

EFFECTS OF PRESSURE* AND TEMPERATURE CHANGES ON THE FLAGELLAR MOVEMENT OF *CRITHIDIA ONCOPELTI*

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INTRODUCTION

The fundamental processes which underly flagellar activity are molecular in nature and it is to be expected that environmental changes which affect chemical processes will also modify the movement of flagella. In particular, the equilibrium state and the rate of a chemical reaction depend on the hydrostatic pressure and temperature to which the system is exposed, and it is possible to derive changes in certain thermodynamic properties such as volume, enthalpy and entropy from the variation of equilibrium and rate constants with pressure and temperature. If some characteristic associated with flagellar movement can be related to the equilibrium and rate constants of chemical reactions within the flagellum, the dependence of flagellar activity on pressure and temperature could be used to determine thermodynamic properties associated with the reactions and hence to obtain information about the reaction mechanisms.

In the interpretation of experimental results concerned with the variation with temperature of flagellar activity (Holwill & Silvester, 1965, 1967; Holwill, 1969), the frequency of beating was assumed to be proportional to the rate constant of a chemical reaction within the flagellum. On the basis of their temperature studies, Holwill & Silvester (1967) suggested that the rate-limiting step within a flagellum is the breakdown of a complex formed between adenosine triphosphate (ATP) and an enzyme.

The effects of pressure on ciliary movement have been studied, but the investigations were limited by technical difficulties to the observation of complete organisms or compound organelles (Ebbecke, 1935 *a, b*; Pease & Kitching, 1939; Kitching, 1957). Some of these results have been interpreted in thermodynamic terms by Johnson, Eyring & Polissar (1954) who calculated an activation volume associated with an unspecified enzymic reaction which was assumed to occur within the organism.

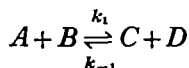
The development of a pressure chamber suitable for high-resolution light microscopy (Coakley & Holwill, 1972) has allowed us to study the responses of individual flagella to changes in both pressure and temperature. In this study we have attempted to characterize the pressure-temperature relations, and their time dependence, of the rate and form of flagellar beating in an intact protozoan flagellate. The results are

* Throughout this paper the S.I. unit of pressure, the pascal (Pa), will be used. $1 \text{ Pa} = 1 \text{ N m}^{-2}$; $1 \text{ atmosphere} = 101\,325 \text{ N m}^{-2} \approx 10^5 \text{ Pa} = 0.1 \text{ MPa}$.

interpreted on the basis of reaction kinetics, making use of what is known about chemically extracted flagella and the structural ATPase dynein which is believed to be intimately involved in flagellar movement.

REACTION KINETICS

It is convenient to summarize here those aspects of reaction kinetics which will be used for the interpretation of the experimental results. The equilibrium constant K for a reaction of the type



is given by

$$K = k_1/k_{-1}, \quad (1)$$

where k_1 , k_{-1} are the rate constants in the forward and reverse directions respectively. The standard change, ΔG , in the Gibbs free energy associated with the reaction is

$$\Delta G = -RT \ln K, \quad (2)$$

where R is the gas constant and T is the thermodynamic temperature. In the transition-state theory of reaction rates (Eyring, 1935; Glasstone, Laidler & Eyring, 1941) the reaction proceeds through an intermediate activated complex X^+ which is assumed to be in equilibrium with the reactants:



This equilibrium assumption appears to be valid in many cases of practical interest and is used implicitly in most theoretical discussions of chemical reaction rates. For this reaction an individual rate constant, k , has the form

$$k = \frac{\tau k T K^+}{h}, \quad (4)$$

where K^+ is the 'equilibrium constant' between initial and activated states, k , h are the Planck and Boltzmann constants respectively while the transmission factor τ is customarily taken to be unity.

The dependence of a rate constant on temperature and pressure can be expressed in terms of thermodynamic parameters by making use of the equations

$$\left. \begin{aligned} \Delta G^+ &= \Delta H^+ - T \Delta S^+ \\ \Delta H^+ &= \Delta E^+ + P \Delta V^+, \end{aligned} \right\} \quad (5)$$

where ΔH^+ , ΔS^+ , ΔE^+ , ΔV^+ are respectively the partial molar changes of enthalpy, entropy, internal energy and volume associated with the transition from the initial to the activated states. It is found that

$$\ln \left(\frac{k}{T} \right) = \ln \left(\frac{\tau k}{h} \right) + \frac{\Delta S^+}{R} - \frac{\Delta E^+}{RT} - \frac{P \Delta V^+}{RT}, \quad (6)$$

from which the relationships

$$\left(\frac{\partial \ln k}{\partial P}\right)_T = -\frac{\Delta V^\ddagger}{RT} \quad (7)$$

and

$$\left(\frac{\partial \ln (k/T)}{\partial (1/T)}\right)_P = -\frac{\Delta H^\ddagger}{R} \quad (8)$$

may be derived. From equations (7) and (8) it follows that, if volume and enthalpy increases are associated with the formation of the activated complex, i.e. ΔV^\ddagger and ΔH^\ddagger are both positive, the reaction rate will be decreased by an increase in the pressure and a decrease in the temperature. If ΔV^\ddagger and ΔH^\ddagger are constant, linear relationships are indicated between $\ln k$ and pressure at constant temperature, and between $\ln (k/T)$ and the reciprocal of the thermodynamic temperature at constant pressure. From the slopes and intercepts of such graphs the values of the various thermodynamic parameters can be found. Relations similar to equations (5)–(8) also describe the pressure and temperature dependence of equilibrium constants.

In earlier work (Holwill & Silvester, 1965, 1967) results consistent with the interpretation that the flagellar frequency is equal to or proportional to a rate constant, equilibrium constant or a product of such constants were reported. This approach will also be used in this paper, as it allows a convenient presentation of the experimental data, but it will be shown that the formal kinetic aspects of the variation of frequency with pressure and temperature are more complex than those suggested by this simple relation and require other mechanisms for their explanation.

MATERIALS AND METHODS

Cultures of *Crithidia oncopelti* were grown in 15 cm³ aliquots containing 3% bacteriological peptone, 0.5% glucose and 0.5% sodium chloride in distilled water with the pH adjusted to 7.4 by addition of sodium hydroxide (Newton, 1957). The organisms were normally used after 4–7 days, when the pH had fallen to between 6.5 and 7.0, but older cultures (11–14 days) were also studied for comparison.

The experimental chamber in which the micro-organisms were exposed to high pressures is described in detail elsewhere (Coakley & Holwill, 1972) so that a brief account only will be given here. The chamber was designed for use with a modified Wild M 11 binocular microscope and was surrounded by a constant-temperature jacket. The accuracy of the pressure gauge was about 1%, and (above atmospheric pressure) the pressure could be maintained constant to within $\pm 7 \times 10^5$ Pa during a 3 h experiment. Temperatures were measured by means of a thermocouple in the pressure chamber using a direct-reading potentiometer circuit (Holwill & Silvester, 1965) which gave a maximum calibration error of ± 0.3 °C. The large thermal capacity of the cell acted as a buffer against small fluctuations of external temperature; during 3 h experiments the temperature was periodically monitored and showed a maximum variation of ± 0.5 °C. The largest temperature variations occurred when the pressure was changed suddenly so that essentially adiabatic compression was achieved. For example, an increase of about 7×10^6 Pa in 5 s caused a temperature rise of 0.5 °C; the temperature fell to that obtaining before the pressure increase after a period of between 1 and 2 min. The range of pressures and temperatures used during the experiments were, respectively, 1×10^6 Pa to 6.89×10^7 Pa and 3.5–40.5 °C.

The cell dead space and pump feed were filled with liquid paraffin B.P., although olive oil was occasionally used in the dead space as it had the advantage of matching the refractive index of the cell windows more closely than liquid paraffin. Specimens in culture medium mounted on the upper window either in a small hanging drop or under a coverslip showed no significant differences in behaviour when exposed to increased pressure, and the latter method was used more often than the former since it facilitated observation of the cells.

Observations were made using dark-field optics at magnifications of between $\times 150$ and $\times 625$. At the lower magnifications a single field of view typically contained 50 organisms. Stroboscopic illumination was provided by a Chadwick Helmuth Point Source Strobex unit modified to give three times the normal flash energy. This lamp emits little infra-red radiation and continuous 3 h observation was possible without damaging the organisms. Frequencies were measured stroboscopically and recorded by means of a digital pulse counter with print-out. Individual frequency measurements were accurate to ± 0.25 Hz. Variations of waveform were recorded photographically using single-flash and multiple-flash exposures on respectively Ilford FP4 and HP4 films which were subsequently developed for maximum contrast.

Three types of experiment were performed on a sample:

(a) A single exposure to one selected pressure in the range 10^5 Pa to 6.89×10^7 Pa at a number of constant temperatures.

