Biochem. J. (1968) **106**, 237
Printed in Great Britain

Influence of Growth Condition on the Concentration of Potassium in *Bacillus subtilis* var. *niger* and its Possible Relationship to Cellular Ribonucleic Acid, Teichoic Acid and Teichuronic Acid

By D. W. TEMPEST, J. W. DICKS and D. C. ELLWOOD
Microbiological Research Establishment, Porton, nr. Salisbury, Wilts.

(*Received* 13 *July* 1967)

1. Mg^{2+}-limited *Bacillus subtilis* var. *niger*, growing in a chemostat in a simple salts medium, contained considerably more potassium and phosphorus than Mg^{2+}-limited *Aerobacter aerogenes* growing in a similar medium at corresponding dilution rates. 2. Growth of the bacillus in a K^+-limited environment did not lower the cellular potassium and phosphorus contents, the molar proportions of cell-bound magnesium, potassium, RNA (as nucleotide) and phosphorus being approximately constant at 1:13:5:13 (compared with 1:4:5:8 in Mg^{2+}-limited or K^+-limited *A. aerogenes*). 3. Growth of *B. subtilis* in a phosphate-limited environment caused the cellular phosphorus content to be lowered to a value similar to that of Mg^{2+}-limited *A. aerogenes*, but the potassium content was not correspondingly lowered; the molar potassium:magnesium ratio varied from 14 to 17 with changes in dilution rate from 0·4 to 0·1 $hr.^{-1}$. 4. Whereas over 70% of the cell-bound phosphorus of Mg^{2+}-limited or K^+-limited *A. aerogenes* was contained in the nucleic acids, these polymers accounted for less than 50% of the phosphorus present in similarly limited *B. subtilis*; much phosphorus was present in the walls of the bacilli, bound in a teichoic acid-type compound composed of glycerol phosphate and glucose (but no alanine). 5. Phosphate-limited *B. subtilis* cell walls (from organisms grown at a dilution rate of 0·2 $hr.^{-1}$) contained little phosphorus and no detectable amounts of teichoic acid, but 40% of the cell-wall dry weight could be accounted for by a teichuronic acid-type compound; this contained a glucuronic acid and galactosamine, neither of which could be detected in the walls of Mg^{2+}-limited *B. subtilis* grown at a corresponding rate. 6. It is suggested that the high concentration of potassium in growing *B. subtilis* (compared with *A. aerogenes*) results from the presence of large amounts of anionic polymer (teichoic acid or teichuronic acid) in the bacillus cell walls.

Probably all organisms require potassium, magnesium and phosphorus for growth. Recently it has been reported (Tempest, Dicks & Hunter, 1966; Dicks & Tempest, 1966) that, although the concentration of each of these substances in growing *Aerobacter aerogenes* organisms varied (with growth rate and with temperature), the molar magnesium:potassium:RNA (as nucleotide):phosphorus proportions remained almost constant at 1:4:5:8. This stoicheiometry indicated a functional relationship between these substances in the growing cell and, because the bulk of the RNA was found to be in the ribosomes, it was suggested (Tempest & Dicks, 1967) that the bulk of the cellular magnesium and potassium also may be concentrated in, or associated with, ribosomal structures. If this hypothesis is correct, then one might expect a similar relationship between these substances to exist in other organisms, particularly since bacterial ribosomes do not seem to vary greatly from species to species (Taylor & Storck, 1964). In this connexion no significant differences were found between the intracellular RNA and magnesium contents of *A. aerogenes* and *Bacillus subtilis* when grown at identical rates in similar environments (Tempest, Dicks & Meers, 1967*b*). Now we have extended our observations on *B. subtilis* to include changes in bacterial potassium and phosphorus contents with growth conditions. The results obtained are considerably different from those for *A. aerogenes* and evidence is presented indicating that the differences result from the presence of anionic polymers, other than RNA, in the *B. subtilis* organisms.

Brief reports of the essential findings have been published (Tempest, Dicks & Ellwood, 1967*a*; Ellwood & Tempest, 1967*a*).

MATERIALS AND METHODS

Organism. *B. subtilis* var. *niger* was obtained from Fort Detrick, Md., U.S.A., and originally called *B. globigii*; A.T.C.C. 9372 is probably the same strain. This was maintained by monthly subculture on tryptic-meat-digest agar slopes containing glucose (0·2%, w/v).

