acting as a sideromycin for sensitive strains of *P. pseudotuberculosis*. This suggestion is attractive because *P. pestis* can grow slowly in nonhemolyzed serum whereas *P. pseudotuberculosis* does not. Both species grow rapidly after saturation of serum transferrin with ferrous ion (*12*). These results would be expected if *P. pestis* possesses a unique ability to obtain iron in vivo by chelation. However, the results shown in Table 3 indicate that loss or normal absence of this proposed activity is of little consequence to bacteria injected intravenously. An alternative suggestion is that strains lacking pesticin I are sensitive to a normal antibacterial component of serum that is inactivated by ferrous ion. An antirespiratory 7*S* globulin that fits this description has been described (*13*).

The major physiological determinant of virulence in *P. pestis* appears to be its ability to survive and multiply within phagocytes, especially within fixed macrophages of the reticuloendothelial system (*14*). Intracellular residence affords protection against normal antibacterial components of serum. The PI-deficient cell may fail to obtain a favored anatomical site in the mouse, suitable for intracellular growth, unless administered directly into the vascular system. The ability of wild-type *P. pestis* to obtain such sites after intraperitoneal or subcutaneous injection may be influenced by coagulase and fibrinolytic factor rather than pesticin I. To resolve the roles of these three factors completely, it may prove necessary to isolate PI^- strains that remain C^+ and F^+, or vice versa, by introduction of suitable point mutations on the determinant for pesticin I.

R. R. BRUBAKER
E. D. BEESLEY
M. J. SURGALLA

U.S. Army Biological Laboratories, Fort Detrick, Federick, Maryland

References and Notes

1. T. W. Burrows, *Brit. Med. Bull.* **18**, 69 (1962); T. W. Burrows, *Ergebn. Mikrobiol. ImmunForsch. exp. Ther.* **37**, 59 (1963).
2. S. Jackson and T. W. Burrows, *Brit. J. Exptl. Pathol.* **37**, 577 (1956).
3. R. Ben-Gurion and J. Hertman, *J. Gen. Microbiol.* **19**, 289 (1958); D. A. Smith and T. W. Burrows, *Nature* **193**, 397 (1962).
4. R. R. Brubaker and M. J. Surgalla, *J. Bacteriol.* **82**, 940 (1961).
5. R. R. Brubaker, M. J. Surgalla, E. D. Beesley, *Zentbl. Bakt. ParasitKde. Abt. I, Originale*, in press.
6. D. M. Eisler, *J. Bacteriol.* **81**, 241 (1961).
7. R. Reeves, *Bacteriol. Rev.* **29**, 24 (1965).
8. F. Jacob and E. L. Wollman, *Compt. Rend.* **247**, 154 (1958).
9. V. J. Freeman, *J. Bacteriol.* **61**, 675 (1951); N. B. Groman, *ibid.* **66**, 184 (1953); S. E. Blair and M. Carr, *ibid.* **83**, 984 (1961); R. A. Altenbern, *Biochem. Biophys. Research Commun.* **9**, 109 (1962).
10. S. Iseki and T. Sakai, *Proc. Japan Acad.* **29**, 127 (1953); P. W. Robbins and T. Uchida, *Biochemistry* **1**, 323 (1962); B. Holloway and G. Cooper, *J. Bacteriol.* **84**, 1321 (1962).
11. T. W. Burrows, personal communication.
12. S. Jackson and B. C. Morris, *Brit. J. Exptl. Pathol.* **42**, 363 (1961).
13. G. H. Bornside, G. B. Merritt, A. C. Weil, *J. Bacteriol.* **87**, 1443 (1964).
14. W. A. Janssen, G. M. Fukui, M. J. Surgalla, *J. Infect. Diseases* **103**, 183 (1958); W. A. Janssen, W. D. Lawton, G. M. Fukui, M. J. Surgalla, *ibid.* **113**, 139 (1963).
15. L. T. Goldberg, H. M. S. Watkins, M. S. Dalwatz, N. A. Schlamn, *ibid.* **94**, 9 (1954).