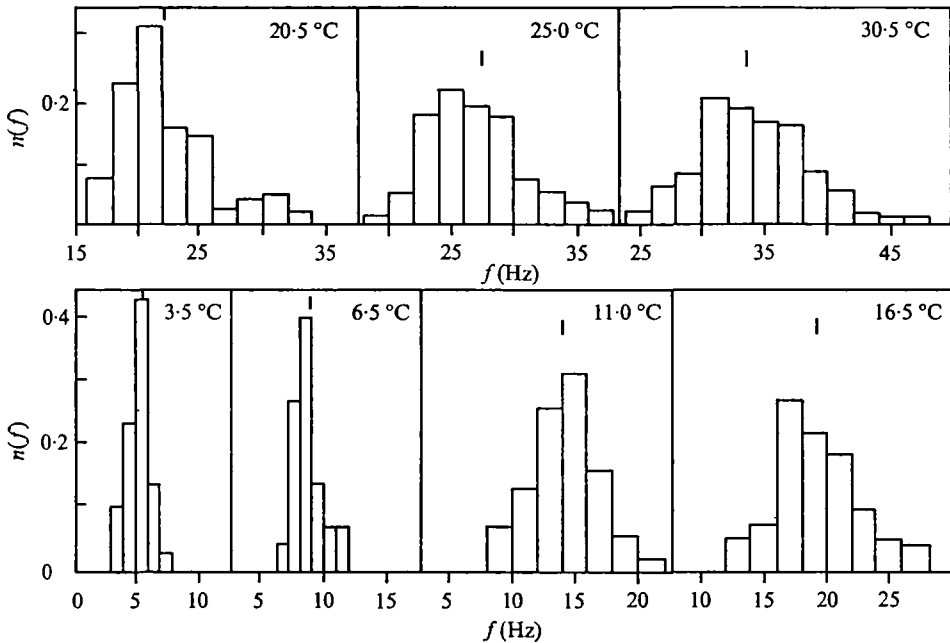
(b) Exposure to pressure increases in increments of 6.89×10^6 Pa and 13.8×10^6 Pa from 10^5 Pa up to 6.89×10^7 Pa at a number of constant temperatures.

(c) Exposure to varying temperature at a number of constant pressures.

Only a few experiments of type (c) were performed to test whether the temperature variation could correctly be predicted from the other two types of experiment. To ensure that thermal equilibrium had been reached samples were allowed to stand for 30 min before increasing the pressure or making measurements. The required pressure was usually reached by increasing the pressure at a rate of about 1.5×10^6 Pa s^{-1} to minimize disturbance of the sample and temperature fluctuations. The effects of rate of change of pressure on a sample were investigated by using several faster rates up to a maximum of 1.5×10^7 Pa s^{-1} .

RESULTS

The movement of *Crithidia oncopelti* at atmospheric pressure and a temperature of 22 °C has been described previously (Holwill, 1965), but a brief description will be given here of those features relevant to the present study. The normal cell body resembles a truncated cone with a small apex angle and measures about 8 μm in length and has an average diameter of about 3 μm . The average length and diameter of the flagellum are 20 and 0.2 μm respectively. Waves are propagated along the flagellum from its tip towards the base with mean values of wavelength, amplitude and frequency of, respectively, 14.4 μm , 2.4 μm and 17 Hz. The organism swims with the flagellum preceding the body and, since the wave is not absolutely planar, a freely swimming organism rotates at 1–2 Hz about its axis of progression. Individual organisms are able to reverse the direction of wave propagation and hence to move 'body first' through their liquid environment.

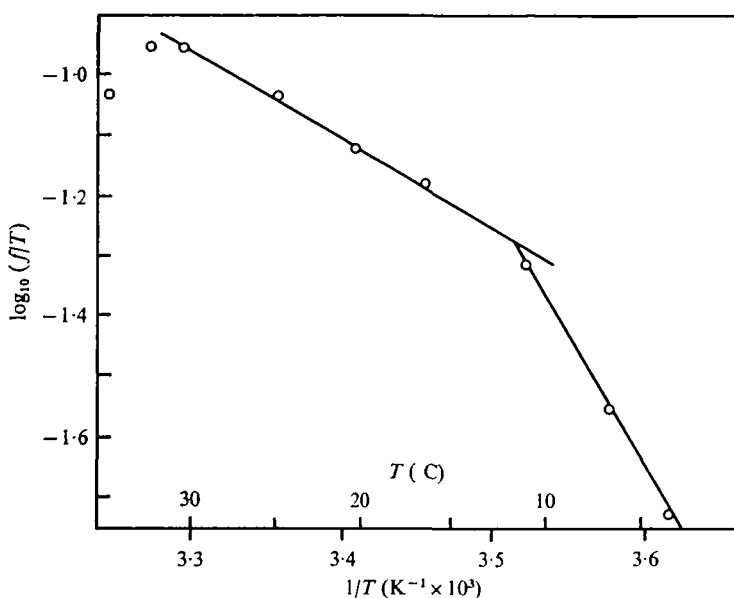


Text-fig. 1. Distributions of frequency (f) in *Crithidia oncopelti* at various temperatures and atmospheric pressure. Each histogram is normalized ($\sum n(f) = 1$) and the mean frequency is indicated by a short vertical line.

Effects of temperature at atmospheric pressure

Preliminary experiments were performed to assess the effects of confining organisms in the pressure chamber for a period of 3 h at atmospheric pressure. The average frequency, determined at half-hourly intervals from measurements on about 20 organisms, remained essentially constant over the 3 h period at a number of temperatures in the range 3.5–30.5 °C. There were no significant visually observable changes in waveform at temperatures between 11.0 and 30.5 °C, but below 11.0 °C the flagellar movements became more sporadic. The proportion of the population which propagated regular waves of definite frequency varied considerably from one sample to another and progressively decreased, by essentially the same fractional amount at a given temperature for all samples, as the temperature was raised above 30.5 °C. It was, however, possible to obtain meaningful values of frequency up to 34.5 °C, at which temperature the proportion of organisms which propagated regular waves had fallen to about 10%. At temperatures between 34.5 and 40.5 °C almost all flagella were curved into a smooth arc with a radius of about 15 μm . Roughly 10% of the flagella executed a localized flexing movement, but no bend propagation was observed. After exposure for about 30 min to a temperature within the range 34.5–40.5 °C all movement ceased. No structural changes in the flagellum or cell body were observed below 40.5 °C. Exposure to temperatures above 40.5 °C killed the organisms.

A return to ambient temperature (*ca.* 22 °C) following prolonged exposure of organisms to a temperature in the range 3.5–30.5 °C resulted in immediate recovery by the organisms of motility characteristic of the ambient temperature. The recovery of behaviour typical of 30.5 °C took up to 1 h after reducing the temperature to this



Text-fig. 2. Dependence on thermodynamic temperature (T) of mean frequency (f) at atmospheric pressure.

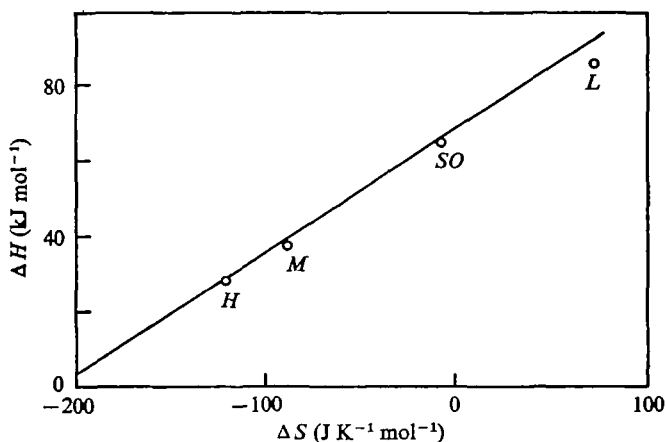
Table 1. *Changes of enthalpy (ΔH) and entropy (ΔS) associated with the flagellar movement of Crithidia oncopelti at atmospheric pressure*

	ΔH (kJ mol ⁻¹)	ΔS (J K ⁻¹ mol ⁻¹)	Key for Text-fig. 3
Mid-temperature region in Text-fig. 2 ($\Delta H_{\frac{1}{2}}^{\dagger}$, $\Delta S_{\frac{1}{2}}^{\dagger}$)	29	-121	<i>H</i>
Low-temperature region in Text-fig. 2 (ΔH_l , ΔS_l)	84	74	<i>L</i>
Mean for Text-fig. 2	38	-89	<i>M</i>
Low-temperature denaturation (ΔH_d , ΔS_d)*	-55	-195	
Holwill & Silvester (1965)	64.5	-3.8	<i>SO</i>

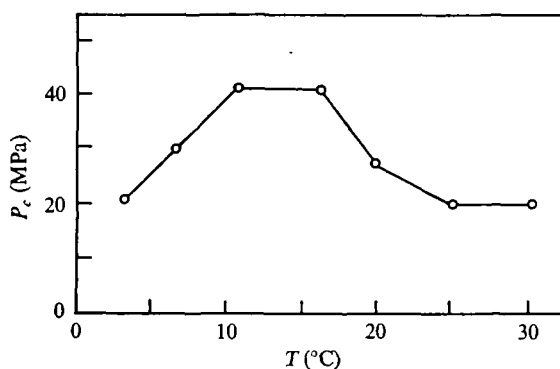
$$\bullet \Delta H_d = \Delta H_{\frac{1}{2}}^{\dagger} - \Delta H_l; \Delta S_d = \Delta S_{\frac{1}{2}}^{\dagger} - \Delta S_l.$$

value following exposure of a sample for 1 h to a temperature of 34.5 °C. When organisms were exposed to a temperature of 40.5 °C for 1 h about 20% recovered on lowering the temperature, and these organisms had normal waveforms but propagated waves at lower frequencies than that characteristic of the reduced temperature.

In Text-fig. 1 distributions of frequency at a number of temperatures up to 30.5 °C are plotted. The width of the distribution increased with temperature, although the fractional width remained approximately constant at about 25%. The distributions are slightly skew, an effect particularly noticeable at the higher temperatures, with the mode frequency less than the mean above about 16 °C and roughly equal to it at lower temperatures. It is interesting to note that, at all temperatures, the highest recorded frequency was approximately twice the lowest. The dependence of mean frequency (f) on the thermodynamic temperature (T), obtained from these and later experiments



Text-fig. 3. Relation of the enthalpy changes (ΔH) and entropy changes (ΔS) from Table 1 to the regression line obtained for various organisms by Holwill & Silvester (1967). The key is in Table 1.