Growth conditions. Continuous cultures of *B. subtilis* were maintained in 0·5l. 'Porton-type' chemostats (Herbert, Phipps & Tempest, 1965) with the temperature regulated at 35° and the pH controlled at 7·0±0·1. The Mg^{2+}-limited medium was that detailed by Tempest *et al.* (1967*b*). The K^+-limited medium contained: Na_2HPO_4, 5·0mM; $NH_4H_2PO_4$, 45mM; $(NH_4)_2SO_4$, 25mM; K_2SO_4, 1·5mM; citric acid, 1·0mM; $MgCl_2$, 1·25mM; $CaCl_2$, 0·1mM; $FeCl_3$, 0·1mM; $ZnCl_2$, 25μM; $MnCl_2$, 25μM; $CuCl_2$, 5·0μM; $CoCl_2$, 5·0μM; Na_2MoO_4, 5·0μM. The pH value was about 5·5. After sterilization (121° for 30min.) a sterile solution of glucose, slightly acidified and sterilized by autoclaving, was added to the bulk medium to give a final concentration of 30g. of glucose/l. The phosphate-limited medium contained: NaH_2PO_4, 1·5mM; $(NH_4)_2SO_4$, 50mM; K_2SO_4, 3·0mM; citric acid, 1·0mM; $MgCl_2$, 1·25mM; $CaCl_2$, 0·1mM; $FeCl_3$, 0·1mM; $ZnCl_2$, 25μM; $MnCl_2$, 25μM; $CuCl_2$, 5·0μM; $CoCl_2$, 5·0μM; Na_2MoO_4, 5·0μM. The pH value was about 3·5. After sterilization (121° for 30min.) a sterile solution of glucose was added to give a final concentration of 30g. of glucose/l. With all the media the pH value was adjusted, in the growth vessel, by addition of 2N-NH_3. In each case the medium was prepared with distilled water that had been further deionized by passage through a mixed-bed ion-exchange resin column.

Experimental procedure. Chemostat cultures were established by inoculating the medium in the growth chamber with about 100ml. of a broth culture of *B. subtilis* (grown for about 6hr. at 37° in an Erlenmeyer flask on a reciprocating shaker). The dilution rate was set immediately at about 0·3hr.$^{-1}$ and the culture left to equilibrate overnight. The following day the dilution rate was adjusted to the required value and the culture left to equilibrate a further 2 days. Foaming of the culture was suppressed by a regular addition of small volumes (0·1–0·2ml.) of sterile PPG-2000 antifoam (Shell Chemical Co. Ltd.). Small samples of culture (up to 25ml.) were drawn directly from the growth vessel, but larger samples (1–2l.) were collected over a period of time from the overflow tube, by using an ice-cooled receiver. Organisms were separated from the culture fluids by centrifugation, washed with deionized water and either freeze-dried or stored as a frozen paste (−20°).

Analytical procedures. The culture bacterial concentration (dry wt. of organisms/ml. of culture), and the bacterial RNA, DNA, protein and carbohydrate contents, were determined by methods described by Tempest, Hunter & Sykes (1965). The potassium contents of growing organisms were determined by the procedure described by Tempest *et al.* (1966). Magnesium contents were determined with an EEL model 140 Atomic Adsorption Spectrophotometer on bacterial samples that had been digested with boiling 60% (w/v) $HClO_4$, and these samples were also used for determining the bacterial phosphorus contents (method of King, 1951). Uronic acid was determined by reaction with H_2SO_4 and carbazole (Dische, 1947). Glucose and galactosamine were determined enzymically, by using the Glucostat and Galactostat reagents of the Worthington Biochemical Corp. (Freehold, N.J., U.S.A.).

