The Effect of Growth Conditions on Adenylate Cyclase Activity and Virulence-related Properties of *Bordetella pertussis*

By R. M. BROWNLIE,* R. PARTON AND J. G. COOTE

Department of Microbiology, University of Glasgow, Alexander Stone Building, Garscube Estate, Bearsden, Glasgow G61 1QH, UK

(Received 23 February 1984; revised 9 June 1984)

Growth of *Bordetella pertussis* in Stainer & Scholte medium in which the NaCl had been replaced by one of several inorganic or organic salts resulted in a large decrease in adenylate cyclase activity, histamine-sensitizing activity and in the amounts of two cell-envelope polypeptides of *M*, 28000 and 30000. Although some variation between strains was observed, there was never a case where one of these properties was lost independently of the others. Cultures in which these properties were lost had decreased amounts of extracellular cAMP when compared to NaCl-grown cultures. Adenylate cyclase activity was detected in three locations of *B. pertussis* cultures (extracellular, extracytoplasmic but cell-associated, and cytoplasmic). After growth in medium containing high concentrations of MgSO_4 , enzyme activity was decreased to a similar extent in all three locations.

INTRODUCTION

Lacey (1960) reported that *Bordetella pertussis* underwent an antigenic transition when grown on Bordet-Gengou medium in which NaCl was replaced by MgSO_4 . This was a freely reversible phenotypic change which Lacey termed antigenic modulation. Growth on medium containing NaCl gave the virulent or so-called X-mode phenotype, whereas growth with MgSO_4 gave the avirulent or C-mode phenotype. Antigenic modulation could be induced by one of several inorganic or organic salts. Some ions clearly favoured X-mode growth whereas others favoured C-mode growth, and the outcome of any combined mixture of ions depended on the sum of their individual potencies for modulation.

More recently, work has concentrated on MgSO_4 -induced modulation or nicotinic acid-induced modulation (the latter originally described by Pusztai & Joó, 1967). During MgSO_4 -induced modulation several properties of *B. pertussis* are lost: pertussigen, also known as histamine-sensitizing factor and lymphocytosis-promoting factor, because of its multiple activities (Munoz, 1976); heat-labile toxin; two major cell-envelope polypeptides (*M*, 28000 and 30000, X polypeptides); filamentous haemagglutinin; agglutininogen 1; adenylate cyclase activity and cytochrome d_{629} (Holt & Spasojevic, 1968; Livey *et al.*, 1978; Dobrogosz *et al.*, 1979; Wardlaw & Parton, 1979). Also, pertussigen-related activities, heat-labile toxin and the X polypeptides are lost during nicotinic acid-induced modulation (Wardlaw *et al.*, 1976; Schneider & Parker, 1982). While some of these components, such as pertussigen, heat-labile toxin and filamentous haemagglutinin may play a role in the pathogenesis of whooping cough (Pittman, 1979; Wardlaw & Parton, 1983), the functions of others, such as adenylate cyclase and the X polypeptides, are not known.

Two unusual features of the adenylate cyclase of *B. pertussis* are its activation by calmodulin,

Abbreviation: HSA, histamine-sensitizing activity.

which is present in mammalian tissues and as a contaminant in many commercial protein preparations (Hewlett *et al.*, 1978; Goldhammer *et al.*, 1981), and its largely extracytoplasmic location: a large portion of the total activity can be detected using culture supernates or intact cells as enzyme sources (Hewlett *et al.*, 1976). In view of the regulatory role that adenylate cyclase plays in other prokaryotes (Pastan & Adhya, 1976; Botsford, 1981) it has been pointed out that loss of adenylate cyclase during modulation of *B. pertussis* may have a causal effect in the loss of virulence factors (Parton & Durham, 1978; Hewlett *et al.*, 1979a; Wardlaw & Parton, 1979). The purpose of this investigation was twofold: firstly, to determine the extent to which the loss of adenylate cyclase activity correlated with the loss of other properties when modulation was induced by alteration of growth conditions, and secondly to explore the possibility that certain growth conditions might promote the loss of some properties independently of others.

