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THERMODYNAMIC AND HYDRODYNAMIC STUDIES RELATING TO THE MECHANOCHEMICAL CYCLE IN THE FLAGELLUM OF *CRITHIDIA ONCOPELTI*

BY M. E. J. HOLWILL AND J. WAIS*
Physics Department, Queen Elizabeth College, London

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SUMMARY

1. The motile response of the flagellum of *Crithidia oncopelti* to changes in the temperature, pressure and viscosity of the environment were studied.

2. The dependence of beat frequency on temperature and pressure can be interpreted in terms of the theory of reaction rates by making the assumption that the frequency is proportional to the rate constant of a chemical reaction within the flagellum.

3. For viscosities below a characteristic value, which depends on the temperature, increasing the pressure causes a reduction in the beat frequency. At the characteristic value, pressure changes do not affect the frequency while above this value an increase in pressure causes an increase in the frequency.

4. Pressure and temperature changes directly affect chemical reactions within the flagella whereas alterations in viscosity produce changes which can be attributed to a modification of the microtubule sliding rate.

5. The time for which detergent-treated flagella remain active following addition of ATP depends upon the environmental conditions. It appears that these *in vitro* preparations can convert only a limited amount of chemical energy into mechanical work.

INTRODUCTION

The energy required for the movement of flagella is derived from chemical reactions occurring within the organelles, so that changes in the environmental conditions such as temperature and pressure, which affect chemical processes, will also modify the movement of flagella. The behaviour of flagella in response to the changing conditions can therefore provide information about the mechanochemical processes which underlie movement. In previous work the effects of temperature on *in vivo* and *in vitro* flagellar systems (Holwill & Silvester, 1965; Holwill, 1970) and of pressure on organisms *in vivo* (Coakley & Holwill, 1974) have been interpreted in terms of the theory of absolute reaction rates (Johnson, Eyring & Stover, 1974). Such an interpretation is possible if it is assumed that the flagellar beat frequency is proportional to a rate constant, as would appear reasonable if the mechanochemical process occurs

* Present address: Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, New York.

once (for example) at each active site along the flagellum for each passage of a wave.

Such a model is an oversimplification of the situation which exists in real flagella, but it provides a basis on which to present data and has achieved some interpretive success. The results of the earlier work are consistent with the hypothesis that the effects of temperature and pressure on flagellar beat frequency are due to two effects: (*a*) changes in the equilibrium of a reversible enzyme denaturation process and (*b*) changes in the rate of breakdown of an enzyme-substrate complex. Under normal environmental conditions for the cell, the breakdown of the complex plays a dominant role in the determination of the frequency, but when the environmental conditions are altered, the denatured form of the enzyme is favoured and the shift in equilibrium of the denaturation process has a profound effect on the frequency.

In the present paper we report the response of the flagellum of *Crithidia oncopelti* to changing the viscosity of the medium in addition to altering the temperature and pressure of the environment. It is well established (e.g. Brokaw, 1975; Holwill, 1965) that the viscosity of the fluid influences the motility of flagella. The primary effect of increasing the fluid viscosity is to provide an increased resistance to movement which leads, generally, to a reduction in the flagellar beat frequency. This effect has been useful in the study of hydrodynamic and kinetic aspects of flagellar movement. We will show that the effects on flagellar activity of altering the pressure, temperature and viscosity in concert provide information which will lead to a greater understanding of the mechanisms responsible for motility.

MATERIALS AND METHODS

The trypanosomid flagellate *Crithida oncopelti* was grown axenically in a synthetic medium described by Newton (1957). The experimental apparatus and techniques used for the observation of micro-organisms under pressure at controlled temperatures are described in detail elsewhere (Coakley & Holwill, 1972) but it is convenient to provide a short summary here. The apparatus consisted of a stainless-steel pressure cell surrounded by a constant temperature jacket mounted on the stage of a modified Wild M11 binocular microscope. Pressure was developed in the system by the action of a P228 Enerpack hand-pump. The temperature, which could be controlled to within $\pm 0{\cdot}5$ °C, was measured by means of a thermocouple in the pressure chamber using direct-reading potentiometer circuit (Holwill & Silvester, 1965). The ranges of pressure and temperature used during experimentation were respectively about 0·1 MPa (i.e. 1 atm) to 70 MPa and 5 °C to 30 °C. Observations were made using dark field microscopy with stroboscopic illumination provided by a Xenon flash lamp.

A valve in the pressure line allowed the pressure to be applied in either of two modes: (*a*) gradually, with the valve open, so that the working pressure was reached in about 5 s, or (*b*) rapidly, by suddenly opening the valve so that the cell and its contents were exposed to the higher pressure. Pressurization of the specimen caused a transient increase in temperature by a maximum of 0·5 °C.

Several substances, e.g. methyl cellulose, polyvinyl pyrrolidone and Ficoll, can be used to increase the viscosity of the medium in the study of cell motility. A number of materials was tested to investigate their suitability as additives to the medium in which *Crithidia* swim. Of those studied, methyl cellulose was the only one which produced

Table 1. *Time (in s) for sphere to fall 1 cm in methyl cellulose solutions at various pressures*

	Viscosity of solution at atmospheric pressure		
Pressure (MPa)	17×10^{-3} Pa s	37×10^{-3} Pa s	59×10^{-3} Pa s
0·1	1·01 ± 0·01	2·48 ± 0·01	4·58 ± 0·03
10	0·98 ± 0·01	2·46 ± 0·01	4·51 ± 0·02
20	0·98 ± 0·01	2·49 ± 0·02	4·54 ± 0·03
30	1·01 ± 0·01	2·46 ± 0·02	4·58 ± 0·03
40	1·06 ± 0·01	2·48 ± 0·02	4·50 ± 0·04
50	1·04 ± 0·01	2·44 ± 0·02	4·54 ± 0·03

Each value is the mean, with standard error, of 30 observations made by two observers.

no obvious aberrations in the motile behaviour of the cells and was therefore used in the present study. Viscous media were prepared by adding methyl cellulose in varying concentrations (all less than 0·5 % weight per volume) to Ringer solution and adjusting the pH to 7·0. The viscosity of the medium was measured using a rotating cylinder viscometer (Brookfield Synchro-electric). Adjustment to the viscosity as a result of temperature changes were made using calibration curves obtained for a range of initial viscosities (see also Holwill, 1965). The effects of pressure on the viscosity of methyl cellulose solutions is small for the range of pressures used in the present study (see Results) and no adjustment was necessary. To study the effect of pressure on the viscosity of the methyl cellulose solution, a simple falling sphere method was used. The pressure cell used for observation of organisms was filled with the solution under test, and a nylon ball 2 mm in diameter placed into the solution. By turning the cell on edge, the time taken for the sphere to fall through a measured distance could be recorded. Careful manipulation ensured that the sphere would fall centrally through the chamber.