Isolation of bacterial cell walls. About 20g. wet wt. of bacteria was suspended in 400ml. of water and the concentration of organisms determined accurately by drying (105°, 15hr.) and weighing triplicate 10·0ml. samples. Sixteen samples (10ml.) of the suspension were mixed with Ballotini glass beads (no. 16 grade, 10ml./sample) and shaken (at 4°) in a vertical shaker of 10cm. travel, 450 oscillations/min., until less than 2% of the bacteria remained unbroken (usually 1½–2hr.); the temperature of the suspension rose slightly during this treatment, but never exceeded 10°. The samples were combined and heated (100° for 10min.) to inhibit enzymic degradation of the cell walls. The beads were then separated by filtration through a sintered-glass filter (grade 2) and washed free of cell debris with water; the filtrate and washings were collected and combined. The bacterial walls were separated from the soluble fraction by centrifugation (10000*g* for 30min.), washed three times with 0·85% (w/v) NaCl and three times with water, and checked for purity by electron microscopy. The washed cell walls were then transferred quantitatively to weighed bottles, freeze-dried and reweighed. The dry weights of duplicate samples invariably agreed within ±2%. The bacterial cell-wall content was calculated from the total weight of wall material and the initial bacterial dry weight.

Extraction of polymers from cell-wall preparations. Samples of dry bacterial cell walls (100mg.) were dispersed in 10% (w/v) trichloroacetic acid (10ml.) and stirred slowly at 37° for 18hr. The undissolved residue was removed by centrifugation (10000*g* for 30min.) and the supernatant fraction poured into 5vol. of acetone cooled to 0°. The solution was further cooled to −10° and kept at this temperature for about 18hr. The precipitate that formed was separated by centrifugation (3000*g* for 30min.), washed with cold acetone, dissolved in water, freeze-dried and weighed.

Examination of the polymers. The polymers were hydrolysed by heating in a sealed tube (100° for 3hr.) with 2N-HCl. The HCl was removed by repeated evaporation *in vacuo* over NaOH pellets, and substances present in the hydrolysates were separated by chromatography with the solvent of Ellwood, Kelemen & Baddiley (1963) for teichoic acids and that of Janczura, Perkins & Rogers (1961) for teichuronic acids. Components of the polymers were tentatively identified by comparison with authentic substances, chromatographed simultaneously, with the detecting reagents of Ellwood *et al.* (1963) and Janczura *et al.* (1961).

RESULTS

Although the RNA and magnesium contents of *B. subtilis* var. *niger* were found to be very similar to those of *A. aerogenes*, when grown at identical rates in similar environments (Tempest *et al.* 1967*b*), the potassium and phosphorus contents of the two organisms were much different. With a progressive change in dilution rate from 0·1hr.$^{-1}$ to 0·4hr.$^{-1}$, the potassium content of Mg^{2+}-limited *B. subtilis* organisms increased from 2·6% to 4·7%, and the phosphorus content from 2·6% to 3·6%, of the bacterial dry weight; corresponding values for Mg^{2+}-limited *A. aerogenes* were 0·9% to 1·5% and

Table 1. *Influence of the dilution rate on the steady-state concentration of bacteria, and on potassium, magnesium and phosphorus contents, of* K^+*-limited* B. subtilis *var.* niger, *growing in a chemostat*

The organisms were cultured in a simple salts medium (plus glucose) in a 500ml. 'Porton-type' chemostat. Culture bacterial concentrations and compositions were determined by procedures indicated in the Materials and Methods section. The results given are average values obtained from analysis of two to four samples (collected and processed on separate days) grown at each dilution rate for at least 2 days before collection of the first sample.

Dilution rate ($hr.^{-1}$)	Dry wt. of bacteria (mg./ml.)	Content (g./100g. of dried bacteria)		
		Potassium	Magnesium	Phosphorus
0·05	4·9	2·3	0·13	1·9
0·1	3·9	2·7	0·15	2·7
0·2	3·0	3·7	0·19	3·2
0·4	1·8	4·9	0·22	3·5

Table 2. *Influence of dilution rate on the macromolecular composition of* K^+*-limited* B. subtilis *var.* niger, *grown in a chemostat*

The organisms were grown in a simple salts medium in a 500ml. 'Porton-type' chemostat (at 35° and pH 7·0). Samples, for analysis, were collected from the overflow line in an ice-cooled receiver overnight; they were separated from the culture fluids and washed by centrifugation and then freeze-dried. Suspensions containing 10mg. of dried bacteria/ml. of distilled water were prepared for analysis by procedures described in the Materials and Methods section.

