80% was seen when Purinethol was administered. With prolonged remission the CLA level was markedly reduced. The daily urinary output of CLA was between 17 and 235 mg d^{-1}. CLA may thus serve as an invaluable indicator of the response to therapy in certain kinds of leukaemias (G. L., F. W. T., W. W., and M. M. T., in preparation).

The nature of cationic leukocyte antigen is unclear. Its electrophoretic mobility in two-dimensional SDS gels indicates that it has a higher molecular weight than lysozyme. Using *Escherichia coli* ribosomal proteins of known molecular weights ranging from 9,600 to 65,000 to calibrate the gels[9], we found that the apparent molecular weight of CLA is about 7,000 daltons greater than that of lysozyme.

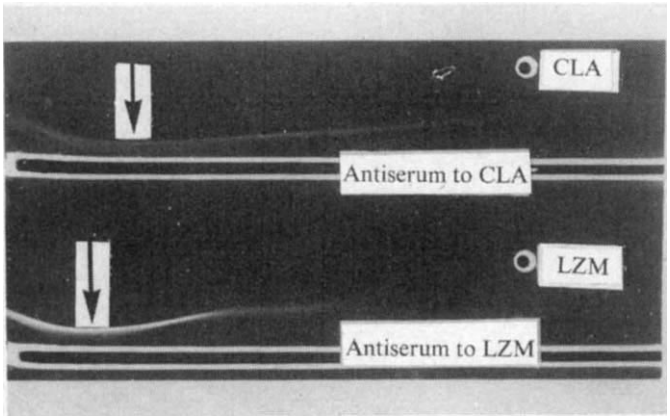

Fig. 2 Immunoelectrophoretic characterisation of human lysozyme (LZM) and cationic leukocyte antigen (CLA). Note the absence of antigenic cross reaction with antiserum to cationic leukocyte antigen. The electrophoretic mobilities of LZM and CLA are indicated by arrows.

CLA was isolated from the urine by elution from 5% polyacrylamide gels (F. W. T., G. L., W. W., G. W. T., M. M. T., and R. Böhm, in preparation). Antisera were prepared by immunising five rabbits with purified CLA using Freund's complete adjuvant. The serum of two rabbits contained antibodies with specificity for CLA. These antisera precipitated only CLA in immunoelectrophoresis (Fig. 2) and Ouchterlony double diffusion experiments as demonstrated by testing with known cationic urinary proteins such as lysozyme and post γ-globulin, and whole normal urine and serum. RNase and DNase gave a negative precipitation reaction. Precipitation-inhibition analysis[10] also failed to demonstrate any cross reaction. The CLA-specific antisera did not inhibit the lytic activity of lysozyme, in contrast to lysozyme-specific antiserum which inhibited lysozyme activity. Purified CLA did not exhibit lytic activity towards the lysozyme substrate, *Micrococcus lysodeikticus*. Tryptic peptide fingerprint patterns of totally reduced and alkylated CLA were strikingly different from those of human lysozyme[11].

As renal function was normal in the two patients, the large quantities of CLA in the urine of these patients probably represent a simple threshold phenomenon and are not due to renal tubular dysfunction. Increased elaboration of CLA in patients with chronic myelocytic leukaemia may result from the increased lysosomal breakdown induced by X irradiation and the cytostatic therapy of the granulocytic cells and their precursors. Patients with chronic myelocytic leukaemia may serve as an invaluable source of homogeneous proteins for studies designed to elucidate the structure, function and genetic control of the cationic proteins of human granulocytes.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft (Forschergruppe 'Leukämie und Tumortherapie'). We thank Mrs K. Kersten and Miss K. Langes for technical assistance.

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Received June 18, 1973.

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Bacteria Swim by Rotating their Flagellar Filaments

IT is widely agreed that bacteria swim by moving their flagella, but how this motion is generated remains obscure[1,2]. A flagellum has a helical filament, a proximal hook, and components at its base associated with the cell wall and the cytoplasmic membrane. If there are several flagella per cell, the filaments tend to form bundles and to move in unison. When viewed by high-speed cinematography, the bundles show a screw-like motion. It is commonly believed that each filament propagates a helical wave[3]. We will show here that existing evidence favours a model in which each filament rotates.