In vitro cell preparations were made by demembranating and reactivating *Crithidia* using a modification of the method described by Holwill & McGregor (1976). The cell membrane was removed using the detergent Nonidet P40 while reactivation was achieved using a solution containing magnesium and ATP. Various other ions were included in the preparation to obtain optimal performance. All solutions, including those used for *in vivo Crithidia oncopelti*, were adjusted to pH 7·0.

RESULTS

Effects of pressure on the viscosity of methyl cellulose solutions

The time taken for a nylon ball 2 mm in diameter to fall a distance of 1 cm under the influence of gravity was recorded for methyl cellulose solutions of different viscosities at a variety of pressures (Table 1). Each result in the table is the mean of 30 readings.

Effect of changed environmental parameters on in vivo Crithidia oncopelti

The separate effects on the flagellar beat frequency of *Crithidia oncopelti* of altering the viscosity, temperature and pressure were found to be similar to those reported elsewhere (Holwill, 1965; Holwill & Silvester, 1965; Coakley & Holwill, 1974). An

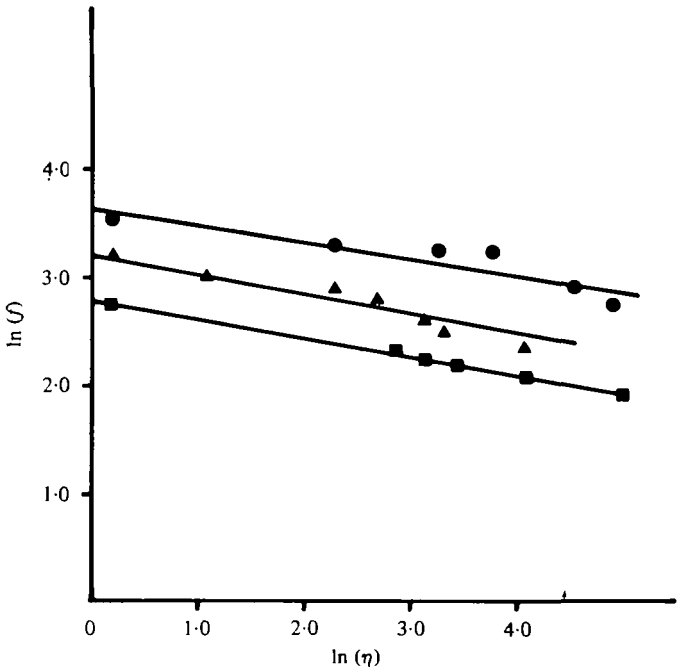

Fig. 1. Variation of beat frequency (f) with viscosity (η) for *Crithidia* at atmospheric pressure and three different temperatures. ■, 10 °C; ▲, 20 °C; ●, 30 °C.

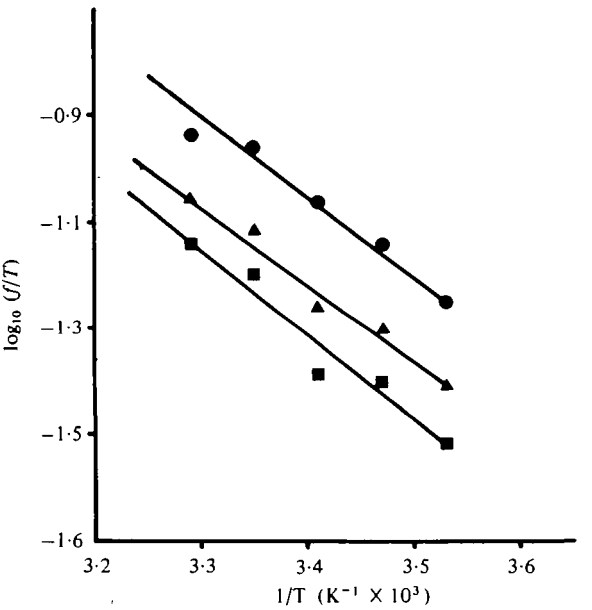

Fig. 2. Variation of beat frequency (f) with temperature (T) for *Crithidia* at atmospheric pressure and three different viscosities. Assuming f to be proportional to a rate constant, reaction rate theory predicts the following relation:

$$\ln (f/T) = \ln (\tau k/h) + \Delta S^{\ddagger}/R - \Delta H^{\ddagger}/RT,$$

where h, k are the Boltzmann and Planck constants, τ is a transmission coefficient customarily taken to be unity, R is the gas constant, T is the thermodynamic temperature and $\Delta S^{\ddagger}$, $\Delta H^{\ddagger}$ are the changes in entropy and enthalpy associated with the reaction characterized by f. Accordingly the data are plotted as $\log_{10} f/T$ *v* $1/T$. ●, $1{\cdot}2 \times 10^{-3}$ Pa s; ▲, 10×10^{-3} Pa s; ■, 40×10^{-3} Pa s.

Table 2. *Entrophy changes,* $\Delta S^{\ddagger}$, *and enthalpy changes,* $\Delta H^{\ddagger}$, *associated with the flagellar beat frequency of* Crithidia oncopelti *in media with different viscosities*

Viscosity (10^{-3} Pa s)	$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})
1·2	−131 ± 8	25 ± 2
10	−122 ± 7	29 ± 2
20	−123 ± 9	29 ± 3
30	−119 ± 11	30 ± 3
40	−118 ± 12	30 ± 4
1·2*	−121	29
1·2†	−4	64

Errors quoted are standard deviations obtained from a regression analysis of lines such as those shown in Fig. 2.

* From Coakley & Holwill (1974).
† From Silvester & Holwill (1965).

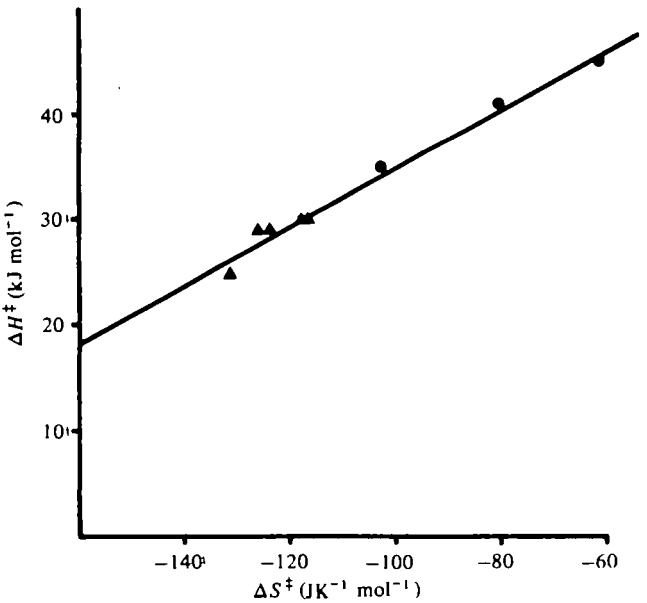

Fig. 3. Variation of activation enthalpy and entropy for *Crithidia* flagella under various conditions. ▲, *in vivo* cells, Table 2; ●, *in vitro* cells, Table 3.

increase in the viscosity or a decrease in the temperature of the environment produced a fall in the beat frequency. The spread of beat frequencies about the mean value was discussed by Coakley & Holwill (1974), who found that the fractional width of a frequency histogram remained approximately constant at about 25% as the temperature was varied but decreased with increasing pressure. In the present study this effect was again observed and it was found that a change in the fluid viscosity had little effect on the fractional width. All frequency measurements obtained are the mean of at least 10 values, and where errors are quoted, they represent standard deviations.