Dilution rate ($hr.^{-1}$)	Content (g./100g. of dried bacteria)			
	Protein	Carbohydrate (as polyglucose)*	DNA	RNA
0·05	65·5	6·5	1·9	8·5
0·1	64·1	8·8	1·9	11·4
0·2	62·9	10·4	1·6	12·8
0·4	58·2	12·6	1·7	14·0

* Measured by the anthrone method of Fales (1951).

1·6% to 2·3% for potassium and phosphorus respectively (Dicks & Tempest, 1966). Thus the intracellular potassium content of the bacillus was almost 300%, and the phosphorus content about 150%, that of *A. aerogenes*, and the molar magnesium:potassium:phosphorus proportions approximated to 1:14:14.

When organisms are limited in their growth by the availability of a particular element, they may be expected to contain the minimum concentration of that element necessary to satisfy their structural and metabolic needs for growth at the imposed rate. Therefore we grew *B. subtilis* in a medium containing K^+ as the growth-limiting component to determine the minimum cellular potassium content necessary for growth of the organisms at each of several different dilution rates.

Influence of K^+*-limitation on the potassium, magnesium and phosphorus contents, and macromolecular composition, of* B. subtilis *var.* niger. When the dilution rate of a K^+-limited chemostat culture of *B. subtilis* var. *niger* was progressively increased, gross changes were observed in the steady-state concentration of bacteria (Table 1). Since, at dilution rates below $0·5 hr.^{-1}$, over 95% of the total culture potassium was present in the organisms, these changes in steady-state concentration of bacteria indicated a progressive increase in bacterial potassium content with growth rate. The values found for bacterial potassium, magnesium and phosphorus contents are given in Table 1; the changes in bacterial content of each of these substances with growth rate were proportionately similar and closely paralleled the changes in bacterial RNA content (Table 2). But it is apparent that the potassium and phosphorus contents are not lower than those in Mg^{2+}-limited *B. subtilis* and again considerably higher than those in K^+-limited *A. aerogenes* (Tempest *et al.* 1966). The molar proportions (particularly potassium:magnesium) varied slightly with dilution rate (Table 3) but averaged 1:13:5:13 for magnesium:potassium:RNA (as nucleotide):phosphorus.

The higher bacterial contents of potassium and phosphorus both in Mg^{2+}-limited and K^+-limited *B. subtilis* (when compared with *A. aerogenes*) suggested the presence in the bacilli of phosphorus-containing compounds, other than RNA and DNA, that presumably had much potassium associated with them. Thus, whereas the nucleic acid content of K^+-limited *A. aerogenes* accounted for over 70% of the total cell-bound phosphorus, at all dilution rates, it accounted for less than 50% of the cellular phosphorus of either Mg^{2+}-limited or K^+-limited *B. subtilis* (Table 4).

If the high potassium content results directly from the presence of non-nucleic acid phosphorus-containing compounds in Mg^{2+}-limited and K^+-limited *B. subtilis* organisms, then any changes in the amounts of these compounds with growth condition should be accompanied by corresponding changes in the potassium content of the organisms. Therefore we attempted to decrease the cellular phosphorus content by growing the organisms in a phosphate-limited environment.

Influence of phosphate-limitation on the potassium, magnesium and phosphorus contents, and macromolecular composition, of B. subtilis *var.* niger. When the dilution rate of a phosphate-limited chemostat culture of *B. subtilis* var. *niger* was

Table 3. *Influence of dilution rate on the interrelationships between potassium, magnesium, phosphorus and RNA contents of* K^+*-limited* B. subtilis *var.* niger, *growing in a chemostat*

The values are derived from results given in Tables 1 and 2 and assuming an average molecular weight for RNA nucleotide of 340.