METHODS

***Bordetella pertussis* strains.** Strain TAB I was isolated pernasally before the death of an infant and was obtained from Professor G. T. Stewart, Ruchill Hospital, Glasgow, UK. Strain 18334 was a vaccine strain obtained from Connaught Laboratories, Toronto, Canada. Strain 18323 was virulent for mice challenged by the intracerebral route and was obtained from Dr F. Sheffield, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, UK. Strains were stored at -70°C as cell suspensions in a medium consisting of Casamino acids (Difco) (10 g l^{-1}), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.1 g l^{-1}), NaCl (5 g l^{-1}), CaCl_2 (16 mg l^{-1}), glycerol (10% , v/v), pH 7.1.

Growth of B. pertussis. Strains were grown on Bordet-Gengou medium (Gibco-Biocult Diagnostics, Paisley, UK) containing 20% (v/v) defibrinated horse blood (Gibco-Biocult) for 72 h at 35°C in a moist atmosphere. A loopful of these cultures was used to inoculate 100 ml Stainer & Scholte medium (SS-X; Stainer & Scholte, 1971) or a modification of SS-X (Table 1) contained in 250 ml dimpled conical flasks. The flasks were incubated at 35°C for 48 h in an orbital incubator at 80 r.p.m.

Adenylate cyclase and cAMP assays. Adenylate cyclase activity was assayed at 30°C using the method of Hewlett & Wolff (1976), but with the ATP regenerating system omitted. The reaction was started by adding 120 μl 2 mM-ATP in 20 mM- MgCl_2 to tubes containing 80 μl 180 mM-Tricine, pH 8.0 and 40 μl of enzyme sample. After 10 min the reaction was terminated by the addition of 1 ml 50 mM-Tris/HCl, 0.5 mM-EDTA, pH 7.5 followed by immersion in a boiling water bath for 10 min. When intact cells were used as an enzyme source, harvested cells (10000 *g* for 15 min) were resuspended in physiological saline. Adenylate cyclase in culture supernate was assayed directly. Lysed cells and cells lysed after treatment with trypsin were used as enzyme samples to determine total cell-associated activity and cytoplasmic activity, respectively.

Samples were assayed for cAMP using the cAMP-binding protein method of Gilman (1970). Binding protein, prepared from rabbit muscle, was donated by Dr J. P. Durham, Department of Clinical Oncology, University of Glasgow, UK. The reliability of the cAMP assays was checked by addition of 3',5'-cyclic nucleotide phosphodiesterase which destroyed all detectable cAMP.

Histamine-sensitizing activity (HSA). This was assayed and evaluated by the probit method, as described by Wardlaw *et al.* (1976).

Slab-gel electrophoresis. Harvested cells were washed once in physiological saline, resuspended to 2 mg protein ml^{-1} and whole cell proteins were analysed by SDS-PAGE using the method described previously (Wardlaw *et al.*, 1976). Briefly, whole cells were solubilized by mixing the cell suspension with an equal volume of 125 mM-Tris/HCl, pH 6.8, 4% (w/v) SDS, 10% (v/v) β -mercaptoethanol, 20% (v/v) glycerol and 0.002% bromophenol blue, and heating the mixture at 100°C for 5 min. Solubilized sample (25 μl) was applied to each track of the gel, which consisted of 10% (v/v) acrylamide in the separating gel and 5% acrylamide in the stacking gel.

Standardization of cell suspensions. Protein was estimated by the method of Herbert *et al.* (1969) with bovine serum albumin as a standard. For convenience, a standard curve relating protein concentration and dry weight to optical density was used. For HSA assays, cell suspensions were standardized (before being heat-killed) by comparison with the Fifth International Reference of Opacity (World Health Organization International Laboratory for Biological Standards, National Institute for Biological Standards and Control, Holly Hill, London NW3 6RB, UK).

Trypsin treatment of cells. Cells in saline were treated with trypsin by incubation with 40 μg trypsin ml^{-1} for 10 min at 37°C ; the reaction was terminated by the addition of lima bean trypsin inhibitor (40 μg ml^{-1}).

