

pressure (6500 atm. at 35°) caused an increase in ATP levels in human amnion cells, presumably by stimulating some ATP-generating reaction(s) (Landau & Peabody, 1963).

Nevertheless, the reduction in the degree of germination which occurred below about 40° as the pressure was increased *above* the optimum of 4000 atm. (Sale *et al.* 1970) is less easily explained, unless one postulates that at these higher pressures the conformation of a critical germination enzyme was so changed as to inhibit reversibly the germination reaction.

An alternative explanation of pressure germination was suggested by the observation that pressure accelerated the racemization of alanine catalysed by spores (Fig. 3). Racemization of alanine does not involve a net change in molecular volume of reactants and products, therefore the reaction rate should not be affected by pressure solely by mass action. The observed acceleration by pressure must have some other cause: for instance, a conformational change in the racemase molecule, leading to more rapid action, or (since whole spores were used as the racemase in these experiments) some change in permeability of spores to the substrate alanine could have allowed more rapid racemization. Pressure has been shown to reduce the stereospecificity of an enzyme reaction, for example the hydrolysis of methyl esters of benzoyl L- and D-alanine by α -chymotrypsin (Gonikberg, Prokhorova & El'yanov, 1968). Major conformational changes are not likely below 1000 atm., thus it is more likely that the lower pressures acted by increasing the permeability of spores. The observed synergism of pressure with low levels of various amino acids can be explained on this basis, i.e. if pressure increased the permeability of spores to these germinative compounds.

Interestingly, pressure influenced the germination initiated by amino acids much more than by ribosides. If a permeability change is the correct explanation of pressure germination, it follows that germination at 1 atm. is normally severely restricted by the low permeability of spores to the germinative α -amino acid, which seems to be an essential endogenous germinant even for those spores which may be caused to germinate by addition of ribosides alone (Black & Gerhardt, 1961; Warren & Gould, 1968). Synergism of ribosides, like inosine, with amino acids as germinants for spores of many aerobic species (Hills, 1949; Foerster & Foster, 1966) would suggest that the riboside may function, like pressure, to increase spore permeability to the amino acid as summarized in Fig. 5. At present, the 'germination site' within the spore remains unrecognized, but may be analogous to the allosteric site postulated by Woese, Vary & Halvorson (1968).

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REFERENCES

- BLACK, S. H. & GERHARDT, P. (1961). Permeability of bacterial spores. I. Characterization of glucose uptake. *Journal of Bacteriology* **82**, 743.
- CLOUSTON, J. G. & WILLS, P. A. (1969). Initiation of germination and inactivation of *Bacillus pumilis* spores by hydrostatic pressure. *Journal of Bacteriology* **97**, 684.
- DOUGLAS, H. E. & JOHNSON, F. H. (1951). The influence of hydrostatic pressure and pH on the rate of lysis of *Micrococcus lysodeikticus* by lysozyme. *Archives of Biochemistry and Biophysics* **31**, 41.
- FOERSTER, H. F. & FOSTER, J. W. (1966). Response of *Bacillus* spores to combinations of germinative compounds. *Journal of Bacteriology* **91**, 1168.

L-alanine,
concentrations)

Pressure causes
reversible increase
in permeability to
L-amino acid

erment (e.g. riboside) or
germinative action of
as a barrier *within* the
also from endogenous

spores

owing incubation†

Bacillus coagulans
at 600 atm.

87
79
0
4
0
24
0
89
85.5
0
0
88
89.5

measured by recording the
coagulans) in 0.1 M-sodium
gonist of pyruvate meta-

and also for the observed
factors. Similar situations
of L-tyrosine ethyl ester
l methyl ester by trypsin
moderate temperatures
ozyme was increased by
complex situation, high