Dilution rate ($hr.^{-1}$)	Molar ratios					
	Potassium: magnesium	Phosphorus: magnesium	Phosphorus: potassium	RNA: potassium	RNA: magnesium	RNA: phosphorus
0·05	11·4	11·9	1·04	0·42	4·8	0·40
0·1	11·0	13·7	1·25	0·48	5·3	0·39
0·2	12·5	13·4	1·08	0·40	4·9	0·37
0·4	14·0	12·6	0·91	0·33	4·6	0·36

Table 4. *Distribution of phosphorus in* A. aerogenes *and* B. subtilis *organisms, growing at different dilution rates in a chemostat*

The values are expressed as g. of (RNA + DNA) P/g. of total P. The values for *A. aerogenes* are derived from the data of Tempest *et al.* (1966), and those for *B. subtilis* from data contained in Tables 1, 2, 5 and 6 of this paper and Table 1 of Tempest *et al.* (1967*b*).

	Distribution of phosphorus				
Dilution rate ($hr.^{-1}$) ...	0·05	0·1	0·2	0·4	0·8
A. aerogenes (K^+-limited)	—	0·67	0·73	0·73	0·71
B. subtilis (K^+-limited)	0·49	0·45	0·41	0·41	—
B. subtilis (Mg^{2+}-limited)	—	0·40	0·44	0·40	—
B. subtilis (phosphate-limited)	—	0·91	0·86	0·74	—

Table 5. *Influence of dilution rate on steady-state concentration of bacteria, and on potassium, magnesium and phosphorus contents of phosphate-limited* B. subtilis *var.* niger, *growing in a chemostat*

The results given are average values obtained from two to four samples (collected and analysed on separate days) grown at each dilution rate for at least 2 days before collection of the first sample.

Dilution rate ($hr.^{-1}$)	Dry wt. of bacteria (mg./ml.)	Content (g./100g. of dried bacteria)		
		Potassium	Magnesium	Phosphorus
0·1	3·5	3·1	0·12	1·3
0·2	2·7	3·8	0·15	1·7
0·4	1·9	4·4	0·20	2·3

progressively increased from 0·1 $hr.^{-1}$ to 0·4 $hr.^{-1}$, the steady-state concentration of bacteria again decreased (Table 5). As with the K^+-limited culture of *B. subtilis*, at dilution rates below 0·5 $hr.^{-1}$ over 95% of the growth-limiting substrate, present in the culture, was contained in the organisms and the changes in steady-state concentration of bacteria indicated corresponding changes in bacterial phosphorus content. The values found for the phosphorus content of phosphate-limited *B. subtilis*, at each of three different dilution rates, and the parallel values for bacterial potassium and magnesium contents, are shown in Table 5. It is apparent that the bacterial phosphorus content of

Table 6. *Influence of dilution rate on the macromolecular composition of phosphate-limited* B. subtilis *var.* niger, *grown in a chemostat*

The results given are mean values from two samples, which were collected on separate days after the culture had equilibrated at the set dilution rate for at least 2 days. Samples, from the overflow tube, were collected overnight into an ice-cooled receiver. Organisms were separated from the culture fluids by centrifugation, washed with distilled water and freeze-dried. Suspensions containing 10mg. of dried organisms/ml. of distilled water were prepared and analysed by the procedures indicated in the Materials and Methods section.

Dilution rate ($hr.^{-1}$)	Content (g./100g. of dried bacteria)			
	Protein	Carbohydrate (as polyglucose)*	DNA	RNA
0·1	61·0	3·1	2·1	10·9
0·2	57·6	3·6	2·0	13·8
0·4	56·5	6·2	2·0	17·0

* Measured by the anthrone method of Fales (1951).

Table 7. *Influence of dilution rate on the interrelationships between potassium, magnesium, phosphorus and RNA contents of phosphate-limited* B. subtilis *var.* niger, *growing in a chemostat*

The values are derived from the data given in Tables 5 and 6, assuming an average molecular weight for RNA nucleotide of 340.

Dilution rate ($hr.^{-1}$)	Molar ratios					
	Potassium: magnesium	Phosphorus: magnesium	Phosphorus: potassium	RNA: potassium	RNA: magnesium	RNA: phosphorus
0·1	16·5	8·6	0·52	0·41	6·4	0·76
0·2	15·7	8·6	0·55	0·42	6·5	0·74
0·4	13·5	8·8	0·60	0·45	6·0	0·68

