The total counts in the original extract were determined as $CaCO_3$ formed subsequent to wet combustion. Correspondence between these counts and the hydrazone counts was checked by wet combustion of several samples of the latter. The routine analyses on the total algal extracts were supplemented by an experiment in which phosphoglyceric acid, with carrier, was first eluted with 0.01 *M* hydrochloric acid as a narrow band from a 100–200 mesh column of Amberlite IRA-400 (Cl^-). This band was then subjected to the usual hydrolysis, oxidation, and isolation procedures. It accounted for 80% of the activity found in phosphoglyceric acid by analysis of the total extract. The discrepancy may be due to the presence of some tagged glyceric acid produced by phosphatase activity of the algae.

Our earlier results in which only 5% (15% in some later experiments) of the fixed tracer was found in phosphoglyceric acid may have been due to a deficit of CO_2 during the tagging. If this had been true, tracer would have been fixed first in phosphoglyceric acid and then rapidly transformed photochemically into other substances. In the more recent experiments leading to the isolation, a large excess of CO_2 was provided, so that a steady-state amount of phosphoglyceric acid typical of normal photosynthesis was always present. Further experiments on the kinetics of phosphoglyceric acid in photosynthesis have been reported elsewhere (*5*).

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A Study of Grinding Techniques for Bacterial Cells[1]

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It has long been a problem of the cell physiologist to get efficient grinding in the preparation of cell extracts. A recent study of this problem, using some of the common techniques and abrasives, yielded certain data that may prove useful. Three types of grinding equipment were used:

[1] Supported in part by a Hormel Institute Fellowship at the University of Minnesota, Austin, and submitted as a Master's thesis to the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis.

1. An ordinary mortar and pestle of a capacity of 20–25 cc for trituration.

2. A Wood-Werkman mill (*1*), a mechanical arrangement whereby the mixture of organisms and abrasive is forced between 2 concentric conical ground-glass surfaces while the inner cone is rotated and the outer one is held stationary by a vicelike clamp. The rotating cone is hollow and can be filled with ice to provide refrigeration. A tube is fused on the small end of the outer cone in such a way as to form a funnel-like piece.

The pasty-fluid mixture of cells and abrasive is forced through the tube by means of a syringelike plunger, easily made by cutting down and ridging a rubber stopper. The mill is best used by first filling the hollow cone with cracked ice and closing the opening that fits over the drive shaft to avoid spilling the ice. The ice-filled cone is then placed in the funnel-like piece, and the grinding mixture poured into the open end of the tube. The plunger is inserted, and the stopper removed from the ice chamber. The assembled mill is then fitted to the clamping and driving mechanisms. A container is inserted under the lip of the funnel to collect the ground material. This can be cooled by means of an ice-water bath. By a slow, *steady* pressure on the plunger, the material is forced between the ground glass surfaces. At least ¼ hp electric motor should be used as a power source. Variable speed pulleys connect the motor to a gearbox, which drives the shaft that operates the mill. The variable-speed pulleys and gearbox allow adjustment of the speed from about 300 rpm to 600 rpm.

3. A modified Potter's homogenizer (*1*), which is constructed by grinding the inside of a pyrex glass tube and preparing a pestle with prongs on the end from another slightly smaller tube, to which a shaft is fused. The sides of the pestle are ground to a tolerance that permits the tube to slide off slowly when not supported. The modification is simple. The prongs are left off the pestle because the nature of the material to be ground does not require their presence. Two sizes proved suitable, but the longer tube and pestle is more satisfactory because it affords greater ease in handling, and the longer pestle presents a larger grinding surface. For grinding, the shaft of the pestle is best connected to a cone-drive laboratory stirring motor by means of a short piece of pressure tubing. This affords some flexibility, which helps prevent breakage of the homogenizer if the pestle should stick or get out of line. The speed of rotation is set at approximately 2,000 rpm. The speed is not constant because the drag of the pestle in the grinding mixture slows it down considerably, and it varies with the adhesiveness of different abrasives. For refrigeration the homogenizer tube is placed in a suitable container filled with cracked ice and water. The two are then moved simultaneously up and down, grinding the cells by forcing the rotating pestle through the mixture.

Commercial abrasives used were Norton's Abrasive Grain Alundum 600X, Norton's Levigated Alumina, Johns-Manville Hyflo Super-Cel, and silicon carbide 940X, which may be obtained from any optical company. Hyflo Super-Cel can be used as it is received, except that it must be washed thoroughly with distilled water. Glass or flint used as the abrasive was prepared by sieving the material through an ordinary kitchen strainer and placing approximately 500 g of this in a 1-gal size ball mill. The ball mill was allowed to run for a period of 200 hr. The abrasive was then harvested by simple liquid levigation. Three-fourths of a gal of distilled water was used to wash the powdered abrasive out of the ball mill. The powder was thoroughly mixed with the water and allowed to settle for varying periods of time, depending on what particle size was desired. It was found that the particles remaining in suspension after 20 hr of settling were of a size suitable for the grinding of bacterial cells. The average particle size was 1.25 μ.

