mortar with pestle is placed in an evaporating dish, and surrounded by and filled with solid carbon dioxide; after thorough cooling of the mortar and pestle, the dry ice is removed from the mortar. The excised tissue, which has been stored in vials over dry ice, is thawed just enough so as to be transferable to the mortar, and dry ice equal to about three times the volume of the tissue is then added. When the tissue is again frozen solid, the mixture is ground until a fine homogeneous powder is obtained. Small bits of dry ice are, if necessary, added during the grinding. The ground powder is then transferred to a vial and the carbon dioxide allowed to evaporate until a pasty mass is obtained. As soon as this change has taken place the tissue is again frozen solid.

Samples of approximately 200 mg of the partially thawed ground tissue are weighed into 50 ml conical centrifuge tubes equipped with a 24/12 ⅋ outer joint.[2] The tube is hung on a balance by an easily made support (*4*). Five ml of 95% ethanol is added and the tube is stoppered and allowed to stand until the entire series of samples have been weighed (usually 6 or 12 samples). The samples are then thoroughly dispersed with the aid of a stirring rod, and sufficient alcohol is added to each tube to wash down the walls and the stirring rod so that a total of 10 ml of solvent is used for each 200 mg of moist sample.

Pyrex #2480 Allihn condensers (200-mm jacket, 19/38 ⅋ joints) are connected to the centrifuge tubes by special adapters. These adapters are provided with an inner 24/12 ⅋ joint and an outer 19/38 ⅋ joint.[3]

The heat source is an electric digestion furnace as described by Miller and Miller (*5*). Rate of boiling may be controlled by using either a variable transformer or by adjusting the height of each tube above the heating element. When the 10-min boiling period is completed, the extraction unit is raised from the heater. After cooling to room temperature, contents of the tubes are swirled to wash down solids that creep up the walls during extraction. After centrifuging 10 min at 3,000 rpm, the clear supernatants are decanted into tared aluminum weighing dishes (Fisher Scientific Co. #8-732). The dishes with contents are placed in a desiccator over calcium chloride under moderate vacuum. The extraction is repeated with an additional 10 ml of ethanol and the resulting supernatants are added to the dishes.

In order to insure complete dryness, the residues are placed on the following day into another desiccator over fresh calcium chloride under high vacuum.

If dispersion during boiling is thorough, the second extraction does not yield more than about 3% of the weight of residue resulting from the first extraction.

Eighteen samples in duplicate of normal and cancer tissue of mice were extracted, with an average difference of 2% between the results of pairs. The average sample weight used was 224 mg, and the evaporation residues ranged from 9.5 to 14.9 mg.

The following additional information may be obtained from a series of extractions. If the insoluble residue is dried in the centrifuge tube at 100° C overnight and weighed, the sum of the weights of the soluble and insoluble portions will give a value which agrees with that of independent drying experiments on the whole tissue ($100 \pm 3\%$). When this insoluble residue is ashed overnight in the centrifuge tube at 450°, and the resulting ash weighed, a correction for soluble ash can be obtained provided the total ash is known. The soluble ash in our series was $58 \pm 5\%$ of the total ash or 14% of the evaporation residue.

[2] These versatile centrifuge tubes may be obtained by special order from either Ace Glass, Vineland, New Jersey, or Corning Glass Works, Corning, New York.

[3] Made by J. F. Uhrig, 4104 North Fifth Street, Philadelphia 40, Pennsylvania.

References

1. Bloor, W. R. *Biochemistry of the fatty acids,* New York: Reinhold, 1943, p. 45.
2. Floyd, N. F. Personal communication.
3. Hsiao, S. C. *Science,* 1948, **107,** 24.
4. Kolb, J. J. *Chemist Analyst,* 1949, **38,** 15.
5. Miller, G. L., and Miller, E. E. *Anal. Chem.,* 1948, **20,** 481.
6. Singher, H. O. *Thesis on muscle protein,* Cambridge: Harvard University Press, 1942, p. 28.

Evidence that Amputation of Bacterial Flagella Does Not Affect Motility

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In previous papers (*5, 6, 8, 10, 11, 12*) the somewhat revolutionary theory was proposed that the motile bacteria which are commonly credited with flagella do not move by means of such flagella, but by means of undulating gyrating contortions of their spiral-shaped bodies. Bacterial flagella would then have to be regarded, not as motor organs, but as mucous twirls peeled off from the mucous capsule, through the very movement of the bodies.

Acceptance of this theory would have far-reaching consequences. When applied to bacteria, the word "flagellum" (which means "whip") would have to be invested with quite a new meaning, or else be given up and replaced by something like "mucous twirl." "Bacillus" and "bacterium" (meaning "rod") would become misnomers in many instances, because although a rod need not be straight, an object which definitely has the shape of a spiral, or rather coil, cannot reasonably be termed a rod. Apart from nomenclature, the new theory would obviously affect classification in a fundamental manner. This might, however, at the same time lead the way to a phylogenetic classification. Teachers might like the new theory, for with less emphasis on the appearance and importance of "flagella," irksome "flagella staining" might be given up!