these organisms was much lower than in either Mg^{2+}-limited or K^+-limited organisms, and only slightly higher than in phosphate-limited *A. aerogenes* organisms, grown at corresponding rates (Dicks & Tempest, 1966). Since the macromolecular composition of phosphate-limited *B. subtilis* (Table 6) was similar to that of K^+-limited *B. subtilis* (Table 2), except with respect to the carbohydrate content of the organisms, the low phosphorus content was not due to a decreased synthesis of RNA and DNA. Obviously some phosphorus-containing compounds, which were present in Mg^{2+}-limited and K^+-limited *B. subtilis*, were absent from phosphate-limited organisms (or present in much diminished concentration) since the nucleic acids now contained over 80% of the total cell-bound phosphorus at all dilution rates (Table 4). However, the potassium content of phosphate-limited *B. subtilis* was not simultaneously lowered (Table 5) and at some dilution rates the potassium content of the organisms was higher than in either Mg^{2+}-limited or K^+-limited organisms (Table 1).

Since the magnesium, phosphorus and RNA contents of phosphate-limited *B. subtilis* varied proportionately and progressively with growth rate (Table 5), their molar proportions (magnesium: RNA: phosphorus) remained relatively constant at about 1:6:9 (Table 7). But the variation in cell-bound potassium, with growth rate, was less proportionate and the potassium: magnesium ratio varied considerably (Table 7).

Teichoic acid and teichuronic acid contents of B. subtilis *var.* niger. The walls of *B. subtilis* organisms are known to contain teichoic acids (Baddiley, 1964) that, since they are phosphorus-containing polymers, could account for the high non-nucleic acid phosphorus content of Mg^{2+}-limited and K^+-limited *B. subtilis* var. *niger* (Table 4). Therefore bacterial cell walls were prepared from Mg^{2+}-limited *B. subtilis* (grown at a dilution rate, *D*, of $0·2 hr.^{-1}$, at 35° and pH 7·0) and analysed. These walls, which amounted to 24% of the bacterial dry weight, contained 6% of phosphorus, sufficient to account for the difference in phosphorus content between Mg^{2+}-limited *B. subtilis* and Mg^{2+}-limited *A. aerogenes* (i.e. 3·0% and 1·7% of the bacterial dry weight respectively at D $0·2 hr.^{-1}$). A single extraction of the wall preparation with trichloroacetic acid, followed by precipitation with acetone, gave a polymer that accounted for 40% of the wall dry weight. Hydrolysis of this polymer and chromatographic analysis revealed the presence of glycerol, glycerol phosphates and glucose only, indicating a teichoic acid-type compound from which alanine was absent. The absence of alanine was confirmed by examination of products released from these walls by treatment with mild alkali.

Bacterial walls prepared from phosphate-limited *B. subtilis* (grown at D $0·2 hr.^{-1}$, at 35° and pH 7·0), which accounted for 20% of the bacterial dry weight, contained only 0·2% of phosphorus, suggesting the absence of teichoic acids. Nevertheless, treatment

Table 8. *Influence of growth-limiting component of the medium on the composition of the cell walls of* B. subtilis *var.* niger

Organisms were grown in chemostats at a dilution rate of $0·2 hr.^{-1}$ (at 35° and pH 7·0), collected into cooled receivers and fractionated as described in the Materials and Methods section. Glucose was determined enzymically on wall preparations that had been hydrolysed with N-H_2SO_4 (at 100° for 3 hr. in a sealed tube) and neutralized (NaOH). Galactosamine also was determined enzymically on samples that had been hydrolysed with 4 N-HCl (at 100° for 4 hr. in a sealed tube) and neutralized (NaOH). Glucuronic acid and phosphorus estimations were carried out on unhydrolysed suspensions of bacterial cell walls (10 mg./ml., in distilled water).

Component	Content (g. of component/100 g. of dry cell walls)	
	Phosphate-limited organisms	Mg^{2+}-limited organisms
Phosphorus	0·2	6·0
Glucose	< 1	28 (27*)
Glucuronic acid	22	< 2
Galactosamine	14	< 2

* Value in parenthesis was determined with anthrone on an unhydrolysed suspension of cell walls.

of these walls with trichloroacetic acid (as above), followed by acetone precipitation, again extracted a polymer amounting to 40% of the bacterial wall dry weight. Acid hydrolysis of this polymer (as above) and chromatographic analysis showed the absence of glycerol, glycerol phosphates and glucose, confirming the absence of the teichoic acid, but the presence of glucuronic acid and galactosamine, indicating the presence of a teichuronic acid-type compound (Janzcura *et al.* 1961). Mg^{2+}-limited *B. subtilis* cell wall contained no detectable amounts of teichuronic acid and this was confirmed by chemical analysis of the wall preparations (Table 8).