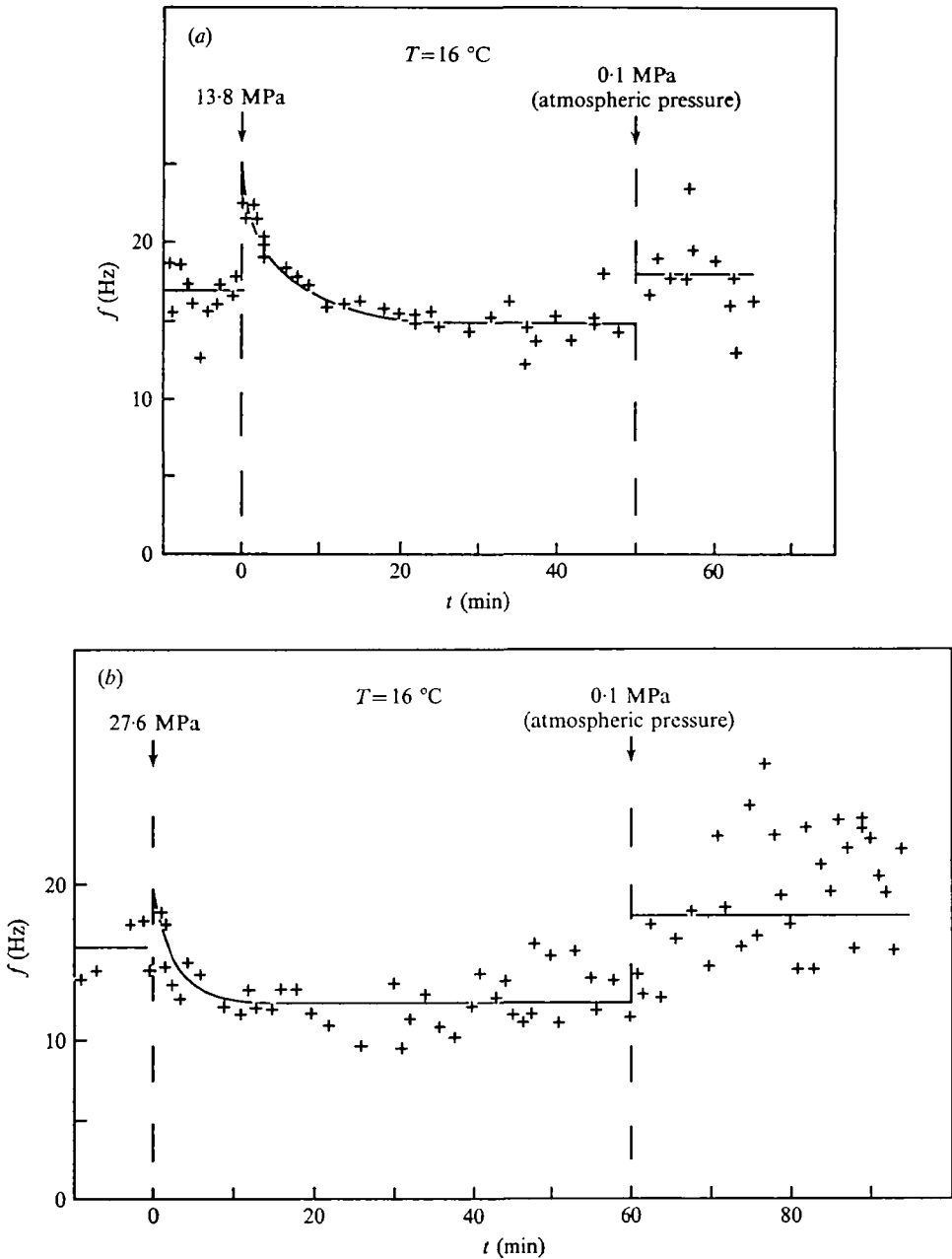
Text-fig. 4. Dependence on temperature (T) of the critical pressure (P_c) (see text for details).

before pressure increases were made, is shown in Text-fig. 2 which is a plot of $\log_{10}(f/T)$ against $1/T$. Such a graph should be linear according to equations (6) and (8), but instead shows two linear regions, one below 10 °C and the other between 10 and 30 °C, and a peak between 30 and 35 °C. The values for the apparent activation enthalpies and entropies derived from the linear portions of the graph are shown in Table 1 and lie close to the line obtained by Holwill & Silvester (1967) by plotting ΔH^* against ΔS^* for a number of cilia and flagella (Text-fig. 3).

Effects of pressure increase

Transient effects

Fast or slow (see Materials and Methods) application of a single pressure, provided it lay below a critical pressure which depended on the temperature, to a sample originally at atmospheric pressure and at a temperature between 6.5 and 30.5 °C, caused a rapid increase (within 10 s) in frequency. The frequency then decayed with time, approximately exponentially, to a steady frequency which was characteristic of



Text-fig. 5. Time (t) course of frequency (f) before, during and after application of various pressures. Each point is the mean of two readings.

the temperature and pressure. The steady level attained was always below the initial frequency. If a pressure greater than the critical value was applied, the frequency showed no transient increase, but decreased to a steady level more rapidly than the decay below the critical pressure. The variation of the critical pressure with temperature is shown in Text-fig. 4 and passes through a maximum between 11 and 16.5°C .

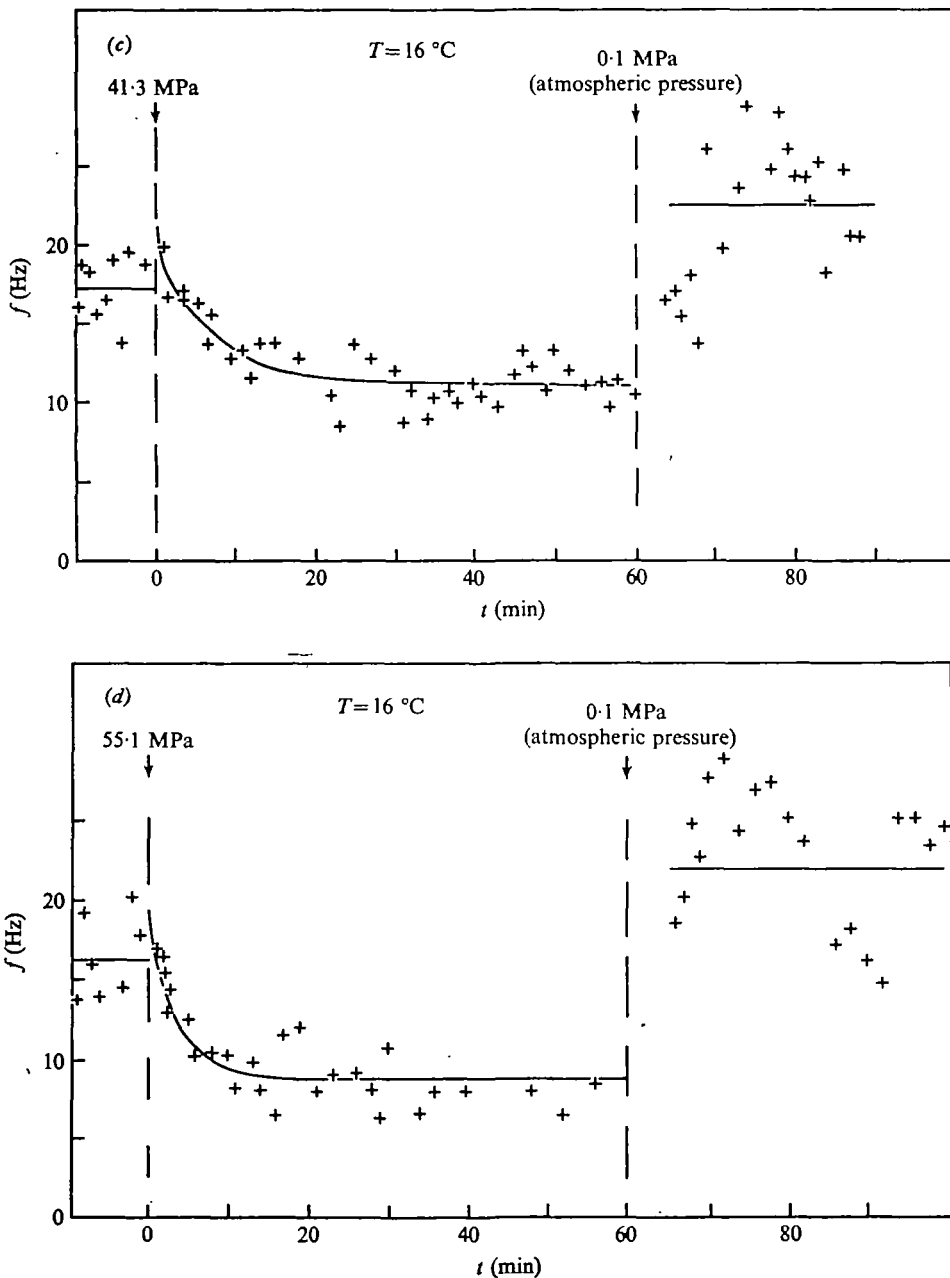


Fig. 5(c) and (d). For legend see opposite.