At a particular temperature a graph of the logarithm of the frequency against the logarithm of the viscosity is essentially linear, as shown in Fig. 1. The graphs corresponding to different temperatures are parallel, within the limits of experimental error, and have a slope of 0·18 ± 0·03 (S.D.). At constant viscosity, beat frequency is related

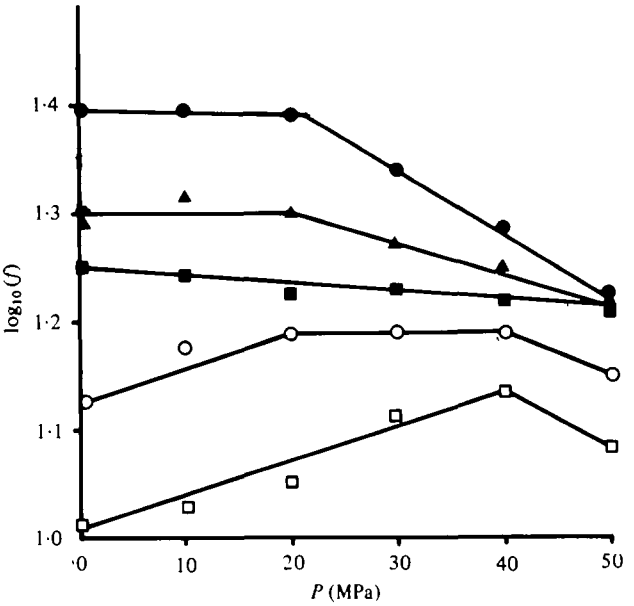

Fig. 4. Variation of beat frequency f with pressure P for *Crithidia* at 20 °C and several different viscosities. Since $\Delta H^{\ddagger} = \Delta E^{\ddagger} + P\Delta V^{\ddagger}$, the equation in the legend of Fig. 2 allows the dependence of frequency upon pressure to be predicted according to reaction rate theory. $\Delta E^{\ddagger}$ is the change in internal energy associated with the reaction characterized by f. Accordingly the data are plotted as $\log_{10} f$ *v.* P. ●, $1{\cdot}2 \times 10^{-3}$ Pa s; ▲, 3×10^{-3} Pa s; ■, 10×10^{-3} Pa s; ○, 23×10^{-3} Pa s; □, 58×10^{-3} Pa s.

to the thermodynamic temperature in the manner predicted by the theory of absolute reaction rates, assuming the frequency to represent a chemical rate constant (Fig. 2). From the slopes and intercepts of the lines drawn in Fig. 2, changes in entropy and enthalpy associated with reactions characterized by the beat frequency can be calculated. These values are shown in Table 2 and plotted in Fig. 3.

The response of *Crithidia oncopelti* to changing pressure has been described in detail by Coakley & Holwill (1974) and their work is confirmed by the present study. The application of pressure, provided this is below a certain critical value which depends on the temperature, causes an initial rise in the beat frequency, which subsequently falls, over a period of several minutes, to a steady value below that obtaining before the application of pressure. The beat frequency remains at the steady level for as long as the pressure is maintained. If the pressure exceeds the critical value referred to earlier, no transient increase in frequency is observed; rather, the frequency falls to its new steady value. The steady level to which the frequency falls is taken to be characteristic of the applied pressure, and suitable graphs show that at a constant temperature, the relationships between pressure and frequency are as expected if the frequency is considered as a rate constant in absolute reaction rate theory, although each graph has two linear sections of differering slope.

The rate at which pressure was applied had no significant effect on the results obtained; the sudden opening of the valve, thus causing a rapid increase in pressure, caused similar changes in the beating behaviour of the flagellum to those observed when the pressure was changed gradually. After preliminary studies had establishe

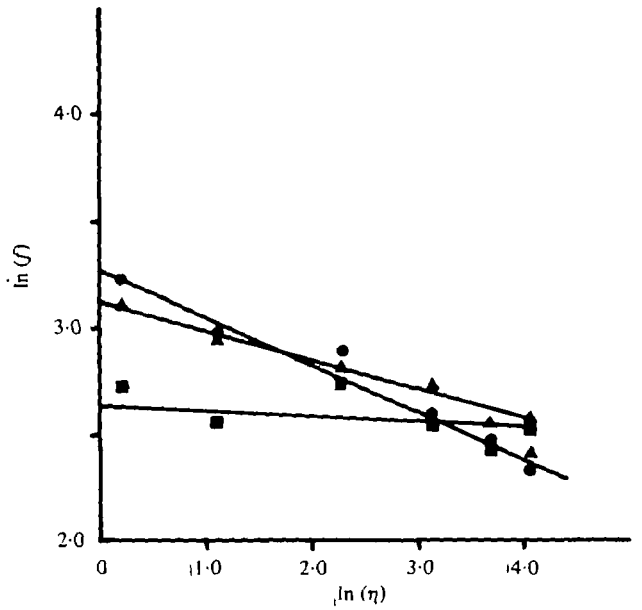

Fig. 5. Variation of beat frequency (f) with viscosity (η) for *Crithidia* at three different pressures. ● Atmospheric pressure, 0·1 MPa; ▲ 30 MPa; ■, 60 MPa.

