected macrophages may be mediated by cyclic AMP of bacterial origin. *Proceedings of the Society for General Microbiology* **2**, 74-75.

- LOWRIE, D. B., ABER, V. R. & CARROL, M. E. W. (1979). Division and death rates of *Salmonella typhimurium* inside macrophages: use of penicillin as a probe. *Journal of General Microbiology* **110**, 409–419.
- NOGUIERA, N. & COHN, Z. (1976). *Trypanosoma cruzi*: mechanism of entry and intracellular fate in mammalian cells. *Journal of Experimental Medicine* **143**, 1402–1420.
- RICKENBERG, H. V. (1974). Cyclic AMP in prokaryotes. *Annual Review of Microbiology* **28**, 353–369.
- TAGESSON, C. & STENDAHL, O. (1973). Influence of the cell surface lipopolysaccharide structure of *Salmonella typhimurium* on resistance to intracellular bactericidal systems. *Acta pathologica et microbiologica scandinavica* **B81**, 473–480.
- TAKEUCHI, A. (1967). Electron microscope studies of experimental salmonella infection. I. Penetration into the intestinal epithelium by *Salmonella typhimurium*. *American Journal of Pathology* **50**, 109–136.
- TODD, W. J. & STORZ, J. (1975). Ultrastructural cytochemical evidence for the activation of lysosomes in the cytotoxic effect of *Chlamydia psittaci*. *Infection and Immunity* **12**, 638–646.
- WEIDNER, E. (1975). Interactions between *Encephalitozoon cuniculi* and macrophages. Parasitophorous vacuole growth and the absence of lysosomal fusion. *Zeitschrift für Parasitenkunde* **47**, 1–9.