27 May 1965

Dynein: A Protein with Adenosine Triphosphatase Activity from Cilia

Abstract. *The adenosine triphosphatase protein from cilia of* Tetrahymena pyriformis *consists of 30S and 14S fractions. The 30S fraction consists of rod-like particles, 70 to 90 angstroms in diameter, which are linear polymers of globular 14S units. The 14S units have a molecular weight of approximately 600,000. The enzymatic properties of the two fractions are similar.*

Cilia isolated from *Tetrahymena pyriformis* contain a protein having adenosine triphosphatase activity (*1*). This protein forms the structures called "arms" which are located on the outer fibers of the cilium (*2*). Density-gradient fractionation (*2*) yields two protein fractions having adenosine triphosphatase activity. The fractions are characterized by their differing sedimentation rates (Fig. 1); extrapolated sedimentation constants ($s^0_{20,w}$) of 14*S* and 30*S*, respectively, have been determined in 0.1*M* KCl.

From our studies of this adenosine triphosphatase protein by physicochemical and enzymatic techniques, we conclude that the 14*S* and 30*S* fractions are related as monomer and polymer. We propose the term "dynein" (*dyne*, force; *-in*, protein) to describe this and other similar adenosine triphosphatase proteins associated with motile structures.

Electron micrographs of 30*S* dynein, shadow-cast with platinum by the method of Hall (*3*), show that it consists of rodlike particles of variable length (Fig. 2*A*). The heights of the particles (determined from shadow length) are 70 to 90 Å; the lengths, in a typical preparation, range between 400 and 5000 Å, 1700 Å for a particle of average weight. When the particles lie obliquely with respect to the direction of shadowing, a repeating globular structure (period approximately 140 Å) can often be seen along their lengths (Fig. 2*B*). Composite pictures, obtained by linear translation and superimposition (*4*) reveal this periodicity even more clearly (Fig. 2*C*).

Electron micrographs obtained by shadow-casting 14*S* dynein show globular particles (Fig. 2*D*). The heights are in the range 70 to 100 Å. Apparent widths are in the range 130 to 180 Å (Fig. 3); correction of the widths for lateral growth of shadowing metal (*3*) indicates the true width to be in the range 90 to 140 Å. Micrographs obtained by the negative-contrast method (*5*) show globular particles of essentially the same size (Fig. 2*E* and Fig. 3). Approximating the shape to an ellipsoid of axes 85, 90, and 140 Å, and assuming an anhydrous protein density of 1.35 g/cm^3, we calculate a molecular weight for 14*S* dynein of 540,000. The possible error in this value is estimated to be ±20 percent.

Molecular weights of several preparations of dynein have been determined by centrifugation. The "Archibald" method (*6*) yielded a value of 5,400,000 ± 1,000,000 for 30*S* dynein. The short-column equilibrium method (*6*) gave a value of 600,000 ± 100,000 for 14*S* dynein. Accuracy was limited by heterogeneity in each case: the 30*S* dynein is intrinsically polydisperse, whereas the preparations of 14*S* dynein contained

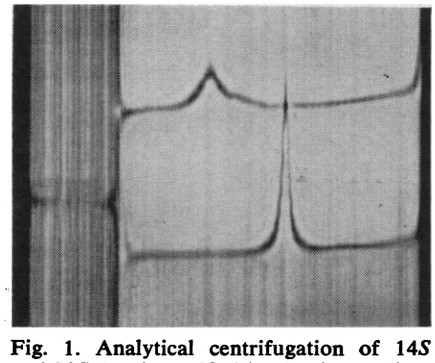

Fig. 1. Analytical centrifugation of 14*S* and 30*S* dynein purified by density-gradient centrifugation. Two cells were run, one with a 1° positive wedge-window to displace its trace upward. The lower trace shows 30*S* dynein (1.8 mg/ml); the upper trace shows 14*S* dynein (1.2 mg/ml). Solvent: 5 m*M* KCl, 0.1 m*M* ethylene diamine tetraacetate, 1 m*M* tris buffer, *p*H 8.2; speed, 59,780 rev/min in the Spinco model E analytical ultracentrifuge; direction of sedimentation is from left to right.

