salt solution suspensions of cells which had evaporated to complete dryness.

Cells were removed from the salt suspension of each species in group three and examined for morphology and Gram staining. In every case, the cells had remained intact and reacted to the Gram stain in the usual manner. In view of the limited data, it appears that, in general, the cultures stored at room temperature retained viability longer than those under refrigeration. Seemingly, one essential to the retention of viability was to maintain sufficient liquid in the tubes to keep the cells in suspension. No experiments have been made to determine the most desirable percentage of salt to use. However, within limits, this may not be too critical since evaporation in many cases increased the percentage of the salt solution to 8 or 9%. Again, no effort has been made to determine whether the pathogenicity of pathogens was retained by this method of storage.

The procedure offers an easy method of storing bacterial cultures for 2 or 3 years, or probably longer, as the limitation of the method is not known. It would appear to be particularly valuable in carrying cultures for class work and in laboratories with limited equipment.

DRY RUPTURE OF BACTERIAL SPORES

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Extensive rupture of bacterial spores has generally been accomplished by violent agitation of slurries in the presence of very large quantities of micron-size glass beads (Powell and Strange, Biochem. J. **54**:205, 1953; Levinson and Sevag, Arch. Biochem. Biophys. **50**:507, 1954; Salton and Marshall, J. Gen. Microbiol. **21**:415, 1959; O'Connor, Doi, and Halvorson, Can. J. Microbiol. **6**:233, 1960). The present communication describes a method for rupturing dry spores which may offer unique advantages in some phases of spore research.

In a typical experiment, 5-mg samples of highly purified, lyophilized spores of *Bacillus cereus* strain T were introduced into stainless-steel vials (⅜ × 1¼ in., 1.5-ml capacity) along with 250 mg of NaCl (−50 mesh, +100 mesh) and a 182-mg chrome-steel ball. The loaded vials were equilibrated at 5 C and secured in a Wig-L-Bug (model 3A) dental amalgamator (Crescent Dental Mfg. Co., Chicago, Ill.) kept in a refrigerator. After agitation for various intervals, the vial contents were transferred to chilled tubes, and suspended in 5 ml of cold 0.13 M tris(hydroxymethyl)aminomethane buffer (pH 7.6); 1 ml of this suspension was removed for turbidity determinations. The residual suspension was centrifuged at 12,000 × *g* for 15 min at 1 C in an angle-head centrifuge. The supernatant liquid was analyzed for dipicolinic acid (DPA) and soluble glucose dehydrogenase, and the sediment was examined by phase microscopy. Results of ana-

TABLE 1. *Influence of agitation time on spore rupture*

Agitation time	Dry wt of spores[a]	Turbidity[b] 2-log G (650 mμ)	Glucose dehydrogenase[c] (units/mg of spores)	DPA[d] (absorbance at 270 mμ)
sec	*mg*			
0	5.02	0.28	0.0	0.05
40	5.14	0.24	6.81	0.465
160	5.04	0.14	15.2	0.902
300	4.99	0.13	12.5	1.142

[a] *B. cereus* T, grown on JL medium (Sacks and Alderton, J. Bacteriol. **82**:331, 1961) in aerobic fermentors at 33 C. Spores were freed of vegetative debris in a polyethylene glycol-phosphate two-phase system (Sacks and Alderton). Dark spores and β-hydroxybutyrate granules were removed by centrifugation in a dense sucrose solution.

[b] Of a 1-ml suspension (containing ca. 1.0 mg of the original spore load, diluted 1:10). Transmittance was determined in 18-mm optically matched tubes, using a Coleman (model 11) spectrophotometer.

[c] Bach and Sadoff, J. Bacteriol. **83**:699, 1962.

[d] Determined from 1 ml of supernatant liquid (representing ca. 1.0 mg of the initial spore load) diluted to 3 ml in a 1-cm path cell. Spectrum recorded in Cary (model 14) spectrophotometer, and the absorbance at 270 mμ measured. The shape of the spectrum-trace of the zero-time agitation indicates that nearly all of the observed value results from scattering from residual debris. The other spectra show similar amounts of scattering.

lytical determinations (Table 1) indicate that conveniently short periods of agitation produce effective spore rupture and liberate high levels of active enzyme (*see* Berger and Marr, J. Gen. Microbiol. **22**:147, 1960; O'Connor et al., Can. J. Microbiol. **6**:233, 1960). Phase microscopy indicated virtually complete disruption of spores after 160 sec. The dry-rupture method employed by Lawrence and Halvorson (J. Bacteriol. **68**:334, 1954) appears to have been much less effective.

Experiments in which NaCl was replaced by the softer KBr showed much slower rupture rates. In an experiment with a series of balls ranging from 16.3 to 263 mg each, the rupture rate of spores increased with ball size. It seems likely that the load of spores could be scaled up considerably by using a heavy-duty model Wig-L-Bug with a 6-ml vial.

In addition to its simplicity and speed, the described method makes possible the elimination of glass beads in spore rupture. Such beads are inconvenient to remove; also, because of their enormous surface, they probably reduce the yield of many spore components by adsorption. Finally, dry rupture of spores may be an effective means of preventing the denaturation and hydrolysis of some of the unique spore components.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

ETHANOL CONVERSION IN THE BOVINE RUMEN

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Pure cultures of some common ruminal bacteria produce ethanol, but ethanol does not accumulate in the rumen. Manometric measurements (Hungate et al., Appl. Microbiol. **9**:554, 1961) and ethanol-1-C^{14} have been employed to determine whether it is an important extracellular intermediate converted in the rumen to other final products.

In the manometric experiments (Table 1), no carbon dioxide was formed, the pressure increase being due to carbon dioxide released by acid, presumably acetic acid. In the conversion of ethanol to acetate, the hydrogen available per μmole is sufficient to reduce 0.5 μmole of carbon dioxide to methane. Only 0.25 μmole of methane was found. The rest of the hydrogen presumably appeared in propionate and butyrate.

Ethanol-1-C^{14} was added to 10 g of ruminal contents plus 30 ml of salt solution, and incubated under the conditions of the manometric experiments. Volatile fatty acids formed were steam-distilled, separated chromatographically, and radioactivity was measured in acetic, propionic, and butyric acid. It was found chiefly in acetic acid. Residual ethanol-1-C^{14} was recovered by distillation and oxidized to acetic acid, which was separated by steam-distillation, concentrated, and assayed for radioactivity. Activity in the residual solids and liquid after removal of acid and ethanol was also determined (Table 2).

On the assumption that the rate of disappearance of ethanol can be expressed as a first-order reaction, rate constants for the four experiments of Table 2 are 0.1, 0.14, 0.34, and 0.2 per hr, respectively. These slow rates, coupled with small pool size, indicate that ethanol is not an important extracellular intermediate.

The explanation for ethanol formation in pure cultures may be that it is essential as a repository for hydrogen. Only limited hydrogen can be accommodated as H_2 since conversion to H_2 be-

TABLE 1. *Fate of ethanol-1-C^{14} added to ruminal contents**

Expt	Incubation time	CO_2 released during run	Acid produced	CH_4 produced
	min	*μmoles*	*μmoles*	*μmoles*
1	70	95	83	28
	100	84	107	20
2	150	140	95	25

* Conditions: 400 μmoles of ethanol; 10 g of bovine-rumen contents plus 30 ml of salt solution per vessel, including 0.5% sodium bicarbonate. Gas phase, CO_2. Mercury in manometers. Vessel volumes, ca. 160 ml. Temperature, 38 C.