This levigation technique is satisfactory for the preparation of most abrasives, and the resulting fractions are quite uniform in size. The time element for settling varies somewhat with the nature of the abrasive; for example, pyrex glass settles out somewhat more slowly than flint. Increase in the ratio of water to powdered abrasive will give more uniformity in size of the settling particles.

Escherichia coli was used as the test organism for this study. The cells were grown in 4 l of 1% lactose broth equally divided into six 2-l flasks, which were placed on a shaking machine for a 24-hr incubation period. They were harvested by centrifugation. The efficiency of the grinding techniques was evaluated as per cent kill by making viable counts before and after grinding.

A series of experiments was conducted using various proportions of cells, abrasive, and water in an effort to establish a suitable set of conditions permitting a grinding efficiency of 99% or better, which could be duplicated in most, if not all, laboratories at a minimal expense with regard to the special equipment required. The mixtures are conveniently made up with an ordinary pan balance or triple beam laboratory balance. The significant data are summarized and recorded in Table 1.

Ratio of cells to abrasive is not as important as the ratio of cells plus abrasive to water. Ratios of cells to abrasive and water content of the grinding mixture vary with the nature of the abrasive and the technique to be used. A ratio of cells to abrasive of 1: 2 or 1: 1, with proper water content to hold the viscosity to the state of a creamy paste, gives high grinding efficiencies with several abrasives. The Potter's homogenizer requires a less viscous mixture than the other two methods used. It is important that the moisture content of the mixture for grinding be kept below the critical level. If the fluid content is too high it appears to act as a mechanical buffer, because the efficiency falls off markedly.

The lethal effect of the various abrasives from contact alone was checked, and the following results noted. The alumina abrasives showed no marked evidence of lethal effect. The pyrex glass and flint showed no noticeable lethal effect, but the data clearly demonstrated the difficulty of redispersing and making a homogeneous mixture with the bacterial cells. The unwashed Hyflo Super-Cel had a marked lethal effect, but on washing with distilled water three times it gave experimental evidence of no lethal effect. Silicon carbide may be suitable if it does not harm the system being studied.

The idea that glass is the best abrasive to use for

TABLE 1

Method of grinding	Ratio of cells to abrasive	Ratio of H_2O to abrasive	Time ground (min)	Kill (%)	Abrasive used
Hand grinding	1 : 2	1 : 2	5	46.3	Norton's 600 × alundum
			20	59.1	
	1 : 4	1 : 2	5	59.1	
			20	90.6	
Potter's homogenizer	1 : 2	3 : 5	5	95.3	
			20	99.3	
	1 : 4	3 : 5	5	93.2	
			20	99.2	
	1 : 8	23 : 40	5	93.3	
			20	99.7	
Wood-Werkman mill	1 : 2	1 : 2	1*	82.6	
			3*	94.3	
	1 : 4	11 : 20	1*	80.9	
			3*	91.8	
	1 : 8	11 : 20	1*	83.0	
			3*	96.3	
Potter's homogenizer	1 : 4	1 : 4	10	98.9	Silicon carbide 940 ×
Wood-Werkman mill	1 : 8	1 : 4	1*	99.6	
Potter's homogenizer	1 : 4	3 : 10	5	97.0	Norton's levigated alumina
			10	99.7	
			20	99.9	
Wood-Werkman mill	1 : 4	3 : 10	1*	83.0	
			2*	89.4	
			3*	99.7	
Potter's homogenizer	1 : 4	3 : 5	5	63.4	Pyrex glass (1–3μ)
			15	73.5	
Wood-Werkman mill	1 : 4	1 : 2	1*	90.0	
			2*	88.0	
	1 : 4	3 : 5	1*	66.7	Flint (0.7–1.2μ)
			2*	74.2	
Potter's homogenizer	1 : 2	1 : 2	10	98.8	Norton's levigated alumina
			20	99.4	
	1 : 1	1 : 1	10	37.7	
			20	56.9	
	1 : 1	1 : 2	20	99.6	
	1 : 1	7 : 10	20	77.8	Pyrex glass (0.6–1μ)
	1 : 2	3 : 1	15	99.9†	Hyflo Super-Cel
	—	—	40	42.0	None

* Instead of time in min, the material was put through the mill once, twice, or three times.