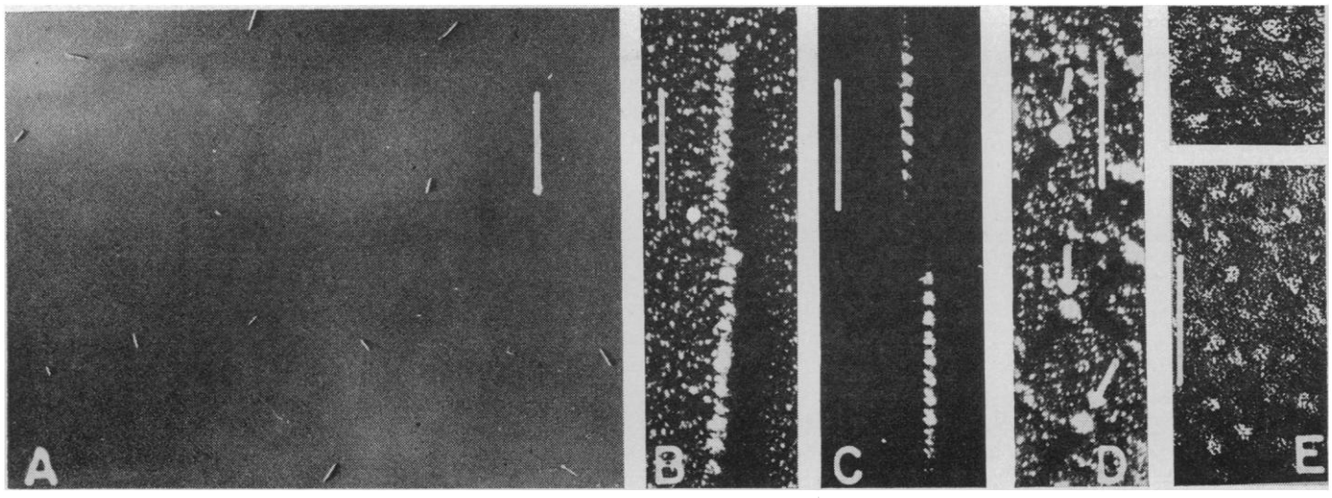

Fig. 2. Electron micrographs of dynein. *A*, 30*S* dynein, shadow-cast with platinum. *B*, Selected shadow-cast particles of 30*S* dynein at higher magnification. *C*, The same particles as *B*, with six images superimposed, translating through a distance equivalent to 140 Å between each exposure. *D*, A selected area from a field containing 14*S* dynein particles shadow-cast with platinum. *E*, Fields showing 14*S* dynein by negative contrast in uranyl acetate. The vertical bars represent 1 μ (*A*) and 0.1 μ (*B* to *E*). The pictures *B* to *E* are at the same final magnification, and the apparently larger size of the 14*S* dynein units in *D* is due to the lateral growth of shadowing metal.

a certain amount of aggregated dynein and also about 25 percent of protein that was not adenosine triphosphatase.

The enzymatic properties of 14*S* and 30*S* dynein are very similar (*7*). Their adenosine triphosphatase activity can be activated by either calcium or magnesium ion, and it is inhibited by an excess of ethylenediamine tetraacetate (EDTA). With Ca-ion activation, the specific activity was about the same for both 14*S* and 30*S* dynein (when the 25 percent of protein other than adenosine triphosphatase protein was allowed for in the 14*S*) and averaged 1.7 ± 0.4 μmole of phosphorus per milligram of protein per minute at 20°C. The ratio of the specific activity with Mg-ion activation to that with Ca ion was about 2.5 for 14S dynein, and 0.7 to 1.1 for 30*S* dynein. The Mg-activated specific activity of 14*S* dynein was thus 2½ to 3 times greater than that of 30*S* dynein.

Both 14*S* and 30*S* dynein are moderately specific for adenosine triphosphate (ATP), since other nucleoside triphosphates are hydrolyzed at only about 10 percent of the rate of ATP. Adenosine diphosphate is hydrolyzed 30 percent as rapidly as ATP, which may reflect the presence of adenylate kinase activity. There is no appreciable hydrolysis of 5′-adenosine monophosphate, sodium pyrophosphate, or *p*-nitrophenyl phosphate.

The evidence that 30*S* dynein is a linear polymer of 14*S* dynein units can be summarized as follows. (i) Electron microscopy reveals that 30*S* dynein consists of a string of globular subunits, indistinguishable in size from 14*S* dynein molecules; (ii) both 14*S* and 30*S* dynein are broken down in alkali to a form sedimenting at about 10*S* (*2*); (iii) the enzymatic properties of the two forms are closely similar. This hypothesis can only be finally proved, however, by a demonstration that the two forms can be interconverted under mild conditions.

The distance between repeating subunits in 30*S* dynein is approximately the same as that shown by the arms on the outer fibers in longitudinal sections of cilia (*8*), which supports earlier evidence (*2*) that the arms consist of dynein. It seems probable, therefore, that the 14*S* dynein molecules form the individual units of the arms and that these units are linked in the longitudinal plane of the cilium. The breaking of links between 14*S* units at random places during preparation would give rise to the observed scatter of lengths among the 30*S* particles.

