IN THE LABORATORY

The Use of Glycine in the Disruption of Bacterial Cells[1]

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Following the addition of glycine to a suspension of living bacteria there results an apparent progressive lysis of the cells. This glycine action was reported by one of us (*1*), who, using thin suspensions of bacteria and following the reaction by turbidimetric methods, studied the effect upon the process of varying some of the factors involved. With the intention of developing the procedure into a method by which chemical fractions could be obtained from larger volumes of cells, attempts have been made to define the conditions optimal for the purpose. Among the factors considered which might influence the lytic process are the concentration of glycine, age of the culture, relative proportions of cell volume to solution volume, and length of time required for lysis.

The procedure consisted in suspending packed saline-washed cells, obtained from 2-liter shake cultures of the desired species, in glycine solutions of different concentrations. The weakest solutions used were of 0.5 M concentration, and the strongest, 3 M. All were adjusted to pH 7.5 before the addition of cells. The age of the cultures was varied from 1 to 10 hrs, and the volume ratios of cells to solution from 1:1 up to 1:10. The period of action was from ½ hr to 18 hrs of incubation at 37°. To determine the efficacy of the lytic process, the viscous mixtures were diluted with saline when necessary to facilitate sedimentation and then centrifuged. A 20-min period at 14,000 rpm was usually sufficient to yield a clear supernate. The amount of protein in this, as well as that in the original cell suspension, was determined by adding equal volumes of 10% trichloracetic acid to aliquots containing about 1 mg of protein nitrogen. The precipitated protein was washed repeatedly by mixing with 5% trichloracetic acid, centrifuging, and removing the supernatant fluid by decantation. The final wash solution was tested for the presence of nitrogen. For nitrogen analysis, the washed precipitate was finally dissolved in M sodium hydroxide and transferred to a Kjeldahl flask.

When suspensions of *Escherichia coli, Aerobacter aerogenes,* or *Bacillus mesentericus* were treated with glycine, as much as 85% of the cellular proteins could be detected in the clear supernatant fluid after 16 hrs of incubation. It was observed that in that period no increase in the amount of protein extracted was effected by increasing the concentration of glycine beyond 1 M. However, the rate of lysis did appear to be more rapid when the more concentrated solutions were employed. The amount of protein material extracted was found also to be a function of the relative proportion of glycine solution to the volume of cells. A ratio of 1 volume of packed cells to 10 volumes of glycine solution proved convenient and effective, although, if it is desired to keep the volume small, even a 1:1 ratio may be used with a somewhat poorer yield. With the latter, however, considerable dilution with saline must be made to permit sedimentation of the undissolved matter in the centifruge.

When cultures of different age were exposed to 1 M glycine for 18 hrs, the degree of lysis, as represented by the amount of protein present in the supernatant fluid, decreased with the age of the culture. The most complete lysis occurred in cultures from 1 to 3 hrs old. On treatment with 1 M glycine, the supernate of a 3-hr culture of *E. coli* showed an increase in protein content, with time, up to the 6th hr of incubation. No further increments were noted. In all cases, the extraction of large amounts of polysaccharide was noted but not quantitated.

TABLE 1

Treatment	Protein extracted (%)	
	E. coli	*B. mesentericus*
Distilled water, 16 hrs, 37°	8.0	4.0
Saline, 0.85%, 16 hrs, 37°	3.0	3.5
Phosphate buffer, 0.05 M, pH 7.5, 16 hrs, 37°	5.5	5.0
Glycine, 3 M, pH 7.5, 16 hrs, 37°	77.0	82.0
Sonic vibration, 9,000 cycles, 10 min, water, 10°	96.0	93.0

The relative efficiency of several procedures for obtaining cellular proteins was compared and evaluated, as before, by the percentage of protein released from the cell. Table 1 lists the results obtained when equal volumes of cells suspended in equal volumes of fluid were treated as described.

The ease and simplicity of effecting the solution of the intracellular contents of bacteria by means of their disintegration or lysis in the presence of glycine suggests many possible uses for this procedure in the separation or isolation of bacterial proteins, including, perhaps, enzymes, nucleic acids, and polysaccharides. Variations in some details may necessarily have to be made in order to obtain certain specific cellular entities. For example, our own efforts to obtain highly polymerized desoxyribonucleic acid were not always successful. The procedure consisted of deproteinizing the clear, viscous glycine extract or lysate with chloroform and pouring the protein-free solution rapidly into 2 volumes of 95% alcohol. Two types of precipitate were thus obtained, a fibrous product which appeared immediately, and a granular precipitate which came down more slowly. It was pos-

[1] Aided by a grant from the Office of Naval Research.

sible to collect the fibrous material by twirling a wooden applicator stick through the suspension of fibers. The fibers adhered to the stick and could be removed before the bulk of the granular substance came out of solution. This fibrous material consisted of much polysaccharide and some nucleic acid, which was determined by the diphenylamine reaction, the phosphorus, or the purine nitrogen content. Younger cultures proved to be better sources of this material than older ones, in which increased amounts of polysaccharide tended to obscure the relatively small quantities of fibrous nucleic acid present.

