in the rayed fluid during the experiment and found not to exceed 38.5°C. after one hour exposure. It is important to mention this point since poliomyelitis virus is attenuated or killed by heating for 30 minutes between 42.5 and 50°C. After raying, one cc. of the virus suspension was injected intracerebrally into monkeys. Duplicate tests were done at all intervals and each experiment was accompanied by control animals injected with one cc. of the unrayed virus suspension. The results obtained are given in Table I.

It may be seen from Table I that raying for periods of time varying from 30 minutes to as little as one minute was evidently sufficient to destroy the virus completely. Even an exposure as short as 5 seconds had possibly caused a certain degree of attenuation of the virus as suggested by the slight paresis developing in one of the 2 monkeys which had received this material.

It follows from these experiments that poliomyelitis virus *in vitro* is incredibly sensitive to the effect of ultraviolet irradiation. The mechanism of this inactivation is unknown, but it is more than likely that oxidation plays an important part. In applying this observation to the epidemiology of the human disease caution is necessary since one is dealing with a complex problem involving operation of the same factor on the virus as well as the host—and the effects produced may not be the same. It is conceivable that exposure to ultraviolet light, under certain conditions, may tend to increase the susceptibility of the host while at the same time causing a diminution in the virulence of the virus.

After completion of this work, a paper by Toomey[10] appeared, who found in repeated tests that exposure for 75 minutes at 18 inches distance from a standard quartz lamp bulb inactivated a 1% suspension of poliomyelitis virus. No shorter periods of exposure were tested.

9493

An Apparatus for Grinding Bacteria at Low Temperatures.

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The disrupting of bacteria by grinding in mills is a well established laboratory procedure, for which various forms of apparatus have been devised. The long grinding required to break most of the bac-

[10] Toomey, J. A., *Am. J. Dis. Child.*, 1937, **53**, 1490.

teria introduced into an ordinary mill changes the material so that some of the immunologically detectable components are no longer present, or at least not identifiable. In order to take advantage of the brittleness of cells at low temperature, Macfadyen and Rowland[1] used a grinding apparatus that was immersed in liquid air. Johlin and Avery[2] constructed a heavy steel cylinder in which a moist bacterial suspension was frozen with CO_2 ice; a tightly fitting plunger was introduced and struck 25 times with an 8 lb. hammer. These authors presented data indicating that this was a very efficient method of rupturing bacteria and obtaining the intracellular contents. For extracting tissues, a somewhat similar apparatus devised by Graeser, Ginsberg, and Friedemann[3] was used by Fox[4] to study the concentration of antibodies in tissues.

Recently Mudd, Shaw, Czarnetsky and Flosdorf[5] have devised an apparatus for disrupting bacteria at low temperatures after desiccation by the lyophil process. The grinding chamber consists of a hollow monel metal disc, milled out in such a manner that 2 large stainless steel balls 1½ inches in diameter fit accurately into the hollow. The bacteria are placed in this mill, frozen and dried by attaching it, with airtight connections, to a condenser which is immersed in a low-temperature freezing bath. Following the drying, the mill is rotated in a similar bath of CO_2 ice and methyl cellosolve. At least 80% of the bacteria are completely disintegrated within a short time. These authors[5] report that the combination of low-temperature desiccation and grinding is specially efficacious in preventing denaturation of the bacterial components. Another feature of the apparatus is that it probably prevents scattering of dried, and living, bacteria during the processes described.*

A few years ago Kreuger[6] devised a mill consisting of a tin-lined steel cylinder of 1-liter capacity, in which were placed 2 or 3 thousand ¼-inch rustless steel balls. Bacteria in suspension, rotated in this mill, at a rate of 130 R.P.M., at room temperature, were disintegrated in 12 to 18 hours so that 30 to 40% of the intracellular

[1] Macfadyen, A., and Rowland, S., *Centr. Bakt. Orig.*, 1903, **34**, 765.

[2] Johlin, J. M., and Avery, R. C., *J. Exp. Med.*, 1930, **52**, 417.