Typical examples of the variation of frequency with time below the critical pressure are shown in Text-fig. 5 and the average frequency recorded within 2 min after application of pressure is given in Table 2. The relative increase in frequency following application of a particular pressure appeared to be greatest at temperatures between 11.0 and 16.5°C , although effects at high temperatures might have been obscured by

Table 2. *Variation of transient wave parameters with pressure at 16 °C.*

Pressure (10^8 Pa)	Amplitude (μm)		Wavelength (μm)		Frequency (Hz)
	Mean	Extremes	Mean	Extremes	
1	2.3	1.4-2.8	14.1	13.0-16.7	16.6
138	3.0	2.2-3.7	14.4	11.2-17.5	21.8
276	3.3	2.3-5.1	14.6	11.1-20.4	18.9
413	3.8	2.8-4.6	15.0	13.0-18.5	18.1
551	3.9	3.2-5.1	16.1	11.1-20.4	17.5
689	3.5	2.8-4.2	14.8	11.1-18.5	15.7

Measurements were made within 2 min. of compression of organisms initially at atmospheric pressure.

the rather broad frequency distributions (Text-fig. 1) and the onset of less readily reversible effects (see later). At a given temperature the initial relative frequency increase was maximal at pressures in the range 1.38×10^7 Pa and 3.44×10^7 Pa, and less readily reversible effects were noticed at the higher pressures of between 4.13×10^7 Pa and 6.89×10^7 Pa. The steady frequencies, which were maintained by samples for at least 60 min, were reached in times which tended to become shorter as the pressure and temperature increased. At the smallest values of these parameters, namely 6.89×10^6 Pa and 6.5 °C, the steady state was attained in 30 min.

The increase in frequency observed on the application of pressure was accompanied by a narrowing of the frequency distribution and an increase in the proportion of organisms which propagated coherent waves. These effects were also noticed at pressures up to 1.38×10^7 Pa above the critical pressure and were greatest at those pressures and temperatures for which the transient frequency increases were greatest. The effect on the frequency distribution was obvious to the observer but was difficult to quantify since it would have required a large number of measurements to be made in a short time interval (about 2 min). The increase in the proportion of organisms which propagated coherent waves varied with the temperature and pressure and was also found to depend on the age of the culture. Thus for a culture 4-7 days old a typical increase in the proportion would be from 50 % at atmospheric pressure to 70 % at a higher pressure. For older cultures the corresponding figures could be 10 and 70 %. Provided the critical pressure was not exceeded, the proportion of organisms exhibiting regular wave propagation decreased to a level similar to, or above, that obtaining before pressure was applied in the time taken for the frequency to fall to its steady level. Above the critical pressure, the proportion decayed to a level below that observed before the application of pressure.

Significant transient changes in the flagellar waveform were also consistently observed following the application of pressure. The average wave amplitude increased by about 50 % of its original value while the average wavelength remained essentially constant, although the spread of wavelengths increased at higher pressures (Pl. 1, figs. 1-6; Table 2). Both the amplitude and wavelength decreased distally to about one-half of their respective values in the proximal region (Pl. 1, figs. 2-6), in contrast to the more uniform wave pattern at atmospheric pressure (Pl. 1, figs. 1, 7, 12). These changes were generally greatest at those pressures and temperatures for which the largest transient frequency increases occurred but were also observed at pressures up

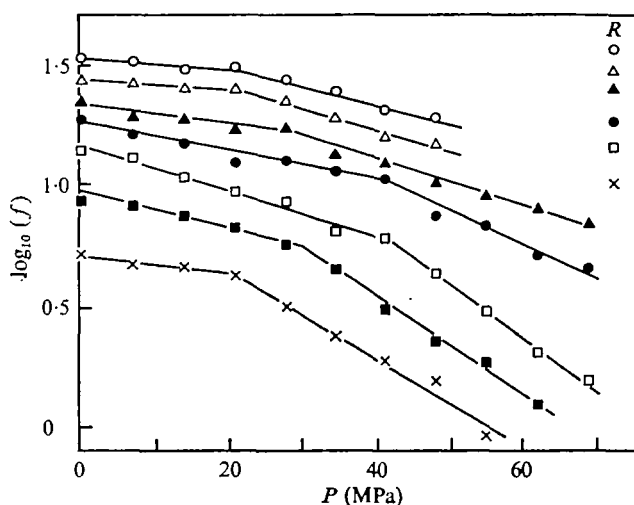
to 1.38×10^7 Pa above the critical pressure. The three-dimensional character of the flagellar waves appeared to be unchanged, but the non-uniform waves (Pl. 1, figs. 13–20) which were produced as a result of the pressure changes caused organisms close to the surface (and therefore unable to rotate freely) to swim in circular paths with radii in the region of $20 \mu\text{m}$. Organisms which were able to rotate about the axis of progression swam in linear paths. The flagella resumed the waveforms obtaining before the pressure increase in a time characteristic of the frequency decay. The immediate effect of compression was thus a stimulation of activity which was more pronounced for pressure increases up to 3.44×10^7 Pa and temperatures between 11 and 16.5°C . It was not possible, however, to establish a simple correlation between the initial frequency changes, either relative or absolute, and the magnitude of the pressure increase.

At a temperature of 34.5°C , where the wave pattern is considerably modified by temperature effects (see previous section), the application of pressures of 1.37×10^7 Pa produced no increase of frequency or other wave parameters, but caused a rapid reduction in the number of active organisms and changed the type of activity. Thus at atmospheric pressure 10% of the organisms propagated coherent waves of low amplitude, while at higher pressures the only activity observed was a low-frequency sporadic localized bending of flagella in about 5% of the organisms.

Steady-state behaviour

In a further series of experiments the pressure was raised in increments of 6.89×10^6 and 1.38×10^7 Pa from atmospheric pressure to 6.89×10^7 at each of seven temperatures in the range 3.5 – 30.5°C . At each pressure change transient effects similar to those described in the previous section were observed provided the critical pressure was not greatly exceeded. The steady frequency levels attained at each pressure were the same no matter which increment was used, and corresponded (within the limits of experimental error) to those observed at the corresponding pressure and temperature in the single-pressure experiments described in the last section. Within a period of 10 min after the steady frequency had been reached measurements were made on a minimum of 20 organisms from a sample at each pressure and the experiments were performed on at least four different samples. A few experiments were performed by varying the temperature of a sample at constant pressure. Again it was found that the steady-state frequencies at a given temperature and pressure were the same, within the limits of experimental error, as those measured in the other two types of experiment. The results from the three experimental methods were therefore used to study the effects of pressure and temperature on the steady-state frequencies.

In Text-fig. 6 the values of $\log_{10} f$, where f is the mean steady frequency, are plotted against the pressure for a number of different temperatures. The graph for a given temperature consists of two essentially linear regions, and the pressure at which the slope changes is (within the limits of experimental error) the same as the critical pressure described in the previous section. Values for the apparent activation volume (eqn 7) were calculated for the two linear parts of each graph and are shown in Table 3 and Text-fig. 7. An alternative presentation of the results is shown in Text-fig. 8 where $\log_{10}(f/T)$ is plotted against $1/T$ at each of a number of pressures. Most



Text-fig. 6. Dependence on pressure (P) of mean steady-level frequency (f) at various temperatures. Key: \circ , 30.5 °C; \triangle , 25.0 °C; \blacktriangle , 20.5 °C; \bullet , 16.5 °C; \square , 11.0 °C; \blacksquare , 6.5 °C; \times , 3.5 °C; R , the frequency levels during recovery from 68.9 MPa.

Table 3. *Changes of volume (ΔV) at various temperatures, derived from Text-fig. 6*

Temperature (°C)	ΔV_i ($10^{-6} \text{ m}^3 \text{ mol}^{-1}$)	ΔV_h ($10^{-6} \text{ m}^3 \text{ mol}^{-1}$)	ΔV_d ($10^{-6} \text{ m}^3 \text{ mol}^{-1}$)
3.5	21	103	-82
6.5	38	107	-69
11.0	50	123	-73
16.5	31	78	-47
20.5	22	53	-31
25.0	13	50	-37
30.5	11	43	-32

ΔV_i and ΔV_h are the volume changes at low and high pressures respectively and $\Delta V_d = \Delta V_i - \Delta V_h$. ΔV_d has the approximate temperature dependence,

$$\Delta V_d = (-644 + 2.07T) \times 10^{-6} \text{ m}^3 \text{ mol}^{-1},$$

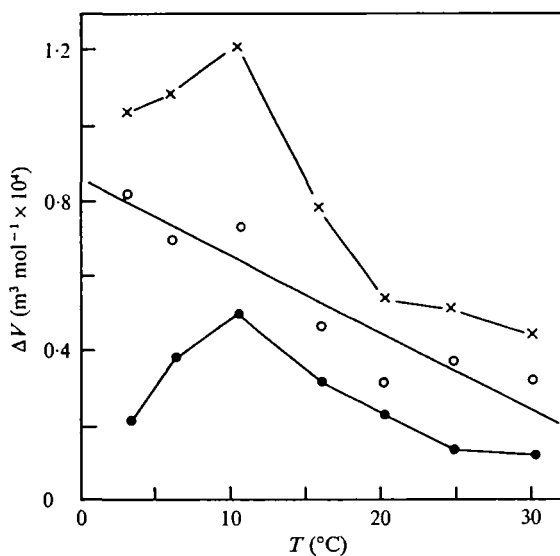
and is interpreted in the text as the volume change associated with a reversible enzyme denaturation. At temperatures above 6.5 °C ΔV_i has an approximate temperature dependence

$$\Delta V_i = (622 - 2.05T) \times 10^{-6} \text{ m}^3 \text{ mol}^{-1},$$

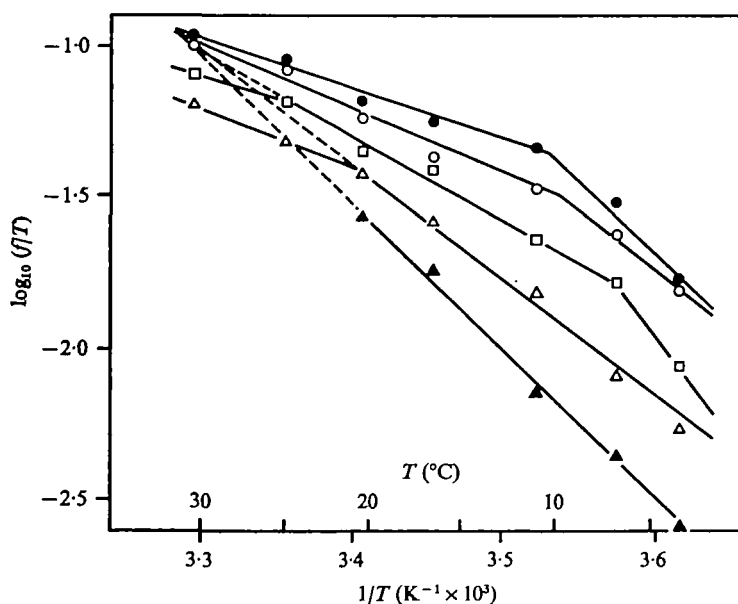
and is interpreted in the text as the activation volume ΔV_i^\ddagger associated with the decomposition of an enzyme-substrate complex.