With the thought that possibly enzymes simultaneously released from the cells might be acting on the extracted cellular materials, attempts were made to increase the yield of desoxyribonucleic acid by adding citrate or phosphate ions to the glycine solution in order to bind magnesium ions, which presumably activate nucleases or polymerases. Contrary to expectation, the yields were considerably diminished. The failure in this instance merely stresses the point that the extraction of each specific substance poses its own problem.

Reference

1. COWLES, P. B. *Yale J. Biol. Med.*, 1947, **19**, 835–838.

A Pipette for the Rapid Transfer of Measured Quantities of Solution

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Measured quantities of solution are transferred frequently in routine analyses. Rapid measurements with a minimum of equipment are desirable when large numbers of determinations are to be made. This is particularly true in the soil-testing laboratory, where a measured quantity of water is mixed with a measured quantity of soil prior to the determination of the pH of the mixture.

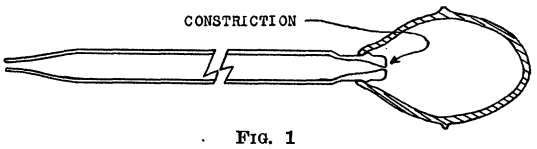

FIG. 1

A rubber bulb attached to a dropping pipette which has been modified by shrinking the upper end to produce an annular constriction with a diameter of about 0.2 mm provides an excellent device for the rapid transfer of liquids (Fig. 1). When water rising in the pipette encounters the constriction, a pronounced click or jerk is produced. The rate of flow of liquid through the constriction is so slow that the pipette may be removed from the liquid with a relatively constant amount of solution. A 200-mm dropping pipette, modified as described above, delivered 5.5 ml with a standard deviation of ± 0.02 ml based on 50 determinations. A Mohr pipette, adjusted to deliver 10 ml of liquid when filled to the constriction, delivered 10.01 ml with a standard deviation of ± 0.06 ml and a maximum deviation of 0.1 ml.

A Method for the Aeration of Liquid Cultures of Microorganisms

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The devices commonly used for aerating and agitating liquid cultures have been found to be not entirely satisfactory for use in culturing certain bacteria in liquid media. These devices are usually extremely difficult to sterilize and keep physically clean; also, because of the size and number of bubbles produced, they may have a low aerating efficiency.

It has been found that air passing at acoustic velocities through an orifice will produce a large number of

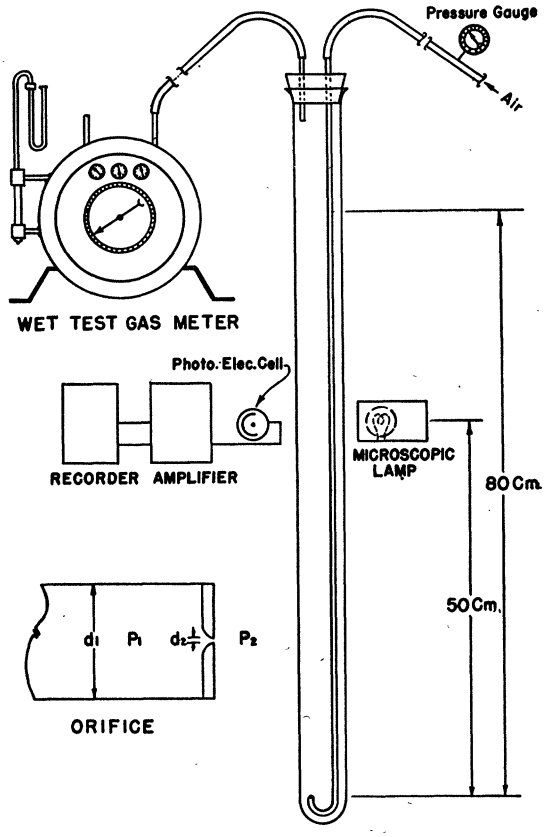

FIG. 1

very small bubbles, many of which are below 10 μ in diameter, and will cause violent agitation of the liquid. This orifice type of sparger was compared with sintered stainless steel, fritted glass, carbon, aloxite, and dishpan type spargers to determine which would produce the highest percentage of small bubbles. This was done by passing an equal amount of air (1 vol of air/vol of liquid/min) through the various types of spargers submerged in 80 cm of a 2% peptone medium contained in a glass tube (8×110 cm). The turbidity was measured, as illustrated in Fig. 1, with a continuous turbidimeter at 50 cm above