Dynein shows little resemblance to any of the contractile proteins of skeletal muscle, but it does closely resemble the protein "myxomyosin" associated with protoplasmic streaming in slime molds (*9*), and it has some similarity to the protein of the mitotic apparatus (*10*). Myxomyosin, which has been studied in more detail, is an adenosine triphosphatase, has a sedimentation constant of 30*S*, and consists of rod-like particles of variable length (up to 7000 Å) having a diameter of 60 to 80 Å. This comparison, although necessarily based on fragmentary evidence, suggests that ciliary dynein is one of a class of proteins of widespread occurrence in relation to cell motility, but distinct from the contractile proteins related to actomyosin (*11*).

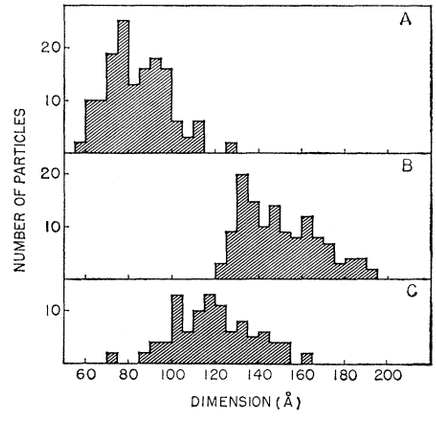

Fig. 3. Measured dimensions of 14*S* dynein particles. *A*, Particle height, estimated from shadow length. *B*, Particle width, estimated from shadow width (not corrected for lateral growth of shadowing metal). *C*, Widths, measured in a fixed, randomly chosen direction, of particles made visible by the negative-contrast technique.

I. R. GIBBONS
A. J. ROWE
Biological Laboratories, Harvard University, Cambridge, Massachusetts

References and Notes

1. F. M. Child, *Exp. Cell Res.* **18**, 258 (1959); S. A. Burnasheva, M. V. Efremenko, M. N. Lyubimova, *Biokhimiya* **28**, 547 (1963).
2. I. R. Gibbons, *Proc. Nat. Acad. Sci. U.S.* **50**, 1002 (1963); *Arch. Biol. Liége*, in press.
3. C. E. Hall, *J. Biochem. Biophys. Cytol.* **7**, 613 (1960). Hall, who measured particle width, reports a correction of 60 Å for lateral growth of shadow-casting metal; in our experiments, we have measured shadow width and have found a correction of 40 Å to be appropriate, ferritin being used as a control.
4. R. Markham, J. H. Hitchborn, G. J. Hills, S. Frey, *Virology* **22**, 342 (1964).
5. H. E. Huxley, *J. Mol. Biol.* **7**, 281 (1963).
6. S. M. Klainer and G. Kegeles, *J. Phys. Chem.* **59**, 952 (1955); K. E. Van Holde and R. E. Baldwin, *ibid.* **62**, 734 (1958).
7. Routine conditions for adenosine triphosphatase assay were 1 m*M* ATP, 1.2 m*M* $MgSO_4$, 0.17 m*M* EDTA, 30 m*M* tris-HCl buffer, *p*H 7.8, 20°C. When appropriate, $CaCl_2$ was substituted for $MgSO_4$, or other substrates were substituted for ATP, on an equimolar basis. Protein assays were performed by the Lowry method, bovine serum mercaptalbumin being used as standard (*2*).
8. I. R. Gibbons and A. V. Grimstone, *J. Biophys. Biochem. Cytol.* **7**, 697 (1960); J. André, *J. Ultrastruct. Res.* **5**, 86 (1961).
9. A. G. Loewy, *J. Cell. Comp. Physiol.* **40**, 127 (1952); P. O. P. Ts'o, L. Eggman, J. Vinograd, *Biochim. Biophys. Acta* **25**, 532 (1957).
10. A. M. Zimmerman, *Exp. Cell Res.* **20**, 529 (19(D. Mazia, R. R. Chaffee, R. M. Iverson, *Proc. Nat. Acad. Sci. U.S.* **47**, 788 (1961).
11. A. G. Szent-Györgyi, in *Structure and Function of Muscle*, G. H. Bourne, Ed. (Academic Press, New York, 1960), vol. 2; H. Hoffmann-Berling, *Biochim. Biophys. Acta* **19**, 453 (1956); M. Bettex-Galland and E. F. Lüscher, *Advan. Protein Chem.* **20**, 1 (1965).

12. We thank Dr. J. T. Edsall and Dr. K. R. Porter for the use of equipment in their laboratories. Supported by PHS grants GM 12124-01 and 1-K3-GM-21,937-01.