Cell disruption. Cell lysates were prepared by passing frozen cell suspensions in physiological saline three times through an LKB X-press (Biotech, Stockholm, Sweden).

Chemicals and reagents. Nucleotides and commercial enzymes were purchased from Sigma and tritiated compounds were purchased from Amersham.

Table 1. *Intact-cell adenylate cyclase activity of B. pertussis strains after growth with various additives*

Strains were grown for 48 h in SS-X medium with NaCl replaced by other additives. Harvested intact cells were assayed for adenylate cyclase activity. Results are means of four determinations $\pm$ S.D.; NT, not tested.

Additive	Final concn (mM)	Strain TAB I			Strain 18334			Strain 18323		
		Adenylate cyclase activity [pmol cAMP min ⁻¹ (mg protein) ⁻¹]	Relative activity (%)*	Adenylate cyclase activity [pmol cAMP min ⁻¹ (mg protein) ⁻¹]	Relative activity (%)*	Adenylate cyclase activity [pmol cAMP min ⁻¹ (mg protein) ⁻¹]	Relative activity (%)*			
NaCl	43	840 ± 156	100	425 ± 25	100	190 ± 10	100			
MgSO ₄	20	16 ± 7	2	17 ± 2	4	21 ± 2	11			
MgCl ₂	43	915 ± 140	109	410 ± 20	96	188 ± 8	99			
Na ₂ SO ₄	43	13 ± 12	2	13 ± 3	3	13 ± 3	7			
Sodium lactate	86	89 ± 12	11	410 ± 30	96	195 ± 5	103			
Sodium succinate	86	27 ± 5	3	NT	NT	NT	NT			
Sodium butyrate	20	14 ± 2	2	15 ± 2	4	14 ± 4	7			
Sodium caprylate†	0.6	NT‡	NT	17 ± 2	4	195 ± 15	103			
Nicotinic acid†	4.1	35 ± 1	4	NT	NT	NT	NT			

* Adenylate cyclase activity of cultures grown in the presence of 43 mM-NaCl was taken as 100%.

† These media also contained 43 mM-NaCl.

‡ Strain TAB I did not grow in this medium.

Table 2. *Adenylate cyclase activity in culture supernates after growth of B. pertussis with various additives*

Strain TAB I was grown for 48 h in SS-X medium with NaCl replaced by other additives. Culture supernate was assayed for adenylate cyclase activity. Results are means of four determinations $\pm$ SD.

Additive	Final concn (mM)	Adenylate cyclase activity (pmol cAMP min ⁻¹ ml ⁻¹)	Relative activity (%)*
NaCl	43	180 $\pm$ 53	100
MgSO ₄	20	16 $\pm$ 2	9
MgCl ₂	43	151 $\pm$ 35	84
Na ₂ SO ₄	43	17 $\pm$ 5	9
Sodium lactate	86	52 $\pm$ 23	29
Sodium succinate	86	34 $\pm$ 10	19
Sodium butyrate	20	15 $\pm$ 2	8
Nicotinic acid†	4.1	19 $\pm$ 5	11

* Adenylate cyclase activity of cultures grown in medium containing 43 mM-NaCl was taken as 100%.

† This medium also contained 43 mM-NaCl.

RESULTS

Effect of growth conditions on adenylate cyclase activity

The adenylate cyclase activity of *B. pertussis* grown in SS-X medium (containing NaCl) depended upon the strain used (Table 1). However, all the strains showed a marked reduction in intact-cell adenylate cyclase activity after growth in any one of several modified versions of SS-X medium containing Na_2SO_4 , MgSO_4 or sodium butyrate (Table 1). Growth in these latter media caused 89–98% loss of adenylate cyclase activity when compared with NaCl-grown cells. Sodium caprylate-induced loss of activity appeared to be strain specific, causing loss for strain 18334 but not for strain 18323. Growth of strain TAB I was inhibited by 0.6 mM-sodium caprylate. Sodium lactate-induced loss of adenylate cyclase activity also appeared to be strain specific and occurred only in strain TAB I. However, this loss of activity was less severe than that caused by MgSO_4 , Na_2SO_4 , sodium succinate, sodium butyrate or nicotinic acid. Unexpectedly, high concentrations of MgCl_2 caused no significant loss of adenylate cyclase activity with any strain. The observed decreases in the adenylate cyclase activity of intact cells were not due to release of the enzyme from the cells since activity in culture supernates paralleled that of intact cells, at least in strain TAB I (Table 2).