of the curves can reasonably be represented by two or three linear portions. The apparent activation enthalpy and entropy at each pressure for the temperature region between about 10 and 20 °C are shown in Table 4 and Text-fig. 9. A more complete interpretation of these figures will be presented in the Discussion.

The patterns of movement observed with increasing pressure can be classified into five types: (i) normal flagellar waves, (ii) low-amplitude waves, often only on the distal half of the flagellum, (iii) rhythmic bending with no wave propagation, (iv) sporadic bending with no wave propagation, and (v) stationary, generally straight, flagella. In the first four groups the frequency did not appear to depend on



Text-fig. 7. Dependence on temperature (T) of the volume changes (ΔV) calculated from Fig. 6. Key: \bullet , from slopes at low pressures identified in text as ΔV_l ; \times , from slopes at high pressures, identified in text as ΔV_h ; \circ , $\Delta V_h - \Delta V_l$, identified in text as $-\Delta V_d$.



Text-fig. 8. Dependence on thermodynamic temperature (T) of mean steady-level frequency (f) are various pressures. Key: \bullet , 6.89 MPa; \circ , 20.7 MPa; \square , 34.4 MPa; \triangle , 48.2 MPa; \blacktriangle , 62.0 MPa.

Table 4. *Changes of enthalpy (ΔH) and entropy (ΔS) at various pressures derived from the temperature region 10 °C to 20 °C in Text-fig. 8*

Pressure (10 ⁶ Pa)	ΔH (kJ mol ⁻¹)	ΔS (J K ⁻¹ mol ⁻¹)
1	29	-121
68.9	32	-112
138	39	-87
207	39	-87
276	46	-65
344	54	-40
413	65	-2
482	73	23
551	87	71
620	93	89
689	104	141

At pressures below 30 MPa the changes of enthalpy and entropy are interpreted as the activation enthalpy ΔH_2^\ddagger and entropy ΔS_2^\ddagger associated with the decomposition of an enzyme-substrate complex and have the approximate pressure dependences:

$$\Delta H_2^\ddagger = (29 + 7.0 \times 10^{-7} P) \text{ kJ mol}^{-1},$$

and

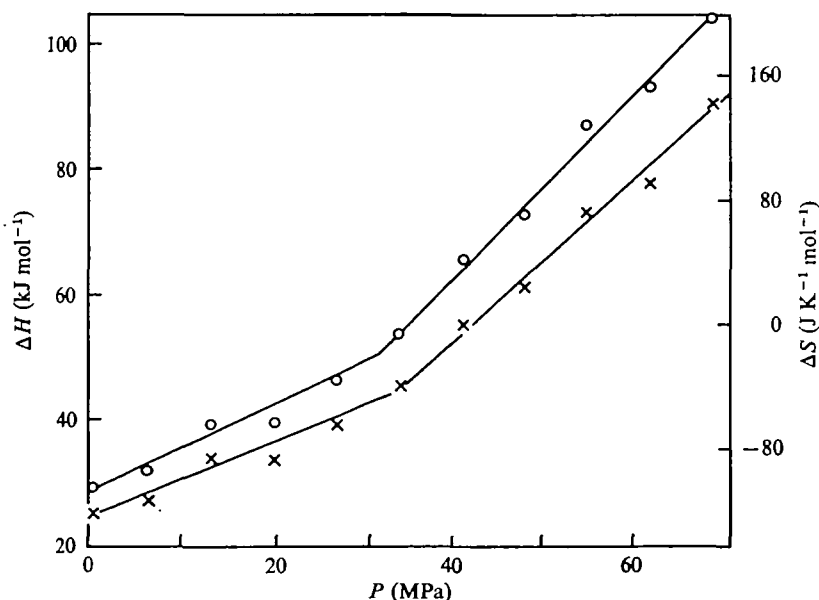
$$\Delta S_2^\ddagger = (-121 + 24.7 \times 10^{-7} P) \text{ J K}^{-1} \text{ mol}^{-1}.$$

At pressures above 30 MPa the changes of enthalpy and entropy have the approximate pressure dependences

$$\Delta H_h = (23 + 9.75 \times 10^{-7} P) \text{ kJ mol}^{-1},$$

and

$$\Delta S_h = (-216 + 51.0 \times 10^{-7} P) \text{ J K}^{-1} \text{ mol}^{-1}.$$



Text-fig. 9. Dependence on pressure (P) of the enthalpy change ΔH (O) and the entropy change ΔS (x) calculated from the mid-temperature region (10–20 °C) of Text-fig. 8. In the text the parameters at low pressures are identified as ΔH_2^\ddagger and ΔS_2^\ddagger , and the parameters at high pressures are identified as ΔH_h and ΔS_h .

Table 5. *Proportion (%) of organisms with various types of movement (described in key below) at a pressure of 68.9 MPa and several temperatures*

Temperature (°C)	Movement type				
	(i)	(ii)	(iii) (iv)	(v)	
3.5	0	0	10	90	
16.5	10	10	20	60	
25.0	0	0	10	90	
30.5	0	0	0	100	

Key. Movement type: (i) normal flagellar waves; (ii) low-amplitude waves; (iii) rhythmic bending, no wave propagation; (iv) sporadic bending, no wave propagation; (v) stationary flagella.

the type of movement. The detailed correlation between the proportion of organisms with each type of movement is complex and will be presented elsewhere. Below the critical pressure the proportion of organisms exhibiting movements of types (ii)–(v) increased as the temperature was raised or lowered from about 16.5 °C but was not markedly affected by pressure changes. Above the critical pressure the proportion of organisms with movement types (ii)–(v) increased with the pressure. The proportion of organisms which propagated normal waves at pressures above the critical value was greatest for temperatures between 11.0 and 20.5 °C. Table 5 contains a summary of the proportion of organisms with the five types of movement at a pressure of 6.89×10^7 Pa and a number of different temperatures. The number of organisms which propagated waves from base to tip was not significantly changed at any of the pressures or temperatures to which the organisms were exposed.

Effects of decompression

On decompression of a sample at a temperature below 30.5 °C from a pressure of or below 4.13×10^7 Pa to atmospheric pressure a brief period of decreased flagellar activity and irregular wave propagation was observed and no measurements were possible during this period. This period was maximal following decompression from 4.13×10^7 Pa when it lasted for about 5 min. When coherent wave propagation was established once more, the distribution of frequencies was found to be very broad, with frequencies generally in excess of those recorded before the application of pressure (Text-fig. 5). It was difficult to determine a significant average for the increase because of the broad distribution, but the relative increase was greatest at the higher temperatures. The increased level of activity following decompression lasted for up to 30 min, after which time the frequencies had decreased to the levels measured before the application of the pressure.

Decompression from 6.89×10^7 Pa to atmospheric pressure at temperatures below 30.5 °C was followed by a complete loss of any residual activity in the sample. The first sign of recovery appeared within 2–5 min and took the form of a low-amplitude sporadic flagellar wave propagation in a small proportion (about 1 %) of the organisms (Pl. 1, fig. 22). Between 10 and 20 min later, at least 50 % of the organisms had recovered normal activity. An increase of frequency above that characteristic of atmospheric pressure was observed during recovery (Text-fig. 6). Recovery of normal activity at temperatures of 3.5 and 30.5 °C was slower than at other temperatures, but

less than about 10% of the population failed to recover in 90 min. Older samples, which behaved as normal samples under pressure, took longer to recover. Little wave propagation was observed 1 h after decompression and sometimes more than 50% of the population failed to recover even after a period of 12 h.

Decompression from pressures of between 4.13×10^7 and 6.89×10^7 Pa to atmospheric pressure immediately produced levels of activity intermediate between those described above (Pl. 1, figs. 9–11). Lowering the pressure from any value to a value above atmospheric caused a loss of activity which was generally greater the larger the pressure decrement. At a temperature of 34.5 °C a decompression following 10 min of pressurization (see previous section) produced an increase to 50% of the proportion of organisms which propagated coherent waves and the observed waveforms were of increased frequency and amplitude; the frequency was about 15% greater than before application of pressure. The activity decreased to the level which obtained before the application of pressure in about 20 min.