† Hyflo Super-Cel consistently gave results of 99.9% kill with 15 min grinding time.

grinding bacteria is not necessarily correct. If there is justification for using glass abrasives, it is because the adsorption of cellular material to the surface is less with glass than with other abrasives. However, Hyflo Super-Cel is believed to be quite free of adsorptive properties (*2*) and is easily prepared for use as an abrasive; it would thus seem more desirable than glass. Hyflo Super-Cel gives grinding efficiencies of 99.9% with 1 part cells, 2 parts abrasive, and 6 parts water.

Efficiency of grinding bacterial cells is chiefly dependent on the total abrasive action applied to the cells. In general, all techniques tried tended to support this concept. Evidence for this is exhibited by the higher efficiency of long grinding periods over short grinding periods. Additional evidence is the fact that the Wood-Werkman mill supplies more abrasive action than the Potter's homogenizer when pyrex glass is used as an abrasive. This is due to the gearbox and more powerful motor on the Wood-Werkman mill setup, which overcomes the friction of the glass paste more readily than the smaller motor on the homogenizer. When grinding large volumes of material and/or when using glass as the abrasive, the Wood-Werkman mill is more advantageous.

The two hardest abrasives tried, flint and pyrex glass, are not as efficient as the others, probably because of the coefficients of friction, as well as the character of the particle surfaces, and are more difficult to prepare. Levigated alumina as an abrasive gives efficiencies of 99% or better, with no lethal effect on prolonged contact with the cells in mixtures of these proportions:

a) 2 parts cells, 2 parts abrasive, and 1 part water
b) 1 part cells, 2 parts abrasive, and 1 part water
c) 5 parts cells, 20 parts abrasive, and 6 parts water

On the basis of viable cell counts, the Potter's homogenizer is an efficient grinding method to use for bacterial cells and possesses a capacity that yields adequate amounts of preparations for most experiments. It does not necessarily follow, however, that the resulting cell-free extracts will have high enzyme activity. The advantages over the Wood-Werkman mill are:

a) It requires less special equipment and storage space in the laboratory.

b) The initial cost of the material is a fraction of the cost of the Wood-Werkman mill setup.

c) It is more versatile and can be used with greater facility.

d) It is easier to refrigerate.

e) Sterile technique can be more easily applied.

f) Less material is lost in grinding, and smaller amounts can be used with satisfying results.

g) It offers easy modification to grind under anaerobic conditions simply by attaching a jet tube and flooding the upper portion of the tube with the desired inert gas.

h) High grinding efficiency can be obtained in less time.

i) Cell destruction can be obtained without addition of abrasive material.

The final trial of grinding without an abrasive in the Potter's homogenizer compared very favorably with the efficiency of the Booth-Green mill (*3*), which gives a 50% kill in 2 hr without refrigeration. The utility of grinding without an abrasive is questionable because of the long time required and the relatively low efficiency.

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The Fluorine Content of Some Miocene Horse Bones

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In view of the recent successful application of fluorine analysis in determining the relative ages of various human and animal remains from the well-known Galley Hill and Piltdown sites, it occurred to the author that this method might advantageously be extended to fossil bones from still earlier horizons. The following paragraphs present a few such data obtained through analysis of Lower Miocene horse material from the Raeford Thomas farm, Gilchrist County, Florida.[1]

Inquiry into the fluorine content of both recent and fossil bone dates back at least to the beginning of the 19th century. The French mining engineer A. Carnot (*2*) has furnished an interesting account of these early procedures and determinations. In order to make their work quantitative, however, pioneers in this field were under the necessity of employing rather lengthy and tedious routines. The modern technique, a modification of which was applied to the British specimens, was published in 1933 by Willard and Winter (*3*). The method is both sensitive and specific. It is typical of the growing tendency to employ organic agents in the determination of inorganic ions. It has, moreover, the advantage, often inestimable to the anthropologist or paleontologist, of requiring only small amounts of tooth or bone, the analysis of which by ordinary gravimetric methods would entail a high percentage of error. In every essential it is the method that has been followed in the present analysis of horse remains.

Since the value of the test for this purpose depends upon its indication of relative, rather than absolute, amounts of fluorine, every effort was made to follow precisely the same routine in each trial of every sample and thus to secure consistent results. The bones were first

[1] Permission to examine these fossils was kindly accorded by A. S. Romer, director of the Museum of Comparative Zoology. The author also wishes to thank Arthur Loveridge, of the same institution, for calling his attention to the article by Kenneth P. Oakley on "Relative Dating of the Piltdown Skull" (*1*).