When activator was included in the assay mixture, intact-cell adenylate cyclase activity increased about 55-fold in cells of strain TAB I grown in unmodified SS-X medium (Table 3). The enzyme was responsive to activation whether growth conditions had or had not induced loss of adenylate cyclase activity, although the extent of activation was generally decreased in the former cases.

Cultures which showed a marked decrease in adenylate cyclase activity associated with intact cells when compared to NaCl-grown cultures also showed a marked decrease in the amounts of cAMP in culture supernates (Table 4). Apparently most of the cAMP produced during growth was excreted into the medium, as cAMP concentrations were the same whether determined in whole culture (heat-treated at 100 °C for 10 min to free intracellular cAMP and then centrifuged at 12000 *g* for 10 min to remove cell debris), culture supernate or heat-treated (100 °C for 10 min) culture supernate (data not shown). Attempts to determine the intracellular concentration of cAMP in *B. pertussis* using the charcoal concentrating method of Epstein *et al.* (1975) were unsuccessful, suggesting that the intracellular concentration was very low. However, the amount of cAMP in the culture supernate may be a useful indicator of intracellular adenylate cyclase activity during growth, as the substrate ATP is most likely to be exclusively intracellular. Growth in medium containing a high concentration of MgCl_2 , where intact-cell activity was similar to that of NaCl-grown cultures, resulted in a 1.4-fold increase in extracellular cAMP compared to NaCl-grown cultures. This may be related to the fact that optimum activity of the enzyme requires an optimum concentration of Mg^{2+} ions (Hewlett & Wolff, 1976).

HSA and the X polypeptides

Growth in media which resulted in severe loss of adenylate cyclase activity also caused a similar loss of HSA (Table 5). Growth of strain TAB I in medium containing sodium lactate resulted in a less severe loss of HSA when compared to MgSO_4 -grown cultures and this paralleled the similar, less severe loss of adenylate cyclase activity (Table 1). Again, a high concentration of MgCl_2 was ineffective in suppressing HSA in any of the three strains tested.

The SDS-PAGE profiles of washed whole cells of strain TAB I after growth in modified SS-X medium (Fig. 1) showed that loss of the X polypeptides correlated with loss of adenylate cyclase activity and HSA. This was also true for strains 18334 and 18323 (results not shown). Moreover, the corresponding X bands were detectable, although fainter, in gels of cells of strain TAB I grown in medium containing sodium lactate (Fig. 1), where extensive but incomplete loss of adenylate cyclase activity and HSA occurred.

Location of adenylate cyclase activity

Hewlett *et al.* (1976) demonstrated at least three compartments of adenylate cyclase activity in *B. pertussis* cultures: culture supernate, cell-associated but extracytoplasmic, and cytoplasmic.

Table 3. *Effect of activator on adenylate cyclase activity of B. pertussis after growth with various additives*

Strain TAB I was grown for 48 h in SS-X medium with NaCl replaced by various additives. Harvested intact cells, with and without activator, were assayed for adenylate cyclase activity. Bovine liver catalase was used as a convenient source of activator (1 mg ml⁻¹ final concentration). Results are means of four determinations $\pm$ SD.

Additive	Final concn (mM)	Adenylate cyclase activity [nmol cAMP min ⁻¹ (mg protein) ⁻¹]		Degree of activation (fold)
		Activator absent	Activator present	
NaCl	43	0.840 $\pm$ 0.16	45 $\pm$ 20	54
MgSO ₄	20	0.016 $\pm$ 0.007	0.36 $\pm$ 0.12	23
MgCl ₂	43	0.915 $\pm$ 0.14	52 $\pm$ 5.0	56
Na ₂ SO ₄	43	0.013 $\pm$ 0.002	0.33 $\pm$ 0.03	25
Sodium lactate	86	0.089 $\pm$ 0.012	4.1 $\pm$ 0.60	46
Sodium succinate	86	0.027 $\pm$ 0.005	0.17 $\pm$ 0.01	6
Sodium butyrate	20	0.014 $\pm$ 0.002	0.32 $\pm$ 0.03	23
Nicotinic acid*	4.1	0.035 $\pm$ 0.001	0.96 $\pm$ 0.10	27

* This medium also contained 43 mM-NaCl.