[3] Graeser, J. B., Ginsberg, J. E., and Friedemann, T. E., *J. Biol. Chem.*, 1934, **104**, 149.

[4] Fox, J. P., *J. Immunol.*, 1936, **31**, 293.

[5] (a) Mudd, S., Shaw, C. E., Czarnetsky, E. J., and Flosdorf, E. W., *J. Immunol.*, 1937, **32**, 483; (b) Mudd, S., Czarnetsky, E. J., Pettit, H., and Lackman, D., *Proc. Am. Philosophical Soc.*, 1937, **77**, 463.

* We are indebted to Dr. Stuart Mudd for sending us the plans and specifications for this apparatus.

[6] Kreuger, A. P., *J. Infect. Dis.*, 1933, **53**, 185.

nitrogen was made soluble. No detailed data were presented concerning the antigenic state of these soluble substances.

For many years we have used a mill designed by White[7] for grinding bacteria which had been previously desiccated after freezing. This mill consists of a heavy glass flask of 1-liter capacity containing 6 or 8 agate balls; the flask rotates on an axis of 45° from horizontal. Several weeks of grinding are required for complete disruption of hemolytic or non-hemolytic streptococci which have been previously frozen and dried, and still longer for those that had been simply dried. As already noted, the material obtained after this long grinding had lost many of the antigenic properties of the original bacteria.

After Mudd and his co-workers reported their success in obtaining type-specific substances of hemolytic streptococci by disrupting of the "lyophil" bacteria in a cold mill, we thought that the advantages of the various mills might be combined in a form which could be easily and inexpensively assembled.

The flask-holder, C, of the White mill† is mounted on one end of a countershaft, about a foot long, which runs horizontally in 2 bearings (not shown), one of which is situated about 3 inches from the holder; and on the middle of the shaft is placed a grooved pulley of such size that it will reduce the speed from an electric motor to about 230 R.P.M. The grinding unit, which may be sterilized with dry heat, consists of a heavy short-necked Pyrex flask, F, of 1-liter capacity, containing about 500 ¼-inch stainless steel balls. Less effective grinding is obtained with 1000 balls because the larger mass does not oscillate so freely. After the material to be ground is inserted, the flask is closed as follows: A gum-rubber stopper of suitable size is placed inside a piece of sterile gauze or oiled silk, and pushed into the neck of the flask until the inner end of the stopper is even with the inside surface of the latter; a second slightly larger stopper is also inserted; this usually projects a short distance from the top of the neck. The gauze is trimmed flush with the second stopper, over which is placed a gum-rubber milk-bottle cap, K, held closely to the neck of the flask with tightly stretched wide rubber bands. This prevents the methyl cellosolve from making contact with the stopper. The flask is held firmly in place by 3 windings of spring belting fastened by wire loops slipped over the end of the heavy wire arms of the flask-holder. A smaller holder is available for a flask of 200 cc. capacity.

[7] White, B. J., *Centr. Bakt. Orig.*, 1909, **48**, 254.

† This is a stock article obtainable from Arthur H. Thomas Company, Philadelphia.