K^+-limited *B. subtilis*, as well as Mg^{2+}-limited organisms (Tempest *et al.* 1967*b*), contain larger amounts of carbohydrate (measured as glucose by the anthrone method) than similarly limited *A. aerogenes* (Tempest & Dicks ,1967). This difference can be mostly accounted for by the presence of glucose in the teichoic acid of the bacillus cell wall (Table 8). Thus phosphate-limited *B. subtilis*, which contain no wall teichoic acid, have a total carbohydrate content (Table 6) similar to that of carbon-limited *A. aerogenes* (Dicks & Tempest, 1967).

DISCUSSION

In a chemostat culture, the growth rate of bacteria can be varied between wide limits; always this has been found to result in parallel changes in intracellular RNA content, provided that the temperature was maintained constant (Schaechter, Maaløe & Kjeldgaard, 1958; Neidhardt, 1963; Tempest & Hunter, 1965). With variously limited cultures of *A. aerogenes*, changes in RNA content also were accompanied by proportionately equivalent changes in bacterial magnesium, potassium and phosphorus contents (Tempest *et al.* 1966; Dicks & Tempest, 1966), indicating a functional relationship between them in the growing cell. Proportionately similar changes in bacterial magnesium, potassium, RNA and phosphorus contents also were observed when K^+-limited cultures of *B. subtilis* were grown at different rates (Tables 1–3), but the molar proportions [1:13:5:13, for magnesium:potassium:RNA (as nucleotide):phosphorus] were substantially different from those reported for *A. aerogenes* (i.e. 1:4:5:8). If, as suggested by Tempest & Dicks (1967), the stoicheiometric relationship in *A. aerogenes* resulted from these substances being associated in the functioning ribosome, then the different relationship between them in growing *B. subtilis* organisms must indicate either a different ribosomal requirement for magnesium and potassium, or the presence in the bacilli of substance(s), other than RNA, containing much phosphorus and potassium.

Since preparations of bacterial ribosomes *in vitro* do not show any significant species differences (Taylor & Storck, 1964), it seems unlikely that the *B. subtilis* and *A. aerogenes* ribosomes have different requirements for magnesium and potassium. Further, it is apparent (Table 4) that K^+-limited and Mg^{2+}-limited *B. subtilis* organisms do contain much more non-nucleic acid phosphorus than *A. aerogenes*. Clearly, this 'extra' phosphorus is contained in the cell wall, largely as a teichoic acid (a polymer that accounts for at least 40% of the wall dry weight and that contains about 10% of phosphorus). Since this teichoic acid does not contain alanine it is likely to be strongly acidic, but whether this polymer accounts for the correspondingly high bacterial potassium content is problematical. Phosphate-limited *B. subtilis* organisms were devoid of teichoic acid but still contained much 'extra' potassium. However, the presence of a different anionic polymer in the walls of these bacteria (i.e. teichuronic acid, which was not present in detectable concentration in the walls of Mg^{2+}-limited *B. subtilis*, grown at the same dilution rate) could account for the continued presence of high potassium contents in these teichoic acid-free organisms.

In Gram-positive organisms, the cell-wall structures are separate from, and exterior to, the plasma membrane (Salton, 1964). Therefore, assuming specificity for K^+ accumulation to reside at the plasma membrane, a specific association between potassium and cell-wall anionic polymers would not be expected, particularly so when the organisms were cultured in a K^+-limited environment where the extracellular K^+ concentration was low and amounted to a very small fraction of the total extracellular cation concentration. However, little cell-bound sodium could be detected in any of the growing *B. subtilis* cultures, even when they were K^+-limited, suggesting that potassium may indeed be bound selectively at the cell surface.