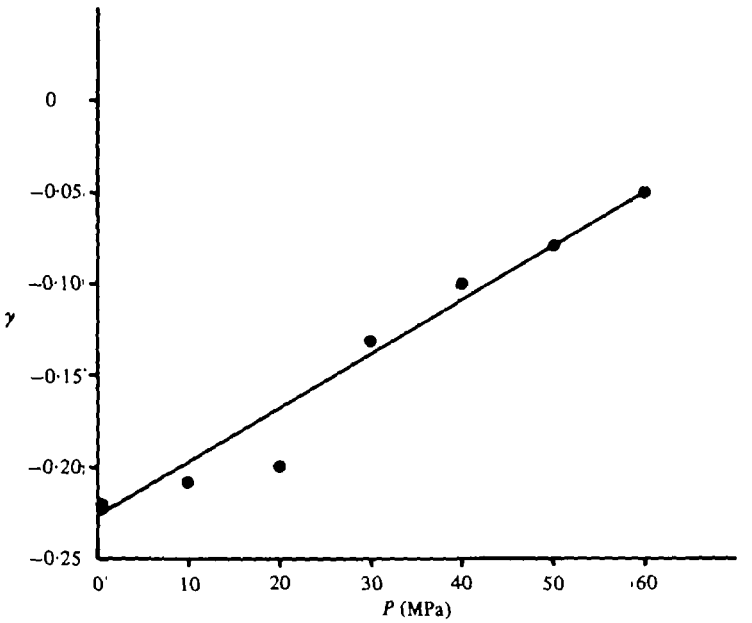

Fig. 6. Variation of the slopes (γ) of $\ln f$ v. $\ln \eta$ curves, examples of which are shown in Fig. 5.

Table 3. *Entropy changes* $\Delta S^{\ddagger}$, *and enthalpy changes* $\Delta H^{\ddagger}$, *associated with the flagellar beat frequency of detergent treated* Crithidia oncopelti *reactivated at three ATP concentrations*

ATP concentration (mol m^{-3})	$\Delta S^{\ddagger}$ (J K^{-1} mol^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})
4	-80 ± 15	40 ± 6
1	-102 ± 13	35 ± 3
0·2	-71 ± 13	45 ± 4

Errors quoted are standard deviations obtained from a regression analysis of lines such as those shown in Fig. 7.

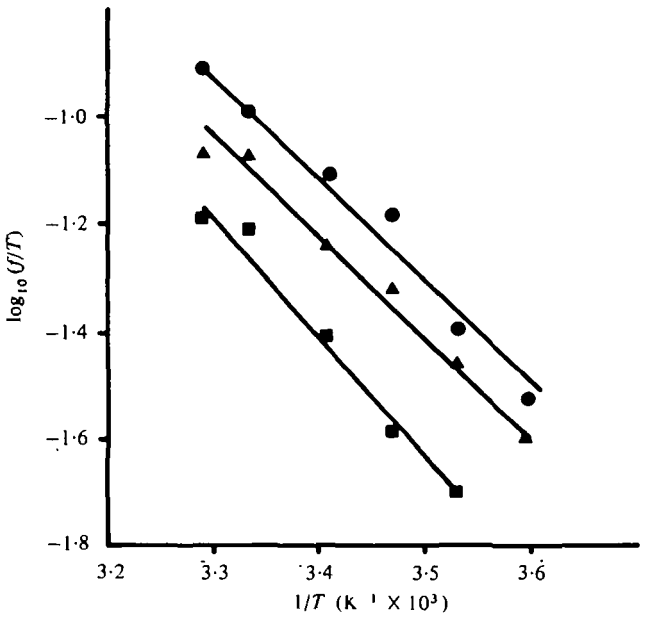

Fig. 7. Variation of beat frequency (f) with temperature (T) for reactivated *Crithidia* at atmospheric pressure and different ATP concentrations. ●, 4 mol m^{-3}; ▲, 1 mol m^{-3}; ■, 0·2 mol m^{-3}. See legend of Fig. 2 for explanation of axes.

this result, further work was performed using a slow application of pressure, to reduce both the temperature rise in the specimen and the risk of failure in the pressurized apparatus.

The effects of altering the pressure on the beat frequency of cells exposed to different viscosities are shown in Fig. 4. Each curve corresponds to a given viscosity and it can be seen that for viscosities lower than a characteristic value, which lies between 10 and 23×10^{-3} Pa s, increasing the pressure causes a decrease in the steady level frequency. As the viscosity increases, the decrease in frequency for a given pressure rise becomes smaller, until at the characteristic viscosity the beat frequency remains unaltered as the pressure is changed. At viscosities greater than the characteristic value, an increase in pressure causes the frequency to rise, and the higher level is maintained while the higher pressure is applied.

At a given pressure, a double logarithmic plot of frequency against viscosity is linear; typical examples are shown in Fig. 5 and the slopes of these lines are linearly related to the pressure as shown in Fig. 6.

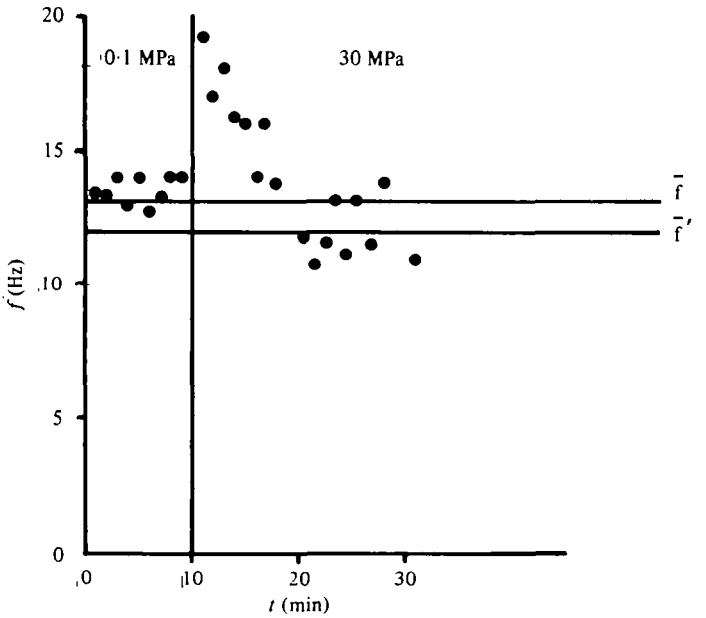

Fig. 8. Time (t) course of beat frequency (f) before, during and after the application of pressure to reactivated *Crithidia*. The solid vertical line at 10 min indicates the time at which pressure was applied. $\bar{f}$ is the mean frequency before the application of pressure and $\bar{f}'$ is the mean value which the frequency attained after the transient rise.

Effects of changed environmental parameters on reactivated demembranated Crithidia oncopelti

The response of demembranated *Crithidia oncopelti* to changing the concentration of ATP and other ions was discussed by Holwill & McGregor (1976) and has been verified in the present study. The direction of wave propagation can be controlled by altering the concentration of calcium ions in the reactivating solution, but this is possible only during the first two minutes of reactivation; thereafter, waves propagate only towards the tip. In the experiments described here, observations were made on cells with distally propagating waves. At a given temperature, the relationship between beat frequency and ATP concentration (more strictly Mg-ATP concentration) follows Michaelis–Menten kinetics. For a particular ATP concentration the relationship between beat frequency and temperature follows the kinetic relationship discussed earlier as shown in Fig. 7. Changes in enthalpy and entropy derived from this figure are shown in Table 3 and Fig. 3.