21 April 1965

Radiation-Induced Increases in Fitness in the Flour Beetle Tribolium confusum

Abstract. *Polygenic mutations were induced in an inbred strain of* Tribolium confusum *by exposure to 500 roentgens of gamma radiation. Female progeny of irradiated males bearing induced mutations in the heterozygous state produced significantly more viable offspring than control female progeny of nonirradiated males.*

Wallace (*1*) demonstrated that the genetic fitness of x-irradiated organisms under certain conditions is increased. He found that mutations, induced in the second chromosome of *Drosophila* by an x-ray dose of 500 r, brought about a significant net increase in viability for the bearer in the heterozygous condition. Wallace used a Cy L/Pm balanced lethal stock to facilitate the isolation and maintain the integrity of individual second chromosomes from wild populations of *Drosophila*. He compared the viability of flies homozygous for a specific wild-type second chromosome with that of flies carrying wild-type second chromosomes identical except for mutations induced in one of the homologues. He interpreted this observed net increase in viability as indicating that radiation-induced polygenic mutations, most of which would be deleterious in the homozygous condition, produced sufficient cumulative heterotic effects in the heterozygous condition to more than counterbalance induced, dominant, deleterious mutations (*1–3*). Contrary views and experimental evidence interpreted as a contradiction of Wallace's results have been reported by Falk (*4*) and Muller and Falk (*5*). We have tested the hypothesis of Wallace in a different genetic system, that of *Tribolium confusum* (*6*). Heterozygosis was induced by irradiating males of an inbred strain (Berkeley CF I 1) and crossing them with full-sibling nonirradiated females. Female progeny of this cross, having received an irradiated set of chromosomes from the male parent, would be heterozygous for any mutations induced in the gonial cells at loci homozygous in the inbred strain and transmitted to them.

Irradiated males and their mates were taken as pupae from the 38th generation of a strain perpetuated by crosses between full siblings with a theoretical coefficient of inbreeding (F) far in excess of 0.99. Experimental and control beetles were taken from a series eclosing during a single week. Between 1 and 2 weeks after eclosion, 16 male parents were irradiated in a single dose with 500 r from a Co^{60} source of γ-rays. An equal number of control male parents received identical treatment except for irradiation itself. Immediately after irradiation, irradiated and control males were placed with genetically marked females for a period of 2 weeks to permit mating and consequent exhaustion of irradiated spermatozoa, spermatids, and spermatocytes. Then, the irradiated and control male parents were paired with full-sibling females. Pairs were maintained in individual vials with whole-wheat flour as the medium, and were transferred to fresh medium weekly for 7 weeks. The sex of the progeny of irradiated and nonirradiated males was determined in the pupal stage; the sexes were isolated and allowed to mature for 1 week following eclosion. Before the test cultures were established, beetles were maintained at approximately 29°C and 70 percent relative humidity on a diet of whole-wheat flour supplemented with killed and dried yeast (5 percent), except when being irradiated, transferred, or otherwise handled.

Test cultures were set up in petri dishes (60 mm in diameter by 15 mm deep) in 7 g of medium; each culture consisted of three males (of nonirradiated parents) and three females. The females in experimental cultures were progeny of irradiated males; those in control cultures were progeny of nonirradiated males. In order to maintain identical relationships between mates in experimental and control cultures, males and females within a culture were never siblings but were descendants of the same grandparents—that is, double first cousins. Fifty experimental and fifty control cultures provided the basis for comparison of productivity. Cultures were established as rapidly as young adults became available, with no attempt to have all parental pairs equally represented in cultures. Thus, parental pairs provided progeny for cultures in direct proportion to their own productivity.

Adults of test and control cultures were transferred weekly to petri dishes containing fresh medium. The measure of fitness recorded was total live progeny (adults and pupae) surviving 8 weeks after the parents were removed from a given culture. By this time, and under the experimental conditions employed, all but a very few pupae had completed metamorphosis. Accordingly, the component of fitness measured was compounded of adult fecundity, egg fertility, and progeny viability under conditions which allowed minimum competition and reduced the possibility of cannibalism.

Two points of importance are noted here: (i) test and control cultures were maintained purposefully at suboptimal conditions (25°C and low, variable room humidity), and (ii) the progeny counts that were compared were based on the productivity of cultures during the first 3 weeks.

On the basis of total productivity for the 3-week test period, cultures containing female progeny of irradiated males produced a mean of 135.28 progeny per culture. By comparison, control cultures containing female progeny of nonirradiated males produced a mean of 118.34 progeny per culture.