

as potentiators of germination under pressure (Table 6). Of the ribosides tested inosine (which is the most effective riboside germinant at 1 atm.) most strongly potentiated the germination of spores of *Bacillus cereus* caused by pressure. Of the other ribosides, adenosine, guanosine and deoxyinosine caused weak potentiation; these three were also weakly active as germinants at 1 atm. (i.e. when used at higher concentrations than in Table 6).

The ribosides, which do not germinate *Bacillus coagulans* spores at 1 atm., did not potentiate the germination by pressure either.

None of the possible metabolites tested, which could be formed more or less directly by breakdown of inosine or alanine, was a potentiator of germination caused by pressure.

Table 5. *Effect of O-carbamyl-D-serine (OCDS) on the germination of spores of Bacillus cereus by pressure*

Amino acid§	Concentration	Germination (%)* following incubation† in			
		Absence of OCDS		Presence of OCDS‡	
		1 atm.	200 atm.	1 atm.	200 atm.
Control (no addition)		0	0	0	0
L-Alanine	50 µM	9	100	85.5	100
	1 µM	0	7	0	100
D-Alanine	50 µM	0	100	0	4
	10 µM	0	14	0	0
L-Phenylalanine	50 µM	0	22.5	0	81.5
β-Alanine	250 µM	0	5	0	5.5
Glycine	20 µM	0	7.5	0	8
L-α-Aminobutyric acid	10 µM	0	5.5	0	6
L-Cysteine	500 µM	0	74	0	74

* Spores were activated before use (70°, 30 min.); germination was measured by recording the percentage of phase-dark spores.

† Spores were incubated at 30° for 30 min. in 0.1 M-sodium phosphate (pH 8.0) plus the indicated amino acids.

‡ *O*-Carbamyl-D-serine was used at a concentration of 100 µM.

§ In addition to those amino acids in the Table, OCDS did not influence the potentiation of pressure germination caused by L-tyrosine, L-serine, L-threonine or L-tryptophan.

Inhibition of pressure germination

In addition to low temperatures (Table 3) and extreme pH values (Fig. 2), pressure germination was inhibited by various metabolic poisons and antimetabolites known to inhibit germination at 1 atm. (Table 7). Further experiments showed that, just as the potentiators (e.g. amino acids, above) became less effective in *accelerating* germination, so the various inhibitors became less effective in *preventing* germination as the pressure employed was increased.

DISCUSSION

The fact that inhibitors of germination initiated by nutrients (octyl alcohol, mercuric chloride etc.; Table 7) also inhibited the germination initiated by pressure, suggested that pressure caused germination *via* some enzyme reaction(s) rather than by simple physical distortion of the spore (e.g. 'mechanical germination'; Rode & Foster, 1960).