The idea that the flagellar filaments might rotate relative to the cell body as rigid or semi-rigid helices has appeared intermittently in the literature, but a convincing case for such a model has not been made. Rigid rotation was noted as a possibility by Stocker in 1956[4]. Doetsch argued for it in 1966[5,6], but he abandoned the idea in 1969[2,7] in favour of one in which the flagella "wobble" and only appear to rotate. Recently, Mussill and Jarosch[8] photographed preparations of *Spirillum volutans* squeezed between slides in which the flagellar bundles remain "motionless" while the wave movement appears in the cell body. They conclude that "the body must rotate around the point of insertion of the flagellar bundle". Rotation of this kind is assumed in a hydromechanical analysis of *Spirillum* by Chwang *et al.*[9]. Since the flagellar filaments of *Spirillum* arise separately[10], an alternative possibility is that each filament rotates individually. This alternative, which we favour, makes sense for peritrichously flagellated bacteria as well. The fiagellar bundles of these cells cannot rotate as a unit, because their filaments arise at widely distant points on the surface of the cell.

It is possible to envisage a biological rotary motor consistent with electron micrographs of structures at the base of a flagellum. In their model of the basal end of a flagellum

extracted from *Escherichia coli*, DePamphilis and Adler[11] show a rod extending from the end of the proximal hook and enclosed by rings of diameter 22.5 nm. Suppose that the ring associated with the cytoplasmic membrane, the M ring[12], is rigidly coupled to the rod yet free to rotate in the membrane, and that the rod is able to rotate freely within the other components through which it passes. Rotation of the flagellum would then be possible if the periphery of the M ring was linked to the cell wall by cross bridges of the kind found in skeletal muscle.

Such a rotary motor would be capable of delivering the power required to drive a flagellum. Coakley and Holwill[13] estimate the power dissipation of an isolated filament running at 50 Hz to be about 4×10^{-10} erg s^{-1}. If the cross bridges step along the periphery of the M ring with a step length of 8 nm (ref. 14), the ring will rotate once about every nine steps, and the filament can be driven at 50 Hz if each cross bridge steps at a rate of about 450 s^{-1}, which is a reasonable figure[15]. Huxley and Simmons[14] judge the external work which can be done per cycle of attachment and detachment of a cross bridge to be about 3×10^{-13} erg. Therefore, three cross bridges stepping at the above rate can generate the necessary power. If there are three cross bridges, the average force which each must exert is about 3.8×10^{-7} dyne, a value only slightly larger than the isometric force exerted by a cross bridge in the experiments of Huxley and Simmons[14]. There is room for five cross bridges along the periphery of the M ring, if their spacing is similar to that in skeletal muscle[15].

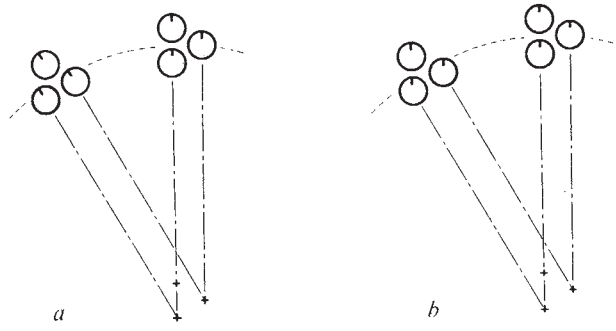

Fig. 1 A schematic cross-sectional view of a bundle of three flagellar filaments rotating individually (*a*) or propagating helical waves (*b*) at two successive instants of time. The section is in a plane normal to the helical axes (+), and the motion is in the frame of reference of the body of the cell. Each filament is marked at a fixed point. In (*a*) the filaments rotate relative to one another; in (*b*) they do not. The thrusts generated are the same.

Although cross bridges of the kind found in skeletal muscle have not been seen at the basal end of a flagellum, they may have been washed away in the procedure of DePamphilis and Adler[16] or obscured in thin-sectioned material by components of the cell wall. Of course, the construction of the motor may be entirely different. We have made the above calculations only to demonstrate the plausibility of a biological rotary motor. Indeed, there appears to be as much, if not more, structural evidence for this model than for one in which the flagellar filaments propagate helical waves[1,2], especially if the energy for the external work which each element of the filament must do is provided by a driving mechanism at the base[3,17].