Provided the organisms remained active after application of pressure (see below), compression produced an increase in the proportion of organisms propagating symmetrical waves. Generally the application of pressure produced no change in the beat frequency of reactivated flagella, although in a few instances the beat frequency showed a transient increase following pressurization, but fell to a value close to that characteristic of atmospheric pressure within a few minutes (Fig. 8). We have been unable to create reproducibly the conditions under which the transient increase occurs and cannot therefore characterize this effect further.

Reactivated cells do not retain their capacity for movement indefinitely, and cease

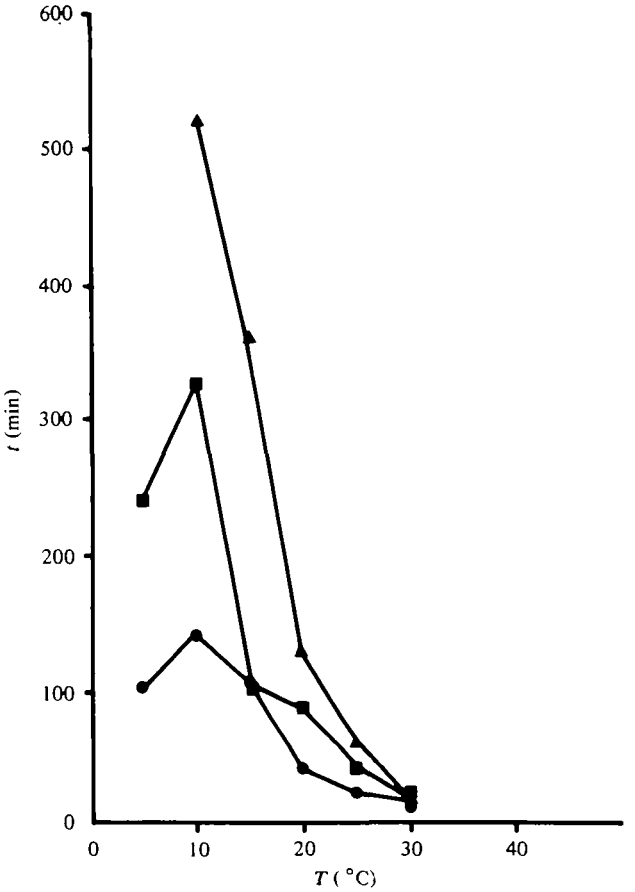

Fig. 9. Variation of active time (t) with temperature (T) at atmospheric pressure and different ATP concentrations. ●, 4 mol m^{-3}; ■, 1 mol m^{-3}; ▲, 0·2 mol m^{-3}.

moving after a period of time even if the supply of ATP is maintained. The time for which the cells remain active (referred to henceforth as the active time) depends on the environmental conditions and is reduced when the viscosity, pressure or ATP concentration are raised. If other conditions remain constant an increase in temperature in the range 5 °C to 10 °C causes an increase in the active time while this parameter is reduced on further increasing the temperature (Fig. 9). For specified values of viscosity, temperature and ATP concentration, an increase in pressure causes the active time to decrease, until a characteristic pressure is reached at which the active time is essentially zero. Experimentally, the application of the characteristic pressure did not cause all movement to cease immediately and it proved most expedient to estimate the magnitude of this pressure by extrapolating a graph of pressure against active time (Fig. 10). The characteristic pressure is dependent on temperature and ATP concentration as shown in Fig. 11. Restoration of motility was observed on decompression from the characteristic pressure to atmospheric pressure unless the cells had remained under compression for longer than a period which depended on the experimental conditions. This period of time was shorter the higher the pressure, being about 5 min for cel

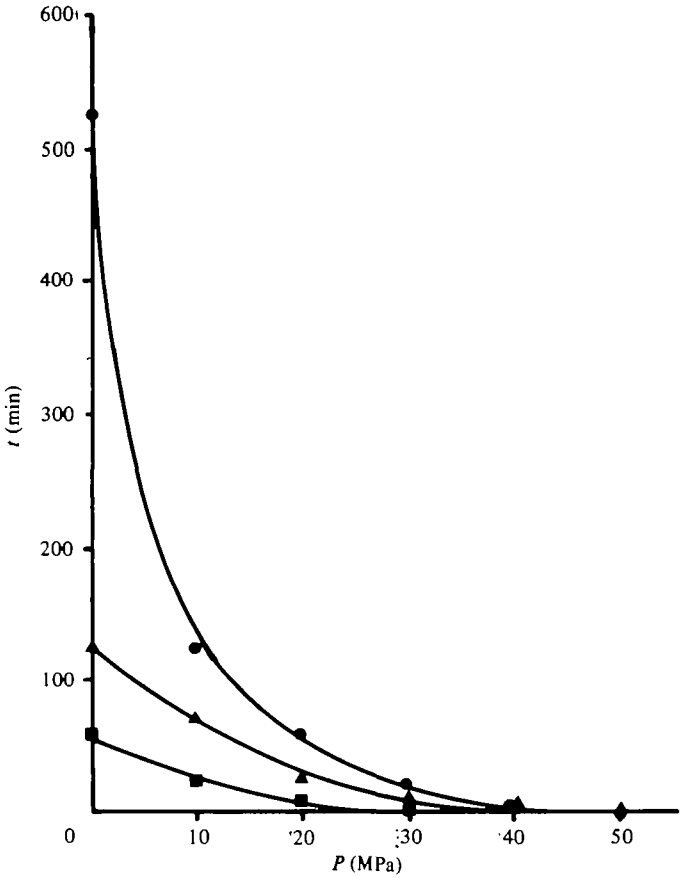

Fig. 10. The effects of pressure (P) on the active time (t) for detergent treated *Crithidia* reactivated with 0·2 mol m^{-3} ATP at 10 °C (●), 20 °C (▲) and 25 °C (■). By extrapolating each curve to zero time, the characteristic pressure (P_{ch}) is obtained.

reactivated with 1 mol m^{-3} ATP at 20 °C and compressed to 30 MPa. Exposure of the cells to high pressure before reactivating them also affected their reactivation capabilities. A 15 min exposure to 30 MPa allowed reactivation if ATP was added after decompression, but if this pressure was maintained for 20 min, reactivation was not possible. At atmospheric pressure, detergent-treated cells remain capable of reactivation for a period of about 5 h, after which the addition of ATP does not induce movement. If cells at 20 °C in ATP at a concentration of 1 mol m^{-3} were exposed to a pressure of 100 MPa, activity ceased within 10 s and motility was not restored after decompression, even when this was effected directly after the cessation of all flagellar movement.