DISCUSSION

Since changes in pressure and temperature are known to alter the viscosity of liquids it is necessary to determine whether the variation in flagellar frequency described in this paper can be accounted for by the change in viscosity of the culture medium. Over the temperature range 0–30 °C the viscosity of pure water changes from 1.8 to 0.8 mNsm⁻²; at a given temperature in this range a maximum variation of $\pm 4\%$ in the viscosity is to be expected for pressure changes in the range from atmospheric pressure to 7×10^7 Pa (Horne & Johnson, 1966). The change in the viscosity is brought about by the destruction of regions of structured water, an effect which is impeded by the addition of electrolyte (Horne & Johnson, 1967). Since the culture medium in which the organisms were grown for this study contains sodium chloride, the viscosity change which results from an alteration of pressure will be less than for pure water exposed to the same conditions. The experimental variation of frequency of *Crithidia oncopelti* with viscosity (Holwill, 1965) suggests that the pressure-induced viscosity changes would alter the frequency of this organism by 0.07 Hz, an amount well within the error of individual frequency measurements. Over the entire ranges of pressure and temperature to which organisms were exposed, the maximum alteration of frequency due to the viscosity change would be about 2 Hz, a figure small when compared to the changes (*ca.* 20 Hz) actually observed. The application of pressure produces a transient change in the temperature, but this change alone is not sufficient to account for the transient frequency variations observed. For example, a rapid increase in pressure to 1.38×10^7 Pa produced a temperature increase of 1 °C, which would increase the frequency by about 1 Hz at 15 °C (Text-fig. 2). The actual increase observed was about 5 Hz. Furthermore, the transient frequency effects were similar in magnitude for both rapid and slow increases in the pressure and the decay times of the frequency were much less than the time taken for the temperature to come to equilibrium.

It is therefore concluded that, as far as flagellar movement is concerned, the dominant effects of pressure and temperature occur within the organisms. The effects may be on the physical structure of the flagellum or on the chemical reactions which

underly its movement. In terms of the structural properties of the flagellum, a variation in the viscosity of the flagellar matrix could be produced by pressure and temperature changes. The precise nature of this material is unknown but it plays a role in the theoretical model proposed by Brokaw (1972). In the sliding filament model suggested by Brokaw, internal viscosity acts to regulate the flagellar wavelength. A variation of the internal viscosity would be expected to produce a greater frequency change than a similar change in the external viscosity, but large changes in the wavelength would also be expected. Such changes were not observed in the experiments described earlier in this paper. The viscosity of most cellular gel-sol systems such as the cytoplasm in amoeba and sea-urchin eggs (Brown & Marsland, 1936; Marsland, 1970) is reduced by increased pressure and reduced temperature. If the flagellar matrix exhibits similar properties, and its changes in viscosity due to the applied temperature and pressure control flagellar behaviour, then increased pressure or decreased temperature would be expected to produce frequency increases rather than the observed decreases. This does not mean that the model proposed by Brokaw is incorrect, but it emphasizes the need for further theoretical and experimental investigations of the internal physical behaviour of flagella so that the magnitude of changes in the wave parameters can be predicted. A further physical property which might influence the frequency and waveform and which could be affected by pressure and temperature changes is the elasticity of the microtubules. The magnitude of the effect is difficult to estimate, but if the flagellum is regarded as a bending beam (Machin, 1958) decreases of its elasticity would be expected to reduce the frequency if other parameters, such as wavelength, remain constant. Since increased pressure or decreased temperature are known to depolymerize protein subunit structures (Kettman *et al.* 1966; Murayama, 1966; Josephs & Harrington, 1968), it is possible that these effects are important. However, large changes in volume and entropy would be expected to accompany such processes. Furthermore, electron microscope studies of *C. oncopelti* (C. J. Coakley & M. E. J. Holwill, unpublished observations) and similar organisms (Marsland, 1970; Brown & Bouck, 1973) exposed to pressures up to 6.89×10^7 Pa show no significant gross changes in microtubule arrangement or structure. From this brief discussion it is apparent that the effects of changed pressure and temperature on flagellar waveforms cannot be interpreted quantitatively in terms of modified physical characteristics of the flagellum.

As mentioned earlier (see Reaction Kinetics), to interpret the reported experimental observations in terms of chemical reactions within the flagellum, the frequency will be treated initially as a rate constant (k). Rate constants are essentially averaged quantities and describe: (a) how many molecular events occur among a large number of molecules in a short time interval ($< 1/k$) at any instant, or (b) how many events on average a single molecule would experience in a time much greater than $1/k$. Since flagellar activity is evidently dependent on discrete molecular events within a structurally ordered array of enzyme molecules care should be exercised in the application to the system of a statistical treatment of reaction rates, based as it is on random collision processes. McClare (1971) has discussed a related problem and has discounted many theoretical models for the discrete molecular events which occur in muscular activity on the basis that the 'useful' free energy in these models would be thermally dissipated before the required work was done. While we adopt no specific

model here for the sequence of molecular events which occur within a flagellum, the thermodynamic quantities obtained from the results are based on classical ideas and thus represent averaged quantities. The relationship between these quantities and the molecular reactions in the flagellum may not be as simple as that implied by the assumption that the frequency is proportional to a rate constant, and this aspect of motile cellular processes in general deserves further theoretical study.

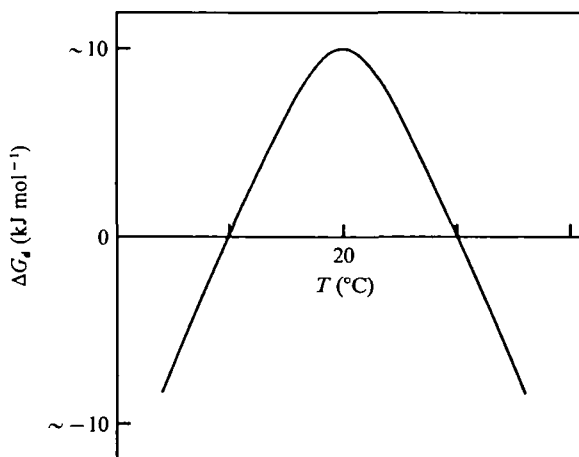
The enzymic reaction which underlies flagellar activity and which has been discussed under Reaction Kinetics in terms of the general reaction (3), can, in its simplest form, be written



where M represents a metal (probably Mg) while E_a is the flagellar enzyme (probably dynein) in an active form and P_i is inorganic phosphate. This scheme is the simplest that can account for the frequency response of chemically extracted flagella to different concentrations of ATP (see, for example, Brokaw, 1967; Brokaw & Benedict, 1968; Holwill, 1969; Gibbons & Gibbons, 1972; Douglas & Holwill, 1972). The frequency follows Michaelis-Menten kinetics, and at high substrate concentrations ($[MATP^{2-}] \gg (k_2 + k_{-1})/(k_1)$) the frequency is given by

$$f = a[E_a]k_2, \quad (10)$$

where a is a constant. It appears that several organisms may supply a sufficient excess of ATP along the flagellum by diffusion from the cell body for equation (10) to hold (Brokaw & Goldstein, 1965; Nevo & Rikmenspoel, 1970). Scheme (9) then indicates that the variation of frequency with pressure and temperature reflects the behaviour of the rate constant k_2 for the rate-determining step of enzyme-substrate complex decomposition. It is only when such simple relationships as (10) exist that the temperature or pressure dependence of macroscopic characteristics may necessarily reflect the behaviour of a rate limiting step at the molecular level. Studies on the enzymic activity of dynein from *Tetrahymena* cilia (Gibbons, 1966) indicate that the factor $a[E_a]$, which may also be influenced by pressure and temperature, is close to unity. This means that the calculated entropy changes (equation 6) are absolute values. Before proceeding with an analysis of scheme (9) we may note that at low substrate concentrations small deviations from this scheme have been reported (Brokaw & Benedict, 1971; Gibbons & Frank, 1972). If more than one substrate molecule is involved in complex formation then equation (10) is unaltered but if complex decomposition is complicated by metal ion activation or if intermediate steps are involved additional rate constants will appear in equation (10). The possibility that pressure and temperature influence the available substrate (ATP) levels is unlikely since Holwill (1969) has found that temperature influences intact and extracted flagella in a similar way, and Landau & Peabody (1963) have shown that steady ATP levels are unchanged or elevated by pressure increases. If the frequency-controlling reaction in intact flagella were to be found to take place at low substrate concentrations then the frequency would also be determined by a rate constant for the formation of an enzyme-substrate complex.



Text-fig. 10. Schematic diagram of the possible dependence on temperature (T) of the free-energy change (ΔG_d) in a two-state reversible denaturation of an enzyme with a maximum stability at 20 °C (see text for details).

We shall consider the steady-level frequency to be represented by equations (9) and (10). This interpretation is supported by observations on another flagellated organism (C. J. Coakley & M. E. J. Holwill, unpublished results) which shows no transient response to pressure increases, and will be further justified later in the Discussion. A contribution to the decrease in flagellar activity could be the inhibition by pressure of protein-protein interactions within the organelle (Penniston, 1971). This author reports that the major cause of inhibition of multimeric enzymes is the dissociation of the enzyme. Little structural information is available for the enzyme associated with flagellar movement, although the activity will surely involve interactions between proteins. Thus, although we interpret our results in terms of an enzyme reaction, an alternative explanation may lie in the dissociation, and hence inactivation of a multimeric enzyme. Further information about the flagellar enzyme *in vivo* is required to determine the importance of this effect.