The model in which the filaments rotate individually may not be argued against on the basis of the greater power required to rotate a helical filament than to propagate a helical wave. Coakley and Holwill[13] find that most of the power dissipation is due to the lateral displacement of the fluid, which is the same in both models. For an isolated filament the power dissipation for rotation is only 0.5% larger than the power dissipation for wave propagation; for a bundle of 200 filaments separated by aqueous fluid of mean thickness equal to the filament radius, the figure is 50%. The latter increase in dissipation may be unrealistically large, since there is no reason *a priori* to suppose that the filament-to-filament separation is as small as the filament radius. Nevertheless, the dissipation per filament for the bundle is relatively small, because the filaments work together in displacing the fluid laterally. For the bundle of 200 filaments rotating individually and in phase, it is only 1.5% of the dissipation for the isolated filament[13].

One cannot distinguish the various models by the fact that the body of a freely swimming cell rotates about an axis parallel to the direction of motion in a sense opposite to the rotation (or the apparent rotation) of the flagellar bundle. As discussed by Taylor[18], the torque responsible for the rotation of the body is generated, in the main, by the lateral displacements. This effect is an essential component in more recent theories[13,19,20].

One can, however, distinguish the models by experiments sensitive to the rotation of filaments relative to one another. This occurs when the filaments rotate individually (Fig. 1) but not when they propagate helical waves, "wobble"[7], or rotate as a group[8]. If the filaments rotate individually and two are linked together, it is likely that the entire bundle will stop. The linked filaments will stop, because they can no longer rotate relative to one another; the others will stop, because their lateral motion will be blocked by the first two.

Cross-linking experiments have been done, and the results clearly favour individual rotation. Greenbury and Moore[21] find that the peritrichous bacterium *Salmonella typhimurium* remains motile when as many as 10^5 univalent flagellar antibodies are adsorbed per cell, a mass of antibody four times that of the mass of the flagella. About half of the cells are immobilized when about 200 bivalent antibodies are adsorbed per cell. They conclude that the bivalent antibody acts by linking the filaments together. An alternative interpretation is that flagellin molecules loaded with univalent antibodies still function, but molecules in the same filament cross linked together do not. This possibility is eliminated in an experiment by DiPierro and Doetsch[22], who compare the effect of univalent and bivalent flagellar antibodies on *E. coli*, which is peritrichous, and on *Pseudomonas fluorescens*, which has only one filament. In the presence of univalent flagellar antibodies both kinds of bacteria remain motile. In the presence of bivalent antibodies *E. coli* stops, but *P. fluorescens* remains motile until the cells are linked together. "Very few non-motile single cells were observed in the *Pseudomonas* culture being immobilized, whereas with the *Escherichia* culture, large numbers of *single* non-motile organisms were observed, and in both cases this condition existed *before* the appearance of microscopically visible clumps"[22].

Results of experiments with bacteriophages that attack flagella provide additional evidence for the individual rotation model. Meynell[23] notes that rapidly swimming *Salmonella abortus-equi* stop abruptly on swimming into a drop of χ phage at high titre, even after the phage has been inactivated by radiation or over-centrifugation. The adsorption of the phage did not cause any morphological changes in the flagella, as far as could be seen by electron microscopy. Raimondo *et al.*[24] conclude that the adsorption of one PBS1 phage to one flagellum of *Bacillus subtilis* is sufficient to render the entire complement of flagella non-functional. They obtain the same results with phage ghosts treated with DNase, and conclude that "the functioning of all the flagella of *B. subtilis* is under the control of one 'motor'". These results can be explained if the filaments rotate. If the tail fibres of PBS1 wrap tightly enough around even a single filament, the bundle will jam, since the protruding body of

the phage will not be able to rotate past the adjacent filaments.

The individual rotation model suggests a mechanism by which flagellotropic phages are able to infect cells. The phage χ attaches to the filament with one tail fibre and then travels to the base of the flagellum where it injects its DNA; if the filament is inactive, the phage fails to reach the site of injection[25]. Assuming that the binding of the phage to the filament is not completely rigid, so that some relative motion is possible, it is conceivable that the phage moves down the filament like a nut on a bolt, the grooves between the helical rows of flagellin molecules serving as the threads. If this notion is correct, the phage will move to the base of the flagellum if the filament is rotating and the "threads" are intact; motion of the cell body is not required. There is a mutant of *Salmonella* which is completely non-motile because its filaments are straight[26]. The defect is in the flagellin, not in the components at the flagellar base. The flagellin molecules are still arranged in helical rows[27]. As we would predict, the mutant is fully sensitive to χ (ref. 26). There is a similar mutant of *B. subtilis* which remains sensitive to PBS1 (ref. 28).