DISCUSSION

The results shown in Table 1 indicate that the viscosity of the methyl cellulose solutions examined is affected little by pressure changes up to 50 MPa. This result accords with the work of Horne & Johnson (1967) who showed that the viscosities of water-based solutions may be changed by about 1×10^{-6} Pa s, or 0·1 %, when the

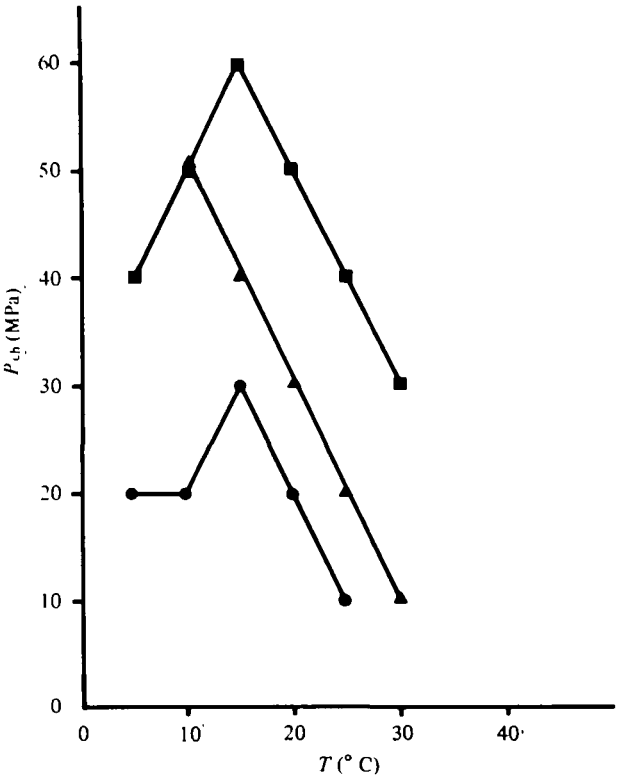

Fig. 11. Variation of characteristic pressure (P_{ch};) (see text for explanation) with temperature (T) for detergent-treated *Crithidia* at different ATP concentrations. ●, 4 mol m^{-3}; ▲, 1 mol m^{-3}; ■, 0·2 mol m^{-3}.

pressure is raised from 0·1 to 60 MPa. Earlier work on methyl cellulose (Suzuki, Taniguchi & Enomoto, 1972) has considered the effect of pressure on the sol-gel transformation in this substance. In the present work the concentrations of methyl cellulose used were much lower than those of Suzuki and his collaborators, and the conditions were not conducive to gel formation. The results of Suzuki could not therefore be used to assess the influence of pressure on the solutions used in the present study. The experiments performed here show that the viscosity of methyl cellulose solution at a concentration below 1 % weight per volume is essentially unchanged by increasing the pressure from 0·1 to 50 MPa. The determinations of viscosity made at atmospheric pressure using the Brookfield viscometer were therefore applicable to the higher pressures.

Certain responses of *in vivo* and *in vitro Crithida oncopelti* to alterations in temperature and pressure are consistent with the concept that the flagellar beat frequency reflects a rate constant of a chemical reaction within the organelle. It is of interest to interpret the appropriate results in terms of this concept and to compare the conclusions derived from the two systems so that similarities and differences between them can be established and functional implications of particular structures determined. Inspection of Table 2 shows that the entropy and enthalpy changes for *in vivo Crithidia* remain essentially constant over a range of viscosities and are the same as th

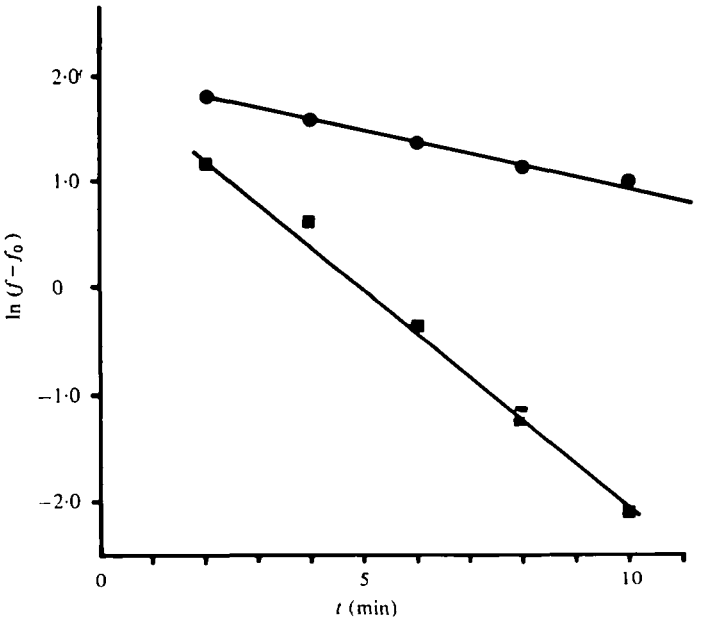

Fig. 12. Showing that the decay of frequency (f) to the steady level value (f_0) is exponential in time for both *in vivo* (■) and *in vitro* (●) *Crithidia*. (■, 16 °C and 27·6 MPa; ●, 15 °C, 30 MPa and 1 mol m^{-3} ATP.)

recorded by Coakley & Holwill (1974). The differences between the values obtained by Holwill & Silvester (1965) and those of the present work could be associated with the fact that the pH of the medium in the present study was different from that in the earlier work. Changes in the chemical environment are known to affect the enthalpy and entropy changes associated with a chemical reaction (e.g. Johnson *et al.* 1974). In the present case the pH of the medium could influence the membrane potential which, in turn, could modify the ionic strength within the cell. For detergent treated cells (Table 3), the entropy and enthalpy changes show little variation over the range of ATP concentrations used, but are different from the values found for *in vivo* cells. This difference could again be due to the different chemical environments to which the active components of the axoneme are exposed in the living and extracted cells. This view is supported by the linear relationship between the entropy and enthalpy shown in Fig. 3. Such a relationship can suggest that the reactions represented are identical but occur under different chemical conditions. It is also interesting to note that the line in Fig. 3 is that derived by Holwill & Silvester (1967) from the behaviour of a variety of cilia and flagella.

The effects of pressure on the flagellar beat frequency of *in vivo Crithidia oncopelti* were interpreted by Coakley & Holwill (1974) in terms of reaction rate theory. Their results are consistent with the hypotheses that the flagellar response is due to (*a*) changes in the equilibrium of a reversible denaturation process and (*b*) changes in the rate of decomposition of an enzyme-substrate complex. The response of demembranated cells to pressure variations does not usually include a transient rise in frequency such as that observed for living cells. The *in vitro* system generally shows changes only in the time for which the cells remain active, rather than in the beat frequency.

The fact that, in a few instances, transient frequency changes *were* observed suggests that this feature could be characteristic of the demembranated cells under the appropriate conditions. In the preparations to date, however, this response has not been reproducible and the characteristics required for it are therefore not available. Despite the apparent absence of the transient, the flagellar beat patterns were normal in appearance, so that the basic machinery responsible for flagellar action was still intact.

