brated 24 hr at room temperature with glycerol (controls without), then frozen at -25 C and stored at this temperature for 2 days before thawing. A further reduction in the glycerol concentration to 1 per cent was undesirable for most strains.

Motile organisms showed all degrees of activity, from feeble twitching to normal spinning movements. Survival was equally good at -25 C and at -50 C, but use of a Dry ice chest for the latter temperature necessitated sealing the cultures in glass to exclude carbon dioxide, which was found to be toxic. Although positive subcultures were sometimes obtained from materials which showed no motility after thawing, only once was there failure to obtain growth when 0.5 per cent motility or greater was observed upon thawing. Subcultures from thawed, glycerol protected materials usually attained dense growth rapidly at 33 C, an indication of numerous surviving organisms.

Since it has frequently been observed that storage of glycerol treated microorganisms at temperatures above -70 C is detrimental to long-term survival (Polge and Solty, Trans. Roy. Soc. Trop. Med. Hyg., 51, 519-526, 1957; Hollander and Nell, Appl. Microbiol., 2, 164-170, 1954), it is suggested that glycerol protected leptospirae should be stored at -70 C or lower, after initial freezing at a relatively slow rate.

**Figure 1.** Relation between the percentage of dead cells of *Sarcina lutea* and the equilibrium dye concentration. Cell concentration = 1.19 g/liter; initial dye concentration = 105.6 mg/liter.

**Figure 2.** Relation between the percentage of dead cells of *Sarcina lutea* determined by the proposed method (*Y*) and the real percentage of dead cells (*X*). Experimental equation (*Y* = 2.3 + 0.947 *X*) and theoretical equation (*Y* = *X*) are represented.

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and was grown on glucose agar. The methods described previously were used (Borzani and Vairo, Stain Technol., 35, 77, 1960).

Figure 1 shows that the adsorption of methylene blue by mixtures of dead and live cells of *S. lutea* follows Freundlich’s law when the percentage of dead cells is greater than about 40 per cent. This fact, already observed with yeasts (Borzani and Vairo, J. Bacteriol., 76, 251, 1958) can be explained, since live cells of *S. lutea* also adsorb the dye.

Figure 2 shows the results obtained in typical experiments carried out to test the adsorption method for determining the percentage of dead cells, similar to the method proposed for yeasts (Borzani and Vairo, J. Bacteriol., 76, 251, 1958). This method does not apply to *Bacillus subtilis, Serratia marcescens*, and *Escherichia coli*, because dead and live cells of these bacteria adsorb practically the same quantity of methylene blue.

Experiments are in progress in our laboratory to study the applicability of the adsorption method to other dyes and other bacteria.

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