Although bundle formation by peritrichously flagellated bacteria is not well understood, the forces responsible are thought to be hydrodynamic. If the viscosity of the medium is increased slightly, for example by the addition of methylcellulose, the bundles scatter more light[4]. The bacteria also swim more rapidly[29]. We find in tracking experiments[30] that they swim less erratically. These effects can all be explained, if, as argued by Stocker[4], the increased viscous stress causes more filaments to join bundles. If hydrodynamic forces, in fact, cause bundle formation, the observation that the mutant of *Salmonella* possessing straight flagella forms bundles[26] provides further evidence for the rotation of the filaments, since in this case interactions due to lateral displacements are absent. We suggest that the bending of the filaments around the sides of the cell is facilitated by the proximal hook, which serves as a flexible coupling or universal joint.

The hydrodynamic basis for the synchronization of filaments within a bundle is more firmly established. Flagella of adjacent spermatozoa[31] or flagellar bundles of adjacent bacteria[4] tend to move in phase. Taylor[31] calculated the forces acting to synchronize parallel undulating sheets and found these forces to be large for sheet spacings smaller than the wavelength of the undulations. Although explicit calculations of this kind have not been made for thin filaments, the work of Coakley and Holwill cited earlier[13] indicates a large reduction in power dissipation for synchronous rotation within a bundle.

Work by Silverman and Simon[32] on "polyhook" mutants of *E. coli* which provides strong support for the rotation model has recently come to our attention. When cells which have abnormally long proximal hooks but no filaments are treated with anti-hook antibody, they form pairs which counter-rotate. This result can be explained if the proximal hooks are driven by rotary motors and the antibodies link a single hook from one cell to one or more hooks from another.

If, as suggested by existing evidence, bacterial flagella rotate, the structures at the base of the flagellum deserve more attention than they have received thus far.

This work was supported by grants from the National Science Foundation and the US Atomic Energy Commission.

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Received July 2; revised August 23, 1973.

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Insect Epoxide Hydrase Inhibition by Juvenile Hormone Analogues and Metabolic Inhibitors

EPOXIDE rings are known to be enzymatically hydrated by numerous organisms[1–3] to give predominantly the corresponding *trans*-dihydroxy compounds and the epoxide hydrases involved can moderate various types of bioactivity. Thus, they are involved in the deactivation of certain labile aromatic epoxides which may be responsible for carcinogenesis[2] and in the detoxication of insecticides such as 1-naphthyl-N-methylcarbamate (carbaryl) or the cyclodiene dieldrin.

The epoxide ring of dieldrin is only slowly attacked by epoxide hydrases[4], but that of the analogue HEOM (I, Fig. 1) varies in stability according to the species. Thus, HEOM is toxic to tsetse flies, which have poor hydrative ability, but innocuous to other insects having high epoxide hydrase activity[5].

For cecropia juvenile hormone (JH; III, Fig. 1) and its mimics containing terminal epoxide rings, hydrative epoxide ring cleavage (and ester hydrolysis, when this function is present) is a significant metabolic deactivation route in several species[6,7] and the epoxide ring has a variable influence on the activity of analogues containing it that may partly reflect differences in its biodegradability[8].

A search for inhibitors of the epoxide hydrase which rapidly converts HEOM[3] into the corresponding *trans*-diol (II, Fig. 1) in several insects has revealed that synthetic cecropia hormone (mixed isomers), as well as methyl epoxyfarnesoate (MEF) and the synthetic mimics EPGE[9] (Table 1) and EGSE[10] (V, Fig. 1), are competitive inhibitors of cyclodiene epoxide hydrase in pupal homogenates of the blowfly, *Calliphora erythrocephala*, and the mealworm, *Tenebrio molitor*, indicating that these compounds are possibly alternative substrates for HEOM-hydrase. In