various Bacillus and Clostridium species, and in particular describes the strongly synergistic effect of pressure with some of the more often studied 'physiological' germinants. The pressure range was limited to 1000 atm. when we found that the synergistic effect disappeared at higher pressures.

METHODS

Organisms and production of spores. Bacillus subtilis MARBURG ATCC6051, *B. brevis* NCTC7577 and *B. pumilis* S3 (laboratory isolate) were grown at 37° on the surface of potato, glucose, yeast-extract agar. Procedures for recovery and 'cleaning' of spores were as described previously (Sale *et al.* 1970). Suspensions were stored at 4°, in water, and were activated by heating (70°, 30 min.) before use unless stated otherwise in Results.

Pressure treatment. Preparation of samples and the method of application of pressure were as described by Sale *et al.* (1970). Pressures up to 600 atm. were generated directly by an electrically driven hydraulic pump connected by a pipe to the vessel in which the sample sachets were treated. The vessel was immersed in a water bath for control of temperature. The pressure was measured by a Bourdon tube pressure gauge.

Measurement of germination. Spores for germination studies were suspended at a concentration of about 10^8/ml. in 0·1 M-sodium phosphate buffer (pH 8·0) unless stated otherwise in Results. Germination was measured in three ways. (1) The optical density (O.D.) of suspensions was measured using an absorptiometer ('Biochem'; Hilger & Watts Ltd., Camden Road, London), fitted with a 580 mμ peak transmission filter; germination was accompanied by a fall in O.D. (2) Samples were examined microscopically using phase contrast optics, when ungerminated spores appeared bright and germinated spores appeared dark. About 250 spores were examined from each sample and the percentage germination recorded. These two methods were insensitive when the amount of germination exceeded 98 % or so, in which case the third method was used. (3) Samples (1 ml.) were sealed in thin walled glass ampoules which were heated by total immersion in a water bath at 70° for 30 min. The surviving heat-resistant (i.e. ungerminated) spores were then enumerated by poured plate viable counts using nutrient agar (Oxoid).

Chemicals. Amino acids, nucleosides and related compounds were obtained from British Drug Houses Ltd. (Poole, Dorset) or Koch-Light Laboratories Ltd. (Colnbrook, Bucks.). *O*-Carbamyl-D-serine was a gift from Dr P. H. Hidy (Commercial Solvents Corp., Terre Haute, Indiana). Other chemicals were of Analar grade.

Alanine racemase. Racemization of alanine by whole spores was measured in the direction L → D by the method of Yoshimoto (1958) using D-amino acid oxidase (British Drug Houses Ltd.; Wood & Gunsalus, 1951) as described by Jones & Gould (1968).

RESULTS

Initiation of germination by pressure: optimum temperature and pressure

The optimum temperature for initiation of spore germination differed at different pressures. As the pressure was increased, the optimum temperature for germination, as measured by phase-darkening of spores or by viable counts of heat-resistant survivors, increased also. This trend is shown in Fig. 1 for spores of *Bacillus coagulans*, *B. subtilis* and *B. cereus*.