As shown in Fig. 12, the decay to the steady level frequency following a transient rise is exponential in time and has a decay constant of about $7 \times 10^{-3}\ s^{-1}$ Similar exponential decays were reported by Coakley & Holwill (1974) for *in vivo Crithidia oncopelti* and by Pease & Kitching (1939) for gill cilia of the mussel *Mytilus edulis*. The results of Pease and Kitching have been interpreted by Johnson *et al* (1974) in terms of the perturbation of a steady state process by the application of pressure. A similar argument to that employed by Johnson *et al.* can be applied to the enzymic reaction which has been used by Coakley & Holwill (1974) in the kinetic intepretation of flagellar beat frequency data. This reaction is

$$MATP^{2-} + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} MATPE \xrightarrow{k_2} MADP^{-} + E + P_i^{-}, \qquad (1)$$

where M is a metal (probably Mg), E is the flagellar enzyme and P_i is inorganic phosphate. k_1, k_{-1} and k_2 are rate constants for the various reactions.

To simplify the presentation of the equations in the mathematical argument to follow it is convenient to re-write equation (1) as

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} N + E + P, \qquad (2)$$

in which equivalent symbols are clear. The rate of the reaction, which is assumed to reflect directly the flagellar beat frequency, is proportional to the concentration of ES, which remains constant when a steady state is achieved. If the system is perturbed the concentration of ES changes at a rate given by

$$\frac{\partial[ES]}{\partial t} = k_1[E][S] - k_{-1}[ES] - k_2[ES]. \qquad (3)$$

Making the reasonable assumption that [E] and [S] remain constant this equation can be integrated to yield

$$(k_{-1} + k_2)[ES] = k_1[E][S] - C\exp -(k_{-1} + k_2)t \qquad (4)$$

where C is the value of $\partial[ES]/\partial t$ at $t = 0$. If the concentration of ES is proportional to beat frequency it is clear from equation (4) that, following a perturbation, the frequency will vary exponentially with time. Although the value for $(k_{-1} + k_2)$ is shown to be $7 \times 10^{-3}\ s^{-1}$, further discussion of its significance would be inappropriate here as little is known about the detailed kinetics of the reaction. The reaction scheme which occurs in flagella is doubtless more complex than that shown in equation (1), so that the kinetic parameters in equation (4) are probably combinations of the reaction constants derived from several steps in the real reaction. The difference between the slopes obtained for *in vivo* and *in vitro* cells may be associated with the somewhat different chemical environments which obtain in the two cases. More detailed criti

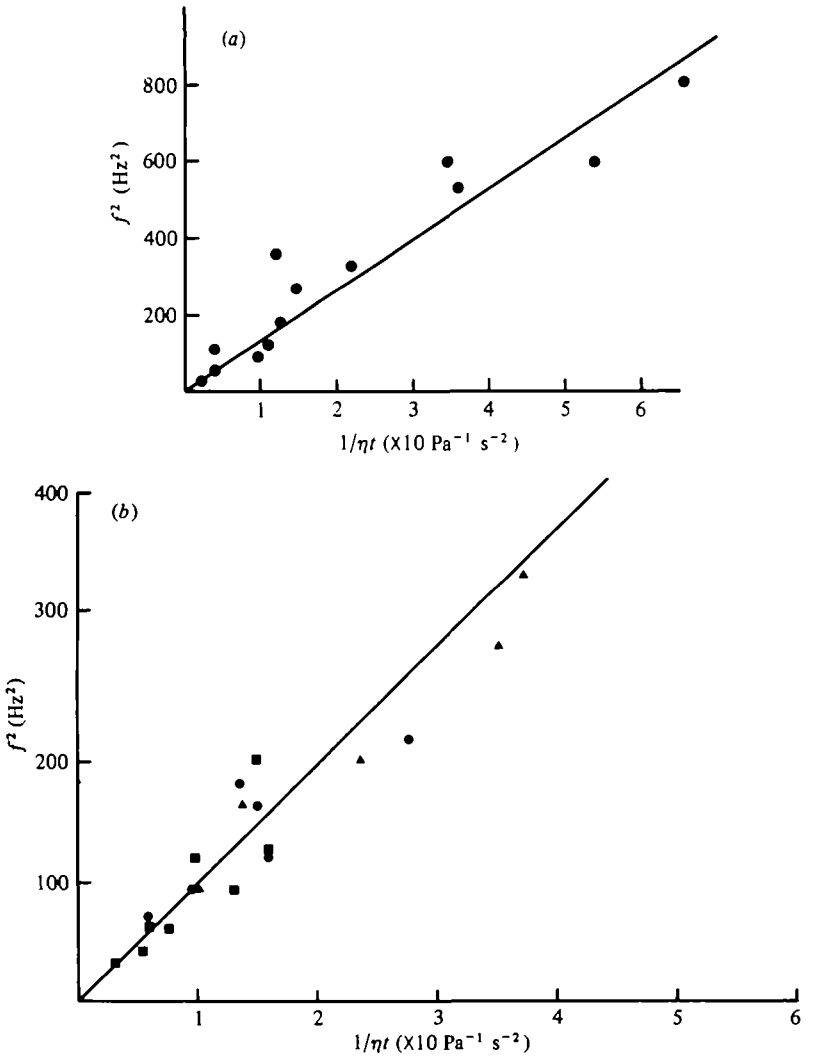

Fig. 13. Showing that the behaviour of *Crithidia* is consistent with equation (8). (*a*) At the viscosity of water and (*b*) at higher viscosities (▲, 15×10^{-3} Pa s; ●, $27{\cdot}6 \times 10^{-3}$ Pa s; ■, 46×10^{-3} Pa s).

studies of the transients are required to provide information about this aspect of the problem.

It is possible to use the absolute theory of reaction rates to interpret the frequency data recorded as a function of pressure at any given viscosity. Examination of Fig. 4 shows that corresponding sections of the various curves have rather different slopes and hence yield different volume changes, since these are given by $-RT(\partial(\ln f)/\partial p)_T$ according to reaction rate theory. The ideas developed earlier in this discussion suggest that the volume changes are characteristic of a chemical reaction occurring within the flagellum and which plays a major role in motility. Changes in the viscosity of the medium *per se* would not be expected to influence the volume change associated with an internal chemical reaction, although as noted by Miles & Holwill (1971) the forces

generated by the flagellum to overcome increased viscous drag could influence the kinetics of the reaction.