The variations of the steady-level frequency with pressure and temperature (Text-figs. 2, 6, 8) strongly suggest that the flagellar frequency of *C. oncopelti* is not proportional to a single rate constant over the entire ranges of temperature and pressure used in the experiments. The form of Text-fig. 2 above 30 °C is similar to those observed for other organisms (Holwill, 1969) and is characteristic of enzyme denaturation, an effect which may explain the behaviour of flagella in this region. The process of denaturation would inactivate enzyme molecules in a random manner along the flagellum, and could thereby produce unco-ordinated flagellar movements, as observed above 30 °C. Sporadic wave propagation observed below about 10 °C was similar to that above 30 °C, and may also be due to the reversible denaturation of an enzyme. High-temperature and low-temperature enzyme denaturations have been discussed by Brandts (1964, 1967), who has shown that a number of effects such as hydrogen bonding, hydrophobic interactions and internal rotational freedom of the molecule can give rise to a temperature-dependent free-energy change for a two-state denaturation such as that shown in Text-fig. 10. The enzyme is clearly most stable at

the temperature for which $[d(\Delta G_d)]/dT = 0$, where ΔG_d is the free energy increase accompanying denaturation, and denaturation is more favoured when the temperature is raised or lowered from this value. The linear region of Text-fig. 2 between 10 and 30 °C would, on this interpretation, reflect the behaviour of the stable form of the enzyme and hence indicates the variation of the rate constant k_2 (eqn 10) with temperature. If we assume that the enzyme denaturation is independent of the enzyme reaction and that the denaturation of a certain fraction of the flagellar enzyme reduces the frequency by the same fraction, then $f = (a[E_a]k_2)/(1 + K_d)$, where K_d is the equilibrium constant for the denaturation process. The denaturation enthalpy and entropy in the low-temperature region are approximately given by

$$\Delta H_d = \Delta H_2^* - \Delta H_1 = -55.5 \text{ kJ mol}^{-1}$$

$$\text{and } \Delta S_d = \Delta S_2^* - \Delta S_1 = -195 \text{ J K}^{-1} \text{ mol}^{-1} \quad (\text{see Table 1}).$$

Although the activation parameters ΔH_1 and ΔS_1 fall close to the line of Text-fig. 3 and may thus suggest a change in the rate-determining step at lower temperatures, the large negative values obtained above for ΔH_d and ΔS_d support the view that they characterise a low-temperature enzyme denaturation (Brandts, 1967). The results taken at the higher temperatures were not obtainable over a sufficient temperature range to make a similar determination of the thermodynamic parameters associated with the high-temperature denaturation. However, both the enthalpy and entropy changes are clearly large and positive.

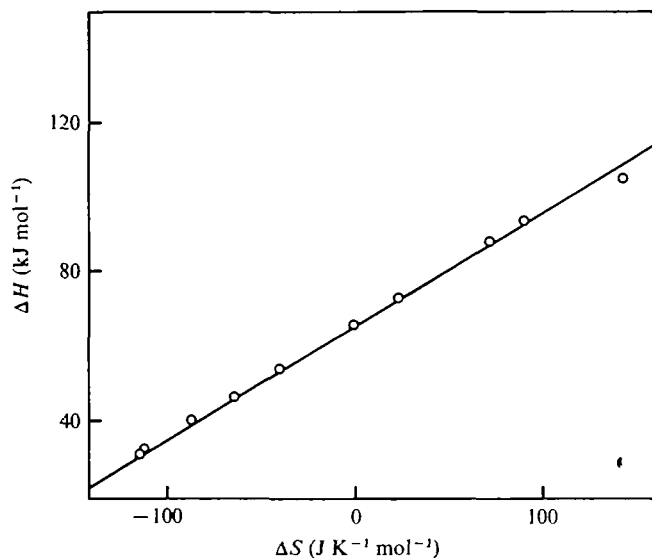
Text-figs. 6 and 8 indicate that for *C. oncopelti* the variations of steady-level frequency with pressure and temperature do not follow the predictions of equation (6) if the rate constant k is replaced by the frequency f . At a given temperature, above the critical pressure, flagellar movement becomes sporadic and is similar in this respect to the behaviour at high and low temperatures for a particular pressure. It is therefore reasonable to consider that flagellar behaviour above the critical pressure results in part from reversible enzyme denaturation while below this pressure the variation in the steady frequency solely reflects the behaviour of the rate constant k_2 . If the enzyme reaction and denaturation of the enzyme are independent, the volume change (ΔV_d) for denaturation is approximately given by

$$\Delta V_d(T) = \Delta V_1(T) - \Delta V_h(T).$$

The volume change ΔV_d is always negative and has an approximately linear relationship with temperature, becoming less negative with increasing temperature (Text-fig. 7). The denatured form of the enzyme thus occupies a smaller volume than the active form and is more favoured at high pressures and lower temperatures.

The results presented here are consistent with the hypothesis that the effects of pressure and temperature on flagellar activity result from changes in the equilibrium of a reversible thermal enzyme denaturation in the flagellum. Only over rather limited pressure and temperature ranges, within which inactivation of the enzyme is unimportant, may the frequency variation be attributed to changes in the rate constant (k_2) of the enzymic reaction in scheme (9).

The values for the enthalpy change ΔH_2^* obtained in the present study (Table 1) are of the order commonly observed for enzymic reactions in solution and indicate that no overall change in covalent bonding, for which $\Delta H = 200\text{--}400 \text{ kJ mol}^{-1}$, occurs



Text-fig. 11. Relation between the enthalpy change (ΔH) and the corresponding entropy change (ΔS) at various pressures. The data are plotted from Text-figs. 8 and 9.

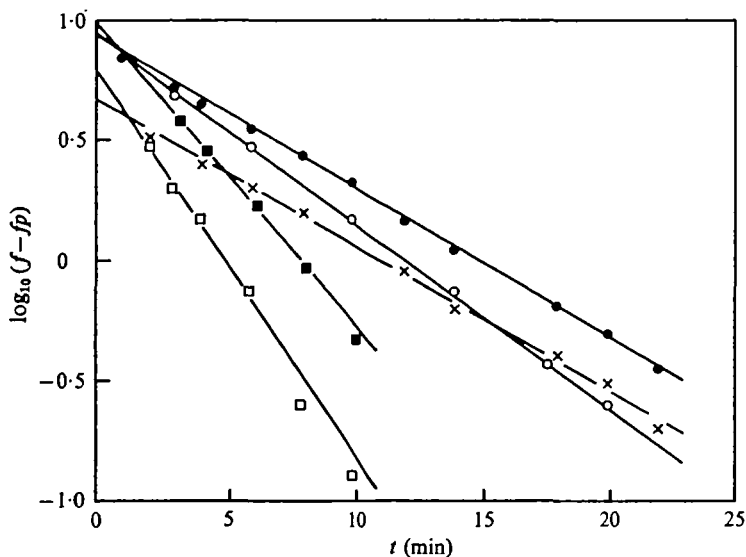
during the reaction. One effect of the enzyme, apart from its important structural role, is to lower the activation enthalpy of the non-catalysed reaction. The enthalpy associated with the non-enzymic hydrolysis of ATP varies between 88 and 122 kJ mol⁻¹ depending on the pH (Friess, 1953; Laidler, 1958). The results of the present study suggest that a flagellar enzyme associated with movement reduces this enthalpy to 29 kJ mol⁻¹.

The enthalpy change at a series of pressures (Table 4) is linearly related to the corresponding entropy change (Text-fig. 11) by the relation

$$\Delta H = T_c \Delta S + 65 \text{ kJ mol}^{-1},$$

where $T_c = 305 \text{ K}$. This is not a thermodynamic necessity and indicates that at, and close to, temperatures of 32 °C variations with pressure of the enthalpy change are compensated by similar variations in the entropy change. Compensation effects have also been noted in the thermodynamic parameters derived from the flagellar activity of different organisms (Holwill & Silvester, 1967) while other types of compensation phenomena have been discussed by Lumry & Rajender (1970).

After a transient frequency change caused by a change of pressure (Text-fig. 5), the variation of frequency with time is approximately exponential (Text-fig. 12) and the relaxation time implies an effective rate constant of about 10^{-3} sec^{-1} . A significant quantitative correlation between the pressure change and the relaxation time or the frequency change relative to the initial frequency was not possible. It may be noted, however, that the difference between the frequency immediately after a pressure change and the subsequent steady frequency was approximately constant and independent of pressure. This provides some justification of the assumption in the previous interpretation of the variations of steady frequency with pressure and temperature that the transient changes were superimposed effects. These facts and the other



Text-fig. 12. Demonstration of the exponential decay of the transient increased frequency (f) to the steady level frequency (f_s). The points are taken from the curve in Text-fig. 5. Key: \circ , 13.8 MPa, 16 °C; \square , 27.6 MPa, 16 °C; \times , 55.1 MPa, 16 °C; \times , 27.6 MPa, 12 °C; \square , 41.3 MPa, 16 °C.

transient changes (see Results) are not readily susceptible to analysis in terms of a kinetic mechanism in which a steady state process is perturbed by a pressure change and then relaxes to another steady level.

Several explanations, possibly combined, of the transient effects can be envisaged. A pressure change might (a) temporarily alter the concentrations of available substrates (e.g. ATP), activating ions or other as yet unknown chemical species; (b) temporarily change the rates of diffusion processes by modifying the structure of the material through which substrate and other necessary materials diffuse; (c) perturb the behaviour of enzyme systems such as the ATPase reaction, an adenylate kinase or other enzymes responsible for controlling the wave parameters, or induce the removal of inhibition present under normal conditions; (d) cause a time-dependent denaturation process; or (e) cause a mechanical or electrical stimulation. It is difficult to discriminate positively between these mechanisms since additional biochemical evidence and a more detailed evaluation of the events immediately following a pressure change are needed for rigorous tests.