Table 4. *Concentration of extracellular cAMP in B. pertussis cultures after growth with various additives*

Strains were grown for 48 h in SS-X medium with NaCl replaced by other additives. Culture supernates were assayed for cAMP. Results are means of four determinations $\pm$ SD; NT, not tested.

Additive	Final concn (mM)	cAMP in supernate [pmol cAMP (mg dry wt cells) ⁻¹]		
		Strain TAB I	Strain 18334	Strain 18323
NaCl	43	112 $\pm$ 24	96 $\pm$ 21	116 $\pm$ 27
MgSO ₄	20	<5*	<5	<5
MgCl ₂	43	183 $\pm$ 37	130 $\pm$ 20	182 $\pm$ 38
Na ₂ SO ₄	43	<5	<5	<5
Sodium lactate	86	20 $\pm$ 6	85 $\pm$ 31	127 $\pm$ 47
Sodium succinate	86	<5	NT	NT
Sodium butyrate	20	<5	<5	<5
Sodium caprylate†	0.6	NT‡	<5	130 $\pm$ 25
Nicotinic acid†	4.1	<5	NT	NT

* Limit of detection.

† These media also contained 43 mM-NaCl.

‡ Strain TAB I did not grow in this medium.

In the present study, adenylate cyclase was uniformly lost from all three compartments during MgSO₄-induced modulation (Table 6).

DISCUSSION

In the three strains of *B. pertussis* examined, loss of adenylate cyclase activity, HSA and the X polypeptides occurred during modulation induced by a variety of growth conditions. Although some strain specificity was observed, in no case was one property lost and another retained. This is consistent with the hypothesis (Wardlaw & Parton, 1979) that a common regulatory mechanism, which is grossly perturbed during modulation, governs the synthesis of these components.

Table 5. *HSA of B. pertussis after growth with various additives*

Strains were grown for 48 h in SS-X medium with NaCl replaced by various additives. Harvested cells were heat-killed at 56 °C for 30 min and assayed for HSA. Results are expressed as the dose (opacity units ml⁻¹) required to kill 50% of mice after histamine challenge (HSD₅₀). Potency was calculated relative to NaCl-grown cells and numbers in parentheses refer to 95% confidence limits; NT, not tested.

Additive	Final concn (mM)	Strain TAB I		Strain 18334		Strain 18323	
		HSD ₅₀	Relative potency (%)	HSD ₅₀	Relative potency (%)	HSD ₅₀	Relative potency (%)
NaCl	43	1.1	100	1.0	100	1.4	100
MgSO ₄	20	22.7	4.6 (1.4, 10)	23.8	4.3 (1.4, 10)	15.2	9.3 (4.3, 19)
MgCl ₂	43	1.4	76 (33, 170)	1.4	72 (31, 160)	1.2	117 (59, 238)
Na ₂ SO ₄	43	24.7	4.3 (1.9, 8.6)	18.5	5.6 (2.0, 12)	38.9	3.6 (1.2, 9.0)
Sodium lactate	86	6.0	18 (9.2, 36)	NT	NT	NT	NT
Sodium succinate	86	16.1	6.5 (3.2, 13)	NT	NT	NT	NT
Sodium butyrate	20	15.7	7.9 (3.9, 14)	22.5	4.6 (1.5, 10)	14.6	9.7 (4.5, 20)
Sodium caprylate*	0.6	NT†	NT	30.1	3.4 (1.0, 8.3)	1.2	119 (60, 240)
Nicotinic acid*	4.1	17.4	6.0 (3.0, 11)	NT	NT	NT	NT

* These media also contained 43 mM-NaCl.
† Strain TAB I did not grow in this medium.