Notwithstanding my several publications on the subject, and the production of a cinemicrographic film in

which the new ideas are worked out step by step,[1] the new theory has received scant attention. Stuart Mudd (*4*) merely stated that it was not so. With the exception of the new edition of Zinsser's *Textbook of bacteriology* (*14*), which gave it "honorable mention" in the form of a very adequate description, bacteriologists seem reluctant to make up their minds. Conn and Elrod (*2*) have published some criticisms, mostly based on electron photomicrography with its unavoidable artifacts, but they left most of my arguments unanswered, and in their final summing up the question was still not settled. Van Iterson (*16*) based her criticism mostly on electron micrographs of the large spirilla, like *Spirillum volutans*, and I have just been able to show (*13*) that in this particular group of microbes the supposed flagella are of an unusual kind and have nothing in common with those of what are usually termed motile bacteria. The new edition of Bergey's Manual (*1*) and the new textbook of Frobisher (*3*) avoid all reference to my divergent views. Tanner and Tanner (*15*) in the new edition of their textbook mention the possibility of "a contractile membrane, or a snake-like movement" in motile bacteria, but at the same time they copy a drawing which shows *Salmonella typhosa* surrounded by more than a hundred flagella. This is of course in keeping with the old belief that motile bacteria "lead a double life," one inside the cell wall and another one outside it, unless one subscribes to the still stranger assumption that a cell wall may be pierced by flagella in more than a hundred places.

In this note I bring a further argument, based on a recent experiment, in support of the new theory.

By using my *sunlight* dark ground technique (*7*, *9*) I have shown that *Salmonella typhosa* (chosen because it is a typical representative of motile bacteria) when grown in suitable broth, exhibits a long thin tail when it is moving fast (Fig. 1). This tail can be seen on occasion to untwist itself into a number of wavy threads which arrange themselves in irregular fashion around the bacterial body and then after a while float away or disappear. All this is illustrated in the motion picture mentioned before. Their appearance and arrangement correspond to the traditional pictures of stained peritrichous flagella and the supposed flagella of electron micrographs. Obviously then, the tails of Fig. 1 are twisted bundles of what are commonly called bacterial flagella, and if these "flagella" were motor organs then the tail would be a motor organ.

On a previous occasion (*10*) I have shown that *Salmonella typhosa*, when grown in unsuitable broth or in simple peptone water, does not *show* tails and yet is perfectly motile. From this it would seem to follow that motility is not dependent on the presence of the tail, or in other words, that *Salmonella typhosa* can propel itself without outside motor organs. As a counterargument one might say that in such unsuitable culture media

[1] This motion picture is available from Prof. Harry E. Morton, Chairman of the Committee on Materials for Visual Instruction in Microbiology, Society of American Bacteriologists, University of Pennsylvania, School of Medicine, Philadelphia 4, Pennsylvania.

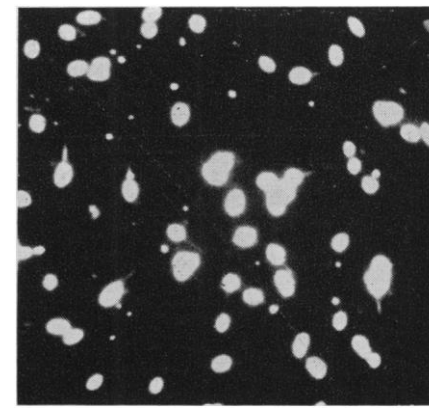

FIG. 1

the tail might have become too thin to be visible. I have therefore this time gone a step further and taken a culture of *Salmonella typhosa* in suitable broth, wherein nearly every individual was very motile and showed a good tail in the sunlight dark ground microscope, and then I have literally taken off the tails. This can be done by subjecting a portion of the culture to vigorous shaking for 15 minutes. The apparatus used was a Griffin & Tatlock Microid Flask Shaker, and it really vibrates more than it shakes. After this treatment the culture was again examined under the microscope; practically all the tails had disappeared but motility was as good as before the treatment, and as good as in the untreated portion, which still showed excellent tails. The bacteria in both portions exhibited their curious gyrating, undulating movement, which shows that during active life they are spiral-shaped, and evidently this movement is quite adequate to propel them. The whole experiment was repeated six times on different cultures, always with the same result.

The fact that one can, as it were, amputate the so-called flagella from motile bacteria and not interfere with their motility shows conclusively that such "flagella" are not motor organs.

References

1. BERGEY'S *Manual of determinative bacteriology*. (6th ed.) Baltimore: Williams & Wilkins, 1948.
2. CONN, H. J., and ELROD, R. P. *J. Bact.*, 1947, **54**, 681.
3. FROBISHER, M. *Fundamentals of bacteriology*. Philadelphia: W. B. Saunders, 1947.
4. MUDD, S. *Nature, Lond.*, 1948, **161**, 200.
5. PIJPER, A. *J. Path. Bact.*, 1938, **47**, 1.
6. ———. *J. biol. Photog. Ass.*, 1940, **8**, 158.
7. ———. *J. Path. Bact.*, 1946, **58**, 325.
8. ———. *J. Bact.*, 1947, **53**, 257.
9. ———. *J. biol. Photog. Ass.*, 1947, **16**, 3.
10. ———. *A motion picture of bacterial shape and motility*. Pretoria: Wallachs', 1947.
11. ———. *Photog. J.*, 1948, **88B**, 2.
12. ———. *Nature, Lond.*, 1948, **161**, 200.
13. ———. *Flagella of Spirillum volutans*, in press.
14. SMITH, D. T., and MARTIN, D. S. *Zinsser's textbook of bacteriology*. New York: Appleton-Century, 1948.
15. TANNER, F. W., and TANNER, F. W., JR. *Bacteriology* (4th ed.) New York: Wiley, 1948.
16. VAN ITERSON, W. *Biochem. Biophys. Acta.*, 1947, **1**, 527.