An explanation of these observations can be given in terms of the sliding filament model of flagellar activity developed by Satir (1968), and for which there is now considerable supporting evidence (see Satir & Sale, 1977, for references). In the model, the dynein arms form cross-bridges between adjacent peripheral microtubules, thereby developing forces tending to cause relative sliding between the doublets. The forces generated at any given time are likely to depend, as in muscle, on the number of cross-bridges formed, a number which will depend on chemical and mechanical events within the system. For a cross-bridge to be formed, it should have the appropriate disposition relative to an active site on the microtubule with which it will interact. Cross-bridge formation will therefore depend on the chemical environment of the dynein arm and the active site, and also on the spatial relationship between the two. The rate at which relative sliding occurs will influence the time for which interaction is possible; the slower the relative sliding velocity the greater the probability of cross-bridge formation. Since an increase in viscosity causes a decrease in the beat frequency of flagella, the relative sliding rate of the microtubules is thereby reduced and the probability of cross-bridge formation consequently increased. The number of cross-bridges attached at any time is governed by an equilibrium constant, K (possibly k_1/k_{-1} in equation (1)), which depends on the relative sliding rate of the microtubules and on other physical parameters, in particular the external pressure. An increase in the applied pressure causes a reduction in K, and hence in the rate of cross-bridge formation whereas an increase in viscosity has the opposite effect. As the viscosity is increased its effects on the beat frequency increase, thereby making the beat frequency less sensitive to changes in the pressure, until at a certain viscosity the pressure variation has little influence on the beat frequency. For higher viscosities the effect of viscosity may be to increase the number of attached cross-bridges to such an extent that the action of some is against that of others, thereby reducing the total force generated by the cross-bridges and hence causing the frequency of beat to fall. The effect of increased pressure then is to reduce the number of attached cross-bridges, so reversing the effect of high viscosities and causing an increase in the frequency of beat (see Fig. 4).

On this basis, the slope of each line in Fig. 4 reflects the constant volume change of a chemical reaction associated with cross-bridge formation together with a viscosity-dependent term. The linear sections of the curves in Fig. 4 can be represented by the equation

$$\ln f = \left[\frac{\Delta V^{\ddagger}}{RT} + \phi(\eta)\right] p + \alpha, \tag{5}$$

where the pressure term is that derived from the theory of absolute reaction rates, $\phi(\eta)$ is an undertermined function of the viscosity and α is a constant term containing the temperature.

Fig. 6 suggests that $\phi(\eta)$ has the form

$$\phi(\eta) = a \ln (\eta/\eta_0) + b, \tag{6}$$

where a, b and η_0 are constants having values $3{\cdot}1 \times 10^{-3}$ MPa^{-1}, $-0{\cdot}23$ MPa^{-1} and

MPa s respectively. There is no clear theoretical basis for equation (6), and alternative forms for $\phi(\eta)$ may be possible. Further studies are needed to provide more information on this point. From the graphs of $\ln f$ *v.* P, values of $\Delta V^{\ddagger}$ can be obtained, and these are similar to the results obtained by Coakley & Holwill (1974). The change in the slope of the $\ln f$ v. P curves was interpreted by Coakley & Holwill (1974) in terms of a reversible enzyme denaturation characterized by an activation volume given by the difference between the volumes calculated from the high and low pressure regions of the curve. The volume changes which accompany denaturation were calculated by the method suggested by Coakley & Holwill (1974), and the possible significance of the changes were discussed in detail by them. The results presented here suggest that changes in the viscosity and pressure affect different aspects of the mechanochemical machinery which bends a flagellum, thereby providing a tool for separating components of the mechano-chemical reaction.

The effects of increased ATP concentration, increased temperature and/or reduced viscosity were to produce an increase in the beat frequency but a decrease in the active time of detergent-treated *C. oncopelti*. These observations suggest that the energy produced by flagella during their active period may be limited by the experimental conditions. It is possible to estimate the energy dissipated by the flagellum against the viscous resistance of the medium using an equation given by Taylor (1952) for an oscillating sinusoidal filament:

$$P = \frac{4\pi^3 \eta f^2 A^2 L}{0{\cdot}6159 - \ln(2\pi\rho/\lambda)}. \tag{7}$$

Here, P is the power expended, A the wave amplitude, L the length of the flagellum, ρ the flagellar radius and λ the wavelength. The waveform of the flagellum of *C. oncopelti* is not strictly sinusoidal (Johnston, Silvester & Holwill, 1978) but this convenient approximation provides a reasonable estimate of the power expenditure (e.g. Silvester & Holwill, 1972). In the case of *C. oncopelti*, the flagellar shape parameters A and λ remained approximately the same over the entire range of conditions to which the cells were exposed, so that the energy, E, used by the flagellum in time t to overcome viscous resistance is:

$$E = \int_0^t P\,\partial t = Cf^2 t\eta, \tag{8}$$

where C has the form

$$\frac{4\pi^3 A^2 L}{0{\cdot}6159 - \ln(2\pi\rho/\lambda)}.$$

An alternative expression for the power expenditure has been given by Carlson (1959) and leads to equation (8) after appropriate analysis, although the constant A has a slightly different form. A plot of f^2 against $1/\eta t$ (Fig. 13) yields two straight lines, one corresponding to the behaviour of cells in a medium with the viscosity of water, the other to that in media of higher viscosities. The total energy, E, dissipated against external viscous forces during the active period is therefore constant in the two cases and has the value $9 \pm 2 \times 10^{-12}$J at the low viscosity and $55 \pm 6 \times 10^{-12}$J at the higher viscosities.

Energy is also required to overcome elastic forces within the flagellum, but these are difficult to estimate reliably, since it is not certain which structural components of the

system resist bending. A major contribution to the elasticity is likely to be made by [redacted] intermicrotubular linkages, some of which undergo cyclic attachment and detachment, so that the elastic properties of the flagellum may vary with position and time. Given a constant elasticity, the energy dissipated against elastic forces would be proportional to the beat frequency (Machin, 1958). It is convenient here to assume, as in other studies, that the energy needed to overcome elastic forces in significantly less than that required to overcome the viscous resistance.

The fact that the hydrodynamic energy dissipation remains constant in the two cases indicates that the mechanisms within flagella responsible for motility convert only a particular amount of chemical energy into work throughout their period of activity. This may occur because the solutions used in the reactivation process cause progressive damage to the system responsible for mechanochemical coupling. It seems unlikely that the effect is caused by progressive solubilization of the dynein arms by ATP, as in this case a reduction in beat frequency with time would be expected (see, for example, Gibbons & Gibbons, 1973), whereas in practice a constant frequency was recorded throughout the active time. Experiments are being undertaken to ascertain which step of the mechanochemical cycle is broken during the active time and is therefore responsible for the cessation of beating at the end of the active period.

In this paper we have presented results which show that the combined effects on flagellar movement of pressure, temperature and viscosity provide information about the mechanochemical cycle which bends the flagellum. Pressure and temperature changes primarily affect the chemical reaction while alterations in the viscosity produce changes which can be attributed to a modification of the microtubule sliding rate. Detergent treated flagella appear to be limited in respect of the amount of chemical energy which can be converted into mechanical energy.

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