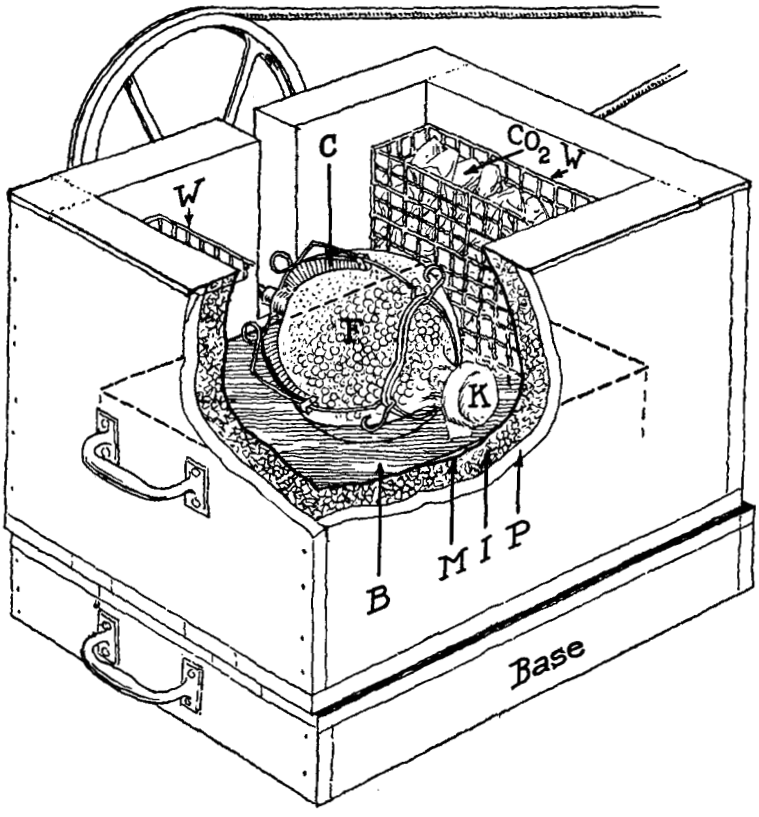

FIG. 1.

The flask is rotated in a bath, B, of methyl cellosolve cooled with CO_2 ice. A lower temperature is maintained and a smaller amount of the ice is used if the bath is properly insulated. A copper box, M, 13 inches long, 10 inches wide and 9 inches deep, with a slot 1 inch wide and 4 inches deep in the middle of one side, is a convenient size for the bath. Around this metal box is one inch of cork board, I, which, in turn, is covered with a casing of pine, P. The metal extends over the top of the cork and pine and also lines the slot, in order to prevent the methyl cellosolve from coming in contact with the cork. About the upper side of the box is nailed a strip of board, 3 inches wide, which thus forms a recess into which fits a removable metal top similarly insulated with cork and pine (not shown in the figure). In each end of the metal box is placed a removable wire basket, W, 2½ inches wide, 10 inches long and 8 inches deep, which is filled with pieces of CO_2 ice that are thus kept from contact with the rotating flask. The refrigerating box

stands on a removable base; this permits convenient separate manipulation of the countershaft, flask-holder and box.

Before grinding, the bacteria are frozen and dried, either in the lyophil apparatus of Flosdorf and Mudd,[8] or by the desiccator-method.[9] Weighed amounts are then placed in a flask, which is tightly stoppered as previously described, and cooled to —75°C. in the bath used for the mill. If the flask is stoppered before chilling and opened while still cold, there will be a rush of air which will blow the bacterial powder inward from the neck of the flask, instead of into the surrounding atmosphere, an occurrence that will take place if this sequence is not followed.

After being properly chilled the flask is firmly fastened in the holder,† so that it is immersed about an inch in the methyl cellosolve. The top is placed on the box and the motor started. The large masses of dried bacteria are quickly broken up; and the bacteria form a coating over the inner surface of the flask and around the balls. The latter act like hundreds of little hammers which hit and grind the cold, brittle bacteria. It has been found that hemolytic streptococci, in a quantity of 1 gm. dry weight, are effectively disrupted after one to 3 hours' grinding in this cold mill.

This technic has been found superior to grinding bacteria which have been frozen in a thin film on the inner surface of the flask without preliminary drying, a method which is somewhat comparable to that described by Macfadyen and Rowland or by Johlin and Avery. The results of studies of material obtained from desiccated bacteria ground in this mill will be reported later.

9494 P

Photodynamic Action of Methylene Blue on Poliomyelitis Virus.*

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Reitz[1] reviewed the effects of the combined action of light and dyes on a number of bacteria, and found that methylene blue, among other dyes, was a very active photodynamic agent. More recently,

[8] Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1935, **29**, 389.

[9] Swift, H. F., *J. Bact.*, 1937, **33**, 411.

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[1] Reitz, A., *Zentralbl. f. Bakt.*, 1908, **45**, 270, 374, 451.