Table 6. *Distribution of adenylate cyclase activity in X- and C-mode cultures of B. pertussis*

Strain TAB I was grown for 48 h in SS-X medium and SS-X medium with NaCl replaced by 20 mM-MgSO₄ to give X- and C-mode cultures, respectively. Fractions of cultures were assayed for adenylate cyclase activity. Extracytoplasmic activity is defined as that portion of total cell-associated activity destroyed by trypsin treatment before cell disruption, whereas cytoplasmic activity is that portion destroyed by trypsin treatment only after cell disruption.

Location	Adenylate cyclase activity [pmol cAMP min ⁻¹ (ml culture) ⁻¹]		Activity relative to X-mode (%)
	X-mode	C-mode	
Culture supernate	221	17	7.6
Cell-associated extracytoplasmic	221	7.6	3.4
Cytoplasmic	40	1.3	3.3

Fig. 1. SDS-PAGE profiles of washed cells of *B. pertussis* after growth with various additives. Strain TAB I was grown for 48 h in SS-X medium (a) and in SS-X medium in which the NaCl was replaced by the following: 20 mM-MgSO₄ (b); 43 mM-MgCl₂ (c); 43 mM-Na₂SO₄ (d); 86 mM-sodium lactate (e); 86 mM-sodium succinate (f); 20 mM-sodium butyrate (g); 4.1 mM-nicotinic acid; 43 mM-NaCl (h). The arrows point to the cell-envelope polypeptides (X polypeptides) with M_r 28000 and 30000.

Growth in medium containing 43 mM-MgCl₂ did not cause modulation for any of the strains. Lacey (1960) reported that this concentration of MgCl₂, and other Mg salts present in Bordet-Gengou medium, caused modulation in terms of antigenic changes, although he reported MgCl₂ to be a weak pro-C-mode salt compared to MgSO₄. In our accompanying study, the critical concentration of MgSO₄ required to induce loss of the X polypeptides in strain TAB I was 10 to 11 mM (Brownlie *et al.*, 1984). These observations suggest that the important component of MgSO₄ for inducing modulation is the SO₄²⁻ anion. Whether or not higher concentrations of MgCl₂ could induce modulation was not investigated. It is possible that MgCl₂ may induce antigenic changes, as demonstrated by Lacey (1960), without causing loss of other properties such as those studied here. Schneider & Parker (1982) reported that when their strain of *B. pertussis* was subcultured twice in medium containing 20 mM-MgCl₂, it underwent antigenic changes and lost the X polypeptides.

Sodium caprylate (C₈) was a potent pro-C-mode compound for strain 18334. When present at 0.6 mM this compound caused substantial loss of adenylate cyclase activity, HSA and the X polypeptides. This is consistent with Lacey's (1960) findings that fatty acids were potent pro-C-mode compounds with potency increasing with chain length. Sodium lactate (86 mM) caused a less severe loss of adenylate cyclase activity, HSA and the X polypeptides in strain TAB I, but had no effect on the expression of these activities for strains 18334 or 18323. This partial loss presumably represents intermediate expression of these factors throughout the population of cells, although a mixed culture of X- and C-mode cells can not be excluded. The intermediate mode in strain TAB I may be similar to the intermediate mode described by Lacey (1960), which contained both X- and C-mode antigens. The reason for the strain specificity of modulation induced

by sodium lactate and sodium caprylate is not known, but may be because the regulatory mechanism, which underlies modulation, varies between strains in its sensitivity to salts. Perhaps continuous subculture in 'unnatural' conditions (e.g. laboratory media or the mouse brain) selects for strains less responsive to modulating compounds.

After growth in media where cells underwent a marked decrease in intact-cell adenylate cyclase activity, enzyme activity still responded to activator but activation was reduced by at least 50% compared to activation of enzyme from NaCl-grown cells. Hewlett *et al.* (1979*b*) reported that during purification of adenylate cyclase, responsiveness to the activator was lost, suggesting the requirement for an additional factor for activation. It is possible that during modulation the production of this factor, as well as adenylate cyclase activity, is decreased.