With cultures of *Escherichia coli*, bacterial potassium content was found to vary markedly with the osmolarity of the medium (Epstein & Schultz, 1965), from which it was concluded that the medium osmolarity is a major determinant of the cell potassium concentration. But the changes in bacterial potassium content associated with changes in growth rate, both in *B. subtilis* (Tables 1 and 5) and in *A. aerogenes* (Tempest & Dicks, 1967) obviously do not result from changes in the osmolarity of the growth media; the culture osmolarity did not vary greatly with dilution rate. Further, the culture osmolarity would tend to increase with decreasing dilution rates owing to the greater proportionate input of saturated sodium chloride solution (through the reference-electrode 'bridge') and titrant needed for pH control; this should cause the bacterial potassium content to increase instead

of decrease, with progressive lowering of the dilution rate.

Presumably the synthesis of cell-wall polymers is genetically controlled, but the cell-wall contents of organisms have been found to vary considerably with the growth condition (Sud & Schaechter, 1964; Ellwood & Tempest, 1967*b*). It is apparent here that the polymer content of *B. subtilis* cell walls changes extensively with the nature of the growth limitation. The physiological significance of bacterial teichoic acid is not yet known, but the fact that teichoic acid is replaced by another anionic polymer in phosphate-limited organisms suggests that cell-wall anionic polymers may play an essential role in cation uptake by this bacillus (Archibald & Baddiley, 1966).

We are indebted to Mr R. E. Strange for many valuable discussions and criticism of this work, and to Mr T. H. Dunham and Mr G. R. G. Moody for their skilled technical assistance.

REFERENCES

Archibald, A. R. & Baddiley, J. (1966). *Advanc. Carbohyd. Chem.* **21**, 323.

Baddiley, J. (1964). *Endeavour*, **23**, 33.

Dicks, J. W. & Tempest, D. W. (1966). *J. gen. Microbiol.* **45**, 547.

Dicks, J. W. & Tempest, D. W. (1967). *Biochim. biophys. Acta*, **136**, 176.

Dische, Z. (1947). *J. biol. Chem.* **167**, 189.

Ellwood, D. C., Kelemen, M. V. & Baddiley, J. (1963). *Biochem. J.* **86**, 213.

Ellwood, D. C. & Tempest, D. W. (1967*a*). *Biochem. J.* **104**, 69P.

Ellwood, D. C. & Tempest, D. W. (1967*b*). *Biochem. J.* **105**, 9P.

Epstein, W. & Schultz, S. G. (1965). *J. gen. Physiol.* **49**, 221.

Fales, F. W. (1951). *J. biol. Chem.* **193**, 113.

Herbert, D., Phipps, P. J. & Tempest, D. W. (1965). *Lab. Practice*, **14**, 1150.

Janczura, E., Perkins, H. R. & Rogers, H. J. (1961). *Biochem. J.* **80**, 82.

King, E. J. (1951). *Micro-analysis in Medical Biochemistry*, p. 65. London: J. and A. Churchill Ltd.

Neidhardt, F. C. (1963). *Annu. Rev. Microbiol.* **17**, 61.

Salton, M. R. J. (1964). *The Bacterial Cell Wall*, p. 12. Amsterdam: Elsevier Publishing Co.

Schaechter, M., Maaløe, O. & Kjeldgaard, N. O. (1958). *J. gen. Microbiol.* **19**, 592.

Sud, I. J. & Schaechter, M. (1964). *J. Bact.* **88**, 1612.

Taylor, M. M. & Storck, R. (1964). *Proc. nat. Acad. Sci., Wash.*, **52**, 958.

Tempest, D. W. & Dicks, J. W. (1967). *Microbial Physiology and Continuous Culture*, p. 140. London: Her Majesty's Stationery Office.

Tempest, D. W., Dicks, J. W. & Ellwood, D. C. (1967*a*). *Biochem. J.* **104**, 68P.

Tempest, D. W., Dicks, J. W. & Hunter, J. R. (1966). *J. gen. Microbiol.* **45**, 135.

Tempest, D. W., Dicks, J. W. & Meers, J. L. (1967*b*). *J. gen. Microbiol.* (in the Press).

Tempest, D. W. & Hunter, J. R. (1965). *J. gen. Microbiol.* **41**, 267.

Tempest, D. W., Hunter, J. R. & Sykes, J. (1965). *J. gen. Microbiol.* **39**, 355.