Although there are no reports in the literature on the combined effects of pressure and temperature on ciliary or flagellar activity, a few accounts describe the effects of pressure or temperature separately, and it is of interest to compare the results of these investigations with the present work. Although the authors of the various papers did not interpret their results using the kinetic approach adopted in this study, it is possible to plot the appropriate graphs from their results and hence to obtain thermodynamic parameters for comparison with those obtained for *C. oncopelti*. In general, the graphs obtained are similar in form to those for *C. oncopelti* described in the Results, and the derived parameters (Tables 6 and 7) are of the same order of magnitude. The values calculated for *Spirostomum ambiguum* (Table 6) and *Mytilus edulis*

Table 6. *Changes of volume (ΔV) for several organisms*

Organism	Measured activity	ΔV_d^\ddagger ($10^{-6} \text{ m}^3 \text{ mol}^{-1}$)	ΔV_d ($10^{-6} \text{ m}^3 \text{ mol}^{-1}$)	Temperature (°C)	Critical pressure (10^5 Pa)	Reference
<i>Stentor polymorphus</i>	Membranellar frequency	8.7	-44	13-20	170	Kitching (1957)
<i>S. polymorphus</i>	Membranellar frequency	2.2	-22	20 (?)	440	Sleigh (1962)
<i>Spirostomum ambiguum</i>	Forward swimming speed	37	-190	20 (?)	440	Kitching (1969)
<i>Epistilis plicatilis</i>	Ciliary activity	-12	—	19-21	—	Kitching (1957)

The significance of ΔV_d^\ddagger , ΔV_d and the critical pressure is explained in the text.

Table 7. *Changes of enthalpy (ΔH) and entropy (ΔS) at atmospheric pressure for several organisms*

Organism	Measured activity	ΔH_d^\ddagger (kJ mol ⁻¹)	ΔS_d^\ddagger (J K ⁻¹ mol ⁻¹)	ΔH_d (kJ mol ⁻¹)	ΔS_d (J K ⁻¹ mol ⁻¹)	Reference
<i>Mytilus edulis</i>	Fluid velocity near gill cilia	54	Indeterminate	-23	(ΔS_d^\ddagger - 54)	Gray (1923)
<i>Stentor polymorphus</i>	Membranellar frequency	28	-102	40 (high temperature) -18 (low temperature)	153 (high temperature) -45 (low temperature)	Sleigh (1956)
<i>Paramecium</i>	Frequency of peristomal cilia	57	-19	57 (high temperature) (low temperature denaturation begins at about 6 °C)	196	Machemer (1972)

The significance of ΔH_d^\ddagger , ΔS_d^\ddagger , ΔH_d and ΔS_d is explained in the text.

(Table 7) were obtained from observations on forward swimming speed or fluid current velocity and therefore do not necessarily represent thermodynamic parameters associated with ciliary activity. In this respect it is significant that similar preliminary experiments with *C. oncopelti* revealed that an increase of pressure produced a significant decrease in the average swimming speed with an apparent activation volume of $8 \times 10^{-6} \text{ m}^3 \text{ mol}^{-1}$. The decrease in swimming speed was shown later to be due to the variations of flagellar activity described earlier so that the parameters derived solely from a study of the effects of pressure or temperature on swimming speed are not necessarily those associated with the fundamental flagellar movement. It would be interesting to study the behaviour of these and other organisms by using the methods discussed in this paper.

In this paper we have presented results which are consistent with the hypotheses that the response of flagella to changes in pressure and temperature reflect the effect of these factors on (a) the equilibrium of a reversible enzyme denaturation process, and (b) the rate of decomposition of an enzyme-substrate complex. Further information would be obtained by investigating the response to pressure and temperature changes of other organisms, of chemically extracted flagella and of isolated dynein, adenylate kinase and possibly other flagellar enzymes responsible for wave initiation

and control. Further study of pressure-induced transient responses is desirable and its discovery in more elementary systems or isolated components will be needed for its explanation.

SUMMARY

1. The combined effects of hydrostatic pressure and temperature on the flagellar movements of *Crithidia oncopelti* were studied.

2. Provided that a critical pressure, which depended on the temperature, was not exceeded, the application of pressure produced an initial increase in flagellar frequency; the frequency then fell to a steady level which was below the value obtaining before the application of pressure.

3. The compression also caused a transient increase in the number of organisms propagating coherent waves, an increase in the average wave amplitude, and asymmetric waveforms with decreasing wavelength and amplitude towards the flagellum tip.

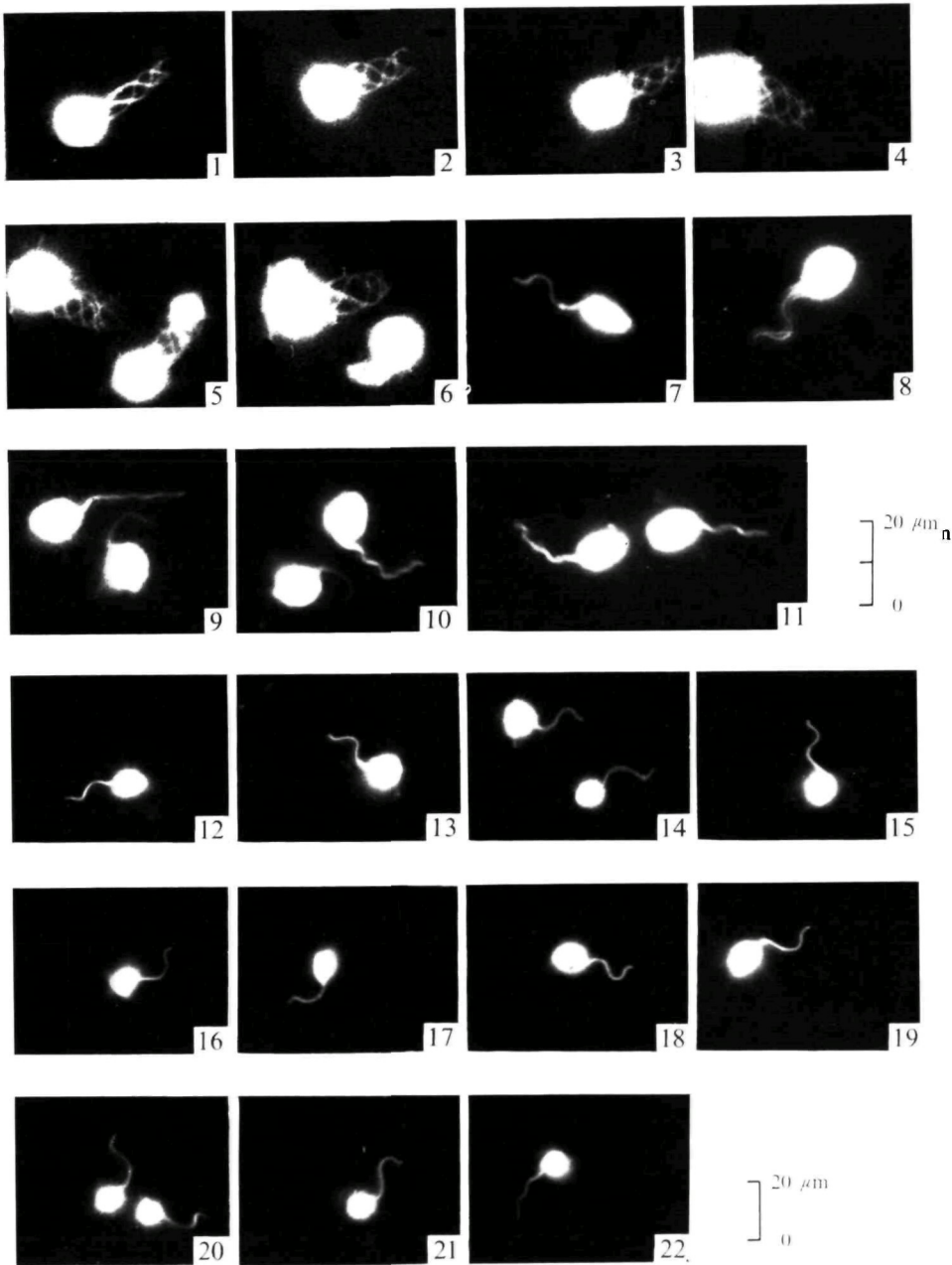
4. The results are consistent with the hypotheses that the observed flagellar response is due to (a) changes in the equilibrium of a reversible enzyme denaturation process, and (b) changes in the rate of decomposition of an enzyme-substrate complex.

5. Values of the volume, enthalpy and entropy changes associated with each process are derived and their significance for mechanism is discussed.

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EXPLANATION OF PLATE I

Dark-field photographs of the flagellum of *Crithidia oncopelti* taken within 2 min after pressure changes at 16 °C. (The excess background on some photographs has been blackened.)

Figs. 1-6. Multiple exposures showing amplitude increases and their decay towards the flagellum tip. Flash rate 40 s⁻¹. Fig. 1. Atmospheric pressure. Figs. 2, 3. 27.6 MPa. Figs. 4-6. 55.1 MPa.

Figs. 7-11. Single exposures showing recovery after decompression. Fig. 7. Atmospheric pressure. Fig. 8. 27.6 MPa. Figs. 9-11. Decompression to atmospheric pressure from 55.1 MPa.

Figs. 12-22. Single exposures showing symmetric and asymmetric waveforms. Fig. 12. Atmospheric pressure. Fig. 13, 14. 27.6 MPa. Figs. 15-17. 41.3 MPa. Figs. 18, 19. 55.1 MPa. Figs. 20, 21. 68.9 MPa. Fig. 22. Decompression to atmospheric pressure from 68.9 MPa.