In addition to loss of adenylate cyclase activity in harvested cells, the concentration of cAMP in the culture supernate was greatly decreased after growth where loss of HSA and the X polypeptides also took place. This suggests that intracellular adenylate cyclase activity occurring during growth is also considerably decreased during modulation. The concentration of cAMP in the culture supernate presumably reflects intracellular adenylate cyclase activity during growth, as the substrate ATP is probably exclusively intracellular.

Hewlett *et al.* (1976) reported that adenylate cyclase activity was located in at least three compartments in *B. pertussis* cultures: the cytoplasm (10%), the cell envelope (70%) and the culture supernate (20%). Results presented here confirm this, although a greater proportion of adenylate cyclase activity was associated with the culture supernate (50%). Perhaps this is due to differences in the assay methods. Hewlett *et al.* (1976) included creatine phosphokinase in the assay mixture to regenerate ATP. Several-fold more cAMP is produced in such assays, as commercial creatine phosphokinase is contaminated with calmodulin (Goldhammer *et al.*, 1981). However, adenylate cyclase in culture supernate is much less responsive to activator than is cell-associated adenylate cyclase (Hewlett *et al.*, 1979*b*), and this may explain the differences in the enzyme activities observed in the two studies. In all three compartments (culture supernate, extracytoplasmic but cell-associated, and cytoplasmic) adenylate cyclase activity was substantially decreased after MgSO₄-induced modulation. This would suggest that the observed loss of activity of intact-cell adenylate cyclase was not due to inhibition of translocation from one compartment to another.

Hewlett *et al.* (1979*a*) and Wardlaw & Parton (1979) have pointed out that loss of adenylate cyclase activity during modulation may have a causal relationship to the loss of the other X-mode specific properties. Results of this study, where loss of HSA and the X polypeptides occurred concomitantly with the loss of adenylate cyclase activity during modulation induced by different inorganic and organic salts, does not detract from this suggestion. However, further studies (Brownlie *et al.*, 1984) indicate that this enzyme does not play a causal role in the loss of virulence-related factors during modulation, but rather that both are influenced by the same control mechanism which underlies the modulation phenomenon.

We thank Professor G. T. Stewart and Dr F. Sheffield for *B. pertussis* strains and Dr J. P. Durham for supplies of cAMP binding protein. R. M. B. was the recipient of a Science and Engineering Research Council studentship.

REFERENCES

- BOTSFORD, J. L. (1981). Cyclic nucleotides in pro-caryotes. *Microbiological Reviews* **45**, 620-642.
- BROWNLIE, R. M., COOTE, J. G. & PARTON, R. (1984). Adenylate cyclase activity during phenotypic variation of *Bordetella pertussis*. *Journal of General Microbiology* **131**, 27-38.
- DOBROGOSZ, W. J., EZZELL, J. W., KLOOS, W. E. & MANCLARK, C. R. (1979). Physiology of *Bordetella pertussis*. In *International Symposium on Pertussis*, pp. 86-93. Edited by C. R. Manclark & J. C. Hill. Washington, DC: US DHEW Publication no. (NIH) 79-1830.
- EPSTEIN, W., ROTHMAN-DENES, L. B. & HESSE, J. (1975). Adenosine 3':5'-cyclic monophosphate as mediator of catabolite repression in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 2300-2304.
- GILMAN, A. G. A. (1970). A protein binding assay for adenosine 3',5'-cyclic monophosphate. *Proceedings of the National Academy of Sciences of the United States of America* **67**, 305-312.
- GOLDHAMMER, R., WOLFF, J., COOK, G. H., BERKOWITZ, S. A., KLEE, C. B., MANCLARK, C. R. & HEWLETT, E. L. (1981). Spurious protein activators

- of *Bordetella pertussis* adenylate cyclase. *European Journal of Biochemistry* **115**, 605–609.
- HERBERT, D., PHIPPS, P. J. & STRANGE, R. E. (1969). Chemical analysis of microbial cells. *Methods in Microbiology* **5B**, 209–344.
- HEWLETT, E. & WOLFF, J. (1976). Soluble adenylate cyclase from the culture medium of *Bordetella pertussis*: purification and characterisation. *Journal of Bacteriology* **127**, 890–898.
- HEWLETT, E. L., URBAN, M. A., MANCLARK, C. R. & WOLFF, J. (1976). Extracytoplasmic adenylate cyclase of *Bordetella pertussis*. *Proceedings of the National Academy of Sciences of the United States of America* **73**, 1926–1930.
- HEWLETT, E. L., WOLFF, J. & MANCLARK, C. R. (1978). Regulation of *Bordetella pertussis* extracytoplasmic adenylate cyclase. *Advances in Cyclic Nucleotide Research* **9**, 621–628.
- HEWLETT, E. L., UNDERHILL, L. H., VARGO, S. A., WOLFF, J. & MANCLARK, C. R. (1979a). *Bordetella pertussis* adenylate cyclase: regulation of activity and its loss in degrading strains. In *International Symposium on Pertussis*, pp. 81–85. Edited by C. R. Manclark & J. C. Hill. Washington, DC: US DHEW Publication no. (NIH) 79-1830.
- HEWLETT, E. L., UNDERHILL, L. H., COOK, G. H., MANCLARK, C. R. & WOLFF, J. (1979b). A protein activator for the adenylate cyclase of *Bordetella pertussis*. *Journal of Biological Chemistry* **254**, 5602–5605.
- HOLT, L. B. & SPASOJEVIC, V. (1968). The role of surface antigens in the protective potency of *Bordetella pertussis* suspensions as measured by the intracerebral challenge technique in mice. *Journal of Medical Microbiology* **1**, 119–126.
- LACEY, B. W. (1960). Antigenic modulation of *Bordetella pertussis*. *Journal of Hygiene* **58**, 57–93.
- LIVEY, I., PARTON, R. & WARDLAW, A. C. (1978). Loss of heat-labile toxin from *Bordetella pertussis* grown in modified Hornibrook medium. *FEMS Microbiology Letters* **3**, 203–205.
- MUNOZ, J. J. (1976). Pertussigen: a substance from *Bordetella pertussis* with many biological activities. *Federation Proceedings* **35**, 813.
- PARTON, R. & DURHAM, J. P. (1978). Loss of adenylate cyclase in variants of *Bordetella pertussis*. *FEMS Microbiology Letters* **4**, 287–289.
- PASTAN, I. & ADHYA, S. (1976). Cyclic adenosine 3',5'-monophosphate in *Escherichia coli*. *Bacteriological Reviews* **40**, 527–551.
- PITTMAN, M. (1979). Pertussis toxin: the cause of the harmful effects and prolonged immunity of whooping cough. A hypothesis. *Reviews in Infectious Diseases* **1**, 401–412.
- PUSZTAI, Z. & JOÓ, I. (1967). Influence of nicotinic acid on the antigenic structure of *Bordetella pertussis*. *Annales immunologicae hungaricae*. **10**, 63–67.
- SCHNEIDER, D. R. & PARKER, C. D. (1982). Effect of pyridines on phenotypic properties of *Bordetella pertussis*. *Infection and Immunity* **38**, 548–553.
- STAINER, D. W. & SCHOLTE, M. J. (1971). A simple chemically defined medium for the production of Phase I *Bordetella pertussis*. *Journal of General Microbiology* **63**, 211–220.
- WARDLAW, A. C. & PARTON, R. (1979). Changes in envelope proteins and correlation with biological activities in *B. pertussis*. In *International Symposium on Pertussis*, pp. 94–98. Edited by C. R. Manclark & J. C. Hill. Washington, DC: DHEW Publication no. (NIH) 79-1830.
- WARDLAW, A. C. & PARTON, R. (1983). *Bordetella pertussis* toxins. *Pharmacology and Therapeutics* **19**, 1–53.
- WARDLAW, A. C., PARTON, R. & HOOKER, M. J. (1976). Loss of protective antigen, histamine-sensitizing factor and envelope polypeptides in cultural variants of *Bordetella pertussis*. *Journal of Medical Microbiology* **9**, 89–100.